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Welcome Remarks

Dear conference participant,

On behalf of the conference committee, we cordially welcome you at the

3rd Annual Meeting of NGFN-Plus and NGFN-Transfer in the
Program of Medical Genome Research
25th – 27th November 2010 at Henry-Ford Building, FU Berlin.

An impressive range of top results from the genome research community is shown in **symposia** and **accompanying poster exhibitions** entitled *Genomics of Common Disease, Animal, Cellular & Tissue Models, Systems Biology, Transfer from Genomics to Application,* and *New Technologies*. It is this range of topics and the gathering of the community that is attracting hundreds of scientists that registered already for the NGFN Meeting 2010.

To all members of NGFN-Plus and NGFN-Transfer, this meeting is an excellent opportunity to meet collaborators and friends, to reinforce existing co-operations and to launch new ones for future projects. We are also pleased to welcome many former members of NGFN-1 and NGFN-2, and all scientists and visitors to take actively part in the scientific discussion and exchange.

The conference starts on November 25 with **two satellite symposia** that focus on hot topics: *Next-Generation Sequencing* and *small RNAs.* Here, novel results and powerful cutting-edge technology relevant for genome research will be discussed.

In the main program on November 26 and 27, internationally renowned keynote speakers will open each symposium with an overview lecture. In each symposium, four scientists of the NGFN will present their latest results on selected topics. On Friday the highlight **Evening Lecture** will be given by Prof. Regine Kollek on *Gene Diagnostics* and its future impact with respect to the technological achievements and general conditions of ethics and justice.

The top three posters will be awarded the *Annemarie Poustka* ***Poster Award*** *for Medical Genome Research 2010* sponsored by Roche Diagnostics in order to acknownledge the achievements of young scientists, and in memory of the late Prof. Dr. Annemarie Poustka. Annemarie Poustka made outstanding achievements in the field of Genome Research and was a visionary scientist for the NGFN.

Company satellite lunch sessions complement the programm and the **industrial exposition** offers comprehensive information on latest technology developments

We are happy to welcome all experts in German genome research in the pleasant atmosphere of the Henry Ford Building at FU Berlin. For their generous support to host this conference in Berlin, we thank Prof. Hans Lehrach and Prof. Martin Vingron, Max Planck Institute for Molecular Genetics, and Prof. Volker A. Erdmann, Freie Universität Berlin.

This conference convenes outstanding scientists in the field of medical genome research. The event offers the exceptional opportunity of information about the latest developments, presentation of scientific results, and discussion and interaction with most competent researchers in a highly dynamic atmosphere.

Finally: Don't miss the **get-together** on Friday evening with the scientific community in a relaxed ambience with good wine, tasty finger food and nice music!

Heidelberg, November 15, 2010

PD Dr. Stefan Wiemann Prof. Dr. Hugo A. Katus

(As Spokespersons for the Project Committee of NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research)

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PD Dr. Stefan Wiemann
DKFZ Heidelberg

Prof. Dr. Wolfgang Wurst
Helmholtz Zentrum München

Program-at-a-glance

Thursday, November 25th

12.00 – 1.45 pm	**Satellite Symposium I - Session I: Next-Generation Sequencing**
1.45 – 2.00 pm	***Coffee Break***
2.00 – 4.00 pm	**Satellite Symposium I – Session II: Next-Generation Sequencing**
4.00 – 4.30 pm	***Coffee Break***
4.30 – 7.00 pm	**Satellite Symposium II: Small RNAs**
7.00 pm	***Supper***

Friday, November 26th

8.30 – 10.15 am	**Symposium I – Genomics of Common Disease I** Mark Lathrop (Keynote) – Thomas W. Mühleisen – Susanne Lucae – André Reis – Vera Kalscheuer
10.15 – 10.45 am	**Coffee Break**
10.45 – 11.15 am	**Welcome:** **Hugo A. Katus,** Heidelberg University Hospital, Germany, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research **Frank Laplace,** Federal Ministry of Education and Research Germany **Hans Lehrach,** Max Planck Institute for Molecular Genetics, Berlin, Germany
11.15 – 1.00 pm	**Symposium I – Genomics of Common Disease II** Xavier Estivill (Keynote) – Andre Franke – Christian Kubisch – Anja Bauerfeind – Jeanette Erdmann
1.00 – 3.00 pm	***Lunch Break and Poster Session I***
1.00 – 3.00 pm	**Company Satellite Lunch Sessions** Fluidigm Europe BV - Illumina– Applied Biosystems Europe
3.00 – 4.45 pm	**Symposium III – Animal, Cellular and Tissue Models** Andrea Ballabio **(**Keynote) – Jens Siveke – Kaomei Guan – Jan Deussing – Bernd Timmermann
4.45 – 5.15 pm	**Coffee Break**
5.15 – 7.00 pm	**Symposium IV – Systems BIology** Anne-Claude Gavin (Keynote) – Reinhold Schäfer – Özgür Sahin – Michaela D. Filiou – Jean-Fred Fontaine
7.00 – 8.00 pm	**Evening Lecture:** Regine Kollek
8.00 – 10.00 pm	***Get-Together (Wine, fingerfood, music)***

Saturday, November 27th

9.00 - 10.45 am	**Symposium V – Transfer from Genomics to Application** Max Hasmann (Keynote) – Michal-Ruth Schweiger – Katja Werner – Heike Bruck – Elena Syurina
10.45 – 12.45 pm	***Lunch Break and Poster Session II***
10.45 – 12.45 pm	**Company Satellite Lunch Sessions:** Affymetrix Europe – Sequenom
12.45 – 1.00 pm	**Ceremony: "Annemarie Poustka Poster Award of Medical Genome Research 2010" sponsored by Roche Diagnostics GmbH**
1.00 – 2.45 pm	**Symposium VI: New Technologies** Philip Rosenstiel– Ralf Sudbrak – Norman Klopp – Sebastian Eck – George Church (Keynote)
2.45 – 3.00 pm	**Concluding Remarks:** Stefan Wiemann, German Cancer Research Center - DKFZ, Heidelberg, Germany, Speaker Project Committee of NGFN-Plus/NGFN-Transfer in the Program of Medical Genome Research

Program

Thursday, November 25th, 2010	
12.00 – 1.45 pm	**Satellite Symposium I - Session I: Next-Generation Sequencing**
1.45 – 2.00 pm	***Coffee Break***
2.00 – 4.00 pm	**Satellite Symposium I – Session II: Next-Generation Sequencing**
4.00 – 4.30 pm	***Coffee Break***
4.30 – 7.00 pm	**Satellite Symposium II: Small RNAs**
7.00 pm	***Supper***

Friday, November 26th, 2010

Symposium I: Genomics of Common Disease I

8.30 – 9.15 am	**Keynote: Mark Lathrop**, Centre National de Génotypage, Evry, France *Medical and public health applications of genomics*
9.15 – 9.30 am	**Thomas W. Mühleisen**, Institute of Human Genetics, University of Bonn, Germany *Genome-wide association study and comprehensive follow-up strongly supports Neurocan (NCAN) as a novel susceptibility gene for bipolar disorder*
9.30 – 9.45 am	**Susanne Lucae,** Max Planck Institute of Psychiatry, Munich, Germany *The neuronal transporter gene SLC6A15 confers risk to major depression*
9.45 – 10.00 am	**André Reis**, University Erlangen-Nuremberg, Germany *MRNET – German Mental Retardation Network – a platform for systematic identification of genes for mental retardation*
10.00 – 10.15 am	**Vera Kalscheuer**, Max Planck Institute for Molecular Genetics, Berlin, Germany *Systematic mutation search in families with XLMR by next-generation sequencing*
10.15 – 10.45 am	***Coffee Break***

Welcome

10.45 – 11.15 am	**Hugo A. Katus,** Heidelberg University Hospital, Germany, Speaker Project Committee of NGFN-Plus/NGFN-Transfer in the Program of Medical Genome Research **Frank Laplace,** Federal Ministry of Education and Research Germany **Hans Lehrach,** Max Planck Institute for Molecular Genetics, Berlin, Germany

Symposium II: Genomics of Common Disease II

11.15 – 12.00 pm	**Keynote: Xavier Estivill**, CRV – Center for Genomic Regulation, Barcelona, Spain *Structural variation analysis by large-scale human genome sequencing*
12.00 – 12.15 pm	**Andre Franke**, Christian-Albrechts-University Kiel, Germany *Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci*
12.15 – 12.30 pm	**Christian Kubisch,** University of Ulm, Germany *Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1*
12.30 – 12.45 pm	**Anja Bauerfeind**, Max Delbrueck Center, Berlin, Germany *A conserved trans-acting regulatory locus underlies an inflammatory gene network and susceptibility to autoimmune type 1 diabetes*
12.45 – 1.00 pm	**Jeanette Erdmann**, University of Luebeck, Germany *CARDIoGRAM: Thirteen novel genetic loci affecting risk of coronary artery disease*
1.00 – 3.00 pm	***Lunch Break and Poster Session I*** *(1.00 – 2.00 pm odd numbers, 2.00 – 3.00 pm even numbers)*

1.00 – 3.00 pm Company Satellite Sessions

1.15 – 1.45 pm	**Simon Margerison,** Senior Sales Application Specialist**, Fluidigm Europe B.V.,** Amsterdam, Netherlands *High Throughput Gene Expression Profiling of Single Cells*
1.50 – 2.20 pm	**Richard Henfrey**, Associate Director Marketing Europe & Stephanie Brooking, Sequencing Segment Specialist Europe, **Illumina Europe** *Advances in Illumina Next-Generation Sequencing and Array Solutions that Enable Biological Discovery*
2.25 – 2.55 pm	**Raimo Tanzi,** Director, Business Development Next Generation Sequencing**, Applied Biosystems Europe**, Darmstadt, Germany *From Proton Sequencing to Achieving 99.99% Accuracy: Recent Advances in the Life Technologies Sequencing Portfolio*

Symposium III: Animal, Cellular & Tissue Models

3.00 – 3.45 pm	**Keynote: Andrea Ballabio,** Telethon Institute of Genetics and Medicine – TIGEM, Naples, Italy *The genetic control of lysosomal function and of cellular clearance*
3.45 – 4.00 pm	**Jens Siveke**, TU – Munich, Germany *Mouse models endogenous pancreatic cancer – role of EGFR and Notch signalling*
4.00 – 4.15 pm	**Kaomei Guan**, Georg-August-University of Goettingen, Germany *Generation of functional cardiomyocytes from patient-specific induced pluripotent stem cells*
4.15 – 4.30 pm	**Jan Deussing**, Max Planck Institute of Psychiatry, Munich, Germany *CRHR1, a key regulator of stress, regulates anxiety in opposite directions by controlling glutamatergic and dopaminergic neurons*
4.30 – 4.45 pm	**Bernd Timmermann**, Max Planck Institute for Molecular Genetics, Berlin, Germany *Identification of a new peroxiredoxin allele with a phenotype of oxidant-resistance and premature aging by whole genome resequencing of yeast.*
4.45 – 5.15 pm	***Coffee Break***

Symposium IV: Systems Biology

5.15 – 6.00 pm	**Keynote: Anne-Claude Gavin**, EMBL, Heidelberg, Germany *Biochemical approaches to biomolecular networks*
6.00 – 6.15 pm	**Reinhold Schäfer**, Charité, Universitätsmedizin Berlin, Germany *Identification of Y-box binding protein 1 as a core regulator of MEK/ERK pathway-dependent gene signatures in colorectal cancer cells*
6.15 – 6.30 pm	**Özgür Sahin**, German Cancer Research Center (DKFZ), Heidelberg, Germany *Genome-wide miRNA level regulation of ErbB receptor protein network in breast cancer*
6.30 – 6.45 pm	**Michaela D. Filiou**, Max Planck Institute of Psychiatry, Munich, Germany *Proteomics and metabolomics analysis for biomarker discovery in a trait anxiety mouse model*
6.45 – 7.00 pm	**Jean-Fred Fontaine**, Max Delbrueck Center for Molecular Medicine, Berlin, Germany *Orthology-based phenotypic inference from disease models to human applications*

Evening Lecture

7.00 – 8.00 pm	**Regine Kollek**, University of Hamburg, FSP BIOGUM, FG Medizin, Hamburg, Germany *Genomes and people: How do they come together?*
8.00 – 10.00 pm	***Get-together (Wine, Cheese, Music)***

Saturday, November 27th, 2010

Symposium V: Transfer from Genomics to Application

9.00 – 9.45 am	**Keynote: Max Hasmann**, Roche Diagnostics GmbH, Penzberg, Germany *Targeted combination therapy of HER2-positive breast cancer*
9.45 – 10.00 am	**Michal-Ruth Schweiger**, Max Planck Institute for Molecular Genetics, Berlin, Germany *A next generation genome-wide view of (epi)genetic alterations in clinically distinct colon cancers.*
10.00 – 10.15 am	**Katja Werner**, Can GmbH, Hamburg, Germany *PROCEED SP10: Molecular tumor imaging using antibody-coated nanoparticles; Construction of antibody conjugated fluorescent nanoparticles for in vivo imaging of prostate cancer cells.*
10.15 – 10.30 am	**Heike Bruck**, University of Duisburg-Essen, Germany *NTCVD-Consortium identified known and new proinflammatory and profibrotic biomarkers in patients with chronic kidney disease*
10.30 – 10.45 am	**Elena Syurina**, Maastricht University, The Netherlands *Identification of the gaps in integration of genome-based knowledge in the International Public Health legislation*
10.45 – 12.45 pm	***Lunch Break and Poster Session II*** *(10.45 - 11.45 am odd numbers, 11.45 - 12.45 pm even numbers)*

10.45 – 12.45 pm	**Company Satellite Sessions:**

10.55 – 11.25 am **Dr. Nick Brain**, **Affymetrix Europe**
Technology advances:
- Powerful custom genotyping from 50K to 5M SNPs
- Unparalleled performance for allele-specific copy number detection in FFPE samples

11.40 – 12.10 pm **Dr. Caren Vollmert, Dr. Henning Gohlke, Sequenom GmbH,** Hamburg, Germany
Translating Genomic Discovery into Human Health –
The MassARRAY® for DNA Methylation and Somatic Mutation Profiling

12.45 – 1.00 pm	**Poster Award Ceremony:**

12.45 – 1.00 pm **Ceremony: "Annemarie Poustka Poster Award of Medical Genome Research 2010" sponsored by Roche Diagnostics GmbH**

Symposium VI: New Technologies

1.00 – 1.15 pm **Philip Rosenstiel,** Institute of Clinical Molecular Biology, University Kiel, Germany
Whole genome sequence of a Crohn disease trio – a paradigm for etiology discovery in complex disease?

1.15 – 1.30 pm **Ralf Sudbrak**, Max Planck Institute for Molecular Genetics, Berlin, Germany
A map of human genome variation from population scale sequencing (the 1000 Genomes Project)

1.30 – 1.45 pm **Norman Klopp**, Helmholtz Zentrum München, Germany
Whole genome amplification in large biobanks

1.45 – 2.00 pm **Sebastian Eck**, Helmholtz Zentrum München, Germany
Analysis pipeline for exome sequencing data

2.00 – 2.45 pm **George Church,** Harvard Medical School, Boston, USA (Live broadcast videoconference)
Technologies for Collecting and Integrating Genome, Environment and Trait data

2.45 – 3.00 pm **Concluding Remarks: Stefan Wiemann**, German Cancer Research Center (DKFZ), Heidelberg, Germany, Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

National Genome
Research Network

Satellite Symposia

3rd Annual Meeting NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research

Henry Ford Building, FU Berlin

Satellite Symposium I
Next-Generation Sequencing

November 25th, 2010

Scientific Organization: Bernhard Korn, Institute for Molecular Biology (IMB), Mainz, Germany

12:00 pm	Introduction & Chair	**Bernhard Korn**
Session 1		
12:05 pm	Microdroplet-based PCR Amplification for Large Scale Targeted Sequencing **Steve Picton, RainDance Technologies, Inc.,Lexington MA, USA**	
12.25 pm	Fully automated library preparation for the Illumina Genome Analyzer – a comparison with the manual method **Kathryn Stemshorn, Cologne Center for Genomics, Germany**	
12.45 pm	Private Variants' – a common cause of disease **Alexander Hoischen, Nijmegen Centre for Molecular Life Sciences, The Netherlands**	
1.05 pm	NGS to Examine the Transcriptome: A Focus on ncRNAs and Viruses" **John Castle, TrOn GmbH, Mainz, Germany**	
1.25 pm	Sequencing using Semiconductor Technology **Armin Winands, Ion Torrent, Inc., San Francisco, USA**	
1.45 – 2.00 pm	***Coffee Break***	
Session 2		
2.00 pm	Complete Human Genome Sequencing for Large-Scale Disease Studies **Rick Tearle, Complete Genomics, Inc., Mountain View, CA, USA**	
2.20 pm	Cool Runnings with the Roche 454 GS Junior **Ralph Oehlmann, IMGM Laboratories GmbH, Martinsried, Germany**	
2.40 pm	High performance genomics with Galaxy on the Ground and in the Clouds **Nate Coraor, PennState University, Pennsylvania, USA**	
3.00 pm	NGS Data Analysis: Genomic and Transcriptomic Variants Identification – Characterization – Visualization **Martin Seifert, Genomatix Software GmbH, Munich, Germany**	
3.20 pm	A comprehensive platform for full-genome data analysis **Roald Forsberg, CLC bio, Aarhus, Denmark**	
3.40 pm	From third generation sequencing to a multi-scale biology approach to understanding and treating human disease **Tobias W.B. Ost, Pacific Biosciences, Menlo Park, CA, USA**	
4.00 – 4.30 pm	***Coffee Break***	

3rd Annual Meeting NGFN-Plus and NGFN-Transfer in the Program of Medical Genome Research

Henry-Ford Building, FU Berlin

Satellite Symposium II
Small RNAs

November 25th, 2010

Scientific Organization & Chairs:
Jürgen Brosius, University of Münster, Germany
Jürgen Haas, Ludwig-Maximilians University Munich, Germany

Sessions	
4:30 pm	OncomiR addiction in a mouse model of cancer **Pedro Medina, Yale University, New Haven, USA**
5.15 pm	Deep sequencing of small RNAs, mRNA targets, and primary transcriptomes in bacterial pathogens **Jörg Vogel, University of Wuerzburg, Germany**
5.45 pm	Role of cytomegalovirus microRNAs in virus pathogenicity **Lars Dölken, Ludwigs-Maximilians University, Munich, Germany**
6.15 pm	Sustained overexpression of microRNA mimics using a lentiviral delivery system **Stephanie Urschel, Thermo Scientific, Bonn, Germany**
6.35 pm	LNA™ based Universal RT microRNA PCR system **Adam Baker, Exiqon, Vedbaek, Denmark**
7.00 pm	End of Workshop
7.00 pm	**Supper**

Satellite Symposium I
Next-Generation Sequencing

Microdroplet-based PCR Amplification for Large Scale Targeted Sequencing

Presenting Author: Dr. Steve Picton

RainDance Technologies, Inc., Lexington, MA, USA

RainDance Technologies' (RDT) innovative RainStorm™ microdroplet-based technology produces picoliter-volume droplets at a rate of 10 million per hour, avoiding complex automation solutions. Each droplet is the functional equivalent of an individual test tube. Droplets are processed on a disposable chip that has no moving parts or valves. When this technology is applied to targeted sequence enrichment we create a workflow that minimize the process-induced bias or error commonly associated with multiplexed technologies.
RainDance's Sequence Enrichment application focuses on the targeted sequencing of the human genome. Leveraging the RainStorm™ microdroplet-based technology, this application enables the high-resolution analysis of genetic variation between individuals within populations at a level unmatched by current methodologies. Next-generation sequencing technologies are rapidly advancing the researcher's measurement capability but in the sequence enrichment step, there is a critical need for cost-effective solutions that target specific genomic regions with high specificity and sensitivity, enabling the detection of both rare and common variants.
We will present and discuss the technology in detail and review its use as well as giving an insight to future applications including base specific Methylation analysis

Fully automated library preparation for the Illumina Genome Analyzer – a comparison with the manual method

Presenting Author: Dr. Kathryn Stemshorn
Janine Altmüller, Christian Becker, Peter Frommolt, Elisabeth Kirst & Peter Nürnberg

Cologne Center for Genomics (CCG), Universität zu Köln, Zülpicher Straße 47, 50674 Köln

The next generation sequencing platform at the CCG currently consist of three Illumina GAIIx sequencers and one GS FLX sequencer from Roche. Sequencing projects include whole genome shotgun sequencing, targeted sequencing, de novo sequencing, RNA-Seq, ChiP-Seq und smallRNA-Seq on organisms ranging from Arabidopsis over nematodes to humans.
In order to streamline our library preparation especially for Illumina sequencing we were on the search for an automated library prep system and implemented the SPRIworks system from Beckman Coulter in our sequencing pipeline.
In our first experiment, the following libraries were prepared with the SPRIworks system: two paired-end libraries which were subsequently whole-exome-enriched using AgilentSure select, two RainDance-enriched libraries and four standard paired-end libraries.
The SPRIworks system consistently produced libraries of good quality (high yield, narrow size-selection, no adaptor peaks) and similar yields for the specific samples. Generally size-selection was more accurate than during manual preparation and a gel-size-selection step, which can potentially lead to contaminations and is also time-consuming, was not necessary anymore.
The cluster densities on the flowcell ranged from 244192 – 306983 clusters per tile, which lies in the same area as manually produced libraries when the same quantity of library is loaded (7 pM). The percentage of clusters that passed filters ranged from 80 % to 92 % which is also comparable to manually produced libraries. Of the sequences produced for the whole-exome enriched libraries 91 % mapped to the target region on average. This is a high percentage compared to previous experiments with manually produced whole-exome enriched libraries, where only 76 % mapped to the target region. The error rates range from 0.17 – 2.2 %, which is again comparable to manually produced libraries.
Generally handling of the SPRIworks system is easy and due to its full automation very time-saving. Hands-on time to start the machine is 0.5 h as compared to 4.5 h to manually conduct fragment-end polishing, A-tailing, adapter-ligation and size-selection for 10 libraries. Library yields are very consistent. Therefore there are clear benefits of this system over manual library preparation, making up for the higher costs per library.

'Private variants' - a common cause of disease

Presenting Author: Dr. Alexander Hoischen

Department of Human Genetics, Nijmegen Centre for Molecular Life Sciences,

Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

We applied large scale human exome sequencing as a genomewide mutation detection method to identify the genes underlying monogenic Mendelian disorders. This unraveled the cause of a dominant disorder by analyzing exomes of 4 affected individuals with Schinzel-Giedion syndrome (Hoischen et al.; Nat Genet. 2010 Jun;42(6):483-5). We identified heterozygous de novo mutations in SETBP1. All missense mutations clustered to a ultra-high conserved 11bp exonic region, which suggests a dominant-negative or gain-of-function effect. Interestingly SETBP1 overlapping CNVs are known to cause different phenotypes, which explains why conventional approaches did not reveal any candidate loci. More recently we showed that for a recessive condition even a single individual can be sufficient to identify a disease causing mutation, it is possible to find the causative gene by sequencing the exome of a single sporadic patient (Gilissen et al. Am J Hum Genet. 2010 Sep;87(3):418-23). Exome sequencing is particularly useful for identifying these types of mutations for which no other genomewide approach is applicable. One limitation is the current inability to reliably identify structural genomic variation associated with disease, however we have first indications that coverage data from exome sequencing experiments can be used to identify CNVs. De novo mutations and structural variations may be a frequent cause of rare syndromes but also other sporadic conditions with reduced fecundity such as congenital malformations, mental retardation, and psychiatric disorders.

NGS to Examine the Transcriptome: A Focus on ncRNAs and Viruses

Presenting Author: Dr. John Castle

TrOn GmbH, Mainz, Germany

Next-generation sequencing (NGS) combines classical nucleic acid sequencing, such as to determine transcript sequences, with an ability to extract millions of such sequence reads in an unbiased fashion and in proportion to their underlying abundance. Here, we probe the transcriptome using NGS with a simple amplification protocol that preserves the original RNA strand and that amplifies all RNA except rRNA. In eleven human tissues, we explore not only mRNAs but also ncRNA, tRNA, and lincRNAs and find ncRNAs 7SL, U2, 7SK, and HBII-52 are expressed at levels far exceeding mRNAs; C/D and H/ACA box snoRNAs, which are associated with rRNA methylation and pseudouridylation, respectively: are both expressed in spleen, while hypothalamus expresses mainly C/D box snoRNAs, and testes show enriched expression of H/ACA box snoRNAs along with RNA telomerase TERC.
Further, we examine the transcriptome of mice +/- SARS viral infection and demonstrate how NGS sensitively and specifically detects the SARS vRNA.

Sequencing using Semiconductor Technology

Presenting Author: Dr. Armin Winands

Ion Torrent Inc., San Francisco, US

Ion Torrent Systems has developed a DNA sequencing system that directly translates chemical signals into digital information on a semiconductor chip. This approach leverages a trillion dollars of investment from the semiconductor industry taking advantage of existing state-of-the-art chip fabrication technology, and the entire semiconductor design and supply chain. Unprecedented scalability and cost reduction result from decades of Moore's Law advances in semiconductor technologies that are brought to bear within a few years for DNA sequencing.

Ion Torrent sequencing takes place in semiconductor microchips that contain sensors which have been fabricated as individual electronic detectors, allowing one sequence read per sensor. Current configurations have 1.5 million sensors in a 1 cm^2 chip, with proof of principle to enable densities over 100 million sensors per chip.

The sequencing chemistry itself is remarkably simple. Native nucleotides are incorporated into the growing strand by native DNA polymerase. As a base is incorporated, a direct electrical measurement of the incorporation event is made and the sequence is read out directly into the digital domain. Thus, sequencing is direct, efficient, and massively parallel, requiring no specialized reagents and no optical systems. Using native DNA chemistry with real time detection enables run times to be very short, on the order of an hour or two with a throughput on the order of 100 Megabases per run.

Complete Human Genome Sequencing for Large-Scale Disease Studies

Presenting Author: Dr. Rick Tearle

Complete Genomics, Inc. Mountain View, CA, USA

Complete Genomics has developed a novel sequencing platform intended specifically for large-scale sequencing of complete human genomes for biomedical research. Hundreds of genome sequences have now been generated using this method, including germ-line sequences of affected and normal individuals as well as tumor genome sequences. We will briefly review technical aspects of this platform and discuss scientific results and data analysis strategies from recent family studies of inherited disorders as well as studies of somatic mutations in tumor-normal comparisons.

Cool Runnings with the Roche 454 GS Junior

Successful Setup and Validation of Targeted Re-sequencing Applications

Presenting Author: Dr. Ralph Oehlmann
Dr. Ralph Oehlmann, Dr. Carola Wagner, Dipl. Biol. Stefan Kotschote

IMGM Laboratories GmbH, Martinsried, Germany

With the advent of small, powerful and affordable Next Generation Sequencing (NGS) systems, such as the Roche 454 GS Junior, NGS is now no longer reserved for large sequencing centers, but is now also available for laboratories in universities and industrial companies.
Due to its long read length, short run time and the flexibility to analyze multiple samples in parallel, the 454 GS Junior is a powerful tool for next generation targeted re-sequencing applications.
However, with hardly any re-sequencing kits currently available, all users have to setup their own NGS workflows from assay development and target enrichment to sequence generation and data analysis.
At IMGM Laboratories, we have successfully implemented efficient workflows for amplicon-based targeted re-sequencing applications based on the GS FLX and GS Junior systems. Our target enrichment approach uses the IMGM Assay Development Pipeline in combination with the Fluidigm Access Array platform. With Access Arrays, target amplification and 454-specific library preparation can be combined elegantly in one single step. In just under 4 hours, 2,304 nanoliter PCR reactions can be carried out to generate 48 individual bar-coded sequencing-libraries consisting of 48 target specific amplicons each. These bar-coded libraries can then be combined and subjected to emulsion PCR and consecutive parallel sequencing. Upon mapping of the reads to a reference sequence, secondary data analysis tools are applied to identify sequence variations present in the samples analyzed.
In practice, before a targeted re-sequencing assay can be run as a routine application, it has first to pass a thorough validation process. Among other parameters checked, NGS data have to match or exceed the quality of data generated by conventional Sanger sequencing. Other important factors such as the minimum required sequence coverage or specific software analysis settings need to be addressed before an assay can go live.

High performance genomics with Galaxy on the ground and in the clouds

Presenting Author: Dr. Nate Coraor
Nate Coraor, Enis Afgan, Dannon Baker, Dan Blankenberg, Greg Von Kuster, Jeremy Goecks, Anton Nekrutenko, James Taylor

PennState University, Pennsylvania, Emory University, Atlanta, USA

We have developed a solution that allows experimentalists to perform large-scale analysis using a variety of high performance computing resources (including the Cloud) with nothing more than a web browser (http://usegalaxy.org). Using Galaxy, a user without computational expertise can instantiate an analysis environment on a cloud, and can add storage and compute resources to this environment as needed. Because the solution is built on the Galaxy framework, analyses using this solution are accessible, transparent, and reproducible. Popular tools and workflows for analyzing of next generation sequence data from various types of experiment are built-in and ready to run.

NGS Data Analysis: Genomic and Transcriptomic Variants Identification - Characterization - Visualization

Presenting Author: Dr. Martin Seifert
Jochen Supper, Matthias Scherf, Martin Seifert

Genomatix Software GmbH, Munich, Germany

Next Generation Sequencing (NGS) has enabled researchers to generate large amounts of genome wide data, with genotyping (DNAseq) and expression analysis (RNAseq) being two main application areas. Here, we present NGS analysis strategies which take the following aspects into account:
Genotyping:
- Uniqueness of mapped reads/ paralogous
- Dependency of copy numbers and SNP ratio expectation
- Functional SNP and structural variant characterization

Expression Analysis:
- Differential gene expression
- Splice- and genefusion analysis
- Transcriptome viewer

The strategies will show the possibilities for biological interpretation and are based on real live examples taking advantage of the background data available within the Genomatix databases.

A comprehensive platform for full-genome data analysis

Presenting Author: Dr. Roald Forsberg

CLC bio, Aarhus, Denmark

High-throughput sequencing of DNA is presently becoming technologically and financially accessible to many areas of biomedical research. The new technologies and applications that have emerged offer great promise of exciting new scientific discoveries and are heralding a general shift in biomedical research from a gene centric to a genome centric research paradigm. However, a major bottleneck in the dissemination of these technologies is the bioinformatics handling and analysis of sequencing data. CLC bio's software platform is developed as an answer to these challenges and offers researchers an integrated analysis solution that can assist in removing the bioinformatics bottleneck and in optimizing the use of bioinformatics personnel- and hardware resources within organizations.

National Genome
Research Network

Satellite Symposium II
Small RNAs

OncomiR addiction in a mouse model of cancer

Presenting Author: Prof. Dr. Pedro Medina

Yale University, New Haven, U.S.A.

MicroRNAs (miRNAs) belong to a recently discovered class of small RNA molecules that regulate gene expression at the post-transcriptional level. miRNAs have crucial functions in the development and establishment of cell identity, and aberrant metabolism or expression of miRNAs has been linked to human diseases, including cancer. Components of the miRNA machinery and miRNAs themselves are involved in many cellular processes that are altered in cancer, such as differentiation, proliferation and apoptosis. Some miRNAs, referred to as oncomiRs, show differential expression levels in cancer and are able to affect cellular transformation, carcinogenesis and metastasis, acting either as oncogenes or tumour suppressors. MicroRNA-21 (miR-21) is a unique miRNA in that it is overexpressed in most tumour types analysed so far. Despite great interest in miR-21, most of the data implicating it in cancer have been obtained through miRNA profiling and limited in vitro functional assays. To explore the role of miR-21 in cancer in vivo, we used Cre and Tet-off technologies to generate mice conditionally expressing miR-21. Overexpression of miR-21 led to a pre-B malignant lymphoid-like phenotype, demonstrating that mir-21 is a genuine oncogene. When miR-21 was inactivated, the tumours regressed completely in a few days, partly as a result of apoptosis. These results demonstrate that tumours can become addicted to oncomiRs and support efforts to treat human cancers through pharmacological inactivation of miRNAs such as miR-21.

Deep sequencing of small RNAs, mRNA targets, and primary transcriptomes in bacterial pathogens

Presenting Author: Prof. Dr. Jörg Vogel

Max Planck Institute of Infection Biology, Berlin, Germany; Institute of Molecular Infection Biology, University of Würzburg, Germany

Experimental strategies based on deep sequencing of cDNA (RNA-seq) have become increasingly important for the discovery of both small noncoding RNAs (sRNAs) and their cellular targets (1). I will present results of genome-wide screens in the model pathogens, Salmonella typhimurium and Helicobacter pylori. To determine post-transcriptional regulons governed by Salmonella sRNAs, we developed a generic method analysing the RNA that is targeted by the common bacterial Sm-like protein, Hfq (2). Sequencing of RNA derived from coIP with epitope-tagged Hfq showed that ~20% of all Salmonella mRNAs were Hfq-bound in vivo. The thus predicted sRNA targets include transcripts of horizontally acquired pathogenicity islands, alternative sigma factor regulons, and the flagellar gene cascade. To explore the transcriptional organization and noncoding RNA output of Helicobacter, we used a novel differential approach (dRNA-seq) selective for the 5' end of primary transcripts (3). dRNA-seq discovered hundreds of transcriptional start sites within operons, indicating uncoupling of polycistrons, and revealed massive genome-wide antisense transcription. Importantly, Helicobacter lacking an Hfq homolog was found to express an unexpected wealth of sRNAs including the ε-proteobacterial counterpart of the regulatory 6S RNA and associated pRNAs, and potential regulators of cis- and trans-encoded mRNA targets. This study established a paradigm for mapping and annotating the primary transcriptomes of many living species. References:

(1) Sharma CM, Vogel J (2009) Experimental approaches for the discovery and characterization of regulatory small RNA. Current Opinion in Microbiology, 12(5):536-46
(2) Sittka A et al. (2008) Deep sequencing analysis of small noncoding RNA and mRNA targets of the global post-transcriptional regulator, Hfq. PLoS Genetics 4(8):e1000163
Sharma CM et al. (2010) The primary transcriptome of the major human pathogen Helicobacter pylori. Nature 464(7286):250-5

Role of Cytomegalovirus MicroRNAs in Virus Pathogenicity

Presenting Author: Dr. Lars Dölken

Ludwig-Maximilians Universität München, Germany

MicroRNAs (miRNAs) are small, non-coding RNA molecules that regulate gene expression at post-transcriptional level in virtually all eukaryotic organisms. The recent discovery of miRNAs of viral origin has dramatically changed our view on virus-host interaction, but their real importance during infection of their hosts remains elusive. The human cytomegalovirus (HCMV) is the major cause of morbidity in immunocompromised patients and allogenic bone-marrow or organ-transplant recipients and the leading cause of congenital birth defects. HCMV encoded miRNAs may thus provide valuable targets for new urgently needed antiviral drugs.

To elucidate the role of CMV miRNA function in vivo we identified and characterized viral miRNAs encoded by the murine cytomegalovirus (mCMV) during lytic infection and latency using next generation sequencing. In addition, we performed a two-sided approach to elucidate their function. On the one hand, knock-out mutants of individual MCMV miRNAs were generated and extensively studied in vivo. On the other hand, Ago2-RISC immunoprecipitation followed by microarray analysis (RIP-Chip) was performed to identify their targets. Together, our results point towards miRNA-based immunoevasion mechanisms important for long-term virus persistence and host-to-host transmission.

Keywords: host genetics, cytomegalovirus, miRNAs, in vivo, MCMV

Sustained Over-expression of microRNA Mimics using a Lentiviral Delivery System

Presenting Author: Dr. Stephanie Urschel

Thermo Fisher Scientific, Bonn, Germany

microRNA (miRNA) mimics and inhibitors are frequently employed to better understand the contributions of non-coding RNAi to cell physiology. Currently, the predominant method for over-expressing mature miRNAs involves delivery of synthetic mimics into target cells that can be readily transfected. To further expand the collection of tools available to researchers, and expand into cell lines that are difficult to transfect, we have developed a new lentiviral-based miRNA expression platform. This uses a unique miRNA scaffold for efficient processing and improved functionality. Strategies used to optimize robust miRNA expression will be presented along with data describing their overall performance in mammalian cell culture models.

LNA™ based Universal RT microRNA PCR system. A new generation high throughput QPCR platform optimized for development microRNA based molecular diagnostic assays on clinical FFPE and blood serum and plasma

Presenting Author: Dr. Adam Baker

Exiqon A/S, Diagnostic Product Development, Vedbaek, Denmark

Background
Blood derived serum or plasma are important bio-fluids that potentially hold critical biomarker information about disease diagnosis and prognosis. miRNAs are altered in many types of cancer. They are found superior to mRNA for classification of cancer and other diseases. Lately, miRNAs are emerging as a new class of blood-based biomarkers. However, their small size and the limiting amount of sample available present a challenge for the sensitivity of the detection system.
Method
Using a Locked Nucleic Acid (LNA™) based miRNA detection technology we have developed a high throughput QPCR system for genome wide detection of miRNAs in clinical paraffin-embedded tissue as well as blood derived plasma or serum. The use of the LNA™ bases adds critical specificity and sensitivity creating a more robust system for more rapid assay generation in the clinical and diagnostic assay development.
Results
Our LNA™-based PCR system provides a truly sensitive miRNA genome wide screening technology from extremely small volumes of blood derived serum or plasma. In addition, the system is ideally suited for screening laser captured and macro-dissected tissue specimens allowing us to build extremely accurate and sensitive miRNA expression profiles from critical tumor biopsies.
Conclusions
We used the LNA™-based PCR system to screen miRNAs in colorectal cancer patient plasma samples and their matching tumor samples. We have been able to identify miRNAs in both the blood derived plasma and tumors that are differentially expressed between patients and healthy controls.

Main Program

Main Program

26th to 27th November 2010

Program (with speakers' biosketch)

Friday, November 26th, 2010

Symposium I: Genomics of Common Disease I

8.30 – 9.15 am ***Opening Keynote Presentation***

Medical and public health applications of genomics

Mark Lathrop

Mark Lathrop, Director of the Centre National de Génotypage, Evry, France

Positions and Honours

2005-	Scientific Director, Centre d'Etude de Polymorphism Humain, Paris, France
1998-	Scientific Director, Centre National de Génotypage, Evry, France
1996-1998	Professor of Human Genetics, University of Oxford, Oxford, UK
1994-1998	Scientific Director, Wellcome Trust Centre for Human Genetics, Oxford, UK
1994-1998	Wellcome Trust Principal Fellow
1988-1994	Directeur de recherche, INSERM, Paris, France
1986-1988	Howard Hughes Medical Institute, University of Utah, Salt Lake City, USA
1983-1988	Chargé de recherche, INSERM, Paris, France
1982-1983	Maître de Conférence, Musée de l'Homme, Paris, France
1980-1982	MRC Post-doctoral fellowship, Musée de l'Homme, Paris, France
1977-1980	Ph.D. in Biomathematics, University of Washington, Seattle, USA

9.15 – 9.30 am

Genome-wide association study and comprehensive follow-up strongly supports Neurocan (NCAN) as a novel susceptibility gene for bipolar disorder

Thomas Mühleisen

Thomas W. Mühleisen is a post-doctoral scientist at the Department of Genomics at the Life & Brain Center in Bonn, Germany. His scientific career began in 2002 at the Max-Planck-Institute for Brain Research in Frankfurt, Germany in the Department of Neuroanatomy, where he investigated differential gene expression in the developing retina using a cDNA microarray screen as part of his PhD thesis.

Since he joined the Department of Genomics in 2005, Thomas has developed a strong interest in methods or the genetic analysis of complex neuropsychiatric phenotypes. His research focus is on the identification of genetic risk variants in bipolar disorder and schizophrenia using genome-wide association studies, analysis of copy number variants, and re-sequencing analyses.

His work has already resulted in a number of scientific presentations and publications. At the World Congress on Psychiatric Genetics 2010, he received an Early Career Investigator Programme award for presenting genetic findings in bipolar disorder coming from the MooDS network.

9.30 – 9.45 am

The neuronal transporter gene SLC6A15 confers risk to major depression

Susanne Lucae

Susanne Lucae, MD, PhD

Susanne Lucae studied biology at the University of Vienna, Austria, and received her medical training at the University of Vienna Medical School. She performed her PhD thesis at the Institute of Pathophysiology, University of Vienna, where she worked in the field of molecular biology of autoimmune disorders. She received her clinical training in psychiatry at the Max Planck Institute of Psychiatry in Munich, Germany. Presently she is staff member of the outpatient clinic of psychiatry. Since 2007 she is head of the research group “Psychiatric Pharmacogenetics” at the Max Planck Institute of Psychiatry.

9.45 – 10.00 am ***MRNET – German Mental Retardation Network – a platform for systematic identification of genes for mental retardation***

André Reis

André Reis studied medicine and received his faculty affiliation from Charité, Humboldt University Berlin in 1995. He was appointed group leader at the Max-Delbrück-Centre where he founded and led the "Gene Mapping Centre" from 1995-2000. In 1998 he became associate professor in Berlin and since 2000 holds the chair of Human Genetics at the University of Erlangen-Nuremberg. He served as member of the steering committee of the German Human Genome Project (DHGP) from 2001-2004. In Erlangen he is currently speaker of the Interdisciplinary Centre for Clinical Research (IZKF) and chairman of the University Senate. Moreover he serves as president of the German Society of Human Genetics (GfH) and is a member of Leopoldina National Academy of Sciences. Research in his laboratory focuses on elucidation of the molecular basis of both Mendelian and complex traits using positional cloning and on detection of genotype-phenotype correlations. Current topics include the genetic underpinnings of psoriatic arthritis, glaucoma and intellectual disability.

10.00 – 10.15 am ***Systematic mutation search in families with XLMR by next-generation sequencing***

Vera Kalscheuer

Vera Kalscheuer graduated with a Ph.D in Biochemistry from the Free University Berlin in 1992. She was a post-doctoral fellow at the Department of Human Genetics, University Hospital Nijmegen, The Netherlands. After this she became a group leader in the Department of Prof. H.H. Ropers at the Max Planck Institute for Molecular Genetics, Berlin. Her current research focuses on the elucidation of genes involved in the pathogenesis of cognitive and developmental disorders.

10.15 – 10.45 am ***Coffee Break***

Welcome

10.45 – 11.15 am **Hugo A. Katus**, Heidelberg University Hospital, Speaker Project Committee of NGFN-Plus/NGFN-Transfer in the Program of Medical Genome Research

Frank Laplace, Federal Ministry of Education and Research, Germany

Hans Lehrach, Max Planck Institute for Molecular Genetics, Berlin, Germany

Symposium II: Genomics of Common Disease II

11.15 – 12.00 pm

<u>Opening Keynote Presentation</u>

Structural variation analysis by large-scale human genome sequencing

Xavier Estivill

Xavier Estivill gained the doctorate in Medicine by the Autonomous University of Barcelona, and in Philosophy by the University of London for studies on the molecular genetics of cystic fibrosis. He is Senior Investigators and Coordinator of the Genes and Disease Program at the Centre for Genomic Regulation (CRG) and Investigator of the Public Health and Epidemiology Network Biomedical Research Center (CIBERESP), Associate Professor of the Pompeu Fabra University, and Director of the Barcelona Spanish Genotyping Center. Achievements of his group are the identification of the genes and mutations that cause several monogenic disorders, and the identification of genes involved in Down syndrome. He has published over 400 research papers and the group of his group has received several awards. Current work of his group is focused on the analysis of complex disorders, with special interest in the role of structural variants, epigenetic changes and non-coding RNAs. His group is applying next generation sequencing technologies to reveal the genetic basis of complex traits and diseases. He coordinates an EU action to analyze the genetic variability of disease (GEUVADIS). Recent achievements of his group have been on the identification of genetic variants associated with psoriasis and several psychiatric diseases.

12.00 – 12.15 pm

Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci

Andre Franke

Andre Franke earned his doctorate at the Christian-Albrechts-University Kiel in 2006. Since then, he has been leading the genetics and bioinformatics group within the institute of Clinical Molecular Biology (ICMB). He is also in charge of the high-throughput facilities at the ICMB. In August 2008, being 29 years old, Dr. Franke became Juniorprofessor within the DFG excellence cluster "Inflammation at Interfaces". In December 2008, Dr. Franke was awarded with the 100,000 Euro large "Hensel prize". In April 2010, he received the W2 Peter Hans-Hofschneider endowment professorship of the foundation for Experimental Biomedicine.

Dr. Franke's main interests are the development and establishment of novel high-throughput genetic/genomic technologies, the inherent bioinformatic integration and the application of both to identify the genetic and epigenetic causes of chronic inflammatory diseases like Crohn's disease and psoriasis. Having worked primarily on genome-wide association studies for the last years, Dr. Franke's research agenda currently focuses on targeted enrichment strategies, whole-genome and -exome resequencing, and copy number variation analyses.

12.15 – 12.30 pm ***Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1***

Christian Kubisch

Christian Kubisch is Professor for Human Genetics and Director of the Institute of Human Genetics at the University of Ulm since 08/2010, before he was Professor for Medical Genetics at the University of Cologne since 2004. From 1988-1995, he studied medicine in Bonn, his thesis dealt with molecular mechanisms of cardiac hypertrophy. From 1995-1999 he was involved in the identification and functional analyses of human disease genes in hereditary forms of human epilepsy and deafness as a post-doc in the ZMNH in Hamburg (AG T. Jentsch). In 1999, he became Assistant Professor in the Institute of Human Genetics at the University of Bonn. During the next years in Bonn and Cologne he and his group were involved in the further identification of disease genes in different monogenic and complex inherited human disorders. He finished his medical specialisation in Human Genetics in 2003. His current projects cover diverse genetic studies in mainly neurological/sensory diseases with a specific focus on the genetics of migraine, neurodegeneration, and hearing loss. He received the Heinz-Maier-Leibnitz Award of the German Research Foundation (DFG) in 2000 and the Early Career Award of the National Academy of Sciences Leopoldina in 2010.

12.30 – 12.45 pm ***A conserved trans-acting regulatory locus underlies an inflammatory gene network and susceptibility to autoimmune type 1 diabetes***

Anja Bauerfeind

Anja Bauerfeind is currently working as a research scientist in the group for Experimental Genetics of Cardiovascular Diseases headed by Prof. Norbert Hübner at the Max-Delbrück-Centrum in Berlin, Germany. Her primary research interest is in the analysis of common complex diseases, in particular in integration of experimental findings with human GWAS and large-scale biological datasets.

Anja Bauerfeind studied Mathematics at the University of Applied Science in Berlin, Germany. After she got her Diploma in 1997 she worked as a research associate at the Institute of Pharmacoepidemiology, FU Berlin. In 2001, she moved to the MDC and graduated with a PhD degree in the Bioinformatics group of Prof. Jens Reich.

12.45 – 1.00 pm	***CARDIoGRAM: Thirteen novel genetic loci affecting risk of coronary artery disease***
	Jeanette Erdmann

Jeanette Erdmann, born 1965, is head of the working group „Cardiovascular Genomics" at the University of Lübeck. Together with Heribert Schunkert she is coordinating several large national and international consortia (Atherogenomics (NGFNplus), Cardiogenics, and CARDIoGRAM). The aim is to identify and to understand the genetic basis of cardiovascular diseases. In the past years the group was very successful in identifying genetic variants associated with CAD/MI, indeed the majority of CAD/MI related SNPs have been identified with a contribution of the Lübeck group. In the next years the group will expand the scientific profile and focus more on system biological aspects, integrating genomic, transcriptomic and metabolomic data. In addition, the group has established a pipeline to generate and phenotype transgenic mouse models to further understand the role of the newly identified risk genes.
Jeanette Erdmann has studied Biology at the University of Köln (1985-1990) and finished her PhD thesis at the Institute of Human Genetics in Bonn (1995). She has worked as PostDoc at the DHZB in Berlin (1995-2000) and the University of Regensburg (2000-2003). Since 2004 she is working at the University of Lübeck.

1.00 – 3.00 pm	***Lunch Break and Poster Session I*** ***(1.00 – 2.00 pm odd numbers, 2.00 – 3.00 pm even numbers)***

1.00 – 3.00 pm	**Company Satellite Sessions**
1.15 – 1.45 pm	**Simon Margerison,** Senior Sales Application Specialist**, Fluidigm Europe B.V.,** Amsterdam, Netherlands *High Throughput Gene Expression Profiling of Single Cells*
1.50 – 2.20 pm	**Richard Henfrey**, Associate Director Marketing Europe & **Stephanie Brooking**, Sequencing Segment Specialist Europe, **Illumina Europe** *Advances in Illumina Next-Generation Sequencing and Array Solutions that Enable Biological Discovery*
2.25 – 2.55 pm	**Raimo Tanzi,** Director, Business Development Next Generation Sequencing**, Applied Biosystems Europe**, Darmstadt, Germany *From Proton Sequencing to Achieving 99.99% Accuracy: Recent Advances in the Life Technologies Sequencing Portfolio*

Symposium III: Animal, Cellular & Tissue Models

3.00 – 3.45 pm

Opening Keynote Presentation

The genetic control of lysosomal function and of cellular clearance

Andrea Ballabio

Andrea Ballabio was born in Naples, Italy on January 27th, 1957. After his graduation in Medicine in 1981 at the University of Naples, Italy, he took residency training in Pediatrics at the same university. He has spent several years working in the field of genetic diseases, first in Italy, then in the UK and subsequently in the USA where he held the positions of Associate Professor of Molecular Genetics and of Co-director of the Human Genome Center at the Baylor College of Medicine, Houston, Texas. He is the founder and director of the Telethon Institute of Genetics and Medicine (TIGEM) in Naples, Italy, He is also Professor of Medical Genetics at the Faculty of Medicine of the University of Naples “Federico II” and Professor of Molecular and Human Genetics at Baylor College of Medicine in Houston, Texas.
Prof. Ballabio’s research interests are the elucidation of the biological mechanisms underlying genetic diseases, using both traditional and state of the art genomic approaches, and the development of innovative therapeutic approaches. Prof. Ballabio’s team identified numerous genes whose mutations cause human inherited diseases, leading to the unraveling of their pathogenetic mechanisms. Among his recent discoveries is the identification of a gene network regulating cellular clearance (Sardiello et al., *Science*, 2009). He has published over 250 papers in prestigious, international scientific journals. Prof. Ballabio was the President of the European Society of Human Genetics and is a Council member of the European Molecular Biology Organization (EMBO). He has received numerous national and international awards for research and culture, among which the 2007 International Award of the European Society of Human Genetics. In 2007 he was appointed “Commendatore of the Italian Republic” by the President of Italy Giorgio Napolitano. In 2010 he was awarded the Advanced Investigator grant of the European Research Council.

3.45 – 4.00 pm

Mouse models endogenous pancreatic cancer – role of EGFR an Notch signalling

Jens Siveke

Jens Siveke Date & Place of Birth: May 8th 1973; Hamburg, Germany
Current address: 2. Medical Department, Klinikum rechts der Isar, Technische Universität München

Education

1994-2001	Medical School, University of Hamburg
1996-1999	M.D. thesis (*summa cum laude*): “T helper 1/2 cells: chemokine-induced chemotaxis and chemokine receptor expression”
1999-2000	Biomedical Exchange Program, New England Medical Center, Tufts University, Boston, MA, USA

Professional Positions & Qualifications

10/2009	Oberarzt (consultant)
09/2009	Habilitation, TU München
01/2009	Board for Internal Medicine
02/2003-09/2009	Fellow & lab head, 2. Med. Department, Klinikum rechts der Isar, TUM
2001-2003	Resident, Med. Policlinic, Klinikum Innenstadt, LM

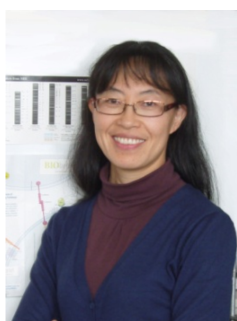
Awards

1999	Research Award, Biomedical Exchange Program
2006 & 2007	Conference Prize, DGVS
2007	Research Prize of the Society of Gastroenterology Bavaria
2008	Rising Star of the ASNEMGE (Association of National European and Mediterranean Societies of Gastroenterology)

4.00 – 4.15 pm

Generation of functional cardiomyocytes from patient-specific induced pluripotent stem cells

Kaomei Guan



Kaomei Guan obtained her Master of Science in 1993 at Beijing Normal University, and then worked as a research assistant there. In 1995 she joined the lab of Prof. Dr. Anna M. Wobus in IPK Gatersleben, Germany. In 1998 she received her Ph.D. from Berlin Humboldt University. From 1999 to 2000, K. Guan worked as a Postdoc in Prof. Wobus's lab. In 2001, she moved to Goettingen, and established the "Stem Cell Lab" at Department of Cardiology and Pneumology at Georg-August-University. The major part of her research focuses on studying pluripotent stem cells and their applications in regenerative medicine. Currently, she is in charge of several projects supported by the German Research Foundation (GU595/2-1) and the Federal Ministry of Education and Research (01GN0822, 01GN0957). She has received several research prizes including the "August Wilhelm und Lieselotte Becht-Forschungspreis" from Deutschen Stiftung für Herzforschung. In addition, she is also a member of Editorial Board of European Journal of Heart Failure.

4.15 – 4.30 pm

CRHR1, a key regulator of stress, regulates anxiety in opposite directions by controlling glutatmatergic and dopaminergic neurons

Jan Deussing

Jan Deussing studied Biology at the University of Goettingen. He graduated at the University of Freiburg in 1999. After a postdoc at the Institute of Molecular Medicine and Cell Research in Freiburg, he joined the Max Planck Institute of Psychiatry in Munich as a postdoctoral researcher in 2001. Since 2004 Dr. Jan Deussing is the head of the research group Molecular Neurogenetics at the Max Planck Institute of Psychiatry.

In his current research he focuses on the molecular and cellular networks underlying mood and anxiety disorders. Based on clinical observations, which have implicated the neuropeptide corticotropin-releasing hormone

(CRH) and its receptors in major depression, his group dedicates major research efforts to the CRH/CRH receptor systems. Furthermore, he is addressing potential novel entry points into molecular mechanisms and pathways relevant to psychiatric diseases, which are emerging from genome-wide association studies. To this end, his group takes particular advantage of the mouse as a model organism, which is accessible to sophisticated genetic engineering and allows to address behavioral endpoints that model endophenotypes of human mood and anxiety disorders).

4.30 – 4.45 pm

Identification of a new peroxiredoxin allele with a phenotype of oxidant-resistance and premature aging by whole genome resequencing of yeast

Bernd Timmermann

Bernd Timmermann, Study of molecular biology at the University Osnabrück (1989-94); Scientist in the Genome Research Group at the Max Delbrück Centrum, Berlin (1995-98); PhD in molecular medicine at the Charite, Berlin; Group Leader DNA Sequencing at the GenProfile AG, (1998-2001), Senior Scientist at the Max Planck Institute of Molecular Genetics (2002-2006),
Currently head of the *Next Generation Sequencing* Group at the Max Planck Institute for Molecular Genetics (since 12/2007) and of the MPG Sequencing Core Facility in Berlin (since 01/2010).
Selected ongoing projects: 1000Genomes Project, *de novo* sequencing of the Canary Genome, involved in different NGFN projects (Mutanom, Modifier)

4.45 – 5.15 pm

Coffee Break

Symposium IV: Systems Biology

5.15 – 6.00 pm

Opening Keynote Presentation

Biochemical approaches to biomolecular networks

Anne-Claude Gavin

Anne-Claude Gavin is Group Leader in the Structural and Computational Biology Unit at the European Molecular Biology Laboratory, Heidelberg. Before joining the EMBL she was a scientific director at Cellzome AG. In 2002 she received the Genome Technology All-Stars Award in proteomics. Her group has been proponent and pioneer of more general strategies aiming at understanding complex biological systems. With more than 1'700 citations, the system-wide characterization of protein complexes in a model eukaryote, *Saccharomyces cerevisiae*, by affinity purification and mass spectrometry is generally considered as a breakthrough. Her main research interest includes the study of biomolecular interaction, the understanding of the principles that govern the assembly and dynamics of protein networks.

6.00 – 6.15 pm

Identification of Y-box binding protein 1 as a core regulator of MEK/ERK pathway- dependent gene signatures in colorectal cancer cells

Reinhold Schäfer

Reinhold Schäfer graduated from the University of Bonn, Germany, in 1972. He received a PhD degree in biology from the University of Bonn in 1976. He did postdoctoral work at the Max-Planck-Institute for Experimental Medicine and the West German Cancer Center, University of Essen, between 1976 and 1985. From April 1985, he was principal investigator at the Ludwig Institute for Cancer Research in Bern, Switzerland, and was appointed Chief of the Division of Cancer Research, Department of Pathology, University Hospital Zürich in 1988. In 1996, he was appointed Professor of Molecular Tumor Pathology at Charité University Medical School Berlin. Since 2001, he is coordinator of the Laboratory for Functional Genomics, a core facility at the Charité and since 2009 Reinhold Schäfer is Deputy Director for Translational Studies at the Charité Comprehensive Cancer Center. Reinhold Schäfer's teaching activities include Molecular and Cellular Biology, Experimental Cancer Research and Molecular Medicine.

6.15 – 6.30 pm **_Genome-wide miRNA level regulation of ErbB receptor protein network in breast cancer_**

Özgür Sahin

Özgür Sahin is a group leader in the Division of Molecular Genome Analysis, DKFZ, Heidelberg
He studied Molecular Biology and Genetics (B.Sc.) at Middle East Technical University (METU) in Ankara, Turkey. He then moved to University of Heidelberg where he received his M.Sc. degree from the International Molecular and Cell Biology (MCB) Program in 2005. He obtained his PhD for his work on the systems level approach to identify novel drug targets in trastuzumab/Herceptin resistant breast carcinoma at German Cancer Research Center (DKFZ) in 2008. During his PhD, he also developed an RNAi-based method to study cross-talk events in signaling pathways controlling tumor cell invasion. Since September 2008, he is the head of Receptor Tyrosine Kinase (RTK) Signaling Group in the Division of Molecular Genome Analysis at DKFZ. His research focuses on how tumor cells metastasize; develop resistance to targeted therapies - especially to the ErbB receptor targeted drugs- and whether certain biomarkers to predict these processes beforehand can be identified. Since the biological processes in the cells are developed as networks, he has been applying systems analysis approaches to elucidate above-mentioned cancer-related processes along his scientific career.

6.30 – 6.45 pm **_Proteomics and metabolomics analysis for biomarker discovery in a trait anxiety mouse model_**

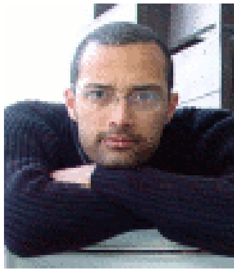
Michaela D. Filiou

Michaela D. Filiou holds a BSc in Biological Applications and Technologies from the *University of Ioannina*, Greece and a MSc in Human Molecular Genetics from *Imperial College London*, United Kingdom. She is enrolled at the International Max Planck Research School for Molecular and Cellular Life Sciences and is currently completing her PhD at the *Max Planck Institute of Psychiatry* in Munich, Germany under the supervision of Prof. Chris W. Turck. Her work focuses on elucidating the neurobiological underpinnings and identifying biomarkers for psychiatric disorders using mouse models and quantitative proteomics and metabolomics methodologies.

6.45 – 7.00 pm

Orthology-based phenotypic inference from disease models to human applications

Jean-Fred Fontaine



Jean-Fred Fontaine has an Engineer degree in computer science, a Master degree in Bioinformatics (Université de la Méditerranée, France), and a PhD in Computational Biology (Université d'Angers, France). His doctoral research was dedicated to the discovery of biological markers in thyroid cancer. He taught Computer Science and Bioinformatics to under graduate students for 4 years in Angers. He works currently in the Computational Biology and Data Mining group at the Max Delbrück Center for Molecular Medicine (Germany) where he develops methods and computer tools for the analysis of biological and text data. Collaborations with biologists and biomedical scientists help him to define new projects to integrate experimental data to biological databases and eventually to create novel algorithms for the analysis of microarray, deep sequencing, sequence motifs, and bibliographic text for example (see http://cbdm.mdc-berlin.de/tools).

7.00 – 8.00 pm

Evening Lecture

Genomes and people: How do they come together?

Regine Kollek

Regine Kollek, PhD, received her doctoral degree in biology from the University of Würzburg in 1979, before she spent two years at the medical school of the University of California, San Diego. From 1981 through 1984 she was senior researcher at the Heinrich-Pette-Institute at the University of Hamburg, before she became a member of the scientific staff of the Enquete-Commission on "Chances and Risks of Gene-Technology" of the German parliament (1985 - 1987). After that she joined the Hamburger Institute for Social Research. Since 1995 she is professor and head of a research group dedicated to the study of the medical, social and ethical implications of modern biotechnology in medicine at the „Research Centre on Biotechnology, Society and Environment", University of Hamburg. From 1999 to 2001 she was chairing the ethics advisory group of the German Ministry of Health. Since June 2001 she is a member of the German National Ethics Council (former national Ethics Council). From March 2002 until Summer 2010 she also was a member of the International Ethics Council of the UNESCO.

Her research focuses on the ethical and societal implications of modern biotechnology and genetics in medicine with special emphasis on individualized medicine, biobanking, genetic testing, stem cell technology, and reproductive medicine. A second focus of her scholarly work concerns sociological and epistemological questions of scientific development.

8.00 – 10.00 pm

Get-together (Wine, Cheese, Music)

Saturday, November 27th, 2010

Symposium V: Transfer from Genomics to Application

9.00 – 9.45 am

Opening Keynote Presentation

Targeted combination therapy of HER2-positive breast cancer

Max Hasmann

Max Hasmann (Roche Diagnostics GmbH, Pharma Research and Early Development (pRED), Penzberg, Germany)

Dr. Max Hasmann is a Preclinical Science Leader and Research Program Manager at Roche's pRED unit in Penzberg.

After studying biology at the Technical University of Munich and earning his Diploma degree in 1982, he worked on his Ph.D. thesis at the Institute for Microbiology at the Technical University of Munich (Prof. K.-H. Schleifer, 1982 – 1985). From 1985 – 1987, he held a post-doc position at the Max-Planck-Institute for Biochemistry, Martinsried, Department of Cancer Cell Research (Prof. G. Valet) working on multidrug resistance mechanisms of tumour cells. In 1987 he joined the pharmaceutical company Klinge Pharma GmbH in Munich as Head of Cell Culture Laboratory focusing on cancer research. Since 2002, Dr. Hasmann has worked on various early and late stage cancer research programs and held several positions with increasing responsibility at the Pharma Research unit of Roche Diagnostics GmbH in Penzberg, Germany.

9.45 – 10.00 am

A next generation genome-wide view of (epi)genetic alterations in clinically distinct colon cancer

Michal-Ruth Schweiger

Michal-Ruth Schweiger is a junior group leader in the department of Vertebrate Genomics at the Max Planck Institute for Molecular Genetics in Berlin. As a fellow of the 'Studienstiftung des deutschen Volkes' she received her M.D. in 2001 (*summa cum laude*) and her Ph.D. in Biochemistry in 2005 (Dr.rer.nat., *summa cum laude*). For her work on Papillomaviruses she received the Robert Koch Dissertation award from the Charité. During her graduate and post-graduate time she joined for four years Prof.P.M.Howley's group at the Harvard Medical School in Boston. Major research areas are epigenetic transcriptional regulation mechanisms in cancer with a focus on pathomechanisms of cervical (human papillomavirus infections), colorectal and prostate cancers.

10.00 – 10.15 am

PROCEED SP10: Molecular tumor imaging using antibody-coated nanoparticles; Construction of antibody conjugated fluorescent nanoparticles for in vivo imaging of prostate cancer cells.

Katja Werner

Katja Werner grew up in Berlin, Germany, and is currently based in Hamburg where she leads the Biofunctionalization Unit in the Centre for Applied Nanotechnology. For the last 17 years she acquired broad knowledge in different fields of proteinbiochmistry. She worked at the MPI for Molecular Genetics in Berlin on the identification of signal transduction pathways, in the Zoological Department of the RWTH Aachen on light receptor proteins from photosensoric membranes and in the Biological Chemistry Institute, at the University Düsseldorf, where she analyed photoinhibition of photosynthesis and the structure and function of the F-type ATPase. A nine years period she spent at EMBL-Hamburg in the field of Proteincrystallisation, mainly implementing rational protein mutagenesis. In 2008 she joined the CAN GmbH in Hamburg, where she links her background in protein biochemistry to the novel field of medical nanoparticles, generating bioconjugates for medical research, diagnosis and in prospect for therapy

10.15 – 10.30 am

NTCVD-Consortium identified known and new proinflammatory and profibrotic biomarkers in patients with chronic kidney disease

Heike Bruck

Heike Bruck, Priv.-Doz. Dr. med
Department of Nephrology, University Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, D-45147 Essen/Germany

Dr. Bruck is consultant nephrologist with special interest in clinical pharmacology and the cardiovascular system. She received her degree of medicine and MD from the University of Essen. She started her postdoctoral research in the Department of Pharmacology at the Martin-Luther-University of Halle-Wittenberg studying the impact of genetic polymorphisms on adrenoceptor function. Thereafter she specialized for internal medicine and nephrology at the University Hospital Essen. Alongside her clinical work she is interested in non-invasive techniques to characterize cardiovascular function in humans and has performed several clinical studies in the cardiovascular and nephrology field. After she achieved habilitation she focussed on the cardiorenal syndrom and joined the BMBF/NGFN-Transfer-Alliance „New Tools for the Prevention of Cardiovascular Disease in Chronic Kidney Disease"- NT^{CVD}, which conducts preclinical and clinical studies to investigate mechanisms why patients with chronic kidney disease suffer from increased cardiovascular morbidity and mortality.

10.30 – 10.45 am | ***Identification of the gaps in integration of genome-based knowledge in the International Public Health legislation***

Elena Syurina

Elena V. Syurina, MSc (born 1986, Moscow), PhD candidate at the Institute for Public Health Genomics (IPHG) within the Faculty of Health, Medicine and Life Sciences at Maastricht University, the Netherlands. Her PhD topic is about "Translational research in Public Health Genomics (PHG) Ways to adapt policy-making and public policy analysis tools to the changing situation in Public Health due to the introduction of PHG"). Elena Syurina is manager of the EU funded project PHGEN II (Public Health Genomics European Network), member of the section Public Health Genomics within the European Public Health Association (EUPHA); member of the Editorial Office of the international journal Public Health Genomics with Karger Publishers, and partner in the DATAproBio joint EU project of the JRC-IPTS of the EC in Seville and the IPHG, in Maastricht on data protection in biobanking and public health. Elena Syurina is specialist in Public Administration and former researcher of the Laboratory for Local Self-Governance at the Higher School of Economics at the State University in Moscow, Russia, as well as former board member and event organiser of the local division of the European Students' Forum, AEGEE. Elena Syurina also holds a degree as a Master in Science in Public Administration from the University of Twente, Enschede, the Netherlands (topic "Difference in framing the policy issue of child oncology in Russia and in the Netherlands", Cum laude).

10.45 – 12.45 pm | ***Lunch Break and Poster Session II***
(10.45 - 11.45 am odd numbers, 11.45 - 12.45 pm even numbers)

10.45 – 12.45 pm | **Company Satellite Sessions:**

10.55 – 11.25 am | **Dr. Nick Brain**, **Affymetrix Europe**

Technology advances:

- Powerful custom genotyping from 50K to 5M SNPs
- Unparalleled performance for allele-specific copy number detection in FFPE samples

11.40 – 12.10 am | **Dr. Caren Vollmert, Dr. Henning Gohlke, Sequenom GmbH,** Hamburg, Germany

Translating Genomic Discovery into Human Health –
The MassARRAY® for DNA Methylation and Somatic Mutation Profiling

12.45 – 1.00 pm | **Poster Award Ceremony:**

12.45 – 1.00 pm | **Ceremony: "Annemarie Poustka Poster Award of Medical Genome Research 2010" sponsored by Roche Diagnostics GmbH**

Symposium VI: New Technologies

1.00 – 1.15 pm

Whole genome sequence of a Crohn disease trio – a paradigm for etiology discovery in complex disease?

Philip Rosenstiel

Philip Rosenstiel studied medicine in Kiel, Germany, and in Boston, USA. During a research scholarship at the Jackson Laboratory and his doctoral thesis at the Laboratory of Jobst Sievers, he worked on the impact of inflammation in neurodegenerative diseases. He joined the mucosal research laboratory of Stefan Schreiber as a postdoctoral researcher with a clinical background in internal medicine, concentrating on the interplay of intestinal immune responses and epithelial barrier integrity, with a focus on the functional genomics and evolution of NLR protein signalling. In 2007, he joined the faculty of the University Kiel as Professor of Molecular and Marine Medicine, where he became deputy director of the Institute for Clinical Molecular Biology. Current research focuses on the molecular pathophysiology and etiology of immune disorders using next generation sequencing and cell biological approaches . He serves as a member of the steering boards of the Cluster of Excellence “Inflammation at Interfaces” and the ICGC Consortium “Malignant lymphoma”

1.15 – 1.30 pm

A map of human genome variation from population scale sequencing (the 1000 Genomes Project)

Ralf Sudbrak

Ralf Sudbrak studied Biology at the Westfälische Wilhelms-Universität Münster and earned his doctorate in 1996. From 1996 to 1998 he worked as a post-doc research fellow in the group of Tony Monaco at the Wellcome Trust Centre for Human Genetics in Oxford, UK. Since 1998 he is group leader in the department of Hans Lehrach at the Max Planck Institute for Molecular Genetics in Berlin. His work focused on mapping and sequencing at a genome-wide scale and several disease gene identification projects. He was involved in the Human Genome Project and is currently project leader in the 1000 Genome Project.

1.30 – 1.45 pm **_Whole genome amplification in large biobanks_**

Norman Klopp

Norman Klopp studied Biology at the University of Bielefeld where he graduated in 1996 in developmental biology. In 1997 he came to the Helmholtz Center Munich. There he worked on his doctoral thesis in the Institute of Mammalian Genetics performing the genetic dissection of mouse mutants and earned his doctorate at the TU Munich in 2000. In 2001 he joined the Institute of Epidemiology where he works as senior scientist in the Genome Analysis Center leading the SNP genotyping platform of the institute. He was strongly involved in several published genome wide association studies for various traits related to metabolic and cardiovascular diseases. The focus of his scientific research is on genomics strategies to unravel the molecular basis of complex diseases such as diabetes and allergies. His field of expertise additionally encompasses biobanking and epigenetics. Currently he is working in the NGFN transfer project "Whole genome and transcriptome amplification in large biobanks".

1.45 – 2.00 pm **_Analysis pipeline for exome sequencing data_**

Sebastian Eck

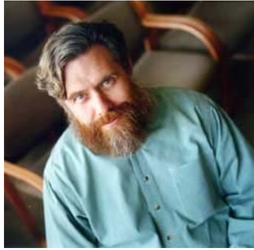
Sebastian Eck is currently a third year PhD student at the Helmholtz Zentrum Munich in the Department of Human Genetics. He studied Bioinformatics jointly at the Technical University Munich and The Ludwig-Maximilians-University of Munich and did his Diploma work in the field of Evolutionary Biology. For his PhD he switched to human genetics. The main focus of his work is the detection of rare and common genetic variation from next generation sequencing data. These variation include single nucleotide variants (SNVs), as well as small insertion and deletion events (indels) and large structural variation. He is primarily responsible for the development of analysis algorithms and pipelines for next generation sequencing data. Currently their main focus lies on the identification of causative variants of rare heritable disease via whole exome sequencing. Further projects include whole Genome sequencing, RNA-Seq expression profiling and ChIP-Seq

2.00 – 2.45 pm

<u>Keynote Presentation (Live broadcast videoconference)</u>

Technologies for collecting and integrating genome, environment and trait data

George Church



George Church, Professor of Genetics, Harvard Medical School, Director of the Center for Computational Genetics. 1984 Harvard PhD included the first direct genomic sequencing method, molecular multiplexing tags, which lead to automation & software used at Genome Therapeutics Corp. for the first genome sequence -- pathogen, Helicobacter in 1994. This multiplex solid-phase sequencing evolved into polonies (1999), ABI-SOLiD (2005) & open-source Polonator.org (2007). Innovations in DNA reading & writing & allele replacement in cells lead to current research & commercialization in human genomics(Complete Genomics Inc, PersonalGenomes.org, 23andme, Knome) & synthetic biology (SynBERC, Joule, LS9) & new ethics/security strategies. Received the 2010 Hoogendijk and 2010 Franklin-Bower Awards.

2.45 – 3.00 pm

Concluding Remarks: Stefan Wiemann, German Cancer Research Center (DKFZ), Speaker Project Committee of NGFN-Plus / NGFN-Transfer in the Program of Medical Genome Research

Overviews

National Genome
Research Network

Oral Presentations

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112	I-Keynote	Mark Lathrop	Medical and public health applications of genomics	
113	O-I-1	Thomas W. Mühleisen	Genome-wide Association Study and Comprehensive Follow-up Strongly Supports Neurocan (NCAN) as a Novel Susceptibility Gene for Bipolar Disorder	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
115	O-I-2	Susanne Lucae	The neuronal transporter gene SLC6A15 confers risk to major depression	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
116	O-I-3	André Reis	MRNET – German Mental Retardation Network – a platform for systematic identification of genes for mental retardation	IG German Mental Retardation Network (MRNET)
117	O-I-4	Vera Kalscheuer	Systematic mutation search in families with XLMR by next-generation sequencing	IG German Mental Retardation Network (MRNET)
Symposium II - Genomics of Common Disease II				
120	II-Keynote	Xavier Estivill	Structural Variation Analysis by Large-Scale Human Genome Sequencing	
121	O-II-1	Andre Franke	Genome-Wide Meta-Analysis Increases to 71 the Number of Confirmed Crohn's Disease Susceptibility Loci	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
122	O-II-2	Christian Kubisch	Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1	IG Epilepsy and Migraine Integrated Network (EMINet)
123	O-II-3	Anja Bauerfeind	A conserved trans-acting regulatory locus underlies an inflammatory gene network and susceptibility to autoimmune type 1 diabetes	IG Genetics of Heart Failure
125	O-II-4	Jeanette Erdmann	CARDIoGRAM: Thirteen novel genetic loci affecting risk of coronary artery disease	IG Genomics of Atherosclerosis
Symposium III - Animal, Cellular & Tissue Models				
128	III-Keynote	Andrea Ballabio	The Genetic Control of Lysosomal Function and of Cellular Clearance	
129	O-III-1	Jens Siveke	Mouse models endogenous pancreatic cancer – role of EGFR and Notch signaling	IG Genome Research Network in Pancreatic Cancer
130	O-III-2	Kaomei Guan	Generation of functional cardiomyocytes from patient-specific induced pluripotent stem cells	IG Genetics of Heart Failure
131	O-III-3	Jan Deussing	CRHR1, a key regulator of stress, regulates anxiety in opposite directions by controlling glutatmatergic and dopaminergic neurons	IG Genetics of Alcohol Addiction
132	O-III-4	Bernd Timmermann	Identification of a new peroxiredoxin allele with a phenotype of oxidant-resistance and premature aging by whole genome resequencing of yeast	

Page	Abstract	Presenting Author	Abstract Title	Consortium
Symposium IV - Systems Biology				
136	IV-Keynote	Anne-Claude Gavin	Biochemical approaches to biomolecular networks	
137	O-IV-1	Reinhold Schäfer	Identification of Y-box binding protein 1 as a core regulator of MEK/ERK pathway- dependent gene signatures in colorectal cancer cells	IG Systems Biology of Genetic Diseases (Mutanom)
138	O-IV-2	Özgür Sahin	Genome-wide miRNA level regulation of ErbB receptor protein network in breast cancer	IG Cellular Systems Genomics in Health and Disease
139	O-IV-3	Michaela D. Filiou	Proteomics and metabolomics analysis for biomarker discovery in a trait anxiety mouse model	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
140	O-IV-4	Jean-Fred Fontaine	Orthology-based phenotypic inference from disease models to human applications	IG Neurodegenera-tive Diseases Networks (NeuroNet)
141	Evening Lecture	Regine Kollek	Genomes and people: How do they come together?	
Symposium V – Transfer from Genomics to Application				
144	V-Keynote	Max Hasmann	Targeted combination therapy of HER2-positive breast cancer	
145	O-V-1	Michal-Ruth Schweiger	A next generation genome-wide view of (epi)genetic alterations in clinically distinct colon cancers	IG Modifiers of Intestinal Tumor Formation and Progression
146	O-V-2	Katja Werner	PROCEED SP10: Molecular tumor imaging using antibody-coated nanoparticles; Construction of antibody conjugated fluorescent nanoparticles for in vivo imaging of prostate cancer cells	IG Integrated Genome Network of Prostate Cancer
147	O-V-3	Heike Bruck	NTCVD-Consortium identified known and new proinflammatory and profibrotic biomarkers in patients with chronic kidney disease	IA New Tools for the Prevention of Cardiovascular Diseases and Disorders in Chronic Kidney Disease
148	O-V-4	Elena Syurina	Identification of the gaps in integration of genome-based knowledge in the International Public Health legislation	
Symposium VI - New Technologies				
152	O-VI-1	Philip Rosenstiel	Whole Genome Sequence of a Crohn disease trio – a paradigm for etiology discovery in complex disease?	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
153	O-VI-2	Ralf Sudbrak	A map of human genome variation from population scale sequencing (the 1000 Genomes Project)	1000 Genomes Project
154	O-VI-3	Norman Klopp	Whole genome amplification in large biobanks	IA Whole genome and transcriptome amplification in large biobanks
155	O-VI-4	Sebastian Eck	Analysis Pipeline for Exome Sequencing Data	IG German Mental Retardation Network (MRNET)
156	VI-Keynote	George Church	Technologies for Collecting and Integrating Genome, Environment and Trait data *Live broadcast videoconference*	

List of Poster Abstracts

sorted by symposia

All posters will be displayed continuously throughout the duration of the meeting. Autors will be present at their posters for discussion during the designated time.

Poster Session I:
Friday, November 26th, 2010
Odd numbers: 1.00 – 2.00 pm
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Poster Session II:
Saturday, November 27th, 2010
Odd numbers: 10.45 – 11.45 am
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Symposia I and II – Genomics of Common Disease				
162	P-I/II-001	Anna-Lena von dem Knesebeck	RANK (TNFRSF11A) is epigenetically inactivated and acts as a tumor suppressor in human gliomas	IG Brain Tumor Network
163	P-I/II-002	Sebastian Barbus	Retinoic acid signaling activates different targets in the tumors of long-term compared to short-term survivors of glioblastoma	IG Brain Tumor Network
164	P-I/II-003	Valentina Vladimirova	Death associated protein 3 contributes to intrinsic and extrinsic pathways for apoptosis in high grade gliomas of childhood	IG Brain Tumor Network
165	P-I/II-004	Chris Lawerenz	Extending iCHIP, the NGFNplus Brain Tumor Net database to ICGC	IG Neuroblastoma Genome Interaction Network
166	P-I/II-005	Martje Tönjes	Molecular signatures classify astrocytic gliomas by IDH1 mutation status	IG Brain Tumor Network
167	P-I/II-006	Chris Lawerenz	Verified specimen for research: iCHIP-based management of bio-resources within NGFNplus-PaCaNet	IG Neuroblastoma Genome Interaction Network
168	P-I/II-007	Abdelouahid Maghnouj	Functional characterization of the potentially “drugable” candidate gene NT5E identified within the PACANet consortium	IG Genome Research Network in Pancreatic Cancer
169	P-I/II-008	Jörg Hoheisel	Analysis of protein isoforms that are relevant to diagnostics and drug activity by means of antibody microarrays; application to pancreatic cancer and lymphoma.	IG Genome Research Network in Pancreatic Cancer
170	P-I/II-009	Bo Kong	Haplotypes in melanoma inhibitory activity 2 correlate with survival and chemoresistance in pancreatic cancer	IG Genome Research Network in Pancreatic Cancer
171	P-I/II-010	Daniela Stangel	Knockdown of kinesin motor protein Kif20a leads to growth inhibition in pancreatic ductal- and neuroendocrine-cancer cells.	IG Genome Research Network in Pancreatic Cancer
172	P-I/II-011	Antje Krohn	Chromosomal deletions, tumor phenotype and prognosis in prostate cancer	IG Integrated Genome Network of Prostate Cancer
173	P-I/II-012	Marc Johannes	Integration of Pathway Knowledge into a Support Vector Framework using Reweighted Recursive Feature Elimination	IG Integrated Genome Network of Prostate Cancer
174	P-I/II-013	Stephan Gade	Linking parallel measurements of high-throughput miRNA and gene expression data	IG Integrated Genome Network of Prostate Cancer
175	P-I/II-014	Jan Christoph Brase	Gene and protein expression profiles associated with prostate cancer specific TMPRSS2-ERG fusion	IG Integrated Genome Network of Prostate Cancer
176	P-I/II-015	Ruprecht Kuner	Functional relevance and downstream signaling of specific microRNAs in prostate cancer cells	IG Integrated Genome Network of Prostate Cancer
177	P-I/II-016	Melanie Isau	Next generation sequencing of coding regions in prostate cancer patients	
178	P-I/II-017	Alexandra Farrall	Searching for Epigenetic Modifiers of APCmin-induced Initiation of Intestinal Oncogenesis in Mice using Chromosome Substitution Strains	

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179	P-I/II-018	Michael Nothnagel	Statistical inference of allelic imbalance from transcriptome data	IG Integrated Genomic Investigation of Colorectal Carcinoma
180	P-I/II-019	Laura Tolosi	Prediction of tumor phenotype from copy number aberrations	IG Deciphering Oncogene Dependencies
181	P-I/II-020	Christof Winter	Prediction of Survival and Response to Adjuvant Therapy in Pancreatic Cancer	IG Genome Research Network in Pancreatic Cancer
182	P-I/II-021	Stefan Börno	A DNA methylation signature for prostate cancer identified by next generation genome-wide profiling.	
183	P-I/II-022	Christina Röhr	Next-Generation Sequencing of (micro)RNAs in Colorectal Cancers	
184	P-I/II-023	Felix Broecker	Regulation of the Death-Associated Protein 3 Gene by a Retroviral Antisense Transcript	
185	P-I/II-024	Martin Kerick	Genetic profiles of MSS and MSI colorectal cancers identified by whole exome sequencing	IG Systems Biology of Genetic Diseases (Mutanom)
186	P-I/II-025	Andrea Wunderlich	Functional characterization of Brd4 as a transcriptional regulator	
187	P-I/II-026	Julia Starmann	Quantification of cancer pathways in a recombinant cellular system	IG Systems Biology of Genetic Diseases (Mutanom)
188	P-I/II-027	Cristina Cadenas	Role of thioredoxin reductase 1 and thioredoxin interacting protein in prognosis of breast cancer	IA Breast Cancer Kit
189	P-I/II-028	Franziska Freund	Identifying Novel Expressed Gene Fusions in MCF-7 Cell Line Using Next Generation Sequencing	
190	P-I/II-029	Cindy Horwedel	A novel large scale screen to identify regulators of the cancer-relevant microRNAs miR-31 and miR-155	IG Cellular Systems Genomics in Health and Disease
191	P-I/II-030	Sarah Jurmeister	MicroRNA-200c represses migration of breast cancer cells by targeting a network of Rho GTPases	IG Cellular Systems Genomics in Health and Disease
192	P-I/II-031	Anika Jöcker	SNPtator: A web service pipeline to predict the functional consequences of SNVs	IG Cellular Systems Genomics in Health and Disease
193	P-I/II-032	Milan Hiersche	Snp2gene and GwasPlot: New toolset for the visualization and annotation of GWA results	IG Genetics of Heart Failure
194	P-I/II-033	Christopher Hardt	Molecular Characterisation of Uremic Toxins in silico	IA New Tools for the Prevention of Cardiovascular Diseases and Disorders in Chronic Kidney Disease

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195	P-I/II-034	Anika Sietmann	The Use of Comparative Genomics for the Analysis of Cardiac Organ damage in Hypertension	IG Genetics of Heart Failure
196	P-I/II-035	Stephan E. Lehnart	Gene expression analysis in a model of cardiac ryanodine receptor calcium leak	IG Genetics of Heart Failure
197	P-I/II-036	Inka Boomgaarden	MicroRNA-582 is differentially expressed in the heart of MLP-knockout mice	IG Genetics of Heart Failure
198	P-I/II-037	Christina Loley	How to Test for Association on the X Chromosome – A Comparison of Suggested Test Statistics	IG Genomics of Atherosclerosis
199	P-I/II-038	Seraya Maouche	Counter-regulation of macrophage phenotype by M-CSF and GM-CSF. Possible implication in atherosclerosis.	IG Genomics of Atherosclerosis
200	P-I/II-039	Janja Nahrstaedt	Comprehensive genetic analyses in an extended family with myocardial infarction	IG Genomics of Atherosclerosis
201	P-I/II-040	Seraya Maouche	Transcriptional profiling of monocytes and MCSF-driven macrophages in a large multi-centre collaborative study	IG Genomics of Atherosclerosis
203	P-I/II-041	Hendrik B Sager	Temporal transcriptional changes in human monocyte RNAs following myocardial infarction: The GerMIFs monocyte expression study	IG Genomics of Atherosclerosis
204	P-I/II-042	Jennifer Freyer	MRas - A Strong Candidate Gene for Coronary Artery Disease (CAD)	IG Genomics of Atherosclerosis
205	P-I/II-043	Jens Baumert	Associations between variations in the TLR4 gene and incident coronary heart disease (CHD) in middle-aged men and women: Results from the MONICA/KORA Augsburg case-cohort study, 1984-2002	IG Genomics of Atherosclerosis
206	P-I/II-044	Ingrid Braenne	Sparse Linear Regression for Interpretive Risk Assessment on GWA Data	IG Genomics of Atherosclerosis
207	P-I/II-045	Jens Baumert	Eight Genetic Loci Associated with Variation in Lipoprotein-Associated Phospholipase A2 Mass and Activity and Coronary Heart Disease: Meta-analysis of Genome-wide Association Studies from Five Community-based Studies	IG Genomics of Atherosclerosis
209	P-I/II-046	Christian Hengstenberg	Genome-wide association study on HDL subclass traits	IG Genomics of Atherosclerosis
210	P-I/II-047	Eva Albrecht	Genome-wide association study identifies two novel regions at 11p15.5-p13 and 1p31 with major impact on acute-phase Serum Amyloid A	
211	P-I/II-048	Ivonne Jarick	Novel common copy number variation for early onset extreme obesity on chromosome 11q11 identified by a genome-wide analysis	IG Molecular Mechanisms in Obesity
212	P-I/II-049	Chung-Ting Han	Sequencing out Effects of Resveratrol on Chromatin	IG Molecular Mechanisms in Obesity
213	P-I/II-050	Anke Hinney	Lack of synthetic association for obesity at the melanocortin 4 receptor gene coding region	IG Molecular Mechanisms in Obesity

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215	P-I/II-052	Josef Frank	Genome wide association study of alcohol dependence	IG Genetics of Alcohol Addiction
217	P-I/II-053	Wolfgang Sommer	Circadian Rhythm and the Development of Alcoholism: Whole-Genome Expression Profiling in the Brain of Short-term and Long-term Alcohol Drinking Rats	IG Genetics of Alcohol Addiction
218	P-I/II-054	Katrin Charlet	Altered implicit neuronal processing of emotional faces (fMRI) in alcohol-dependent patients?	IG Genetics of Alcohol Addiction
219	P-I/II-055	Bernadeta Walaszek	Metabolic changes in alcohol-dependent patients' brains – a magnetic resonance spectroscopy study	IG Genetics of Alcohol Addiction
220	P-I/II-056	Anne Beck	Cue-induced brain activation mediates subsequent relapse in abstinent alcohol-dependent patients	IG Genetics of Alcohol Addiction
221	P-I/II-057	Antje Salg	Screening of DNA & drug libraries for recovery of potent ADAM10 stimulators for Alzheimer's disease therapy	IG Gene Identification and Functional Analyses in Alzheimer's Disease
222	P-I/II-058	Thomas M. Feulner	Genome-wide analysis of quantitative markers associated with Alzheimer's disease	IG Gene Identification and Functional Analyses in Alzheimer's Disease
223	P-I/II-059	Gilles Gasparoni	Epigenetic characterisation of Alzheimer's disease brain samples	IG Gene Identification and Functional Analyses in Alzheimer's Disease
224	P-I/II-060	Thomas M. Feulner	Genome-wide association study (GWAS) of Alzheimer's disease	IG Gene Identification and Functional Analyses in Alzheimer's Disease
225	P-I/II-061	Tobias Hartmann	Sphingolipids and Alzheimer's disease - vicious cycle of physiological and pathophysiological function of the Amyloid Precursor Protein	IG Gene Identification and Functional Analyses in Alzheimer's Disease
226	P-I/II-062	Florian Mittag	Identifying functional units in Genome-wide association studies (GWAS) data	IG Functional Genomics of Parkinson
227	P-I/II-063	Stefan Schmid	Cerebrospinal fluid fatty acid composition in healthy elderly seems widely independent of plasma composition	IG Functional Genomics of Parkinson
228	P-I/II-064	Florian Mittag	A machine learning pipeline for disease risk assessment of Genome-wide association studies (GWAS) SNP data	IG Functional Genomics of Parkinson

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230	P-I/II-066	Katharina Pernhorst	Compromized expression control of genes involved in serotonin and GABA metabolism in human epileptic tissue	IG Epilepsy and Migraine Integrated Network (EMINet)
231	P-I/II-067	Yvonne Weber	The Glut1 syndromes	IG Epilepsy and Migraine Integrated Network (EMINet)
232	P-I/II-068	Tobias Freilinger	Genome-wide association study in migraine without aura – an update	IG Epilepsy and Migraine Integrated Network (EMINet)
233	P-I/II-069	Jasmin Remmes	Genetic basis of Levetiracetam pharmacoresistance and side effects in human epilepsy	IG Epilepsy and Migraine Integrated Network (EMINet)
234	P-I/II-070	Holger Trucks	Recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 predispose to idiopathic generalized epilepsies	IG Epilepsy and Migraine Integrated Network (EMINet)
235	P-I/II-071	Costin Leu	Genome-wide association mapping of susceptibility alleles predisposing to idiopathic generalized epilepsy	IG Epilepsy and Migraine Integrated Network (EMINet)
236	P-I/II-072	Stephanie H. Witt	Genome-wide supported risk variants for bipolar disorder in Ank3 alter anatomical connectivity in the human brain	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
237	P-I/II-073	Vanessa Nieratschker	The Catechol-O-Methyl Transferase (COMT) gene and its potential association with schizophrenia: findings from a large German case-control and family-based sample	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
238	P-I/II-074	Manuel Mattheisen	Association between genetic variation in a region on chromosome 11 and schizophrenia in large samples from Europe	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
240	P-I/II-076	Thomas Mühleisen	Resequencing of neurexin 1 (NRXN1) and Follow-up in schizophrenia	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
241	P-I/II-077	Fitnat Buket Basmanav	Re-Sequencing analysis of the schizophrenia-associated microdeletion region on 1q21.1	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
242	P-I/II-078	Jana Strohmaier	Sexual dysfunction during antidepressant treatment and the 5-HTTLPR	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)

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247	P-I/II-083	Lutz Priebe	Suggestive evidence for an involvement of copy number variants in a German patient-control sample of major depressive disorder	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
248	P-I/II-084	René Breuer	Different approaches to disentangle major psychoses: moving beyond categorical diagnoses	IG Molecular Causes of Major Mood Disorders and Schizophrenia (MooDS)
249	P-I/II-085	Karl Hackmann	MRNET TP7 - Molecular karyotyping of patients with mental retardation – Identification of regions containing candidate genes	IG German Mental Retardation Network (MRNET)
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251	P-I/II-087	Karl Hackmann	Search for mutations and pathogenic haplotypes in hemizygous genomic regions of patients with mental retardation (MR)	IG German Mental Retardation Network (MRNET)
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253	P-I/II-089	Juliane Hoyer	Microaberrations in 16p11.2 influence language acquisition and body height	IG German Mental Retardation Network (MRNET)
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258	P-I/II-094	Volker Endris	Homozygous Loss of CHRNA7 on Chromosome 15q13.3 Causes Severe Encephalopathy With Seizures and Hypotonia	IG German Mental Retardation Network (MRNET)
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260	P-I/II-096	Maja Hempel	Cohen syndrome diagnosis using whole genome arrays	IG German Mental Retardation Network (MRNET)
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263	P-I/II-098	Kristin Hofmann	Partial NFIA gene deletion in a patient with mild intellectual deficiency	IG German Mental Retardation Network (MRNET)
264	P-I/II-099	Andreas Kuss	Homozygosity mapping in consanguineous Iranian families with autosomal recessive mental retardation identifies numerous single linkage intervals, more than 10 novel loci and several mutation hotspots	IG German Mental Retardation Network (MRNET)
266	P-I/II-100	Alexandra Dittmer	Distribution of host and viral micro RNAs in MCMV infection	IG Pathogenic Role of mi-RNA in Herpes-Infections
267	P-I/II-101	Georg Malterer	Systematic Analysis of Viral MicroRNA Targets in Cells Latently Infected with Human gamma-Herpesviruses by RISC Immunoprecipitation Assay.	IG Pathogenic Role of mi-RNA in Herpes-Infections
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269	P-I/II-103	Esther Schmidt	GenomeRNAi: A Database for Cell-based RNAi Phenotypes and Integration of Phenotypes Across Species	IG Neurodegenerative Diseases Networks (NeuroNet)
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274	P-I/II-108	Eva Ellinghaus	Genome-wide association study identifies a novel susceptibility locus for Psoriasis	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
276	P-I/II-109	Heidi Schaarschmidt	Identification of common genetic susceptibility loci in three chronic inflammatory barrier diseases through a “phenotype overlap analysis” (PoA)	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
277	P-I/II-110	Robert Häsler	Micro-RNA Signatures in Response to Proinflammatory Stimuli in Primary Human Monocytes	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
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279	P-I/II-112	Sandra May	Resequencing of 10 susceptibility loci in 56 individuals using Next Generation Sequencing based on Long Range PCR enrichment	IG Systematic Genomics of Chronic Inflammatory Barrier Diseases
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296	P-III-05	Mark Laible	Functional characterisation of cancer-relevant mutations using recombinant cancer cell line technology	IG Systems Biology of Genetic Diseases (Mutanom)
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299	P-III-08	Kamila Adamczyk	Insights from proteomic analysis of tumor cell secretomes: Release of EGFR via exosomes	IG Genome Research Network in Pancreatic Cancer
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310	P-III-19	Sabine Hölter	German Mouse Clinic: the Behaviour screen	IG German Mouse Clinic - Deciphering Comorbidity in Human Disease Models
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331	P-III-41	Daniel Milkereit	A severe epileptic phenotype due to a moderate loss of M-current in the KCNQ2NMF134 mouse model	IG Epilepsy and Migraine Integrated Network (EMINet)
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Oral Presentation Abstracts

Oral Presentation Abstracts

Symposium I

Genomics of Common Disease I

Medical and public health applications of genomics

Presenting Author: Mark Lathrop

Centre National de Génotypage, Evry, France

Genome-wide Association Study and Comprehensive Follow-up Strongly Supports Neurocan (NCAN) as a Novel Susceptibility Gene for Bipolar Disorder

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Bipolar disorder (BD) is a highly heritable and chronic disorder of mood, characterized by recurrent episodes of mania and depression. We had initially conducted a genome-wide association study (GWAS) in a clinically well-characterized discovery sample of 682 patients with a DSM-IV life-time diagnosis of BD (type 1, n=679) and 1,300 controls, all of German ancestry. The top association signals (autosomes: p=7.57E-5; X-chromosome: p=1.89E-4) were tested in BD samples from Europe (Germany, Poland, Spain, Russia, Romania, Bosnia-Herzegovina, Serbia), comprising 1,729 patients (type 1, n=1,409) and 2,313 controls. A common single-nucleotide polymorphism in the gene neurocan (NCAN) on chromosome 19p13.11 was associated with BD in all follow-up samples and meta-analysis across these and the GWAS sample showed p=3.02E-8 (OR=1.31).

In a second replication step, we have now sought after support for this genome-wide significant finding in BD samples from Europe (WTCCC, deCODE, Germany, France, Norway), from the USA (GAIN-EA, TGEN1), and Australia, totaling 6,030 patients (type 1, n=5,044) and 31,749 controls. In all of these samples, the risk allele was overrepresented in patients and meta-analysis demonstrated a p=1.37E-4 (OR=1.12).

Our overall study, comprising 8,441 patients and 35,362 controls, provides strong support that genetic variation in NCAN is a novel, common risk factor for BD. NCAN encodes an extracellular matrix glycoprotein, supposed to be involved in cell adhesion and migration. Our expression studies in mice provided further support for a role of NCAN in BD since its transcript expression is localized to brain areas (cortex, hippocampus) in which abnormalities have been identified in BD. These abnormalities may be indicative of disturbances in key neuronal circuits.

This study was supported by the German Federal Ministry of Education and Research (BMBF), within the context of the National Genome Research Network plus (NGFNplus), and the MooDS-Net.

The neuronal transporter gene SLC6A15 confers risk to major depression

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Major depression is one of the most prevalent psychiatric disorders and a leading cause of loss in work productivity. A combination of genetic and environmental risk factors likely contributes to this disorder. We present data from a genome-wide association study revealing a neuron-specific neutral amino acid transporter (SLC6A15) as a novel susceptibility gene for major depression. Risk allele carrier status in humans and chronic stress in mice were associated with a downregulation of the expression of this gene in the hippocampus, a brain region implicated in the pathophysiology of this disorder. The same polymorphisms also showed associations with alterations in hippocampal volume and neuronal integrity in this brain region. Thus, decreased SLC6A15 expression, due to genetic or environmental factors might specifically alter neuronal circuits related to the susceptibility for major depression. Our convergent data from human genetics, expression studies, brain imaging and animal models suggest a novel pathophysiological mechanism for major depression that may be accessible to drug targeting.

MRNET – German Mental Retardation Network – a platform for systematic identification of genes for mental retardation

Presenting Author: André Reis
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Mental retardation (MR) or intellectual disability has a prevalence of 2-3%. In recent years it has become clear that genetic factors are the major contributor to the aetiology of MR, at least in industrialised countries. In more than 50% of cases the causative genetic basis remains unknown impeding optimal health care and targeted help for special needs of patients and families. The German Mental Retardation Network (MRNET) aims to systematically identify and characterise genes involved in MR and to correlate genetic and phenotypic findings in individual patients. A better understanding of the molecular basis of MR and its phenotypic consequences is a prerequisite for developing future therapeutic options. MRNET involves 9 basic research centres with specialised MR clinics and one group dedicated to pathway dissection in Drosophila as model system.
As of October 2010, a total of 2,330 patients were recruited, for whom clinical data from 1,825 index patients (1,081 males, 744 females) and photographic material from 922 patients was already entered into the central database. Copy number profiling using microarrays in 1,581 patients revealed apparently disease causing aberrations in about 15 % of them and resulted in the identification of both, novel autosomal dominant and recessive genes for mental retardation such as MEF2C, SHANK2, NRXN1, CNTNAP2, CCDC88C, FOXP1, GRIN2A and GRIN2B. In addition, genome partitioning and next generation sequencing (NGS) in >150 patients with X linked MR revealed nonsense, frameshift and non-synonymous mutations in 10 genes that had not been firmly implicated in XLMR before. Autozygosity mapping in more than 100 consanguineous families with autosomal recessive MR followed by NGS is ongoing. Finally, an efficient Drosophila phenotype analysis pipeline was established for a large-scale synapse screen of 250 evolutionarily conserved MR genes.

Systematic mutation search in families with XLMR by next-generation sequencing

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X-linked mental retardation (XLMR) affects 1-2/1,000 males and accounts for 10% of all forms of mental retardation. Recent screening of X-linked genes has revealed truncating mutations in 25% of the families studied (Tarpey et al, 2009. Previous studies had indicated that mutations in known XLMR genes account for at least 42% of XLMR families (de Brouwer et al, 2007). To resolve this discrepancy and to shed more light on the molecular causes of XLMR, we have combined genome partitioning techniques and Next Generation Sequencing (NGS) to find the causative gene defect in another 245 families from the European MRX Consortium. In this study group, 18% of the families carry apparently pathogenic mutations in known XLMR genes, including nonsense and missense mutations. About 4% of the families carry deleterious mutations in novel XLMR genes and 8% carry truncating or convincing missense/in frame deletion mutations in novel candidate XLMR genes. Many other families carry non-synonymous, possibly pathogenic changes in candidate XLMR genes. Thus, the majority of genes whose loss gives rise to non-syndromic XLMR may already be known. In about 40% of the families the causative gene defect is not detected yet. There are several plausible explanations for this, including that the fundamental defect may not reside in coding regions of the X-chromosome or missed upon enrichment and NGS.
Supported by BMBF and Max-Planck-Society.

Oral Presentation Abstracts

Symposium II

Genomics of Common Disease II

Structural Variation Analysis by Large-Scale Human Genome Sequencing

Presenting Author: Xavier Estivill

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Structural variations are genomic changes that can have an important impact on gene function and expression. Some structural variations have already been associated to human disease, including complex traits and common disorders. We have performed a systematic analysis of structural variation in the human genome using different platforms. While array-based platforms define a large number of deletions and duplications, only whole genome and exome sequencing can provide a comprehensive characterization of the whole set of changes (duplications, deletions, inversions and translocations) that are present at the individual, organ, tissue and cellular levels. Depth of coverage and paired-end mapping sequencing approaches allow a complete characterization of genomic variability, with detection of different layers of genomic changes. In this presentation I'll summarize our progress in the analysis of structural variation in complex human disorders, including normal variability and its phenotypic consequences.

Genome-Wide Meta-Analysis Increases to 71 the Number of Confirmed Crohn's Disease Susceptibility Loci

Presenting Author: Andre Franke
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We undertook a meta-analysis of six Crohn's disease (CD) genome-wide association studies (GWAS) comprising 6,333 cases and 15,056 controls, and followed up the top association signals in 15,694 cases, 14,026 controls and 414 parent/offspring trios. Thirty new susceptibility loci meeting genome-wide significance (P-value $<5\times10^{-8}$) were identified. A series of in silico analyses highlighted particular genes within these loci and, together with manual curation, implicated functionally interesting candidate genes including SMAD3, ERAP2, IL10, IL2RA, TYK2, FUT2, DNMT3a, DENND1B, BACH2 and TAGAP. Combined with previously confirmed loci, the results described here identify a total of 71 distinct loci with genome-wide significant evidence for association with Crohn's disease.

Genome-wide association study of migraine implicates a common susceptibility variant on 8q22.1

Presenting Author: Christian Kubisch
International Headache Genetics Consortium

International Headache Genetics Consortium

Migraine is a common episodic neurological disorder, typically presenting with recurrent attacks of severe headache and autonomic dysfunction. Apart from rare monogenic subtypes, no genetic or molecular markers for migraine have been convincingly established. We identified the minor allele of rs1835740 on chromosome 8q22.1 to be associated with migraine ($P = 5.38 \times 10^{-9}$, odds ratio = 1.23, 95% CI 1.150–1.324) in a genome-wide association study of 2,731 migraine cases ascertained from three European headache clinics and 10,747 population-matched controls. The association was replicated in 3,202 cases and 40,062 controls for an overall meta-analysis P value of 1.69×10^{-11} (odds ratio = 1.18, 95% CI 1.127–1.244). rs1835740 is located between MTDH (astrocyte elevated gene 1, also known as AEG-1) and PGCP (encoding plasma glutamate carboxypeptidase). In an expression quantitative trait study in lymphoblastoid cell lines, transcript levels of the MTDH were found to have a significant correlation to rs1835740 ($P = 3.96 \times 10^{-5}$, permuted threshold for genome-wide significance 7.7×10^{-5}). To our knowledge, our data establish rs1835740 as the first genetic risk factor for migraine.

A conserved trans-acting regulatory locus underlies an inflammatory gene network and susceptibility to autoimmune type 1 diabetes

Presenting Author: Anja Bauerfeind
Matthias Heinig 1,2, Enrico Petretto 3,4, Chris Wallace 5, Leonardo Bottolo 3,4, Maxime Rotival 6, Han Lu 3, Yoyo Li 3, Rizwan Sarwar 3, Sarah R. Langley 3, Anja Bauerfeind 1, Oliver Hummel 1, Young-Ae Lee 1,7, Svetlana Paskas 1, Carola Rintisch 1, Kathrin Saar 1, Jason Cooper 5, Rachel Buchan 3, Elizabeth E. Gray 8, Jason G. Cyster 8, Cardiogenics Consortium, Jeanette Erdmann 14, Christian Hengstenberg 10, Seraya Maouche 6, Willem H. Ouwehand 11,12, Catherine M. Rice 12, Nilesh J Samani 13, Heribert Schunkert 14, Alison H Goodall 13, Herbert Schulz 1, Helge Roider 2, Martin Vingron 2, Stefan Blankenberg 16, Thomas Münzel 15, Tanja Zeller 15, Silke Szymczak 16, Andreas Ziegler 16, Laurence Tiret 6, Deborah J. Smyth 5, Michal Pravenec 17, Timothy J. Aitman 3, Francois Cambien 6, David Clayton 5, John A. Todd 5, Norbert Hubner 1, Stuart A. Cook 3,18

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Introduction: Although genome-wide association studies have uncovered many common genetic variants associated with human diseases, the molecular mechanisms by which DNA variation affects disease risk remain poorly characterized. Combined analyses of gene networks and DNA sequence variation have the potential to provide new insights into the aetiology of common diseases.
Methods: A panel of HxB/BxH rat recombinant inbred strains was used to study transcription-factor (TF-) driven gene networks and their regulatory loci. We combined expression quantitative trait loci (eQTL)-data with TF- binding site enrichment to identify core gene networks centred on transcription factors. The core networks were expanded to co-expressed genes across rat tissues and isolated human monocytes obtained from population-based individuals from the Gutenberg-Heart-Study and the Cardiogenics Study. A combined network was constructed and analysed for association with T1D.
Results: We defined a gene network centred on the interferon response transcription factor Irf7, which is regulated in multiple rat tissues by a single locus on chromosome 15q25. At this locus, Epstein-Barr virus induced gene 2 (Ebi2), which we localised to macrophages and controls B cell migration, directly regulates the Irf7 network that represents a molecular

biomarker for macrophages in tissues. The IRF7 network is conserved in human monocytes and network genes were significantly more likely to associate with T1D than randomly selected genes ($P = 2.9 \times 10^{-8}$). The human chromosome 13q32 locus that is orthologous to the Ebi2 rat region controlling the network was associated with risk of T1D at SNP rs9585056 in two independent large human cohorts (combined $P = 7.0 \times 10^{-10}$, odds ratio = 1.15).

Summary: Our data implicate IRF7 network genes and their regulatory locus in the pathogenesis of T1D and demonstrate how integration of gene networks and genome-wide association analyses can advance knowledge of disease pathogenesis.

CARDIoGRAM: Thirteen novel genetic loci affecting risk of coronary artery disease

Presenting Author: Jeanette Erdmann
Jeanette Erdmann, Inke R. König, Michael Preuss, Christian Hengstenberg, Sekar Kathiresan, Muredach P. Reilly, Themistocles L. Assimes, Hilma Holm, Panos Deloukas, John R. Thompson, Kari Stefansson, Robert Roberts, Christopher J. O'Donnell, Ruth McPherson, Nilesh J. Samani, Heribert Schunkert on behalf of CARDIoGRAM

University of Lübeck

BACKGROUND
Coronary artery disease has a significant heritability that is incompletely characterized.
METHODS
We combined data from 14 genomewide association studies and compared allele frequencies of more than 2 million single nucleotide polymorphisms in 22,233 cases with coronary disease and 64,762 controls, all of European ancestry. Promising variants were followed-up in up to 49,343 additional individuals. Novel loci confirmed to be associated with risk of coronary disease were further investigated for correlation with traditional cardiovascular risk factors in population-based samples, with gene expression in multiple tissues, and for co-localization with other traits.
RESULTS
We established firm association ($P<5x10^{-8}$) with coronary disease for 23 chromosomal loci, 13 of which are novel. At the 13 novel loci, risk allele frequencies range from 13% – 91% and the odds ratios for coronary disease range from 1.06 – 1.17 per copy. Only three of these loci display significant association with traditional risk factors. Risk alleles at four of these loci are strongly associated with transcript levels of nearby genes including TCF21 (6q23.2), RASD1, SMCR3 and PEMT (17p11.2), SMG6 (17p13.3), and UBE2Z, GIP, ATP5G1, and SNF8 (17q21.32). Risk alleles at five novel loci are, in addition to coronary disease, strongly associated with various other human disease traits.
CONCLUSIONS
The genetic predisposition for coronary disease is mediated in part by multiple common genetic variants of small to moderate effect size. Many appear to act via biological mechanisms that are independent of traditional risk factors.

Oral Presentation Abstracts

Symposium III

Animal, Cellular & Tissue Models

The Genetic Control of Lysosomal Function and of Cellular Clearance

Presenting Author: Andrea Ballabio

Telethon Institute of Genetics and Medicine and Baylor College of Medicine

The lysosome is involved in numerous diseases including lysosomal storage disorders (LSDs) and neurodegenerative diseases. All these diseases share as a common feature the progressive accumulation of undigested macromolecules within the cell that are either intermediates of the cellular catabolism, or proteins that tend to form pathogenic aggregates. To facilitate the coordination of complex functions, such as cellular clearance, the cell has developed a strategy by which these processes occur in specialized organelles. As the degradative requirements of the cell vary depending upon tissue type, age and environmental conditions, it is reasonable to expect the presence of systems that allow regulation of lysosomal function. We discovered a gene regulatory network (CLEAR: Coordinated Lysosomal Enhancement And Regulation) that controls lysosomal biogenesis and function. The transcription factor TFEB acts as a modulator of the CLEAR network and it is physiologically activated by lysosomal storage. Upon activation, TFEB translocates into the nucleus, binds to CLEAR target sites in the promoter of lysosomal genes, induces lysosomal biogenesis and increases the ability of the cell to degrade complex molecules, such as glycosaminoglycans (GAGs) (Sardiello et al. Science 2009). More recent studies in our laboratory revealed that TFEB is directly involved in the regulation of two important cellular processes mediated by the lysosome: autophagy and lysosomal exocytosis. Notably, activation of lysosomal exocytosis in four different types of lysosomal storage diseases leads to the clearance of the pathological storage both in vitro and in vivo in mouse models of the human diseases, and to a striking rescue of the cellular morphological abnormalities. These results unveil a novel mechanism that exerts a global control on the cellular degradation machinery and provide a tool to promote cellular clearance in human disease.

Mouse models endogenous pancreatic cancer – role of EGFR and Notch signaling

Presenting Author: Jens Siveke
Jens Siveke, Roxanne Brodylo, Pawel Mazur, Barbara Grüner, Irina Heid, Marija Trajkovic-Arsic, Roland M. Schmid

II. Med. Klinik & Poliklinik

Klinikum rechts der Isar der TU München

Pancreatic ductal adenocarcinoma (PDAC) is an invariably lethal disease. The lack of effective therapies underscores the need for better preclinical models with high predictive value. In addition, novel therapeutic approaches include targeting of developmental pathways. Notch signaling is a key regulator of cell fate specification and pancreatic cancer development and closely interacts with EGFR signaling, which is commonly activated in pancreatic cancer.
We here demonstrate the role of Notch and EGFR/Ras-dependent pathway members using conditional knockout and knockin approaches for activation and inactivation of the respective pathways. These genetically engineered mouse models were phenotyped using histomorphological, molecular and bioinformatic analyses.
We show that alterations in these pathways lead to distinctive phenotypes demonstrating the high plasticity of the pancreatic progenitor and exocrine compartment. We provide evidence for EGFR- and Notch-dependent signaling pathways as key regulators of pancreatic carcinogenesis, which generate a complex signaling network and determine the development and progression of distinctive preneoplastic lesions to pancreatic cancer. Using multimodal MRT, PET/CT and molecular imaging, these models can be non-invasively characterized and are utilized for preclinical therapeutic studies.
In conclusion, genetically engineered mouse models are highly suitable for analysis of key signaling events in pancreatic carcinogenesis and for preclinical therapeutic studies with high predictive value.

Generation of functional cardiomyocytes from patient-specific induced pluripotent stem cells

Presenting Author: Kaomei Guan
Kaomei Guan1, Frieder Wolf1, Katrin Streckfuss-Bömeke1, Azadeh Azizian1, Stefan Wagner1, Poh Loong Soong2, Simin Chen2, Ralf Dressel1, Michael Stauske1, Wolfram H. Zimmermann2, Gerd Hasenfuss1

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The recent discovery of reprogramming somatic cells to induced pluripotent stem cells (iPSCs) provides a unique opportunity for generation of patient-specific (ps) cells for use in disease model and screening teratogens. Aim of this study was to generate functional cardiomyocytes from ps-iPSCs. Methods and Results: We transduced bone marrow-derive cells of patients with four factors (Oct4, Sox2, Nanog and Lin28) using a lentiviral system. The generated ps-iPSCs showed phenotypical characteristics similar to human embryonic stem cells. RT-PCR analysis showed that they expressed pluripotency markers Oct4, Nanog, Sox2 and Lin28. Immunocytochemistry studies showed that they were positive for alkaline phosphatase, Nanog, Oct4, Sox2, Tra-1-60 and SSEA4. Our reprogramming efficiency is 0.05% comparable to the previously published data. After injection of the cells into SCID mice, teratomas were formed. When cultivated as embryoid bodies (EBs), the cells spontaneously differentiate into derivatives of three embryonic germ layers. During differentiation in vitro, tissue-specific genes and proteins were expressed in a developmentally controlled manner as demonstrated by RT-PCR and immunocytochemical staining. Action potential analyses (n=35 cells) demonstrate the presence of pacemaker-, atrial-, ventricular- and Purkinje-like cardiomyocytes. Confocal microscopy analysis of intracellular Ca exhibits that calcium increases homogenously throughout the iPSC-derived cardiomyocyte. Using line scan mode, rhythmic Ca transients are found and even Ca sparks, which are mainly due to the sarcoplasmic reticulum Ca release through a cluster of RyRs, appear. Conclusion: we report for the first time that the four defined factors are sufficient to reprogram bone marrow-derived cells of patient to iPSCs. These ps-iPSCs can be differentiated into functional cardiomyocytes. The findings open new possibilities for basic research on heart diseases as well as cardiac regeneration.

CRHR1, a key regulator of stress, regulates anxiety in opposite directions by controlling glutatmatergic and dopaminergic neurons

Presenting Author: Jan Deussing
Damian Refojo1, Martin Schweizer1,2, Claudia Kuehne1, Stefanie Ehrenberg1, Christoph Thoeringer1, Annette M. Vogl1, Nina Dedic1, Marion Schumacher1, Gregor von Wolff1, Charilaos Avrabos1, David Engblom3 Günther Schütz3, Evan Deneris4, Klaus-Armin Nave5, Matthias Eder1, Carsten T. Wotjak1, Inge Sillaber1, 2, Wolfgang Wurst1,6-8, Florian Holsboer1, Jan M. Deussing1

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Corticotropin-releasing hormone (CRH) and its type 1 high affinity receptor (CRHR1) are widely distributed throughout the CNS. Together they orchestrate the neuroendocrine and behavioral adaptation to stress. Chronic stress-associated dysfunction of CRH/CRHR1-related neuronal circuitries has been implicated in the etiology of mood and anxiety disorders. However, owing to the unavailability of reliable antibodies, the neurotransmitter (NT) identity of neurons expressing CRHR1 and mediating its effects remains largely unknown. This is a fundamental question because CRH, as neuroregulator, does not exhibit a robust effect upon membrane potential per se, but acts via facilitating or depressing the actions of “true” neurotransmitters.
To this end, we established a systematic neurochemical map of CRHR1 expression in different NT systems using a sensitive double ISH method and a newly generated CRHR1 knock-in GFP reporter mouse line. To ultimately dissect the NT circuits, which mediate the effects of CRH/CRHR1 on emotional behavior, we applied conditional mutagenesis in the mouse to selectively disrupt CRHR1 function in (i) glutamatergic, (ii) GABAergic, (iii) dopaminergic and (iv) serotonergic neurons. Comprehensive behavioral testing revealed a decrease in anxiety-like behavior in glutamatergic CRHR1 KO mice and an unexpected increase in anxiety-like behavior in mice lacking CRHR1 in dopaminergic neurons. Moreover, mechanistic studies demonstrated that CRHR1 regulates aspects of glutamatergic and dopaminergic neurotransmission that are highly relevant for controlling anxiety-like behavior. The observed dual role of CRHR1 suggests, that under physiological conditions, CRH/CRHR1-controlled glutamatergic and dopaminergic systems might function in an antagonistic manner to keep anxiety-related responses in balance. This opens up the hypothesis that the role of CRHR1 in mood disorders might be secondary to an imbalance of these two NT circuits.

Identification of a new peroxiredoxin allele with a phenotype of oxidant-resistance and premature aging by whole genome resequencing of yeast.

Presenting Author: Bernd Timmermann
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The combination of functional genomics with next generation sequencing facilitates new experimental strategies for addressing complex biological phenomena. Here, we report the identification of a gain-of-function allele of peroxiredoxin (thioredoxin peroxidase, Tsa1p) via whole-genome re-sequencing of a dominant Saccharomyces cerevisiae mutant obtained by chemical mutagenesis. Yeast strain K6001, a screening system for lifespan phenotypes, was treated with ethyl methanesulfonate (EMS). We isolated an oxidative stress-resistant mutant (B7) which transmitted this phenotype in a background-independent, monogenic and dominant way. By massive parallel pyrosequencing, we generated an 38.8 fold whole-genome coverage of the strains, which differed in 12,482 positions from the reference (S288c) genome. Via a subtraction strategy, we could narrow this number to 13 total and 4 missense nucleotide variations that were specific for the mutant. Via expression in wild type backgrounds, we show that one of these mutations, exchanging a residue in the peroxiredoxin Tsa1p, was responsible for the mutant phenotype causing background-independent dominant oxidative stress-resistance. These effects were not provoked by altered Tsa1p levels, nor could they be simulated by deletion, haploinsufficiency or over-expression of the wild-type allele. Furthermore, via both a mother-enrichment technique and a micromanipulation assay, we found a robust premature aging phenotype of this oxidant-resistant strain. Thus, TSA1-B7 encodes for a novel dominant form of peroxiredoxin, and establishes a new connection between oxidative stress and aging. In addition, this study shows that the re-sequencing of entire genomes is becoming a promising alternative for the identification of functional alleles in approaches of classic molecular genetics.

Oral Presentation Abstracts

Symposium IV

Systems Biology

Biochemical approaches to biomolecular networks

Presenting Author: Anne-Claude Gavin

EMBL, Heidelberg

Since the sequencing of the first eukaryotic genome, Saccharomyces cerevisiae, more than 10 years ago, explosion of new analytical tools in the fields of transcriptomics, proteomics and metabolomics contributes ever-growing molecular repertoires of the building blocks that make up a cell. Biology does not rely on biomolecules acting in isolation. Biological function depends on the concerted action of molecules acting in protein complexes, pathways or networks. Biomolecular interactions are central to all biological functions. In human, for example, impaired or deregulated protein-protein or protein-metabolite interaction often leads to disease. Recent strategies have been designed that allow the study of interactions more globally at the level of entire biological systems. We will discuss the use of these biochemical approaches to genome-wide screen in model organisms.

Identification of Y-box binding protein 1 as a core regulator of MEK/ERK pathway- dependent gene signatures in colorectal cancer cells

Presenting Author: Reinhold Schäfer
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Transcriptional signatures are an indispensible source of correlative information on disease-related molecular alterations on a genome-wide level. Numerous candidate genes involved in disease and factors of predictive as well as of prognostic value have been deduced from such molecular portraits, e.g. in cancer. However, mechanistic insights into the regulatory principles governing global transcriptional changes are lagging behind extensive compilations of deregulated genes. To identify regulators of transcriptome alterations, we used an integrated approach combining transcriptional profiling of colorectal cancer cell lines treated with inhibitors targeting the receptor tyrosine kinase (RTK) / RAS / mitogen-activated protein kinase (MEK/ERK) pathway, computational prediction of regulatory elements in promoters of co-regulated genes, chromatin-based and functional cellular assays. We identified commonly co-regulated, proliferation-associated target genes that respond to the MAPK pathway. We recognized E2F and NFY transcription factor binding sites as prevalent motifs in those pathway-responsive genes and confirmed the predicted regulatory role of Y-box binding protein 1 (YBX1) by reporter gene, gel shift and chromatin immuno-precipitation assays. We also validated the MAPK-dependent gene signature in colorectal cancers and provided evidence for the association of YBX1 with poor prognosis in colorectal cancer patients. This suggests that MEK/ERK-dependent, YBX1-regulated target genes are involved in executing malignant properties.

Genome-wide miRNA level regulation of ErbB receptor protein network in breast cancer

Presenting Author: Özgür Sahin
Stefan Uhlmann1, Heiko Mannsperger1, Jitao David Zhang1, Emoke-Agnes Horvath2, Christian Schmidt1, Moritz Küblbeck1, Nina Zweig2, Stefan Wiemann1, Ulrike Korf1, Özgür Sahin1

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Aberrant EGFR (also known as ErbB receptor) signaling can induce development of many human cancers e.g. breast, lung, brain and gastric carcinomas. Particularly, EGFR and ErbB2 belong to the most oncogenic kinases in humans, and are in the center of targeted therapy. A novel class of post-transcriptional regulators, microRNAs (miRNAs), emerged recently as important regulators of tumorigenesis of different cancer entities. Although there have been several miRNAs (e.g. miR-7 and miR-205) shown to target and regulate components of the ErbB receptor network and cancer-related cellular phenotypes, there is no systematic genome-wide level study of miRNA regulation of ErbB network. Therefore, we aimed at elucidating how miRNAs regulate the ErbB receptor protein network, and investigating whether they could act as potential targets for breast cancer therapy. We started with constructing the ErbB receptor network including all ErbB receptor family members, downstream signaling intermediates, negative feedback regulators, key TFs and cell cycle proteins. Using miRNA mimic library containing 810 miRNAs, we screened the effects of each miRNA on the expression levels of around 40 ErbB network proteins using Reverse Phase Protein Arrays (RPPAs). We observed very interesting co-regulation patterns of network components by miRNAs using different clustering approaches, and currently testing the capacity of these “node” miRNAs as potential targets in certain subsets of breast cancer. Hence, combining bioinformatics, functional genomics, proteomics as well as molecular and cellular biology approaches, we integrate miRNAs into well-studied ErbB receptor network for breast cancer therapy.

Proteomics and metabolomics analysis for biomarker discovery in a trait anxiety mouse model

Presenting Author: Michaela D. Filiou
Michaela D. Filiou, Yaoyang Zhang, Larysa Teplytska, Stefan Reckow, Philipp Gormanns, Elisabeth Frank, Melanie Kessler, Boris Hambsch, Markus Nussbaumer, Alexander Yassouridis, Giuseppina Maccarrone, Rainer Landgraf, Chris W. Turck

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Although anxiety disorders are the most prevalent psychiatric disorders, no molecular biomarkers exist for their premorbid diagnosis, accurate patient subcategorization or treatment efficacy prediction. To unravel the neurobiological underpinnings and identify candidate biomarkers for anxiety disorders, we interrogated the mouse model of high (HAB), normal (NAB) and low (LAB) anxiety-related behavior employing a quantitative proteomics and metabolomics discovery approach. We compared the cingulate cortex synaptosome proteomes of HAB and LAB mice by in vivo 15N metabolic labeling and quantitative LC-ESI-MS/MS. In addition, we quantified the cingulate cortex metabolome profiles of HAB/NAB/LAB mice by GC-TOF-MS. The combined datasets were used to identify divergent protein and metabolite networks by in silico pathway analysis. Our data reveal alterations in energy metabolism, mitochondrial import and transport, oxidative stress and neurotransmission suggesting a previously not highlighted role of mitochondria in modulating anxiety-related behavior. Our results provide the basis for the establishment of a biomarker panel for anxiety disorders and offer insights toward a systemic understanding of anxiety pathophysiology with a focus on mitochondrial contribution.

Orthology-based phenotypic inference from disease models to human applications.

Presenting Author: Jean-Fred Fontaine
Jean-Fred Fontaine and Miguel A Andrade-Navarro

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Although many ortholog genes and proteins can exist between two different species, their respective complex molecular systems can not be considered similar. The translation of disease studies performed on animal models to human is not trivial and requires extensive validation experiments. Such studies may benefit from advanced data mining on public biological databases and private datasets, especially by using orthology information for phenotypic inference. It would allow the selection of the best human-animal orthologous genes even if literature information is incomplete or missing.
Novel algorithms were implemented to process the Entrez Gene, Homologene, MeSH and MEDLINE databases to derive orthology-based gene and phenotypic data. First, gene-phenotype associations were automatically set for entire genomes of more than 300 species with high precision on disease phenotypes using Fisher's statistics (range: 70%-100%). Then, orthology-based data transfer and phenotype analysis was set between 20 species. Integration of gene-expression experiments on heart development showed high precision in the prediction of functional genes, and highlighted the high potential of this method to bring novel candidates to study human cardiovascular diseases in animal models.
This novel method has exclusive and more extended features than existing methods, and it showed the best performances on several benchmarks. Data and literature mining on entire databases has the potential to systematically and precisely associate human and animal model genes in relation to a phenotype. Such method may help in selecting genes or proteins that are more likely to be validated in confirmatory studies.

Genomes and people: How do they come together?

Presenting Author: Regine Kollek

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After completion of the Human Genome Project, the availability of affordable high throughput technology for parallel genotyping has opened the field of genetics to genome-wide association studies (GWAS). Such studies produce detailed, genotype information, and the results of more focused studies can potentially be used to determine genetic variation for a wide range of conditions and traits. Insofar they are – in ethical and social terms – extremely challenging. In addition, information derived from GWA scans is a powerful personal identifier; it provides information not just on the individual, but also on his or her relatives.

The use of such studies in biomedical research and the increased ability to share data create a need to update the discourse on some of the ethical questions which have – up to now – mainly been debated in the context of more conventional genetic and genomic research. Among them are questions of participant information and consent, feedback of research results, privacy and confidentiality, and of the waning distinction between research and clinical care, just to name a few.

The lecture will take up some of these issues and discuss them in the German legal context. How does the German Law on Genetic Diagnosis (GenDG) affect the work of genome researchers? Do gene expression studies fall under the GenDG? What kind of implications do GWAS studies have for patient information and informed consent? What may be the future ethical and legal challenges of clinic-genomic research? The lecture will close with some reflections on the questions what may happen if we fail to cope with these challenges adequately? Could this lead to an impairment of trust on the part of potential research participants? Since genomic and associated clinical research fundamentally depends on human subject participation, this should be avoided by all means.

National Genome
Research Network

Oral Presentation Abstracts

Symposium V

Transfer from Genomics to Application

Targeted combination therapy of HER2-positive breast cancer

Presenting Author: Max Hasmann

Roche Diagnostics GmbH, pRED Penzberg, Germany

Among the most extensively investigated biological targets in breast cancer (BC) are members of the human epidermal growth factor receptor (HER) family, HER1 – HER4. At least some of these receptors are often dysregulated. For example, amplification and overexpression of HER2 occurs in approximately 20%-25% of human BCs and is associated with a relatively poor prognosis, with faster time to relapse or progression of disease. The introduction of trastuzumab for the treatment of HER2-positive BC in different lines of therapy, from early BC to metastatic disease in first and later lines, has recently changed the natural course of this aggressive cancer. Unfortunately, some patients suffer disease recurrence despite trastuzumab or do not respond to first line therapy. One potential explanation is that HER2 may interact with other, ligand-activated HER family members like HER3 enabling heterodimer signalling which is not disturbed by trastuzumab. To overcome this problem, several concepts are being tested in clinical trials and some promising results are already available: combination treatment of HER2-positive BC with trastuzumab and pertuzumab, another monoclonal antibody binding to a different epitope on HER2, demonstrated synergistic activity in patients whose tumour progressed on trastuzumab therapy. On the other hand, trastuzumab-DM1 (T-DM1), an antibody-drug conjugate that delivers the cytotoxic agent DM1, a maytansine derivative, to HER2-overexpressing cancer cells, has shown encouraging single-agent efficacy and a good safety profile in patients with heavily pre-treated HER2-positive metastatic BC in two Phase II clinical trials. Perspectives of these new treatment options by mechanism-based combination targeted therapy including T-DM1 and pertuzumab will be discussed.

A next generation genome-wide view of (epi)genetic alterations in clinically distinct colon cancers.

Presenting Author: Michal-Ruth Schweiger
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The chemical modification of the C5 position of the cytosine ring (5mC) in CpG dinucleotides alters the structure of cytosine without changing its base-pairing properties. These changes are locus - specific with certain methylation patterns found in a diverse array of complex human diseases such as cancer, systemic autoimmune and psychiatric diseases; first epigenetic biomarkers are under development. On the other side, specific inter-individual variations exist which also seem to be age-dependent. Loci which are prone to age-related variations are also thought to be the primary sites of epigenetic changes in cancer. However, due to a lack of genome profiling technologies, these interconnections have been primarily investigated with hypothesis – driven experiments. Here, we utilized an antibody-based immunoprecipitation of 5mC followed by next generation sequencing (MeDIP-seq) to gain an in principle unbiased view of the genome wide methylation patterns of colorectal cancer patients. We performed MeDIP-seq experiments on matched sample pairs (normal mucosa and tumour tissue) and generated approximately $4x10^8$ unique mappable reads and identified several significantly differentially methylated regions which may serve as epigenetic biomarkers. We also took advantage of genetic information generated from different next generation sequencing (NGS) applications on the same samples such as CNVs, gene expression and mutational profiles to identify tumor-specific subclasses. These subclassifications are useful for further elucidation of the pathogenesis of colorectal cancer.

PROCEED SP10: Molecular tumor imaging using antibody-coated nanoparticles; Construction of antibody conjugated fluorescent nanoparticles for in vivo imaging of prostate cancer cells.

Presenting Author: Katja Werner
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The technical challenge of in vivo imaging lies in the quality of the conjugates presenting a reporter assisted biomolecule for specific cell targeting. In the first period of the project we developed an IP protected method for the highly reproducible synthesis of top quality semiconductor nanoparticles based on a continuous flow approach. For the enhancement of the quality of water soluble quantum dots (QD) we additionally developed a rigid phase transfer method for the encapsulation of nanoparticles. In benefit we achieved non-toxic, highly stable and water soluble fluorescent nanoparticles in highly reproducible quality. The polymer used for the encapsulation offers a variety of endgroups for different bioconjugation strategies. We established standart operation procedures for IgG antibody conjugation to to fluorescent nanoparticles. Currently we are screening a wide range of bioconjugation methods on different biomolecules to develop a conjugation matrix predicting the most promising strategy for a defined application. For applications in the PROCEED project we conjugated three different monoclonal PSMA antibodies to our semiconductor nanoparticles for the proof of principle in vitro experiments. We realised, that the most important part of work is the construction of a homogenous conjugate sample basically free of clusters, un-conjugated antibody and nanoparticle. We analyzed the conjugates in vitro in 96-well plate assays using an anti-host antibody and recombinant PSMA antigen as targets and the fluorescent readout of the reporter. Our constructs proved to be highly sensitive and specific in these recombinant assays. Even though, the targeting kinetik of the molecules needs further optimisation. We are investigating strategies to increase the accessibility of the biomolecule without losing specificity and stability, which seems to be the technical challenge in this attempt.

NTCVD-Consortium identified known and new proinflammatory and profibrotic biomarkers in patients with chronic kidney disease

Presenting Author: Heike Bruck
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Background:
Patients with chronic kidney disease (CKD) suffer from increased cardiovascular morbidity and mortality, but mechanisms are not clearly understood. Thus, accelerated atherosclerosis, fibrosis and inflammation-related vascular damage seem to play a role.
Methods:
Cystatin C and different proinflammatory (CRP, resistin, tenascin-C FNIIIB) and profibrotic (osteopontin, t-PAI-1 protein) biomarkers were measured in blood samples collected from 93 patients with chronic kidney disease (n=51 CKD 3-4, eGFR 15-59 ml/min and n=42 CKD 5/D, eGFR <15ml/min including patients on hemodialysis) and 64 control subjects with normal renal function (controls, eGFR =60 ml/min). Classical cardiovascular risk factors, cardiovascular history and events were documented and cardiovascular and renal function were clinically studied including pulse wave analysis, ankle-brachial-index, electro- and echocardiography, exercise-testing.
Data were analysed with ANOVA and t-test and were presented as mean ± standard deviation.
Results:
With decreasing kidney function different biomarker levels significantly increased (Cystatin C controls: 0,84±0,22 vs. CKD 3-4: 1,43±0,74, CKD 5/D: 3,4±1,1 mg/l, p<0.00; CRP controls: 0,22±0,5 vs. CKD 3-4: 0,43±0,66, CKD 5/D: 1,34±3,58 mg/dl, p= 0.012; resistin controls: 9,3±3 vs. CKD 3-4: 15,6±7,2, CKD 5/D: 33,7±7,8 ng/ml, p<0.00; tenascin-C FNIIIB controls: 510±226 vs. CKD 3-4: 519±156, CKD 5/D: 775±207 ng/ml, p<0.00; osteopontin controls: 76,63±20,87 vs. CKD 3-4: 102,43±67,09, CKD 5/D: 216,36±97,54 ng/ml, p<0.00) while t-PAI-1 protein levels significantly decreased (controls: 6,49±5,78 vs. CKD 3-4: 5,33±3,16, CKD 5/D: 3,53±2,26 ng/ml, p=0.006).
Conclusion:
The NTCVD-Consortium identified known and unknown profibrotic and proinflammatory biomarkers potentially involved in progression of chronic kidney disease and its complications (particularly cardiovascular disease).

Identification of the gaps in integration of genome-based knowledge in the International Public Health legislation

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Background
Genome-based knowledge is growing rapidly, but a gap exists between basic science research and the translation of findings into Public Health. Besides, many controversies, such as reimbursement of personalised drugs are to be resolved. For successful introduction of the genome-based knowledge into Public Health tasks, policy actions must be taken on all levels. The PHGEN II project (Public Health Genomics European Network) aims to provide guidance for introduction of genome-based information in Public Health policy at the EU level. We analysed existing legislation dealing with the use of genome-based knowledge in order to identify existing gaps.
Methods
We collected existing evidence-based policies by using major online search resources. Included documents should satisfy the following criteria: international, issued in the last 20 years, dealing with genome information\technologies, not cancelled or reissued. The expert opinion of the partners of the project was required to reflect on and grade the collected evidence.
Documents were categorized by the area of coverage using a list of important policy areas for successful introduction of genome-based technologies into public health 1.
Results
Serious inequalities in coverage of important issues of Public health Genomics were found. Clinical utility and clinical validity of the screening and testing of human objects were most often addressed. Areas such as trade agreements, public adoption of technologies, behavior modification in response to genomics results were not covered at all.
Conclusion
For the successful adoption of genome-based information in the system of Public Health it is necessary to pay attention to all aspects of Public Health Policy-making. For the successful adoption of the new technologies on the public health level the focus should be not only on the translation to clinical practice, but the translation from bench to public health policy and back, a more integrative model is needed.

Oral Presentation Abstracts

Symposium VI

New Technologies

Whole Genome Sequence of a Crohn disease trio – a paradigm for etiology discovery in complex disease?

Presenting Author: Philip Rosenstiel
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Gathering knowledge on entire individual disease genomes is a logical advancement of GWAS as it includes rare and potentially causative variants "hidden" on common haplotypes. For this benchmarking study, a Falk-Rubinstein trio with an extreme phenotype was selected, i.e. early, severe onset in the affected child. Genome sequencing was carried out on an Applied Biosystems SOLiD System. We analyzed approx. 1.5 billion shotgun mapped reads from 50+25 bp paired end libraries and 0.5 billion 50+50 mate pair reads for each of the individuals. With a minimum mapped individual throughput of 90 Gb, over 99% of the reference genome was covered with at least one uniquely placed pair of reads. The genome data sets resulted in approx. 3.3 million SNP calls in each individual with high confidence, approx. 20% of which are not annotated in current databases. An Illumina SNP-Chip (HumanOmni1-Quad_v1-0) was applied as a benchmark for the SNP identification methods. SNP calling by sequence analysis and SNP-chip data showed a correlation of >99.3% for all three individuals. Categorization of the SNPs in relation to functional elements in the genome was performed and identified >10,000 novel SNPs in gene associated regions. We utilized the pedigree structure to prioritize putatively functional SNPs which follow a recessive mode of inheritance (gain of homozygosity or compound heterozygous SNPs). The individual genomes are related to corresponding RNAseq data from peripheral blood mononuclear cells and intestinal tissue resulting in a comprehensive map of expression levels, alternative splicing and allelic imbalance that was further used to filter the genomic information. In conclusion, we present the first study aiming for the extraction of the full genetic variability in a disease trio and utilizing this data together with genetical genomics analyses by RNAseq as a basis for personalized understanding of the genetic variants that cause human disease.

A map of human genome variation from population scale sequencing (the 1000 Genomes Project)

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The international Human Genome Project has, with participation of German groups, established the reference sequence of the human genome. With the advent of next generation sequencing technologies, it has become feasible to extend this analysis to a detailed characterisation of the genomes of individual humans, an essential basis for the discovery and understanding of the genetic variants that influence human disease. The analysis of individual genomes will provide the missing link to translate the wealth of recent association findings into an individual understanding of how the phenotypes are generated. In response to this, the 1000 Genomes Project (www.1000genomes.org) has been launched in 2008 by a number of international centres. The aim of the project is to discover genotype and provide accurate haplotype information on all forms of human DNA polymorphism in multiple human populations. During the project pilot phase three studies were conducted to test multiple strategies to produce a catalogue of genetic variants that are present in 1 percent or greater frequency in the different populations (Nature in press) chosen for study (European, African and East Asian). The adjacent production phase of the full project combines low coverage whole genome sequencing, array based genotyping, and deep targeted sequencing of all coding regions in 2,500 individuals from five large regions of the world (five population samples of 100 in or with ancestry from each of Europe, East Asia, South Asia and West Africa, and seven populations totalling 500 from the Americas. We increased the low coverage average depth to over 4x per individual. We successfully finished the phase 1 of the full project by sequencing 50 samples to coverage of 4x. In phase 2 of the full project 75 additional samples and the exomes of all 125 samples will be analysed. This project will be key for a further understanding of genetic variation and hence have a significant importance for commercial exploitation.

Whole genome amplification in large biobanks

Presenting Author: Norman Klopp
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Biobanks are a key resource for unravelling the molecular basis of diseases and the identification of new targets for disease therapy. One challenge of biobanks is to maintain huge collections of limited samples sizes for future studies. In consequence, there is a need for a high quality amplification of limited amounts of nucleic acids. In this project we focused on the standardization and qualification tools of whole genome amplification (WGA) in the context of biobanks. A new simple quantitative PCR based test system was developed, which enables the standardization of the complete whole genome amplification process including (1) the quality and quantity of the template DNA and (2) the resulting WGA products. For validation of the performance, the system was tested in different laboratories using template DNAs of different quality. Consistent results could be achieved in the quality system test of the WGA products reflecting the amount and the fragmentation degree of the initial template DNA. The system was also implemented in the KORA-Biobank with SNP genotyping technology. The results of the WGA test system were correlated with the concordance of genome wide SNP genotypes from genomic template DNA and from the corresponding WGA products. Based on this result, a general standardized protocol for WGA in biobanking will be developed.
Furthermore, the concept of this project is to make the WGA solution available to other national and international biobanks. The development of the proposed, innovative and specialized tools and customized solutions will help to expand and improve the used of biobanks.

Analysis Pipeline for Exome Sequencing Data

Presenting Author: Sebastian Eck
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Enrichment techniques for targeted sequencing of coding regions are currently applied to identify rare variants. We developed a pipeline to analyze exome sequencing data. The pipeline is a collection of Perl scripts which start with the sequence files generated by the Illumina software. It calculates quality metrics and performs read alignment to the reference sequence, variant calling, variant annotation and selection of candidate variants according to the genetic model. Variants are stored in a database, which allows user queries through a web interface and enables pedigree or gene based searches. Exomes deposited in the database can be used as controls.
Alignment and variant calling is performed with BWA and SAMtools. First, quality metrics are calculated which include base quality, % mapped reads, % duplicates, % reads overlapping the target regions and read depth an a single base level. As a second task, variants are further filtered and annotated. Annotation includes presence in dbSNP, type of mutation and - if applicable - amino acid change. In addition, the frequency of the variants in our exome samples is determined. These information is then used, in conjunction with optional information such as inheritance model, affected siblings or a linkage region, to identify putative causative variants.
The pipeline has a modular composition and subsets of components may be run in an arbitrary combination. For manual inspection of the results, bed- and html-files are provided.
We applied the analysis pipeline to approximately 50 exomes. From an average of ~7 GB of aligned sequence, the pipeline calls ~16,000 coding variants. Approximately 7,500 of these are non-synonymous variants of which ~700 along with ~30 splice site variants and ~60 indels are not present in dbSNP (version 130). Depending on the number of affected individuals and the underlying inheritance model, we are able to confine this list to 1-10 putatively disease causing variants.

Technologies for Collecting and Integrating Genome, Environment and Trait data

Presenting Author: George Church

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Live broadcast videoconference

The human genome draft completed in 2004 (at 8-fold coverage) was a milestone, but at $3 billion it was not applicable to routine research or diagnostics. Expensive "common variant" association studies also generally failed to produce highly predictive and actionable diagnostics. Since 2004, we have pushed the cost of sequencing down by over a million-fold to about $2000 per 40-fold genome today[1]. This also enables time-series studies of epigenomic and immunogenomic responses to cancers, microbes, allergens, vaccines, etc. Sharing personal fibroblasts and stem cell lines and associated genome, environment and trait (GET) data greatly enables commercial and academic research, open-source software and data for interpreting whole and partial genome sequences [2] as well as community tools for diverse phenomics. As the utility of cells, genes, and traits increases, insights come from highly integrative approaches – evaluating individuals in cohorts holistically and computationally, often from outside the clinical specialty of the study, e.g. computer scientists, systems biologists, or educational communities. Progress has been made by consenting volunteers with the understanding of full disclosure [3] (including tests of comprehension of the consenting materials). Technologies for analysis of single-chromosome haplotypes and single-cell epigenomics include dilution libraries and in situ sequencing.

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Poster Presentation Abstracts

Poster Presentation Abstracts

Symposium I/II
Genomics of Common Disease

RANK (TNFRSF11A) is epigenetically inactivated and acts as a tumor suppressor in human gliomas

Presenting Author: Anna-Lena von dem Knesebeck
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Alterations of DNA methylation play an important role in carcinogenesis and influence the transcription of tumor suppressor genes in many tumor entities. Using a microarray based technology we have identified novel candidate genes that are de novo methylated in gliomas. One of these candidate genes is TNFRSF11A, also known as Receptor Activator of NF-κB (RANK), which after binding of the ligand RANKL activates several signaling pathways (e.g. NF-κB, JNK, ERK, p38 and Akt/PKB). Using pyrosequencing we have confirmed strong hypermethylation of the RANK 5'region in 2 of 8 (25%) astrocytomas (WHO grade II), 3 of 9 (33%) anaplastic astrocytomas (WHO grade III), 32 of 69 (46%) glioblastomas (WHO grade IV) and 5 of 6 glioma cell-lines (83%). Within the investigated patient cohort, a significant increase in methylation frequency as well as methylation values with malignancy was observed. In addition, strong methylation of RANK was detected in primary stem cell-enriched glioblastoma cultures (n = 8) but not in normal brain tissues (n = 4). Treatment of glioblastoma cell lines with the demethylating agent 5-aza-2'-deoxycytidine significantly reduced methylation and resulted in an activation of RANK transcription as measured by RT-PCR. To investigate the functional role of RANK in gliomas we have transfected and overexpressed the gene in glioblastoma cell lines. A significant reduction of focus formation and an elevated apoptotic rate was observed. Mutant forms of the receptor that prevent binding of TRAF proteins retain tumor suppressor function, indicating the existence of an TRAF independent signaling function of the receptor in gliomas. We conclude that RANK is a novel and frequent target for de novo methylation in gliomas that via influencing cell growth and apoptotic potential contributes to the molecular pathogenesis of gliomas.

Retinoic acid signaling activates different targets in the tumors of long-term compared to short-term survivors of glioblastoma

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Glioblastoma is the most common and most malignant primary brain tumor. The prognosis is poor as indicated by a median survival of less than 12 months in a population-based study (Ohgaki and Kleihues 2005). However, a small fraction of patients (3-5%), so-called "long-term survivors" (LTS), survive for < 36 months after diagnosis. LTS typically are younger patients who are in good clinical condition and frequently have MGMT-hypermethylated tumors (Krex et al. 2007). Nevertheless, the relevant pathomechanisms underlying the LTS phenotype are as yet unknown. To study differences in biochemical pathways, we performed microarray-based expression profiling of primary glioblastomas from 11 long-term (LTS) and 12 short-term survivors (STS; survival <6 months). Our results confirm that glioblastomas from LTS demonstrate more frequent MGMT promoter methylation and IDH1 mutation. In addition, our results suggest an important role of the retinoic acid pathway in long-term survival as indicated by significantly lower expression of RBP1, RARRES2 and FABP5 in tumors from LTS. Consequently, the ratio of FABP5 to CRABP2 is small in LTS and therefore associated with an activation of the anti-oncogenic retinoic acid receptors (RARs) whereas in STS the ratio is significantly higher ($p<0.001$, t-test), suggesting that the primary target of retinoic acid signaling in these tumors is the pro-oncogenic nuclear receptor peroxisome proliferator-activated receptor delta (PPARD). Using immunohistochemical staining we demonstrate that high FABP5 protein expression in STS tumors is linked to highly proliferating tumor cells of astrocytic origin. The affected cells express high levels of phosphorylated PDK1, an indicator of AKT-pathway activation.
These data suggest that retinoic acid signaling activates different targets in glioblastomas from LTS and STS patients, which is of paramount importance for therapeutic strategies aiming at a targeted modulation of the retinoic acid pathway.

Death associated protein 3 contributes to intrinsic and extrinsic pathways for apoptosis in high grade gliomas of childhood

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The death-associated protein 3 (DAP3) is localized on the chromosomal region 1q21-22 that has been previously described as a region of frequent chromosomal gain associated with poor outcome in pediatric high grade gliomas. It encodes a 46 kDa mitochondrial ribosomal GTP-binding pro-apoptotic protein. DAP3 has been reported to be overexpressed in glioblastomas and to protect glioblastoma cells from camptothecin-induced apoptosis. In the current study we investigated the functional role of DAP3 in high grade gliomas and found that repression of DAP3 by shRNA induced mitochondrial fragmentation and apoptosis in pediatric glioma cell lines while in adult glioma cell lines it only reduced cell proliferation. Specific chemical inhibition of the PKCa and PKCß significantly reduced the expression of DAP3 protein in both pediatric and adult glioma cell lines. The inhibition of PKCa changed morphology, mitochondrial status and significantly suppressed proliferation of both pediatric and adult glioma cell lines without associated caspase activation indicating that DAP3 may mediate nonapoptotic form of cell death in high grade gliomas. The suppression of PKCß rendered pediatric glioma cells more sensitive to the apoptotic effect of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) through a caspase-dependent process as compared with adult glioma cells suggesting a possible implication of DAP3 in intrinsic and extrinsic pathways for apoptosis in pediatric high grade gliomas. Our findings confirmed the anti-apoptotic role of DAP3 in glioma cell biology and pointed out the specific functional role of this protein in mitochondrial maintenance. The results suggest that post-translation modification of DAP3 mediated by differential activation of specific PKC isoforms in glioma cells is required for functional suppression of apoptosis by DAP3 in high grade gliomas. DAP3 may represent a novel target for the treatment of gliomas.

Extending iCHIP, the NGFNplus Brain Tumor Net database to ICGC

Presenting Author: Chris Lawerenz
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Our database platform iCHIP partially funded by NGFN is the basis for new applications in cancer research. The management platform is the central repository for experimental, clinical and histological specifications for the entire Brain Tumor Net (BTN) project funded by NGFNplus. Clinical probe annotation and experimental data are integrated and correlated after data curation and congruency checks. The patient and tumor sample information including follow-up data, surveyed by the institute of neuropathology in Duesseldorf, is version controlled uploaded into iCHIP. We assigned the highest possible relevance to standardization of data annotation and analysis as a prerequisite for subsequent valid and reproducible interpretation of research results.
iCHIP has been subsequently extended to the need of the three German ICGC projects. In the ICGC pediatric brain tumor project (PedBrain) we are providing, e.g, histopathological reference entries from the university hospitals in Duesseldorf and Heidelberg together with the corresponding histological images. Additionally our database is on the way to manage all sequence data of PedBrain. This includes the incorporation of whole DNA-, Exome-, RNA- and methylation sequences. Considerable whole genome DNA sequences from different sequence locations (GATC, EMBL, DKFZ) are already integrated into our new LSDF (large scale data facility). The results from mutation and aberration analysis as well as alignments will be provided by easy-to-use, user-friendly interfaces for precise queries and graphical displays. Statistical information and Metadata will allow fast data access to enable re-calculations over the full range of sequences.

Molecular signatures classify astrocytic gliomas by IDH1 mutation status

Presenting Author: Martje Tönjes
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In order to identify novel glioma-associated pathomechanisms and molecular markers, we carried out an aCGH analysis of 131 diffuse astrocytic gliomas. The cohort comprised 87 primary glioblastomas (pGBIV), 13 secondary glioblastomas (sGBIV), 19 anaplastic astrocytomas (AAIII), and 12 diffuse astrocytomas (AII). Microarray-based expression profiling was performed for 74 tumors (42 pGBIV, 11 sGBIV, 13 AAIII, 8 AII). All tumors were screened for MGMT methylation, IDH1/IDH2 and TP53 mutations. Unsupervised and supervised bioinformatic analyses revealed distinct genomic and expression profiles separating pGBIV from the other entities. Classifier expression signatures were strongly associated with the IDH1 gene mutation status. Within pGBIV, the rare subtype of IDH1 mutant tumors shared expression profiles with IDH1 mutant sGBIV and was associated with longer overall survival compared with IDH1 wild-type tumors. A special case was represented by a group of IDH1 mutant primary glioblastomas (pGBIV-IDH1mut). Although based on only 7 tumours, our data suggest pGBIV-IDH1mut as a separate group of glioblastomas characterised by a unique pattern of genomic imbalances, distinct clinical features, in particular younger age of onset and more favourable prognosis than pGBIV-IDH1wt. In patients with IDH1wt pGBIV, PDGFRA gain or amplification as well as 19q gain were associated with patient outcome. aCGH analysis additionally revealed homozygous deletions of the FGFR2 gene in two pGBIV, with reduced FGFR2 mRNA levels being frequent in pGBIV and linked to poor outcome suggesting FGFR2 as a novel glioma-associated candidate tumor suppressor gene. Furthermore, the RPRM gene, which encodes the p53-induced protein reprimo, was frequently hypermethylated and transcriptionally down-regulated in TP53wt gliomas, including most pGBIV. We suggest that epigenetic silencing of RPRM could constitute a molecular mechanism by which TP53wt gliomas escape from p53 dependent growth control.

Verified specimen for research: iCHIP-based management of bio-resources within NGFNplus- PaCaNet

Presenting Author: Chris Lawerenz
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The creation of a common resources platform is an integral part of any consortium committed to genomic research. Meaningful exploitation of a shareable specimen collection is dependent on the access to standardized sets of comprehensive pathological and clinical annotations. Pancreatic Cancer Network – PaCaNet – employed an iCHIP-based technology to establish a web-based biobank allowing a central management (Heidelberg) of decentralized clinical specimens (Heidelberg, Bochum, Tübingen, Munich and Marburg). This platform integrates all workflow steps beginning with patient registration, specimen quality assessment, pathological validation, visual presentation of information and ending with the possibility of an on-line database searches, ordering and tracing of the samples, which are distributed to participating location for high-throughput screenings. Special attention is being paid to confidentiality and safety issues. The introduction of PID (personal identifier)-generator enables the early pseudonymization of patients and warrants secure transfer and use of the data deposited on the dedicated server. The PaCaNet-bio-resources platform provides a pool of verified well-annotated specimens, thus allowing a rapid and effective serving the scientific needs of consortium's members. This platform has an excellent exploitation potential because of capability to upload and store the dataflow coming from diverse profiling, sequencing, imaging and other high-throughput screening procedures in parallel to permanently updated clinical information.

Functional characterization of the potentially “drugable” candidate gene NT5E identified within the PACANet consortium

Presenting Author: Abdelouahid Maghnouj
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Pancreatic ductal adenocarcinoma (PDAC) is one of the most deadly cancers. Novel molecular targeted therapies are urgently needed. In silico screening of our large PDAC expression database for drugable candidate genes and subsequent qRT-PCR validation in microdissected tissues allowed among others the identification of Ecto-5’-nucleotidase (CD73), a cell surface protein that hydrolyzes extracellular AMP into adenosine and phosphate, is highly expressed in pancreatic cancer tissues compared to adjacent normal tissues of the pancreas. Previous studies reported that CD73 is widely expressed in many tumor cell lines and is up-regulated in solid tumors. However, its role in cancer remains uncharacterized. To better understand the role of CD73 in PDAC, we silenced the expression of CD73 using a specific lentiviral-mediated small hairpin RNA (shRNA)-CD73 in the human pancreatic cancer cell line Panc1. We showed that knockdown of CD73 expression reduced cell proliferation and increased cellular apoptosis according to real time cell (xCELLigence/Roche) and flow cytometry analyses. To identify mechanisms potentially responsible for the observed cellular effects, microarray analyses were performed. We compared gene expression of CD73-knock-down Panc1 cells with control cells. A total of 179 genes were differentially expressed. Of these 43 were up-regulated and 136 genes were down-regulated. In line with our in-vitro functional data, silencing of CD73 increased the expression of several pro-apoptotic genes (HIC2, E2F2, EIF4EBP2, CDKN2C), and decreased the expression of cell cycle progression genes (BNIP2, YAP1, NGFRAP1, BIRC3, PPP1CB, ITGAV, CYCS, ANXA1, ITGB1). A selected set of these genes were subsequently validated by qRT-PCR. In summary, attenuation of CD73 via shRNA led to suppressed cell proliferation and induction of apoptosis, suggesting that CD73 may be a novel targeted therapy candidate for PDAC.

Analysis of protein isoforms that are relevant to diagnostics and drug activity by means of antibody microarrays; application to pancreatic cancer and lymphoma.

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A set of 100 antibody pairs was generated that bind with high specificity to either the wildtype form of a protein or a cancer-specific isoform. The binders were arranged in a microarray format and used for the analysis of samples from patients with pancreatic cancer or lymphoma as well as appropriate control samples. Complex protein extracts isolated from both serum and tissue were studied. Apart from the utility as a means of diagnostics, and particularly early detection of disease during pathogenesis, protein isoforms were examined, against which drugs may exist that interact with one isoform while not affecting the other one.

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Haplotypes in melanoma inhibitory activity 2 correlate with survival and chemoresistance in pancreatic cancer

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Background: Response rates of pancreatic ductal adenocarcinomas (PDAC) to chemotherapy are low; identification of a subgroup of patients who are more likely to benefit from chemotherapy might constitute a rational approach to improve prognosis.
Methods: Expression of melanoma inhibitory activity 2 (MIA2) was examined in pancreatic tissues and pancreatic cancer cell lines. MIA2 polymorphisms were defined by conventional PCR sequencing and high-resolution melting curve analysis. To assess the functional relevance of MIA2, loss-of-function and gain-of-function studies were performed in pancreatic cancer cell lines.
Results: MIA2 staining in cancer cells was found in 71% (43 out of 61) of the PDAC tissues. Though MIA2 expression was found in ASPC-1, Capan-1 and Colo-357, MIA2 was secreted only by ASPC-1 and Capan-1. Sequencing results revealed a homozygous Met141/His547 allele in Colo-357 but a homozygous Ile141/Asp547 allele in ASPC-1 and Capan-1 cells. PDAC patients heterozygous or homozygous for Met141/His547 (n=75/223 patients) survived significantly shorter than patients homozygous for Ile141/Asp547 (15 vs. 21 months). Functionally, silencing of MIA2 Ile141/Asp547 in Capan-1 and ASPC-1 conferred resistance to gemcitabine treatment, while silencing of MIA2 Met141/His547 in Colo-357 had no such effect. Correspondingly, overexpression of MIA2 Ile141/Asp547 in Su86.86 cells increased sensitivity to gemcitabine which was specifically rescued by MIA2 RNAi. However, overexpression of the MIA2 Met141/Asp547, Ile141/His547 or Met141/His547 variants had no such effect. Furthermore, the different MIA2 polymorphisms were associated with specific activation patterns of AKT and ERK signalling pathways following gemcitabine treatment.
Conclusion: Haplotypes in MIA2 are associated with survival and chemoresistance in pancreatic cancer.

Knockdown of kinesin motor protein Kif20a leads to growth inhibition in pancreatic ductal- and neuroendocrine-cancer cells.

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Aim: To characterize potential molecular drug targets in pancreatic cancer, we investigated Kif20a, which belongs to the kinesin superfamily involved in trafficking of molecules and organelles.
Methods: Detection of Kif20a as a druggable candidate was made by; combined evaluation of various high-throughput gene analysis panels. In vitro analysis were made in pancreatic ductal adenocarcinoma (PDAC) and neuroendocrine cancer (NEC) cell lines using quantitative realtime-PCR, immunohistochemisty, immumofluorescence, immunoblot methods and MTT assay.
Results: Immunohistochemical analysis of paraffin embedded pancreatic tumor samples showed a stronger staining in cancer than in healthy pancreatic tissues. Stronger immunostaining was also observed in several altered acinar cell cluster in chronic pancreatitis.
Immunofluorescence analysis of pancreatic ductal- and neuroendocrine cancer cell lines showed nuclear and cytoplasmic localization. mRNA and protein expression of Kif20a was comparable in three PDAC and three NEC cell lines. Knockdown of Kif20a with small interfering RNA molecules leads to 35-40% and 15-30% reduction of proliferation in PDAC and NEC cell lines, respectively.
Conclusion: With an upregulation of more than 10-fold in pancreatic cancer cells, Kif20a appears as a likely candidate for development of drugs to treat pancreatic ductal adenocarcinoma at the molecular level.

Chromosomal deletions, tumor phenotype and prognosis in prostate cancer

Presenting Author: Antje Krohn
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Deletions of chromosomal material are frequent in prostate cancer. This project aimed at the identification of frequent and new deletions in prostate cancer and to study their association with tumor phenotype and PSA recurrence.
Array CGH was performed on 77 advanced prostate cancers. Alterations of interest, including TMPRSS2:ERG fusion and deletion at 10q23 (PTEN) and 3p14 (FOXP1) were analyzed on a tissue microarray containing more than 2000 prostate cancers with clinical follow-up data using fluorescence in situ hybridization (FISH).
The most frequent deletions found by array CGH were 21q (representing TMPRSS2-ERG fusion) in 18%, 10q23 (PTEN) and 3p14 (including FOXP1) in 18%, 5q31 in 16%, 5q21 in 14%, 6q13 in 21%, 6q21 in 19%, 6q26 in 14%, 8p11 in 17%, 12p13 in 14%, 13q14 in 14%, and 16q24 in 22%.
TMPRSS2-ERG fusions, PTEN and FOXP1 deletions were selected for FISH analysis. TMPRSS2-ERG fusion was observed in 394 of 947 interpretable cases (41.6%), and was unrelated to stage, Gleason grade, and PSA recurrence. PTEN deletions were seen in 8.9% of 1844 interpretable cases and were associated with advanced tumor stage ($p<0.0001$), high Gleason grade ($p<0.0001$), and early recurrence ($p<0.0001$). FOXP1 deletions were seen in 5% of 619 cases. FOXP1 deletions were unrelated to tumor phenotype and outcome. Both PTEN and FOXP1 deletions were strongly linked to TMPRSS-ERG fusions. TMPRSS-ERG fusion positive tumors had PTEN deletions in 15.4% and FOXP1 deletions in 10.7% while Fusion negative cancers had PTEN deletions in only 5.8% and FOXP1 deletions in only 2% of cases ($p<0.0001$ each).
The TMPRSS2 ERG fusion determines a genetically distinct subgroup of prostate cancers. Our data provide no evidence for a particular clinical behaviour of TMPRSS2-ERG fusions in radically operated prostate cancer. Both PTEN and FOXP1 alterations are preferentially found in fusion type cancers but may contribute to progression of both fusion positive and fusion negative prostate cancers.

Integration of Pathway Knowledge into a Support Vector Framework using Reweighted Recursive Feature Elimination

Presenting Author: Marc Johannes
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Motivation: Classification methods are often used to identify gene signatures that have the potential to predict the clinical outcome of cancer patients. However, standard methods merely rely on gene-expression data and assume the genes to be independent. Including pathway knowledge a priori into the classification process has recently been indicated as a promising way to increase classification accuracy as well as the interpretability and reproducibility of prognostic gene signatures.

Results: We propose a new method called Reweighted Recursive Feature Elimination. It is based on the hypothesis that a gene with a low fold-change should have an increased influence on the classifier if it is connected to differentially expressed genes. We used a modified version of Google's PageRank algorithm to alter the ranking criterion of the SVM-RFE algorithm. Evaluations of our method on an integrated breast cancer data set comprising 788 samples showed a significant improvement of the area under the receiver operator characteristic curve as well as an enhanced reproducibility and interpretability of selected genes.

Linking parallel measurements of high-throughput miRNA and gene expression data

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The parallel measurement of different types of high throughput expression data, like gene, miRNA and protein data, requires new tasks of data integration. Different strategies have been formulated, which perform the integration on different levels of the analysis process.
miRNAs are small non-coding RNAs, which regulate gene expression and translation and therefore play an important role during cancer development. Here, we propose a graph-based work flow to integrate miRNA and gene expression data.

We applied this workflow to global miRNA and mRNA expression data sets encompassing 47 prostate cancer and 48 normal samples. By combining the correlation structures of the two data sets with target predictions, we were able to construct a bipartite graph with connections between miRNAs and genes. Meta informations were added and used to distinguish subclusters in this graph. This structure was used to perform an over-representation analysis of targets and effected pathways. We were able to identify clusters of miRNAs which are significantly associated to the KEGG pathways 'Cell cycle' and 'p53 signaling pathway'.
The bipartite graph is interpretable as a pathway linking different kinds of features. Similar to pathways derived from gene expression data alone, it is suitable as an additional knowledge for classification tasks.

Gene and protein expression profiles associated with prostate cancer specific TMPRSS2-ERG fusion

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Prostate cancer is the most frequently diagnosed malignancy in men with a remarkably differing clinical course due to the molecular heterogeneity of the tumors.
Recurrent gene fusions between the androgen responsive promoter of TMPRSS2 and the ETS transcription factor ERG have been identified in a subset (~ 50%) of prostate cancer patients. Although TMPRSS2 - ERG fusion seems to be a critical and early event in prostate cancer, the precise clinical and functional role is still unclear. In order to learn more about the consequences of TMPRSS2 – ERG fusion, we studied gene expression profiles and analyzed selected proteins by reverse phase protein arrays. RNA and proteins were isolated from prostate cancer tissues taken from men undergoing radical prostatectomy. Extracted RNA was processed and hybridized onto Affymetrix GeneChip Exon 1.0 ST exon array. In order to detect TMPRSS2 – ERG gene fusion events, expression levels of the ERG exons were analyzed and putative fusion events were verified by RT-PCR. 1635 genes were found to be significantly deregulated on the RNA level between the fusion positive and negative patients. Additionally, some proteins from cancer relevant pathways were found to be deregulated by the reverse phase array analysis. In conclusion, our study demonstrates deregulated gene and protein expression differentiating fusion positive and negative samples, which might improve the understanding of the underlying molecular mechanism of recurrent gene fusions in prostate cancer.

Functional relevance and downstream signaling of specific microRNAs in prostate cancer cells

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Prostate cancer is the most frequent solid cancer in men and the second most frequent cancer-related death cause in western countries. The accumulation of genetic alterations during the development and progression of prostate cancer is very complex and leads to changes in gene, microRNA and protein expression. We extracted genomic DNA, total RNA including microRNA and protein from 50 high-grade tumors and 50 benign prostate tissue samples. By using low-density array technology we identified more than 100 differentially expressed microRNAs (p-value <0.05; fold change <0.5 or >2) between prostate cancer and benign epithelium. Thirty microRNAs have been introduced into functional analysis in three different prostate cancer cell lines (PC3, LNCaP, RWPE1) to screen for consistent effects on cell viability. For five microRNAs alterations in proliferation across different cell lines were observed after activation (MIMIC molecules) or after inhibition (inhibitor molecules). Downstream targets have been detected by subsequent microarray experiments, by the integration of exon array data from the same sample collective to focus the correlation structure, and by the mining of microRNA target prediction databases. These downstream genes and signatures were searched for links to cellular processes which may explain the observed functional consequences in the cellular models. In summary, microRNA expression data was explored for their relevance in prostate cancer and can be integrated into broad molecular analysis to highlight interactions with genomic, transcriptomic and proteomic data.

Next generation sequencing of coding regions in prostate cancer patients

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Genetic and epigenetic factors play an important role in the origin and/or progression of prostate cancer. Previous studies using linkage analyses and positional cloning experiments highlight a number of potential cancer susceptibility genes and cancer candidate loci with risk effects. However, to date none of the identified genes has consistently been shown to be clinically relevant for large populations and systematic analyses have just recently developed.
To this end, we have performed targeted exon sequencing experiments of 29 prostate cancer patients using solution-based sequence capturing of 12.366 (3.9 Mb) independent regions followed by massively parallel sequencing using the Illumina Genome Analyser II Sequencer. For each patient we sequenced benign and tumour prostate tissue and developed analyses pipelines especially adapted to tumour tissues. We found approximately 2.600 small nuclear variants of which 18% were unknown to dbSNP. Overall approximately 18% of the variants represent non-synonymous substitutions within the coding sequence. The analysis of cancer specific signal transduction pathways suggests a multi-hit strategy for tumour development. This project is one of the first to investigate private mutations in a relevant number of cancer patients using next generation sequencing technologies and it will be the basis for additional clinical studies.

Searching for Epigenetic Modifiers of APCmin-induced Initiation of Intestinal Oncogenesis in Mice using Chromosome Substitution Strains

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Genetic variation in human individuals is intimately linked to disease susceptibility &/or prognosis, as found with colon cancer. However, the nature of cancer susceptibility is polygenic and complex, and remains to be fully understood. The APCmin mouse model of human colon cancer has provided insight into the genetic requirements for cancer initiation and development, and differing genetic backgrounds can influence its disease phenotypes, such as tumour multiplicity or spectrum. To further elucidate how this is conferred, we have set up a screen to identify novel global modifiers of the tumour phenotype induced by APCmin in the mouse intestine using chromosome substitution strains (CSSs), which carry single chromosomes of the PWD/Ph (PWD donor) strain on a C57BL/6 (B6) background. Analysis of B6/PWD F1 animals provides evidence for the existence of multiple modifier genes on different chromosomes. We are now using high density SNP analysis to direct back-cross breeding programs to map these modifiers in subcongenic lines. A focus of our program is the isolation of modifiers of epigenetic regulation and tumour progression in the intestine. We therefore also study changes in the epigenetic and transcriptional landscapes during tumour initiation. We are utilising primary intestinal crypt culture for in vitro functional and validation studies of candidate modifiers, and to link changes in the epigenome to gene expression. By combining these data, we aim to isolate key epigenetic modifiers involved in colon cancer initiation, progression and stem cell function. We anticipate the identification of multiple genetic traits affecting tumour initiation and progression, and that this work will facilitate the definition of low- and high-risk groups among patients for whom treatment regimes can be tailored accordingly, and also provide novel targets for the design of chemopreventative therapeutics.

Statistical inference of allelic imbalance from transcriptome data

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Next-generation sequencing has facilitated an analysis of somatic and meiotic mutations at unprecedented level. In this context, the study of allelic imbalance in intermediate RNA phenotypes may prove a useful means to elucidate the likely effects of DNA variants of unknown significance. We developed a statistical framework for the assessment of allelic imbalance in next-generation transcriptome sequencing (RNA-seq) data that requires knowledge neither of an expression reference nor of the underlying nuclear genotype(s), and that allows for sequencing errors. Both extensive simulations under a wide range of practically relevant scenarios and application to publicly available whole-transcriptome data with auxiliary genotype information showed superior power of our approach in terms of both genotype inference and allelic imbalance assessment, compared to the more naïve approach of completely ignoring allele miscalls, particularly at low sequencing coverage. The ability to assess somatic mutations and allelic imbalance in one and the same RNA-seq data set will make our framework particularly well suited for the analysis of somatic genetic variation in cancer studies.

Prediction of tumor phenotype from copy number aberrations

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Next generation sequencing or even older technologies like microarrays afford very high resolution measurements of the state of the genome, epigenome or transcriptome of tumor cells. However, common clinical practice demands simple and cost-efficient tests targeting key molecular changes that are relevant for tumor phenotype. On the way from large scale experimental studies to treatment improvement therefore lays the need for automated computational tools that can select important biomarkers and show how they influence phenotype.

In this work, we present automatic tools for predicting tumor phenotype from genome-scale copy number aberrations [1]. We use robust statistical models in order to learn from existing tumors models that can link specific patterns of aberrations to particular phenotypes. Using these models, it is possible to predict stage, grade or subtype for new tumors, or decide if a treatment is likely to succeed or not. Apart from prediction, our models can provide with a ranked list of the most relevant copy number aberrations, such that simple diagnosis tests can be constructed, using only a few markers.

We present an extensive study on Neuroblastoma tumors, using the methodology mentioned above. The data come from two independent studies [2, 3]. We identify the most discriminative aberrations between stages 1-3, stage 4 and stage 4S and also between tumors of children of very young age and older. We consider the most relevant aberrations as events and we infer the probable order in which they occur during tumor progression.

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Prediction of Survival and Response to Adjuvant Therapy in Pancreatic Cancer

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Despite all efforts, patients with pancreatic ductal adenocarcinoma have a poor prognosis with a median survival between one and two years. To further improve patient survival, new approaches are needed to individualise therapy based on molecular prognostic signatures. In a multicenter study, we performed a genome-wide screen for gene expression markers for predicting survival after resection in pancreatic cancer patients. To identify genes prognostic for survival, we devised a novel computational approach that incorporates background knowledge in form of a network. This approach identified seven prognostic genes which were validated using quantitative RT-PCR and immunohistochemistry on an independent set of formalin-fixed, paraffin-embedded tumour samples. Their predictive accuracy is comparable to studies that were performed in breast cancer or lymphatic malignancies. Yet, this is one of first studies of this kind for pancreatic cancer. Reliable survival prediction will have implications for the correct choice of therapy: whereas some patients might benefit from extensive therapy such as surgery and chemotherapy, others might not. For the latter, less aggressive or different treatment could result in better life quality.

A DNA methylation signature for prostate cancer identified by next generation genome-wide profiling.

Presenting Author: Stefan Börno
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Without doubt, epigenetic alterations play an important role in prostate cancer (PCa) development and progression. Here we used a novel technology, immunoprecipitation of methylated DNA followed by SOLiD next generation sequencing (MeDIP-Seq) and present its application for the discrimination of normal and tumour tissues.
So far we have applied this approach to 53 normal and 51 tumour prostate tissues and generated over 60Gbp of unique sequence information. We were able to identify more than 20.000 significantly differentially methylated regions that can serve as potential biomarkers for PCa including already described markers such as GSTP1. Using support vector machines we were able to detect a small set of markers with the potential to perfectly discriminate tumour and normal samples.
As a partner of the IG Prostate Cancer project we have access to CNV, miRNA, mRNA and protein data and are now in the progress to integrate these different levels of information. This will further allow us to gain insight into tumour promoting processes.

Next-Generation Sequencing of (micro)RNAs in Colorectal Cancers

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Colorectal cancer (CRC) is the third most prevalent malignant neoplasm and a major cause of cancer mortality worldwide. It is known that miRNAs play a critical role in oncogenic signaling pathways, including oncogenesis, progression, invasion, metastasis and angiogenesis. Previous studies of miRNA expression patterns in CRC elucidated a strong association between expression levels of microRNAs and the tumor stage as well as the survival prognosis for cancer patients. To follow up these encouraging results we performed a genome-scale analysis of miRNA expressions using Illumina next-generation sequencing technologies. We sequenced small RNAs of different colon cancer patients and determined the differences of miRNA expression in matching tumor and metastasis-tissues compared to normal colonic mucosa. We identified several alterations in the miRNA expression patterns; some of which are already known and present a quality criteria for our experimental design and bioinformatics analysis pipeline. We are in the progress to evaluate or findings in a large number of samples and to establish them as new biomarkers in cancer diagnosis. In addition, we are analysing small RNA species to identify new, not yet annotated microRNAs.

Regulation of the Death-Associated Protein 3 Gene by a Retroviral Antisense Transcript

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Human Endogenous Retroviruses (HERVs) mediate gene regulation by numerous mechanisms [1]. HERVs comprise Long Terminal Repeat (LTR) sequences that potentially act as promoters able to generate regulatory transcripts [2]. In a genome-wide analysis we identified a HERV of the HERV-K(C4) family located in antisense orientation in intron 1 of the human Death-Associated Protein 3 (DAP3) gene. This HERV negatively regulates DAP3 gene expression by providing LTR-primed cis-natural antisense transcripts (cis-NATs). Promoter activity of the HERV-K(C4) 5'LTR was verified by luciferase reporter assays. Targeting cis-NATs with specific antisense-oligonucleotides (termed "antisense-antisense-ONs") caused an up to approximately 11-fold increase of the DAP3 mRNA level in HeLa cells. DAP3 is a mediator of the extrinsic pathways of apoptosis and its over-expression causes cell death [3]. Consistently we found that knockdown of cis-NATs induces apoptosis in HeLa cells, and enhances susceptibility to Interferon-gamma and Tumor Necrosis Factor-alpha induced cell death. Due to its deregulation in numerous human cancers, DAP3 has been proposed as target in tumor therapy [4]. Activation of HERV LTR transcriptional activity is a common feature of different tumors [5]. Hence, the cis-NATs identified in this study may be especially abundant in tumor cells and thereby mediate their resistance to apoptosis via down-regulation of DAP3. Targeting the identified cis-NATs might specifically affect tumor cells and enhance their susceptibility to apoptosis. We conclude that the DAP3-specific antisense-antisense-ONs described here are novel promising candidates for cancer therapy.

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Genetic profiles of MSS and MSI colorectal cancers identified by whole exome sequencing

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Colorectal cancer (CRC) is the third most common cancer, with about 1 million cases worldwide. Here, we performed whole exome sequencing of matching normal and tumor tissues from microsatellite stable (MSS) and microsatellite instable (MSI) colon cancer patients and identified more than 50,000 small nucleotide variations for each tissue. Microsatellite stability is one criterion to predict the heredibility of the cancer risk and to classify colorectal cancers and we sought to determine the profile of genomic aberrations linked to these statuses. Using next generation sequencing technologies we identified more than seven times more somatic non-synonymous variations in MSI cancers than in MSS cancers. Along this line we were able to distinguish the subsets of cancers based on the amount of somatic mutations found and we were able to identify aberrations of mismatch repair system genes in parallel. Our bioinformatics filtering approaches narrowed down the rate of most significant mutations to approximately 400 for MSI CRC and 50 for MSS CRC with predicted altered protein function. Interestingly, we repeatedly identified somatic mutations in the intracellular kinase domain of bone morphogenetic protein receptor 1A, BMPR1A, and show that the mutations impair the protein function. We conclude that deep sequencing of tumor exomes reflect the microsatellite status of CRC and it enables the identification of clinically relevant mutations.

Functional characterization of Brd4 as a transcriptional regulator

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The bromodomain containing protein 4 (Brd4) associates with acetylated histones and is known to regulate mRNA transcription via interaction with mediator complex proteins and the recruitment of pTEFb to promote RNA polymerase II activation. A knock-out of the gene in mice is lethal. A fusion gene of Brd4 is found in highly aggressive lung cancers and it was stated that the protein level correlates with the survival rate in breast cancer patients. Finally, Brd4 is involved in the regulation of transcription of human papilloma viruses (HPV) which are the underlying cause of cervical and anal cancers. This HPV transcriptional regulation is due to the interaction of Brd4 and the viral E2 protein which itself binds to the viral genome and therefore recruits the regulator. This protein protein interaction also serves as the link to viral persistence through the transduction of the viral genome to the next generation of cells. This mechanism is furthermore known in the tumor DNA viruses Eppstein-Bar and Human Herpes virus where Brd4 interacts with the viral proteins LANA1 and EBNA1 respectively.
To understand the function of Brd4 in transcriptional regulation and therefore to gain insight into the mechanisms underlying viral cancer pathogenesis and virus infections we are performing ChIP-Seq experiments as well as RNA-Seq experiments. In addition we will present data on different pathways Brd4 seems to play major functional roles in.

Quantification of cancer pathways in a recombinant cellular system

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Somatic mutations are major molecular determinants underlying tumor development and progression. However, the complex patterns of genetic alterations within tumors provide a considerable challenge to the understanding of changes in cell signaling pathways that promote cell growth and proliferation. In order to analyse molecular changes associated with potentially cancer-relevant mutations, a site-specific recombination system for rapid generation of highly standardized, isogenic cancer cell lines was established. Using this recombination system a library of cell lines stably overexpressing wild type and corresponding mutated genes was generated. In an initial screen of the breast cancer cell library, candidate genes were analysed for effects on the viability of the tumor cells. Genes showing either a highly increasing or decreasing effect on cell viability were chosen for further analysis by the reverse-phase protein array (RPPA) technology. The measurement of key regulators offers the possibility to discover differences in cancer cell signaling pathways varying from normal cell lines.
Overexpression of B-RAFV600E mutant as well as of a variety of HRAS mutants led to significant changes in protein expression. B-RAFV600K showed increased MAPK but decreased AKT signalling. In contrast, phosphorylated AKTSer473 and pERKThr202/Tyr204 were strongly increased in HRAS mutants. Phosphorylated p70S6K, a marker for bad prognosis and metastasis in breast cancer, is also more abundant in B-RAFV600K and HRAS mutant overexpression cell lines.
Taken together, over 1000 protein lysates from different isogenic cell lines were analysed by RPPA. The analysis of different cancer-relevant mutations in parallel provides us with an overview of their effect on cell signalling in cancer cell lines. In addition, these data will be useful for estimating the relationship between frequently occurring mutations and tumor progression.

Role of thioredoxin reductase 1 and thioredoxin interacting protein in prognosis of breast cancer

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Introduction: The purpose of this work was to study the prognostic influence in breast cancer of thioredoxin reductase 1 (TXNRD1) and thioredoxin interacting protein (TXNIP), key players in oxidative stress control that are currently evaluated as possible therapeutic targets.

Methods: Analysis of the association of TXNRD1 and TXNIP RNA expression with metastasis-free interval (MFI) was performed in 788 patients with node-negative breast cancer, consisting of three individual cohorts (Mainz, Rotterdam and Transbig). Correlation with metagenes and conventional clinical parameters (age, pT stage, grading, hormone and ERBB2 status) was explored. MCF-7 cells with a doxycycline-inducible expression of an oncogenic ERBB2 were used to investigate the influence of ERBB2 on TXNRD1 and TXNIP transcription.

Results: TXNRD1 was associated with worse MFI in the combined cohort (hazard ratio = 1.955; P <0.001) as well as in all three individual cohorts. In contrast, TXNIP was associated with better prognosis (hazard ratio = 0.642; P <0.001) and similar results were obtained in all three subcohorts. Interestingly, patients with ERBB2-status-positive tumors expressed higher levels of TXNRD1. Induction of ERBB2 in MCF-7 cells caused not only an immediate increase in TXNRD1 but also a strong decrease in TXNIP. A subsequent upregulation of TXNIP as cells undergo senescence was accompanied by a strong increase in levels of reactive oxygen species.

Conclusions: TXNRD1 and TXNIP are associated with prognosis in breast cancer, and ERBB2 seems to be one of the factors shifting balances of both factors of the redox control system in a prognostic unfavorable manner.

Identifying Novel Expressed Gene Fusions in MCF-7 Cell Line Using Next Generation Sequencing

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Chromosome aberrations, especially gene fusions, are implicated in the initiation of tumorigenesis. High throughput RNA-Seq allows characterization of gene expression and exon splicing patterns of expressed transcripts. We developed a new junction finder algorithm to identify and quantify exon splicing events using paired end RNA-Seq. With this algorithm, single read and paired end splice junction evidence are stored in directed splicing graphs. We investigate two MicroArray Quality Control (MAQC) samples and breast cancer cell line MCF-7.

We sequenced 129,950,066 and 113,147,501 uniquely mapped pairs of RNA-Seq reads from MAQC samples Human Brain Reference (HBR) and Universal Human Reference (UHR). Compared to RefSeq, we found 136,671/14,271 and 138,536/14,083 known/putative exon junctions in UHR and HBR, respectively. Increasing mapping stringency slightly increases the ratio of known/putative exon junctions. We compared sequencing depth required to distinguish low-expressed variants by progressively mapping barcoded libraries and report that ~70% of alternative splicing events were accessible with ~20M and ~82% with ~40M qualified read pairs. Remaining junctions are detectable only at higher depth. Of 36 fusion junctions called for UHR, we verified 16 with TaqMan assays.

We sequenced breast cancer cell line MCF-7, obtaining 192,246,750 uniquely mapped pairs of RNA-Seq reads. We identified 123,386 known and 17,317 putative junctions, called 60 fusions, of which 10 were between genes from different strands or chromosomes, including 5 recently discovered MCF-7 fusions. We detected 5 novel fusions at lower expression levels (undergoing validation) which suggest enrichment in the number of translocation events between Chromosomes 1, 17 and 20 during evolution of MCF-7 genome.

Easy and low-cost genome-wide detection of novel gene fusions allows interrogation of tumor samples and discovery of biologically important gene fusions.

A novel large scale screen to identify regulators of the cancer-relevant microRNAs miR-31 and miR-155

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Recent evidence has shown that alterations in microRNA (miRNA) expression contribute to tumor growth by modulating critical genes. Several miRNAs are up- or downregulated in cancer and have been proven to correlate with tumor stage. Two examples for cancer-related miRNAs are miR-31 and miR-155. MiR-31 is found to be downregulated in aggressive breast cancers leading to an increase in invasiveness of these tumor cells as suppression of the migration-suppressive target genes is released. In contrast, miR-155 is known to be upregulated in several cancer types including breast cancer. It targets a number of tumor suppressor genes thereby causing increased proliferation and chemosensitivity of cells overespressing this miRNA. To identify genes involved in the regulation of cancer-relevant microRNAs we screened in breast epithelial cells (MCF10A) a siRNA-library of 4500 selected siRNAs, chosen based on their potential to regulate microRNA biogenesis and function. A first primary screen was carried out with a luciferase reporter plasmid that contains target sites of miR-31 and miR-155 in the 3'UTRs of firefly and renilla luciferase, respectively. Hits will be validated in several luciferase- and qRT-PCR-based secondary screens. The new candidate genes should then be further analyzed in functional assays to determine their mechanism of action. Thus, by this screen we aim to identify and characterize novel tumor-promoting or tumor-suppressing genes, which modulate tumor growth by regulating those two miRNAs or miRNA function in general. These genes may serve as therapeutic targets. Moreover, the identification of general regulators of miRNA biogenesis and function may lead to a deeper understanding of miRNA processing and regulation.

MicroRNA-200c represses migration of breast cancer cells by targeting a network of Rho GTPases

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Complications arising from metastasis are responsible for the vast majority of cancer-related deaths. During recent years, microRNAs have emerged as potential regulators of the metastatic process. Among these so-called metastamirs is the miR-200 family, which was shown to suppress metastasis by inhibiting epithelial-to-mesenchymal transition (EMT). However, little is known about mechanistic understanding of how the miR-200 family contributes to the regulation of metastasis. Therefore, our work focuses on the identification of protein networks regulated by miR-200c, one of five miR-200 family members. Using a combination of gene expression profiling and computational target prediction, we were able to identify potential targets of miR-200c. Strikingly, genes regulating cell motility were found to be functionally enriched among these predicted targets. Validation of microRNA targeting by luciferase reporter assay together with site-directed mutagenesis confirmed that miR-200c directly targets six genes that regulate the activity of small GTPases of the Rho family or their downstream effectors, which are responsible for the rearrangement of the actin cytoskeleton and for adopting a polarized shape by defining a leading edge and a contractile rear. Consistent with these findings, we could show that overexpression of miR-200c in the highly metastatic breast cancer cell line MDA-MB-231 significantly reduces migration and abolishes cell polarization as well as the formation of actin filaments. Similarly, individual knock-down of the six candidate genes also affects cell motility and polarization. Thus, we propose that miR-200c regulates breast cancer cell migration by targeting a network of Rho GTPases. Further experiments aim at confirming the contribution of these candidate genes to the suppressive effect of miR-200c on metastasis in breast cancer patient samples.

SNPtator: A web service pipeline to predict the functional consequences of SNVs

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High-throughput technologies enable the investigation of abnormalities in the DNA sequence (e.g. SNVs), which are responsible for human diseases like cancer. However, a big bottleneck in the analysis is the differentiation between functional important variants which caused the disease and neutral variants which have no influence at all. There are already different kinds of tools available to investigate the functional consequences of SNVs in the coding region of a gene and in some regulatory regions. However, the local installation of all these tools is time consuming and usually not straight forward. Furthermore, sometimes unknown SNVs not included in public databases, intronic SNVs, intragenic SNVs and synonymous SNVs are not considered in available workflows. In addition, the influencing factors which lead to the final result (e.g. “functional damaging”) are not listed in the output.
Here we present SNPtator a web service pipeline which is able to predict the functional consequences of SNVs in exonic, intronic as well as intragenic regions. SNPtator is easy scalable and extensible and it can also handle unknown SNVs and synonymous SNVs. As input a GFF3 file is required. At the moment the pipeline includes web services to predict changes in protein domains as well as known motifs (regulatory motifs and motifs in the coding region), prediction of functional changes based on evolutionary constraints (conservation level (SIFT)), changes in poly-A signals, changes in alternative splicing and functional changes of transcription factor binding sites. Furthermore, all SNVs are checked in public databases like dbSNP and Cosmic to identify known SNPs. The outputs are the results from the different programs which can be easily combined and compared.
We have tested SNPtator on a set of SNVs for which the functional consequence is known and showed that SNPtator is able to predict functional relevant SNPs.

Snp2gene and GwasPlot: New toolset for the visualization and annotation of GWA results

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Genomewide association analysis has become an established and succesful method for correlating phenotypic traits with genomic loci. Several tools for the interpretation of GWA results are available (HapMap, UCSC, snp.plotter). However, these toolsests generally lack the ability to meaningful annotate subsets of significant SNPs and their genomic location in an automated fashion. Here, we present a new toolset which (1) annotates genes beyond the closest not only in terms of base position but also by information on linkage disequilibrium (2) produces a printable summary plot showing windows with genes, LD and p-values from multiple analysis for a list of significant SNPs on a glance.
The toolset (Snp2gene and GwasPlot) are implemented as easy to use and embedable functions in the statistical programming language R. The packages will be made available via the CRAN/Bioconductor repositories.

Molecular Characterisation of Uremic Toxins in silico

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In uremia the kidney has restricted functions what results in an abnormally high concentration of certain pathophysiologic active mediators in the blood. These retention solutes can cause severe organ damage, atherosclerosis and vascular remodeling. Recent reviews have identified at least 115 uremic retention solutes that were detected in increased concentrations in patients with renal failure across multiple studies. Although it had been pointed out that the retention of these toxins might constitute potent risk factors, summarized in the term uremic syndrome, a detailed molecular characterization is still missing. Furthermore, it remained clear that many more substances might exert toxic functions that have not yet been detected. In order to address these points we have performed a comprehensive in silico analysis. To expand the body of potential uremic toxins, we applied network analysis and over-representation analysis with respect to known pathways, gene ontology and other annotation in order to identify common interaction partners of the known uremic toxins and associated functional modules. Furthermore, we used statistical classification with respect to physico-chemical properties in order to find discriminating features between uremic retention solutes and other substances taken from the PubChem database. We then used this classifier to predict new potential markers for the uremic syndrome. We used literature mining in order to assess co-association of these molecules with relevant terms. In summary, this work presents the first in silico study of the uremic syndrome which allows us to prioritize known uremic toxins on the molecular level as well as to predict new potential toxins and is, thus, a complement to ongoing and future experimental studies.

The Use of Comparative Genomics for the Analysis of Cardiac Organ damage in Hypertension

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Finding susceptibility genes for common diseases including arterial hypertension or subsequent hypertensive end organ damage such as left ventricular (LV) hypertrophy (LVH) remains a difficult task due to the complex, multifactorial nature of the disease. The underlying genetic basis of LVH was investigated in a genome-wide association study (GWAS) using a case-control design in a well phenotyped German population with arterial hypertension and heart disease including LVH (i.e. ESTher cohort). Subsequently, integration of available linkage data from a rat model for LVH under high blood pressure conditions, the spontaneously hypertensive rat stroke prone (SHRSP) and its normotensive reference strain F344 combined with a comparative mapping approach was used to dissect the underyling mechanisms for developing hypertensive LVH.
As a result of our comparative mapping approach, combining human GWAS data with linkage results from an SHRSP/F344 rat intercross showing a quantitative trait loci (QTL) for relative LV weight on rat chromosome 1 (LOD score 8.34), we were able to identify two replicable single nucleotide polymorphisms (SNPs; human chromosome 11q13, $P=1.6*10^{-7}$ and $P=6.6*10^{-6}$) on human chromosome 11 implicating 4 genes as potential candidates. Fine mapping was performed addressing the attributable genetic variance harboured within this genomic region revealed one further significantly associated SNP.
In summary, our approach offers new candidate genes influencing the development of LVH in arterial hypertension, functional studies will be required to dissect the molecular mechanisms of their putative role.

Gene expression analysis in a model of cardiac ryanodine receptor calcium leak

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In heart failure, altered intracellular calcium signaling is a major mechanism of depressed contractile function and ventricular arrhythmias. However, if specific intracellular calcium abnormalities contribute to remodeling and progressive cardiomyopathy is not well understood. We have investigated if intracellular calcium leak from the cardiac ryanodine receptor (RyR2) causes abnormal cardiac remodeling through changes in gene expression.
RyR2-R2474S (RS/WT) and littermate wild-type (WT) mice were treated with the beta-adrenergic receptor agonist isoproterenol up to 28 days (ISO; 20 mg/kg/d s.c. by osmotic minipump). Following different durations of ISO treatment, left ventricle structure and function has been assessed by echocardiography and global gene expression by Affymetrix Gene Chip Mouse Gene 1.0 ST arrays.
Following ISO treatment for 3 days both RS/WT and WT hearts showed a significant upregulation of hypertrophy pathways as indicated by NPPA (3-fold change) and of fibrosis pathways as indicated by Col3a1 (3-fold change ISO vs. control), and Fbn1 (fold change +2.73 ISO vs. control). However, differential regulation between RS/WT and WT has occurred for genes associated with: 1) the Z-line including Myo18b (RS/WT significant fold change +2.71 ISO vs. control) and Myoz2 (RS/WT significant fold change -2.77 ISO vs. control); 2) the cardiac ryanodine receptor regulatory protein triadin (Trdn, RS/WT significant fold change +2.97 ISO vs. control; each $p<0.001$). In addition, RS/WT echocardiography indicated significant changes due to structural remodeling as indicated by a diastolic increase in anterior wall thickness after ISO treatment (RS/WT 0.87 ± 0.04 mm, n=18; WT 0.78 ± 0.02 mm, n=16; p=0.05). In summary, our data show differential changes in pathway expression and the cardiac phenotype of RS/WT hearts indicating that stress-induced RyR2 dysfunction may lead to specific patterns of cardiac remodeling.

MicroRNA-582 is differentially expressed in the heart of MLP-knockout mice

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The homozygous loss of muscle LIM protein (MLP) in a transgenic mouse model results in dilated cardiomyopathy. Furthermore, mutations of the MLP-encoding CSRP3 gene have been shown to be associated with hypertrophic and dilated cardiomyopathy (DCM). Nevertheless, the molecular mechanisms leading to this phenotype are still not satisfactorily explained. MicroRNAs have emerged as important regulators controlling gene expression and a number of microRNAs have been shown to play a role in the pathophysiology of cardiomyopathies.
We hypothesized that microRNAs might be involved in the MLP knockout ($MLP^{-/-}$) phenotype, therefore providing further insights into the molecular mechanisms underlying this DCM model.
Total RNA from the ventricles of MLP knockout and wild type control mice (N=4 per group) was used to profile microRNA expression with an Illumina BeadArray, interrogating 656 microRNA loci. Results were verified in additional samples ($MLP^{-/-}$ N=10 and WT N=9) with quantitative Real-Time PCR. The microRNA showing highest expression changes when compared to WT was mmu-miR-582, which is an intronic microRNA located within the Pde4d locus, a cAMP-specific phosphodiesterase. Both mature miR-582 microRNAs were upregulated (miR-582-3p 18.5 ± 4.0-fold change ± SD, miR-582-5p 8.2 ± 3.0-fold change) and these results were verified in the control samples, with 7.1 ± 0.27-fold change ± SEM (P=0.003) and 4.1 ± 0.64 (P=0.003), respectively.
In conclusion, we have identified the murine miR-582 as differentially regulated in a murine knockout model of DCM, implicating a possible role of this microRNA in the etiology of cardiomyopathies. The genomic location of mmu-miR-582 within the Pde4d gene locus, which has been previously shown to play a critical role in cardiac ß adrenoreceptor signaling, further emphasizes the importance of this finding.

How to Test for Association on the X Chromosome – A Comparison of Suggested Test Statistics

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Genetic association studies were successful to elucidate the genetic background of complex diseases. However, X chromosomal data have usually not been analyzed (ERDMANN et al. 2009; SAMANI et al. 2007), so that information from the X chromosome is neglected. A reason for this is that there is so far no standard instrument for the statistical analysis.

While females carry two copies of the X chromosome, males have only one. Thus, for loci not in the pseudo-autosomal region, special tests are required. Another peculiarity is inactivation of one of the female X chromosomes which may be a mechanism of dosage compensation, resulting in equal effects for one copy of the X chromosome in males and two copies in females. But since this X inactivation is not complete one might either neglect it entirely (ZHENG et al,2007). Thus, the influence of one risk allele in males is equated with one in females. Alternatively, inactivation may be considered (CLAYTON 2008, WTCCC 2007) by treating men with one risk allele like females homozygous for this allele.

This contribution evaluates the test statistics regarding type one error rates and power. Hereto we performed extensive simulation studies covering a wide range of different settings.

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Counter-regulation of macrophage phenotype by M-CSF and GM-CSF. Possible implication in atherosclerosis.

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BACKGROUND: Monocyte-derived macrophages (MØ) play a key role in the inflammatory response and in atherosclerosis. These cells display different phenotypes characterized by morphology, transcriptional profile, and function according to environment and the type of stimulation. GM-CSF and M-CSF are the major prosurvival/mitogenic factors for macrophage lineage with the capacity to activate and to induce differentiation from monocytes.
METHODS: We characterized the transcriptional profiles of GM-CSF induced macrophages (GM-MØ) and M-CSF induced macrophages (M-MØ) using the RNG/MRC arrays. We used Tissue MicroArrays (TMA) and quantitative immunohistochemistry (IHC) to investigate in situ a subset of differentially expressed genes and their products in human and mouse atherosclerosis lesions. We then investigated the interplay of GM-CSF and M-CSF by varying their ratio and identifying the genes sensitive to this variation.
RESULTS: We found that 31 genes were over-expressed by GM-MØ and 26 genes were over-expressed by M-MØ. We selected a subset of modulated genes based on their possible role in atherosclerosis and further studied their expression by RT-qPCR, TMA and IHC in human aortic lesions, and human carotids vs. mammary non-atherosclerotic arteries. Finally, we studied expression of these genes in normal and atherosclerotic aortas of apoE null mice. We showed that, STAB1, SEPP1 and CD163L1 (M-MØ sensitive genes) and PPBP (GM-MØ sensitive gene) were expressed in both human arterial and apoE null mice atherosclerotic tissues. By varying the M-CSF/GM-CSF ratio, we show that the expression of STAB1, SEPP1, ADAMDEC1, CD163L1, FUCA1, ALOX5AP, PPBP and MRC1 was very sensitive to this variation.
CONCLUSION: Our findings suggest that the in vivo ratio between M-CSF and GM-CSF may affect the function and heterogeneity of MØ in atherosclerotic tissues. The genes identified as specific to each MØ phenotype might be used as new markers and/or targets for each phenotype.

Comprehensive genetic analyses in an extended family with myocardial infarction

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Our group established a large collection of myocardial infarction (MI)-families with 2-5 affected first-degree relatives. Based on a genome-wide linkage analysis with 805 microsatellites we found a peak in one of the families on 8q24 spanning approx 12 Mb.
The aim is to confirm and narrow down the linkage signal by genotyping the Illumina 10K array and to study the corresponding region in CARDIoGRAM. Furthermore, Whole-Exome sequencing (WES) of 2 affected cousins may identify a mutation in this family.
305 single nucleotide polymorphisms (SNPs) from the 10K array on chromosome 8 were used for linkage-analysis. Both linkage analyses were model-based assuming an autosomal dominant inheritance pattern with a disease frequency of 1% and incomplete penetrance allowing for phenocopies. The results were compared with those from a large meta-analysis of genome-wide association studies on coronary artery disease in the CARDIoGRAM consortium. This consortium included 22,233 patients and 64,762 controls mostly from population-based studies. Genome-wide genotyping was performed using high-density SNP arrays. Furthermore, WES in two affected cousins was performed.
The linkage region could be narrowed down to a 7 Mb haplotype cosegregating with the disease in the family. Corroborating the linkage findings, few SNPs in the region on 8q42 were found to be associated in the CARDIoGRAM data. Specifically, these SNPs are located within an intergenic region. WES on two affected family members identified several potential deleterious mutations. No mutation in the region on 8q24 was found, however this could be due to incomplete exon coverage of only 84%.
A comprehensive genetic analysis using linkage, GWAS and next-generation sequencing allowed to narrow down a locus for MI in an extended family to a 7 Mb region. Further sequencing, esp. an intergenic region of 0,1 Mb is of greatest interest because of an association signal in the CARDIoGRAM data may identify the underlying causal mutation.

Transcriptional profiling of monocytes and MCSF-driven macrophages in a large multi-centre collaborative study

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BACKGROUND: The global transcriptional profile of cells involved in the pathogenesis of coronary artery disease (CAD) such as monocytes and macrophages is poorly characterized. We thus aimed to identify biological processes and transcription factors (TF) involved in the maturation of monocyte to macrophages.
METHODS: 1,533 monocyte and macrophage RNAs from 917 individuals (healthy controls and patients diagnosed with MI/CAD) recruited in five European centres within the Cardiogenics project were analysed on the Illumina expression arrays.
RESULTS: The arrays used in this study contain 18,311 genes represented by 24,526 probes. Among them 46.55 % (9,465 genes) and 46.89 % (9,584 genes) were expressed in monocytes and macrophages, respectively. We showed that macrophages express slightly more genes than monocytes which are mainly involved in cholesterol metabolism such as APOE, PLAU, A2M and SPP1. Genes specific to monocytes are mainly involved in defense response such as LILRA1 and NLRP1. We then identified the transcriptional changes accompanying monocyte-to macrophages differentiation. We found that this differentiation process involves a

massive change in gene expression levels with 4,181 genes being significantly (adjusted P<10-3) modulated. At this level of statistical significance, fold change values for gene over-expressed in macrophages ranged from 1.08 (DZIP1L gene) to 154.9 (GPNMB). For under-expressed genes, fold change values ranged from -1.08 (ZNF699) to -58 (CX3CR1).The differentiation process is mainly characterized by repression of genes involved in the immune response and over-expression of genes involved in lipid metabolism. 155 TFs were modulated between monocytes and macrophages. Among which 84.5% were down-regulated in macrophages.
CONCLUSION: The findings are remarkable because they show that macrophage differentiation involves lipid handling genes even in the absence of exposure to respective molecules.

Temporal transcriptional changes in human monocyte RNAs following myocardial infarction: The GerMIFs monocyte expression study

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BACKGROUND: Monocytes are believed to play a pivotal role at all stages of acute myocardial infarction (MI) from initiation of fatty lesions to atherosclerotic plaque rupture and the inflammatory response that follows acute MI. We hypothesized that gene expression profiling in circulating monocytes will mirror pathophysiological events occurring at various stages of MI.
METHODS: Circulating monocytes were isolated from venous blood of 28 MI patients at three time points: t1: within 6 hours of onset of chest pain at the start of PCI (acute phase), t2: 3 days after MI (subacute phase) and t3: 90 days after MI (stable phase). We also used gene expression from an additional control group of age and sex matched patients with stable CAD. RNA samples were analyzed using the Illumina gene expression arrays and a subset of modulated genes was validated by RT-qPCR.
RESULTS: The acute phase was characterized by a large number of significantly modulated expression levels (1 857 out of 10 273 analyzed genes). Expression levels of the majority of these genes were modulated in opposite direction between 3 and 90 days after MI going back to baseline. Only 0.35% of the transcripts analyzed were changed at the acute phase. Gene Set Enrichment Analysis using pre-defined pathways and functional categories revealed perturbation of focal adhesion ($P < 10^{-4}$) and multiple related pathways. 46 % of the 109 genes mapped to this pathway were overexpressed in t1 samples compared to control. The majority of these genes were underexpressed at the subacute phase. Importantly, we identified novel genes previously not known to be involved in molecular mechanisms of MI.
CONCLUSION: We characterized the temporal changes in the expression of human monocytes following MI and we show that gene expression profiling of circulating blood monocytes during acute MI reflects crucial pathophysiological events such as cell adhesion, invasion of the sub-endothelial space and secretion of inflammatory molecule.

MRas - A Strong Candidate Gene for Coronary Artery Disease (CAD)

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Background: Our recent genome-wide association study (GWAS) identified the MRas gene on human chromosome 3q22 with genome-wide significance for CAD. Atherosclerotic manifestations like CAD reflect a chronic inflammation of the vessel wall with accumulation of lipid-laden macrophages in the large arteries. M-Ras is a member of the Ras superfamily of small GTPases; many of which function as molecular switches in diverse cellular functions and thereby regulate a variety of biological processes. M-Ras has been implicated in the regulation of TNFa-stimulated LFA-1 activation and integrin-mediated leukocyte adhesion downstream of various inflammatory cytokines.
Aim of the study: We aimed to perform a functional analysis of M-Ras related to atherosclerosis in mice to further understand the pathogenesis of CAD.
Methods and Materials: First, we confirmed the association of the Mras locus in the CARDIOGRAM data set involving 20.000 cases and 60.000 controlls (p= 7,4x10-13). We than determined the expression of MRas/M-Ras in several tissues. Second, we obtained an MRas-KO mice and started adhesion and enzyme-linked immunosorbent assays with macrophages, monocytes, B- and T-cells to study the influence of the MRas-KO on adhesion and migration as a potential pathomechanisms of atherosclerosis.
Results: RT-PCR and immunfluorescence analyses demonstrated that MRas is expressed in several tissues, including mouse and human aorta and heart, tissues that are involved in atherosclerosis. We found that leukocyte adhesion in the MRas-KO mice is about 20% reduced in comparison with leukocytes out of WT mice. Preliminary data also show an increased TNF-a secretion after LPS stimulation in vitro of MRas-KO macrophages in comparison with WT macrophages.
Conclusion: In our preliminary experiments we show that MRas has a functional relevance in leukocytes and macrophages. Both cell types are involved in the onset and development of CAD making a pathogenetic role of MRas very likely.

Associations between variations in the TLR4 gene and incident coronary heart disease (CHD) in middle-aged men and women: Results from the MONICA/KORA Augsburg case-cohort study, 1984-2002

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Background: Toll-like receptor 4 (TLR4), the signalling receptor for lipopolysaccharides, is an important member of the innate immunity system. Since several studies have suggested that atherosclerosis might be associated with changes in the innate immune response, and a SNP in the TLR4 gene (Asp299Gly; Kiechl et al, N Engl J Med 2002;347:185-92) was shown to be associated with a decreased risk of atherosclerosis, we sought to investigate the impact of gene variants in the TLR4 gene on incident CHD.
Methods: A case-cohort study was conducted in initially healthy, middle-aged men and women based on data from the MONICA/KORA Augsburg studies collected between 1984 and 2002, with a mean FU of 10.2±4.8 years. The present analyses are based on 318 case subjects with incident CHD and 1,727 non-case subjects. Seven SNPs (rs2770150; rs6478317; rs1927911; rs2149356; rs4986791; rs7873784; rs1927906) were systematically selected in the TLR4 gene, and haplotypes were constructed.
Results: TLR4 genotype distribution did not significantly differ among subjects with incident CHD and non-case subjects. No significant interactions between any of the SNPs and major cardiovascular risk factors on CHD risk were found. There was no consistent association between the 7 different SNPs within the TLR4 gene and incident CHD in crude and in multivariable adjusted analyses, neither for men and women separately, nor in a model that included all study participants.
Conclusions: In contrast to an earlier and smaller prospective study, we could not confirm an association between various SNPs within the TLR4 gene and incident CHD. The presence of various alleles of the TLR4 gene, including Asp299Gly, does not seem to exert a major influence on the progression of atherosclerosis in the general population.

Sparse Linear Regression for Interpretive Risk Assessment on GWA Data

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Genome-wide association (GWA) studies, which typically aim to identify SNPs associated with a disease, yield large amounts of high-dimensional data.Most GWA studies deal with complex diseases. Complex diseases are caused by a variety of genetic factors, and these factors, e.g. SNPs, may interact positively or negatively to increase or reduce the effect of the individual factors; indeed, an appreciable disease effect may only come about through such an interaction. Since, so far, most of the identified genetic variants have only a limited effect on disease risk, it suggests itself to analyse several SNPs simultaneously. A common approach to predicting risk using multiple SNPs is to compute a genotype score. This is done by selecting SNPs that are individually associated with the disease, then counting the number of unfavorable alleles per individual. However, such an analysis is still based on individual SNPs, and effects that come about only through the interaction of multiple SNPs are not captured.

Support Vector Machines (SVMs) can be used to infer interactions between SNPs; recent studies using SVMs have been successful in improving the risk prediction for Type I and II diabetes. However, SVMs have drawbacks: the resulting classifier is hard to interpret, all the more so the larger the number of SNPs used for classification. A feature selection is thus required to reduce the number of SNPs on which the SVM operates. A common way of doing this is to choose SNPs that are individually associated with the disease, but this discard interacting SNPs without single effects.

In this work, we propose an algorithm that does not require a feature selection step and predicts risk using only a small number of SNPs, thus allowing a genetic interpretation of the resulting classifier. The algorithm searches for the best risk predicting pattern among the complete set of SNPs by applying a method for sparse linear regression problems; the method can be applied to large data sets.

Eight Genetic Loci Associated with Variation in Lipoprotein-Associated Phospholipase A2 Mass and Activity and Coronary Heart Disease: Meta-analysis of Genome-wide Association Studies from Five Community-based Studies

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Aims: Lipoprotein-associated phospholipase A2 (Lp-PLA2) generates proinflammatory and proatherogenic products in the arterial vascular wall, and is a potential therapeutic target in coronary heart disease (CHD). We searched for genetic variants related to Lp-PLA2 mass or activity, via a genome-wide association study in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.
Methods and Results: In meta-analyses of findings from five population-based studies, comprising up to 13,664 subjects, variants at two loci (PLA2G7, CYP39A1 CETP) were associated with Lp-PLA2 mass. The strongest signal was at rs1805017 in PLA2G7 ($p=2.4\times10^{-23}$, log Lp-PLA2 difference per allele [beta]: 0.043). Variants at six loci were associated with Lp-PLA2 activity (PLA2G7, APOC1, CELSR2, LDLR2, ZNF259, SCARB1), among which the strongest signals were at rs4420638, near the APOE–APOC1–APOC4–APOC2 cluster ($p=4.9\times10^{-30}$; log Lp-PLA2 difference per allele [beta]: –0.054). There were no significant gene–environment interactions on Lp-PLA2 mass or activity between these eight polymorphisms and age, sex, body mass index, or smoking status ($P > 0.08$). In secondary analyses, four of the polymorphisms (in APOC1, CELSR2, LDLR, ZNF259), but not PLA2G7, were significantly associated with CHD ($P < 0.002$).
Conclusion: Levels of Lp-PLA2 mass and activity were associated with PLA2G7, the gene coding for this protein. Lp-PLA2 activity was also strongly associated with genetic variants in genes that have been related to low-density lipoprotein cholesterol levels and CHD.

Keywords: epidemiology; genome-wide association; inflammation; lipoprotein-associated phospholipase A2; single nucleotide polymorphism.

Genome-wide association study on HDL subclass traits

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Background: High-density lipoprotein cholesterol (HDL) is discussed as a prognostic marker of cardiovascular events. However, HDL is not a homogenous class, but can be further divided into subclasses. Heritable large HDL particles have been associated with a favorable cardiovascular risk profile. The genetic regulation of HDL subclass distribution is not fully understood. We therefore conducted a genome-wide association study with traits of the HDL subclass profile in subjects of the KORA (Cooperative health research in the Region of Augsburg) F4 cohort.
Methods: Genotypes and nuclear magnetic resonance (NMR) spectroscopy measurements of lipid profile were available for 1,797 individuals. We analyzed the traits HDL mean particle size and HDL particle number in relation to genotypes derived from genome-wide arrays. Association analyses were performed assuming an additive model of inheritance and adjusted for age and gender.
Results: A high correlation coefficient (Pearson) between conventional laboratory measurement of HDL concentration was found with NMR-derived HDL concentration (0.82), HDL mean size (0.68), and to a lower extend with HDL number (0.43). Genetic association analyses revealed genome-wide significant signals ($p<5x10^{-8}$) between HDL size and polymorphisms in LIPC and CETP genes. However, for HDL number, none of the polymorphisms reached genome-wide significance level.
Conclusion: In the KORA F4 cohort, polymorphisms in LIPC and CETP genes are strongly associated with HDL size. Larger samples are required for a comprehensive analysis of the genetic basis for HDL size and HDL number. Therefore, in the future additional cohorts will be included in the analysis. In a next step, additional NMR-derived lipoprotein subclasses will be used for genetic association studies and their prognostic value will be tested in a case-cohort design.

Genome-wide association study identifies two novel regions at 11p15.5-p13 and 1p31 with major impact on acute-phase Serum Amyloid A

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Serum amyloid A (SAA), an acute-phase protein, is causally involved in the pathogenesis of amyloidosis and has been found to be implicated in obesity, atherosclerosis and its clinical complications, and various malignancies.
We conducted the first genome-wide association study on baseline acute-phase SAA (A-SAA) concentrations in three population-based studies (KORA, TwinsUK, Sorbs) and one prospective case cohort study (LURIC) including a total of 4,212 participants of European descent and identified two genetic susceptibility regions at chromosome 11 and 1.
The region at chromosome 11 contains serum amyloid A1 (SAA1) and the adjacent general transcription factor 2 H1 (GTF2H1), Hermansky-Pudlak Syndrome 5 (HPS5), lactate dehydrogenase A (LDHA), and lactate dehydrogenase C (LDHC). The high degree of explained variance (10.84%) of the total variation of A-SAA suggests that the region is of key importance in the regulation of inflammation.
The second region encloses the leptin receptor (LEPR) gene at chromosome 1. As this region has been found to be associated with other acute-phase proteins in previous studies, our finding indicates a close interplay between A-SAA, leptin, and other inflammatory proteins and a larger role of the LEPR gene locus in inflammatory processes as it has been assumed in the past.

Novel common copy number variation for early onset extreme obesity on chromosome 11q11 identified by a genome-wide analysis

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Heritability of obesity is substantial and recent meta-analyses of genome-wide association studies (GWAS) have been successful in detecting several robustly associated genomic regions for obesity using single nucleotid polymorphisms (SNPs). However, taken together the SNPs only explain a small proportion of the overall heritability. Copy number variations (CNVs) might contribute to the ‘missing heritability’.
We searched genome-wide for association between common CNVs and early onset extreme obesity. 424 case-parents obesity trios and an independent sample of 453 extremely obese children and adolescents and 435 normal-weight and lean adult controls were genotyped by the Affymetrix Genome-Wide Human SNP Array 6.0. We detected 20 common copy number variable regions (CNVRs) which were associated to obesity. The most promising CNVRs were followed-up in an indepedent sample of 365 obesity trios.
We identified a common CNVR exclusively covering the three olfactory receptor genes OR4P4, OR4S2, OR4C6 to be associated with obesity (combined p-value = 0.015 in a total of 789 families; odds ratio for the obesity effect allele = 1.19; 95% confidence interval = 1.016-1.394). We also replicated two common deletions (near NEGR1 and at chromosome 10q11.22) that have previously been reported to be associated with body weight. Additionally, we support a rare CNV on chromosome 16 that has recently been reported by two independent groups. However rare CNVs had not been the focus of our study.
We conclude that common CNVs are unlikely to contribute substantially to the genetic basis of early onset extreme obesity.

Sequencing out Effects of Resveratrol on Chromatin

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Sirt1, an NAD^+-dependent deacetylase, acts as a master regulator of metabolic adaptations to nutrient availability. Recent studies have revealed an obscure activation of Sirt1 by resveratrol in vitro. Nonetheless, how does resveratrol exert its caloric restriction mimetic effect in vivo, possibly through Sirt1, remains to be elucidated. To decipher the effects of resveratrol on chromatin, a known deacetylation site of Sirt1 – acetylated histone H1K26 mark – was examined by chromatin immunoprecipitation followed by sequencing (ChIP-Seq). Surprisingly, striking acetylated histone H1K26 mark is detected on the exons of Sirt1 in resveratrol-treated C2C12 skeletal muscle cells. This exon marking is in accordance with an up-regulated Sirt1 gene expression. Despite a global deacetylation of histone H1K26 in resveratrol-treated cells, the majority of this mark tends to center on the Sirt1 gene itself. Furthermore, most of the 4,200 Sirt1 binding sites are found ~200 bp around TSS. Many of the Sirt1-bound regions contain a sequence motif that is most similar to Ctcf, an insulator which can also act as a chromatin barrier by preventing the spread of heterochromatin. Genes associated with Sirt1 binding sites show a highly significant enrichment for certain Gene Ontology and KEGG Pathway classifications, including cell cycle, and MAPK, p53, insulin, and mTOR signaling pathways. Strikingly, increased gene expression for oxidation/reduction is in accordance with known instability of resveratrol in fluids that contain hydrogencarbonate and pyruvate, which eventually leads to degradation of resveratrol activity and increased concentrations of hydrogen peroxide. From our data to date, we propose that resveratrol may induce mild stress to the cell, activate Sirt1 and thereby influence chromatin modification, thus subsequently mediating metabolic adaptations in response to environmental stimuli.

Lack of synthetic association for obesity at the melanocortin 4 receptor gene coding region

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Background
Independent genome-wide association studies (GWAS) showed an obesogenic effect of two single nucleotide polymorphisms (SNP; rs12970134 and rs17782313) more than 150 kb downstream of the melanocortin 4 receptor gene (MC4R). It is unclear if the SNPs directly influence MC4R function or expression, or if the SNPs are on a haplotype that predisposes to obesity or includes functionally relevant genetic variation (synthetic association). As both exist, functionally relevant mutations and polymorphisms in the MC4R coding region and a robust association downstream of the gene, MC4R is an ideal model to explore synthetic association.
Methodology/Principal Findings
We analyzed a genomic region (364.9 kb) encompassing the MC4R in GWAS data of 424 obesity trios consisting of an extremely obese child or adolescent and both parents. SNP rs12970134 showed the lowest p-value ($p = 0.004$; relative risk (RR) for the obesity effect allele: 1.37); conditional analyses on this SNP revealed that 7 of the 78 analyzed SNPs provided independent signals ($p = 0.05$). Analyses on these 8 SNPs were used to derive two-marker haplotypes. The three best (according to p-value) haplotype combinations were chosen for confirmation in 363 independent obesity trios. The confirmed obesity effect haplotype includes SNPs 3' and 5' of the MC4R. Including MC4R coding variants in a joint model had almost no impact on the effect size estimators expected under synthetic association.
Conclusions/Significance
We demonstrate that a haplotype reaching from a region 5' of the MC4R to a region at least 150 kb from the 3' end of the gene showed a stronger association to obesity than single SNPs. MC4R coding variants had almost no impact on the association signal under a model of synthetic association. Carriers of the haplotype should be enriched for relevant mutations outside the MC4R coding region and could thus be used for re-sequencing approaches to elucidate the association signal of the non-coding region.

Genomic imprinting in a family-based genome-wide association study

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Introduction: The results of genome-wide association studies (GWAS) have had a dramatic impact on our understanding of genetic factors involved in common complex disorders. Genomic imprinting is an epigenetic process in which the copy of a gene inherited from one parent is expressed at a significantly lower level than the copy from the other parent. Imprinting has not yet been analysed systematically in early onset extreme obesity.
Material and Methods: We performed a genome-wide imprinting analysis of 705 German nuclear families with extremely obese offspring based on Affymetrix Genome-Wide Human SNP Array 6.0 data. We analysed the data set by stratified transmission-disequilibrium-tests (TDT) as available in PLINK 1.07 (option '--tdt –poo'; Purcell et al., 2007), by the parental-asymmetry tests (PAT, Weinberg, 1999) and finally explored the inclusion of a priori information on imprinted human genes using both data-base knowledge from http://www.geneimprint.com/ or information from Gregg et al. (2010a, b) who recently reported new imprinted mouse genes within a Bayesian framework.
Results: We observed no genome-wide significant imprinting signal at a level a of $5x10^{-8}$. Comparing the results of the stratified TDT and the PAT the single SNP results were in some cases very different. Among the top 30 imprinting signals (according to PAT p-value) we observed no SNP in genomic regions (gene coding region ± 100kb) with previous evidence for imprinting.
Discussion: Our analyses demonstrate that the choice of the test statistics for addressing parent-of-origin effects in GWAS is important. Moreover, the formal inclusion of a priori information within a Bayesian framework requires further attention though the choice of the prior distributions remains the biggest challenge.

Genome wide association study of alcohol dependence

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Context: Identification of genes contributing to alcohol dependence will improve our understanding of the mechanisms underlying this disorder. Four GWASs have been reported so far, one of them by us.

Objective: To substantially extent our previous GWAS and perform a combined analysis

Methods: GWAS 1 included 487 male inpatients with DSM-IV alcohol dependence with an age at onset below 28 years and 1,358 population based control individuals. All subjects were of German descent. The GWAS tested 524,396 single nucleotide polymorphisms (SNPs) genotyped using Illumina HH550v3 Bead chip. GWAS 2 included 900 male inpatients with DSM-IV alcohol dependence and 862 controls, all of German descent, and was performed using Illumina 610-Quad and the 660W Bead chips for patients and HH550v3 Bead chip for controls. The pooled dataset after quality control included 462,776 autosomal SNPs that were used for association testing.

Results: The combined GWAS comprising 1342 patients and 2172 controls after quality control, provided 19 SNPs with nominal p<10-5. One SNP located between ADH1B and ADH1C genes met genome-wide significance (rs1789891: p=3.3x10-8).
Conclusion: The ADH gene cluster is the most replicated finding from earlier linkage analyses (Prescott et al., 2006) as well as association studies (Birley et al., 2009) of alcohol dependence in populations of European origin. Our genome-wide significant SNP rs1789891 is in complete LD (D´=1.0, r^2=0.27) with an amino acid exchange in the ADH1C gene (rs1693482), which was biochemically shown to have functional relevance.
The German study is the first to report genome wide significant association with alcohol dependence. Our finding represents a proof of principle that a GWAS—conducted in homogeneous samples—is a promising and valid approach to systematically explore susceptibility genes involved, even in as heterogeneous a disorder as alcohol addiction.

Circadian Rhythm and the Development of Alcoholism: Whole-Genome Expression Profiling in the Brain of Short-term and Long-term Alcohol Drinking Rats

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Drug abuse clearly affects the circadian rhythm of many behavioural and physiological functions such as sleep and food intake. As well, drugs of abuse can affect the expression of the so-called clock-genes to a different extent depending on the time of the day. Furthermore, recent findings revealed the importance of clock-genes in the development of drug addiction such as alcoholism. However, the molecular targets of the clock-genes in this regard are still unknown.
Therefore, we here aimed at comparing whole-genome expression profiles at different times of the day during critical phases in the development of alcohol addiction. We thus used a specific animal model of alcohol addiction, i.e. alcohol self-administration with repeated deprivation phases, and compared the gene expression profiles of rats having had short-term versus long-term access to alcohol. Alcohol drinking rats withdrawn from alcohol for 2.5 weeks after either the 1st or the 6th deprivation phase were sacrificed, together with age-matched control rats, at four different times of the day (n = 6/group at ZT5, ZT11, ZT17 and ZT23 where ZT12 corresponds to the onset of the dark period under a 12h/12h light-dark cycle). Brains were immediately deep frozen. Since the nucleus accumbens is crucial for the reinforcement effect of alcohol, we chose to look at the expression differences in this region specifically. Total RNA was prepared from microdissected nucleus accumbens and hybridised on Illumina RatRef-12 Expression BeadChips.
Microarray data analysis revealed highly significant expression differences between experimental groups at the various time points. Further data mining will include cluster analysis to find groups of co-regulated genes. Identified candidate genes will be validated using quantitative PCR. Among the candidate genes we expect to find new molecular targets that may be useful for medication development in alcoholism.

Altered implicit neuronal processing of emotional faces (fMRI) in alcohol-dependent patients?

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Introduction:
Alcohol-dependent patients (ADP) experience deficits in the processing of emotional facial stimuli due to inadequate recognition (Frigerio et al., 2002). The accurate perception and processing of emotional information is highly relevant for social interaction and interpersonal relationships (Kornreich et al., 2002). One fMRI-investigation of ADP showed that patients vs. controls displayed reduced functional brain activation while evaluating emotional facial expressions (Salloum et al., 2007).
Aim:
This study investigated the implicit processing of emotional facial cues in ADP in order to assess neuronal activation patterns in ADP versus healthy control subjects (CON).
Methods:
19 right-handed ADP (age (mean/SD)=49.84/9.62, sex (male=15/female=4), education years (mean/SD))=15.90/4.32) and 19 CON matched by age, sex and education underwent a modified fMRI cue-comparison-paradigm (two conditions (forms/faces), duration 4:30 min) at 3T. Data were analysed using SPSS 14.0 and SPM8.
Results:
Behavioural data analyses showed no significant differences of the percentages of hits, incorrects or misses in both conditions in ADP compared to CON. Concerning the reaction times of emotional cues (faces) and neutral cues (forms) no significant differences between ADP and CON were observed. Analyses of the fMRI-data will be presented.
Discussion:
We could not confirm reduced implicit facial identification performance in ADP vs. CON. One previous study with an explicit facial recognition task also observed no significant differences in the accuracy or reaction time of facial identification. However, decreased BOLD activation was found in the rostral anterior cingulated cortex while viewing negative vs. positive and neutral facial expressions in ADP vs. CON (Salloum et al., 2007). We will present our imaging data.

Metabolic changes in alcohol-dependent patients' brains – a magnetic resonance spectroscopy study

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Introduction:
Chronic alcohol consumption leads to alterations in the glutamate system which are associated with alcohol withdrawal symptoms and craving in alcohol addicition (Tsai et al., 1998). Subtle biochemical changes, such as those in glutamate (Glu) concentration, may thus underlie the neuropathology within human brain tissue that subsequently gives rise to the cognitive and behavioral impairments associated with alcohol addiction.
An MR spectroscopy (MRS) study by Lee et al. (2007) reported increased glutamate/creatine ratio in the anterior cingulate cortex (ACC) in young alcohol-dependent patients undergoing withdrawal.
Aim:
We investigated changes indicating neurodegenerative or neuroregenerative processes in the ACC in alcohol-dependent patients. In particular, the absolute concentrations of Glu and N-acetylaspartate (NAA) were quantified in the ACC by MRS using dedicated procedures.
Methods:
In a work in progress, 20 alcohol-dependent patients and 20 healthy control subjects underwent MRS at two measurement time points in a 3T Siemens Magnetom Verio, the first MRS being conducted after 5-10 days of detoxification, the second after a follow-up period of 14 days.

We will present the absolute concentration of Glu in the course of the early detoxification to investigate the hypothesized biological role of glutamate in the withdrawal symptomatology. In addition, the association between the magnitude of glutamate change and the severity of withdrawal symptoms, alcohol craving and the days of abstinence will be presented. The results will be referenced to alterations of NAA as a surrogate parameter of disturbed neuronal integrity.

Cue-induced brain activation mediates subsequent relapse in abstinent alcohol-dependent patients

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In alcoholism, one major mechanism contributing to relapse is the exposure to stimuli that have regularly been associated with alcohol intake. Such stimuli can become conditioned cues that elicit conditioned responses like alcohol craving and consumption. In the last decade, considerable progress has been made in identifying the basic neuronal mechanisms that underlie cue-induced alcohol craving. We explored whether functional brain activation during exposure to alcohol-associated cues would be related to the prospective relapse risk in detoxified alcohol-dependent patients.
46 detoxified alcoholics and 46 age- and gender-matched healthy controls participated in a functional magnetic resonance imaging (fMRI) study using a cue reactivity paradigm, in which visual alcohol-related and neutral control stimuli were presented. Patients were followed for 3 months, and alcohol intake was recorded. Afterwards data was analysed regarding the subsequent relapse, which divided the group of patients into 16 abstainers and 30 relapsers.
Visual alcohol-related versus neutral stimuli activated a frontocortical-limbic network including inferior, medial and middle frontal gyrus as well as putamen in the patient group relative to healthy controls. Comparing the two groups of patients, abstainers showed a stronger activation of orbitofrontal cortex as well as midbrain during the presentation of alcohol-related cues whereas relapsers revealed a stronger activation of cingulate gyrus.
This study suggests that cue-induced activation of the orbitofrontal cortex and dopaminergic innervated midbrain is negatively associated with the prospective relapse risk in alcohol-dependent patients. This could indicate a more pronounced and conscious processing of alcohol cues which might serve as a warning signal and a behavioural controlling function. In contrast, prospective relapsers showed a stronger activation of the cingulate gyrus, a region involved in the attribution of motivational value.

Screening of DNA & drug libraries for recovery of potent ADAM10 stimulators for Alzheimer's disease therapy

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In the pathogenesis of Alzheimer's disease (AD) the amyloid precursor protein (APP) plays a pivotal role. It can be processed by either the ß-secretase BACE1 (beta-site APP cleaving enzyme 1) which leads to generation of neurotoxic Aß-peptides or the a-secretase ADAM10 (a disintegrin and metalloproteinase 10). ADAM10 cleaves inside the Aß-sequence, thus prevents the release of toxic Aß and provides neurotrophic and neuroprotective APPsa. Therefore, increasing ADAM10 gene-expression and subsequently the amount of enzyme by activating the promoter of ADAM10 represents a promising target in the therapeutic treatment of AD.
We established a cell-based reporter assay with the human promoters of ADAM10 and BACE1 integrated into luciferase-reporter-vectors and performed a cotransfection screening of 704 human transcription factors (TFs). The 50 hits identified by significant alteration of gene expression in this first screening were revised on a second level for their occurrence in the CNS of adults. Afterwards it was examined if the remaining TFs, evaluated as candidate stimulators of ADAM10 gene expression, have an effect on the human APP promoter. In addition, 640 compounds of a FDA approved drug library were screened in order to detect drug candidates for AD therapy based on regulation of the transcriptional activity of either ADAM10 or BACE1.
The in vivo-evaluation of drug candidates will be examined in the future in an ADAM10-promoter-reporter mouse. This mouse model will use a firefly-luciferase integrated in the endogenous ADAM10 gene locus under the control of the ADAM10 promoter as a reporter for enhanced promoter activity. The firefly-luciferase and ADAM10 coding sequence will be connected via a small picornavirus-peptide (T2A) coding sequence which should lead to a co-expression of the reporter-luciferase and an intact and functional ADAM10 from the murine gene locus. The feasibility of the T2A based strategy was investigated in neuroblastoma cells.

Genome-wide analysis of quantitative markers associated with Alzheimer's disease

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With the advent of technologies that allow simultaneous genotyping of thousands of single nucleotide polymorphisms (SNPs) across the genome, the genetic contributions to complex diseases can be explored at an unprecedented detail. Recent large genome-wide association studies and meta analyses of case/control sample sets have successfully identified several new risk genes of Alzheimer's disease (AD). However, these results do not sufficiently explain the heritability of AD.
We have performed genome-wide SNP genotyping of 709 patients with AD using Illumina Infinium technology and used several quantitative clinical parameters available for these patients to identify possible associations with these endophenotypes. To serve as an ideal endophenotype of AD these parameters need to be linked very close to the neurodegenerative process or need to be part of the diagnostic procedure. As such, we have included several neuropsychological test results, such as the MMSE and CERAD-NP battery, as well as cerebrospinal fluid levels of Tau and A-beta proteins, and measures of lipid and iron metabolism.
A linear regression algorithm was applied, and for some of the parameters investigated, several SNPs or genetic regions showing significant associations at a genome-wide level have been identified. For most of the associations, validation studies using independent samples have been performed or are currently on the way. In conclusion, we have successfully identified several new candidate genes contributing to different aspects of the disease such as cognitive decline and neurodegeneration. These results will help us to understand the complex interaction of biological processes leading to the clinical syndrome of AD.

Epigenetic characterisation of Alzheimer's disease brain samples

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Alzheimer's disease (AD) is the most common form of dementia affecting millions of people world-wide. Despite tremendous research efforts the etiology of AD remains largely unknown. So far, many genome-wide studies have been performed to identify genetic factors contributing to the development of AD. However, despite the identification of several new risk factors the genetic contribution and the heritability of AD cannot be completely explained. As a consequence we aimed to explore the contribution of epigenetic components such as DNA methylation and histone modifications to the (i) development of AD and (ii) clinical features such as disease onset and progression. We performed a comprehensive analysis of DNA methylation and histone modifications in 3 areas (cerebellum, frontal cortex, temporal cortex) of AD (n = 43) and control brains (n = 25). Using Illumina's Infinium Methylation arrays, we determined methylation levels for more than 27.000 CpG sites located at approx. 14.000 genes. For the first time we identified significant differentially methylated loci only in the temporal cortex (which is severely affected in the AD-brain), but not in the cerebellum (which usually is not affected). Next, we measured global DNA methylation/hydroxymethylation as well as histone modification levels using an ELISA-based approach. Accordingly, we found significantly altered DNA hydroxymethylation in AD affected temporal cortex compared to control brain areas. Again no differences were observed for the cerebellum.
Our preliminary results demonstrate that epigenetic alterations do occur in AD and may play a considerable role not only for the development of AD, but also for onset and progression of the disease. Future studies will focus on the validation of the observed changes in methylation using larger sample sizes and will also aim at functional investigations. In particular, possible effects on mRNA, miRNA and protein expression levels in AD-brain need to be investigated.

Genome-wide association study (GWAS) of Alzheimer's disease

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disease and most recently several risk genes were identified by GWAS. We performed a GWAS that includes 709 well characterized patients with AD and 971 population based healthy controls from popgen biobank in Kiel, Germany, and the Cooperative Health Research in the Augsburg Region (KORA). Genotyping was performed using Illumina's Infinium technology analysing 550 and 610 thousand SNPs, respectively. Statistical analysis of association was performed using logistic regression methodologies. Several SNPs and genetic regions were identified showing strong association signals above the threshold of genome-wide significance including APOE. In a second stage, we performed a validation of the top 100 SNPs showing the strongest association derived from three different statistical models (additive, dominant, recessive) using an independent genotyping methodology (Sequenom's iPlex assay) and an independent sample set of 513 AD cases and 377 age-matched controls. We were able to validate a couple of loci that are currently being replicated using the already available GWAS data of the international AD GWAS consortium.
In addition, we compared association signals and allele frequencies of previously reported associations by Lambert et al. and Harold et al. 2009 in Nature Genetics (CR1: rs3818361; CLU: rs11136000; PICALM: rs3851179; BIN1: rs7561528, rs744373) with the results obtained for our German cohort. Although our association results for these SNPs did not achieve the criteria for genome-wide significance, we obtained similar allele frequencies, except of rs11136000 (CLU), and nominal significant associations which clearly indicate that (i) our German cohort is comparable to the others and (ii) GWAS results are reproducible across different populations.

Sphingolipids and Alzheimer's disease - vicious cycle of physiological and pathophysiological function of the Amyloid Precursor Protein

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Alzheimer’s disease (AD) is the major neurodegenerative disorder. Simple overproduction of a small peptide, the amyloid beta peptide 42 (Aß42), is sufficient to cause amyloid plaques build-up and cognitive deficits in mice and AD in humans. Aß production is very sensitive to changes in the lipid composition of cellular membranes and 5 out of the 6 recently identified risk genes for AD are lipid related.
We found that physiological Aß levels in brain function as potent signaling molecules that alter the activity of lipid metabolic enzymes and that APP processing is involved in the regulation of lipid metabolic pathways. Theses pathways involve feed-back regulation, where the lipids which are targeted by APP processing themselves influence Ab production. As expected from physiological regulatory cycles, APP processing typically responds already to small changes in membrane composition, but extreme (non-physiological) alterations in lipid composition are required to abolish Ab generation.
Although the vast majority of lipids are not affected by APP, some of the most important and best-studied lipid classes are, among these are sterols and sphingolipids. On both of these the knock-out of APP results in drastic changes in lipid levels and the activity of several lipid biosynthetic enzymes. Several observations suggest that sphingolipids might be closely linked to AD ethiology. Homeostasis of sphingomyelin is largely controlled by the activity of sphingomyelinases which are activated by Aß42, but not by the closely related Aß40 (Aß40 is not involved in AD pathogensis). Moreover, mutations that cause familial AD increase sphingomyelinase activity. Inversely, slightly increased sphingomyelin levels are sufficient to strongly decrease Aß production. In agreement with the previous observation that APP driven lipid regulation involves feed-back regulation, we found that ganglioside production is another target of APP mediated lipid homeostasis.

Identifying functional units in Genome-wide association studies (GWAS) data

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GWAS are commonly used to identify disease risk genes. To this end, gene variants, i.e. Single Nucleotide Polymorphisms (SNPs) of healthy and diseased people are compared and assigned with a p-value as a result of a standard case/control association analysis. A SNP can, but does not necessarily have to be the linked to the outbreak of a disease. In some cases the SNP is just a biomarker in a high linkage disequilibrium with the actual genetic cause or the effect results from the interplay with other SNPs. To identify associated sets of SNPs in GWAS data we developed a four step meta-analysis procedure as follows: (i) We collect further information about the SNP, like gene loci, gene products, gene interaction partners and pathways information from UniProt, KEGG, DOMINE and SwissPfam. (ii) We define functional units of possibly associated SNPs depending on pathway and interaction information collected in the first step. Here we distinguish pathway, pathway-gene, and pathway-interacting units. Pathway units contain SNPs in genes occurring in one pathway. In contrast the pathway-gene units consider only SNPs in genes occurring exclusively in one pathway. The pathway-interaction units involve only SNPs in genes assigned to a specific gene interaction class. There are four different gene interaction classes created referring to the DOMINE database: experimentally validated (PDB), predicted with high (HC), medium (MC) and low (LC) confidence. The advantage of using predicted interaction information, too, is the possible identification of previously unknown interactions which might influence the disease. (iii) We compute a set specific p-value with the program SLAT for each functional unit. This score is based on combined statistics with the null hypothesis, that the SNPs are not associated. (iv) Finally, we create a graphical representation of the results. The best functional units are listed and the pathways with the affected genes are visualized.

Cerebrospinal fluid fatty acid composition in healthy elderly seems widely independent of plasma composition

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The relevance of fatty acids (FA) in physiological and pathological processes is increasingly recognized. They are proposed to be particularly involved in brain diseases such as Alzheimer's and Parkinson's disease. However, their distribution and regulation in the cerebrospinal fluid (CSF) is not readily understood. Twelve common FA were determined in 25 healthy elderly using gas chromatography. We compared absolute and relative levels of saturated (SFA), monounsaturated (MUFA) and polyunsaturated FA (PUFA) with corresponding plasma levels, and with demographic variables. CSF FA levels were 10-200 times lower, and ranges commonly smaller compared to corresponding plasma FA levels. CSF PUFA levels were significantly higher, and CSF SFA levels significantly lower compared to plasma levels. The "de novo" lipogenesis index was lower, and stearoyl-CoA desaturase (SCD) indices were higher in CSF than in plasma. We conclude that CSF FA composition seems locally regulated, and highly independent of plasma FA composition. This should be considered in future studies on the association of neurodegenerative diseases with fatty acid metabolism.

A machine learning pipeline for disease risk assessment of Genome-wide association studies (GWAS) SNP data

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Using machine learning methods, GWAS data can be analysed for more complex relations between single nucleotid polymorphisms (SNPs) and diseases than simple statistical methods that look at each SNP separately. However, this often requires the use of several tools and the cexessity of intensive data conversion and user interaction. We developed an automated pipeline that uses state-of-the-art machine learning algorithms to create a disease risk model based on a given GWAS SNP dataset and assesses its predictive performance for unseen datasets. The pipeline can either use a first dataset for training the model and a second for validation, or perform a nested k-fold cross-validation on a single dataset. For each training set a basic case/control association analysis is performed to estimate the association between each single SNP and the phenotype. Using this information the dataset is filtered to create multiple subsets that contain only SNPs below a certain p-value threshold and for each subset a model is trained using a support vector machine (LIBSVM: linear and RBF kernel) and tested on its corresponding validation subset. The prediction performance is measured as the area under the ROC curve (AUC) and visualized in a plot showing average AUC and standard deviation for each p-value threshold. The only required input for this pipeline is the SNP data, all other parameters use default values, but can be specified by the user, if wanted. During the whole process, the pipeline takes care of the necessary conversions between different data formats and stores all intermediate data and final results to allow for subsequent analysis of single steps. Additionally, if a second analysis is performed on the same dataset with different parameters, e.g., adding another p-value threshold, the pipeline will not repeat steps to create data that is still valid.

Modulation of Protein Complex Composition and Function involved in Neurodegenerative Diseases

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Many efforts have been made to increase our understanding of the cause, progression and mechanism leading to neurodegenerative disorders like Alzheimer disease, Parkinson disease or amyotrophic lateral sclerosis. Whereas most of these diseases are sporadic and of unknown cause, a series of different gene mutations have been identified to cause familial variants of several neurodegenerative disorders. It is the aim of this project to identify neurodegenerative disease relevant protein-protein interactions, and to characterise the identified protein complexes for modelling disease related signalling pathways on a biochemical and functional level.
To achieve this goal we have adapted an inducible Flp-In T-REx HEK 293 cell culture system to the GATEWAY high-throughput cloning system for transfection and efficient generation of stable cell lines. So far we produced 32 stable cell lines expressing a C- or N-terminal TAP-fusion protein for SOD1, SNCA, APP, ATXN1, PARK2, PARK7, PSEN1, HTT and TARDBP (wild type and mutant forms). Native protein complexes are isolated by tandem affinity purification (TAP) and analysed via mass spectrometry. Functional assays are further employed to study the morphological and cellular traits as a consequence of neuronal disease related protein overexpression of wild type and mutant variants. In the future, disease-relevant cues such as cellular stress or co-expression of upstream regulatory kinases will be applied in our cellular system to investigate the consequence of such modulation on protein complex composition and function.

Compromized expression control of genes involved in serotonin and GABA metabolism in human epileptic tissue

Presenting Author: Katharina Pernhorst
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Temporal lobe epilepsy (TLE) is frequently associated with pharmacoresistance. Neurosurgical removal of the epileptogenic focus in many pharmacoresistant TLE patients results in seizure control. Based on such hippocampal biopsy specimen from epilepsy surgery (n>170), we have addressed if allelic promoter variants previously found to be augmented in epilepsy or migraine patients alter the expression of corresponding genes. We observed a significantly increased expression of SSADH-mRNA in brain tissue of individuals, who are carriers of an SSADH promoter variant associated with idiopathic generalized epilepsy. Further, the HTR1a (5-HT-serotonin) receptor mRNA we found as abundant in individuals with the GG-genotype of the 5-HTR1a receptor promoter polymorphism associated with avoidance of physical activity during migraine. The GABA(A) receptor beta 3 (GABRB3) mRNA was reduced in TLE patients homozygous for the G/G SNP genotype. Subsequently, we used functional promoter assays addressing potentially impaired binding affinity of transcription factors (TFs) due to gene promoter variants of the three genes. Our recent data revealed the prediction of an increased binding affinity of the EGR-3 TF to the SNP- (74%) versus the wildtype sequence (59%) in the SSADH promoter. This, we confirmed by overexpressing EGR-3 in a respective luciferase assay. Corresponding analyses are currently carried out for HTR1a and GABRB3. Our study provides new insights in the role of promoter variants in episodic brain disorders.

The Glut1 syndromes

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Glut1 is the glucose transporter of the blood brain barrier, thus a crucial molecule to deliver the most important energy carrier to the brain. Mutations in SLC2A1 coding for Glut1 have been found in the classical Glut1-deficiency syndrome (Glut1-DS), a severe syndrome of early childhood with drug resistant epilepsy, microcephaly and progressive mental retardation. Recently, we detected mutations in SLC2A1 in patients with paroxysmal exercise-induced dyskinesia (PED) and, in cooperation with others, also in patients with a special form of an idiopathic generalized epilepsy, the early onset absence epilepsy (EOAE) and patients with a permanent and slightly progressive paraparesis combined with PED (CSE). The symptoms respond well to a ketogenic diet providing ketone bodies instead of glucose to the brain as the main energy source. Thus, the clinical spectrum of the Glut1 syndromes was dramatically enlarged in the last three years. All detected mutations were functionally tested and reduced the uptake of glucose by the transporter significantly. The abstract summarizes the clinical, genetic and functional data of the Glut1 syndromes and gives hints to genotype-phenotype correlations.

Genome-wide association study in migraine without aura – an update

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Background. Migraine is a common primary headache disorder, characterized by attacks of severe, throbbing headache and autonomic nervous system dysfunction. In some patients, headaches are preceded by transient neurological disturbances (i.e. migraine with aura, MA), while the majority of affecteds suffer from migraine without aura (MO). Recently, the International Migraine Genetics Consortium (IMGC) by means of a genome-wide association study (GWAS) for the first time identified a common susceptibility variant for migraine on chromosome 8q22.1 (Anttila et al., Nature Genetics 2010). Based on these results, we aimed at the identification of variants associated specifically with the MO subtype of migraine.
Patients. A cohort of 1238 clinic-based German MO patients was recruited at the Department of Neurology of Munich University. All patients were subjected to an extensive previously validated headache questionnaire. For diagnosis of MO, the criteria of the International Headache Society were used. Population-matched controls (n= 840) were recruited from the KORA study as well as from the GSK and the MPIPSYKL study.
Genetic analysis. Genotyping was performed using the Human610-Quad v1 array (n= 838) or the Human660W-Quad v1 array (Illumina). Mean call rate for the cases was 99.40%.
Statistical analysis. Association analysis was performed using logistic regression (allelic/additive model). Cases and controls were imputed to Hapmap III using Impute v2 and all Hapmap II reference panels.
Preliminary results. We identified a total of 16 variants with genome-wide significance.
Future perspectives. As a next step, we are planning to perform a meta-analysis, combining our results with the data from another MO GWAS performed in the context of the IMGC. Finally, for replication purposes, the identified variant(s) will be tested in other clinic-bases MO cohorts available through the IMGC.

Genetic basis of Levetiracetam pharmacoresistance and side effects in human epilepsy

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Pharmacoresistance and substantial side effects represent serious problems in the treatment of epilepsy patients with anti-epileptic drugs (AEDs). To date, the molecular basis of drug resistance in epilepsy remains elusive. Only recently, with the identification of the synaptic vesicle protein SV2A as high-affinity binding site for the AED Levetiracetam (LEV), the presynapse has come into focus as a target for AEDs. However, it is still unknown how LEV affects the function of SV2A. LEV is an effective AED in many epilepsy patients refractory to other AEDs. Intriguingly, 90% of all LEV-treated patients can be divided into two distinct groups: “a-priori non-responders” versus “primary responders”. In this study, we have classified 503 patients according to their initial response to LEV. Using genomic DNA from blood of these patients we performed a target gene analysis of the SV2A gene as well as a genome wide SNP and CNV analysis in order to identify genomic variants associated with an a-priori non-response. As a subgroup of these patients underwent surgical treatment to alleviate the seizure phenotype we analyzed the resulting hippocampal biopsy specimens with regard to SV2A expression levels by quantitative real time RT-PCR and immunofluorescence and microscopy. We found that SV2A expression was significantly reduced in the a-priori non-responder group. We furthermore examined if the expression of other genes is altered in these samples between the two groups using an mRNA expression array and thereby identified 17 differentially expressed synaptic genes.

Recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 predispose to idiopathic generalized epilepsies

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Idiopathic generalized epilepsies (IGEs) accounts for 30% of all epilepsies. Despite a predominant genetic aetiology, the genetic factors predisposing to IGE remain elusive. Studies of structural genomic variations have revealed a significant excess of recurrent microdeletions at 15q11.2, 15q13.3, 16p13.11, and 22q11.2 in various neuropsychiatric disorders. The present study investigates the impact of these four microdeletions on the genetic risk to common IGE syndromes. Initially, candidate microdeletions were assessed by high-density single nucleotide polymorphism (SNP) arrays. Loss-of-heterozygosity (LOH) pre-screening using STR and SNP panels across the consensus region of the recurrent microdeletions were performed with a larger cohort, at which STR-panels represent a sensitive and cost-efficient tool for screening large recurrent microdeletions. Overall, the study sample comprises 3151 IGE patients and 3564 controls of European descent. Potential deletions were validated by quantitative PCR and breakpoints were refined by array comparative genomic hybridization. In total, 57 IGE patients (1.8%) carried one of the four microdeletions compared with 14 controls (0.4%) (OR = 4.6; 95% CI: 2.6-8.3, $P=2.1x10^{-7}$). Parental transmission could be examined in 27 trios, showing eight de novo and 19 inherited microdeletions. Of those, 17 were transmitted by clinically unaffected parents, suggesting that the microdeletion itself is not sufficient to cause epilepsy. Based on our data the microdeletions at 15q11.2, 16p13.11 and 15q13.3 display an incomplete penetrance ranging from 1%-10%. Accordingly, there is a low predictive value for genetic counseling. The 15q13.3 microdeletion merged as major genetic risk factor for IGE (OR>50, 95% CI 5.3-8). Although the microdeletions investigated are individually rare (<1%) in patients with IGE, they collectively seem to account for a significant fraction of the genetic variance in common idiopathic generalized epilepsy syndromes.

Genome-wide association mapping of susceptibility alleles predisposing to idiopathic generalized epilepsy

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Idiopathic generalized epilepsies (IGEs) affect about 0.2% of the general population and account for 30% of all epilepsies. Genetic factors play a predominant role in the etiology of common IGE syndromes. However, the vast majority of IGE patients display a complex-polygenic etiology. Up to date, the genetic variants predisposing to common IGE syndromes remain elusive. To search for common genetic risk factors, we are carrying out an ongoing two-staged genome-wide association study (GWAS) on common IGE syndromes in 1426 IGE patients of Western-European ancestry and 2883 German population controls. Stage-1 association screening was conducted for the IGE sample and two subsamples, comprising 668 IGE probands with idiopathic absence epilepsy (IAE) and 539 probands with juvenile myoclonic epilepsy (JME). Subsequently, Stage-2 replication analysis of 93 Stage-1 association hints ($P<10^{-3}$) was performed in an independent study sample of European ancestry, including 507 IGE parent-offspring trios (IAE: n=353; JME: n=104), 503 IGE singletons (IAE: n=174; JME: n=250) and 905 ethnically matched controls. We observed one significant association at 5q32 ($P=4.0x10^{-4}$) exceeding the Bonferroni threshold for multiple testing (Stage-1 $P=6.0x10^{-6}$, combined $P=3.3x10^{-8}$). A second locus at 17q21.32 displayed a suggestive association lead (Stage-2 $P=9.1x10^{-3}$, Stage-1 $P=1.1x10^{-7}$) with a strong association signal in the combined analysis ($P=2.9x10^{-9}$). Although replication analysis did not confirm a strong Stage-1 association signal at 2p16.1 (Stage-1 $P=1.2x10^{-8}$, Stage-2 P=0.06), the combined analysis still supports an association with absence seizures ($P=2.4x10^{-8}$). We are still in process to increase the sample sizes for both GWAS stages aiming for a higher statistical power to identify common susceptibility alleles for IGE which will provide deeper insights into molecular pathways of epileptogenesis.

Genome-wide supported risk variants for bipolar disorder in Ank3 alter anatomical connectivity in the human brain

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Bipolar disorder is a devastating, highly heritable mental disorder related to disconnectivity between limbic and frontal brain areas. Meta-analyses of genome-wide association studies showed rs10994336 in ANK3 as currently the best supported variant and also discovered rs9804190 in ANK3 as independent variant associated with bipolar disorder. Using an imaging genetics approach employing diffusion tensor imaging, we show decreased white matter integrity, indicated by lower fractional anisotropy and longitudinal diffusivity in healthy carriers of rs10994336 risk genotype in the anterior limb of the internal capsule. This result suggests disruption of fronto-limbic circuits, related to increased risk-taking in carriers of a risk variant for bipolar disorder. Furthermore, in carriers of rs9804190 risk genotype, we identified increased fractional anisotropy in the anterior thalamic radiation. Higher fractional anisotropy in the anterior thalamic radiation might either result from less crossing fibers in this region and would thus underline disruption of anatomical connectivity in risk allele carriers or might be interpreted in the context of the differential susceptibility hypothesis stressing the importance of environmental factors for a favorable or disadvantageous gene effects. In sum, our findings confirm altered connectivity as neurogenetic risk mechanism for bipolar disorder.

The Catechol-O-Methyl Transferase (COMT) gene and its potential association with schizophrenia: findings from a large German case-control and family-based sample

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The aim of the present study was to investigate possible associations between schizophrenia and 13 SNP markers in COMT. No association was observed in 631 cases, 207 nuclear families, and 776 controls. A cognitive performance phenotype (Trail Marking Test) was available for a subgroup of the patients. No association was found between the 13 markers and this phenotype. Four clinically-defined subgroups (early age at onset, negative symptoms, family history of schizophrenia, and life-time major depressive episode) were also investigated. Associations were observed for 3 of these subgroups, although none withstood correction for multiple testing. COMT does not appear to be a risk factor for schizophrenia in this population.

Association between genetic variation in a region on chromosome 11 and schizophrenia in large samples from Europe

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Recent molecular studies have implicated common alleles of small to moderate effect and rare alleles with larger effect sizes in the genetic architecture of schizophrenia (SCZ). It is expected that the reliable detection of risk variants with very small effect sizes can only be achieved through the recruitment of very large samples of patients and controls (i.e. tens of thousands) , or large, potentially more homogeneous samples that have been recruited from confined geographical areas using identical diagnostic criteria. Applying the latter strategy, we performed a genome-wide association study (GWAS) of 1,169 clinically well-characterized and ethnically homogeneous SCZ patients from a confined area of Western Europe (464 from Germany, 705 from The Netherlands) and 3,714 ethnically matched controls (1,272 and 2,442, respectively). In a subsequent follow-up study of our top GWAS results, we included an additional 2,569 SCZ patients and 4,088 controls (from Germany, The Netherlands, and Denmark). Genetic variation in a region on chromosome 11 that contains the candidate genes AMBRA1, DGKZ, and CHRM4 was significantly associated with SCZ in the combined sample (n= 11,540; P= 3.89×10^{-9}, OR= 1.25). This finding was replicated in 23,970 independent samples of European ancestry (P= 0.0029, OR= 1.11). In a subsequent imaging genetics study, healthy carriers of the risk allele exhibited altered activation in the cingulate cortex during a cognitive control task. The area of interest is a critical interface between emotion-regulation and cognition that is structurally and functionally abnormal in SCZ and bipolar disorder.

Resequencing of neurexin 1 (NRXN1) and Follow-up in schizophrenia

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Large rare deletions in NRXN1 increase the risk for schizophrenia. The aim of the present study was to determine whether small rare sequence changes in exons and splice sites contribute to the development of schizophrenia in a high-penetrance manner. Complete coding regions and splice sites were resequenced in 94 patients and 94 controls. Among the 16 rare sequence variants, two missense substitutions (E201G and I1068V) were observed in single patients but not in controls. Investigation of DNA samples from family members and in silico analysis of possible effects on protein function provided no obvious evidence for high-penetrance genetic effects. To better assess the effect size and statistical significance, we are currently conduction genotyping and association analysis of these rare variants using >1000 schizophrenia patients and >1000 psychiatrically screened controls.

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Re-Sequencing analysis of the schizophrenia-associated microdeletion region on 1q21.1

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We and others have previously identified rare recurrent microdeletions on chromosome 1q21.1 as a strong genetic risk factor for schizophrenia. In the present study, we aimed to explore to what extent rare sequencing variants in the region might contribute to the disease allele spectrum and which of the several genes spanned by the deletion might be real schizophrenia susceptibility genes.

We performed exon-based Sanger re-sequencing covering seven RefSeq genes lying in the region in 94 DSMIV-diagnosed schizophrenia patients and 94 sex-matched controls. A total 5.3 Mb sequence information was generated and screened for rare variants filtered for a combined case/control minor allele frequency of 1%. These variants were subjected to in silico analysis by use of various bioinformatical tools. Novel exonic single base exchanges with a potential functional effect were numerically over-represented in patients in comparison to controls, several of them being patient specific. In silico analysis provided further support for a subset of patient specific variants to have a higher likelihood of conferring risk for schizophrenia. This subset was distributed throughout three of the seven genes. Transcriptional expression analysis from pre-mortem human hippocampus, in situ hybridization analysis in mice and our literature search has provided further support for a possible involvement of the three genes in schizophrenia development.

As a preliminary conclusion; we hypothesize that some of the rare and potentially functional alterations we identified may contribute to the schizophrenia development pointing out three genes as potential schizophrenia susceptibility genes. As the next step, all rare variants will be assessed in a large cohort of patients and controls to be verified as real schizophrenia risk variants.

F.B.Basmanav and A.J.Forstner contributed equally to this work.

Sexual dysfunction during antidepressant treatment and the 5-HTTLPR

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Objectives: Sexual dysfunction (SD) is a frequently reported side-effect of antidepressant treatment, particularly of selective serotonin reuptake inhibitors (SSRI). In the multicenter clinical and pharmacogenetic GENDEP study (Genome Based Therapeutic Drugs for Depression), the effect of the serotonin transporter gene promoter polymorphism (5-HTTLPR) on sexual function was investigated during treatment with escitalopram (SSRI) and nortriptyline (a tricyclic antidepressant).
Methods: Four hundred seventy-three subjects aged 19-72 with an episode of DSM-IV major depression were randomly assigned to baseline-controlled treatment with escitalopram or nortriptyline. Over twelve weeks, depressive symptoms and SD were measured weekly with the Montgomery-Asberg Depression Rating Scale, the Antidepressant Side-Effect Checklist, the UKU Side Effect Rating Scale, and the Sexual Functioning Questionnaire.
Results: The incidence of reported SD after twelve weeks of treatment was relatively low, and did not differ significantly between antidepressants (14.9% escitalopram, 19.7% nortriptyline). There was no significant interaction between 5-HTTLPR and antidepressant. Improvement in depressive symptoms and younger age were associated with lower SD. The effect of age on SD may have been moderated by the 5-HTTLPR.
Conclusions: In GENDEP, the rates of reported SD during treatment were lower than those described in previous reports. The 5-HTTLPR is not a major determinant of SD during pharmacological treatment for depression.

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Symptom dimensions across the major psychoses and evidence for their usefulness in genetic studies

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Background: Most psychiatric genetic studies rely on categorical diagnoses, which define psychiatric disorders as distinct disease entities. However, these disorders are in fact clinically heterogeneous and there is a substantial overlap in clinical symptoms between disorders. .Successful attempts have been made to reduce biological heterogeneity by sub-grouping patient samples according to clinical symptoms. Few studies have applied a symptom dimension approach.
Aims: The aims of the present study were (i) to examine symptom dimensions across the diagnoses of schizophrenia, bipolar disorder, and major depression, and (ii) to test the usefulness of the derived dimensions in molecular genetic studies.
Methods: A principal component analysis was performed for 69 items from the OPCRIT checklist using a sample of 1718 patients with a DSMIV diagnose of schizophrenia (n=480), bipolar disorder (n=641), or major depression (n=597). Association analyses were performed using both categorical and dimensional phenotypes for rs1064395, a variant in the neurocan gene for which genome-wide significant association with bipolar disorder has been reported).
Results: Five factors were identified in the overall patient sample: reality distortion, mania, depression, disorganisation, and drug abuse/dependence. In the overall sample, patients carrying the rs1064395 risk allele scored higher on the mania dimension than non-carriers. This finding was supported by analysing the three disorders separately.
Conclusions: Factor analytical approaches in psychiatric genetic studies enhance the identification of the clinical core-phenotypes that underlie the associations.

Identification of DNA variants with functional background for human hippocampal gene expression (eQTL analyses)

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Recent studies have demonstrated that much of the genetic component of human phenotypic diversity has been proposed to be the result of cis-acting influences on gene expression. Also trans effects which are able to affect the transcription machinery contributes to human phenotypic variation. In particular, allele-specific expression is relatively common among non-imprinted autosomal genes. In the present study, we aim to identify genetic factors that influence gene expression in the human hippocampus in cis and in trans. For systematic mapping of determinants for hippocampal gene expression, we isolated genomic DNA and RNA of hippocampus samples (Bonn tissue bank) and performed an expressed quantitative trait loci (eQTL) analysis. Each individual DNA sample is genotyped with >600,000 SNPs. Individual gene expression levels for more than 99,9% of all known human genes (~25,000 annotated RefSeq and UniGene genes with > 48.000 probes) are interrogated with microarrays. Gene expression levels are then systematically correlated with individual genotype information to identify cis and trans eQTLs. After quality control (SNP call rate >98%, MAF >0.01, HWE $1x10^{-5}$; probe detection p-value >0.01) 530777 SNPs and 15426 probes were used for eQTL analyses. As a first analysis step, Bonferroni correction of the data set ($>10^{-12}$) results in 284 cis and 60 trans associations (top eQTLs) with high potential to be eQTLs in the human hippocampus. Due to the fact that this is too conservative for cis-regulating elements, we are currently performing a permutation based analysis. The next step for follow up experiments will be lymphocyte cell assays to characterize regulatory elements via Chromatin immune-precipitation (ChIP). One important application of our findings will be the interpretation of SNP association findings for brain phenotypes, in particular neuropsychiatric disorders.

Efficient Approach for Genome-Wide Interaction Analysis including all Pair-Wise Interaction Tests

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Nowadays genome-wide association studies (GWAS) represent an effective strategy for the analysis of variations across the genome and the detection of risk and protective factors for human diseases. Nevertheless often a huge part of the heritability of complex diseases cannot be explained by conventional GWAS examination due to the low effect of single SNPs. Hence a more promising approach is the examination of interactions between genetic variants on a trait by genome wide interaction analysis (GWIA). Since the interaction analysis of all pairs of SNPs is computationally challenging, it was necessary to develop a sophisticated GWIA approach including various advancements in data compression, specific data representation and interleaved data organization.
The approach is developed on the basis of a case-control study of idiopathic generalized epilepsy. The data are derived from 1,601 German epilepsy patients and controls genotyped with the Affymetrix Genome-Wide Human SNP Array 6.0. After the exclusion of individuals and markers due to quality control criteria, 1,559 individuals and 620,726 SNPs were considered. Within the interaction analysis all possible combinations of any two SNPs were examined. For this step 1.9 x 10^{11} contingency tables were generated. The calculation was parallelized on a multi-processor system. Since it is not reasonable to store all contingency tables, a threshold for the p values was empirically determined. Tables with p-values above 4.33 x 10^{-8} were discarded. The TOP HIT interaction list reveals some promising findings. The list contains genes (DNER, CTNNA3), which are involved into neuronal migration, synaptogenesis, and the formation of neuronal circuits. Hence the findings indicate a possible interaction of these genes in epileptogenesis. Our results demonstrate the capability of genome wide interaction analysis to become an efficient tool in genetic epidemiology, which may help to close the increasingly recognized gap in heritability.

Screening for copy number variants in a German patient-control sample of schizophrenia

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Schizophrenia is a severe, disabling psychiatric disorder with a prevalence of around 1% worldwide. Several recent studies showed association of copy number variants (CNVs) with schizophrenia. However, the contribution of such genetic variants has yet to be entirely elucidated. In the present study, we conducted a systematic genome-wide survey for CNVs in a sample of 1,479 patients with a DSM-IV diagnosis of either schizophrenia or schizoaffective disorder and 1,338 population-based controls. All individuals were of German descent and genotyped on Illumina's HumanHap550, Human610 or Human660W arrays, sharing about 550,000 markers. To identify potential CNVs, we analyzed each individual's SNP fluorescence intensity data with QuantiSNP, an algorithm using a Hidden-Markov model. We systematically filtered for intensity changes (indicative of a putative CNV) with a minimum of 30 consecutive SNPs and a log Bayes Factor (lBF) of at least 30. The lBF, calculated by QuantiSNP, is an indicator for the confidence of each predicted CNV, with higher values indicating higher statistical reliability. Using PLINK, we ran a permutation-based test for association of specific chromosomal regions with schizophrenia. We identified overlapping microdeletions and microduplications in a region on chromosome 6q26 to be significantly overrepresented in schizophrenia patients compared to controls. Furthermore, we screened the previously described regions on 1q21, 2p16, 2q32, 7q34, 15q11, 15q13, 16q11, 17p12 and 22q11 for CNVs. Validation experiments are currently being performed.

Suggestive evidence for an involvement of copy number variants in a German patient-control sample of major depressive disorder

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Major depressive disorder is the third leading cause of global burden of disease world-wide. Microdeletions and microduplications, also known as copy number variants (CNVs), contribute to the genetic differences among humans. Several studies showed that CNVs play a role in the etiology of psychiatric disorders, such as schizophrenia and autism. To the authors' knowledge no genome-wide screening for CNVs has been conducted for major depressive disorder, so far. In the present study, we performed a systematic genome-wide survey for CNVs in a sample of 579 patients with a DSM-IV diagnosis of major depression and 1,333 population-based controls. All individuals were of self-reported German ancestry and were either genotyped on Illumina's HumanHap550 or Human610 arrays, with a consensus set of approximately 550,000 markers. To identify potential CNVs, we analyzed each individual's SNP fluorescence intensity data with QuantiSNP, an algorithm using a Hidden-Markov model. In order to reduce the number of false-positive CNV calls we applied stringent quality criteria, requiring CNVs to have a minimum of 30 consecutive SNPs and a log Bayes Factor (lBF) of at least 30. The lBF, calculated by QuantiSNP, is a factor indicating the confidence of each predicted CNV, with higher values pointing towards higher statistical reliability. Using PLINK, we performed a permutation-based test for association of specific chromosomal regions with major depressive disorder. We discovered CNVs in the chromosomal region 16p11.2 that were significantly overrepresented in patients suffering from major depressive disorder and which are currently subject to validation experiments.

Different approaches to disentangle major psychoses: moving beyond categorical diagnoses

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Genome-wide association studies in the psychiatric disorders major depression, bipolar disorder, and schizophrenia so far resulted in first genome-wide significant findings. Still the majority of genetic variation contributing to these disorders remains undiscovered. Apart from limited sample size this is due to the heterogeneity of each of these disorders. One possibility to address this problem is refinement of the phenotype. Refined phenotypes are thought to be closer to genes and biological pathways than classical diagnoses thus leading to better association signals. This refinement is difficult however as it is not clear which phenotypes to choose. Novel approaches, looking particularly for complex phenotype-genotype interactions, are necessary. Here, we focus on the concept of reverse phenotyping to find such interactions by using genetic marker data to form the basis of new definitions of phenotypes. We systematically explore which phenotypic features (e.g. clinical characteristics, or environmental variables) or phenotype clusters show the strongest relationships with genetic vulnerability factors. As a first method, we used biclustering. The crucial feature here is the simultaneously clustering of individuals as well as their genotypic and phenotypic information. In detail, we applied the consecutive ones model (Oswald and Reinelt, 2004) and rather cHawk (Cheng and Church, 2000) to capture phenotype-genotype clusters. As a second method, we applied transactional analysis. Originally this data mining concept was used to identify meaningful purchase habits in large warehouse databases. For genetic studies, the goal is to identify phenotypic groupings that are distinguished by more deviant allele frequencies than are seen in traditional diagnostic categories. We have compared our methods through simulation and by application to data of 3 independent bipolar disorder studies. Methods and results of the reverse phenotyping approach will be presented.

MRNET TP7 - Molecular karyotyping of patients with mental retardation – Identification of regions containing candidate genes

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Molecular Karyotyping was performed in 156 patients with mental retardation using high resolution Agilent arrays (244k or 2x400k chips). For speeding up the analysis procedures we developed a software tool based on the open-source framework Grails (http://grails.org/). Validation was carried out by FISH analysis, customized array CGH, quantitative PCR, or quantitative genotyping. The clinical data sets of 188 patients including 24 patients with specific syndromes/malformations were imported into the MRNET database.
In 16 patients we found de novo deletions/duplications of which 5 have not been described to date: Deletion 1p36; 1q22; 1q25.2; 4q11-q13.3; 4q24; 6q27; Duplication 12q12; Deletion 16p11.2; Duplication 17q21.33; 17q25.3; 18q22.1; and Deletion 21q22.11. We identified a compound heterozygous deletion on 15q13.3 in one patient with severe encephalopathy and seizures and contributed to the identification of the possible candidate gene CHRNA7 in collaboration with MRNET TP9 (Heidelberg).
Other candidate loci we are currently investigating include one locus with four genes for frontotemporoparietal polymicrogyria and one gene for perisylvian polymicrogyria, one candidate gene for Shprintzen-Goldberg syndrome/Loeys Dietz syndrome type 1 and one gene for microcephaly.
About 80 patients showed familial copy number variants of unclear relevance. We are interested in studying 42 candidate genes in these regions.
Finally, we have started to use Next Generation Sequencing of the exome in one family with autosomal dominant MR and plan on analyzing 7 families with unaffected parents and two affected siblings. We will also continue to employ Sanger DNA Sequencing for selected candidate genes in close cooperation with the other MRNET centres.

Mental retardation, cleft palata and unusual face: A new autosomal dominant syndrome?

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Cleft palate as well as congenital ptosis and also a combination of both are common clinical findings in syndromal diseases. For example, an LMD search with both features results in a list of 93 conditions, all of them associated with additional specific minor and/or major abnormalities.
We describe a family (mother and daughter) with developmental delay, cleft palate, and distinct facial features: thick hair, low hairline, flat midface, puffy eyes and increased distance between the alae nasi as well as small, low set, and posteriorly rotated ears. Extensive examination of the mother did not reveal any malformations of inner organs or of the nervous system. A skeletal survey showed a normal bone anatomy except a mild pectus excavatum. Both, mother and daughter had a history of pronounced feeding difficulties (even after surgical correction of the cleft palate). Despite the distinct phenotype we could not establish a diagnosis or find a similar case in the literature. Recently, another unrelated male patient with similar facial features, developmental delay, and congenital malformations was referred to our clinic.
All analyses performed (Chromosome banding analysis, Molecular Karyotyping with 244A Agilent chip, sequencing of known genes in the RAS-MAPK pathway) were normal. Exome analysis using Next Generation Sequencing is in progress. The clinical findings of the three patients will be presented.

Search for mutations and pathogenic haplotypes in hemizygous genomic regions of patients with mental retardation (MR)

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Background: Molecular Karyotyping in a large number of mentally retarded children from healthy parents has revealed family-specific deletions which do not represent known CNVs. Such deletions could either be harmless familial variants or recessive mutations. If the latter is true, the affected child should carry an additional mutation on the homologous chromosome. Since autosomal recessive inheritance is supposed to account for nearly a quarter of all individuals with non-syndromic MR, the likelihood of finding a second mutation in children with an inherited deletion is substantial.
Experimental approach: Sanger-sequencing of coding exons of selected candidate genes. Criteria for gene selection: complete or partial deletion of one allele plus expression in the mammalian brain. Selected genes: SYT11, NRXN1, ARPP-21, HIST1H2AG, HIST1H2AH, HIST1H2BJ, HIST1H2BK, HIST1H4I, ANLN, EXOC4, LINGO2, VCL, ALDOA, DOC2A, ASPHD1, KCTD13, RAB37, and SULT4A1.
Results: Besides several known SNPs in various candidate genes we identified two unclassified variants in DOC2A (p.Gly48Ser) and LINGO2 (p.Arg369Gln), respectively. Since both variations affect the amino acid sequence, they may represent the recessive mutations we have been looking for.
Future work: LINGO2 belongs to a family of neuronal growth modulating proteins. Furthermore, down-regulation of the closely related LINGO1 in cell culture and knockout mice has been shown to be critical for CNS myelination. Thus LINGO2 may also be critical for mammalian brain development. As a very first step to elucidate a clinical relevance of LINGO2 mutations, we want to search for “second hits” in five additional children with MR and an (inherited) LINGO2 deletion. In parallel, we are going to quantify LINGO2 expression on cDNA level in MR-patients with a LINGO2 deletion, as well as in the corresponding healthy family members. Analogous experiments will be performed for DOC2A which is involved in neurotransmitter release and synaptic activity.

Mutations in MEF2C from the 5q14.3q15 Microdeletion Syndrome Region Are a Frequent Cause of Severe Mental Retardation and Diminish MECP2 and CDKL5 Expression

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The etiology of mental retardation remains elusive in the majority of cases. Microdeletions within chromosomal bands 5q14.3q15 were recently identified as a recurrent cause of severe mental retardation, epilepsy, muscular hypotonia, and variable minor anomalies. By molecular karyotyping we identified two novel 2.4- and 1.5-Mb microdeletions of this region in patients with a similar phenotype. Both deletions contained the MEF2C gene, which is located proximally to the previously defined smallest region of overlap. Nevertheless, due to its known role in neurogenesis, we considered MEF2C as a phenocritical candidate gene for the 5q14.3q15 microdeletion phenotype. We therefore performed mutational analysis in 362 patients with severe mental retardation and found two truncating and two missense de novo mutations in MEF2C, establishing defects in this transcription factor as a novel relatively frequent autosomal dominant cause of severe mental retardation accounting for as much as 1.1% of patients. In these patients we found diminished MECP2 and CDKL5 expression in vivo, and transcriptional reporter assays indicated that MEF2C mutations diminish synergistic transactivation of E-box promoters including that of MECP2 and CDKL5. We therefore conclude that the phenotypic overlap of patients with MEF2C mutations and atypical Rett syndrome is due to the involvement of a common pathway. Further studies including genomewide expression analyses to investigate the interaction between MEF2C and other genes are ongoing.

Microaberrations in 16p11.2 influence language acquisition and body height

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Microaberrations of chromosome 16p11.2 are associated with a recurrent syndrome of mental retardation and autism. Here we describe 20 more cases from Germany with microdeletion or microduplication 16p11.2.
Screening a cohort of 694 patients with mental retardation using a 6.0 Affymetrix SNP array platform and 234 patients with an Agilent 244A array we identified 13 patients harbouring the typical 593 kb deletion and 7 patients with the according duplication.
Segregation could be assessed in most cases. The deletion occurred de novo in 9 index patients and was inherited from a normal father and three affected mothers in the other cases. Five duplications were inherited while two duplications could not be assessed.
Both microdeletions and microduplications in 16p11.2 were found to be associated with mild to moderate mental retardation as well as behavioural problems, dysmorphic features and congenital anomalies. All patients showed a delayed language acquisition, especially microduplication patients (median age at first words 36 months compared to 19.5 months in microdeletion patients). High body measurements were observed in patients with microdeletion whereas low body measurements occurred in microduplication cases. As the phenotypical spectrum is not easily recognizable and overlaps with other microdeletion syndromes such as 15q13.3 microdeletion syndrome clinical diagnosis is hampered. Clinical variability might be due to other genetic aberrations that we observed in some of our patients. Two patients in addition had FGFR3 mutations and one patient had an additional 16p13 microduplication syndrome. Rare copy number variants that are suspected to be pathogenic were detected in further six patients.

A de novo t(5;14)(p15.33;q12) Translocation Associated with Severe Mental Retardation

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We report on a 5-year-old female patient diagnosed with microcephaly, partial agenesis of the corpus callosum, hypoplasia of the temporal lobe and a delayed myelination. The patient suffers from severe mental retardation and a pronounced developmental delay including speech impediment. Seizures were additionally diagnosed at the age of 15 months. The facial gestalt of the patient was considered to fit into the spectrum of Cri-du-Chat-syndrome. Cytogenetic analysis using high resolution GTG-banding revealed an apparently balanced de novo translocation described as t(5;14)(p15.33;q12). Using fluorescence in situ hybridization (FISH) mapping and subsequent "mini-FISH" refinement, a delineation of the breakpoint regions to 27.5 and 25.5 kb respectively could be achieved, but sequencing of a breakpoint-spanning long-template PCR product was not successful. Additional copy number variation studies using the Affymetrix 6.0 SNP platform were without pathological findings. Both chromosomal breaks were located in regions without annotated genes and the breakpoint on chromosome 5 corresponds to the mild mental retardation region of Cri-du-Chat syndrome, hence it is unlikely to explain the phenotype. SYBR-Green based expression analysis of selected genes from the breakpoint regions revealed a decreased expression of nine genes located in the affected region on chromosome 5 (SEC6L1, BDR9, NDUFS6, MRPL36, PDCD6, ZDHHC11, CEP72, POLS, TPPP). Interestingly, the chromosomal break on chromosome 14 was found to be located in a relatively gene poor region only 0.5 Mb downstream of FOXG1, which encodes a brain-specific transcription factor. FOXG1 is an important factor of brain development and was previously associated with autosomal dominant Rett-like syndrome. Due to the comparable severity of our patient´s phenotype we hypothesize the pathomechanism is based on FOXG1 haploinsufficiency. This hypothesis is under further investigation.

Shox2 mediates Tbx5 activity by regulating Bmp4 in the pacemaker region of the developing heart

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Heart formation requires a highly balanced network of transcriptional activation of genes. The homeodomain transcription factor Shox2 is essential for the formation of the sinoatrial valves and for the development of the pacemaking system. The elucidation of molecular mechanisms underlying the development of pacemaker tissue has gained clinical interest as defects in its patterning can be related to atrial arrhythmias. We have analysed putative targets of Shox2 and identified the Bmp4 gene as a direct target. Shox2 interacts directly with the Bmp4 promoter in ChIP assays and activates transcription in luciferase reporter assays. In addition, ectopic expression of Shox2 in Xenopus embryos stimulates transcription of the Bmp4 gene and silencing of Shox2 in cardiomyocytes leads to a reduction in Bmp4 expression. In $Tbx5^{del/+}$ mice, a model for Holt-Oram syndrome, and $Shox2^{-/-}$ mice we show that the T-box transcription factor Tbx5 is a regulator for Shox2 expression in the inflow tract and that Bmp4 is regulated by Shox2 in this compartment of the embryonic heart. In addition, we could show that Tbx5 acts cooperatively with Nkx2.5 to regulate Shox2 and Bmp4 expression. This work establishes a link between Tbx5, Shox2 and Bmp4 in the pacemaker region of the developing heart and thus contributes to the unravelling of the intricate interplay between the heart-specific transcriptional machinery and developmental signalling pathways.

Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation

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Using microarrays, we identified de novo copy number variations in the SHANK2 synaptic scaffolding gene in two unrelated individuals with autism-spectrum disorder (ASD) and mental retardation. DNA sequencing of SHANK2 in 396 individuals with ASD, 184 individuals with mental retardation and 659 unaffected individuals (controls) revealed additional variants that were specific to ASD and mental retardation cases, including a de novo nonsense mutation and seven rare inherited changes. Our findings further link common genes between ASD and intellectual disability.

Identification of FOXP1 deletions in three unrelated patients with mental retardation and significant speech and language deficits

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Mental retardation affects 2-3% of the population and shows a high heritability. Neurodevelopmental disorders that include pronounced impairment in language and speech skills occur less frequently. For most cases, the molecular basis of mental retardation with or without speech and language disorder is unknown due to the heterogeneity of underlying genetic factors. We have used molecular karyotyping on 1523 patients with mental retardation to detect copy number variations (CNVs) including deletions or duplications. These studies revealed three heterozygous overlapping deletions solely affecting the forkhead box P1 (FOXP1) gene. All three patients had moderate mental retardation and significant language and speech deficits. Since our results are consistent with a de novo occurrence of these deletions, we considered them as causal although we detected a single large deletion including FOXP1 and additional genes in 4104 ancestrally matched controls. These findings are of interest with regard to the structural and functional relationship between FOXP1 and FOXP2. Mutations in FOXP2 have been previously related to monogenic cases of developmental verbal dyspraxia. Both FOXP1 and FOXP2 are expressed in songbird and human brain regions that are important for the developmental processes that culminate in speech and language.

Homozygous Loss of CHRNA7 on Chromosome 15q13.3 Causes Severe Encephalopathy With Seizures and Hypotonia

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Since the introduction of array techniques small chromosomal imbalances have increasingly been recognised as a (possible) cause of mental retardation (MR) and other neurodevelopmental disorders. Recurrent deletions on 15q13.3 have been identified as a predisposition to mental retardation, epilepsy and psychiatric disease. We report compound heterozygous deletions on 15q13.3 in two unrelated patients with severe encephalopathy and seizures. Both patients have a recurrent 1.5 Mb deletion on one chromosome 15, and different-sized deletions (680 kb and 3.4 Mb, respectively) on the second chromosome, resulting in nullisomy for at least the small 680 kb segment. Our data support the hypothesis that CHRNA7, the gene for the a-subunit of a neuronal nicotinic acetylcholine receptor, within 15q13.3 conveys the phenotype of heterozygous and homozygous 15q13.3 deletions.

Cytogenetic and molecular karyotyping of 130 patients with mental retardation: The German Mental Retardation Network (MRNET) Subproject Münster

Presenting Author: Albrecht Röpke
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We present our results of cytogenetic analysis and molecular karyotyping (array CGH) of 130 patients with non-specific or syndromal mental retardation. The study was performed in conjunction with The German Mental Retardation Network.
Patients with Fragile X syndrome as well as other phenotypically noticeable syndromes were excluded. 130 patients were pre-diagnosed by conventional cytogenetic and/or subtelomeric fluorescence in situ hybridisation (FISH) analyses by standard techniques. In all patients, array CGH analyses using Agilent`s 244A and 400K microarray platforms were performed and the results were evaluated using the Database of Genomic Variants of known polymorphisms. Aberrations not listed in this database were validated by FISH or quantitative PCR in the patients and in their parents to distinguish between inherited and de novo aberration.
In our cohort of 130 patients we could identify chromosomal aberrations in 29 patients (22.3%) with altogether 34 de novo imbalances. Two of these patients demonstrated larger de novo balanced chromosomal translocations, detected by conventional cytogenetic analysis. Both patients did not show additional pathogenic CNV by array CGH. In six patients we were able to confirmed unbalanced subtelomeric rearrangements previously detected by FISH analyses. In further 21 patients, array CGH revealed 26 de novo aberrations including 10 gains and 16 losses. The de novo alterations varied in size from 53.0Kb to 7.9Mb.
Our results demonstrated that array CGH is a powerful method to identify small deletions and duplications in patients with mental retardation that were not detectable by standard cytogenetic and molecular cytogenetic techniques. Furthermore, we detect new candidate loci that are currently under further investigations. Sequence analyses in patients with inherited microdeletions or microduplications not described as polymorphism, are in progress to detect possible mutations in the non-deleted or non-duplicated alleles.

Cohen syndrome diagnosis using whole genome arrays

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Background: Cohen syndrome is a rare autosomal recessive disorder with a complex phenotype including psychomotor retardation, microcephaly, obesity with slender extremities, joint laxity, progressive chorioretinal dystrophy/ myopia, intermittent isolated neutropenia, a cheerful disposition, and characteristic facial features. The COH1 gene, which contains 62 exons, is so far the only gene known to be associated with Cohen syndrome. Point mutations, deletions and duplications have been described in this gene. Oligonucleotide arrays have reached a resolution which allows the detection of intragenic deletions and duplications especially in large genes such as COH1.
Results: We have analysed high density oligonucleotide array data from patients with unexplained mental retardation (n=1523) and normal controls (n=1612) for copy number variation (CNV) changes. We detected intragenic heterozygous deletions in the COH1 gene in three patients but no such changes in the controls. Subsequent sequencing of the COH1 gene revealed point mutations in the second allele in all three patients analysed.
Conclusion: Genome wide CNV screening with high density arrays provides a tool to detect intragenic deletions in the COH1 gene. We present an example how microarrays can be used to identify autosomal recessive syndromes and to extend the phenotypic and mutational spectrum of recessive disorders.

Mutations in GRIN2A and GRIN2B encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes and are a relatively frequent cause of mental retardation

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N-methyl-D-aspartate (NMDA) receptors are neurotransmitter-gated ion channels involved in regulation of synaptic function in the CNS. They are heterotetrameric assemblies of two glycine-binding NR1 and two glutamate-binding NR2 subunits. In two patients with mental retardation (MR), behavioral anomalies, and abnormal electroencephalogram we identified de novo chromosome translocations and a common breakpoint in 12p13.1, directly disrupting GRIN2B which encodes the NR2B subunit. Screening 468 patients with mild to severe MR for GRIN2B alterations identified heterozygous de novo mutations in four of them. Another translocation, t(16;17)(p13.2;q11.2), was found to affect the GRIN2A gene encoding the NR2A subunit and co-segregates in a family with epilepsy and variable degree of cognitive impairment. Sequencing GRIN2A in a cohort of 127 patients with idiopathic epilepsy and/or abnormal EEG and variable degree of MR identified two mutations, a heterozygous nonsense mutation in a three-generation family with mild MR and a history of childhood seizures and the heterozygous de novo missense mutation p.N615K in a girl with early-onset epileptic encephalopathy and severe developmental delay. A unique property of NMDA receptors is its voltage-dependent activation as a result of an ion channel block. We analyzed voltage dependence of NR1/NR2AN615K receptor currents and identified a voltage-independent ion flux. We conclude that loss-of-function mutations in GRIN2A are associated with a relatively mild phenotype, while the severe phenotype in the patient with p.N615K can be explained by a dominant-negative effect on NMDA receptor function.
Our findings suggest that disturbances in the neuronal electrophysiological balance during development result in variable neurological phenotypes, depending on which NR2 subunit of NMDA receptors is affected. While GRIN2A mutations seem to be mainly associated with epilepsy, GRIN2B mutations cause non-syndromic MR in about 1% of patients.

Partial NFIA gene deletion in a patient with mild intellectual deficiency

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NFIA is a transcription factor which controls the transition from neurogenesis to gliogenesis in the developing spinal cord and Nfia knockout mice show perinatal lethality, hydrocephalus and agenesis of corpus callosum. Lu et al. (2007) suggested that NFIA haploinsufficiency causes a distinct brain and kidney malformation phenotype with agenesis of corpus callosum and developmental delay of unspecified severity as consistent features and variable other brain and spinal cord malformations, seizures and kidney anomalies in human. However, all 6 patients known in the literature in addition to NFIA haploinsufficiency have other genes interrupted or deleted with deletion sizes ranging from 2-12 Mb (Lu, 2007; Koehler, 2010). NFIA mutational analysis in a total of 219 patients with variable combinations of the observed malformation spectrum revealed no apparently pathogenic mutation.
We now report the first patient with a de novo microdeletion limited to the NFIA gene. This patient, a 12 years old girl, showed mild intellectual deficiency with macrocephaly and unspecific minor anomalies, but in contrast to the published cases normal MRI scans. Molecular karyotyping using a 6.0 GeneChip array showed a 128 kb deletion on chromosome 1p13.3. This in frame deletion removes exon 3 to 6 of the NFIA gene and thus destroys the nuclear localization signal. MLPA analyses with self-designed probes for the deleted region confirmed the deletion in the patient and excluded it in the parents, thus suggesting de novo origin. A deletion of NFIA was not observed in 667 control individuals. By sequencing the coding exons of all 4 isoforms in 190 patients with mild mental retardation and in 95 non-microcephalic patients with severe mental delay we detected no obvious mutation, but a few variants, which are currently under further investigation.
In summary we suggest that defects limited to the NFIA gene cause only a mild mental phenotype.

Homozygosity mapping in consanguineous Iranian families with autosomal recessive mental retardation identifies numerous single linkage intervals, more than 10 novel loci and several mutation hotspots

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There is reason to believe that autosomal recessive mental retardation (ARMR) is more common than X-linked MR, but it has so far received considerably less attention. This is partly due to small family sizes and low consanguinity rates in industrialized societies, both of which have so far hampered identification of causative gene defects. To shed more light on the causes of ARMR, we have set out in 2003 to perform systematic clinical and molecular studies in large consanguineous Iranian families with with non-syndromic ARMR (NS-ARMR). As reported previously (Najmabadi et al., Hum Genet. 2007, 121:43-8.), this led us to the identification of 12 novel ARMR loci, eight of which showed a significant LOD score (OMIM: MRT 5-12). In the meantime, others and we have found causative gene defects in two of these intervals. Here we report on the results we obtained by tripling the size of our family cohort, which led to the identification of at least 25 additional unrelated families with NS-ARMR and single linkage intervals. More than ten of these define novel loci for this condition. In our total cohort clustering of single linkage intervals was observed at 1p34, 4q27, 5p15, 9q34, 11p11-q13 and 19q13, respectively. Five of these clusters consist of two significantly overlapping linkage intervals, and on chr 1p34, two single linkage intervals coincide with the previously described MRT12 locus. By Monte Carlo simulation we could show that the probability for this distribution to be due to chance is only 1.15×10^{-5}. Thus, in contrast with our previous conclusions, these novel data indicate that molecular causes of NS-ARMR with increased frequency do exist, and in the Iranian population, the most

frequent ones may well account for several percent of the patients. Mutation screening to elucidate the underlying gene defects is ongoing and the outcome of our project will broaden the basis for genetic counselling and provide insight into the molecular basis of brain function.

Distribution of host and viral micro RNAs in MCMV infection

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The goal of viral infection is to reprogram the host cell to optimize viral replication. As part of this process, viral miRNAs may compete for or modify distribution of components of the miRNA/siRNA pathway as well as regulate cellular targets.
Mouse Cytomegalovirus (MCMV), a ß-Herpesvirus, was used as a model system to answer the following questions:
How do viral miRNAs modify the host cell small RNA biogenesis system?
Is there evidence for sorting of viral small RNAs into specific Ago-proteins?
Is there a cellular small RNA response against the viral genome?
Deep sequencing analysis of MCMV infected cells showed app. 10% of all detected miRNAs to be of viral origin both in total RNA. All 18 known MCMV miRNAs with the exception of miR-m88-1* were present and for MCMV miR-m01-1 an additional miRNA, designated as miR-m01-1-5p, was found. Its presence was confirmed by qPCR. Deep sequencing after RISC IP with antibodies specific for either Ago1 or Ago2 showed that all MCMV miRNAs are loaded into both RISC complexes with most of them having slightly more reads in the Ago2 IP. The ratio of MCMV to mouse miRNAs was similar to that found in total RNA. Viral miRNAs therefore do not appear have a global impact on mouse miRNAs.
A downregulation of the mouse miRNAs miR-26a and miR-27a as well as an upregulation of miR-7a could be observed by deep sequencing and was confirmed by qPCR and Northern Blot. Furthermore, a symmetric and continuous association of reads with entire MCMV genome was observed.

Systematic Analysis of Viral MicroRNA Targets in Cells Latently Infected with Human gamma-Herpesviruses by RISC Immunoprecipitation Assay.

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The mRNA targets of microRNAs (miRNAs) can be identified by immunoprecipitation of Argonaute (Ago) protein-containing RNA-induced silencing complexes (RISCs) followed by microarray analysis (RIP-Chip). Here we used Ago2-based RIP-Chip to identify transcripts targeted by Kaposi's sarcoma-associated herpesvirus (KSHV) miRNAs (n = 114), Epstein-Barr virus (EBV) miRNAs (n = 44) in six latently infected or stably transduced human B cell lines. For the viral miRNA targets we identified, predicted binding sites were predominantely overrepresented in target 3’UTRs. Of six KSHV miRNA targets chosen for validation, four showed regulation via their 3'UTR, while two showed regulation via binding sites within coding sequences. Two genes governing cellular transport processes (TOMM22 and IPO7) were confirmed to be targeted by EBV miRNAs. A significant number of viral miRNA targets were upregulated in infected cells, suggesting that viral miRNAs preferentially target cellular genes induced upon infection. Transcript half-life both of cellular and viral miRNA targets negatively correlated with recruitment to RISC complexes, indicating that RIP-Chip offers a quantitative estimate of miRNA function.

Reference: Doelken et al., Cell Host & Microbe, Volume 7, Issue 4, Pages 324-334

Interactions of Varicella Zoster Virus with the cellular miRNA machinery

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MicroRNAs (miRNAs) are approximately 22nt long, non-coding RNA molecules which regulate expression of target genes at a posttranscriptional level. A wide range of organisms including plants, mammals and viruses has been found to encode miRNAs. These small molecules play important roles in many cellular processes such as development, differentiation, apoptosis and tumorigenesis. Moreover, cellular miRNAs are thought to represent a defence mechanism against pathogens while viruses, e.g. herpesviruses, employ miRNAs to modulate the host cell in support of viral replication or persistence. Several herpesviral miRNAs are proposed to function in establishment or maintenance of latent infection. Varicella Zoster Virus (VZV), a member of the alphaherpesvirus subfamily, causes chickenpox and – upon reactivation – gives rise to shingles. Intriguingly, so far no VZV-derived miRNAs could be identified, suggesting that VZV might use cellular miRNAs to promote its own replication and spread. Alternatively, VZV might impair the cellular miRNA-mediated defence machinery by a counterdefence mechanism. In order to test these hypotheses, we set up a high-throughput screen aimed at identifying miRNAs with inhibitory or promoting effect on virus growth. Several candidates showing significant effects have been subjected to further analysis.

GenomeRNAi: A Database for Cell-based RNAi Phenotypes and Integration of Phenotypes Across Species

Presenting Author: Esther Schmidt
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RNA interference (RNAi) has emerged as a powerful tool to generate loss-of-function phenotypes in a variety of organisms. Combined with the sequence information of almost completely annotated genomes, RNAi technologies have opened new avenues to conduct systematic genetic screens for every annotated gene in the genome.
As increasingly large datasets of RNAi-induced phenotypes become available, an important challenge remains the systematic integration and annotation of functional information. Genome-wide RNAi screens have been performed in human, Drosophila melanogaster and C.elegans for a variety of phenotypes and several RNAi libraries have become available to assess phenotypes on a large scale. These screens were performed using different types of assays from visible phenotypes to focused transcriptional readouts and provide a rich data source for functional annotation across different species.
The GenomeRNAi database provides access to phenotypes obtained from cell-based screens in drosophila and human cells with image- or luminescence-based readouts as well as manually annotated phenotypes. It currently holds 97600 entries for human and 99700 for drosophila phenotypes from 136 published RNAi screens. In addition it contains sequence and efficiency data for 300000 and 118000 RNAi reagents for human and drosophila, respectively. The database can be searched by phenotype, by gene or by RNAi probe and is publicly accessible at http://www.genomernai.org. Our aim is comprehensive coverage of all published genome-wide RNAi screening data in GenomeRNAi. To this end we are currently developing an annotation guide and a curation strategy. An update on new features on the GenomeRNAi website will also be presented.

miRNA let-7 regulates cytokine expression in macrophages upon microbial challenge

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miRNAs have been reported to regulate innate immunity-related signaling. Studies so far mainly focused on the behavior of single miRNAs such as miR-155 or miR-146 upon challenge of immune-cells with purified bacterial antigens or extracellular bacteria such as Helicobacter pylori. However, a systematic screen for miRNAs associated with bacterial infection of immune cells such as macrophages has not been done so far. We applied deep sequencing to characterize the miRNA response of macrophages to Salmonella typhimurium infection. We observed induction of the miRNAs known to be regulated upon LPS-treatment (i.e. miR-146, miR-155 and miR-21), but also reduction of the levels of mature let-7 upon infection. Intriguingly, the miRNA response pattern was not influenced by the Salmonella type III secretions systems since infections carried out with deletion mutants lacking the corresponding genomic islands induced the same miRNA regulations as wild-type infection. This is surprising since Salmonella is well known to employ its secretion systems to interfere with various intracellular signaling pathways. All miRNA regulations could be recapitulated with purified lipopolysaccharide, whereas purified flagellin previously described as a strong inducer of systemic inflammation as well did not regulate miRNAs in macrophages. Luciferase reporters harbouring the 3’ UTRs of IL-6 and IL-10 were used to discover that reduction of let-7 levels upon infection directly influences the expression of cytokines with important roles in bacterial infections. Our data suggest that miRNAs are important players in the regulation of systemic innate immune response outcomes.

Biophysics of malaria parasite motility

Presenting Author: Friedrich Frischknecht
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Plasmodium sporozoites are the forms of malaria parasites transmitted to the mammalian host by an infected mosquito. During a mosquito bite they enter the skin where they migrate to find a blood vessel, which is then invaded to continue the journey to the liver. The ability of the parasites to migrate is essential for progression through the life cycle. The parasites move on substrates in an actin and myosin-dependent fashion. Our goal is to better understand the molecular and physical mechanisms of parasite adhesion, motility and differentiation in the host cell. To this end we combine the generation of transgenic parasites lacking specific genes or expressing fluorescent fusion proteins with the use of quantitative imaging and image-analysis methods. We will present some of our insights from cryo-electron tomography and quantitative light microscopy studies as well as the use of new material science approaches.

INTERREG 4 Syddanmark-Schleswig-K.E.R.N. Transnational research on the genetics of healthy ageing

Presenting Author: Rabea Kleindorp
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Human longevity is a polygenic phenotype with a genetic contribution of approximately 25-30%. The aim of this German-Danish cooperation is to identify genetic determinants that lead to a long life in good mental and physical health. The project is based on a close collaboration between the Institute of Clinical Molecular Biology at the Christian Albrechts University in Kiel and the Institute of Epidemiology at the University of Southern Denmark in Odense. Both institutes have already collected a large number of biological samples of long-lived individuals and younger controls. The Danish 1905 cohort study is a prospectively designed examination of the birth-cohort of the year 1905. The German case-control study sample consists of ~1600 long-lived individuals (LLI) and 1100 younger controls. The combination of both collections results in one of the largest data- and biobank of this kind worldwide (> 5000 long-lived individuals). The collaboration is based on two parts: 1) analysis of genome-wide SNP Chip data of Affymetrix 6.0 Chip (763 German LLI and 1085 younger controls) and Illumina Omni Express Chips analyzing >600 Danish individuals (92-93 years), and 2) comparison of mRNA expression profiles using transcriptome sequencing. This data will be obtained from 100 monozygotic and dizygotic Danish twins (83-92 years, controls: 57-59 years) and >50 German LLI (90+ years) and 50 controls (30-50 years). Expected results of this second part will be age-specific molecular patterns that reflect the progression of the ageing process. The findings of this collaboration may provide new targets for the diagnosis, prevention and pharmacological therapy of age-related diseases.

This project is co-financed by the INTERREG 4 A programme Syddanmark-Schlewig-K.E.R.N. by EU funds from the European Regional Development Fund.

A Genome-wide Association and Imputation Software Pipeline for The Identification of Shared Susceptibility Loci

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Genome-wide association studies (GWAS) and subsequent meta-analysis projects have been successful in identifying novel genetic susceptibility factors for several autoimmune and inflammatory diseases. Although many examples exist that different disorders share common genetic risk loci (e.g. IL23R in Crohn's disease, ulcerative colitis and psoriasis; NOD2 in Crohn's disease, sarcoidosis and leprosy), systematic and genome-wide approaches are scarce. We therefore developed a fast and flexible genome wide association and imputation software pipeline for an automated processing of genome-wide association data. Different and complementary analysis strategies are currently developed to identify common disease loci and to prioritize associated loci for follow-up studies: GWAS datasets will be combined via confirmed-marker-, joint-, ranking-, meta- and difference-analyses. The software pipeline is implemented in Python and R in an object-oriented style, makes use of the open source PLINK library and the BEAGLE software package, and runs on a Linux compute cluster with a batch processing system. Nearly all parameters, options and datasets can be combined in a flexible way. An attached comprehensive and fully automated plotting pipeline facilitates a fast evaluation of candidate loci (regional association plots) or summary statistics (IBS-, Manhattan-, and QQ-plots).

We have recently completed GWAS for various complex diseases. More than 8,000 controls and 16,000 cases for 15 distinct diseases were genotyped on Affymetrix or Illumina SNP arrays and were prepared for combined analyses. The project takes a systematic, genome-wide approach by studying an overlay of GWAS data sets from different diseases in clinically relevant combinations. Association analysis results for the combined analyses will be made publicly available through a UCSC custom track. The current status of the project will be presented at the conference and the software will be publicly released.

Genome-wide association study identifies a novel susceptibility locus for Psoriasis

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Psoriasis is a multifactorial skin disease that affects up to 3% of the Caucasian population. The most common form is psoriasis vulgaris (PsV) which is characterized by red, raised, scaly plaques that commonly occur on the elbows, knees, scalp and lower back. The first identified and now well-confirmed susceptibility locus is PSORS1 on chromosome 6 in the MHC-class1-region. In addition, part of the genetic susceptibility can be explained by SNPs at the established susceptibility loci IL12B, IL23R, IL23A, IL4/IL13, TNIP1 and TNFAIP3.

To identify additional psoriasis susceptibility loci, we performed a genome-wide association study of 2,339,118 SNPs in 472 PsV cases and 1146 controls from Germany with follow-up of

the 147 most significant SNPs in 2746 PsV cases and 4140 controls from three independent replication panels. We identified a genome-wide significant association at a gene on 6q21 that encodes a protein involved in IL-17 signaling and interacting with members of the Rel/NF-κB transcription factor family ($P=7.31\times10^{-9}$). We genotyped two SNPs at this locus in two additional replication panels which further corroborated the association (combined discovery and replication panels: 6487 cases and 8037 controls; $Pcomb.=2.36\times10^{-10}$ for intronic SNP and $Pcomb.=1.24\times10^{-16}$ for missense SNP). Logistic regression analysis revealed that the associations at this gene could be fully accounted for by the missense SNP in exon 2. In a subset of German cases and controls (993 cases and 2277 controls) for which HLA-Cw6 status was available, we tested for the presence of a statistical interaction between HLA-Cw6 carriership and the missense SNP but found no evidence for it (P=0.77). About 15% of psoriasis cases develop psoriatic arthritis (PsA). We compared all PsA cases within our combined sample (1919 cases) versus 1919 randomly selected PsV cases and obtained no significant difference (P=0.13), suggesting that our locus represents a shared susceptibility for PsV and PsA.

Identification of common genetic susceptibility loci in three chronic inflammatory barrier diseases through a "phenotype overlap analysis" (PoA)

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Atopic dermatitis (AD), Asthma (Ast) and Inflammatory Bowel Disease (IBD) are chronic inflammatory barrier diseases, which are characterized by epithelial barrier deficiencies and aberrant immune reactions against common environmental antigens. In recent years many distinct genetic risk factors were identified, some of which appear to impact several of these diseases. In order to systematically screen for susceptibility loci relevant across several chronic-inflammatory phenotypes, we re-analyzed existing genome-wide data as to carry out a "phenotype overlap analysis" (PoA) using 5,299 population controls, 878 AD patients, 685 Ast patients and 1,525 IBD patients.
Chip data of three independent scans (AE, Ast and IBD) typed either with the Affymetrix 1000k GeneChip or with the Illumina 550k Chip were imputed using the HapMap Phase II haplotype data. Then different combinations of phenotypes were analyzed jointly (AE-Ast / AE-IBD / Ast-IBD / AE-Ast-IBD). After applying conventional quality control criteria (minor allele frequency >1%, Hardy-Weinberg equilibrium in controls pHWE =0.001 and SNP call rate >95%) ~2.5 Mio SNPs remained for analysis per combination. For the selection of the replication markers, different criteria were applied. SNPs were selected if they had shown a nominally significant association (p =0.05) and an effect size into the same direction in the single analyses, as well as a lower p-value in the combined analysis as compared to the single analyses. All markers fulfilling these criteria were plotted and these plots were checked visually. Subsequently from each combination a set of SNPs was selected. Replication typing of these marker sets in an independent replication panel of 1,500 controls and different case cohorts has been started.

Micro-RNA Signatures in Response to Proinflammatory Stimuli in Primary Human Monocytes

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Micro RNAs (miRNAs) are short, non-coding RNAs that regulate gene expression post-transcriptionally. Several studies have demonstrated the relevance of miRNAs for a wide range of cellular mechanisms, however, the current knowledge on how miRNAs respond to relevant external stimuli, e.g. in disease scenarios is very limited. Here, we quantified the levels of 330 miRNAs upon stimulation with a panel of pro-inflammatory factors (flagellin, diacylated lipopeptide, Listeria monocytogenes, lipopolysaccharide, muramyl-dipeptide and tumor necrosis factor alpha) in primary human monocytes (n=4 individuals) at different timepoints (0h, 1h, 2h, 4h) using quantitative real time PCR. As a result, we found distinct miRNA response clusters for each stimulus used. Our findings on highly co-regulated clusters of miRNAs support the hypothesis, that miRNAs act in functional groups. Interestingly, these functional groups do not reflect the genomic origin of the miRNA. A target prediction analysis for the significantly differentially regulated miRNAs (n=61) indicates that a large proportion of target genes of the miRNAs are associated to processes regulating mRNA transcription. Additionally, we identified potential target genes of three exemplary miRNAs which were part of the response clusters by transfecting cell lines with the corresponding pre- or anti-miRNAs, many of which were known to be involved in pathways associated to the previously used stimuli. Controlling and fine-tuning inflammatory processes is one of the key elements to balance the host organism's response between appropriate defense and excessive immune reaction, which may be harmful. This comprehensive assessment of the miRNome demonstrates for the first time that response signatures of miRNAs in primary human monocytes to pro-inflammatory stimuli may provide a regulatory network to adjust the inflammatory process, while further research may lead to new concepts for anti-inflammatory or anti-allergic therapies.

Copy number variant in the human beta-defensin gene cluster (8p23) is not associated with atopic dermatitis

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Atopic dermatitis (eczema) is a common chronic inflammatory skin disease affecting 10 – 20 % of children in the industrialized countries. Atopic dermatitis is a complex disease arising from multiple genetic and environmental factors interacting to determine disease susceptibility. Affected individuals often suffer from recurrent bacterial skin infections, especially infections with Staphylococcus aureus, pointing to a defect in the defense mechanisms of the skin in atopic dermatitis patients. Beta-defensins are small cationic molecules of the innate immune system that act as a first line of defense against many infectious agents. Recently, a common copy number polymorphism of the beta-defensin gene cluster on 8p23 was identified. Differences in copy numbers of the human beta-defensin gene DEFB4 were shown to be associated with other chronic inflammatory disorders including psoriasis and Crohn's disease. Here, we investigated the relationship between DEFB4 gene copy number and the susceptibility to atopic dermatitis. In this study we determined the number of DEFB4 copies in a group of 750 German children with atopic dermatitis and 750 German control individuals using the paralog ratio test, and tested for association with the Mann-Whitney-U-Test. We found no significant association of the DEFB4 copy number variant with atopic dermatitis. The power to detect an association with a reduced number of DEFB4 copies in this study group was approximately 90%, assuming a disease prevalence of 10%, a genotype relative risk of 1.25 and an allele frequency of the disease allele of 0.248.
We conclude that the DEFB4 copy number variant does not play a major role in determining susceptibility to atopic dermatitis.

Resequencing of 10 susceptibility loci in 56 individuals using Next Generation Sequencing based on Long Range PCR enrichment

Presenting Author: Sandra May
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One of the key applications of Next Generation Sequencing is to study the genetic variation between healthy individuals and patients using whole – genome or targeted resequencing. The perhaps best-established approach for high throughput population-based sequencing of targeted intervals in the human genome is to amplify the regions of interest using long-range PCR followed by Next Generation sequencing.
In our current project we resequenced with the SOLiD platform 10 validated disease susceptibility loci in 56 individuals (30 cases, 20 controls and 6 HapMap control samples). In order to enrich the samples for genetic diversity at the locus of interest, haplotype analyses were carried out using dense genotyping data from available genome-wide association scans. Cases and healthy controls carrying the associated risk haplotypes were preferentially selected and individuals with the remaining haplotypes were used for filling the pools. This means that patient sets that are resequenced vary between targets so we obtained full sequence variation – i.e. in coding and non-coding genomic sequence - and individual haplotypes for these 10 disease-associated genes. For these ten loci, more than 5500 long-range PCR amplicons have been successfully generated. This large number requires a high degree of automation and logistics. On the conference, we will show first results of our sequencing data and we will give a demonstration of the employed workflow.

Genomewide search for imprinting and maternal effects in eczema

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Eczema is a frequent inflammatory skin disease with complex etiology. Epidemiological studies have shown that children of eczema-affected mothers have a higher risk to develop the disease than children of eczema-affected fathers. This parent-of-origin (POO) effect may be due to genomic imprinting, feto-maternal interaction during pregnancy/lactation (maternal effect), or to maternal inheritance of the mitochondrial genome.
We aimed to detect POO effects in eczema by analyzing 270 complete nuclear families with eczema, from which genome-wide high-density array SNP data is available (Esparza-Gordillo et al. 2009). We have analyzed the genotype data according to the method of Weinberg (Weinberg, 1999). This method classifies families by mating types regarding the number of alleles carried by the father, mother and sibs and carries out a logistic regression over strata of the mating types containing information on imprinting and maternal effects. The independent imprinting and maternal effects can then be identified by appropriate likelihood ratio tests.
Additionally, we have used gene expression arrays to quantify the total mRNA levels in peripheral blood mononuclear cells (PBMCs) in 47 families. We are currently aiming to find POO effects at the expression level by detecting expression differences among reciprocal heterozygotes with a regression model which includes a main allelic effect and an imprinting effect (Belonogova et al. 2009).
Based on the genomewide scan for POO effects in eczema we will select 100 SNPs for replication in a set of 1200 independent complete nuclear families with eczema. We will prioritize hits which simultaneously show an imprinted pattern on disease risk and on gene expression or those located in imprinted regions. This is the first study to date performing a genome-wide POO association analysis in eczema.

Keywords: Eczema, parent-of-origin, genomic imprinting, maternal effect, genome wide association, imprinted expression.

The Population Risk of a Common Variant on Chromosome 11q13 (rs7927894) on Childhood Eczema, Asthma, and Hay Fever

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In a genome-wide association study, a common variant on chromosome 11q13.5 (rs7927894[T]) has been identified as a susceptibility locus for eczema. We aimed to analyze the effect of this risk variant on various allergic phenotypes, and to determine its impact on the population level in over 9,300 individuals of the ALSPAC birth cohort.
We replicated the effect of rs7927894[T] on eczema in the prospectively evaluated ALSPAC cohort (OR=1.17; 95% CI, 1.08-1.28; $p=4.9x10^{-5}$) and demonstrate that the main effect was due to the subgroup of individuals with allergic sensitization (atopic eczema). In addition, we detected an association of the risk allele with allergic asthma and hay fever, yielding the strongest effects on the combined phenotypes allergic asthma plus eczema (OR=1.50; 95% CI, 1.20-1.88; $p=3.7x10^{-4}$) and hay fever plus eczema (OR=1.40; 95% CI, 1.19-1.65; $p=7.6x10^{-5}$). The estimated population attributable risk fractions for eczema, associated allergic asthma or hay fever were 9.3%, 24.9%, and 23.5% respectively.
The rs7927894 risk allele confers a moderate individual risk for eczema, but carries a substantial effect on concomitant eczema and allergic airways disease on the population level. The association with eczema, allergic asthma, and hay fever may point to a joint molecular mechanism underlying allergic diseases.

Genetic variants in Toll like receptor pathway genes influence asthma

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Background: Toll like receptors (TLR) respond to pathogens associated molecular patterns by activating a complex intracellular signaling cascade. Common genetic variants in TLR are associated with atopic and allergic conditions, but very little is known about the relevance of polymorphisms in TLR regulatory and signaling molecules inside the cell. We systematically investigated the association of genetic variation in intracellular TLR signaling on asthma development using GWAS and regenotyping.

Methods: We identified genes involved in TLR signaling pathway by systematic literature and database search. Polymorphisms in these genes were extracted from HapmapII) database. Association with asthma was investigated by statistical analysis using gPLINK software package, on our GWAS dataset (comprising of 681 controls and 718 asthamtics) comprising 317,00 genotypes and 1 million imputed datapoints. Ranking system was applied to prioritize the associated genes and mapped to TLR signalling network. In-silico analysis for putative functional relevance of the associated SNPs was performed.

Results: We identified 48 genes harbouring 1723 polymorphism with minor allele frequency of >5%, involved in the TLR signaling pathway. This genetic information could be covered by analysing 493 tagging polymorphisms. Twenty two out of 48 investigated genes showed significant association with asthma with p-value <0.05. By functional pathway mapping of the top ranked genes, we identified two clusters in TLR signalling network: MAPK pathway genes such as ERKs and MKKs, and TLR regulatory genes like IL1RL1. In-silico analyses of the associated SNPs revealed that 185 polymorphisms in TLR pathway could putatively affect

respective gene function (e.g. by alternate splicing, transcription factor binding).
Conclusion: Variation in TLR pathway genes are associated with asthma and map to two distinct functional clusters in the TLR signaling network.

Gene polymorphisms in the heme degradation pathway and outcome of severe human sepsis

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Heme oxygenase (HO) breaks down heme yielding equimolar amounts of biliverdin, iron, and carbon monoxide (CO). Among the isoenzymes cloned to date, only heme oxygenase-1 (HMOX1) can be induced by panoply of stimuli linked by their ability to provoke oxidative stress. Heme degradation products deeply affect the stress response and manipulating the pathway confers protection against tissue injury in rodent models. Regulatory elements present in human heme oxygenase 1 (HMOX1) and biliverdin reductase A/B (BLVRA/B) genes might impact outcome in severe human sepsis.
To test whether a highly polymorphic (GT)n microsatellite within the HMOX1 locus and several single nucleotide polymorphisms in HMOX1 and BLVRA/B genes are associated with outcome and morbidity in severe sepsis.

534 patients with severe sepsis/septic shock were genotyped for SNPs and the microsatellite. Using database mining, DNA amplification, cloning and sequencing techniques the HMOX1 gene structure was reevaluated.

Based on mean-SOFA scores patients homozygous for the rs2071746 A-allele or medium length (GT)n microsatellites (27-33 units) of HMOX1 showed higher degree of organ dysfunction as well as higher 28day and 90day mortality rates (mean and 95% CI: AA 44.4% (36.6-52.3), TT 32.3% (25.7-38.9), A/T 32.9% (21.9-43.9) and s/s: 32.7% (19.9-45.4), s/m: 30.4% (23.7-37.1), s/l: 25.0% (3.8-46.2), m/m: 44.5% (36.7-52.3), m/l: 40.0% (15.2-64.8)) compared to the other genotypes. SNPs within BLVRA/B showed no association with sepsis related organ dysfunction. A novel HMOX1 alternative first exon upstream of the currently annotated exon1 and alternative splice variants, that might be influenced by the length of the (GT)n repeat, were observed.

Conclusions: Short (GT)n repeats, which are in linkage disequilibrium with the T-allele of rs2071746 in HMOX1, are strongly associated with favorable outcome. Remarkably, the protective effect becomes apparent only in the postacute phase of sepsis.

Quantifying the effect of sequence variation on regulatory interactions.

Presenting Author: Thomas Manke
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The increasing amount of sequence data provides new opportunities and challenges to derive mechanistic models that can link sequence variations to phenotypic diversity. Here we introduce a new computational framework to suggest possible consequences of sequence variations on regulatory networks. Our method, called sTRAP, analyses variations in the DNA sequence and predicts quantitative changes to the binding strength of any transcription factor for which there is a binding model. We have tested the method against a set of known associations between SNPs and their regulatory consequences.
Our predictions are robust with respect to different parameters and model assumptions.Importantly we set an objective and quantifiable benchmark against which future improvements can be compared. Given the good performance of our method, we developed a publicly available tool which can serve as an important starting point for routine analysis of disease-associated sequence regions.

Genome-wide meta-analysis identifies 70 loci associated with platelet count and platelet volume in the HaemGen consortium

Presenting Author: Christian Gieger
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Platelet count (PLT) and mean platelet volume (MPV) are highly heritable quantitative traits that are widely used in the clinic. MPV has been associated in epidemiological studies with an increased risk of myocardial infarction and stroke. In a previous meta-analysis encompassing about 5,000 individuals we identified 12 and 3 loci associated with MPV and PLT, together accounting for approximately 8 percent of phenotypic variation in MPV. In a subsequent analysis we showed that one of the PLT loci is associated with coronary artery disease and myocardial infarction. Here we extend such previous analysis to up to 23 world-wide cohorts, including over 50,000 (PLT) and 20,000 (MPV) samples. We discovered 70 independent loci reaching genome-wide significance ($P<=5x10^{-8}$). Of these, 15 were described previously and 55 are novel. Some of the top loci found in our present analyses are rs1354034 in ARHGEF3 and rs3184504 in SH2B3 and rs10914144 in DNM3. The further characterizations of the loci were carried out including pleiotropy in the haematopoietic pathway. We also did a network analysis of the genes associated with the lead loci. Finally, we supplemented our work in populations of European ancestry by extensive inter-ethnic comparisons. Taken together this meta-analysis with its follow-up analyses constitutes the first comprehensive picture of the genetic architecture of platelet count and volume.

Novel association to the proprotein convertase PCSK7 alongside HFE and TMPRSS6 gene loci revealed by analysing soluble transferrin receptor (sTfR) levels

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Serum iron parameters indicate the availability of and the demand for iron. This is the first meta-analysis of genome-wide association studies (GWAs) on the soluble transferrin receptor (sTfR). This parameter seems to be a more reliable index of iron deficiency in anemia, compared to other serum iron parameter such as ferritin which may be disproportionately influenced by inflammation or neoplasia. The meta-analysis of five GWAs on sTfR and ferritin serum levels revealed a novel association to the PCSK7 locus on chromosome 11. Additionally, two genes, TMPRSS6 and HFE, which are known to be associated with iron parameters such as iron, ferritin or transferrin, have also been found to be associated with sTfR. The association with the PCSK7 locus was the most significant (rs236918, $p = 1.1 \times 10^{-27}$) suggesting that proprotein convertase 7, the gene product of PCSK7, is involved in sTfR generation and/or iron homeostasis.

Common variants at ten genomic loci influence hemoglobin A1C levels via glycemic and non-glycemic pathways

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Glycated hemoglobin (HbA(1C)), used to monitor diabetes, is influenced by average glycemia over 2-3 months. Genetic factors affecting expression, turnover and abnormal glycation of hemoglobin could also be associated with increased levels of HbA(1C). It is uncertain to what extent such genetic variation influences diabetes classification based on HbA(1C) levels. We studied associations with HbA(1C) in up to 46,368 non-diabetic adults of European descent. from 23 genome-wide association studies (GWAS) and 8 cohorts with de novo genotyped single nucleotide polymorphisms (SNPs). We estimated the global effect of HbA(1C) loci using a multi-locus risk score, and used net reclassification to estimate genetic effects on diabetes screening. Ten loci reached genome-wide significant association with HbA(1C), including six new loci near FN3K (lead SNP/P-value, rs1046896/P=1.6×10^{-26}), HFE (rs1800562/P=2.6×10^{-20}), TMPRSS6 (rs855791/P=2.7×10^{-14}), ANK1 (rs4737009/P=6.1×10^{-12}), SPTA1 (rs2779116/P=2.8×10^{-9}) and ATP11A/TUBGCP3 (rs7998202/P=5.2×10^{-9}), and four known HbA(1C) loci: HK1 (rs16926246/P=3.1×10^{-54}), MTNR1B (rs1387153/P=4.0×10^{-11}), GCK (rs1799884/P=1.5×10^{-20}) and G6PC2/ABCB11 (rs552976/P=8.2×10^{-18}). We show that associations with HbA(1C) are partly a function of hyperglycemia associated with three of the ten loci (GCK, G6PC2 and MTNR1B). The seven non-glycemic loci accounted for a 0.19 (% HbA(1C)) difference between the extreme 10% tails of the risk score, and would reclassify ~2% of a general white population screened for diabetes with HbA(1C). GWAS identified ten genetic loci reproducibly associated with HbA(1C). Six are novel and seven map to loci where rarer variants cause hereditary anemias and iron storage disorders. Common variants at these loci likely influence HbA(1C) levels via erythrocyte biology, and confer a small but detectable reclassification of diabetes classification by HbA(1C).

Efficient whole-genome DNA methylation analysis of the Human Reference Genome (HuRef)

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Aberrant DNA methylation is characteristic of many cancers and differences in methylation have been observed in a wide variety of genomic contexts; both within “classic” promoter-associated CpG islands and in distal, non-CpG island regions. Establishing a method to broadly and efficiently survey DNA methylation patterns genome-wide is the objective of the work presented. The method combines the power of methyl-CpG binding domain (MBD) proteins to sensitively and selectively bind methylated DNA sequences with the coverage, precision, and accuracy provided by high-throughput sequencing. Notably, MBD-affinity capture can also be used to sub-fractionate genomic DNA based on its average methyl-CpG content. Human Reference Genome (HuRef) DNA was enriched and salt-fractionated with a commercial MBD-based affinity reagent and high-throughput sequencing libraries, both bisulfite converted and unconverted, were prepared from each of the three fractions. The libraries were sequenced using error correcting codes and paired-end technology that yielded 75 bp read-lengths from one end and 30 bp read-lengths from the opposite end on a SOLiD 4 System. Peak analysis of the distribution of mapped unconverted reads permitted discovery of thousands of locations of putative methylation in different genomic locus classes; low salt fractions were depleted of CpG islands and enriched for exons while the highest salt fraction was enriched for CpG islands, exons, and promoter regions. Methylation at a large number of these positions was confirmed by bisulfite-sequencing of the same libraries. We conclude that such enrichment and fractionation, when coupled to high-throughput sequencing with or without bisulfite conversion, can be used to efficiently survey the majority of DNA methylation marks within samples of genomic DNA and to discover genomic loci of differential methylation. This method and reference dataset are intended to provide a tool for large-scale methylation studies.

Poster Presentation Abstracts

Symposium III

Animal, Cellular & Tissue Models

Differential protein expression analysis of hypoxia-exposed NDRG1 knock-down glioma cells in sublethal hypoxia using proteomics techniques

Presenting Author: Tore Kempf
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Tumor hypoxia is a central issue in glioblastoma, the most common type of primary malignant brain tumor in adults. Adaption of the cellular machinery to hypoxic stress allows glioblastoma cells to survive and develop resistance to treatment. In a comparative proteomics analysis screening for candidate proteins underlying hypoxic signaling using 2D gel electrophoresis and mass spectrometry, we identified and validated N-myc downstream regulated gene 1 protein (NDRG1/CAP43) as being robustly up-regulated with sublethal hypoxia in various human malignant glioma cell lines. Aiming to learn more about the functional relevance of NDRG1 in tumor hypoxia, we performed 2D Fluorescence Difference Gel Electrophoresis (2D DIGE) analyses in 4 independent NDRG1 knockdown LNT-229 glioma cell clones following 72 hours of hypoxic treatment. Scramble-transfected LNT-229 cells served as controls. Computer-assisted image analysis of 2-D DIGE gels allowed to detect differentially expressed proteins. Protein spots with $p < 0.05$ in t-test and regulations between < -1.20 and > 1.20 were localized on a preparative gel and identified by mass spectrometry. NDRG1 was found down-regulated in all 4 NDRG1 knockdown clones by factors between 2.5 and 4.8. Altogether, 78 proteins were found down-regulated whereas 93 proteins were found up-regulated. Based on Ingenuity Pathway Analysis, most of the deregulated proteins were assigned to five cellular networks: „DNA Replication, Recombination, and Repair, Cancer, Cell Death“, „Organismal Injury and Abnormalities, Cell-To-Cell Signaling and Interaction, Hematological System Development and Function“, „Organismal Injury and Abnormalities, Cell Death, Cellular Movement“, „Cellular Development, Hematological System Development and Function, Hematopoiesis“, and „Cellular Development, Cellular Growth and Proliferation, Cancer“. In summary, our data provide further information on NDRG1-dependent hypoxic signaling in glioblastoma.

Co-stimulatory protein 4IgB7H3 drives the malignant phenotype of glioblastoma by mediating immune escape and invasiveness

Presenting Author: Philipp-Niclas Pfenning
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Glioblastoma, the most common and aggressive primary brain tumor in human adults, is armed with effective immunosuppressive properties resulting in an impaired recognition and ineffective attack of tumor cells by the immune system. In addition, extensive and diffuse invasion of tumor cells into the surrounding brain tissue limits the efficacy of surgical resection and radiotherapy. In this regard, recent work points out the role of 4IgB7H3, a member of the B7-family of costimulatory proteins, in conveying immunosuppression and enforced invasiveness in a variety of tumor entities, associated with poor prognosis.
In order to characterize the immune phenotype of glioblastoma we show that 4IgB7H3 is not only expressed in glioma cell lines and glioma initiating cells, but also in human glioma tissue samples positively correlating with the tumor grade. 4IgB7H3 expression by tumor and endothelial cells correlates with the malignancy grade of gliomas. Furthermore, a soluble form of 4IgB7H3 could be identified in the supernatant of glioma cells. Both, intracellular and soluble 4IgB7H3 are functional having the ability to effectively suppress natural killer cell-mediated tumor cell lysis. In addition, we show by gene silencing that 4IgB7H3 and soluble 4IgB7H3 convey a proinvasive phenotype in glioma cells and glioma initiating cells in vitro. These proinvasive properties were confirmed in vivo with xenografted 4IgB7H3 gene silenced glioma initiating cells which invaded significantly less into the surrounding brain tissue compared with control cells. Due to the observed immunosuppressive and proinvasive function 4IgB7H3 may serve as a therapeutic target in the treatment of glioblastoma.

Development of isogenic cell lines stably overexpressing prostate cancer candidate genes

Presenting Author: Lukasz Kacprzyk
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Analyses of global changes in the gene expression patterns have recovered a large number of genes, which are deregulated in prostate cancer, compared to normal prostate tissue. It is now crucial to determine genes that truly contribute to tumor development and to separate them from non-causal anomalies in expression levels. To achieve that, we construct isogenic cell lines libraries derived from well-established prostate and prostate cancer cell lines and subject them to a range of functional assays.

Using technology for rapid generation of stably-transfected isogenic cell lines we have created the isogenic library derived from PC-3 cell line consisting of 78 recombinants. Each recombinant in the library overexpresses one prostate cancer candidate gene in a tet-inducible manner from the same genomic locus. In contrast to random knock-in strategies, our system ensures that readily interpretable phenotypes are obtained and thereby provides a highly standardized resource for functional gene analysis.

To identify modulators of cell proliferation we have developed a new proliferation assay that allows for screening of heterogeneous cell populations in a single flask. Briefly, the library consisting of 78 clones was divided into three parts, each composed of 25-30 individual clones. The resulting three isogenic cell line pools were grown for 5 passages. Changes in the composition of the pool after induction of the transgene expression were monitored by qPCR. Distinct patterns of cellular proliferation were detected in each of three pools with several well-described, cancer-relevant genes serving as controls. Data analysis revealed few genes with so far unknown function that act as classically understood oncogenes and tumor suppressor genes in PC-3 cells. This technology represents a generally applicable resource for the in vitro characterization of any given gene in a prostate cancer background.

Understanding the role of TMEM45B protein in prostate cancer progression

Presenting Author: Azeemudeen Hussain
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Introduction: TMEM45B is a conserved protein of unknown function expressed in human prostate tissue. The genomic localization of TMEM45B on human and mouse chromosomes are 11q24.3 and 9.9, respectively. TMEM45B is made of 275 amino acids (32kDa) and is predicted to have a transmembrane topology. It was first identified as a candidate gene strongly related to the prostate cancer (PCa) in our expression profiling analysis comparing clinical prostate cancer to normal prostate tissue.

Aim: The aim of this study is to establish the role of TMEM45B in normal and tumour prostate development.

Results: Quantitative real-time PCR experiment, comparing normal and tumour prostate tissues, revealed TMEM45B as a strong candidate specific to prostate tumour tissue with 12 fold increase in the expression level. We have found TMEM45B was overexpressed in human prostate cancer cell lines compared to normal prostate cells at transcript levels. Knockdown of TMEM45B using specific siRNAs, decreased the viability of PCa cells, indicating that TMEM45B is essential for the survival of PCa cells. In order to increase our understanding of the role of TMEM45B in PCa progression, we generated TMEM45B knockout mouse to study the involvement of TMEM45B in the progression of PCa. Quantitative real-time PCR showed TMEM45B to be expressed at high levels in mouse small intestine, colon, bladder, prostate and urinary bladder. Detailed characterization of TMEM45B knock out mice is currently in progress.

Conclusion: Given the elevated TMEM45B expression specifically in prostate tumour tissue and its contribution to the survival of PCa cell lines, TMEM45B could be a potential candidate for improved clinical management of prostate cancer.

Functional characterisation of cancer-relevant mutations using recombinant cancer cell line technology

Presenting Author: Mark Laible
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Cancer is the major cause of death in the industrialised world and germline and somatic mutations are widely accepted as the major drivers of this disease. In recent years a series of genetic screens have revealed a large number of genes and gene mutations associated with different types and stages of cancer that found their entry into comprehensive databases (e.g. COSMIC). The functional consequences for some of these mutations have been elucidated and even allow predictions of a patient's response to some anti cancer drugs. However, for most of the cancer associated gene mutations, the detailed functional consequences remain unknown.
In order to analyse molecular changes associated with potentially cancer-relevant mutations, we developed a powerful recombination-based system to generate libraries of stably transfected cell lines. By inserting genes of interest into a pre-defined locus within the cellular genome, we are able to generate highly standardized series of isogenic cell lines. Within the NGFNplus project IG-Mutanom, we use this technology and so far have constructed a breast and prostate cancer cell line library each consisting of ~80 recombinants inducibly overexpressing different mutated cancer genes and their wild type counterparts. In an initial screen of the breast cancer library, this set of candidate genes was analysed for effects on the viability of the tumour cells. The screen showed a number of genes and gene mutations which affected the cell viability. Some of the genes reducing cell growth were further analysed by FACS revealing the mode of growth inhibition. In addition to the cell based assays, the cell line libraries will also be used for analysing the consequences of the transgene expression on the protein- and mRNA level. By combining the results of the functional and gene expression analyses, we aim at deepening our understanding on the complex networks of signalling pathways associated with cancer development and progression.

Genome-wide DNA methylation alterations in the $Apc^{min/+}$ mouse model of intestinal cancer

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Mutations in the APC gene (adenomatosis polyposis coli) lead to a deregulation of the Wnt/beta-Catenin signalling pathway and are common in hereditary and sporadic cases of human colorectal cancer. The $Apc^{min/+}$ mouse represents a model of the early steps of intestinal cancer and we analyzed DNA-methylation in adenoma and normal mucosa of $Apc^{min/+}$ as well as normal mucosa of wild type mice by immunoprecipitation of methylated DNA followed by Illumina sequencing (MeDIP-seq), allowing in principle an unbiased genome wide analysis. About 20x106 single 36mer sequencing reads were generated per sample revealing methylation alterations occurring during adenoma formation in the $Apc^{min/+}$ mouse model. In addition, RNA-expression profiles were generated from the same samples and as expected a major deregulation of the Wnt-pathway was observed in adenoma of $Apc^{min/+}$ mice. To gain insight into the genetic-epigenetic aspects of tumour resistance, DNA-methylation of $Apc^{min/+}$ crosses with B6.PWD chromosome substitution strains showing a reduction in adenoma-load was investigated and compared to the methylation-profiles of the parental adenoma-prone $Apc^{min/+}$ mice. The work presents epigenetic and expression changes occurring during early steps of tumour formation in the $Apc^{min/+}$ mouse model.

Lack of CCR7 expression is rate limiting for lymphatic spread of pancreatic ductal adenocarcinoma

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The mechanisms controlling the spread of tumor cells via blood and lymphatic vessels and the formation of metastases at distant sites from the primary tumor are incompletely understood. Pancreatic ductal adenocarcinoma (PDAC) representing one of the most aggressive human neoplasms has a high potential to metastasize to lymph nodes and to the liver. The main goal of our studies was to define the significance of chemokines/chemokine receptors for the invasion of PDAC cells into lymph vessels and for the formation of metastases in lymph nodes. Chemokines and chemokine receptors known to play an important role in the development, homeostasis, and function of the immune system have also been shown to be involved in the extravasation and directed migration of tumor cells to sites of metastases in several tumor types. We studied the role of the chemokine receptor CCR7 known to be a key molecule in the homing of T cells from peripheral tissues to lymph nodes in the lymphatic spread of PDAC. CCR7 was expressed in the majority (10/12) of PDAC cell lines at mRNA level. FACS analysis revealed low constitutive CCR7 protein expression of monolayer cultures in most cell lines that could be upregulated up to 70 % depending on the PDAC cell line by culturing the cells as spheroids. In transwell migration assays Pt45P1 and BxPc3 PDAC cells that underwent spheroid culture to upregulate CCR7 showed enhanced migration towards CCL21 (the ligand of CCR7 which is expressed in secondary lymphoid organs). Orthotopically injected CCR7 transfected Pt45P1 cells gave rise to significantly larger tumors and showed higher rates of lymph node metastases than mock transfected cells in a nude mouse model. The analysis of immunofluorescence stains revealed a significant upregulation of CCL21 in peritumoral and intratumoral lymph vessels compared with lymph vessels in disease-free pancreata. In conclusion, CCR7 expression significantly promotes lymphatic spread in PDAC.

Insights from proteomic analysis of tumor cell secretomes: Release of EGFR via exosomes

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Aims: The secretome of cultured tumor cells is increasingly being appreciated as a rich source for biomarker discovery. The secretome in a proteomic sense is defined as the entirety of proteins released from the cells irrespective from the underlying molecular mechanism. We have previously shown, that - in addition to classical secretion and cell death – ectodomain shedding of membrane proteins and the release of microvesicles (exosomes) contribute significant fractions to the cellular secretome.
Methods: We now have established catalogues of secretome proteins derived from five human pancreatic carcinoma cell lines. Secretome proteins were resolved on 1D gradient gels, gel lanes were cut into 30 slices, each, and proteins in each gel slice were separately subjected to tryptic digestion and mass spectrometric analysis.
Results: This experimental approach produced partially overlapping catalogues, each of which comprises more than 1000 identified proteins. Through the assignment of the subcellular localization and the estimated experimental size we can define candidate proteins from these lists which are presumably released through ectodomain shedding or through the secretion of exosomes. The transmembrane EGFR was identified in several catalogues in slices which suggested the presence of the full-length protein as well as diverse processed isoforms.
Conclusion: Here we show, that intact EGFR is released from pancreatic carcinoma cells as a constituent of exosomes and is also released in a proteolytically processed form.

Parallelized Functional Characterization of Pancreatic Cancer Candidate Genes on Reverse Transfection Cell Microarrays

Presenting Author: Sandra Melchisedech
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Introduction:
In previous high-throughput analyses, we generated expression profiles of ~2000 candidate genes in primary tissues and model systems of pancreatic cancer. 80 candidate genes with a high probability of being biologically relevant or to be suited as target genes for diagnosis or therapy of pancreatic cancer were selected for further characterization.
This project aims to functionally analyze the 80 genes in transformed and non-transformed cell lines to select highly relevant candidates for further in-depth characterization. In parallelized assays, the candidates are overexpressed as well as downregulated in different cell lines and the effects on differentiation, proliferation, and apoptosis-resistance evaluated.
Methods:
The parallelized analyses are performed in the “reverse transfection” format. In this approach, overexpression- and knockdown-constructs are spotted in regular arrays together with transfection reagents on glass slides. Cells are then cultivated on the slides, incorporating and expressing the different constructs. Overexpression or knockdown effects are analysed by fluorescence-based assays.
Results:
Candidates and controls were expressed as fusion constructs with fluorescence proteins in carcinoma cell lines (PANC-1, SUIT007, EPras) as well as non-transformed cells (HEK293, EP4) in the presence or absence of serum. Several candidates were identified as target genes for serum stimulation (alteration of subcellular localisation) and/or showed influence on proliferation (Ki67-staining) or apoptosis resistance (cleaved caspase 3-assay). Analysis of the effects on cell differentiation (staining for E-Cadherin and Vimentin) as well as experiments in knockdown format are ongoing.
Conclusion:
’Reverse transfection arrays’ are an efficient technology to identify tumor-relevant genes and characterize important functional roles with high throughput.

The ectodomain of the giant cadherin Fat1 is released via ectodomain-shedding and is stable in conditioned media from cultured cells and in blood.

Presenting Author: Nathalie Wojtalewicz
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Aims: The secretome of cultured tumor cells designating the entirety of proteins released from cells is regarded as a promising source for biomarker discovery. A mass spectrometric analysis of the secretomes from five human pancreatic carcinoma cell lines and one control cell line previously indicated a huge amount of the cadherin fat1 in conditioned media. Here we analysed the mechanisms of fat1 release and the proteases implicated in its shedding.
Methods: The secretomes were screened for fat1 via Western blotting. To find the sheddases involved a broad-range inhibitor (Batimastat) and an ADAM10-specific inhibitor (GI254023X) were used. To confirm presumptive sheddases siRNA mediated knockdown of ADAM10 and MMP9 was done. Differential centrifugation methods were established to enrich the 460 kDa fat1 ectodomain in serum samples.
Results: Release of the fat1 ectodomain was shown in secretomes from human pancreatic (BxPc3) and colorectal carcinoma cell lines (SW948, SW620, CaCo2) via Western blotting with antibodies directed against an intracellular and an extracellular epitope. Using mass spectrometric analysis of distinct gel bands we were able to show, that the fat1 ectodomain is present in huge amounts. An analysis of the sheddases involved delivered a complex picture dependent on the respective cells. In SW948 and SW620 cells Batimastat and GI254023X suppressed fat1 shedding. In CaCo2 cells ADAM10 knockdown but not the chemical inhibitors showed an effect. Neither ADAM10 nor MMP9 are involved in fat1 shedding in BxPc3 cells. The fat1 ectodomain could also be shown in a serum sample form a CRC-patient although this was at the detection limit.
Conclusions: Here we show that fat1 ectodomain shedding in part is mediated via ADAM10 and MMP9. The ectodomain also appears to be produced in vivo, as it occurs in blood. Further analyses of its suitability as a biomarker candidate and unraveling its functional relevance will need to establish better tools for analysis.

Pathologic phenotype of the novel Fgf9Y162C mutant mouse is restricted to decreased vision and retarded lens growth

Presenting Author: Oliver Puk
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Background and aim:
Fibroblast growth factor (Fgf) signalling plays a crucial role in many developmental processes. Among the Fgf pathway ligands, Fgf9 has been demonstrated to participate in maturation of various organs and tissues including skeleton, testes, lung, heart, and eye. We recently established the novel Fgf9Y162C missense mutant Aca12 in a dominant N-ethyl-N-nitrosourea (ENU) screen for eye-size abnormalities. Aca12 was originally identified because of its significantly reduced lens thickness. In this study, we investigated eye development and vision of Aca12 in detail and examined putative effects on sex development or skeletogenesis.
Methods:
Prenatal lens core development was histologically investigated at E12.5 and E15.5. Postnatal secondary lens fiber growth of individual lenses was tracked by optical low coherence interferometry (OLCI) at different periods between four and 15 weeks of age. Furthermore, visual properties were investigated by electroretinography (ERG) and in the virtual drum.
Results:
Histological investigations showed that lens growth retardation starts during embryogenesis and continues after birth. Retinal histology as well as its basic functional characterization by ERG did not show any abnormality. However, the analysis of head-tracking response of the Aca12 mutants in a virtual drum indicated a gene-dosage dependent vision loss of almost 50%. In contrast to previously described Fgf9 mutants, Fgf9Y162C carriers were fully viable and did not reveal reduced body-size, male-to-female sexual reversal or skeletal malformations. Taken together, Fgf9Y162C is a novel neomorphic allele that initiates microphakia and reduced vision but does not affect development of testes and skeleton. Our data further point to a role of Fgf9 signalling in primary and secondary lens fiber cell growth.

The European Mouse Mutant Archive - EMMA

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The European Mouse Mutant Archive (EMMA) offers the worldwide scientific community a free archiving service for its mutant mouse lines and access to a wide range of disease models and other research tools. A full description of these services can be viewed on the EMMA website at http://www.emmanet.org.

The EMMA network is comprised of 14 partners who operate as the primary mouse repository in Europe and is funded by NGFN-Plus, institutional funding and the European Commission's FP7 Capacities Specific Programme.

EMMA's primary objectives are to establish and manage a unified repository for maintaining mouse mutations and to make them available to the scientific community. In addition to these core services, the consortium can generate germ-free (axenic) mice for its customers and also hosts courses in cryopreservation.

All applications for archiving and requests for mutant mouse strains are submitted through the EMMA website. Mouse strains submitted for archiving are evaluated by EMMA's external scientific committee. Once approval has been granted depositors are asked to send mice of breeding age to one of the EMMA partners for embryo or spermatozoa cryopreservation. Strains held under the EMMA umbrella can be provided as frozen materials or re-derived and shipped as live mice depending on the customer's needs. However, certain strains that are in high demand are maintained as breeding colonies to facilitate their rapid delivery. All animals supplied by EMMA are classified as SPF in accordance with the FELASA recommendations.

EMMA is a founding member of FIMRe (International Federation of Mouse Resources) and actively cooperates with other leading repositories like TJL and the MMRRC in the US and BRC RIKEN from Japan.

The effects of sample preparation on immuno-phenotyping

Presenting Author: Markus Scheerer
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We established a novel protocol for immunophenotyping of mouse lines by multi-color flow cytometry. Our method enables us, to use only small volumes (2 µl) of peripheral blood. We analysed the effect of erythrocyte lysis on the detected frequency of T cells, B cells, granulocytes and further subsets. We show, that erythrocyte lysis can effect the detection of differences between mutants and controls. Exemplarily, we present data of diabetic Leptin-receptor knockout mice compared to controls.
Our data provide evidence for a specific impact of the blood sample preparation on the results of flow cytometric phenotyping.

Model for human Listeriosis: in vivo monitoring of orally infected mice using bioluminescent Listeria monocytogenes

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Listeria monocytogenes is a Gram-positive bacterium, which causes invasive, often fatal infections in humans and animals. The pathogen infects the host via contaminated food and is able to cross the intestinal, blood-brain and placental barrier which may result in gastroenteritis, meningitis and maternofetal infections which may cause abortion and spontaneous stillbirth.

The primary site of infection is the intestinal epithelium. We have generated a bioluminescent Listeria monocytogenes strain which carries two amino acid substitutions in the bacterial invasion protein Internalin A (InlAS192NY369S). These alterations increase the affinity of Internalin A to murine E-cadherin compared to wildtype Listeria. The bioluminescence of this strain then was used to monitor bacterial dissemination in orally infected mice. For visualization of bioluminescent bacteria during the infection we used the Xenogen IVIS system.

In this study we present data that demonstrate varying severity of infectious disease in different mouse inbred strains (BALB/cByJ, C57BL/6J, CD1, 129P2Ola/Hsd, C3HeB/FeJ). We observed that CD1 and C3HeB/FeJ mice are more resistant to orally transmitted Listeriosis compared to other mouse strains. They showed reduced bacterial dissemination in the early phase of infection (during first 72 hrs) and a fast clearance of the pathogen from day 5 post infection. In contrast BALB/cByJ and C57BL/6J mice we found to be more susceptible after oral infection. This is reflected by slower bacterial clearance and reduced survival.

Furthermore we performed ex vivo imaging to follow Listeria spreading at the cellular organ level. Bacterial load in target organs like liver and spleen were determined. Our experimental system can be used further to obtain a more detailed insight in the process of crossing the feto-placental barrier in pregnant mice as well as the blood-brain barrier after oral infection.

Metabolic phenotyping of mouse mutant lines

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Based on the increase of diseases related to disturbed energy homeostasis (e.g. obesity or diabetes) the identification of genes associated with energy balance is of highest public interest. The Metabolic Module of the German Mouse Clinic (GMC) performs a comprehensive analysis of energy balance in mouse mutant lines (MML). We make use of different methods for the investigation of disturbances in energy metabolism, body weight regulation and body composition. Currently, indirect calorimetry is implemented in the module with the aim to detect alterations in metabolic rate in a high throughput primary screen. Energy expenditure is calculated from gas exchange analysis in an open flow respirometry system allowing the serial measurement of up to 7 mice.
Because most energy metabolism parameters are related to body mass (and also age, sex, size) it is important to account for differences in body weight when comparing metabolic rate between mice. There is an ongoing debate on how to standardize metabolic data to body mass. Very recently, Kaiyala et al. (n=137, PM ID 20413511) reported a much larger effect of body fat mass on energy expenditure than was expected from this metabolically inert tissue. We verified this finding by analysing data of 839 mice (mutants and controls, males and females) from our primary screen to estimate the contribution of body mass, changes in body mass, lean and fat mass, body temperature, locomotor activity and food intake on mean and minimum oxygen consumption using a linear regression model. Interestingly, body fat content had a much weaker effect than expected whereas all the other parameters significantly affected oxygen consumption.

This work was supported by grants from the European Community (EUMODIC LSHG-2006-037188) to the German Mouse Clinic and from the BMBF (NGFNplus) to MK (01GS0869) and to MHA (01GS0850).

Neurological characterization of mutant mouse models in the German Mouse Clinic (GMC)

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Neurological diseases are a common health problem in developed countries and respective mouse models help to understand mechanisms and develop therapeutic strategies. The neurological screen of the German Mouse Clinic (GMC) provides an overview on basic neurological functions of mutant mouse lines. The primary screening includes a general examination according to a modified SHIRPA protocol, measuring grip strength and evaluation of motor coordination with an accelerating rotarod. Dependent on primary results more in-dept-analysis can be performed with more elaborate methods. An automated gait analysis can be applied to characterize gait abnormalities. Electrophysiological methods include electromyography for muscle functions well as nerve conduction studies. Telemetric EEG can be performed to characterize seizure disorders. To determine seizure threshold reactions to pentetrazol injections are analyzed. Running wheels are used to determine locomotor activity levels under standard conditions but it is also possible to measure reactions to modifications of the running wheel in motor learning paradigms. Since many neurological disorders are discussed to be influenced by aging or additional exogenous factors we established a platform for the application of oxidative stress to the mice in order to mimic aging conditions, especially the decline of mitochondrial functions.

Comprehensive phenotyping of mutant lines from clinical networks in the German Mouse Clinic

Presenting Author: Valérie Gailus-Durner
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Next challenges in functional annotation of mammalian genomes are yet of a much larger scope than previous genomics initiatives. Mouse mutant resources must be phenotyped systematically (one after the other) and systemically (assessing all organ systems). In addition, for the next generation of mouse models the "envirotypes", that humans are exposed to need to be modeled.
We established the German Mouse Clinic (GMC) as the first mouse phenotyping platform worldwide with the logistics of systemic, standardized phenotypic analysis and interpretation, with open access for the scientific community on a collaborative basis. The high throughput approach enabled us to screen mouse models for human conditions for clinical networks and IGs within NGFN-Plus. The mutant mouse lines have been requested by and generated for the IG Atherogenomics, IG Genetics of Heart Failure, IG Brain Tumor Networks, IG Cellular Systems Genomics, IG ENGINE, and IG Systematic Genomics of Chronic Inflammatory Barrier Diseases.
To explore the complex relationship between environmental changes and genetic factors, we have been setting up standardized challenge platforms for mouse phenotyping. By simulating specific environmental exposures or life styles we mimic envirotypes that have a strong impact on human health. Five platforms have been implemented in the areas of nutrition, exercise, air, infection and stress. Goal is to decipher their effects on disease etiology and progression, uncovering the physiological and molecular mechanisms of genome-environment interactions. First results will be presented.

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Novel missense mutation of the Pou3f3 gene in mice is associated with small kidney size and impaired renal function

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The incidence of kidney diseases increases globally. In addition, kidney diseases are associated with diabetes and chronic hypertension. Therefore, additional animal models are required for the functional study of nephropathies. The clinical-chemical screen of the phenotype-driven Munich ENU mouse mutagenesis project searched for high plasma urea levels as parameter for nephropathies in a high number of mice and resulted in the establishment of mutant lines exhibiting high plasma urea levels on the genetic background of C3HeB/FeJ (C3H) inbred mice. Line UREHR2 (= HST011) harboring a recessive mutation was successfully bred for several generations according to the plasma urea phenotype. Morphological analysis showed a small kidney size in the mutant animals. Linkage analysis was carried out for the mapping of the causative mutation by using BALB/c mice as second inbred strain. A panel of 116 genome-wide polymorphic markers was used for the genotyping of G2 phenotypically mutant animals. Further fine mapping was carried out using additional polymorphic markers to narrow the chromosomal region of the causative mutation. Pou3f3 on chromosome 1 was chosen as candidate gene for the further analysis. Sequence analysis revealed the point mutation T→C at nt 1268 leading to the amino acid exchange from leucine to proline at position 423. Therefore, line UREHR2 was designated as Pou3f3L423P. Two knockout mouse lines have already been published for Pou3f3 both showing neonatal death and defects in various organs including the kidney. Thus, we successfully established a Pou3f3 mutant line showing postnatal viability and impaired kidney function for the further functional analysis of the gene.

German Mouse Clinic: the Behaviour screen

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The German Mouse Clinic (GMC) (http://www.gsf.de/ieg/gmc) is an open access phenotyping platform, dedicated to the establishment of mouse models of human diseases. Behavioural phenotyping in the GMC is focused on the detection of endophenotypes relevant for human neuropsychiatric dysfunctions and impairments related to neurodegenerative diseases.
In our primary screening, exploratory behaviour in a novel environment is assessed using the open field test. Sensorimotor competence is also examined by pre-pulse inhibition of the acoustic startle reflex. When phenotypes emerge, more tests for that behaviour are applied. For example, to assess motor ability, we use an automated system (Catwalk, Noldus) for detailed gait analysis. With the modified hole-board we further assess exploratory behaviour and the dark-light box and elevated plus-maze tests are used for analysis of an anxiety-related phenotype. For the detection of depression-related behaviours, we apply the forced swim and tail suspension tests. Aspects of cognitive performance are evaluated using tests like the five choice serial reaction time task, fear-potentiated startle, the Y-maze, object and social recognition tests. If we detect a social recognition deficit, we also analyse olfactory function in more detail using a positively reinforced olfactory conditioning procedure.
For certain mutations it is necessary to challenge the animal to unmask phenotypes. Based on this rationale, we have established an acute stress challenge with restraint as the stressor of choice. This manipulation mimics human psychosocial stress in rodents and reliably induces prolonged behavioural changes (i.e. increased anxiety). We are also interested in the functionality of neurogenesis and implement running wheels for its induction to examine its role in mouse behaviour.
With this research, we attempt to contribute to the elucidation of the molecular mechanisms underlying human disorders and development of new therapeutic strategies.

Inhibitory effect of Mg2+ on phosphate-induced vascular calcification

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BACKGROUND: Vascular calcification is known as one of major factors leading to cardiovascular events in uremic patients. One of the major risk factors for vascular calcification in uremic patients is hyperphosphataemia. This study focuses on the potential protective effect of magnesium in the process of vascular calcification due to elevated phosphate blood levels.
METHODS: Aortic segments from male Wister rats were incubated in Dulbecco's Modified Eagle Medium (DMEM) in the presence of 5 Vol-% CO_2/95 Vol-% O_2 for 7 days. The phosphate concentration of the medium was elevated to 3.8 mM by adding either beta-glycerolphosphate (BGP) to induce calcification. Alkaline phosphatase (3 U/ml) was added to inhibit release of pyrophosphate inhibiting calcification in presence of increased phosphate concentrations. The aortic segments were incubated in the absence and presence of 3 mM $MgCl_2$. Calcification was quantified by both van Kossa staining and quantification of tissue calcium by an ELISA.
RESULTS: Incubation of aortic segments in the presence of BGP caused an increased Ca^{2+}-amount in the aortic rings compared to control conditions (63 ± 11 nmol Ca^{2+}/mg tissue vs. 21 ± 3 nmol Ca^{2+}/mg tissue; N=18). The Ca^{2+}-amount in the aortic rings significantly decreased in the presence of Mg^{2+} (54 ± 6 nmol Ca^{2+}/mg tissue; N=18).
DISCUSSION AND CONCLUSION: Medial calcification can be induced in intact rat aorta cultured with alkaline phosphatase in the presence of high phosphate concentration. Magnesium reduced vascular calcification despite increased phosphate concentrations. Magnesium ions may be a new mediator for prevention and therapy of vascular calcification, most likely resulting in a reduction of cardiovascular events in uremic patients.

Core-binding Factor ß is essential to maintain sarcomeric architecture and cardiac contractility

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The cardiac Z-disk hosts specific sensor and transducer proteins that detect mechanical stress and translate it into downstream signaling cascades, enabling the heart to adapt the force of contraction to changing hemodynamic needs.
We identified the novel transcription cofactor Core-binding Factor ß (CBFß) that localizes to nuclei and Z-disks of cardiomyocytes and skeletal muscle cells. Using reverse genetics in zebrafish, we demonstrate that CBFß is essential for Z-disk integrity and cardiomyocyte contractility. Accordingly, CBFß-depleted zebrafish develop both, progressive heart failure and skeletal muscle myopathy (86% of injected embryos; n=100). Since we find predicted AP-1 binding sites in the promoter region of CBFß, we next identified the zebrafish ortholog of JunB, member of the AP-1 transcription factor complex. In JunB-morphant zebrafish, which show a heart failure phenotype similar to that of CBFß-deficient zebrafish, transcript and protein levels of CBFß are severely reduced. Accordingly, overexpression of CBFß can reconstitute cardiac contractility in JunB-morphants, demonstrating for the first time an important role of JunB-CBFß signaling for maintaining cardiomyocyte architecture and function.

Diuridine tetraphosphate: A potent endothelium-derived angiogenic factor

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The secretion of angiogenic factors by vascular endothelial cells is one of the key mechanisms of angiogenesis. Here we report the isolation of a new potent angiogenic factor, diuridine tetraphosphate (Up4U) from the secretome of human endothelial cells. The angiogenic effect of the endothelial secretome was partially reduced after incubation with alkaline phosphatase and abolished in the presence of suramin. In one fraction purified to homogeneity by reversed phase and affinity chromatography, diuridine tetraphosphate (Up4U) was identified by MALDI-LIFT-fragment-mass-spectrometry (MALDI-TOF-TOF-MS), enzymatic cleavage analysis, and retention-time comparison. Beside a strong angiogenic effect on the yolk sac membrane and the developing rat embryo itself, Up4U increased the proliferation rate of endothelial cells and, in the presence of PDGF, of vascular smooth muscle cells. Up4U stimulated the migration rate of endothelial cells via P2Y2-receptors and increased the ability of endothelial cells to form capillary-like tubes. This effect was additive to that of PDGF. Endothelial cells released Up4U after stimulation with shear stress. Mean total plasma Up4U concentrations of healthy subjects (N=6) were sufficient to induce angiogenic and proliferative effects (1.34 ± 0.26 nmol L^{-1}). In conclusion, Up4U is a novel strong human endothelium-derived angiogenic factor.

bungee – a novel regulator of cardiac valve formation in zebrafish

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The molecular mechanisms that guide heart valve formation are not well understood but of immense clinical importance for future biocompatible therapies with tissue-engineered heart valves. To characterize new signaling pathways involved in cardiac valve formation, the zebrafish has become an excellent model organism.
In search for key regulators of cardiac valve formation, we isolated in a large-scale ENU-mutagenesis screen the recessive, embryonic lethal zebrafish mutation bungee (bngjh177). bng mutant zebrafish embryos fail to develop AV valves due to impaired endocardial notch1b signaling. By positional cloning, gene knock-down and rescue experiments we demonstrate that the bng phenotype is caused by a missense mutation within a gene encoding a zebrafish Serine/Threonine protein kinase, leading to a substitution of a Thyrosine to an Asparagine within the highly conserved kinase domain. In vitro kinase assay reveals a severely reduced kinase activity of bng mutant kinase. Injection of wild-type mRNA in bng mutant embryos restores the wild-type phenotype, while injection of a kinase-dead variant is unable to rescue the bng mutant phenotype. Additionally, Morpholino-modified antisense oligonucleotides directed against a splice-donor site within the kinase-domain phenocopies the bng mutant phenotype. Whole-mount antisense RNA in situ hybridization reveals that expression of zebrafish bungee within the embryonic heart is restricted to the endocardial cell layer of the atrioventricular canal.
Here we show for the first time that the bungee kinase activity is crucial for proper development of the atrioventricular canal and the formation cardiac valves.

Myomasp/LRRC39, a heart- and muscle-specific protein, is a novel component of the sarcomeric M-band and is involved in stretch sensing

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The M-band represents a transverse structure in the centre of the sarcomeric A-band and provides an anchor for the myosin-containing thick filaments. In contrast to the sarcomeric Z-disc, only few M-band-specific proteins have been characterized and its exact molecular composition remains unclear.
Methods and Results: Using a bioinformatic approach we found a previously uncharacterized leucine rich protein, termed myomasp (Myosin-interacting, M-band-associated stress-responsive protein)/LRRC39. RT-PCR, Northern and Western blot analyses confirmed a cardiac-enriched expression pattern, and immunolocalization of myomasp revealed a strong and specific signal at the sarcomeric M-band. Yeast two-hybrid screens as well as co-immunoprecipitation experiments identified the C-terminus of myosin heavy chain (MYH7) as an interaction partner.
Cardiomyocyte stretch in vitro and in vivo led to a significant downregulation of endogenous myomasp, whereas artificial knockdown of myomasp in neonatal rat ventricular myocytes (NRVM) led to a significant upregulation of the stretch-sensitive genes GDF-15 and BNP. Conversely, the expression of MYH7 and the M-band proteins myomesin-1 and -2 was found to be markedly reduced. Mechanistically, knockdown of myomasp in NRVM led to a dose-dependent suppression of SRF-dependent gene expression, consistent with earlier observations linking the M-band to SRF-mediated signaling. Downregulation of myomasp in spontaneous beating engineered heart tissue constructs resulted in significantly lower force generation and reduced fractional shortening. Likewise, in vivo knockdown of myomasp/LRRC39 in zebrafish resulted in reduced contractility, cardiomyopathy and disturbed ultrastructural M-band architecture
Conclusions: These findings reveal myomasp as a previously unrecognized component of an M-band associated signaling pathway that regulates cardiomyocyte gene expression in response to biomechanical stress.

flatline – a novel Myosin interacting protein – is essential for thick filament assembly

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Assembly, maintenance and renewal of sarcomeric units requires highly organized and balanced regulatory mechanisms with respect to protein folding, transport, modification, and degradation. However, mechanisms that regulate these fundamental processes are only poorly understood, but of great clinical importance, since many cardiac and skeletal muscle diseases are associated with defective sarcomerogenesis.
To define novel genetic components of muscle myofibrillogenesis we performed a forward genetic mutagenesis screen in zebrafish and isolated the recessive mutant flatline (fla), which shows disturbed sarcomere assembly exclusively in heart and fast-twitch skeletal muscle. By positional cloning we identified a nonsense mutation within a novel Histone-Methyltransferase (HMT) to be responsible for the fla phenotype. This HMT localizes, next to the nucleus, to sarcomeric M-lines where it physically associates with Myosin. Interestingly, we find that its Myosin-binding capability is crucial for sarcomerogenesis in zebrafish, whereas nuclear Histone Methyltransferase activity is dispensable for sarcomere assembly. In summary, we show for the first time that the flatline Histone-Methyltransferase plays a pivotal role in the processing of sarcomeric Myosin and thereby in the orchestration of thick filament assembly exclusively in zebrafish heart and fast-twitch skeletal muscle cells.

ZFYVE7 promotes autophagy in cardiomyocytes

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Using bioinformatics we aimed to identify novel genes that are predicted to be specifically expressed in heart and skeletal muscle. Thereby we found ZFYVE7 as a candidate gene. We could confirm the expression pattern enriched in striated muscle by real time PCR and Northern blotting. In order to assess the cellular function of ZFYVE7 we performed a yeast two-hybrid screen which revealed LC3 as interacting protein. LC3 is an essential protein in autophagy, a lysosome-dependent degradation and recycling mechanism of the cell. During this process, vesicles termed autophagosomes internalize proteins or even entire organelles and fuse with lysosomes.

Overexpression of ZFYVE7 in cardiomyocytes by adenoviral gene transfer resulted in increased LC3-II levels, a modified form of LC3, which is associated with an increased number of autophagosomes. Under starvation conditions by glucose deprivation for 3h and for 24h, which induces autophagy, this effect was exaggerated. Interestingly, overexpressed and endogenous ZFYVE7 localized to the cytosol in a punctuated pattern like LC3 which is a marker protein for autophagosomes. Taken together, ZFYVE7 might be involved in the autophagic machinery.

In order to examine the cellular function of ZFYVE7 in vivo we knocked it down in Zebrafish. This morpholino mediated knockdown caused heart failure with pericardial edema which could be due to impaired autophagy. To further analyze the effects of ZFYVE7 deficiency in vivo we generated a constitutive knockout mouse model. Homozygous mice were born in the expected ratio and developed normal. Body and heart weight as well as cardiac function assessed by echocardiography were not different to their wildtype littermates. Since autophagy is induced under stress it is conceivable that these mice do not develop a pathological phenotype under baseline, but under stress conditions. This will be part of upcoming research.

Strong up-regulation of Runx2 in DCC-susceptible C3H/He mice after freeze-thaw-injury

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Introduction: Mice were used as model for Dystrophic Cardiac Calcification (DCC) using freeze-thaw-injury. DCC shares many features with osteogenesis.

Aim: The aim of this study was to analyze the expression-level of transcription-factors involved in osteogenesis and to identify respective target-genes of these for a better understanding of the initiation and development of cardiac and vascular calcification.

Methods: DCC-susceptible C3H/He and DCC-resistant C57BL/6 mice (n=3 in each group and time point) were subjected to freeze-thaw-injury to induce calcification. Early at 24 and 72 hours necrotic and healthy myocardium was separated. tRNA and cryo-sections from each tissue were prepared for histological analysis and relative-real-time-PCR quantification using the delta-deltaCt-method.

Results: Using Calcein-staining calcification-like deposits appear in resistant and susceptible mice 1 day after injury. Calcification progresses in C3H/He but not in C57BL/6 mice 2 days later. Among the tested transcription-factors a 30.3-fold up-regulation of Runx2 was detected in calcified tissue of C3H/He as compared to healthy myocardium. Low expression was found for Sox9, Vdr, Nfkb, Msx1, Smad1, Smad2 and Smad4. Based on this finding we further tested downstream-genes of Runx2: Vdr, Dmp1, Phex, Osterix, Col1a2, IBSP, MMP2, MMP8, MMP9, MMP13, Bglap II, Opn and Akp2. In both strains we found an up-regulation of Vdr, Col1a2, MMP8, MMP9, MMP13 and a significant up-regulation of Opn (372.55-fold of induction) in C3H/He compared to C57BL/6 (64.44-fold of induction, p= 0,028).

Conclusion: Infiltrating cells that differentiate into osteoblast-like-cells following injury display high expression of Runx2, which may activate Opn and in turn the MMPs-pathway to cleave collagen (type-I, -II, -III). This study suggests that Opn may play a determinant role in myocardial calcification as a downstream gene of Runx2.

Potential transcriptional regulatory element in the MRas 3`untranslated region (UTR)

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Background: Our recent genome-wide association study (GWAS) identified SNP rs9818870 located the in the 3`UTR of the MRas gene on human chromosome 3q22 with genome-wide significance for coronary artery disease (CAD) and myocardial infarction (MI).
Atherosclerotic manifestations like CAD reflect a chronic inflammation of the vessel wall with accumulation of lipid-laden macrophages in the large arteries. M-Ras is a member of the Ras superfamily of small GTPases; many of which function as molecular switches in diverse cellular functions and thereby regulate a variety of biological processes.
Aim of the study: We aimed to perform a functional analysis of MRas 3`UTR related to a possible regulatory element located in this region.
Methods and Materials: We performed luciferase assays to find first hints of the regulatory relevance of the 3`UTR. For this we cloned four fragments (each app 200bp in length) located in the MRas 3`UTR: one covers the position of rs9818870, three additional fragments cover highly conserved regions in the 3`UTR. We cloned and analysed these fragments from mice and human in mice and human cells, respectively.
Results: Luciferase assays showed a clear down regulation of the luciferase activity in human cells due to the human constructs.
Conclusion: In our preliminary experiments we show that the 3`UTR of human MRas has a functional relevance for gene regulation. It seems that in this region a possible gene silencer is located. Further analyses will get more insights into the regulatory impact of this region.

Lethal neonatal cardiomyopathy in Nexilin$^{-/-}$ mice

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Background: Dilated cardiomyopathy (DCM) is a leading cause of heart failure. Recently, we identified nexilin (Nex) gene mutations in patients with DCM and showed that loss of Nex in zebrafish led to perturbation of the cardiac contractile apparatus with instability of the Z-disk and subsequent cardiac dilatation. Here we further investigate the role of Nex in DCM in a murine knockout model.

Material and Methods: A constitutive nexilin knockout mice was generated by TaconicArtemis GmbH. Neonatal hearts were studied in vivo by echocardiography and analysed histologically by electron microscopy at different time points. Furthermore, we measured heart and body weights.

Results and Discussion: Heterozygous (Nex$^{+/-}$) mice displayed only subtle phenotypes when analysed in the primary screen at the German Mouse Clinic. Homozygous (Nex$^{-/-}$) mice were produced by mating Nex$^{+/-}$ mice. At birth, the ratio of Nex$^{-/-}$: Nex$^{+/-}$:wild-type (WT) mice approximated the expected ratios of 1:2:1. After postnatal day 7, survival of Nex$^{-/-}$ mice decreased progressively, none lived beyond postnatal day 10. Moreover, the heart-to-body weight ratio was 1.2-fold and 2.8-fold higher in Nex$^{-/-}$ than in WT mice at postnatal days 4 and 6, respectively. Between days 4 and 6, Nex$^{-/-}$ mice developed a rapidly progressive cardiomyopathy with left ventricular dilation and wall thinning as determined by echocardiography. Histological analysis in hearts from neonatal Nex$^{-/-}$ mice at day 2, 4 and 6 confirmed a progressive left ventricular dilation first at day 4 and more pronounced at day 6. Electron microscopy showed a blurring of the boundary between the I and A bands within the cardiac myocytes.

Conclusion: Nex$^{-/-}$ mice exhibited neonatal lethality caused by a fulminant dilated cardiomyopathy starting at postnatal day 4 and leading to fatal heart failure after day 6 due to malfunction of the contractile apparatus.

Role of the GTPase ARFRP1 for hepatic glucose metabolism

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ADP-ribosylation factor related protein 1 (ARFRP1) is an ubiquitiously expressed Ras-related GTPase. In the active GTP-bound form, ARFRP1 associates with Golgi membranes and recruits ARL1 and its effector Golgin-245 to the trans-Golgi. The aim of this study was to scrutinize the role of ARFRP1 in energy metabolism and glucose homeostasis.
In order to elucidate the function of ARFRP1 in the liver, we generated hepatocyte-specific knockout mice (Arfrp1$^{liv-/-}$). In the liver of these mice the organization of the trans-Golgi was markedly affected since the trans-Golgi marker TGN38 was only rarely detectable in Arfrp1$^{liv-/-}$ hepatocytes. The Arfrp1$^{liv-/-}$ mice showed a postnatal growth retardation (week 5: Arfrp1$^{flox/flox}$ 22.1 ± 2.5 g; Arfrp1$^{liv-/-}$ 16.0 ± 2.1 g) accompanied by a significantly reduced liver weight. Plasma and hepatic IGF1 levels were diminished, most likely leading to the reduced growth. In contrast, Igf1 mRNA and other liver-specific transcripts (Alb, Ahsg, Gys2, Pygl) were unaltered indicating that hepatocyte differentiation per se was not affected in the absence of ARFRP1. In addition, liver glycogen content was lower in Arfrp1$^{liv-/-}$ mice in consequence of a reduced glucose uptake into the liver. Immunohistochemical staining of the glucose transporter GLUT2 revealed a reduction of GLUT2 in the plasma membrane of Arfrp1$^{liv-/-}$ hepatocytes.
In conclusion, the growth retardation of Arfrp1$^{liv-/-}$ mice is caused by reduced IGF1 levels and an impaired glucose metabolism. ARFRP1 is required for an undisturbed structure of the trans-Golgi network. The proposed mechanisms leading to these effects on protein trafficking involve the indirect recruitment of golgin proteins which are downstream targets of ARFRP1 and ARL1. Therefore, ARFRP1 might be essential for the undisturbed trafficking of GLUT2 and IGF1 in the liver.

Characterisation of a putative obesity gene encoding an enzyme of beta-oxidation

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Background: Obesity, as a central component of the metabolic syndrome, is associated with type 2 diabetes and cardiovascular complications. By comparing two genome wide screenings performed in C. elegans a polygenic obesity mouse model, the New Zealand obese (NZO) mouse we have recently identified Adp3 (synonym) on murine chromosome 3 which encodes an enzyme of the mitochondrial beta-oxidation.
Methods: For the characterisation of the putative obesity gene we generated an Adp3-knockout mouse using the gene trap approach. In order to examine the metabolism of this mouse we investigated development of body weight, body composition, food intake and locomotor activity under high-fat-diet conditions.
Results: Under high-fat-diet conditions knockout mice displayed a lower body weight and a reduced fat mass in comparison to their wild-type littermates but did not differ in food intake. However, respiratory quotient of the knockouts was decreased indicating an elevated fat oxidation. Furthermore, the higher body temperature of knockout mice and a significantly increased locomotor activity during the light phase might be responsible for the reduced body weight of these mice.
Conclusion: We identified a gene of beta-oxidation which is involved in alterations of the body weight and fat mass development presumably by elevating fat oxidation.

Positional cloning of Nob3: A QTL protecting from adiposity

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Several chromosomal regions (QTL) and gene variants (Tbc1d1, Zfp69, Abcg1, Nmur2) associated with different degrees of obesity and related metabolic disorders were previously identified by our group with outcross populations derived from the New Zealand obese (NZO) mouse. A major obesity QTL (Nob3) was identified on distal mouse chromosome 1 responsible for a body weight increment of approx. 13 g in week 22. To define the critical segment of the QTL Nob3 with a conventional strategy of positional cloning, we introgressed a 38 Mbp fragment corresponding with the distal peak of Nob3 from NZO into the C57BL/6J strain (B6.NZO-Nob3.38). Homozygous carriers of Nob3.38 exhibited a significantly higher body weight, body fat, and lean mass than their littermates carrying B6 allels of Nob3. Moreover, this recombinant-congenic line showed a significantly reduced body temperature, increased running-wheel activity, and higher plasma levels of IGF-1. Additional congenic lines were generated and characterized defining a critical genomic interval with 42 genes that is required for elevated body weight. Expression analysis of genes located in the critical fragment revealed the most striking difference for a gene cluster; mRNA levels were markedly reduced in tissues of NZO-allele carriers in comparison to B6-allele carriers of the congenic line Nob3.38. In contrast, one gene of this cluster (Nob3 gene) showed a completely different pattern since transcripts were not detectable in tissues of B6.NZO-Nob3.38B6 mice but highly expressed in skeletal muscle, liver, and adipose tissue of B6.NZO-Nob3.38NZO mice. However, it has to be clarified if the difference in body weight between the allele carriers is the consequence of a single-gene defect (Nob3 gene) or due to a dysregulation of the whole gene cluster.
In summary, with the generation and characterization of congenic lines we were able to identify a gene cluster on chromosome 1 which plays a crucial role in the development of adiposity.

The onset of voluntary exercise influences body weight development in genetically obese mice

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Obesity is the result of interactions between genetic predisposition and the environment, such as sedentary lifestyle and excess nutrition. We have established the Berlin Fat Mouse Inbred (BFMI) line as a novel model for juvenile obesity, which has been selected for high fatness. In this study we investigated the influence of the onset of voluntary exercise on body composition. For this purpose animals of the BFMI line were provided a running wheel for voluntary usage at three weeks (childhood, after weaning) and at nine weeks (early adulthood), respectively. The control group did not have the possibility of additional exercise.
Animals who had access to running wheels from three weeks had lower body weights than animals without exercise. Mice with physical activity from week nine onwards exhibited higher food consumption than animals with exercise from three weeks on and animals without exercise. Therefore, mice with exercise from nine weeks on had only a marginally decrease in body weight and fat content compared to the control group without exercise.
To identify differentially expressed proteins in fat tissues between the different exercise groups a proteome study has been performed. A first PCA analysis of the proteome revealed that samples of mice with exercise from nine weeks on and the control group cluster together, indicating no significant difference between these groups. The proteomes of mice with exercise from three weeks on of mice without exercise differed in about 300 expressed proteins.
The data indicated that physical activity in early childhood has lasting effects on body composition and on molecular basis of fat tissue.

Does diet-induced obesity cause 'oxidative stress' in the mouse brain? - Validation of the candidate mitochondrial superoxide dismutase (SOD2)

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In a previous proteome analysis comparing hypothalamic extracts from C57BL6/N mice that had been fed a high-fat or a chow diet for 18 weeks, Superoxide Dismutase 2 (SOD2, mitochondrial form) was found to be higher abundant in the high-fat diet group. This candidate was selected for further validation on gene expression, protein and activity level. SOD2 catalyzes the dismutation of superoxide to hydrogen peroxide and molecular oxygen. Consequently, the potential upregulation of SOD2 indicated a hypothalamic stress response towards a high-fat diet. We therefore examined whether isolated hypothalamic mitochondria from high-fat diet fed mice exhibit enhanced superoxide production rates.

C57BL6/N mice were fed the same high-fat and chow diet as in the proteome study for 12 weeks. We did not detect any significant changes in Sod2 transcript levels as determined by quantitative real-time PCR. Moreover, western blot analysis showed no differences of SOD2 protein amount between the diet groups. The same was true for SOD2 or total SOD activity. Mitochondrial superoxide production in the hypothalamus of mice was investigated using fluorescent Amplex red measurements of hydrogen peroxide. No effect of the 12-weeks high-fat diet on superoxide production in mitochondria isolated from hypothalamus or brain could be detected.

Taken together, an upregulation of SOD2 in the hypothalamus after feeding a high-fat diet was not confirmed in the experiments performed here. However, the feeding trial was not an exact replicate of the former proteome study as the mice were older and the feeding period shorter. Hence, the increase of SOD2 is not a robust marker for oxidative stress in mice with diet-induced obesity. Further studies on mitochondrial ROS generation and the expression of ROS defence systems in the brain are required to clarify the impact of a high fat diet on "oxidative stress".

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Phenotyping of the Melanocortin-4-Receptor Polymorphism V103I in vitro and in vivo

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The melanocortin-4-receptor (MC4R) is a G-Protein coupled receptor which is mainly expressed in the CNS. Loss-of-function mutations in the MC4R gene are the most common cause for monogenic obesity. Other than the most MC4R mutations the polygenic MC4RV103I allele is negatively associated with obesity. Herein we aim to characterize the MC4RV103I variant in vitro and in vivo to identify possible mechanisms underlying the protective effect of this allele against weight gain.
In a cell culture based assay we revealed an increase in basal activity of Mc4rV103I in comparison to Mc4rwt indicating a gain of receptor function. Moreover we generated a Mc4rV103I knockin mouse line by gene targeting in embryonic stem cells. Phenotyping data demonstrated no difference between mutant and wild-type mice concerning body weight and energy intake. However, homozygous $Mc4r^{V103I/V103I}$ mutants had a higher rectal body temperature compared to $Mc4r^{wt/V103I}$ and $Mc4r^{wt/wt}$ mice. Despite an elevated body temperature body weight and energy intake were unaffected in mutant mice suggesting an altered partitioning in the energy budget. Detailed measurements monitoring O_2-consumption, activity and daily body temperature profiles will provide insights into energy utilization in the Mc4rV103I knockin mouse line.
qRT-PCR revealed down regulation of Mc4r transcript levels in $Mc4r^{V103I/V103I}$ knockin mice. Thus, normal energy balance is maintained despite reduced Mc4r expression. Using primer extension assays we measured the ratio of the Mc4rV103I and Mc4rwt allele in heterozygous males and observed a lower Mc4rV103I mRNA abundance. Possible explanations for this effect range from side effects of the gene targeting strategy to autoregulatory mechanisms on Mc4r gene expression.
The Mc4rV103I knockin mouse line is the first mouse model harboring a protective polygenic allele against obesity. Further experiments will help to understand the role Mc4rV103I on energy homeostasis.

Function of BACE1 and Neuregulins in the developing and adult nervous system

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BACE1 (beta-site APP cleaving enzyme), an aspartyl protease, plays a critical role in the production of amyloid Abeta peptides; insoluble plaques containing Abeta constitute the molecular basis of pathogenesis in Alzheimer's disease. One principal physiological function of BACE1 is the cleavage of Neuregulins (Nrg), EGF-like growth factors that are highly expressed in the nervous system. Previously we showed that BACE1 is required for cleavage of axonally-expressed type III Nrg1, which is essential for peripheral nerve myelination by signaling to apposing Schwann cells. We have further characterized the functions of BACE1 in Nrg1 signaling in the neonatal and adult mouse nervous system by the use of mice carrying compound mutations in BACE1 and specific isoforms of Nrg1. As well as revealing genetic synergy between BACE1 and type III Nrg1 in peripheral nerve myelination, analysis of compound mutant mice has shown that BACE1 is also necessary for the function of other Nrg1 isoforms. Nrg1 controls the differentiation of the muscle spindle, and the type I isoform (an immunoglobulin domain containing isoform) has been implicated in this process. We established a new floxed Nrg1 allele, in which the immunoglobulin-containing isoforms of Nrg1 can be mutated in a tissue-specific manner. Conditional ablation of the Ig-isoforms in sensory neurons resulted in an almost complete loss of muscle spindles. Conversely, neuronal overexpression of the Type I isoform under control of the Thy1-promoter resulted in a doubling of the number of muscle spindles, an effect which was entirely dependent on BACE1. Furthermore, we observed reduced numbers of muscle spindles in $BACE1^{-/-}$, and a further loss in $BACE1^{-/-}$; $Nrg1^{+/-}$ mice, as well as in compound $BACE1^{-/-}$; $Nrg1^{Ig/+}$ mice in which there is haploinsufficiency of the Ig-isoforms of Nrg1. Our studies demonstrate thus that the biological activity of the type I Nrg1 isoform depends on cleavage by BACE1.

Generation and analysis of a Pink1 deficient mouse model for Parkinson's disease

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder with a typical onset in the 6th decade of life. The major pathological hallmarks of the disease is the progressive degeneration of dopaminergic neurons in the substantia nigra. Clinically the disease is characterized by severe motor symptoms. Over the recent decade several genes have been shown to be associated with familial forms of PD, amongst which Pink1 leads to a recessive form of PD. To determine its role in the pathogenesis of the disease we generated and analysed Pink1 deficient mice. Pink1 deficient mice are viable and fertile without any obvious signs of abnormality in body weight, posture, morbidity or mortality. Morphologically no degeneration of dopaminergic neurons could be observed, which is reflected by the absence of gross motoric symptoms. Further analysis revealed an impairment in olfaction, a major non-motoric symptom of PD. By analysing the olfactory system of these mice we found a decrease in the serotonergic innervation of the olfactory bulb. However, the functional significance of this finding still has to be evaluated. The absence of major PD symptoms in this mouse model prompted us to analyse mitochondrial morphology in these mice in order to evaluate whether the cellular phenotype observed in vitro is compensated in vivo. Indeed, in primary neurons acute knock-down of Pink1 leads to a fragmentation of mitochondria, which is, however, compensated within five days. Even more, in the mutant mice the mitochondria are elongated and not fragmented. Both observations suggest that Pink1 deficiency is readily compensated in vivo. These observations are in full accord with the multiple hit theory of the pathoetiology of PD in which only the presence of additional environmental stressors leads to the outbreak of the disease. Thus the Pink1 mice are reflecting the situation in humans and are therefore true and valuable models to study the pathoetiology of PD.

The role of Parkinson's disease associated protein DJ-1 (Park7) for mitochondrial trafficking in neurons

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Loss of function of the protein DJ-1 is a rare cause for inherited Parkinson's disease (PD). In addition to its known functions in transcription and its antioxidant properties, we recently found that DJ-1 plays also a major role in the lysosomal degradation of mitochondria and mitochondrial dynamis in fibroblasts. However, the role of DJ-1 has never been validated in human dopaminergic neurons due to a lack of appropriate cell models. The iPS-cell (induced pluripotent stem cells) technology provides the possibility to generate human dopaminergic neurons in vitro. iPS-cells from PD-patients carrying a homozygous DJ-1 E64D mutation serve as an ideal base to model and analyze DJ1-function in dopaminergic neurons.
Here we report the generation of iPS-cells from a PD-patient carrying a homozygous mutation within the DJ-1-gene (E64D). As we could show that DJ-1 E64D is nearly undetectable by immunoblotting due to instability of mRNA, which was confirmed by RT-PCR. Additionally, we successfully generated a rescue cell line by genetic correction through lentiviral transduction of DJ-1 WT. This cell-line stably expresses DJ-1 in amounts comparable to iPS-cells derived from healthy individuals.
As our focus is mainly on mitochondrial function of DJ-1, the generation of dopaminergic neurons from these iPS-cells offers the possibility to validate known effects and to study new pathomechanisms of DJ-1 in these cells, especially on mitochondrial transport.
We expect that results from this study will expand our view on the role of mitochondria in PD and will further give insight in the molecular mechanism of PD causing and PD associated genes.

Dissecting the role of the mitochondrial chaperone mortalin in Parkinson's disease – functional impact of disease-related variants on mitochondrial homeostasis

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Mortalin is an intramitochondrial stress response protein that regulates mitochondrial homeostasis and has been linked to Parkinson's disease (PD) pathogenesis due to significantly reduced levels in affected brain regions of PD patients. We identified human mortalin as a mitochondrial DJ-1-interacting protein involved in cellular stress response. Recently, first PD-associated mortalin variants have been identified in a Spanish cohort. We identified a novel, highly conserved PD-associated variant in German PD patients. To understand the relevance of mortalin variants for mitochondrial homeostasis, we performed functional studies in various in vitro and ex vivo models.
In different overexpression models, we investigated the effect of mortalin variants on mitochondrial localization, morphology, ROS production and mitochondrial membrane potential in comparison to wild type (wt) mortalin. We found no effect on proper mitochondrial import, but specific alterations within mitochondria including impaired mitochondrial function in stably transfected SH-SY5Y cells overexpressing mortalin variants. Altered mitochondrial integrity was observed in cells transfected with variants as well as in fibroblasts from a carrier of the 'German' variant. Knockdown of mortalin lead to impaired mitochondrial function with complete rescue by wt mortalin but not by any of the known variants.
Our analyses on novel mortalin variants reveal a loss of protective function phenotype including impaired mitochondrial function and disturbed mitochondrial integrity. Our results support the role of this mitochondrial chaperone in neurodegeneration in PD and underscore the concept of impaired mitochondrial protein quality control in the pathogenesis of PD. Current experiments focus on specifying the mechanistic aspects of the described mitochondrial phenotype.

A severe epileptic phenotype due to a moderate loss of M-current in the KCNQ2NMF134 mouse model

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Mutations in the human KCNQ2 and KCNQ3 genes encoding M-channel subunits are associated with an epilepsy syndrome of newborns (BFNC). The KCNQ2NMF134 (Nmf134) mouse line (Jackson lab) that carries a V182M-mutation in the S3-helix of KCNQ2 was identified by a reduced seizure threshold in response to electrical stimulation. We used the Nmf134 mouse line as novel M channel-deficient model complementary to our dominant negative KCNQ2 transgenic mouse line (Peters et al, 2005).
Homozygous Nmf134 mice were smaller and lighter than their wildtype littermates and died from spontaneous seizure activity during the first postnatal weeks with a mortality rate of about 70% after 6 weeks. No gross morphological changes were observed in hippocampal brain sections. However, in preliminary immunostains, augmented c-fos immunoreactivity pointed to increased neuronal activity, and the presence of reactive astrocytes indicated an ongoing pathological process.
Electrophysiological analysis of the V182M mutation in Xenopus leavis oocytes showed a 50% reduction in KCNQ2/KCNQ3 channel-mediated current amplitudes and a significant shift of voltage-dependent gating to more positive membrane potentials. Similar results with respect to M-channel amplitudes were obtained from CA1 pyramidal neurons of Nmf134 mice, leading to increased cellular excitability upon current injection. The sensitivity of mutant M-channels to the anticonvulsive drug and M-channel mimetic retigabine was unaffected, both in oocyte and brain slice recordings.
Our experiments demonstrate the generation of a severe epileptic phenotype by a moderate shift in voltage dependence of the KCNQ2 channel activation. We now use the Nmf134 mouse line as a model for neonatal seizures to evaluate treatment strategies during neonatal brain development.

Two de novo SCN1A mutations associated with sudden unexpexted death in epilepsy

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Sudden unexpected death in epilepsy (SUDEP) is one of most common death causes in epileptic patients. It is defined as a sudden, unexpected death with unkown cause in epilepsy patients. The underlying pathophysiological mechanisms of SUDEP remain unclear. We identified recently two de novo missense mutations in SCN1A (encoding the brain sodium channel NaV1.1) in two families with generalized epilepsy with febrile seizures plus (GEFS+) and an increased rate of SUDEP. The functional consequence of these two mutations were characterized by whole cell patch clamp technique, in mammalian tsA201 cells. Both mutant channels showed a clear loss-of-function, one showing no measurable sodium current, the other a significantly reduced sodium current density and a slowed rate of recovery from fast inactivation. The loss-of-function of NaV1.1 predicts a decrease of sodium current amplitudes in GABAergic interneurons and a reduction of their firing rate, which could explain the occurrence of seizures, as has been shown for other mutations previously. Since expression of NaV1.1 has also been described in the sino-atrial node of the cardiac pacemaking system and also in brain stem nuclei, it would be conceivable that such mutations could alter cardiac rhythm or the central regulation of cardiac rhythm and/or breathing; or even affect neural control of cardiac rhythmicity by aberrant autonomic pathways, which may explain the increased rate of SUDEP in those patients.

Functional alteration of the voltage-gated sodium channel Nav1.1 leads to diminished inhibition in various brain regions

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Voltage-gated sodium channels (NaVs) are essential for the generation and propagation of action potentials. Mutations of the SCN1A gene which encodes the a-subunit of the Nav1.1 neuronal sodium channel have been previously shown to cause neurological disorders like e.g. generalized epilepsy with febrile seizures plus (GEFS+).
One of the first identified human GEFS+ associated SCN1A mutations is R1648H, which is located in the voltage sensor of domain IV. To study the effects of this mutation on the neuronal excitability, we used a knock-in mouse model carrying the human GEFS+ mutation SCN1A-R1648H (M.S. Martin et al., JBC, 2010).
Whole-cell patch clamp recordings were performed in acute brain slices to examine the firing properties of GABAergic interneurons. Both hippocampal stratum oriens and thalamic nucleus reticularis interneurons from heterozygous mice showed a decreased firing rate compared to those from wild-type littermates. The membrane properties such as input resistance and resting membrane potential did expectedly not differ between heterozygous and wild-type interneurons. Excitatory pyramidal cells within the stratum pyramidale of the hippocampal CA1 region were also studied as a control but we did not find any differences between heterozygous and wild-type pyramidal cells. Thus, our data suggest a decreased excitability of interneurons in different brain regions of the SCN1A-R1648H knock-in mice, which may lead to epileptic seizures and this could also be true for GEFS+ patients.

Alterations in the developmental expression of ion channels at axon initial segments associated with age-dependent seizures

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The axon initial segment (AIS) is a site of action potential generation and plays a major role is neuronal excitability. A large number of different ion channels are expressed at high concentrations in this specialized neuronal compartment and some of these channels, like voltage-gated potassium channels Kv7.2 and Kv7.3 (encoded by KCNQ2/3 genes), or voltage-gated sodium channel Nav1.2 (gene SCN2A) have been associated with benign forms of neonatal seizures, starting within the first days (KCNQ2/3) or first days to months of life (SCN2A) and remitting spontaneously after weeks to months. The loss-of-function caused by Kv7.2/7.3 epilepsy mutations and gain-of-function of the Nav1.2 mutations can explain the hyperexcitability of the AIS and the generation of seizures, but the mechanisms underlying their spontaneous remission remain unclear.
Developmental expression of different ion channels at AIS in unfixed mouse brains was analysed in cryosections obtained at postnatal (P) days P1-P40 using specific antibodies against different channel subunits and the Ankyrin G as an AIS marker. We reported that Nav1.2 channels are expressed early in development at AIS of principal neurons and that their expression is diminished and they are gradually replaced, in particular at distal AIS, by Nav1.6 during maturation. We further observed an upregulation of Kv7.2 and Kv7.3 channels at AIS during development and their strong expression in the adulthood. Co-stainings of Nav and Kv7 channels revealed their distinctive localization within the AIS, with Nav1.6 and Kv7.2 lying more distally compared to Nav1.2 and Kv7.3. Furthermore, we observed that gephyrin, a protein important for postsynaptic clustering of GABA(A) receptors within inhibitory synapses, is upregulated at the AIS of pyramidal hippocampal neurons after P15. This dynamic developmental reorganization within the AIS including the changes in ion channel expression may account for the transient epileptic phenotypes.

Pharmacological or genetic elevation of D-serine levels improve mood and cognitive performance

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Background: NMDA receptors are activated after binding of the agonist glutamate to the NR2 subunit and a co-agonist, either L-glycine or D-serine, to the NR1 subunit. While glycine is likely to be the predominant co-agonist in the spinal cord and brain stem, D-serine seems to be the relevant co-activator in forebrain regions. The modulation of D-serine levels influences NMDA receptor activity in these brain regions and thus potentially regulates important behavioral effects of NMDA receptor signaling, such as learning and memory, motivation and affect. D-serine is predominantly produced in astrocytes through isomerization of L-serine by the serine racemase (Srr). D-serine is then released by an activity-dependent mechanism involving secretory vesicles.

Methods: For this study we generated mutant mice (SrrTg) over-expressing the Srr under the human GFAP promoter. This mouse model was biochemically and behaviorally characterized using behavioral paradigms of anxiety, depression and cognition. Furthermore, we investigated the behavioral effects of long-term administration of D-serine as a food supplement in wild type animals.

Results: We show that the elevated brain D-serine levels of SrrTg mice result in a specific behavioral phenotype indicative of a reduced proneness towards depression-related and increased goal-directed behaviors. Basal anxiety was unchanged in SrrTg mice. Additionally, SrrTg mice performed better in cognitive paradigms for spatial and associative learning. Finally we show that a chronic dietary D-serine supplement mimics the behavioral phenotype observed in SrrTg mice.

Conclusions: Our results suggest a beneficial influence of a D-serine treatment in mood disorders such as depression and cognitive disturbances.

Identification and analysis of expression of novel microRNAs of murine gammaherpesvirus 68

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Murine gammaherpesvirus 68 (MHV-68) is closely related to Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV) and provides a small-animal model with which to study the pathogenesis of gammaherpesvirus (γHV) infections. To completely explore the potential of the MHV-68 system for the investigation of γHV microRNAs (miRNAs), it would be desirable to know the number and expression patterns of all miRNAs encoded by MHV-68. By deep sequencing of small RNAs, we systematically investigated the expression profiles of MHV-68 miRNAs in both lytically and persistently infected cells. In addition to the nine known MHV-68 miRNAs, we identified six novel MHV-68 miRNA genes and analyzed the expression levels of all MHV-68 miRNAs. Furthermore, we also characterized the cellular miRNA expression signatures in MHV-68-infected versus non-infected NIH3T3 fibroblasts and in 12-0-tetradecanoyl-phorbol-13-acetate (TPA)-treated versus non-treated S11 cells. We found that mmu-mir-15b and mmu-mir-16 are highly upregulated upon MHV-68 infection of NIH3T3 cells, indicating a potential role for cellular miRNAs during MHV-68 infection. Our data will aid in the full exploration of the functions of γHV miRNAs.

Systematic RNA interference screen identifies novel modulators of NOD2-dependent NF-kappaB activation

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NOD-like receptors (NLRs) are key players in host defence and inflammation and represent cytosolic sensor proteins for endogenous and exogenous danger signals. The NLR member NOD2 recognizes the presence of muramyl dipeptide (MDP) derived from bacterial cell walls within the cytosol and activates pro-inflammatory signalling cascades like NF-kappaB. Genetic variants of NOD2 are associated with increased risk for Crohn disease. In the present study, we used a "druggable-genome" siRNA library (Ambion), targeting 7784 genes, to uncover participants of NOD2-dependent NF-kappaB signalling. For each gene, three different siRNAs were reverse transfected into HEK293 cells. 24h later, plasmids encoding for NOD2 and NF-kappaB-dependent reporter gene (dual luciferase) were added. Cells were stimulated with 10 µg/ml MDP and 48h after siRNA transfection, luciferase activities were determined. Resulting candidates modifiying the NF-kappaB activation were subjected to further screening procedures including altered siRNAs (pooled; changed supplier), stimulus (TNF-alpha), read-out (IL-8) and cell line (Caco-2) in order to stringently select for true positive candidate genes. In conclusion, the screen confirmed known pathway members of NOD2-dependent NF-kappaB activation (e.g. RIPK2 and RELA) and identified putative new interaction partners. Among these are PSMD2, PSMC3, PSMB3 and PSMB4 which highlight the importance of the proteasome for NF-kappaB signal transduction. A further identified candidate gene (ScreenHit13) that has reported function in tight junction formation was found to co-localize with NOD2 in intestinal epithelial cells (Caco-2 cells and human colonic biopsies) and to be implicated in resistance against infection with Listeria monocytogenes. Together, this screen has proven to identify novel players involved in NOD2-dependent signalling and intestinal barrier integrity, confirming the prominent role of NOD2-associated protein networks for epithelial homeostasis.

Poster Presentation Abstracts

Symposium IV

Systems Biology

ICHIP, the NGFNplus ENGINE database in the context of systems biology

Presenting Author: Chris Lawerenz
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Initially developed for medical genome research and subsequently tailored towards the needs of Cell Biology and Systems Biology, iCHIP (http://www.ichip.de) serves as the central data management and data analysis hub for the ENGINE project partners. iCHIP supports effective translational research by combining new technological approaches with consolidated sets of valuable patient sample data. Based on comprehensive clinical information and experimental data it is now feasible to generate model hypotheses about, e.g., the quality of putative targets for drug or therapy development.
The database system iCHIP has been extended with new functionality. In particular, we have integrated in fully automated fashion information about essential relationships between genes, transcripts, proteins and transcriptional regulators based on sequence alignments with ENSEMBL (EBI, Sanger) and RefSeq (NCBI).
Based on our regularly updated molecular annotation system we are now on the way to link experimental data in iCHIP to corresponding models of biological processes in the BioModels repository. Recently a collaboration with the EBI has been started to rework the existing BioModels software. A crucial feature is to connect experimental data in iCHIP with numerical components in BioModels DB. This will enable model-based simulations using experimental data from iCHIP. Joining our systems biology activities is based on an adaption of SysMo DB of the European SysMo consortium.
The broad functionality, technological enhancements and our joining activities are the facts which channel our IT landscape towards systems biology.

ConsensusPathDB – towards a more complete picture of cell biology

Presenting Author: Atanas Kamburov
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ConsensusPathDB is a meta-database that integrates different types of functional interactions from heterogeneous interaction data resources. Physical protein interactions, metabolic and signaling reactions, and gene regulatory interactions are integrated in a seamless functional association network that simultaneously describes multiple functional aspects of genes, proteins, complexes, metabolites, etc. With 155,432 human, 194,480 yeast and 13,648 mouse complex functional interactions (originating from 18 databases on human and 8 databases on yeast and mouse interactions each), ConsensusPathDB currently constitutes the most comprehensive publicly available interaction repository for these species. The Web interface at http://cpdb.molgen.mpg.de offers different ways of utilizing these integrated interaction data, in particular with tools for visualization, analysis and interpretation of high-throughput expression data in the light of functional interactions and biological pathways

Systems biology approach deciphers gene regulatory networks governing tumor-stromal cell communication in the pancreatic cancer

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Aim: The communication between tumor cells and surrounding stroma conveys aggressive progression of the disease and resistance to chemo-, radio- and immunotherapy in pancreatic cancer. Mathematical modelling of the experimental high-throughput screening data and the identification of interaction patterns which govern gene regulatory networks may provide a way of deciphering the double-paracrine information flow between pancreatic tumor and stellate cells. Methods: The in vitro modeling of tumor-stellate cell interactions was performed by genome-wide RNA profiling of cells co-cultered under different conditions in a time-resolved manner. ODE-based high-throughput complexity reduction CTRNN approach was applied to assess processing of information in each cell type and to identify specific clusters and patterns. A ranking of genes led to a selection of central-hub elements and was consequently used for TFBS analyses, reverse engineering and in silico simulations. Results and Conclusion: Our global gene expression analysis, ranking and modeling allowed to i) capture network dynamics and major regulatory elements in tumor (18 core genes) and stellate (4 core genes) cells, ii) to create models simulating intercellular interactions and iii) to test and experimentally validate reverse engineering approach. Identification of key players delivered a set of potential targets for clinical testing towards development of the novel therapeutics and personalized screening tools in pancreatic cancer.
Grant support: NGFNplus Verbundsprojekt Pankreaskrebs (PaCaNet 01GS08114)

Inferring Boolean networks via correlation

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The behavior of genetic regulatory networks is often described and analyzed using Boolean network models. In this kind of model, for each gene only two states are discriminated (active/inactive) and the dynamics of the network are described by Boolean functions. This model works with a very small set of parameters and thus represents a very stringent application of Occam's Razor, which makes it especially suitable for modelling large genetic networks [1].

The reconstruction of such Boolean networks from biological data requires the identification of causal dependencies within the network. To facilitate network inference for genetic regulatory networks we can take advantage of the fact that a specific transcription factor often will consistently either activate or inhibit a specific target gene. In this case, the observed regulatory behavior can be modeled by the use of monotone functions.

We show that Pearson correlation can identify the dependencies in a Boolean network containing only monotone Boolean functions. This approach enables the inference of Boolean networks based on an intuitive correlation measure. In contrast to the best-fit extension algorithm [2], the running time of this approach does not depend on the input degree of the network and only increases quadratically in the number of network nodes. In our experiments, we could reconstruct large fractions of both a published E. coli transcriptional regulatory and metabolic network from simulated data as well as a yeast cell cycle network from microarray data.

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DIPSBC – Data Integration Platform for Systems Biology Collaborations

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Modern biomedical research is often organised in collaborations sometimes involving dozens of labs worldwide. In particular, in genome research and systems biology complex molecular systems are under investigation that need the generation and interpretation of heterogeneous data for their explanation, for example ranging from gene expression studies and mass spectrometry measurements to experimental techniques for molecular interactions and functional assays.
Extensible markup language (XML) has become the most prominent way for representing and exchanging these data. However, besides the development of standards there is still a fundamental lack of data integration systems that are able to utilise these exchange formats, organise the data in an integrated way and link it with applications for data interpretation and analysis.
Here we present DIPSBC, Data Integration Platform for Systems Biology Collaborations, an interactive data integration architecture supporting collaborative research projects. All components of the system are open-source developments, and, thus, can be quickly adopted by researchers. An exemplary installation of such a collaboration platform is provided at http://dipsbc.molgen.mpg.de. DIPSBC is currently in use in five different collaborations projects such as the NGFN-plus projects MUTANOM, Modifiers and the NGFN-transfer project NT-CVD.

Kinase-targeted proteomics after Hsp90 inhibition reveals new clients and differences in the response of primary and cancer cells

Presenting Author: Armin Haupt
Armin Haupt1, Gerard Joberty2, Holger Froehlich2, Marcus Bantscheff2, Henning Stehr1, Michal Schweiger1, Michael Lappe1, Hans Lehrach1, Cayetano Gonzalez3, Gerard Drewes2 and Bodo M.H. Lange1

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The heat shock protein 90 (Hsp90) is required for the stability of many kinases, receptors and mutated oncogenes. Hsp90 is an attractive target of cancer therapy, because its inhibition causes the simultaneous degradation of these proteins affecting multiple signalling pathways in parallel. We investigated the effect of Hsp90 inhibitor geldanamycin on the kinome of human primary fibroblasts and three cancer cell lines using western blotting and isobaric tags (TMT) quantitative mass spectrometry with prior kinase enrichment (Kinobeads (TM)). We quantified in total 148 kinases most of which are degraded after Hsp90 inhibition. In addition down-regulation of many known Hsp90 client proteins underscores the consistency of our data with previous work. We present 51 new potential Hsp90 substrates representing kinases of diverse signalling pathways like MAPK and BMP signalling. A number of the investigated kinases are more strongly affected in cancer cells, an effect that is in part cell line dependent. Second generation sequencing identified missense mutations in seven destabilized kinases. Structural modelling suggests a potential role of mutated residues on the structure of these kinases that possibly affects the interaction with Hsp90. Our work for the first time systematically investigates the differences between primary and cancer cells upon Hsp90 inhibition on the level of regulatory kinases and reveals cancer specific and common responses between cancer cells and primary non transformed cells.

Computational analysis of immunofluorescence images for scoring visual phenotypes relating to cell proliferation and cell division

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The availability of automated fluorescence microscopy has opened the possibility to perform large-scale cellular imaging in functional genomics. The multi-parameter read-outs in microscopy-based high content screening (HCS) assays thereby facilitate the quantitative description of complex phenotypic patterns on a cellular and subcellular level. This provides quantitative und time-resolved data on single gene function and, from complex synthetic screens, information on the role of protein groups in signalling pathways.
Here, we performed an image-based screen to elucidate the functional relevance of centrosomal phosphoproteins throughout the cell cycle. Using a mass spectrometry approach we identified 27 phosphoproteins as being part of the centrosome proteome in Drosophila embryos. Using RNA interference (RNAi) in cultured Drosophila cells and computational image analysis we then investigated the phenotypes after protein depletion. Image-based read-outs such as the number and size of the centrosomes and data on chromosome segregation aberrations revealed redundant and non-redundant functions of the phosphoproteins in centrosome replication and maturation and cell division pathways. Moreover, using a combinatorial RNAi approach, we identified functional interactions of the proteins with four major protein kinases (polo, aur, cdc2 and CkIIß) and demonstrated their integration into the kinases' signaling pathways in a hierarchical manner.
The above study is an example of the potential of image-based screens for the automated and unbiased scoring of complex visual phenotypes in cell proliferation and cell division pathways. Image-based assays will also be employed to study additional cancer related traits relating to DNA damage and cell morphological changes arising as a consequence of disease related protein overexpression of wild type and mutant variants.

Proteomic and functional characterization of oncogenes and tumor suppressors in a cancer tissue culture model in the IG Mutanom project

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International sequencing efforts identified a large number of cancer-associated mutations in a wide variety of tumor types. In spite of these efforts, the functional relevance and molecular consequences of these mutations for tumorigenesis and tumor progression often remain to be investigated. The Mutanom consortium (www.mutanom.org) aims to characterize frequently occurring mutations in a systems biology approach. This project combines genomics, proteomics, cellular assays with data from model organisms and the clinic.
Using inducible isogenic acceptor cell lines expressing wildtype or mutated oncogenes/tumor suppressors, we identify differences in protein complex composition using Tandem Affinity Purification (TAP) followed by mass spectrometry. The use of isobaric labeling allows the quantitative analysis of complex composition, comparing wild type and mutant state. In addition, proteome profiling and KinobeadsTM approaches provide a quantitative readout on the effect of oncogene/tumor suppressor expression for a large cohort of proteins and enriched kinases, respectively.
The main pathways we are currently analyzing are the PI3-kinase pathway, the MAPK pathway and the DNA damage response. Results are integrated in a systems biology approach with data obtained from proteome analyses, second generation sequencing and mRNA profiling experiments to generate a predictive model of cancer pathways.

Proteomic and functional characterization of driver mutations in MAPK signaling pathway - a systems biology approach

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Second generation and classical sequencing approaches have discovered a large number of cancer mutations (http://www.sanger.ac.uk/genetics/CGP/cosmic/). However, the functional and molecular consequence of the majority of these mutations is unclear. The Mutanom consortium (www.mutanom.org) aims to characterize frequently occurring mutations in a systems biology approach. This project combines genomics, proteomics, cellular assays with data collected from model organisms and from the clinic.
Mutations in RAS-ERK signaling pathway often lead to uncontrolled cell growth and tumor expansion. Our initial target list comprises KRAS (wt and 5 mutants), BRAF (wt and 2 mutants) and SRC (wt and 1 mutant). Therefore we generated isogenic cell lines that effectively express inducible wild type or mutated bait protein. Protein complexes are isolated by Tandem Affinity Purification (TAP) and analyzed by quantitative mass spectrometry using isobaric labelling. Comparative analysis of mutants vs. wild type proteins deciphers specific interaction partners. Function of these interactors will be subsequently studied using cell-based approaches (e.g. proliferation, apoptosis, adhesion and migration assays). Additionally, proteome profiling and KinobeadsTM technology are designed to provide a quantitative readout of the effect of oncogene/tumor suppressor expression for a large set of proteins and kinases, respectively.
This project also tests newly synthesized compounds/inhibitors on BRAF wt, BRAF V600E and BRAF V600K. Application of these compounds on isogenic cells will facilitate understanding of cancer specific signaling events.
Results obtained by proteome analyses, second generation sequencing and mRNA profiling experiments will be integrated in a systems biology model, which is aimed to generate a predictive model of cancer pathways.

IG Mutanom - Systems Biology of Genetic Diseases

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Cancer, like many other diseases, is caused by disturbances in the complex networks of biological processes in the organisms. Prevention, diagnosis and therapy of these diseases require a detailed understanding of these processes in health and disease. Application of techniques from the area of functional genomics on the individual patient, combined with the development of systems, that are able to model the disease process are now required. The Mutanom project (WWW.MUTANOM.ORG) is an Integrated Genome Research Network (IG) funded through the NGFN Plus Research initiative. The IG Mutanom aims to characterise the functional consequences of somatic mutations and to develop Systems Biology models that predict the outcome of such genetic alterations on a molecular pathway level, cellular and organism level. Initially our effort will concentrate on characterising „driver“ mutations i.e. mutations that occur in cancer due to selective pressure promoting cancer progression. A core set of mutations that frequently occurs in breast, prostate and gastrointestinal cancer tissues has been identified (COSMIC) and additional mutations have been selected through new generation sequencing approaches. A predictive model is currently been developed from the quantitative molecular information on signalling pathways obtained from combining functional genomics, proteomics, cellular assays, model organism and clinical data. The developed model and pathway information can then be applied to other genetic diseases and will be systematically exploited to identify new drug targets and improve our understanding on the action and side effects of drugs. Hence, we expect this approach and the combined infrastructure to become a key instrument in improving diagnosis and therapy of cancer and many other complex diseases. The aims and overall structure of the project will be reported here.

Systems Level Analysis and Modeling of Cancer Pathways

Presenting Author: Christoph Wierling
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Cancer is known to be a complex disease and its therapy has turned out to be difficult. Much information is available on molecules and pathways involved in cancer onset and progression. By processing literature information and pathway databases of twenty different signaling pathways known to be relevant for cancer (like Wnt, Notch, BMP, Fas, Trail, EGF, IGF, Hedgehog, etc.), we have developed a large mathematical model of these pathways. Although the development of large detailed mathematical models is difficult, the benefit one could gain using their predictive power is tremendous. The development of such detailed mathematical models is not only hampered by a limited knowledge about the topology of the cellular reaction network, but also by a highly restricted availability of detailed mathematical descriptions of the individual reaction kinetics along with their respective kinetic parameters. To overcome this bottleneck we introduce an approach, based on a Monte Carlo strategy, in which the kinetic parameters are sampled from appropriate probability distributions and used for multiple simulations in parallel. Results from different forms of the model (e.g., a model that resembles a certain mutation or the treatment by a drug) can be compared with the unperturbed control and used for the prediction of the effect of the perturbation. The established resources, tools, algorithms and models build a foundation for the application of systems biology strategies in medical and pharmaceutical research and, based on data from high-throughput genome, transcriptome, and proteome analysis, it enables the development of a personalized medicine.

Modelling the differentiation of human embryonic and induced pluripotency stem cells into hepatocyte-like cells

Presenting Author: Andriani Daskalaki
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Embryonic and induced pluripotent stem cell differentiation into hepatocytes is of high importance. These cells can be used in the treatment of severe liver diseases instead of whole organ transplantation.
During the process of in vitro differentiation into hepatocytes, gene expression analysis has led to the identification of numerous potential target genes and associated signaling pathways that might be involved in hepatogenesis in vivo. Ligands such as FGF4, BMP 2 and HGF are considered to play important roles in hepatogenesis. ACTIVIN A induces differentiation of human embryonic stem cells (hESCs) and human induced pluripotency stem cells (hiPSCs) into definitive endoderm defined by the expression of FOXA2, CXCR4, SOX17. Further supplementation with FGF4 and HGF induces hepatic initiation.
Our aim is to simulate the differentiation into hepatocytes including genes encoding proteins that play an important role in differentiation to endoderm based on a model of ordinary differential equations (ODE). Therefore, we implemented a mathematical model of signaling pathways and regulatory networks based on biological pathways that are important in the process of differentiation of hES and hiPS into hepatocytes.
Pathway annotation of the network was performed manulally based on literature information and the Consensus Pathway Database (CPDB). Computational modelling of cell differentiation requires the translation of the pathway design into a computer model that can convey information on the concentrations of the model components and on the kinetics of the reactions these components are involved in. This workflow contains the design of suitable computer objects, the implementation of the reactions, the assignment of kinetic laws to these reactions and the model analysis.
Modelling was performed with the PyBioS software, an object-oriented tool for modelling and simulation of cellular processes.

Modeling Cancer using Deep Sequencing Technologies for Personalized Cancer Treatment

Presenting Author: Alexander Kühn
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Given the reacent increases in the capacity of next-generation sequencing systems from companies like Applied Biosystems, Roche, and Complete Genomics, the integration and analysis of genome, epigenome, transcriptome, and proteome data is an important issue. It will soon be commercially feasible to apply deep sequencing in the clinic at the full-genome level to clinical patient samples. Since the routine clinical application of such technologies is just a few years away, we are presenting a “Virtual Patient” system that has the capacity to integrate these types of data in a “holistic” and comprehensive fashion. This system allows us to create predictive models out of all the information available from any deep molecular characterisation technology, and to make testable predictions about the cell line or patient from which the sample came. In particular for cancer patients, we can now predict in a dose-dependent manner which cancer drugs or drug combinations should be prescribed, and which will have little or no value on a particular patient.

Analysis and Modeling of Hedgehog/GLI Signaling and Regulatory Networks in Cancer

Presenting Author: Hendrik Hache
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Several investigations indicate that persistent activation of the Hedgehog (HH)/GLI signal plays a critical role in the initiation and growth of a number of human malignancies including prostate, breast, brain, skin, and pancreatic cancer. However, the detailed mechanisms of HH/GLI target genes and their associated signaling pathways and regulatory networks in cancer formation are still not well understood. Using the PyBioS modeling system we have developed a model prototype based on knowledge from literature and pathway databases. Our current model comprises more than 830 biological components of 21 different cancer related signaling pathways. The part of the model prototype which represents the current knowledge with regard to HH/GLI related processes is refined within this project. We have performed several global time course measurements of gene expression with thirteen time points under defined stimuli of EGF and GLI. Several known direct EGF and GLI targets show significant response after such stimuli. The Identification of the underlying gene regulatory network is supported by reverse engineering strategies. We will apply a model driven reverse engineering algorithm based on neural networks for the identification of gene regulations. Newly identified targets will be integrated to refine our model. This new model will be used for the generation of predictions and suggestions of further experiments in the transcriptome and interactome by in silico experiments using PyBioS. The refinement and validation is done in an iterative manner. Our results improve the understanding of the complex molecular networks regulated by oncogenic HH/GLI signaling and will accelerate the search for novel molecular targets that represent an opportunity for therapeutic intervention.

Establishing Network-Analysis-Oriented Computational Biology (NAOCB) platform for translational research in oncology

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We describe the establishment of a Network-Analysis-Oriented Computational Biology (NAOCB) platform, focusing on network-analysis-driven approaches to solve computational biology challenges. The potential of this platform has been successfully explored by targeting current key issues in the oncology translational research, for instance data analysis of high-throughput screening for drug targets, MoA analysis of drug resistance, as well as novel biomarker identification.

Here we demonstrate the power of this platform by network analysis principles, practices and outcomes in three biomedical settings. Using data mining approaches, we were able to earn new perspectives in cancer by using existing function/pathway information alone, or map heterogeneous experimental and clinical data (i.e., profiling, NGS and proteomics) to biological networks. By employing qualitative network-analysis skills, we discovered intrinsic regulatory motifs from multiple-output proteomics high-throughput screening. And finally by using quantitative network models, we could gain deeper understanding and novel knowledge from the ErbB system, which is essential to understand to design therapy with better safety and efficacy profiles for several cancer types including breast cancer.

The exploratory data analysis, integration and network analysis using this platform have led to downstream experiment and clinical trials. Works with this platform have produced novel knowledge and findings that have not been possible without systematic exploration with network-based approaches. We believe the Network-Analysis-Oriented Computational Biology (NAOCB) platform will facilitate the discovery and elucidation of patterns hidden under complex networks in highly systematic diseases like cancer, and the information learned from networks will help researchers to improve the life quality of patients.

Catching the dynamics of RNAi phenotypes during a human kinome screen

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Analysis of biological processes is frequently performed with help of phenotypic assays where data is mostly acquired in single end-point analysis. We have carried out a whole kinome screen to capture dynamic RNAi phenotypes employing real-time monitoring of cell growth with help of impedance measurement and a real time cell analysis (RTCA) system. Computing the first derivative of the acquired data, i.e. the slope of phenotypic changes, this system proves suitable for large-scale screening. The kinome screen validated previously identified inhibitor genes and, additionally, identified activators of cell growth. The high-content of RTCA data with respect to time-resolved measurements allowed us to investigate the dynamics of RNAi phenotypes. The data indicates that impedance measurement is able to pick up the timing of RNAi phenotypes. Maximum effect sizes during the assay correspond to cellular functions that are associated with the onset of phenotypes in response to the knockdown of target genes, as we could establish cell cycle genes to be among the first displaying inhibiting effects and migration-related genes for activators. RTCA data thus indicates a biological protein half life, i.e. the time at which the protein's concentration reaches a threshold level that is required to maintain cellular homeostasis. Our data thus on the one hand establishes RTCA technology as a novel tool amenable for the high-throughput screening of dynamic cellular phenotypes that are induced by RNAi and likely also by other perturbations and on the other hand has identified a number of kinases that stimulate cell growth when being downregulated.

A Genome-wide miRNA screen identifies novel regulators of NF-kB signaling pathway

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The Nuclear Factor-kappa B (NF-kB) signaling pathway is involved in a variety of biological processes, including inflammation and cell survival. Deregulation of the NF-kB pathway plays a critical role in many diseases, such as cancer development and progression. MicroRNAs (miRNAs) are small non-coding RNAs regulating post-transcriptional gene expression by translational repression or mRNA degradation. The aim of this study is to identify and characterize miRNAs that regulate the activity of the NF-kB signaling pathway using a miRNA mimic-library covering 810 human miRNAs. For the miRNA screen a reporter plasmid carrying three consensus NF-kB binding sites followed by a luciferase gene was used. Changes in NF-kB activity after transfection of miRNAs were quantified by assaying luciferase activity. The feasability of this system was proven by knocking down main players of the NF-kB pathway as positive controls. The hits from the screen were further validated by nuclear translocation assays. Furthermore, the target genes of these miRNAs in the extended NF-kB network are validated using a dual luciferase assay. Finally, identified miRNAs will be examined in cancer-related assays and also in patient samples to determine their oncogenic potential. The outcome of this project will help to better understand the activation and deregulation of the NF-kB pathway which is of pharmacological importance, mainly because of its role in inflammation and cancer. Moreover, a result of this screen might be the identification of novel drug targets that could be exploited to modulate the NF-kB signaling pathway in disease processes.

Impact of targeted therapeutics on ligand-induced signaling in breast cancer cell lines

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Breast tumors can be categorized into different molecular subtypes. In 20-25 % of breast tumors, the orphan receptor ERBB2, a member of the EGF receptor family, is overexpressed. A targeted therapy applying the ERBB2 targeting antibody trastuzumab is already clinically approved. As only 30 % of the patients benefit from trastuzumab therapy and almost all patients develop resistance within one year, further targeted therapeutics and combinatorial therapies are under investigation. Our aim was to identify novel therapeutic strategies to improve efficacy. Thus, quantitative protein microarray-based proteomics were applied to unravel the impact of the ERBB2 targeting antibodies trastuzumab and pertuzumab on ligand-induced signaling. In addition, the EGFR inhibiting small molecule erlotinib was included. Targeted therapeutics were applied alone and in combination, and the turnover of > 20 phosphoproteins was measured in a time-resolved manner. The data revealed that the combination of trastuzumab and erlotinib showed the strongest inhibitory impact on fast, ligand-induced signaling and on cell cycle progression. Furthermore, the time course data is used to calculate a dynamic model of ERBB receptor signalling with respect to the systems behaviour in order to identify key regulatory elements that could be potential targets for a novel combinatorial therapy.

Oestrogen-receptor pathway signalling and genomics in breast cancer

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Even though the oestrogen-receptor pathway has been the subject of intensive biochemical and genetic studies we are missing largely the relevant molecular information on tumour development and on dynamic protein interactions. In particular more quantitative approaches are required to gain better understanding of the molecular action of compounds that modulate oestrogen pathways and relevant downstream events in mamma cancer on a cellular and organism level. Here, we are applying a tissue culture cell model to elucidate molecular changes of oestrogen receptor (ER) signalling by systematically dissecting the pathway components via RNAi in combination with functional cellular assays. A second focus of this project is to analyse the influence of cancer associated fibroblasts (CAF) on different breast cell lines (e.g. MCF-10A, MCF-7) in the context of ER signalling and relevant chemotherapeutic treatment. Therefore we will characterize the effect of the co-culture of CAF and breast cancer cell line by Reverse Phase Protein Array (RPPA) and analyse their interaction in 3D cell culture on a proteomics, mRNA expression and cytological level.
The primary goals of this project are: (a) to analyse the aberrations of oestrogen receptor pathway in the cell model by mRNA and protein profiling in combination with functional cellular assays and second generation sequencing approaches (b) to evaluate the relevance of anti-cancer therapies through detailed analysis of the response to drug treatment (e.g. tamoxifen, parthenolide, rapamycin, gefitinib) in ER positive and ER negative cell lines on the oestrogen receptor pathway and its signalling components (e.g. mTOR, AKT; PI3K).

QuantProReloaded: Software for the statistical analysis of Microspot Immunoassays

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Methods to acquire quantitative proteomic data allow a comprehensive investigation of cellular signaling pathways and thus a better understanding of molecular pathogenesis as well as the identification of new biomarkers.
Microspot Immunoassays (MIAs) [1][2] enable quantitative and parallel measurements of the abundance and activity levels of many proteins in one experiment. This technology is superior over standard technologies like Enzyme Linked Immunosorbent Assay, because of its low sample consumption, high sensitivity and high specificity. However, data analysis and visualization is still a bottleneck. Thus we introduce QuantProReloaded (http://code.google.com/p/quantproreloaded/) an open source tool designed for the analysis of time-resolved and non-time-resolved data from MIAs. QuantProReloaded is build on ideas of QuantPro [1], but is a completely new implementation with major changes of the analysis workflow and new functions for the analysis of non-time-resolved MIA data. At the moment QuantProReloaded provides two main functions: The performance analysis shows how accurate the concentration can be estimated from the calibrator curve fitted on raw standard data. The other function named measurement analysis estimates the target protein concentration within each spot based on the calibrator curve. The output of QuantProReloaded is a calibrator plot with the estimated concentrations as well as one table including the estimated concentration levels for each spot and another table with the median concentrations. For data derived from time-course experiments, a time-course plot facilitate the direct comparison between median changes of a certain target protein under different conditions at different time points. Analysis functions of QuantProReloaded are written in R. The user interface is written in Java.

LifeDB in a new Design: Get new insights from experimental data

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A different and more comprehensive view is often needed to get new insights from experimental data and to choose the correct candidate genes, proteins and clones for experiments.
Here we present LifeDB, an application which integrates subcellular localization data of proteins, cDNA cloning information, protein interaction and genomic data.

LifeDB enables the fast and easy identification of high quality human full length protein coding ORF clones which can be used for e.g. Subcellular localization experiments. Clones from the division of Molecular Genome Analysis as well as partners of the Orfeome collaboration have been integrated and mapped to various external database identifiers. By this approach users of LifeDB are able to search for clones by entering gene lists containing different kind of gene and protein identifiers. Furthermore, a search for proteins by their subcellular location is provided, which is derived from high content microscopy localization experiments. To identify the most suitable clone, a quality score assigned to each clone is shown, which provides Information about the clone sequence similarity in comparison to annotated human reference genes.

In a new release of LifeDB the possibility to compare protein networks for different cell lines will be available to identify new candidate genes, proteins and cell lines for further experiments. Therefore interaction and pathway data from different external databases, e.g. KEGG, have been integrated in LifeDB and are combined with experimental data from several breast cancer cell lines e.g. from in house Next-Generation sequencing experiments and external data sources. Networks retrieved from gene, protein or clone lists can be visualized, explored and compared of cell line specific pathways by highlighting functional relevant mutations as well as copy number alternations in genes/proteins.

LifeDB is implemented in an easily extensible JavaEE structure and provides an user-friendly web-interface.

miR-200bc/429 cluster targets PLCγ1 and differentially regulates proliferation, apoptosis and EGF-driven invasion than miR-200a/141 in breast cancer

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MicroRNAs (miRNAs) are small non-coding RNAs and regulate the expression of genes post-transcriptionally. The genes of the miR-200 family map to fragile chromosomal regions and are frequently downregulated upon tumor progression. Although members of miR-200 family have been reported to regulate epithelial-to-mesenchymal transition (EMT) and TGF-ß-driven cell invasion, there are no studies until now showing the role of individual members of the miR-200 family, especially of the miR-200bc/429 cluster, on breast cancer tumorigenesis. In this study, we showed for the first time that miR-200 family members differentially regulate EGF-driven invasion, viability, apoptosis, and cell cycle progression of breast cancer cells. We demonstrated that the miR-200 family members regulate EGF-driven invasion, with the miR-200bc/429 cluster showing stronger effects than the miR-200a/141 cluster. Furthermore, expression of the miR-200a/141 cluster results in G1 arrest, whereas that of the 200bc/429 cluster increases G2/M phase and induces apoptosis. Genome-wide microarray profiling in combination with gain-of-function studies identified PLCG1, which was downregulated only by the miR-200bc/429 cluster, as a potential candidate contributing to this difference. Direct targeting of PLCG1 by the miR-200bc/429 cluster was validated via luciferase reporter assay and site-directed mutagenesis. Finally, loss of PLCG1 in part mimicked the effect of miR-200bc/429 overexpression in viability, apoptosis and EGF-driven cell invasion of breast cancer cells. Our results suggest that the miR-200 family has a tumor-suppressor function by regulating EGF-driven cell invasion, viability, apoptosis and cell cycle progression in breast cancer.

Homology model of Abcc6 to provide insight into the function of mutations causing cardiovascular phenotype

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Mutations in the Multidrug resistance-associated protein 6 (Abcc6) have been demonstrated to cause pseudoxanthoma elasticum (PXE) in human as well as dystrophic cardiovascular calcification (DCC) in mice. A case-control study indicates that mutations in the Abcc6 gene are not rare in the general population and contribute to an increased propensity toward premature atherosclerotic vascular disease. Abcc6 is a member of the ATP-binding cassette sub-family C and involved in the transport of molecules using ATP. The exact biological function of Abcc6 is presently still unknown, same as the functional relationship of this transmembrane transporter to the pathogenesis of atherosclerosis or calcification.
Mouse models deficient in Abcc6 confirm the functional role of the gene for cardiovascular phenotypes. Moreover, our group identified several mutations within the Abcc6 gene in mice predisposed to DCC. These mutations lead to amino acid (aa) substitution that in turn may affect the function of Abcc6. Seven aa substitutions were found to severely influence the function of Abcc6.
We have built a homology model of this Abcc6-transporter, and analysed the position of the mutation within the predicted structure. The mutations present in the transmission interface of the transporter, which is involved in domain-domain interaction of Abcc6. This indicates the importance of the region in the function of the transporter. 2009 Fülöp et al. also suggest the importance of this region in context of functionality. As a result of these findings the molecular phenotype should be verified by dynamic simulation and genetic studies.

Potential role of microRNA-29b in atrial AF-promoting fibrotic remodeling

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Introduction
Heart failure (HF) causes an atrial fibrotic substrate for atrial fibrillation (AF) maintenance, but the underlying mechanisms are poorly understood. MicroRNA 29b suppresses collagen production and decreased miR29b expression promotes fibrotic responses. This study assessed the potential contribution of miR29b changes to HF-induced atrial fibrotic remodeling.
Methods
Experiments were performed in a canine HF model of AF and atrial fibrosis induced by ventricular tachypacing (VTP, 240 bpm). We studied 4 groups: 24 h, 1 wk, 2 wk VTP and control (CTL) dogs (n=8/group). Expression of miR29b and target genes collagen COL1A1, COL3A1 and fibrillin (FBN) in left atrial (LA) tissue and in isolated LA fibroblasts (AFBs) was assessed by real time PCR.
Results
After 1 wk, VTP remodeled atria showed increased AF duration and fibrosis, which remained elevated at 2 wk VTP. MiR29b expression fell rapidly after 24 h VTP and remained reduced at 1 wk and 2 wk VTP, while target gene expression showed a significant increase in COL1A1, COL3A1, and FBN after 2 wk VTP. To assess regulation of miR29b by TGFß , we examined the effect of 48 h TGFß treatment on AFB gene expression. TGFß decreased AFB expression of mir29b and increased AFB expression of COL1A1. To further evaluate the potential role of miR29b in altered AFB ECM gene expression, we studied the effects of lentiviral-mediated miR29b-knockdown and miR29b-overexpression in AFBs. Mir29b-knockdown increased the expression of fibrotic genes COL1A1 and FBN, while mir29b-overexpression decreased AFB expression of COL1A1 and COL3A1.
Conclusions
VTP-induced HF causes a rapid and sustained decrease in miR29b expression, which is followed by increased expression of fibrosis-related miR29b-target ECM genes. TGFß induces miR29b downregulation in AFBs. In vitro knockdown of miR29b mimics in vivo changes, suggesting a causal role of mir29b downregulation in CHF-related generation of atrial fibrosis via TGFß signaling.

Physiological aging alters metabolism of glutamate and glutamine in the human brain in a gender-related pattern

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Introduction: Glutamate (Glu) is the major excitatory neurotransmitter in the human brain located predominantly in neurons. Glutamate induced excitotoxicity is involved in various neurodegenerative disorders. Furthermore, there is evidence that the glutamate concentration is altered with the normal aging process of the brain. Glutamine (Gln) is the precursor of Glu and is found primarily in glial cells. Steroidal sex hormones have been shown to influence the cerebral glutamate/glutamine metabolism, suggesting gender-related differences of brain glutamate (Haghighat 2005; Krause et al 2006). An age-related decline of Glu accompanied by an simultaneously rise of Gln has been reported previously, suggesting an altered metabolism of Glu/Gln in the aging human brain (Kaiser et al 2005). Aim of this study was to determine the effects of age cerebral concetrations of Glu and Gln in a large sample of healthy subjects.
Methods: In 118 healthy subjects the absolute concentrations of Glu, Gln and N-acetylaspartate (NAA) in the left hippocampus and the anterior cingulated cortex (ACC) were investigated, using MRS at 3 Tesla and a well established quantification procedure.
Results: An age-related decline of the concentrations of glutamate (ACC: $r=-0.370$, $p<0.001$; hippocampus: $r=-0.227$, $p=0.016$) and NAA (ACC: $r=-0.187$, $p=0.042$; hippocampus: $r=-0.167$, $p=0.074$) in both voxels were observed, whereas glutamine is positively correlated with age in the ACC ($r=0.288$, $p=0.010$). In the female subgroup, the age-related declines of NAA in the hippocampus ($r=-0.430$, $p=0.004$) and of Glu in the ACC ($r=-0.551$, $p<0.001$) were more pronounced than in the male group.
Conclusion: The results demonstrate opposed effects of age on glutamate, glutamine, and NAA concentrations in healthy human brain. The shown age-related alterations were found to be gender-dependent. The results may add to growing evidence for age- and gender-related difference in cerebral neurotransmission, metabolism and structure.

From KEGG to dynamic pathway models: a collection of tools to facilitate the modeling of biochemical networks

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Modeling of biochemical processes has gained lots of attention. In several projects, researchers laboriously gather reaction pathways and kinetic information with the aim to construct large-scale biochemical network models. When dealing with these models dedicated software tools are indispensable to facilitate the complicated and highly error-prone model building, simulation, and calibration process. Here we present a collection of specifically developed tools. (i) SBMLfromKEGG creates a network topology from pathways in the KEGG database and stores it in SBML format together with semantic information in systems biology ontology and MIRIAM format (Minimal Information Required In the Annotation of Models). It also includes all graphical annotation in form of CellDesigner tags. (ii) SBMLsqueezer then equips the reactions within such an annotated network with kinetic equations considering the context of each reaction. The units of all newly introduced parameters are derived automatically and the sizes of compartments are also taken into account to assure consistent models. (iii) The combination of SBMLsimulator and the heuristic optimization framework EvA2 estimates the values of all parameters with respect to given time-series of metabolite or gene expression values. Experimental validation of the model decides if refining previous steps is necessary. (iv) SBML2LaTeX exhaustively documents the resulting model in a PDF report. (v) The Java library JSBML has been especially developed as the basis of all these modeling steps and can be easily adapted and modified if necessary. All programs introduced above are open-source tools and can freely be downloaded for academic purposes and can therefore be applied in a higher-level algorithm. In this way, creation, optimization, and documentation of complex pathway models can be easily performed with this collection of convenient tools.

Confidence scoring and prediction of protein-protein-interactions by integrating network topology with functional protein annotations

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A frequent problem in molecular biology is to determine whether two given proteins physically interact or not. Various computational approaches aim to assist the laborious experimental work by either predicting protein-protein interactions (PPI) de novo or by distinguishing between true interactions and false measurements in large noisy experimental screens. These computational approaches usually exploit protein properties (such as their sequence, structure, domain composition and function) or the graph topology of the network of already known PPIs.
We address here the PPI prediction problem by integrating properties of both the proteins and the network in a novel way. In our ongoing work we generalize the common notion of functional similarity between a protein pair to the transition probability between two protein neighborhoods. This conditional probability is modelled in a Markov chain and trained by performing random walks in a large integrated human PPI network. Additionally, we consider global network topological features to assign scores to protein pairs that reflect the probability of the protein pair to interact under physiological conditions. We show how the subscores alone perform well and can compete with other approaches by integrating them into a machine learning classification framework. We compile a gold standard positive training set consisting of highly reliable interactions for which multiple experimental evidence exists. For the selection of negative non-interacting protein pairs we propose a new random selection procedure that avoids common pitfalls which have been shown to bias the predictive performance of the classifier.
Our solution to the PPI prediction problem is both novel in the exploited features as well as competitive in terms of running time and performance.

Pathway analysis of genome wide association data for unipolar depression

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High density arrays for genome-wide association analysis are a valuable tool to identify common susceptibility variants for complex disorders, including neuropsychiatric disorders. However, due to single-locus case-control comparisons risk factors with small effects are difficult to detect. One way to reduce the complexity of the genetic tests is to include biological and functional pathway and network information. The analysis of SNPs in groups predefined by biological knowledge as the unit of analysis can increase the power to detect association between genes and disease.
A method analyzing data from genome-wide association screens was implemented in the context of biological pathways. The globaltest was originally developed for gene expression data and can be applied to categorical variables. To assign genes to SNPs including regulatory and functional regions, we used all SNPs lying within 20 kb of the 5’ and 3’ ends of the first and last exons, respectively. We applied the globaltest to SNP data coding the SNP effect as allele dosis effect (0,1,2), major homozygote only (0,0,1), heterozygote only (0,1,0). Groups of genes were defined using several pathway databases such as KEGG, Gene Ontology, and Transpath. We analyzed a data set on unipolar depression comparing the genome wide SNP status of 600 affected and 1300 control samples. Results of this analysis will be presented.

Nascent transcriptome analysis (NTA-Chip) reveals distinct functional networks regulated during early cytomegalovirus infection

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During lytic viral infection cellular gene expression is subject to rapid, gross alterations induced by both viral and antiviral mechanisms. These can not be properly resolved by standard expression profiling using total cellular RNA due to its low sensitivity for short-term changes in gene expression. In addition, alterations in RNA synthesis and decay can not be differentiated.
Recently, we developed nascent transcriptome analysis (NTA) to overcome these problems. This method is based on biosynthetic tagging of nascent RNA using 4-thiouridine. Total cellular RNA of 4-thiouridine treated cells is isolated and separated into nascent and untagged pre-existing RNA by thiol-specific biotinylation and streptavidin-coated magnetic beads.
We applied this method to study differential gene expression during the first six hours of infection of murine NIH-3T3 fibroblasts with murine cytomegalovirus using both host and viral microarray analysis. In contrast to microarray analysis of total cellular RNA, NTA-Chip revealed discrete functional networks of genes regulated with distinct kinetics, e.g. we found genes involved in cytoskeleton and extracellular matrix formation to be rapidly and consistently down-regulated upon MCMV infection indicating novel mechanisms by which the virus alters its host cell environment at transcriptional level. In summary, this study provides the first comprehensive analysis of the temporal kinetics and interplay between lytic virus replication and host defence during the early stages of virus infection.

MicroRNA profiling of EBV-associated diffuse large B-cell lymphoma and NK/T-cell lymphomas by deep sequencing

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The Epstein-Barr virus (EBV) is an oncogenic human Herpes virus involved in the pathogenesis of nasal NK/T-cell lymphoma. EBV encodes microRNAs (miRNAs) and induces changes in the host cellular miRNA profile. MiRNAs are short non-coding RNAs of about 19-25 nt length that regulate gene expression by post-transcriptional mechanisms. The microRNA profiles of EBV-positive vs. negative diffuse large B-cell lymphoma (DLBCL), NK/T-cell lymphoma, non-infected T-cell lymphoma and normal thymus were established by deep-sequencing of small RNA libraries. In both EBV-positive DLBCL and NK/T-cell lymphomas, no miRNAs from the BHRF1 cluster were detected in line with the previous finding that these miRNAs are only expressed in tumours arrising under immunosuppression. Furthermore, we identified 10 novel miRNAs from known precursors and two new miRNAs. The comparison of the EBV-positive NK/T-cell vs. EBV-negative T-cell lymphoma revealed 15 up- und 16 down-regulated miRNAs. Of these, only one was up-regulated and only two were reduced at least two-fold in both tumours relative to thymus. In the EBV-positive vs. negative lymphomas, a total of 33 cellular miRNAs were deregulated. The sequencing was confirmed for selected miRNAs by qRT-PCR. We identified various targets for the EBV-deregulated cellular miRNAs. The targets were confirmed by reporter assays involving the wt- and binding-site mutated 3'-UTRs and by down-regulation of the proteins by ectopic expression of the cognate miRNAs.

Prediction of transcription factor-DNA binding affinities using k-mers trained on protein binding microarrays

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Transcription factors (TFs) bind to specific DNA regulatory sequences to control the gene expression. However, only for a small part of TFs the binding preferences are known or sufficiently characterized. Modeling of the TF binding affinity to different individual genomic sequences is dominated by the Position Weight Matrix (PWM) model. The PWM model shows limitations such as the independent dealing of the residues sites or inability of modeling gaps in the binding sites. Recently, Berger et al. [1] developed a new protein binding microarray (PBM) technique, in which binding preferences of all possible k-mers are measured. Using PBMs, a more accurate analysis of high resolution protein-DNA binding data is possible.

We present a computational method called MASH6 for predicting TF binding preferences from PBM data. We study short k-mers (length 6-10bp, allowing for gaps) which additively contribute to the total affinity of the full sequence. In order to find the best k-mer (motif) which correctly describes the TF-binding affinity, we evaluate the models using randomly sampled sequences of the training set. The model giving the highest Spearman correlation is used to predict affinities for different genomic sequences. We have analyzed 66 mouse TFs and for each, looked at the overlap of predicted and measured top-100 probes with the largest affinity. On average, 25 of top-100 probes are correctly identified by the method. We observe an average Spearman correlation of 0.58 which can be favorably compared to other known methods.

[1] Berger, M.F. and Bulyk, M.L., Protein binding microarrays (PBMs) for rapid, high-throughput characterization of the sequence specificities of DNA binding proteins. Methods Mol Biol. 2006;338:245-260

Tracing the derivation of embryonic stem cells from the inner cell mass by single cell RNA-Seq analysis

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The molecular mechanism underlying the transition from inner cell mass (ICM) of blastocysts to pluripotent embryonic stem cells (ESC) is not fully understood, partly because of heterogeneity amongst a small group of cells which poses difficulties in investigation. Using single cell RNA-Seq transcriptome analysis, we analyzed the dynamic molecular network within individual cells from the ICM outgrowth and established ESC. This study shows that key genes which confer the property of self-renewal are up regulated as ICM cells progress to ESC. We detected significant global changes of transcript variants from individual genes, amongst which general metabolism genes are strongly over-represented. There was a global increase in expression of repressive epigenetic regulators with a concomitant decrease in gene activators. The unique ESC epigenotype may thus be sustained while retaining an inherent plasticity for differentiation. Moreover, changes in miRNAs result in one set that targets early differentiation genes and the second set targets ESC specific pluripotency genes to maintain a delicate balance between pluripotency and capacity for rapid differentiation.
In conclusion, our study provides insight into the dynamic and systematic molecular changes that occur during cell fate decisions from identical cells. We demonstrate how the retention of expression of a full set of master genes allows inheritance of pluripotency, while the up/down regulation of other crucial genes permits exit from a normal developmental program, which at the same time confers the key property of unlimited self-renewal. Changes in epigenetic regulators allow for the stable transmission and robust maintenance of the newly acquired epigenotype in ESCs between exceptionally fast cell generations. The conversion is also regulated by miRNAs, with distinct sets of these non-coding RNAs that allow self-renewal while the cells retain the potential to respond rapidly to cause differentiation.

Poster Presentation Abstracts

Symposium V

Transfer from Genomics to Application

Prediction of MHC II loading of tissue restricted antigens

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Central Self Tolerance is the capability of the immune system to tolerate our own tissues, while protecting the body from foreign pathogens. It is established through the negative selection of potentially autoreactive T cells in the thymus. T cells are induced to undergo apoptosis if their specific T cell receptor binds too strong to self antigens presented to them on MHC II molecules of medullary thymic epithelial cells. The self antigens are known to be tissue restricted antigens (TRAs), mirroring virtually all tissues of the body to the T cells. The composition as well as the molecular mechanism of TRA expression and MHC II loading is not well understood yet. Defining the TRAs and test their affinity for MHC II loading could help to shed more light onto the understanding of autoimmune diseases.
TRA detection was done by analyzing publicly available microarray data from the Novartis Foundation, with gene expression of 15,859 genes in 61 tissues of the mouse. Formerly defined TRAs were tested on the Net MHC II Server for their binding affinities to MHC II molecules in three available mouse MHC Alleles.
The binding affinity of tissue restricted antigens to the MHC II molecule varies between different TRAs. Some do not show any epitope which could bind at all, others have up to 20 binding motives in their protein structure that can be uploaded to MHC II. Since only self antigens that are capable of being uploaded to MHC II molecules can be presented to negatively select autoreactive T cells in the thymus, it is very interesting to calculate the number and binding affinities for all tissue restricted antigens (TRAs).
There is a clear difference in the binding affinity of different TRAs, which reveals which TRAs are important in the negative selection of T cells and which are not. This may help to understand better the molecular basis of autoimmune diseases in the future.

Early detection of colorectal cancer from patient blood plasma using microRNA-based RT-qPCR

Presenting Author: Adam Baker
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microRNA
microRNAs (miRNAs) constitute a recently discovered class of small RNAs (typically 21-23 nt) that function as post-transcriptional regulators of gene expression. Current estimates indicate that more than one third of the cellular transcriptome is regulated by miRNAs, and that each miRNA potentially regulates hundreds of different mRNAs. As a consequence, miRNAs have been proposed to be master regulators of cellular state. This hypothesis has been borne out by a large number of studies demonstrating a causal link between miRNA dysregulation and numerous disease states, including a diverse array of human cancers. Furthermore, the high stability of miRNA in common clinical source materials (e.g. FFPE blocks, plasma, serum, urine, saliva, etc.) and the ability of miRNA expression profiles to accurately classify discrete tissue types and disease states have positioned miRNA quantification as a promising new tool for a wide range of diagnostic applications.

Colorectal cancer screening
Colorectal cancer (CRC) ranks 4th in terms of prevalence and second in numbers of deaths among cancers of the western world. Although early detection of CRC leads to a favourable prognosis, and though CRC can easily be detected in the locally restricted state using a variety of diagnostic procedures, frequent late diagnosis means that CRC is still a leading cause of cancer mortality worldwide.
Current procedures suffer from one or more disadvantages within areas such as cost, safety, inconvenience to the patient with the consequence of low compliance, lack of trained personnel, sensitivity etc, thereby precluding their adoption as a population screening tool. There is therefore an unmet need for a generally acceptable CRC screening assay.

qPCR-based biomarker discovery

To facilitate discovery and clinical transfer of miRNA-based diagnostic markers, we developed a genome-wide LNA™-based miRNA RT-qPCR platform with unparalleled sensitivity and robustness. The platform uses a single RT reaction to profile >700 human miRNAs from 2 predefined 384 well plates and thus allows high-throughput profiling of miRNAs from important clinical sources without the need for pre-amplification. Using this system, we have profiled a large number of plasma samples from localized and regional CRC patients, and from matched healthy controls. An extensive QC system has been implemented in order to secure technical excellence and reveal any unwanted bias in the dataset. We will present our approaches to data normalization and the results of signature development using linear classification methods. We show that we can detect the majority of cancer cases with good specificity, using a cross-validation approach. In summary, our results show that minimally invasive early detection of CRC using a clinically viable approach is feasible.

Antibody microarray-based proteomic profiling of 24 pancreatic cancer cell lines revealed new leads characteristic to the disease, its degree of differentiation, source of cells, and the metastatic potential

Presenting Author: Mohamed Alhamdani
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Pancreatic adenocarcinoma is considered as one of the worst of all cancers. The difficulty to predict, detect, and diagnose it sufficiently early as well as its resistance to all current treatments except early surgery is the prime challenges facing this devastating disease. There is controversy about the carcinogenesis of the disease resulting from the observed histogenesis and early biochemical and genetic alterations. In vitro studies offer a useful tool to study physiologic, pathophysiologic, differentiation, and transformation processes of cells. In the present study, the cellular proteome of 24 pancreatic cancer cell lines was investigated using antibody microarray targeting about 800 cancer-related proteins. Network and pathways analysis demonstrated 95 potential new marker proteins that were up- or down-regulated in cancer cells. Additionally, categorizing cancer cells in accordance to their location (primary, liver, and ascites) showed distinctive candidates regulated only in a specific location. The comparison of cell lines on the basis of their degree of differentiation (well-, moderate-, and poor-differentiated) presented unique markers of high relevance to the cellular differentiation grade. One hundred ninety-two proteins, the major of which functionally related to cellular movement, were significantly regulated in a comparison of primary versus metastatic cancer cells. The results obtained from this study may help in the assessment of pancreatic cancer, grading of the disease and identification of its metastatic potential.

Differentiation of multiple histological subtypes of pancreatico-biliary tumors by molecular analysis of clinical specimens

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Introduction: Timely and accurate diagnosis of pancreatic ductal adenocarcinoma (PDAC) is critical in order to provide adequate care to patients. The clinical signs and symptoms of PDAC, however, are shared by other malignant or benign masses in the pancreatico-biliary system, which may thus be difficult to differentiate from PDAC with conventional diagnostic procedures. Among others, this includes ampullary cancers, solid-pseudopapillary tumors, and adenocarcinomas of the distant bile duct, as well as inflammatory tumors developing in chronic pancreatitis. Within the Paca-Net consortium, we have established accurate differentiation between these tumor entities based on molecular analysis of biopsy material.
Methods: 144 bulk tissue and fine needle aspiration biopsy samples were analyzed using a dedicated diagnostic cDNA array. A composite classification algorithm was developed based on linear support vector machine analysis of array data and evaluated through 10x10fold cross validation, thus testing the performance of the classification system under more demanding conditions than encountered in clinical routine.
Results: The five histological subtypes of pancreatic masses were separable with 100% accuracy when using all 144 individual samples for classification. Cross validation (100 independent test runs) revealed correct classification into the five diagnostic groups for 80.4% of 1440 test set predictions. Performance increased to 84.2% diagnostic accuracy when PDAC and distant bile duct carcinomas were combined in a single diagnostic class. Importantly, sensitivity of detection of malignant disease was 92%.
Conclusions: The approach presented here is suitable to significantly aid in the differential diagnosis of undetermined pancreatic masses by molecular analysis of clinical biopsy material. To our knowledge, this is the first study reporting accurate differentiation between several types of pancreatico-biliary tumors in a single molecular analysis procedure.

Differential diagnosis of pancreatic inflammation and cancer by measurement of microRNA abundance in blood and tissue

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Background: Early diagnosis would have an enormous impact on the successful treatment of pancreatic cancer, for which currently the incidence is nearly identical to mortality. MicroRNA signatures could act as a means to such ends, due to the molecules' central role in cellular regulation, their inherent stability and their relatively small molecule number, all of which makes them appropriate for diagnostic assays.

Methods: Variations in the abundance of all known microRNA molecules in total RNA preparations from pancreas tissues and peripheral blood cells were analysed on comprehensive microarrays and validated by real-time PCR.

Findings: From blood, a clear distinction between healthy people and patients with either chronic pancreatitis or cancer could be achieved. However, no discrimination was possible between the diseases. This was accomplished by studying the microRNA signatures obtained from tissue samples.

Interpretation: Utilising a minimally invasive blood test, the occurrence of chronic pancreatitits or a pancreatic tumour could be detected. Since inflammation is highly correlated with the development of pancreatic tumours, this assay has a high likelihood of identifying disease early. Once a medical indication of disease has been established, more discriminative diagnosis can be performed by an analysis of microRNA signatures of tissue samples, alone or in combination with mRNA profiles.

Funding: The work was financially supported by the European Commission and the German Federal Ministry of Education and Research (BMBF) as part of the MolDiagPaCa, BioRN and PaCaNet projects and funded by the National Institute for Health Research Liverpool Pancreatic Biomedical Research Unit.

Protein kinase D2 is a crucial regulator of tumour cell-endothelial cell communication in gastrointestinal tumours

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Tumour angiogenesis is crucially dependent on the communication between the tumour and the associated endothelium. Protein kinase D (PKD) isoenzymes mediate vascular endothelial growth factor-A (VEGF-A) induced endothelial cell proliferation and migration and are also highly expressed in various tumours.
In this study we examined the role of PKDs for tumour proliferation and angiogenesis selectively in pancreatic and gastric tumours and in tumour-associated endothelium in vitro and in vivo. PKD2 was found to be highly expressed in gastrointestinal tumours and in the tumour-associated endothelium. In addition, tumour growth and angiogenesis in the chorioallantois (CAM) model and in tumour xenografts require PKD2 expression in endothelial cells. Conversely, hypoxia activates PKD2 in pancreatic cancer cells and PKD2 was identified as the major mediator of hypoxia-stimulated VEGF-A promoter activity, expression and secretion in tumour cells. Depletion of PKD2 in pancreatic tumours inhibited tumour-driven blood vessel formation and tumour growth in the CAM and in orthotopic pancreatic cancer xenografts Thus, PKD2 regulates hypoxia-induced VEGF-A expression/secretion by tumour cells and VEGF-A stimulated blood vessel formation and is a novel, essential mediator of tumour cell/endothelial cell communication. These features make PKD2 a promising therapeutic target in gastrointestinal cancers.

NTCVD-Consortium studies cognitive disturbances in chronic kidney disease: cardiovascular risk factors and mood disorders as underestimated confounders

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Background:
Cognitive and memory deficits are common findings in patients with chronic kidney disease (CKD). Most often affected domains are verbal memory and cognitive flexibility. There is strong evidence for associations between CKD with cardiovascular risk factors and diseases. The precise link between risk factors, diseases, associated mood disturbances and cognition remains vague.
Methods:
28 patients with CKD (eGFR <60ml/min, 61±13 yrs) were prospectively compared with two separate control groups: 29 patients with cardiovascular diseases without CKD (eGFR >60ml/min, 63±11 yrs) and 10 healthy participants (49±5 years). All participants were assessed with a neuropsychological test battery (memory, information processing, cognitive flexibility and visuospatial abilities); depression and anxiety were assessed with Hospital Anxiety and Depression Scale (german version).
Results:
CKD patients performed significantly worse concerning information processing speed, cognitive flexibility and spatial working memory compared with healthy participants. In contrast, cognitive performance of CKD patients and patients with CVD and normal kidney function was not different. Interestingly, cardiovascular risk factors and performance in neuropsychological tests were significantly negative correlated. CKD patients also showed higher depression and anxiety scores, while depression scores were especially high in dialysis patients and negatively associated with parameters of cognitition.
Conclusion:
Although CKD is associated with deficits in specific cognitive domains, cardiovascular risk factors might influence cognition independent from CKD. In addition, depression is likely to represent a confounder for neuropsychological deficits at least in certain cognitive domains. Moreover, our data reveal for the first time different patterns of cognitive deficits in CKD patients with and without dialysis treatment.

Superoxide dismutase type 1 in monocytes of chronic kidney disease patients

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We analyzed proteomic profiles in monocytes of chronic kidney disease patients and healthy control subjects. Two-dimensional electrophoresis (2-DE) and silver staining indicated differences in protein pattern. Among the analyzed proteins, superoxide dismutase type 1 (SOD1), which was identified both by MS/MS mass-spectrometry and immunoblotting, was reduced in kidney disease.We characterized SOD1 protein amount, using quantitative in-cell Western assay and immunostaining of 2-DE gel blots, and SOD1 gene expression, using quantitative real-time polymerase chain reaction (PCR), in 98 chronic hemodialysis and 211 chronic kidney disease patients, and 34 control subjects. Furthermore, we showed that different SOD1 protein species exist in human monocytes. SOD1 protein amount was significantly lower in hemodialysis (normalized SOD1 protein, 27.2±2.8) compared to chronic kidney disease patients (34.3±2.8), or control subjects (48.0±8.6; mean±SEM; $p<0.05$). Analysis of SOD1 immunostaining showed significantly more SOD1 protein in control subjects compared to patients with chronic kidney disease or hemodialysis ($p<0.0001$, analysis of main immunoreactive protein spot). SOD1 gene expression was significantly higher in hemodialysis (normalized SOD1 gene expression, 17.8±2.3) compared to chronic kidney disease patients (9.0±0.7), or control subjects (5.5±1.0; $p<0.0001$). An increased SOD1 gene expression may indicate increased protein degradation in patients with chronic kidney disease and compensatory increase of SOD1 gene expression. Taken together, we show reduced SOD1 protein amount in monocytes of chronic kidney disease, most pronounced in hemodialysis patients, accompanied by increased SOD1 gene expression.

Phenylacetic acid and arterial stiffness

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Phenylacetic acid is a recently described uremic toxin that inhibits iNOS expression and may thereby affect the remodeling of arteries. These effects were not evaluated yet in patients with end-stage renal failure.
We measured the plasma concentrations of phenylacetic acid using nuclear magnetic resonance (NMR) spectroscopy in 50 patients with end-stage renal failure (37 men, 13 women) on maintenance hemodialysis. Arterial stiffness of small arteries was obtained from radial artery waveforms using applanation tonometry.
During the hemodialysis session the plasma phenylacetic acid concentration was reduced from 3.64 ± 0.39 mmol/L to 2.37 ± 0.18 mmol/L (mean ± SEM; $n = 50$; $p < 0.001$). There was a significant correlation between the phenylacetic acid concentration before and after hemodialysis and arterial stiffness of small arteries (Spearman $r = 0.29$; $p = 0.005$). Multivariate analysis showed that arterial stiffness was significantly associated with phenylacetic acid concentrations ($B = 14.4$; $p = 0.009$), whereas changes of blood urea nitrogen, serum creatinine or serum phosphate had no significant effects.
The study demonstrates an association of phenylacetic acid and arterial stiffness in patients with end-stage renal failure. This finding indicates that the removal of the uremic toxin phenylacetic acid by the hemodialysis session improves vascular functions in patients with end-stage renal failure.

CDKN2BAS is associated with periodontitis in different European populations and is activated by bacterial infection

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Epidemiological studies indicated a relationship between coronary heart disease (CHD) and periodontitis. Recently, CDKN2BAS was reported as a shared genetic risk factor of CHD and aggressive periodontitis (AgP), but the causative variant has remained unknown. To identify and validate risk variants in different European populations, we first explored 150 kb of the genetic region of CDKN2BAS including the adjacent genes CDKN2A and CDKN2B, covering 51 tagging single nucleotide polymorphisms (tagSNPs) in AgP and chronic periodontitis (CP) in individuals of Dutch origin (n=313). In a second step, we tested the significant SNP associations in an independent AgP and CP population of German origin (n=1,264). For the tagSNPs rs1360590, rs3217992, and rs518394, we could validate the associations with AgP prior and after adjustment for the covariates smoking, gender and diabetes, with SNP rs3217992 being the most significant (p=0.0004, odds ratio (OR)=1.48 [95% confidence interval (CI 95%) 1.19-1.85]). We further showed in-vivo gene expression of CDKN2BAS, CDKN2A, CDKN2B, and CDK4 in healthy and inflamed gingival epithelium (GE) and connective tissue (CT), and detected a significantly higher expression of CDKN2BAS in healthy CT compared to GE (p=0.004). After 24 hours of stimulation with P. gingivalis in S. gordonii pre-treated gingival fibroblast (HGF) and cultured gingival epithelial cells (GECs), we observed 25-fold and 4-fold increase of CDKN2BAS gene expression in HGFs (p=0.003) and GECs (p=0.004), respectively. Considering the global importance of CDKN2BAS in the disease risk of CHD, this observation supports the theory of inflammatory components in the disease physiology of CHD.

An Ang-II like peptide causing vasodilatory effects

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The family of angiotensin peptides has been steadily growing in recent years. Most are fragments of angiotensin II (Ang-II) with different affinities to the known angiotensin receptors. Here we describe the novel endogenous octapeptide Pro-Glu-Val-Tyr-Ile-His-Pro-Phe (Angioprotectin), which acts as a strong agonist at MAS receptors. Angioprotectin provides physiological antagonism of vasoconstrictor actions of Ang-II via the AT1 receptor by modulating both Ca^{2+} influx and NO release, via stimulation of the MAS receptor. Plasma concentrations in healthy human volunteers were about 15 % and in renal failure patients up to 50 %, of plasma Ang-II concentrations. A commercially available Ang-II antibody did not discriminate between Angioprotectin and Ang-II and thus Angioprotectin can contribute to Ang-II concentrations measured by antibody-based assays. This novel peptide is likely to be highly relevant component of the human renin-angiotensin-system.

Gene therapy with AAV9-Calsarcin-1 attenuates Angiotensin-II-mediated left ventricular contractile dysfunction and remodeling

Presenting Author: Martin Vogel
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Cardiac remodelling caused by sustained pressure and/or volume overload leads to the development of cardiac hypertrophy. One molecular pathway that is involved in hypertrophic growth is mediated by the calcium- and calmodulin-dependent serine/threonine phosphatase calcineurin, which plays a key role in transducing calcium-dependent signals from the cytosol to the nucleus.
In previous studies we have shown that mice lacking Calsarcin-1 (CS1), a calcineurin-interacting protein, are sensitized to calcineurin signaling. Conversely mice overexpressing CS1 blunt hypertrophic growth when exposed to chronic angiotensin-II (Ang-II) infusion.
To use the protective effect of CS1 we first generate an adeno-associated virus serotype 9 (AAV9) that encompasses the CS1 full length coding sequence under the control of the 0.26kb cardiac myosin light chain promoter fused to the cytomegalovirus immediate-early enhancer (CMV(enh)/MLC0.26). AAV9-CS1 or a Renilla-luciferase control-AAV9 (AAV9-Ren) where systemically injected via the tail vain (dose 2x1011 Vg) in 8-weeks-old male C57Bl/6 wild type (WT) mice. One week after intravenously AAV9 injection mice were subjected to long-term stimulation with Ang-II (1000ng/kg per min) or 0.9% NaCl respectively using subcutaneous minipumps for two weeks.
Ang-II stimulated and AAV9-Ren treated WT mice showed a contractile dysfunction with a significant reduction of the fractional shortening (FS) assessed by echocardiography and a strong trend to a dilation of the left ventricle. AAV9 mediated overexpression of CS1 leads in contrast to a complete removal of LV-dysfunction.
Taken together these results give a further hint that CS1 influence the cardiac remodelling after hypertrophic stimulation in a positive way.

Cellular uptake of transcription factor-neutralising DNA oligonucleotides

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Preclinical animal studies addressing various inflammatory diseases [Hecker et al. (2008) in: Therapeutic Oligonucleotides; Kurreck, Ed. RSC Biomolecular Sciences] demonstrated that the transcription factor decoy oligonucleotide (ODN) technology is a promising therapeutic approach to abrogate disease-related gene expression following local delivery. Uptake of these short double-stranded DNA oligonucleotides was shown to operate without any auxiliary means, thus reducing the risk for adverse reactions. Using human primary cultured endothelial cells and monocytes we now demonstrate that this uptake is mainly brought about by well-defined cellular transport systems for folic acid, i.e. the reduced folate carrier (RFC) and the folate receptor (FR1). By using fluorescence dye or [35S]-phosphothioate-labelled oligonucleotides this notion was supported by the following findings: (i) Like uptake of folic acid via the RFC ODN uptake was sensitive to competitive inhibition by folic acid analogues such as methotrexate and folic acid itself at higher concentrations; (ii) ODN uptake was blocked by anion exchange inhibitors as well as by blocking RFC expression with a specific antisense oligonucleotide; (iii) like RFC-mediated transport of methotrexate ODN uptake into the cells was sodium-independent; (iv) reducing the anion, i.e. chloride concentration in the medium significantly enhanced ODN uptake, which in addition was saturable, time and sequence-dependent; and (v) both fluorescence confocal microscopy and radiometry of different cell fractions confirmed the intracellular localization of the oligonucleotides. Finally, HeLa cells overexpressing RFC or FR1 by way of transient transfection revealed a robust increase in ODN uptake as compared to the non-transduced cells. A better understanding of the cellular transport mechanism for these decoy oligonucleotides may lead to an improved delivery strategy for topical treatment with nucleic acid-based drugs in general.

Biotinylated AAV as tool for attaching nucleic acids on the capsid surface

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Background:
Adeno-associated viruses (AAV) enable an efficient transduction of myocardium. Aim of our study was to develop an approach for cardiac delivery of nucleic acids attached to the AAV surface combining viral and non-viral gene transfer techniques.

Methods:
We loaded plasmid DNA on polylysin coupled to streptavidin. These DNA/polylysin complexes were attached to AAV2 and AAV9 vectors genetically biotinylated using a biotin acceptor protein (BAP) motif within the cap-sequence of AAV and cotransfection of biotin ligase within the vector production.

Results:
Complete biotinylation decreased vector tropism and efficacy. Thus, we determined the amount of biotinylation of capsids that enables the best compromise between preservation of AAV tropism and transfer efficiency of plasmid-DNA by comparing different ratios of wildtype (wt) and BAP-modified capsids for vector production. Plasmid DNA encoding a red fluorescent reporter protein was linked via streptavidin to the vectors harboring an EGFP reporter gene within their genome. A ratio of one biotinylated capisd per three wt capsids showed the highest transduction efficacy and plasmid transfer in HEK293 cells up to 10% transfer.
For in vivo delivery, biotinylated AAV9 vectors were used for packaging a luciferase reporter. 4 weeks after injection in adult mice, luciferase expression in representative organs was determined. Although the in vivo cardiac tropism of AAV9 wt decreased after biotinylation by about 50%, the heart was still the main target.

Conclusion:
Conditions were established for transfer of plasmid-DNA via biotinylated AAV2 in HEK293 cells. Genetically biotinylated AAV9 enabled a preserved cardiac transduction pattern of myocardium in vivo.

Xq27.3-q28 microdeletions including FMR1 and FMR2 in three female patients with mild MR and obesity

Presenting Author: Hartmut Engels
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Trinucleotide repeat expansions, or less frequently point mutations and deletions of the FMR1 gene, are a well known cause of mental retardation (MR) predominantly in males. Additional features of Fragile X syndrome include language delay and behavioral problems. Trinucleotide repeat expansions in the FMR2 gene have been associated with a milder phenotype also including speech, learning and behavioral problems.
We describe three unrelated female patients aged 3 ½, 9 and 16 10/12 years for whom molecular karyotyping detected overlapping microdeletions in Xq27.3-q28 including FMR1 and FMR2, as well as the Z97180.1, FMR1NB, IDS, AF011889.1-1 and AC016940.7 genes (Ensembl release 54, May 2009). De novo occurrence was demonstrated in two of the patients; maternal inheritance was excluded in the third. Common features of all three patients include mild MR and obesity. Two of the patients also have a diminished sense of satiety, muscular hypotonia, hyperextensibility of small joints and macrocephaly. MR is the main clinical feature in common between classical Fragile X syndrome and the phenotype of female patients presented here. Lymphocyte X inactivation studies were performed on two patients, and revealed random inactivation. Thus, no evidence was found for skewed X inactivation as an etiological mechanism.
We are performing currently lymphocyte RNA expression studies of FMR1, FMR2, FMR1NB, and IDS in the commonly deleted region in our patients to elucidate whether haploinsufficiency of only one gene, e.g. FMR1, or of a combination of genes might underly this phenotype.

Excessive glutamatergic neurotransmission during withdrawal from alcohol: A translation magnetic resonance spectroscopy study

Presenting Author: Wolfgang Sommer
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Animal studies suggest that excessive glutamate release during the withdrawal reaction contributes to the pathology of alcohol addiction, but evidence from human studies is lacking. In vivo brain imaging allows for highly translatable investigations of human pathological conditions and animal models thereof.
Here, we measured brain glutamate levels during detoxification in alcohol addicted patients and in healthy controls as well as in a rat model of alcoholism by proton nuclear magnetic resonance spectroscopy (^{1}H-MRS) at 3 and 9.4 T, respectively. Patients were examined by ^{1}H-MRS at admission when breath alcohol has fallen < 1 mg/ml and 2 weeks into controlled abstinence. Healthy subjects were scanned once. Rats were assessed in a within-subject, repeated measurement design before and during ethanol intoxication, in acute withdrawal and after protracted abstinence. The main outcome were glutamate levels in the human anterior cingulate cortex and the rat medial prefrontal cortex as determined by single voxel spectroscopy using LCModel quantification with water scaling and correction for CSF content.
In acute withdrawal, patients had significantly increased glutamate levels ($p < .05$) compared to healthy control subjects. Glutamate levels in patients were significantly correlated to withdrawal severity and decreased over time with abstinence. Alcohol addicted rats showed increased glutamate levels during 12 and 60 hrs withdrawal compared to the intoxicated state ($p < .05$) as well as vs. naïve control rats ($p < .01$). The increase in glutamate was paralleled by reduced glutamine levels. All changes returned to normal levels within 3 weeks of abstinence.
In conclusion, glutamate levels in the prefrontal cortex are increased during withdrawal from alcohol. The inverse relationship of glutamate and glutamine in the rat mPFC suggests increased utilisation of glutamine by accelerating the „glutamate–glutamine cycle“ linking MRS findings to neurotransmission and may comprise a potential biomarker for addiction severity. Thus, MRS is a suitable approach for development of highly translatable biomarkers for monitoring human pathological conditions and animal models thereof.

In-depth characterization of 13 patients with deletions of 5q14.3-q15: Is MEF2C the whole story?

Presenting Author: Hartmut Engels

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Microdeletions of chromosomal bands 5q14.3-q15 have been reported to cause a phenotype including severe mental retardation / developmental delay (MR/DD), epilepsy, muscular hypotonia, and variable minor anomalies. Proximal deletions of this region including the MEF2C gene led to the identification of the transcription factor MEF2C as a frequent autosomal dominant cause of severe MR. Deletions as well as truncating and missense mutations are associated with diminished expression of MEF2C resulting in reduced transactivation of MECP2 and CDKL5.

Here, we present the clinical and genetic characterization of 13 patients with microdeletions concerning 5q14.3-q15, eight of which have not yet been published.

All patients exhibited MR/DD and muscular hypotonia. Patients with proximal deletions displayed severe MR/DD and seizures. Periventricular heterotopias in three patients with 5q14.3-q15 microdeletions as published by Cardoso and colleagues in 2009 (Neurology; 72:784-92) have been excluded specifically for seven of our patients, but a wide range of mostly minor cranial MRI changes was found.

Lymphocyte RNA expression profiling of nine genes from the deleted regions demonstrated diminished expression of several genes in seven patients. Interestingly, reduced expression of MEF2C was found in one patient whose deletion did not encompass the MEF2C gene. However, MEF2C expression levels were unchanged in two patients with more distal deletions who also exhibit a microdeletion 5q14.3-q15 phenotype including severe MR, epilepsy and muscular hypotonia. In order to further elucidate the role of MEF2C haploinsufficiency in different microdeletions of 5q14.3-q15, and to gain insight into which other gene(s) might contribute to the phenotype, further expression analyses are being performed. These include targeted analyses of MEF2C and MECP2 as well as genome-wide expression studies.

Five Patients with Novel Overlapping Interstitial Deletions in 8q22.2q22.3

Presenting Author: Dagmar Wieczorek
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High-resolution microarray technology has facilitated the detection of submicroscopic chromosome aberrations and characterization of new microdeletion syndromes. We present clinical and molecular data of five patients with previously undescribed overlapping interstitial deletions involving 8q22.2q22.3. All deletions differ in size and breakpoints. Patients 1-4 carry deletions between 5.25 Mb and 6.44 Mb in size, resulting in a minimal deletion overlap of 3.87 Mb (from 100.69 Mb to 104.56 Mb; hg18) comprising at least 25 genes. These patients share similar facial dysmorphism with blepharophimosis, telecanthus, epicanthus, flat malar region, thin upper lip, downturned corners of the mouth and a poor facial movement/little facial expression. They have a moderate to severe developmental delay (4/4), absent speech (3/4), microcephaly (3/4), a history of seizures (3/4), postnatal short stature (2/4), and a diaphragmatic or hiatus hernia (2/4). Patient 5 was diagnosed with a smaller deletion of about 1.92 Mb (containing 9 genes) localized within the deletion overlap of the other four patients. Patient 5 shows a different facial phenotype and a less severe mental retardation.

In patients 1-4, COH1 is involved in the deletion (in total or in part), but none of them showed clinical features of Cohen syndrome. In two patients (patients 2 and 4), ZFPM2 (also called FOG2, a candidate gene for congenital diaphragmatic hernias) was partly deleted.

We suggest that patients with a microdeletion of 8q22.2q22.3 may represent a clinically recognizable condition characterized particularly by the facial phenotype and developmental delay. More patients have to be evaluated to establish a phenotype-genotype correlation.

De novo MECP2 duplication as a cause for moderate mental retardation in two females with random X-inactivation

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Xq28 duplications including MECP2 are a well known cause for severe mental retardation in males with encephalopathy, muscular hypotonia, progressive spasticity, seizures, poor speech and recurrent infections that often lead to early death. Usually female carriers show a normal intellectual performance due to skewed X inactivation. We report on two female patients, seven and 18 years old, with a MECP2 duplication who are concurrently the first females with a de novo MECP2 duplication. Both patients have the de novo duplication occurring on the paternal allele and both show a random X-inactivation which can be assumed as the triggering factor for the phenotype. We delineate the phenotype, which might be restricted to mild to moderate mental retardation and autistic features as well as recurrent infections in early childhood, constipation, and spastic hypertonia in early adulthood.

Poster Presentation Abstracts

Symposium VI

New Technologies

Computational analysis of genome-wide methylation with MeDIP-seq

Presenting Author: Lukas Chavez
Lukas Chavez[1], Christina Grimm[1], Jörn Dietrich[1], Matthias Lienhard[1], Kerstin Neubert[1], Marcus Albrecht[1], Alexandra Farrall[2], Jörn Walter[3], Kurt Zatloukal[4], Hans Lehrach[1], Bernhard Herrmann[2], Michal Schweiger[1], and Ralf Herwig[1]

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Advances in the investigation of epigenetic gene control in cancer have demonstrated the fundamental role of epigenetic changes in tumour onset and progression, for example through hypermethylation in promoters of tumour suppressor genes. In the NGFN-plus project "Modifiers of Intestinal Tumor Formation and Progression" we study epigenetic changes in colon cancer with patient samples and mouse models using genome-wide DNA methylation experiments. Sequencing-based DNA methylation data is an emerging technology for analyzing epigenetic modifications. Methylated DNA immunoprecipitation (MeDIP) depends on the use of an antibody specific for methylated cytosines in order to immunocapture methylated genomic fragments which is combined with next-generation sequencing (MeDIP-seq).
So far the major bottleneck of MeDIP-seq experiments was on the computational side. Here, we present our recent software development MEDIPS, the first comprehensive approach for normalization and differential analysis of MeDIP-seq data. MEDIPS is a full pipeline consisting of QC features and methods for data pre-processing and statistical analysis [1]. MEDIPS has been intensively tested on currently more than 1.5 billion sequence reads. In the presentation we describe important factors, such as local CpG density and copy number variation that may influence methylation signals and show how MEDIPS compensates for these factors. We highlight the performance of the pre-processing and statistical analysis with public benchmark data as well as project data. Furthermore, we describe global as well as local correlation of methylation and gene expression. Additionally, we show how epigenetic changes can be mapped onto human pathways using the ConsensusPathDB, our meta-database of heterogeneous molecular interactions [2].

References
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RNA phenotypes from next-generation sequencing

Presenting Author: Karol Szafranski
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A functional interpretation of the large amount of emerging somatic and germ line structural variants and SNPs remains a formidable challenge. An auxiliary approach we suggest for this problem is the assessment of intermediate RNA phenotypes using next-generation sequencing (Illumina GAII) of transcriptomes (RNA-seq) to help in the interpretation of variants of unknown significance. In particular we studied allelic imbalance, which refers to a deviation of active autosomal transcription from parity. Allelic imbalance was assessed on a panel of 10 lymphoma and lymphoblastoid cell lines together with a microarray-based characterization of genotypes and expression profiles. Allelic imbalance was analyzed using a Bayesian statistical model, and the top hits were validated by pyrosequencing. We found that the library preparation method represents an important step in obtaining consistent results. Moreover, Illumina sequencing introduces a sequence context-dependent bias of allele calling specificity. Using filtering and correction strategies, 70% correct calls in the top candidates were achieved.

Modifications of the Illumina library preparation protocol for small RNA-Seq

Presenting Author: Christian Becker
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Small RNAs play an important role in establishing and maintaining cell lineages. We are using next generation sequencing (NGS) for profiling the small RNA transcriptome of many diverse cell types and cell conditions. In contrast to hybridization-based techniques, NGS provides data at an unprecedented depth with almost no bias. It combines a superior dynamic range with the opportunity to discover novel small RNAs.
For small RNA-Seq it is most important to generate reliably and reproducibly high-quality small RNA libraries. At the CCG, we are using the Illumina v1.5 protocol with some modifications that we have introduced.
In brief, input material should be 5µg of total RNA (RIN>8). At first we enrich the small RNA fraction using the Qiagen RNeasy MinElute Cleanup Kit. Prior to library preparation the volume of the eluted small RNAs is reduced to 5µL by evaporation using a SpeedVac-Concentrator. When following the Illumina library preparation protocol, we include a negative control to be able to identify the band of adapter-dimers in the gel. This prevents us from cutting out adapter-only products in the sample lanes. To purify the amplified cDNA construct, we use a 10% Novex TBE PAGE gel, instead of the recommended 6% gel. The purified construct is recovered using the Qiagen MinElute Gel-Extraction Kit. Both quality control steps (input RNA and library) are performed using the Agilent Bioanalyzer machine and Bioanalyzer High Sensitive Kits to check for concentration and fragment size.
We have applied the modified protocol to many samples in a number of different projects and consistently obtained data of very high quality. Hence we strongly recommend our modified version of the Illumina v1.5 protocol for small RNA-Seq library preparation to all other users.

Deep Sequencing of Mitochondrial Genomes and Transcriptomes

Presenting Author: Janine Altmüller
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Objectives: Although mitochondrial genomes are small in size and the sequence for many species is already well known, there are some specific questions that can only (or most effectively) be solved by deep sequencing. We therefore report two projects and their special technological needs to prepare libraries suitable to be analyzed on an Illumina GAIIx sequencing machine.
1. Mitochondrial genome of the human brain
In tissues of patients that suffer from chronic, degenerative diseases caused by disturbed mitochondrial maintenance, the mitochondrial genome shows multiple heteroplasmic deletions that are difficult to characterize by conventional sequencing. We performed a pilot experiment on blood of a healthy individual and skeletal muscle of a patient known to carry multiple mitochondrial DNA deletions. The mitochondrial genome was amplified by long-range PCR, and a modified fragmentation protocol was used to generate fragments for library preparation.
In the case sample, the genome-wide coverage of the mitochondrial genome displays a severe drop in the region, where most deletions overlap. In contrast, the coverage is quite uniform in the control sample.
2. Mitochondrial transcriptome of Physarum polycephalum
RNA transcribed from the mitochondrial genome of Physarum polycephalum (Pp) is heavily edited. This complexity has made even the prediction of genes in the mitochondrial genome and sites of editing in RNAs extremely difficult. We therefore determined the complete Pp mitochondrial transcriptome using Illumina deep sequencing of RNA from gradient-purified Physarum mitochondria.
We have discovered 763 previously uncharacterized editing sites, including the first known A and G insertions in Pp mtRNA, as well as instances of intergenic editing and partial editing. Our findings double the number of known genes in the mitochondrial genome of Physarum polycephalum.

Toward an automated analysis pipeline for deducing functional consequences of sequence variants detected by 2nd generation sequencing

Presenting Author: Holger Thiele
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Sequencing of whole exomes and genomes has become a standard approach to the analysis of complex genetic and Mendelian diseases. A vast number of variations is detected in each experiment. Exome-seq usually yields up to 50,000 variations with about 10% having a potential functional consequence. The assessment of the called variants is the major bottleneck in these kinds of experiments. In an attempt to expedite the analysis of the effects of variations on protein sequences, we are currently developing a database-based evaluation strategy. As the primary biological data sources we use Ensembl, variations found by the 1000 Genomes project and in house generated data.
In the first step, a local data base is generated by query Ensembl for a given species. The subsequent analysis then determines for each variation the overlap for each known protein coding transcript and every known variation. Subsequently, the impact is determined on the protein structure and translated as a mutation, following the recommendations of Human Genome Variation Society. The algorithm used here takes into account SNPs, insertions, deletions and indels which have been found by different variation caller programs. Then filter processes combine the search for known variations, predictions about the pathogenicity for example by Polyphen2, linkage, project-based comparison with other sequence data sets, evolutionary conservation and manual searches. The bioinformatics part is integrated into a NGS project management database to enable optimal information flow between the team members.

Protein microarray-based comparison of HER2, estrogen receptor and progesterone receptor status in core biopsies and surgical specimens from FFPE breast cancer tissues

Presenting Author: Daniela Berg
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Purpose: Currently, core biopsies are routinely used for diagnosis of breast cancer and they are often the only sample for providing prognostic and predictive markers prior to treatment. However, biopsies may not accurately reflect protein expression profiles from the whole tumour. In the last few years, reverse phase protein arrays (RPPA) have become a very promising tool for biomarker profiling allowing quick, precise, and simultaneous analysis of many components of a protein network.
Experimental Design: After extraction of full-length proteins from formalin-fixed and paraffin-embedded (FFPE) tissues, we compared HER2, ERa and PGR expression levels in a series of 35 FFPE breast cancer surgical specimens and their corresponding core biopsies using RPPA.
Results: We found a high concordance between protein expression in core biopsies and surgical specimens with concordance- and k-values of 91.4% and k=0.677 for HER2; 80% and k=0.587 for ERa and 82.8% and k=0.656 for PGR.
Conclusion and clinical relevance: In this study we could demonstrate that HER2, ERa and PGR receptor expression can be assessed reliably on core biopsies of FFPE breast cancer tissues using RRPA. These results might facilitate the implementation of RPPA technology in routine clinical settings.

New insights into uPA / PAI-1 related signaling in breast cancer

Presenting Author: Katharina Malinowsky
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Previous analysis of the interaction of uPA and its inhibitor PAI-1 with cancer related signaling pathways in breast cancer revealed a substantial association of uPA with Erk activation and Stat3 expression, while PAI-1 correlated with Akt activation, EGFR related signaling and the expression of integrin-alphaV. Here we analyzed these pathways in more detail thus shedding light on possible mechanisms by which uPA and PAI-1 influence migration and proliferation of cells in breast cancer.

Proteins from FFPE tissues of 201 breast cancer patients were analyzed by reverse phase protein arrays to determine protein expression. We focused our analysis on interactions of uPA and PAI-1 with molecules either related to Erk and Akt pathways or known to play a role in integrin based signaling. In our analysis we considered only associations with a correlation-coefficient higher than 0.35 as possibly biological relevant.

The correlation of uPA expression with the phosphorylation of Erk could be confirmed. Additionally, uPA correlated with Hsp27 expression and activation of p38. PAI-1 did not only correlate with activation of Akt in this setting but also showed co-expression with PI3K, a known activator of Akt. Expression of PAI-1 could also be correlated with ILK and FAK expression. Both factors interact with integrins and play a role in the Akt pathway. They also influence the regulation of the cytoskeleton, thus further interfering with migration and invasion properties of cells. Interestingly, PAI-1 also could be associated with Hsp27 and pp38 which we already found to correlate with uPA expression.

Our results place uPA and PAI-1 in the middle of main events of cancer development and provide insight in deregulated signaling in primary patient samples by demonstrating their interaction with major cancer related signaling networks. Thus, we can confirm knowledge from cell culture experiments and put it into a more physiological context.

Reverse phase protein array based evaluation of HER2 receptor status in FFPE breast cancer tissues

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Purpose: The determination of HER2 levels has major impact on prognosis and therapy decisions of breast cancer patients. Currently, immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) are the predominant methods for HER2 assessment. However, there is a widespread concern that differences in IHC technique and interpretation can substantially affect the accuracy leading to an unacceptable high rate of misclassification. Here, we analysed the association of reverse phase protein array (RPPA) based HER2 expression levels compared with IHC and FISH.
Experimental Design: Using RPPA technology we established a HER2-concentration threshold to distinguish immunohistochemical HER2-negative (scored as 0, 1+, 2+/FISH-) from immunohistochemical HER2-positive (scored as 2+/FISH+; 3+) breast cancer patients. We compared RPPA based HER2 measurement to HER2 determination by IHC and FISH for 312 FFPE breast cancer tissues. IHC was done at the Institute of Pathology, Technical University of Munich, Germany according to certified standards. For HER2 immunohistochemistry 2+ cases, additional in situ hybridisation was used to determine the final status.
Results: Compared with IHC/FISH, RPPA showed a concordance of 92% (κ= 0.806; 95% CI, 87-97%) for surgical specimens and of 93% (κ=0.860; 95% CI, 86-100%) for core biopsies respectively. Additionally, RPPA allows to distinguish FISH-negative and FISH-positive samples in a high proportion of cases in both surgical specimens (concordance 91%; κ=0.762; 95%CI, 83-99%) and core biopsies (concordance 94%; κ=0.876; 95%CI, 83-105%)
Conclusion: RPPA based HER2 quantification shows a high concordance with IHC/ FISH and thus provides an additional, objective, and quantitative method for HER2 determination in breast cancer patients.

Detection Of Genomic Variation In Glioblastoma

Presenting Author: Sabine Kelkenberg-Schade
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Malignant glioma is the most frequent and lethal cancer originating in the central nervous system. The most aggressive subtype is glioblastoma multiforme.
The appearance of cancer is in most cases a consequence of genetic alterations including structural chromosomal variations of the genome as well as epimutations.
The structural variations include deletions, duplications, insertions, inversions, and complex combinations of rearrangements. DNA methylation is essential for normal development and has been implicated in many pathologies including cancer.
Comprehensive genetic variation studies greatly benefit from the introduction of the next-generation-sequencing platforms. However, this technology is still far from being applicable to whole genome re-sequencing of complex organisms. Therefore it is necessary to focus on individual genomic subsets of interest by reducing the sequence complexity of the sample. Such an approach was established using microarray-based genomic selection. Therefore a genomic DNA library is hybridized to oligonucleotides on-array representing the target DNA regions. For methylation analysis DNA is treated with bisulphite. In comparison to normal tissue, alterations in the methylation pattern should be detected.
For the identification of structural variation, sequencing of mate-pair libraries is accomplished. The capability of this method derives from the way in which the libraries are constructed that allows sequencing of both ends. When the sequences of the two mate-pairs are aligned to the reference genome, their orientation and distance is determined. Because both sequences originated from a single DNA fragment of defined size, aberrations in scaffold of the genome can be detected.

Catching the dynamics of RNAi phenotypes during a human kinome screen

Presenting Author: Jitao David Zhang
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We present the first human kinome screen with a real-time cell analysis system (RTCA) to capture dynamic RNAi phenotypes employing time-resolved monitoring of cell growth via electrical impedance. The screen validated previously identified inhibitor genes and, additionally, identified activators of cell growth. RTCA allowed us to investigate the dynamics of RNAi phenotypes instead of using conventional end-point analysis. Time kinetics of screening hits correspond to cellular functions associated with RNAi target genes, as we could establish mitotic-event related genes to be among the first displaying inhibiting effects as well as migration-associated genes for activators. The profile captured by RTCA thus indicates a biological protein half life, i.e. the time at which the protein's knock-down asserts the maximum effect. Our data establishes RTCA technology as a novel robust tool with biological and pharmacological relevance amenable for high-throughput screening, and identifies kinases that inhibit or stimulate cell growth when being downregulated.

Biomarker discovery in plasma of breast cancer patients using microspot immunoassays

Presenting Author: Johanna Sonntag
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The potential use of biomarkers in breast cancer includes aiding early diagnosis, determining prognosis, and predicting response or resistance to different therapies. The ease with which blood can be sampled makes it a logical choice for biomarker applications.
Over the past few years different protein microarray platforms emerged as experimental tools for biomarker discovery. They allow the simultaneous determination of various parameters from a minute amount of sample within a single experiment. The experimental design of microspot immunoassays is based on antibody pairs specifically recognizing different epitopes of the analytes. One antibody is used to capture the analyte from the complex sample and the second antibody is used for detection.
Various transmembrane proteins are proteolytically released from the cell surface by a process known as ectodomain shedding both under normal and pathophysiological conditions. In addition, soluble receptor isoforms can be generated through alternative splicing. We have developed a microspot immunoassay for the evaluation of biomarker signatures focusing on the ectodomain shedding products/soluble isoforms of the ERBB1, ERBB2, and ERBB3 receptor. In addition, the ectodomain shedding product of the MET receptor is quantified as well. This 4-plex microspot immunoassay has been used to determine target protein concentrations in 150 plasma samples from breast cancer patients taken at primary diagnosis. The study gives an overview of baseline ectodomain shedding product/soluble receptor isoform levels in breast cancer patients at primary diagnosis and serves as a basis for a long term follow up study in these patients.

Methods used in the morphological characterization of mouse models in the German Mouse Clinic

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Introduction
The pathology is an important screen in the GMC examining the NGFN mutant mice and control littermates macro- and microscopically. The continuous enhancements and establishment of techniques enables us to make a contribution to the identification of new phenotypes. The most modern techniques used in human pathology including immunophenotyping and molecular methods have been adapted and established for mouse tissue. This poster will provide an insight into the current practice.

Materials and Methods
In the primary pathology screen the mice undergo a complete morphological analysis of all organs with standard stains. Specific targeted evaluations, such as radiography, quantitative immunohistochemistry (IHC), double immunhistochemistry, electron microscopy (EM) and molecular techniques, can additionally be performed in a second step to provide further insight into the histopathological findings. If vectors including the lacZ gene are used for generation of the mutant mouse line the pathology screen offers whole-mount adult lacZ staining for reporter gene analysis. Virtual slide microscopy is presently used for archiving of samples and promoting online discussions and conferences worldwide.

Results
Representative pictures of different techniques mentioned in the material and methods section are exemplified shown for the NGFN lines analyzed so far.
Of 78 mutant lines analyzed recently, 46 (60%) showed a pathological phenotype and 39 of them (50%) were newly identified using common stains as well as additional techniques in our so called secondary and tertiary screen.

Conclusion
The continuous development and use of advanced techniques in mouse pathology increases the detection of new phenotypes and the ability of the pathology screen to achieve the demands of cooperation partners in a high qualitative way.

Effects of Modified Uremic Nucleosides on Endothelial Cells

Presenting Author: Kerstin Lehmann
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1 Charité - Universitätsmedizin Berlin, Center for Muskuloskeletal Chirurgie / Berlin-Brandenburg Center for Regenerative Therapies, 2 Charité – Universitätsmedizin Berlin, Medizinische Klinik IV-Nephrology, Campus Benjamin Franklin, 3 Bayer Schering Pharma AG, Wuppertal, 4 EXcorLab GmbH, Obernburg

Accumulation of uremic retention solutes during loss of kidney function promotes uremic cardiovascular disease (CVD), a major cause of mortality in patients with chronic kidney disease (CKD). Recent research has allowed to unravel multiple patho-physiologic mechanisms and to identify as yet more than 100 responsible uremic compounds of which modified nucleosides have been found in increased amounts in uremic retention solutes during uremia.
To get insight into the biological mechanisms of uremic toxicity, we focussed on effects of modified uremic nucleosides on activation of distinct signal transduction pathways in human primary endothelial cells (human umbilical vein endothelial cells, HUVEC). Altered functional properties, such as proliferation and apoptosis in endothelial cells were assessed.
The present work shows that modified uremic nucleosides show impaired protection of HUVECs from apoptosis as determined by caspase-3 and caspase-7 activation. Interestingly, only a moderate effect on endothelial cell proliferation as assessed by measuring DNA synthesis by BrdU incorporation could be observed.
Assessing ERK1,2 MAPK signalling and Akt activation, two major pathways accounting for cell proliferation and cell survival, we show, that unmodified uremic nucleosides transiently stimulate ERK1,2 MAPK and Akt activation and that ERK1,2 MAPK and Akt activation is reduced upon treatment with modified uremic nucleosides.
Together these data indicate that nucleosides which exhibit physiological functions might be turned into patho-physiological active compounds upon posttranslational modification promoting the process of chronic kidney disease.

NGFN infrastructure: Expression analysis and methylation typing using the Illumina iScan technology

Presenting Author: Annegret Fischer
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With the Illumina iScan technology the Institute of clinical molecular Biology in Kiel provides further high-throughput infrastructure to academic cooperation partners within the NGFN for conducting large-scale chip-based methylation and expression analyses. The technology is integrated in the well-established high-throughput genotyping workflow at the ICMB. This ensures high-quality data through standardized procedures in sample preparation and the semi-automated processing of the Illumina BeadChips using Tecan robots.
In a pilot project using the Illumina Infinium HumanMethylation27 and HumanHT-12 Expression BeadChips quality issues were extensively considered supported by NGFN grants: Batch effects and inter-platform variation were assessed by calculation concordance rates of technical as well as biological replicates. The overall performance of the technology was controlled by determining call rate and signal distribution. In conclusion, the Illumina iScan technology was confirmed as a highly reproducible and cost-effective method for chip-based analysis of DNA-methylation and expression patterns. This platform already supports the DNA methylation analyses in the NGFN plus initiative “Integrated genomic investigation of colorectal carcinoma”.

Haplotyping and copy number estimation of the highly polymorphic human beta-defensin locus on 8p23 by 454 amplicon sequencing.

Presenting Author: Klaus Huse
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The beta-defensin gene cluster (DEFB) at chromosome 8p23.1 is one of the most copy number (CN) variable regions of the human genome. Whereas individual DEFB CNs have been suggested as independent genetic risk factors for several diseases (e.g. psoriasis and Crohn's disease), the role of multisite sequence variations (MSV) is less well understood and to date has only been reported for prostate cancer. Simultaneous assessment of MSVs and CNs can be achieved by PCR, cloning and Sanger sequencing, however, these methods are labour and cost intensive as well as prone to methodological bias introduced by bacterial cloning. Here, we demonstrate that amplicon sequencing of pooled individual PCR products by the 454 technology allows in-depth determination of MSV haplotypes and estimation of DEFB CNs in parallel.

Six PCR products spread over ~87 kb of DEFB and harbouring 24 known MSVs were amplified from 11 DNA samples, pooled and sequenced on a Roche 454 GS FLX sequencer. From ~142,000 reads, ~120,000 haplotype calls (HC) were inferred that identified 22 haplotypes ranging from 2 to 7 per amplicon. In addition to the 24 known MSVs, two additional sequence variations were detected. Minimal CNs were estimated from the ratio of HCs and compared to absolute CNs determined by alternative methods. Concordance in CNs was found for 7 samples, the CNs differed by one in 2 samples and the estimated minimal CN was half of the absolute in one sample. For 7 samples and 2 amplicons, the 454 haplotyping results were compared to those by cloning/Sanger sequencing. Intrinsic problems related to chimera formation during PCR and differences between haplotyping by 454 and cloning/Sanger sequencing are discussed.

Deep amplicon sequencing using the 454 technology yield thousands of HCs per amplicon for an affordable price and may represent an effective method for parallel haplotyping and CN estimation in small to medium-sized cohorts.

Targeted and whole-exome next-generation resequencing: lessons learned from benchmarking studies

Presenting Author: Abdou ElSharawy
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Until the whole-genome re-sequencing of large numbers of individuals becomes feasible, whole-exome (WEx) and/or targeted re-sequencing (TR) of a complete set of specific genomic loci present the next logic available option. Next generation sequencing (NGS) has revolutionised throughput, but two challenges exist, namely sample-multiplexing at the front end to reduce costs, and evaluating the accuracy of variation discovery in these data sets. We carried out many benchmarking studies aiming at assessing the performance of WEx and multiplexed TR NGS approaches, and evaluating the current bioinformatics tools, in order to establish best practices for our future projects. Here, we established two different multiplexed TR-NGS workflows, employing molecular bar-coding and pooling strategies, to increase throughput, on the ABI SOLiD system. In the first workflow, 68 E. coli and BRCA1/2 human genes were targeted using microarray-based sequence capture (Febit, Germany) at different multiplexing levels in three experimental stages. The results showed adequate enrichment measures and uniform distribution of indexed samples. SNP discovery was depended strongly on tools/settings. Surprising decrease in SNP-detection rate by ~15% was observed, despite improvement achieved in mapping coverage. In the second workflow, 384 human exons were enriched in six gDNA and three matched whole-genome amplified HapMap gDNA samples using microdroplet-based PCR (RainDance, USA). The performance of different pooling scenarios was evaluated at three stages of the SOLiD protocol. The results showed efficient parallel detection of both homozygous and heterozygous SNPs and maintenance of 99.5% average genotype concordance across all tested samples, regardless of applied pooling strategy. Finally, benchmarking of different WEx capturing (solution- vs. solid-phase) approaches of two gDNA samples on three different NGS platforms revealed divergent enrichment efficiencies and variant discovery rates.

Pair End Sequencing of Human Genomes on the SOLiD™ Platform

Presenting Author: Oliver Stephan
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Life Technologies, 500 Cummings Center, Beverly, MA

The SOLiDTM DNA sequencing system utilizes stepwise ligation of oligonucleotide probes and enables high fidelity, high throughput sequencing. Previous sequencing protocols for the SOLiDTM system have only been available in the forward direction (3’ to 5’). However, fragment library paired end sequencing (in both forward and reverse directions) is highly desired to maximize sequencing capacity and to meet special research interests such as whole transcriptome and translocation studies. To achieve this using the SOLiDTM platform, novel ligation chemistries were developed to support 5’ to 3’ read lengths of up to 35 bases. Utilizing this new chemistry, we sequenced an anonymous Caucasian male to an average depth of coverage of 22.4x (2 SOLiDTM sequencing runs) covering 96.55% of the genome. The purpose of any resquencing project is to measure variants against a known reference sequence, so we analyzed this data using our in-house variant detection algorithms to assess data integrity. Approximately 2.97M SNPs were discovered: 1.21M homozygotes (94.4% in dbSNP v129) and 1.76M heterozygotes (73.7% in dbSNP v129). Also detected were 103,027 small indels (73.8% in dbSNP v129). Paired-end sequencing with this novel chemistry presented here is effective at variant-detection in a human genome and these values are similar to variant totals from other large-scale human resequencing.

Analysis of a South African genome using SOLiD error correction codes

Presenting Author: Roland Wicki
Roland Wicki, Vasisht Tadigotla, Christopher Clouser, Tristen Weaver, Jessica Spangler, Marcin Sikora, John Healy, Clarence C. Lee, Timothy Harkins

Life Technologies, Darmstadt, Germany; Foster City, CA and Beverly, MA, USA

The Bushmen of southern Africa are known to be genetically divergent from other humans. An individual from that community (KB1) has recently been sequenced using the 454 GS FLX platform to 10.2 fold coverage and a de novo assembly was created. Here we present the deep sequencing of KB1 using the latest developments with the SOLiD(TM) system and ligation-based sequencing. Specifically, recent improvements in paired-end sequencing have allowed us to achieve longer read lengths in both the forward and reverse direction. Novel error correction codes (ECC) for SOLiD(TM), are based on standard techniques used in modern communication and data storage systems. ECC works by transforming the valuable information and augmenting it with redundancy to make it more resistant to measurement error. Due to ligation based sequencing, the SOLiD system is unique and can employ ECC encoding by using specially-designed probe sets, redundancy is achieved by an additional round of probe ligations. This allows us to improve accuracy with minimal impact on sequencing time, as well as decode the original sequence using a Bayesian inference. In total, ~30x paired coverage of KB1 was achieved by combining longer paired end sequencing reads (75 bp forward, 30 bp reverse) with standard SOLiDTM mate pair libraries (2x50 bp). These libraries have insert sizes of 250 bp and 1.5 kb respectively. When mapped against hg18 with ECC applied, there is a 40% reduction in uncovered regions in KB1 and a higher number of heterozygous SNPs that are present in dbSNP are called. Structural variations as well as the hybrid assembly of the KB1 genome using 454 and SOLiD data will also be discussed.

SOLiD™ ChIP-seq kit for ChIP and ChIP-Sequencing from low number of cell and tissue samples

Presenting Author: Andrea Toeppel
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Life Technologies, Carlsbad, CA 92008

Chromatin Immunoprecipitation (ChIP) assay is the most widely used and powerful method to identify regions of the genome associated with specific proteins. Combined with massively parallel next-generation sequencing technology, ChIP-sequencing provides a high resolution digital solution for genome-wide survey of protein-DNA interactions.

We developed a SOLiD™ ChIP-seq kit, which offers an optimized ChIP workflow and an efficient ChIP-seq library construction from relatively low number of cell or tissue samples. ChIP procedure is usually laborious, time consuming, and typically requires large starting cell numbers or large amount of tissue. We use MAGnify™ Chromatin Immunoprecipitation System, suitable for fast enrichment of chromatin complexes and DNA recovery, for ChIP workflow in the kit. It is able to use lower starting cell numbers (10,000-300,000 cells) for ChIP thus preserving precious samples such as primary cells, stem cells, biopsies. We also developed a novel sample preparation method for a variety of tissues, such as brain, heart, kidney and liver. This new approach allows faster throughput to investigate different chromatin and transcription time-course events as well as enable antibody screening to determine ChIP compatibility. In addition, we develop a sensitive ChIP-seq library construction procedure which enables users to construct a complex library using as low as 1 ng ChIP DNA. Combining with SOLiD's ultra high sequencing throughput, SOLiD ChIP-seq offers a highly sensitive, hypothesis-neutral approach to accurately characterize protein-DNA interactions at genome-wide scale.

Using SOLiD™ ChIP-seq kit, we characterize histone H3 methylation profile in the RSC-1 embryonic stem cells on the SOLiD system. The data demonstrate that SOLiD ChIP-seq kit provides a streamlined, optimized and reproducible assay for the enrichment of chromatin/protein complexes, DNA recovery using magnetic bead capture technology and construction of sequencing library.

Genome-wide methylation data analysis on the SOLiD™ System

Presenting Author: Liqun He
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DNA methylation is an epigenetic modification crucial for development and normal gene regulation. Using the versatile methyl-CpG binding protein-based system (MethylMiner™) for enrichment, followed by SOLiD™ sequencing, allows for focused evaluation of genome-wide methylation patterns. This is a cost effective alternative to shotgun bisulfite sequencing of the entire genome because only ~1% of the human genome is methylated and requires interrogation. The workflow for mapping and analyzing MethylMiner-enriched fractions of genomic DNA as well as bisulfite converted reads sequenced on the SOLiD System, employs available public tools (e.g. Bowtie, SAMtools, MACS) and our own scripts and programs. It enables characterization of methylation patterns at different levels of resolution, from broad genome region comparisons and profile differences between samples to individual methyl C resolution. Functionality provided:

- Mapping of unconverted and bisulfite-converted reads
- Filtering of clonal reads
- Mapping statistics: distribution, coverage and read depth statistics, C and CpG counts in mapped reads
- Methylation analysis: methylation status of C residues and bisulfite conversion efficiency
- Peak-finding in MethylMiner-enriched reads
- Level of enrichment in exons, introns, CpG islands, repeats, etc.
- Visualization of mapped reads and MethylMiner-enriched peaks with publicly available genome browsers (e.g. the UCSC or IGV browser).

We have implemented this analysis pipeline to analyze human data sets (IMR90 and MCF-7 cell lines). Results showed good agreement with publicly available methylation data: peaks in MethylMiner-selected reads have high coverage of genome regions with higher densities of published methyl-CpGs. This analysis workflow is a convenient and flexible solution for users allowing the integration of methylation data with results from other SOLiD™ System applications (e.g. ChIP-seq and RNA-Seq).

Single Molecule Real-time DNA Sequencing on the Surface of a Quantum-dot Nanocrystal

Presenting Author: Raimo Tanzi
Raimo Tanzi(1), Joseph M. Beechem(2),

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2 Genetic Systems, Life Technologies, Carlsbad CA USA

A single molecule, long read-length, real-time sequencing-by-synthesis technology has been developed by building a sequencer directly on the surface of a ~10 nm quantum-dot nanocrystal. Five-color fluorescence resonance energy-transfer technology (FRET) is used for detection; signals from the quantum-dot labeled DNA polymerase plus 4 DNA-base-specific acceptor dyes are simultaneously detected. Acting as the FRET donor, the Qdot™-polymerase generates a correlated “photon-dip” for every inserted based (the “quantum-correlation-signal”), allowing for more accurate base-calling. The sequencer is not physically bound to any solid substrate, so it can be exchanged (like a reagent) during runs, replacing non-functioning polymerases mid-reaction. Each exchange cycle lengthens the effective read-length, which can be continuously extended without “gaps”. After sequencing a particular length of DNA, the newly synthesized strand can be removed. The original genomic DNA strand is re-primed, Qdot-polymerase sequencers rebound, and the identical genomic DNA strand can be sequenced again, increasing the net accuracy and not requiring circularization of genomic templates. The desired accuracy and read-length can be “tuned” by adjusting the number of exchange cycles. Each reaction can be completed in minutes, thus multiple exchange experiments can be performed per sequencing hour. Qdot™ nanocrystals are smaller than currently available materials (to increase FRET signals), have an extinction coefficient ~100X greater than organic-dyes, allowing for very low levels of excitation power while sequencing, yielding a favorable environment for polymerase activity and template integrity. Qdot-polymerase sequencers can bind to ultra-long DNA segments (>10kb) at multiple positions and sequence while moving “horizontally”, enabling “ordered-reads” for long-phased haplotype sequencing. Examples of real-time sequencing of homopolymeric, patterned, and complex templates will be shown.

Advanced Nucleic Acid Fractionation for Next Generation Sequencing Sample Preparation

Presenting Author: Hannes Arnold

Caliper Life Sciences, Lise-Meitner-Str. 11, 55129 Mainz, Germany

Separating nucleic acids by size with subsequent isolation of specific fragments is a fundamental, but tedious, molecular biology technique. As second generation sequencing platforms gain utility, it has become clear that reproducible and scalable sizing and isolation has become a bottle neck for many applications of new sequencing technologies. Caliper Life Sciences has developed and commercialized instruments that utilize microfluidics to achieve rapid and high resolution electrophoretic separations. While we have focused most of our efforts on analysis applications (e.g. DNA, RNA and protein sizing), we have also previously demonstrated preparative applications, such as fractionation of nucleic acid for cloning. We have now developed a commercial solution, the LabChip XT, that will simplify and improve nucleic acid fractionation. By using intersecting microfluidic channels, optical detection and computer control, we can automatically extract a target band during separation and route the selected material to a successfully isolated narrow bands (±5%) in the range of 100-500 bp from a fragmented DNA sample. The isolated nucleic acid is PCR-ready and the fractionation is complete in 30 minutes. The presented material will describe the fundamentals of our microfluidics-based solution, as well as highlight some early data and collaborative work.
Keywords : Next Generation Sequencing, NGS, DNA fractionation

Mapping structural variation at fine scale by population scale genome sequencing

Presenting Author: Jan Korbel
Jan Korbel(1), Ryan Mills(2), Chip Stewart(2), and the Structural Variation Analysis Group of the 1000 Genomes Project

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Genomic structural variants (SVs), such as copy-number variants and balanced rearrangements of the genome, differ from other variation classes in extent, origin, and functional impact. Despite progress in SV characterization the nucleotide resolution architecture of most SVs has remained unknown. We constructed an SV map based on 185 human genomes by integrating evidence from complementary SV discovery approaches with extensive experimental validations. Our map encompasses ~22,000 deletions and ~6,000 additional SVs comprising insertions and tandem duplications. Most SVs were mapped to nucleotide resolution, which facilitated their genotyping and inference of their origin and functional impact. We identified numerous whole and partial gene deletions, with a depletion of gene disruptions amongst high allele-frequency deletions. Furthermore, we observed differences in the size spectra of SVs originating from distinct formation mechanisms, and report a map of SV hotspots formed by common formation mechanisms. Our analytical framework and SV map will serve as a resource for future sequencing-based disease association studies.

Company Satellite Sessions

Abstracts

High Throughput Gene Expression Profiling of Single Cells

Presenting Author: Simon Margerison
Senior Sales Application Specialist

Fluidigm B.V.

Fluctuations in gene expression at the single cell level could be key for generating developmental signals. Data needs to be collected from a statistically significant number of single cells in order to determine the range of gene expression present in a population of cells. Furthermore, transcripts need to quantified for a number of genes in order to obtain meaningful cell signatures. Fluidigm's BioMark™ arrays provide a convenient and cost-effective system for performing multiple RNA expression assays on multiple single-cell samples. The 96.96 array enables running up to 96 real time qPCR assays on up to 96 samples. This system has been used to study single cell gene expression in embryonic stem cells, cancer stem cells, and early stage embryos.
Fluidigm develops, manufactures and markets Integrated Fluidic Circuit (IFC) systems that significantly improve productivity for applications such single cell gene expression, copy number variation and next-generation sequencing. These "integrated circuits for biology" perform thousands of sophisticated biochemical measurements in extremely minute volumes. Fluidigm products are available for research use only.

Advances in Illumina Next-Generation Sequencing and Array Solutions that Enable Biological Discovery

Presenting Authors: Dr. Richard Henfrey – Associate Director Marketing, Europe
Dr. Stephanie Brooking – Sequencing Segment Specialist, Europe

Illumina Europe

At Illumina, our goal is to apply innovative technologies and revolutionary assays to the analysis of genetic variation and function, making studies possible that were not even imaginable just a few years ago. With such rapid advances in technology taking place, it is mission critical to have solutions that are not only innovative, but flexible, scalable, and complete with industry-leading support and service. Visit our workshop to hear about the latest leading-edge solutions for Next Generation sequencing, SNP genotyping (including use of FFPE samples), copy number variation, methylation and gene expression.

From Proton Sequencing to Achieving 99.99% Accuracy: Recent Advances in the Life Technologies Sequencing Portfolio

Presenting Author: Raimo Tanzi
Director, Business Development Next Generation Sequencing

Applied Biosystems Europe, Darmstadt, Germany

The technologies in next generation sequencing are rapidly evolving. There has been a strong focus on increasing data throughput per instrument run, and the leading platforms can readily sequence one to several whole human genomes in a single instrument run, transforming the scope and magnitude of genetically based projects. In addition to the throughput, there is intense efforts to increase the accuracy for single reads with a goal of achieving 99.999% accuracy. Accuracy of this level will better enable the sequencing of heterogeneous samples (such as tumor samples) to readily identify low frequency somatic mutations that are believed to be the "driver mutations" for cancer progression. To achieve this type of accuracy, a new sequencing chemistry that utilizes error correcting codes (ECC) has been developed for the SOLiD platform. As next generation sequencing better enables clinical research, Life Technologies is preparing to launch a fourth generation sequencing platform developed by Ion Torrent. This platform requires no optical detection, detecting pH changes associated with nucleotide incorporation events, and provides a new paradigm for sequencing on a much faster time scale than any current next generation platform. An overview of the ECC development and the new Personal Genome Machine will be presented.

Technology advances: innovative new solutions for custom genotyping, allelic copy number in FFPE samples, and single RNA molecule in situ visualization in single cells

Presenting Authors: Dr. Fiona Brew, Wolfgang Trautwein

Affymetrix Europe

Affymetrix solutions enable analysis from whole genome to single gene. Join us at our short Technology Advances seminar to learn about some recent important additions to our tools for basic and clinical research.

Unmatched scalability for custom genotyping is now available with Axiom™ myDesign™. Design arrays with 50,000 to 2.6M SNPs selected from a validated database of 7.4M rare and common SNPs including HapMap and 1000 Genomes Project variants. Process more than 750 samples per week, without compromising accuracy or reproducibility.

Maximize cancer discoveries in your FFPE samples by integrating copy number, allelic ratio and somatic mutation analysis with OncoScan™ FFPE Express. Obtain unparalleled whole-genome data quality and high sample pass rates with the lowest amount of input DNA. Detect the broadest linear range from 0 to 60 copies to accurately characterize the complexity of the cancer genome.

Enable direct quantitative biology in your laboratory with innovative QuantiGene® branched DNA technology. QuantiGene assays use direct signal amplification to avoid RT and PCR amplification. This enables greater sensitivity, specificity and robustness in any sample type. QuantiGene ViewRNA offers in situ RNA visualization down to one molecule in one cell. QuantiGene 2.0 and QuantiGene Plex enable DNA copy number and RNA expression assays from single-plex to 36 plex. QuantiGene assays are compatible with any sample type including FFPE and other challenging samples.

Affymetrix solutions enable analysis from whole genome to single gene. Join us at our short Technology Advances seminar to learn about some recent important additions to our tools for basic and clinical research.

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Translating Genomic Discovery into Human Health – The Sequenom MassARRAY System for Epigenetics and Rare Mutation Screening

Presenting Author: Caren Vollmert, PhD & Henning Gohlke, PhD, MPH

Sequenom GmbH

Genetic and epigenetic variants play significant role in the development of tumors, pathogenesis, and disease progression. Therefore, oncogene mutations and methylation pattern may be the key to cancer research.
Sequenom® offers different OncoCarta™ Panels for mutation analysis screening of varied cancer tumor samples for biomarker discovery and validation. Combining the high sensitivity of PCR amplification with the precise, accurate, and ultra-high sensitive detector of MALDI-TOF mass spectrometry Sequenom®`s MassARRAY® technology allows rare mutation detection of mutation frequencies <10% with a quantitative precision of 3-4% CVs. This is essential especially in the typically admixed samples of tumor and healthy tissue. The iPLEX™ biochemistry is based on PCR followed by an allele-specific single base extension process. Due to its ability to multiplex assays each of the four OncoCarta™ panels allows you to screen more than 200 mutations at the same time using <500ng of DNA derived from FFPE, frozen or fresh tumor, or cell line samples. The OncoCarta™ Panels are pre-designed and validated ready-to-use kits eliminating any needs for design optimization or primer adjustment.
MassARRAY® EpiTYPER® for quantitative DNA methylation analysis combines base-specific enzymatic cleavage with MALDI-TOF MS. This combination creates a highly accurate, sensitive, and high-throughput method for the quantitative analysis of DNA methylation. The EpiTYPER™ assay is based on proven bisulfite conversion biochemistry, followed by PCR and a proprietary base specific cleavage process. The resulting cleavage pattern depends on the presence of methylated cytosine in the original genomic DNA. The cleavage products are automatically and quantitatively analyzed by MALDI-TOF mass spectrometry. MassARRAY® EpiTYPER® is the method of choice for the quantitative analysis of DNA methylation and identification of differentially methylated CpG sites in any genomic region or candidate gene.

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List of NGFN-Plus Integrated Genome Research Networks and NGFN-Transfer Innovation Alliances

IG Atherogenomics

Koordination: Prof. Dr. Heribert Schunkert

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Erklärung**
Schunkert	Heribert	Prof. Dr. med.	Universität zu Lübeck	A1a, A2a, E1, F1	**A. Explorative Genomics**
Erdmann	Jeanette	Prof. Dr. rer. nat.	Universität zu Lübeck	A1a, A2a C2, F1	A1 Polygenic and monogenic forms of MI
Linsel-Nitschke	Patrick	Dr. med.	Universität zu Lübeck	A2a, D1, D2a	A2 Genomics of coronary artery disease
Aherrarhou	Zouhair	Dr. med.	Universität zu Lübeck	B2	A3 Genomics of sub clinical atherosclerosis
Ehlers	Eva-Maria	PD Dr. med.	Universität zu Lübeck	B2	**B. Comparative Genomics**
Döhring	Lars	Dr. med.	Universität zu Lübeck	B2	B1 Syntenic regions for atherosclerosis in mice and humans
Fischer	Marcus	PD. Dr. med.	Universität zu Regensburg	A2b, D2b	B2 ABCC6 and arterial calcification
Hengstenberg	Christian	Prof. Dr. med.	Universität zu Regensburg	A1b, C1, E1	**C. Population Genetics**
Teupser	Daniel	PD Dr. med.	Universität Leipzig	B1	C1 Cases and population platform (KORA/MONICA; GMIS;PREVENT-IT, LE HEART)
Thiery	Joachim	Prof. Dr. med.	Universität Leipzig	B1	C2 Genetic epidemiology methods platform
Blankenberg	Stefan	Prof. Dr. med.	Klinikum der Johannes Gutenberg-Universität	A3a, D1, E1	**D. Functional Genomics**
Zeller	Tanja	Dr. rer. nat.	Klinikum der Johannes Gutenberg-Universität	A3a, D1, E1	D1 Gene expression profiling Transcriptome of monocytes in subclinical atherosclerosis and MI patients
Steller	Ulf	Dr. rer. nat.	Euroimmun AG	E1	D2 Genomics of plasma lipids
Koenig	Wolfgang	Prof. Dr. med.	Universitätsklinikum Ulm	A3b, C1, E2	**E. Transfer**
König	Inke	PD Dr. rer. nat.	Universität zu Lübeck	C2b	E1 SNP array for atherosclerosis Development of innovative diagnostics
Wichmann	H. Erich	Prof. Dr. rer. nat. Dr. med.	Helmholtz Zentrum München	C1	E2 50 K Vascular Disease SNP Array
Ziegler	Andreas	Prof. Dr. rer. nat.	Universität zu Lübeck	C2b	**F. Organisation**
Meitinger	Thomas	Prof. Dr. med.	Helmholtz Zentrum München	CF	F1 Coordinating office
					Genotyping facility
					CF Genotyping/sequencing facility

IG Genetics of Heart Failure (Genetik der Herzinsuffizienz)

Koordination: Prof. Dr. Hugo A. Katus

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	1a	Genetic Risk of Heart Failure and its Subphenotypes
Hasenfuß	Gerd	Prof. Dr.	Georg-August-Universität Göttingen	1b	Genetic Risk of Heart Failure and its Subphenotypes
Kääb	Stefan	Prof. Dr.	Ludwig-Maximilians-Universität München	1c	Genetic Risk of Heart Failure and its Subphenotypes

Kreutz	Reinhold	Prof. Dr.	Charité Universitätsmedizin, CBF	2	Systems Biology Genomics of Left Ventricular Hypertrophy (LVH) using congenic rat models of polygenic hypertension
Hübner	Norbert	Prof. Dr.	Max-Delbrück-Centrum für molekulare Medizin	3	Gene Regulatory Networks in Cardiac Hypertrophy and Failure
Ivandic	Boris	Dr.	Universitätsklinikum Heidelberg	4	Genetic Modifiers of Heart Failure in Mice
Rottbauer	Wolfgang	PD Dr.	Universitätsklinikum Heidelberg	5	Functional Genomics in Zebrafish to Dissect the Genetics of Human Myocardial Disease
Frey	Norbert	PD Dr.	Universitätsklinikum Heidelberg	6	Novel Molecular Pathways in Cardiac Hypertrophy and Failure
Knöll	Ralph	Prof. Dr.	Georg-August-Universität Göttingen	7	Genetics and Functional Analysis of Cardiac Mechano sensation - Relevance for the Pathophysiology of Diastolic Heart Failure
Guan	Kaomei	Dr.	Georg-August-Universität Göttingen	7	Genetics and Functional Analysis of Cardiac Mechanosensation - Relevance for the Pathophysiology of Diastolic Heart Failure
Lehnart	Stephan	Dr.	Georg-August-Universität Göttingen	8	Molecular Genomics Intracellular Calcium-Handling in Diastolic Dysfunction, Heart Failure and Arrhythmias
Weis	Tanja	Dr.	Universitätsklinikum Heidelberg	9	Coordination Office
Stoll	Monika	Prof. Dr.	Leibniz-Institut für Arterioskleroseforschung an der Universität Münster	10	Genetic epidemiology of Heart Failure: Genetic Epidemio-logical Support for the IG
Eils	Roland	Prof. Dr.	Deutsches Krebsforschungszentrum	11	Bioinformatic Methods
Brors	Benedikt	Dr.	Deutsches Krebsforschungszentrum	11	Bioinformatic Methods
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	12	DNA-Platform
Meder	Benjamin	Dr.	Universitätsklinikum Heidelberg	12	DNA-Platform
Rottbauer	Wolfgang	PD Dr.	Universitätsklinikum Heidelberg	13	High-throughput functional in vivo evaluation of heart failure associated genes and pathways by Morpholino knock-down in zebrafish

IG Molekulare Mechanismen der Adipositas

Koordination: Prof. Dr. Johannes Hebebrand

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Hinney	Anke	PD Dr.	Universität Duisburg-Essen	TP1	Identification of human obesity genes with a focus on developmental aspects
Schürmann	Annette	Prof. Dr.	Deutsches Institut für Ernährungsforschung (DIfE)	TP2	Identification and characterization of obesity genes, gene-gene and diet gene interactions involved in polygenic obesity in mice

Klingenspor	Martin	Prof. Dr.	Technische Universität München	TP3a	Alterations of the mouse brain proteome associated with the early development of diet-induced obesity in the mouse
Stühler	Kai	Dr.	Ruhr-Universität Bochum	TP3b	Alterations of the mouse brain proteome associated with the early development of diet-induced obesity in the mouse
Illig	Thomas	PD Dr.	Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	WB2-Aa	Evaluation of candidate genes for obesity and related disorders in large representative epidemiological cohorts encompassing children and adults - KORA
Reinehr	Thomas	PD Dr.	Institut für Pädiatrische Ernährungsmedizin, Vestische Kinder- und Jugendklinik, Universität Witten/Herdecke	WB2-Ab	Evaluation of candidate genes for obesity and related disorders in large representative epidemiological cohorts encompassing children and adults - Obeldicks
Krude	Heiko	Prof. Dr.	Charité	WB2-B	WB2-BEPOC
Moebus	Susanne	PD Dr.	Universität Duisburg-Essen	WB2-C	WB2-RECALL
Wabitsch	Martin	Prof. Dr.	Universität Ulm	WB2-D	WB2-UPOC
Rosskopf	Dieter	Prof. Dr.	Universität Greifswald	WB2-E	WB2-SHIP
Boeing	Heiner	Prof. Dr.	Deutsches Institut für Ernährungsforschung (DIfE)	WB2-F	WB2-EPIC
Klingenspor	Martin	Prof.Dr.	Technische Universität München	WB3-Aa	Novel mouse models for the evaluation of candidate genes in the physiology of energy balance regulation
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	WB3-Ab	Novel mouse models for the evaluation of candidate genes in the physiology of energy balance regulation
Fischer-Posovszky	Pamela	Dr.	Universität Ulm	WB3-B	Adipogenese
Horsthemke	Bernhard	Prof. Dr.	Universität Duisburg-Essen	WB3-C	Allelische Expression
Biebermann	Heike	Dr.	Charité	WB3-C	Methylierung
Brockmann	Gudrun	Prof. Dr.	Humboldt-Universität zu Berlin	WB3-D	Bioinformatik
Rüther	Ulrich	Prof. Dr.	Heinrich-Heine-Universität Düsseldorf	TP10	Investigation of Fto as a major contributor to obesity
Sauer	Sascha	Dr.	Max-Planck-Institut für Molekulare Genetik (MPIMG)	TP11a	Systematic molecular characterisation of compounds for prevention and therapy of obesity and insulin resistance
Büssow	Konrad	Dr	HZI Braunschweig	TP11b	Systematic molecular characterisation of compounds for prevention and therapy of obesity and insulin resistance
Blüher	Matthias	Prof. Dr.	Universität Leipzig	TP12	Adverse effects of weight cycling on longevity in rodents
Brockmann	Gudrun	Prof. Dr.	Humboldt-Universität zu Berlin	TP14	Implications of diet and exercise with interaction of allelic variations in the Berlin Fat Mouse line
Schäfer	Helmut	Prof. Dr.	Philipps-Universität Marburg	TP15a	Central statistical genomics and data management
Scherag	André	Dr.	Universität Duisburg-Essen	TP15b	Central statistical genomics and data management
Hebebrand	Johannes	Prof. Dr.	Universität Duisburg-Essen	TP16	Coordination and quality management

IG Pathogenic role of mi-RNA in Herpes Infections Koordination: Prof. Dr. Dr. Jürgen G. Haas					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Haas	Jürgen G.	Prof. Dr. Dr.	LMU , München	1	Herpesviral factors modulating the cellular miRNA processing machinery
Koszinowski	Ulrich	Prof. Dr.	LMU	2	Characterization of CMV miRNAs in vitro and in vivo
Adler	Heiko	PD Dr.	Helmholtz-Zentrum	3	In vivo effects of miRNAs inthe murine herpesvirus 68 (mHV-68)
Grässer	Friedrich	Prof. Dr.	Universitätsklinik des Saarlandes	4	Function of EBV-encoded and EBV-induced mirRNA in latency and transformation
Meister	Gunther	Dr.	Max-Planck Institut	5	Identification of cellular targets of viral miRNAs
Förstemann	Klaus	Prof. Dr.	LMU	6	Biochemical interaction of viral and cellular miRNAs
Zimmer	Ralf	Prof. Dr.	LMU	7	Prediction of viral miRNAs targets

IG RNomics in Infections Koordination: Prof. Dr. Jürgen Brosius					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Reinhardt	Richard	Dr.	MPI für Molekulare. Genetik	1	Ultra-High-Parallel Sequencing and Biocomputational Analysis of npcRNA
Vogel	Jörg	Prof. Dr.	MPI für Infektionsbiologie, Göttingen	2	RNomics of bacterial infections
Rudel	Thomas	Prof.	Universität Würzburg	2	RNomics of bacterial infections
Walter	Lutz	Prof. Dr.	Deutsches Primatenzentrum Göttingen	3	RNomics of viral infections
Brosius	Jürgen	Prof. Dr.	Universität Münster	4	RNomics of eukaryotic parasites

IG Systematic Genomics of Chronic Inflammatory Barrier Diseases Koordination: Prof. Dr. Stefan Schreiber					
Projektleiter				**Teilprojekt**	
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Schreiber	Stefan	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T7	Koordination
Franke	Andre	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 1	Genetische Ätiologie des M. Crohn
Rüther	Andreas	Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 5	Genetische Ätiologie der atopischen Dermatitis
Fölster-Holst	Regina	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 5	Genetische Ätiologie der atopischen Dermatitis
Nebel	Almut	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 6	Genetische Ätiologie der Psoriasis
Weichenthal	Michael	PD. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 6	Genetische Ätiologie der Psoriasis
Nikolaus	Susanna	PD Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 7	Genetische Ätiologie der Colitis ulcerosa

Schreiber	Stefan	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 8	Follow up Genotypisierung i.d. Teilprojekten GP 1, 2, 4-7
Rosenstiel	Philip	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 9	Funktionelle Aufklärung
Platzer	Matthias	PD Dr.	FLI- Leibniz-Institut für Altersforschung	GP 9	Funktionelle Aufklärung
Rosenstiel	Philip	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	GP 10	Systematische Aufklärung von Signaltransduktions-wegen: angeborene Immunität
Hofmann	Sylvia	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T1	Qualitätsmanagement
Wittig	Michael	ohne	Uniklinik Schleswig-Holstein, Campus Kiel	T3a	Bioinformatische Unterstützung
Schilhabel	Markus	Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T1	Qualitätsmanagement
Jacobs	Gunnar	Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T5a	Hochdurchsatz zelluläre Screening Assays via RNA Interferenz
Krawczak	Michael	Prof. Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T2	Genetisch-epidemiologische Unterstützung
Nothnagel	Michael	Dr.	Uniklinik Schleswig-Holstein, Campus Kiel	T2	Genetisch-epidemiologische Unterstützung
Kabesch	Michael	Prof. Dr.	Medizinische Hochschule Hannover	GP 2	Genetische Ätiologie des Asthma bronchiale
Horstmann	Rolf	Prof.	Bernhard-Nocht-Institut für Tropenmedizin	GP 4	Genetischer Ätiologie der Tuberkulose
Meyer	Christian	Prof. Dr.	Bernhard-Nocht-Institut für Tropenmedizin	GP 4	Genetischer Ätiologie der Tuberkulose
Lee	Young-Ae	Prof. Dr.	Charité, Campus Virchow-Klinikum	GP 5	Genetische Ätiologie der atopischen Dermatitis
Vingron	Martin	Prof. Dr.	Max Planck Institut für Molekulare Genetik (MPI-MG)	T 3b	Bioinformatische Unterstützung
Albrecht	Mario	Dr.	Max Planck Institut für Informatik (MPI-INF)	T 3c	Bioinformatische Unterstützung
Weidinger	Stefan	PD. Dr.	Technische Universität München, Klinikum Rechts der Isar	GP 5	Genetische Ätiologie der atopischen Dermatitis
Kaufman	Stefan H.E.	Prof. Dr.	Max Planck Institut für Infektionsbiologie	GP11	Systematische Aufklärung von Stoffwechelwegen: Adaptive Immunität
Wiemann	Stefan	PD Dr.	Deutsches Krebsforschungszentrum - DKFZ	T5b	Hochdurchsatz zelluläre Screening Assays via RNA Interferenz

IG Functional and Translational Genomics of Acute Leukemias

Koordination: Prof. Dr. Christian Hagemeier

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Hagemeier	Christian	Prof. Dr.	Charité	TP13 TP14	Intermediate risk childhood ALL, Genes of pathogenetic and therapeutic value in relapsed ALL
Döhner	Hartmut	Prof. Dr.	Uni Ulm	TP1	Pathogenecity in AML

Thiede	Christian	Prof. Dr.	TU Dresden	TP2	Novel abnormalities in AML with normal karyotype
Hubert	Serve	Prof. Dr.	Uni Frankfurt	TP3a	Epigenetics of AML
Müller-Tidow	Carsten	Prof. Dr.	Uni Münster	TP3b	Epigenetics of AML
Kulozik	Andreas	Prof. Dr.	Uni Heidelberg	TP4	NOTCH1 signaling
Marschalek	Rolf	Prof. Dr.	Uni Frankfurt	TP5	MLL and stem cell program
Bohlander	Stefan	Prof. Dr.	LMU München	TP6	CALM/AF10 target gene analysis
Feuring-Buske	Michaela	PD. Dr.	Universität Ulm	TP7	MEIS1 homeobox gene expression
Leutz	Achim	Prof. Dr.	MDC Berlin	TP8	Wnt signaling in leukemic stem cells
Duyster	Justus	Prof. Dr.	TU München	TP9	Genetic basis of imatinib resistance
Grez	Manuel	Prof. Dr.	GSH Frankfurt	TP10	Molecular inhibitors of AML1/ETO
Neubauer	Andreas	Prof. Dr.	Uni Marburg	TP11	Resistance to retinoic acid in AML
Schrappe	Martin	Prof. Dr.	Uni Kiel	TP12	Very high risk childhood ALL
Lottaz	Claudio	Dr.	Uni Regensburg	TP15	Bioinformatics, clinical data, and leukemic cell banks
IG Brain Tumor Network Koordination: Prof. Dr. Peter Lichter					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Lichter	Peter	Prof. Dr.	DKFZ	SP-C	Koordinierung und Lenkung des Netzwerkes BTN^{plus}
Lichter	Peter	Prof. Dr.	DKFZ	SP-1	Hochdurchsatzanalyse von potentiellen Onkogenen und Tumorsuppressorgenen in Gliomen
Wolter	Marietta	Dr.	Heinrich-Heine-Universität	SP-2a	Aberrante miRNA-Expression in Gliomen: Molekulare Mechanismen, funktionelle Konsequenzen und deren klinische Signifikanz
Stühler	Kai	Dr.	Ruhr-Universität Bochum	SP-2b	Aberrante miRNA-Expression in Gliomen: Molekulare Mechanismen, funktionelle Konsequenzen und deren klinische Signifikanz
Brors	Benedikt	Dr.	DKFZ	SP-3	Modellierung und Bioinformatik
Lichter	Peter	Prof. Dr.	DKFZ	SP-4	Funktionelle Charakterisierung der an Hypoxie und Sauerstoffmetabolismus beteiligten Gene *Cited4* und *PRDX1*, die günstiges Therapieansprechverhalten und verbessertes Gesamtüberleben bei Gliompatienten vorhersagen
Acker	Till	Prof. Dr.	Universitätsklinikum Gießen und Marburg GmbH	SP-5	Selbsterneuerungs- und Differenzierungsmechanismen in Gliom-Stammzellen

Wick	Wolfgang	Prof. Dr.	DKFZ	SP-6a	Funktionelle Charakterisierung durch chronische nicht-lethale Hypoxie induzierter Invasions-assoziierter Proteine
Vajkoczy	Peter	Prof. Dr.	Charité - Medizinische Universität Berlin	SP-6b	Validierung hypoxie-regulierter Moleküle für Tumorinvasion und Angiogenese
Hau	Peter	Dr.	Universität Regensburg	SP-7	Dysregulierte Migration und Differenzierung - molekulare und zelluläre Dissektion von Krebsstammzellen in hochgradigen Gliomen
Waha	Andreas	Dr.	Universitätsklinikum Bonn	SP-8	Funktionelle Bedeutung epigenetisch deregulierter Gene in Gliomen
Angel	Peter	Prof. Dr.	DKFZ	SP-9a	Funktionelle Analyse der KLK-ADAM-Achse bei der Zellmigration und Invasion von humanen Gliomen
Pietsch	Torsten	Prof. Dr.	Universitätsklinikum Bonn	SP-9b	Funktionelle Analyse der KLK-ADAM-Achse in der Migration und Invasion von Glioblastomen
Roth	Wilfried	Dr.	DKFZ	SP-10	Neue Funktionen von BCL2-Familien-Proteinen: Invasivität und Autophagie
Reifenberger	Guido	Prof. Dr.	Heinrich-Heine-Universität	SP-11a	Molekulare und funktionelle Charakterisierung von Genen, welche die Zellteilungs-symmetrie in malignen Gliomen kontrollieren
Radlwimmer	Bernhard	Dr.	DKFZ	SP-11b	Molekulare und funktionelle Charakterisierung von Genen, welche die Zellteilungs-symmetrie in malignen Gliomen kontrollieren
Herold-Mende	Christel	PD Dr.	Universität Heidelberg	SP-12a	Funktionelle Analysen von differenzierungsrelevanten Kandidatengenen in Gliomstammzellen
Radlwimmer	Bernhard	Dr.	DKFZ	SP-12b	Funktionelle Analysen von differenzierungsrelevanten Kandidatengenen in Gliom-Stammzellen
Hartmann	Christian	PD Dr.	DKFZ	SP-13	Funktionelle Charakterisierung der putativen Tumorsuppressorgene *EMP3* und *ST13* in Gliomen
Wick	Wolfgang	Prof. Dr.	Universität Heidelberg	SP-14	Klonierung und funktionelle Charakterisierung des murinen Regenerations- und Toleranz-Faktors: ein Glioma-Autoantigen-Kandidat mit immuno-suppressiven Eigenschaften

IG Integrated Genome Network of Prostate Cancer Koordination: PD Dr. Holger Sültmann					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Schlomm	Thorsten	Dr.	Martini-Klinik Prostatakrebszentrum und UKE Hamburg	TP1	Kollektivierung und Bereitstellung von klinischen Proben und Patientendaten
Simon	Ronald	PD Dr.	UKE Hamburg-Eppendorf	TP2	Biologische und klinische Signifikanz von Mikroamplifikationen im Prostatakarzinom
Yekebas	Emre	Prof. Dr.	UKE Hamburg-Eppendorf	TP3	Zytogenetische und molekulare Charakterisierung von Translokations-Bruchpunkten im Prostatakarzinom
Dierlamm	Judith	Prof. Dr.	UKE Hamburg-Eppendorf	TP3	Zytogenetische und molekulare Charakterisierung von Translokations-Bruchpunkten im Prostatakarzinom
Schweiger	Michal-Ruth	Dr. Dr.	MPI für Molekulare Genetik	TP4	Analyse von Mutationen und epigenetischen Veränderungen im Prostatakarzinom
Lehrach	Hans	Prof. Dr.	MPI für Molekulare Genetik	TP4	Analyse von Mutationen und epigenetischen Veränderungen im Prostatakarzinom
Sültmann	Holger	PD Dr.	Deutsches Krebsforschungszentrum	TP5	Splice-Varianten- und miRNA Expression in Tumoren
Balabanov	Stefan	Dr. rer. nat. Dr. med.	Universitätsklinikum Hamburg Eppendorf	TP6	Identifizierung klinisch relevanter Proteine im Prostatakarzinom
Heitmann	Alke	Dr.	Qiagen Hamburg GmbH	TP7	Entwicklung und Kommerzialisierung eines diagnostisch einsetzbaren Tools zur Detektion molekularer Marker im Prostatakarzinom
Haese	Alexander	PD Dr.	Martini-Klinik Prostatakrebszentrum und UKE Hamburg	TP8	Identifizierung und Validierung von diagnostischen und prognostischen Markern für die Therapieentscheidung beim Prostatakarzinom
Korf	Ulrike	Dr.	Deutsches Krebsforschungszentrum	TP9	Proteinarrays zur quantitativen Analyse von Proteinen in Tumoren und in Patientenseren
Weller	Horst	Prof. Dr.	Centrum für Angewandte Nanotechnologie (CAN) GmbH	TP10	Molekulare Tumor-Bildgebung mit Hilfe Antikörper-gekoppelter Nanopartikel
Mollenhauer	Jan	Prof. Dr.	Deutsches Krebsforschungszentrum	TP11	Funktionelle zelluläre Assays in Prostatakarzinom-Zelllinien
Sültmann	Holger	PD Dr.	Deutsches Krebsforschungszentrum	TP12	In vivo Analyse von Genen im Prostatakarzinom
Beissbarth	Tim	Prof. Dr.	Deutsches Krebsforschungszentrum	TP13	Bioinformatik und Systembiologie
Sültmann	Holger	PD Dr.	Deutsches Krebsforschungszentrum	TP14	Koordinierung, Kommunikation und Qualitätsmanagement

IG ENGINE (Extended Neuroblastoma Genome Interaction NEtwork) Koordination: Prof. Dr. Angelika Eggert					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Berthold	Frank	Prof. Dr.	Klinikum der Universität zu Köln, Zentrum für Kinderheilkunde und Jugendmedizin	2a	Central database & tumorbank
				8	Predictive gene signatures and transcription regulatory networks
Brors	Benedikt	Dr.	Deutsches Krebsforschungszentrum	7	Biostatistics for molecular trial design
Deubzer	Hedwig	Dr.	Deutsches Krebsforschungszentrum	3	Identification of NB initiating cells
				14	Targeting class I histone deacetylases
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	1	Project management
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	4a	Proteomics of NB master regulators
Eggert	Angelika	Prof. Dr.	Universitäts-Kinderklinik Essen	9a	NB Toponome
Eilers	Martin	Prof. Dr.	Philipps-Universität Marburg	11	Systematic drug testing
Fischer	Matthias	PD Dr.	Klinikum der Universität zu Köln, Zentrum für Kinderheilkunde und Jugendmedizin	8	Predictive gene signatures and transcription regulatory networks
Ivics	Zoltan	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	5a	Identification of NB initiating genes
König	Rainer	Dr.	Institut für Pharmazie und Molekulare Biotechnologie/Bioquant	12	Refined treatment selection with machine learning techniques
Lawerenz	Christian		Deutsches Krebsforschungszentrum	2b	Central database & tumorbank
Lode	Holger	Prof. Dr.	Charité Campus Virchow-Klinikum	15	Genetic vaccination
Oberthür	André	Dr.	Zentrum für Kinderheilkunde	9b	NB Toponome
Savelyeva	Larissa	Dr.	Deutsches Krebsforschungszentrum	10	NB Fragilome
Schramm	Alexander	PD Dr.	Universitäts-Kinderklinik Essen	4a	Proteomics of NB master regulators
				6	Role of microRNAs in NB pathogenesis
Schubert	Walter	Dr.	Otto-von-Guericke-Universität Magdeburg	9c	NB Toponome
Schulte	Johannes H.	Dr.	Universitäts-Kinderklinik Essen	5b	Identification of NB initiating genes
				6	Role of microRNAs in NB pathogenesis
Schwab	Manfred	Prof. Dr.	Deutsches Krebsforschungszentrum	10	NB Fragilome
				13	Targeting Myc functions
Stühler	Kai	Dr.	Ruhr-Universität Bochum	4b	Proteomics of NB master regulators
Westermann	Frank	Dr.	Deutsches Krebsforschungszentrum	13	Targeting Myc functions
Witt	Olaf	Prof. Dr.	Deutsches Krebsforschungszentrum	3	Identification of NB initiating cells
				14	Targeting class I histone deacetylases

IG Deciphering Oncogene Dependencies in Human Cancer Oncogene Mutation Space Koordination: PD Dr. Roman Thomas					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Thomas	Roman	Dr.	Max-Planck-Institute	0	Coordinating office
Nürnberg	Peter	Prof. Dr.	University of Cologne	1	Evaluation of tools for clinical detection of mutations and copy number changes
Wolf	Jürgen	Prof. Dr.	University Clinic Cologne	2	Analysis of patient mutation space and clinical outcome
Thomas	Roman	Dr.	Max-Planck-Institute	3	Systematic high-throughput analysis of oncogenicity of human oncogene mutations
Ahmadian	Reza	PD Dr.	Heinrich-Heine Universtity Hospital	4a	Functional impact of oncogene mutants and small molecules on the Ras and Rho signaling pathways
Wittinghofer	Alfred	Prof. Dr.	MPI für molekulare Physiologie	4b	Functional impact of oncogene mutants and small molecules on the Ras and Rho signaling pathways
Rauh	Daniel	Dr.	MPI for Molecular Physiology Dortmund	5a	Dissection of oncogene dependencies by small organic molecule perturbations
Waldmann	Herbert	Prof. Dr.	MPI for Molecular Physiology Dortmund	5b	Dissection of oncogene dependencies by small organic molecule perturbations
Rahnenführer	Jörg	Prof. Dr.	University Dortmund	6b	Statistical modeling of drug response and pathway alterations
Lengauer	Thomas	Prof. Dr. Dr.	MPI für Informatik	6a	Statistical modeling of drug response and pathway alterations

IG Systems Biology of Genetic Diseases, Mutanom Koordination: Prof. Dr. Hans Lehrach					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Lange	Bodo	PD Dr.	Max-Planck-Institut für Molekulare Genetik	TP1	Project coordination
Lehrach	Hans	Prof. Dr.	Max-Planck-Institut für Molekulare Genetik	TP1	Project coordination
Brand	Angela	Prof. Dr.	Maastricht University	TP2	Translational Health Research
Schulte in den Bäumen	Tobias	Dr.	Maastricht University	TP2	Translational Health Research
Schweiger	Michal-Ruth	Dr. Dr.	Max-Planck-Institut für Molekulare Genetik	TP3	Mutational analysis
Mollenhauer	Jan	Prof. Dr.	Medical Biotechnology Center University of Southern Denmark	TP4	Recombinant cancer cell libraries & drug target recovery
Sültmann	Holger	PD Dr.	German Cancer Research Center (DKFZ)	TP5	Quantification of cancer pathways
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	TP6	Protein interaction networks
Schäfer	Reinhold	Prof.	Charité Universitätsmedizin Berlin	TP7	Cellular signalling networks
Herrmann	Bernhard	Prof.	Max-Planck-Institut für Molekulare Genetik	TP8	Mouse disease models

Morkel	Markus	Dr.	Max-Planck-Institut für Molekulare Genetik	TP8	Mouse disease models
Lange	Bodo	PD Dr.	Max-Planck-Institut für Molekulare Genetik	TP9	Protein complex composition and function in disease
Wierling	Christoph		Max-Planck-Institut für Molekulare Genetik	TP10	Data integration and modelling
Drewes	Gerard	Dr.	Cellzome AG	TP11	Quantitative Proteomics
Joberty	Gerard	Dr.	Cellzome AG	TP11	Quantitative Proteomics
IG Translational Genome Research Network in Pancreatic Cancer Koordination: Prof. Dr. Thomas M. Gress					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Gress	Thomas M.	Prof. Dr.	Philipps-Universität Marburg	TP0,TP1b, TP2b,	TP0 Koordination TP1b Klinische Ressourcen und Daten TP2b Mausmodelle des Pankreaskarzinoms
Buchholz	Esther		Philipps-Universität Marburg	TP 0	TP 0 Koordination
Giese	Nathalia	Dr.	Universitätsklinikum Heidelberg	TP1a	Klinische Ressourcen und Daten
Tannapfel	Andrea	Prof. Dr.	Ruhr-Universität Bochum	TP1c	Klinische Ressourcen und Daten
Sipos	Bence	Prof. Dr.	Eberhard-Karls Universität Tübingen	TP1d	Klinische Ressourcen und Daten
Sipos	Bence	Prof. Dr.	Eberhard-Karls Universität Tübingen	TP2c	Mausmodelle des Pankreaskarzinoms
Schmid	Roland M.	Prof. Dr.	TU München	TP2a	Mausmodelle des Pankreaskarzinoms
Buchholz	Malte	PD Dr.	Philipps-Universität Marburg	TP3, TP11a	TP3 Parallelisierte funktionelle Charakterisierung TP11 Molekulare Differentialdiagnose
Seufferlein	Thomas	Prof. Dr.	Martin-Luther-Universität Halle-Wittenberg	TP4	Kinasenetzwerke im Pankreaskarzinom
Hoheisel	Jörg	Dr.	DKFZ Heidelberg	TP5, TP12	TP5 Quantitative Analyse von Proteininteraktionen TP12 Epigenetische Analyse zur therapeutischen Patienten-Stratifizierung
Hahn	Stephan	Prof. Dr.	Ruhr-Universität Bochum	TP6	MiRNAs als therapeutische Targets für das Pankreaskarzinom
Friess	Helmut	Prof. Dr.	TU München	TP7	Molekulare Analyse der tumorspezifischen Stromaaktivierung
Kleeff	Jörg	PD Dr.	TU München	TP7	Molekulare Analyse der tumorspezifischen Stromaaktivierung
Schwarte-Waldhoff	Irmgard	PD Dr.	Ruhr-Universität Bochum	TP9a	Entwicklung von molekulardiagnostischen Verfahren zur Früherkennung des Pankreaskarzinoms basierend auf sezernierten/freigesetzten Kandidaten-Proteinen

Schnölzer	Martina	Dr.	DKFZ Heidelberg	TP9b	Entwicklung von molekulardiagnostischen Verfahren zur Früherkennung des Pankreaskarzinoms basierend auf sezernierten/freigesetzten Kandidaten-Proteinen
Kestler	Hans	Prof. Dr.	Uniklinik Ulm	TP11b	Molekulare Differentialdiagnose
Günther	Simone	Dr.	Applied Biosystems	TP11c	Molekulare Differentialdiagnose

IG Modifiers of Intestinal Tumor Formation and Progression

Koordination: Prof. Dr. Bernhard Herrmann

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Schweiger	Michal-Ruth	Dr. Dr.	Max-Planck-Institut für molekulare Genetik	1	Analyse von normalem und Darmtumorgewebe und Validierungsexperimente in menschlichen Zelllinien
Herrmann	Bernhard	Prof. Dr.	Max-Planck-Institut für molekulare Genetik	2	Identifizierung und Feinkartierung von Modulatoren der epigenetischen Genkontrolle und APC-Min induzierter Darmtumore in CSS Mausstämmen
Lehrach	Hans	Prof. Dr	Max-Planck-Institut für molekulare Genetik	3	Immunpräzipitation von methylierter DNA und Gen-Expressionsanalyse mittels der Sequenziertechnik der 2. Generation
Walter	Jörn	Prof. Dr.	Universität des Saarlandes, Campus Saarbrücken	4	Entwicklung einer Bisulphit-Hochdurchsatz-Sequenzierungsplatform in Kombination mit integrierter Bioinformatik
Morkel	Markus	Dr.	Max-Planck-Institut für molekulare Genetik	5	Validierung von Kandidatengenen (Modifier) in transgenen Mausmodellen
Herwig	Ralf	Dr.	Max-Planck-Institut für molekulare Genetik	6	Bioinformatik und Datenintegration

IG Integrated Genomic Investigation of Colorectal Carcinoma (CRC)

Koordination: Prof. Dr. Kari Hemminki

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Hampe	Jochen	PD Dr.	Universitätsklinikum Schleswig-Holstein	TP1	Fine mapping + replication
Hemminki	Kari	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based studies
Schafmayer	Clemens	Dr.	Universitätsklinikum Schleswig-Holstein	TP2	Population-based and prospective validation
Chang-Claude	Jenny	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based and prospective validation
Brenner	Hermann	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based and prospective validation
Burwinkel	Barbara	Prof. Dr.	Deutsches Krebsforschungszentrum	TP2	Population-based and prospective validation
Krawczak	Michael	Prof. Dr.	Universitätsklinikum Schleswig-Holstein	TP3	Statistics and Genetic epidemiology

Brosch	Mario	Dr.	Universitätsklinikum Schleswig-Holstein	TP4	Somatic mutation signature
Platzer	Matthias	Prof. Dr.	Leibniz-Institut für Altersforschung	TP4	Somatic mutation signature
Siebert	Reiner	Prof. Dr.	Universitätsklinikum Schleswig-Holstein	TP5	Somatic genomic imbalances, LOH and methylation
Boutros	Michael	Prof. Dr.	Deutsches Krebsforschungszentrum	TP6	Systems biology of signaling pathways in colorectal carcinomas
Spang	Rainer	Prof. Dr.	Universität Regensburg	TP7	System biology of the cancer cell
Kalthoff	Holger	Prof. Dr.	Universitätsklinikum Schleswig-Holstein	TP8	Pathways: tumor tissue
Hemminki	Kari	Prof. Dr.	Deutsches Krebsforschungszentrum	TP9	Coordination

IG MooDS: Systematic Investigation of the Molecular Causes of Major Mood Disorders and Schizophrenia Koordination: Prof. Dr. Markus Nöthen					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Cichon	Sven	Prof. Dr. rer. nat.	Universitätsklinikum Bonn	1	Genomik bei Bipolarer Störung
Schumacher	Johannes	PD Dr.	National Institute of Mental Health (NIMH)	1	Genomik bei Bipolarer Störung
Holsboer	Florian	Prof. Dr. Dr.	Max Planck Institut für Psychiatrie	2	Genomik bei unipolarer Störung
Lucae	Susanne	Dr. Dr.	Max Planck Institut für Psychiatrie	2	Genomik bei unipolarer Störung
Rujescu	Dan	PD Dr.	Psychiatrische Klinik der LMU	3	Genomik bei Schizophrenie
Maier	Wolfgang	Prof. Dr.	Universitätsklinikum Bonn	3	Genomik bei Schizophrenie
Nöthen	Markus	Prof. Dr. med.	Universitätsklinikum Bonn	4a	Hochdurchsatz-Genotypisierung
Bettecken	Thomas	Dr. rer. nat.	Max Planck Institut für Psychiatrie	4b	Hochdurchsatz-Genotypisierung
Rietschel	Marcella	Prof. Dr. med.	Zentralinstitut für Seelische Gesundheit	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Reinelt	Gerhard	Prof. Dr. med.	Universität Heidelberg	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Schulze	Thomas G.	PD Dr. med.	Unit on the Genetic Basis of Mood and Anxiety Disorders	5	MooDS Phenom Datenbank und Reverse Phänotypisierung
Meyer-Lindenberg	Andreas	Prof. Dr. med. Dipl. math.	Zentralinstitut für Seelische Gesundheit	6a	Imaging Genetik
Walter	Henrik	Prof. Dr. med. Dr. phil.	Universitaetsmedizin Charite, Campus Mitte	6b	Imaging Genetik
Heinz	Andreas	Prof. Dr. med.	Charité– Universitätsmedizin Berlin	6c	Imaging Genetik
Wienker	Thomas F.	Prof. Dr. med.	Universitätsklinikum Bonn	7	Statistische Analysen zu genomweiten Assoziationsstudien
Müller-Myhsok	Bertram	Prof. Dr. med.	Max Planck Institut für Psychiatrie	8	Entwicklung statistischer Methoden für komplexe Gen-Gen Interaktionen in genomweiten Datensätzen
Cichon	Sven	Prof. Dr. rer. nat.	Universitätsklinikum Bonn	9	Allel-spezifische Expression
Becker	Albert	Prof. Dr. med.	Universitätsklinikum Bonn	9	Allel-spezifische Expression

Eils	Roland	Prof. Dr.	DKFZ Heidelberg	10	Methodenentwicklung für biologische Pathway-Informationen in GWA-Analysen
Brors	Benedikt	Dr. rer. nat.	Universität Heidelberg	10	Methodenentwicklung für biologische Pathway-Informationen in GWA-Analysen
Wanker	Erich E.	Prof. Dr. rer. nat.	Max-Delbrueck-Center für Molekulare Medizin Berlin-Buch	11	Protein-Protein Interaktions-Netzwerk
Zimmer	Andreas	Prof. Dr. rer. nat.	Universitätsklinikum Bonn	12a	Funktionelle Untersuchungen mit Hilfe von transgenen Mausmodellen und Proteomanalysen
Wurst	Wolfgang	Prof. Dr. rer. nat.	Helmholtz Zentrum München	12b	Funktionelle Untersuchungen mit Hilfe von transgenen Mausmodellen und Proteomanalysen
Turck	Chris	Prof. Dr. rer. nat.	Max Planck Institut für Psychiatrie	12	Funktionelle Untersuchungen mit Hilfe von transgenen Mausmodellen und Proteomanalysen
Nöthen	Markus	Prof. Dr. med.	Universitätsklinikum Bonn	14	Projekt-Management und Graduierten-Training
Raff	Ruth	Dr. rer. nat.	Universitätsklinikum Bonn	14	Projekt-Management und Graduierten-Training

IG Genetics of Alcohol Addiction

Koordination: Prof. Dr. Rainer Spanagel

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Spanagel	Rainer	Prof. Dr.	Central Institute of Mental Health	1	Coordination Consortium
Eils	Roland	Prof. Dr.	German Cancer Research Center	2a	Gene data mining platform and statistics
Brors	Benedikt	Dr.	German Cancer Research Center	2a	siehe Eils
Wienker	Thomas	Prof. Dr.	University of Bonn	2b	siehe Eils
Matthäus	Franziska	Dr.	University of Heidelberg,	3	Mathematical Modelling and Analysis
Jäger	Willi	Prof. Dr. Dr. h.c. mult	University of Heidelberg,	3	siehe Matthäus
Schütz	Günter	Dr. med.	German Cancer Research Center (DKFZ)	4	Functional analysis I and conditional mouse models
Wurst	Wolfgang	Prof. Dr.	GSF - National Research Center for Environment and Health	5	Functional analysis II and RNAi in vivo application
Deussing	Jan	Dr.	Max Planck Institute of Psychiatry	5	siehe Wurst
Zimmer	Andreas	Prof. Dr.	University of Bonn	6	Functional analysis III
Bartsch	Dusan	Prof. Dr.	Central Institute of Mental Health	7	Transgenic rat models
Zimmer	Andreas	Prof. Dr.	University of Bonn	8	Behavioral analysis of Animal Models
Spanagel	Rainer	Prof. Dr.	Central Institute of Mental Health	8	Behavioral analysis of Animal Models
Gebicke-Haerter	Peter	Prof. Dr.	Central Institute of Mental Health	9	Glutamatergic and epigenetic profiling with microarrays
Hoheisel	Jörg	Dr.	Deutsches Krebsforschungszentrum	9	siehe Gebicke-Haerter
Sprengel	Rolf	Dr.	MPI Med. Forschung Heidelberg	10	Transcriptional and posttranscriptional modifications

Rietschel	Marcella	Prof. Dr.	Central Institute of Mental Health	11	GWA studies in alcohol dependent patients and replication studies
Nöthen	Markus	Prof. Dr.	University of Bonn	11	siehe Rietschel
Dahmen	Norbert	PD Dr.	Universität Mainz	12a	GWA studies in population-based samples for high versus low alcohol consumption and replication studies
Wichmann	H. Erich	Prof. Dr.	GSF Institute of Epidemiology	12b	siehe Dahmen
Heinz	Andreas	Prof. Dr.	University Medical Center Berlin, Campus Charité	13b	Endophenotyping with fMRI: Genetic modulation and treatment response
Walter	Henrik	Prof. Dr.	Universitaetsmedizin Charite, Campus Mitte	13a	siehe Heinz
Kiefer	Falk	Prof. Dr.	Central Institute of Mental Health	13c	siehe Heinz
Mann	Karl	Prof. Dr. Dr.	Central Institute of Mental Health	14a	Endophenotyping with spectroscopy: Genetic modulation and treatment response
Ende	Gabriele	Dr.	Central Institute of Mental Health	14a	siehe Mann
Gallinat	Jürgen	PD Dr.	Psychiatry, Charité, CCM	14b	siehe Mann
Sartorius	Alexander	Dr.	Central Institute of Mental Health	15	Glutamate spectroscopy at 9.4T combined with microdialysis in rodents

IG German Mental Retardation Network (Netzwerk Mentale Retardierung)

Koordination: Prof. Dr. André Reis

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Rauch	Anita	Prof. Dr. med.	Friedrich-Alexander-Universität Erlangen-Nürnberg	1	MR Zentrum Erlangen
Ropers	Hans-Hilger	Prof. Dr. med.	Max Planck Institut für Molekulare Genetik	2	MR Zentrum Berlin
Riess	Olaf	Prof. Dr. med.	Eberhard-Karls-Universität Tübingen	3	MR Zentrum Tübingen
Strom	Tim M	PD Dr. med.	Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH)	4	MR Zentrum München
Engels	Hartmut	Dr. rer. nat.	Rheinische Friedrich-Wilhelms-Universität Bonn	5	MR Zentrum Bonn
Wieacker	Peter	Prof. Dr. med.	Medizinische Fakultät der Westfälischen Wilhelms-Universität Münster	6	MR Zentrum Münster
Schröck	Evelin	Prof. Dr. med.	Medizinische Fakultät Carl Gustav Carus der Technischen Universität Dresden	7	MR Zentrum Dresden
Wieczorek	Dagmar	PD Dr. med.	Universität Duisburg Essen	8	MR Zentrum Essen
Rappold	Gudrun	Prof. Dr. rer. nat.	Ruprechts-Karls Universität Heidelberg	9	MR Zentrum Heidelberg
Schenck	Annette	Dr. rer. nat.	Radboud Universität Nijmegen	10	Modellierung mentaler Retardierung in Fliegen
Reis	André	Prof. Dr. med.	Friedrich-Alexander-Universität Erlangen-Nürnberg	11	Projektkoordination

IG Epilepsy and Migraine Integrated Network (EMINet) Koordination: Prof. Dr. Christian Kubisch					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Kubisch	Christian	Prof. Dr.	University of Ulm	1	Genome-wide association analysis and gene identification in migraine with aura
Dichgans	Martin	Prof. Dr.	LMU Munich	2	Whole-genome association study in migraine without aura and functional characterization of disease associated alleles (TP2)
Sander	Thomas	Dr.	University of Cologne	3	Genome-wide association mapping of gene configurations conferring risk to idiopathic generalized epilepsies (TP3)
Nürnberg	Peter	Prof. Dr.	University of Cologne	4	High-throughput sequencing of functional and positional candidate genes for common forms of migraine and epilepsy (TP4)
Schoch-McGovern	Susanne	Dr.	University of Bonn	5	Genetic basis of Levetiracetam pharmacoresistance and side effects in human epilepsy (TP5)
Lerche	Holger	Prof. Dr.	University of Ulm	6	Functional analysis of human ion channel mutations in cellular and animal models (TP6)
Becker	Albert	PD Dr.	University of Bonn	7	Aberrant transcriptional networks in human epileptic tissue
Beck	Heinz	Prof. Dr.	University of Bonn	8	Mechanisms underlying the development of cellular hyperexcitability in mouse models of human epilepsy
Isbrandt	Dirk	Dr.	University of Hamburg	9	Subthreshold ion channels in epileptogenesis and neuronal synchronization

IG Gene Identification and Functional analyses in Alzheimer's disease Koordination: Prof. Dr. Matthias Riemenschneider					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Riemenschneider	Matthias	Prof. Dr	Universitätsklinikum des Saarlandes	1	Identification of genetic factors in Alzheimer's disease
Krobitsch	Sylvia	Dr.	Max Plank Institut für molekulare Genetik	2	Identification and functional characterization of novel early-onset Alzheimer's genes
Haas	Christian	Prof. Dr.	LMU München	3	The physiological function of BACE1-is BACE1 a safe therapeutic target?
Garratt	Alistair	Dr.	Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch	3	The physiological function of BACE1-is BACE1 a safe therapeutic target?
Müller	Ulrike	Prof. Dr.	University of Heidelberg	4	In vivo analysis of APP functional domains-can we safely abrogate APP/APLP processing?

Hartmann	Tobias	Prof. Dr.	Universität des Saarlandes	5	Functional involvement of Alzheimer's disease candidate risk genes in lipid homeostasis, Ab metabolism and Ab response
Endres	Kristina	Dr.	Johannes Gutenberg Univers. Mainz	6	Regulation of ADAM10 gene expression and neuroprotection
Jucker	Mathias	Prof. Dr.	Hertie-Institut für klinische Hirnforschung	7	Pathomechanism of Cerebral Amyloid Angiopathy
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin-Buch	8	Identification and characterization of modulators of Alzheimer's disease pathogenesis
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	9	Animal models for candidate genes of Alzheimer's disease
Riemenschneider	Matthias	Prof. Dr.	Universitätsklinikum des Saarlandes	10	Scientific administration office of the AD-IG
IG Functional Genomics of Parkinson's disease Koordination: Prof. Dr. Thomas Gasser					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Gasser	Thomas	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP1/TP2	Scientific Coordinating Office
Klein	Christine	Prof. Dr.	Universität Lübeck	TP3	Mutations in recessive Parkinson's disease genes
Höglinger	Günther	PD. Dr.	Philipps-Universität Marburg	TP4	Genome-wide siRNA screen in an α-synuclein-based in vitro model of Parkinson's disease
Schulz	Jörg B.	Prof. Dr.	Universität Göttingen	TP5	Modifier screen in flies overexpressing LRRK2
Zweckstetter	Markus	Prof. Dr.	Universität Göttingen	TP6	Molecular mechanisms of pathogenic misfolding of α-synuclein
Auburger	Georg	Prof. Dr.	J.W. Goethe University	TP7	Biomarkers of the common Parkinson pathway:α-Synuclein induction and synaptic pathology in recessive PD
Riess	Olaf	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP8	Calpain cleavage of α-synuclein in the pathogenesis of Parkinson's disease by cell culture and animal models
Kahle	Philipp	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP9	Regulation of Apoptosis Signal Regulating Kinase Pathways by DJ-1 and Parkin
Krüger	Rejko	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP10	Mitochondrial stress response in neurodegeneration and aging: OMI and DJ-1 mediated signalling pathways
Winklhofer	Konstanze	PD. Dr.	Ludwig-Maximilians-Universität München	TP11	The physiological and pathological function of PINK 1, parkin and LRRK2 in zebrafish and other models
Haass	Christian	Prof. Dr.	Ludwig-Maximilians-Universität München	TP11	The physiological and pathological function of PINK 1, parkin and LRRK2 in zebrafish and other models
Ueffing	Marius	PD. Dr.	TU München	TP12	Functional characterization of LRRK2 in mammalian cells and tissues

Roeper	Jochen	Prof. Dr.	J.W. Goethe University	TP13a	Dopaminergic dysfunction and molecular pathways to selective neurodegeneration: from mouse models to Parkinson disease
Liss	Birgit	Prof. Dr.	Universität Ulm	TP13	Dopaminergic dysfunction and molecular pathways to selective neurodegeneration: from mouse models to Parkinson disease
Schütz	Günther	Prof. Dr.	German Cancer Research Center	TP14	Characterization of genetic mouse models for Parkinson´s disease
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	TP14	Characterization of genetic mouse models for Parkinson´s disease
Marcus	Katrin	Prof. Dr.	Ruhr University Bochum	TP15	Core facility: High-performance proteome analysis for biomarker discovery and elucidation of pathomechanisms
Zell	Andreas	Prof. Dr.	Eberhard-Karls-Universität Tübingen	TP16	Core facility: Bioinformatics: data integration towards a systems level model of Parkinson's disease Generation of a systems biology model
Meitinger	Thomas	Prof. Dr.	Helmholtz Zentrum München	Core facility	Core facility: High throughput genotyping

IG NeuroNet - Verbundprojekt Neurodegeneration

Koordination: Prof. Dr. Erich Wanker

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	1	Protein-Protein Interaktionsnetzwerke bei neurodegenerativen Erkrankungen
Selbach	Matthias	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	2	Protein Interaktionsscreening durch quantitative Massen-spektroskopie
Stelzl	Ulrich	Dr.	Max-Planck-Institut für Molekulare Genetik	3	Modulation von Protein-Protein Wechselwirkungen durch Phosphorylierung
Priller	Josef	Prof. Dr.	Charité - Universitätsmedizin Berlin	4	Klassifikation von Phänotyp-Genotyp-Beziehungen bei - neurodegenerativen Erkrankungen
Lange	Bodo	PD Dr.	Max-Planck-Institut für Molekulare Genetik	5	Modulation von Protein-komplexkomposition und Funktion durch Stress und Neurodegenerative Krankheitssignale
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	6a	Erstellung von Genexpressionssignaturen von neurodegenerativen Krankheitsprozessen
Nietfeld	Wilfried	Dr.	Max-Planck-Institut für Molekulare Genetik	6b	Erstellung von Genexpressionssignaturen von neurodegenerativen Krankheitsprozessen

Bork	Peer	Prof. Dr.	European Molecular Biology Laboratory	6c	Erstellung von Genexpressionssignaturen von neurodegenerativen Krankheitsprozessen
Boutros	Michael	Dr.	Deutsches Krebsforschungszentrum	7	Systematische Analyse von Phänotypen mittels RNAi und kleinen Molekülen
Bork	Peer	Prof. Dr.	European Molecular Biology Laboratory	8a	Datenintegration und Erstellung von Phänotyp-Protein- Wirkstoff Netzwerken
Andrade	Miguel	Dr.	Max-Delbrück-Centrum für Molekulare Medizin	8b	Datenintegration und Erstellung von Phänotyp-Protein- Wirkstoff Netzwerken
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin	9	Management der IG „NeuroNet“
Wanker	Erich	Prof. Dr.	Max-Delbrück-Centrum für Molekulare Medizin		Wissenschaftliche Plattform „Interaktom“ für systematische Protein-Interaktionsstudien

IG From Disease genes to Protein Pathways (DiGTOP)

Koordination: Prof. Dr. Wolfgang Wurst

Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Stewart	Francis	Prof. Dr.	Technische Universität Dresden	1	Genidentifikation und DNA Konstruktproduktion
von Melchner	Harald	Prof. Dr.	Universität Frankfurt	2	In situ Markierung von Krankheitsproteinen in embryonalen Stammzellen mit Genfallen-induzierten Mehrzweckallelen
Wurst	Wolfgang	Prof. Dr.	HelmholtzZentrum München	3	Produktion proteinmarkierter pluripotenter und differenzierter ES Zellen
Hyman	Tony	Prof. Dr.	MPI für Zellbiologie und Genetik Dresden	4	Produktion und Imaging von HeLa und ES Zelllinien
Brüstle	Oliver	Prof. Dr.	Universität Bonn	5	Etablierung und Analyse transgener hES Zelllinien und neuralen Stammzelllinien
Mann	Matthias	Prof. Dr.	MPI für Biochemie, Martinsried	6	Proteininteraktionsstudien mittels massenspektrometrie-basierter Proteomik in in vitro und in vivo Systemen
Hansen	Jens	Dr.	HelmholtzZentrum München	7a	DiGtoP bioinformatics – resource development and application in comparative network analysis
Gibson	Toby	Prof. Dr.	EMBL Heidelberg	7	DiGtoP bioinformatics – resource development and application in comparative network analysis
Kühn	Ralf	Dr.	HelmholtzZentrum München	8	Mausmodelle für die in vivo Validierung von Proteininteraktionen
Buchholz	Frank	Dr.	MPI für Zellbiologie und Genetik Dresden	9	Validierung und Zergliederung der Signalwege von Krankheitsrelevanten Genen mit endoribonucelase präparierter siRNA
Wurst	Wolfgang	Prof. Dr.	HelmholtzZentrum München	10	Management & Training

IG German Mouse Clinic (GMC)

Koordination: Prof. Dr. Martin Hrabě de Angelis

Projektleiter				Teilprojekt	
Nachname	Vorname	Titel	Institution	Nr.	Titel
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	1	Core Facility
Gailus-Durner	Valérie	Dr.	Helmholtz Zentrum München	1	Core Facility
Fuchs	Helmut	Dr.	Helmholtz Zentrum München	1	Core Facility
Wolf	Eckhard	Prof. Dr.	Genzentrum der LMU München	2	Clinical Chemical Screen
Wurst	Wolfgang	Prof. Dr.	Helmholtz Zentrum München	3	Behavioral Screen
Hölter-Koch	Sabine	Dr.	Helmholtz Zentrum München	3	Behavioral Screen
Klopstock	Thomas	PD Dr. med.	LMU München	4	Neurological Screen
Graw	Jochen	Prof. Dr.	Helmholtz Zentrum München	5	Eye Screen
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	6	Dysmorphology Screen
Fuchs	Helmut	Dr.	Helmholtz Zentrum München	6	Dysmorphology Screen
Busch	Dirk	Prof. Dr.	TU München	7	Immunology Screen
Ollert	Markus	Prof. Dr.	TU München	8	Allergy Screen
Adamski	Jerzy	Prof. Dr.	Helmholtz Zentrum München	9	Steroid Screen
Zimmer	Andreas	Prof. Dr.	Universitätsklinikum Bonn	10	Nociceptive Screen
Schulz	Holger	Prof. Dr.	Helmholtz Zentrum München	11	Lung Function Screen
Stöger	Tobias	Dr.	Helmholtz Zentrum München	11	Lung Function Screen
Beckers	Johannes	PD Dr.	Helmholtz Zentrum München	12	Molecular Phenotyping Screen
Klingenspor	Martin	Prof. Dr.	TU München	13	Energy Metabolism Screen
Daniel	Hannelore	Prof. Dr.	TU München	13	Energy Metabolism Screen
Katus	Hugo	Prof. Dr.	Universität Heidelberg	14	Cardiovascular Screen
Bekeredjian	Raffi	Dr.	Universität Heidelberg	14	Cardiovascular Screen
Höfler	Heinz	Prof. Dr.	Helmholtz Zentrum München	15	Pathology Screen
Esposito	Irene	PD Dr.	Helmholtz Zentrum München	15	Pathology Screen
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	16	Data Management
Lengger	Christoph	Dr.	Helmholtz Zentrum München	16	Data Management
Schughart	Klaus	Prof. Dr.	HZI - Helmholtz-Zentrum für Infektionsforschung	17	Host Pathogen Interaction Screen
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	18	EMMA
Hagn	Michael	Dr.	Helmholtz Zentrum München	18	EMMA
Hrabě de Angelis	Martin	Prof. Dr.	Helmholtz Zentrum München	01GS0849	Generierung von Mausmodellen

IG MHC Haplotype Sequencing: An Integrated Approach to Common Disease

Koordination: Dr. Margret Hoehe

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Hoehe	Margret	Dr.	MPI-MG Berlin	1	MHC-Haplotypen-Sequenzierung

IG Cellular Systems Genomics in Health and Disease

Koordination: PD Dr. Stefan Wiemann

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	1	Projekt Coordination
Bechtel	Stefanie	Dr.	DKFZ Heidelberg	2	Functional Genomic Resources for NGFNplus
Tschulena	Ulrich	Dr.	DKFZ Heidelberg	3	Cellular Screening Systems
Arlt	Dorit	Dr.	DKFZ Heidelberg	4	Signalling Network analysis
Gavin	Anne-Claude	Dr.	EMBL Heidelberg	5	TAP - Protein interaction mapping
Pepperkok	Rainer	Dr.	EMBL Heidelberg	6	Protein and Network dynamics
Korf	Ulrike	Dr.	DKFZ Heidelberg	7	Quantitative Proteinarrays
Lange	Bodo	PD Dr.	Max-Planck Institut für Molekulare Genetik	8	Primary Cancer Cell Models
Schneeweiss	Andreas	Prof. Dr.	Uniklinik Heidelberg	9	Clinical validation
Beissbarth	Tim	Dr.	DKFZ Heidelberg	10	Pathway reconstruction & modelling
Joecker	Anika	Dr.	DKFZ Heidelberg	11	Integrated bioinformatics
Wiemann	Stefan	PD Dr.	DKFZ Heidelberg	12	QM & Standards

NGFN Geschäftsstelle

Koordination: Dr. Silke Argo

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Argo	Silke	Dr.	DKFZ Heidelberg	1	Geschäftsstelle des Projektkomitees von NGFN-Plus und NGFN-Transfer im Programm der Medizinischen Genomforschung

KTT

Koordination: Dr. Hubert Müller

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Müller	Hugo	Dr.	Ascenion GmbH	O1GS0901	Nationales Genomforschungsnetz: KompetenzCenter Technologietransfer (KTT) – Fortführung

IA Entwicklung prophylaktisch wirksamer Anti-Malaria Verbindungen

Koordination: Dr. Birte Sönnichsen

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Sönnichsen	Birte	Dr.	Cenix BioScience GmbH	1	Anti Malaria Zielgene und Wirkstoffkandidaten
Matuschewski	Kai	PD Dr.	Universität Heidelberg	2	Zielgene im Parasiten
Frischknecht	Friedrich	Dr.	Universität Heidelberg	3	Imaging von Interaktionen des Parasiten mit Leberzellen

IA Breast Cancer Kit

Koordination: Prof. Dr. Jan Georg Hengstler

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Schmidt	Marcus	Dr. med.	Universität Mainz	1	Chemosensitivity determination, clinical data and tumour tissue banking
Gehrmann	Mathias	Dr.	Siemens Medical Solutions Diagnostic GmbH	2	Identification of gene signatures predicting drug efficacy
Hengstler	Jan Georg	Prof. Dr. med.	Institut für Arbeitsphysiologie an der Technischen Universität Dortmund	3	Oncoprofile-Kit

IA Heart Failure Therapy

Koordination: Prof. Dr. Markus Hecker

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
			AVONTEC GmbH	1	Zielmoleküle für eine Decoy Oligodesoxnukleotid-Therapie der Herzinsuffizienz
Hecker	Markus	Prof. Dr.	Universität Heidelberg	2	Validierung der Decoy Oligodesoxynukleotid-Medikamentenkandidaten in Herzinsuffizienzmodellen
Wagner	Andreas H.	Priv.-Doz. Dr.	Universität Heidelberg	2	Validierung der Decoy Oligodesoxynukleotid-Medikamentenkandidaten in Herzinsuffizienzmodellen
Müller	Oliver J.	PD Dr. med.	Universität Heidelberg	3	Zellspezifischer Decoy Oligodesoxynukleotid-Transfer ins insuffiziente Herz
Bekeredjian	Raffi	PD Dr. med.	Universität Heidelberg	3	Zellspezifischer Decoy Oligodesoxynukleotid-Transfer ins insuffiziente Herz

IA Metabolomics in Heart Failure as a Novel Diagnostic Tool Koordination: Prof. Dr. Hugo A. Katus					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Katus	Hugo A.	Prof. Dr.	Universitätsklinikum Heidelberg	1a	Novel Biomarkers for Heart Failure - Metabolic Signatures (Erster Projektleiter und Ansprechpartner auf der Arbeitsebene)
Fuhrmann	Jens	Dr.	metanomics GmbH	1b	Novel Biomarkers for Heart Failure - Metabolic Signatures (Co-Pi und Ansprechpartner auf der Arbeitsebene)
Frey	Norbert	PD Dr.	Universitätsklinikum Heidelberg	2	Metabolic Profiling in Mouse Models of Heart Failure (Erster Projektleiter und Ansprechpartner auf der Arbeitsebene)
Müller	Oliver J.	PD Dr.	Universitätsklinikum Heidelberg	2	Metabolic Profiling in Mouse Models of Heart Failure (Co-Pi und Ansprechpartner auf der Arbeitsebene)
Weis	Tanja	Dr.	Universitätsklinikum Heidelberg		Coordination

IA New Tools for the Prevention of Cardiovascular Diseases and Disorders in Chronic Kidney Disease Koordination: Prof. Dr. Joachim Jankowski					
Projektleiter				**Teilprojekt**	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Jankowski	Vera	Dr.	Charité – Universitätsmedizin Berlin	1	Bioanalytik der chronischen Niereninsuffizienz
Lehmann	Kerstin	Dr.	Charité – Universitätsmedizin Berlin	2	Effekte auf aktivierte Endothelzellen
Buschmann	Ivo	PD Dr.	Charité – Universitätsmedizin Berlin	2	Effekte auf aktivierte Endothelzellen
Herget-Rosenthal	Stefan	PD Dr.	Universitätsklinikum Essen / Universität Duisburg Essen	3	Patienten und Proben
Herwig	Ralf	Dr.	Max Planck Institut für Molekulare Genetik (MPIMG)	4	Bioinformatik
Lemke	Horst-Dieter	Dr.	EXcorLab GmbH	5	Aktivierung von Neutrophilen durch urämische Proteine
Krahn	Thomas	Dr.	Bayer Schering Pharma	6	CVD Drug Discovery Biomarker & Targets

IA Proteinanalysen in FFPE Brustkrebsgeweben - Brustkrebsmarker

Koordination: Prof. Dr. Karl-Friedrich Becker

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Becker	Karl-Friedrich	Prof. Dr.	Technische Universität München	1	Proteinlysat Mikroarrayanalyse für uPA und PAI-1 von Formalin-fixierten Brustkrebsgeweben
				2	HER2-Rezeptor Expression und Signalwege in Brustkrebsgeweben
Porschewski	Peter	Dr.	Qiagen GmbH	3	Proteomsignaturen in FFPE-Geweben

IA Subgenome Fraktionation for High Throughput Sequencing

Koordination: Dr. med. Benjamin Meder

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Beier	Markus	Dr.	febit AG	1	Development of microarrays for sub-genome preparation
Scharfenberger-Schmeer	Maren	Dr.	DKFZ	2	Cancer Genome Comparisons
Pfeufer	Arne	PD Dr.	TU München	3a	Cardiomyopathy Re-sequencing
Meder	Benjamin	Dr. med.	Universität Heidelberg	3b	Cardiomyopathy Re-sequencing
Strom	Tim	Dr.	Helmholtzzentrum München	4	Coverage and variation detection

IA Whole Genome and Transcriptome Amplification in Large Biobanks

Koordination: Prof. Dr. Dr. H.-Erich Wichmann, Dr. Christian Korfhage

Projektleiter				Teilprojekt	
Nachname	**Vorname**	**Titel**	**Institution**	**Nr.**	**Titel**
Korfhage	Christian	Dr.	Qiagen	1	Development and standardization of new WGA and WTA methods
Wichmann	H.-Erich	Prof.Dr. Dr.	HMGU	2	Provision of biosamples of different quality to test whole genome and transcriptome amplification techniques.
Klopp	Norman	Dr.	HMGU	2	Provision of biosamples of different quality to test whole genome and transcriptome amplification techniques.
Wichmann	H.-Erich	Prof.Dr. Dr.	HMGU	3	Transfer of the results to international organisations in the field of biobanking

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