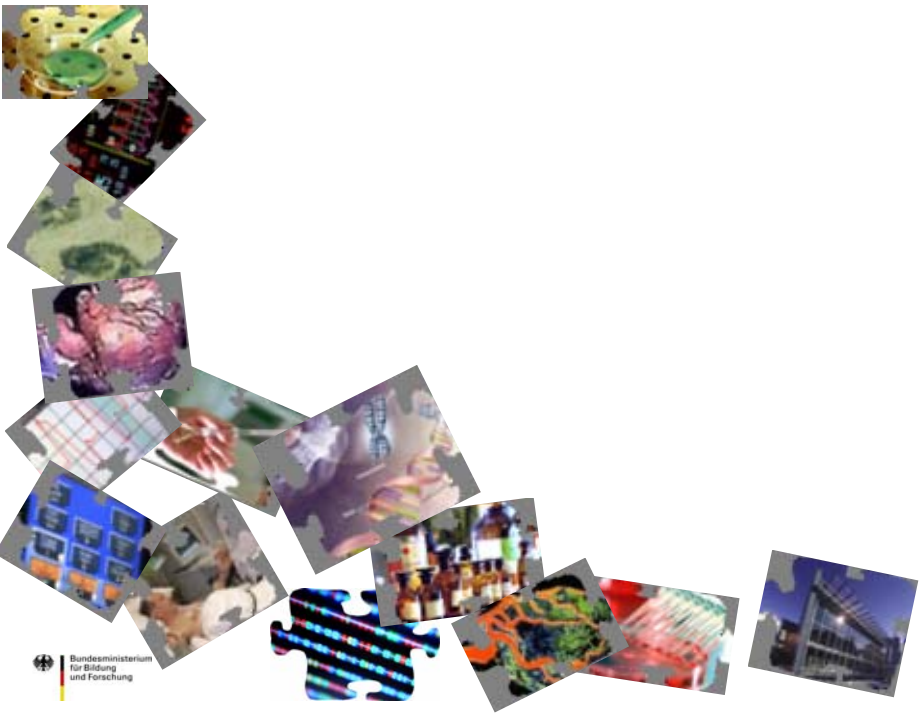
1st Network Meeting
Herz-Kreislauf-Netz in the National Genome Research Network

December 2nd-3rd 2005

University Hospital Heidelberg,
Medical Clinic (Krehl Klinik)



Bundesministerium für Bildung und Forschung

NGFN Nationales Genomforschungsnetz

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Cardiovascular diseases are by far and even more than the malignant diseases the most frequent cause of death in the western developed countries at a world-wide increasing tendency.

We all know the classic risk factors like smoking, obesity, diabetes and unfavorable blood fats. However, the research of the past years clearly revealed that the individual genetic disposition is of utmost importance in the genesis and clinical course of cardiovascular diseases.

Therefore it is a special pleasure for me to welcome you to the first network meeting of the „Herz-Kreislauf-Netz im Nationalen Genomforschungsnetz“ (Cardiovascular Diseases Network in the National Genome Research Network). By means of lectures, poster presentations and workshops we will present our newest data and exciting results in funtional cardiovascular genomics.

I look forward to a interesting meeting and vivid discussions.

Prof. Dr. Hugo A. Katus
(Speaker and Coordinator)

Friday, December 2nd, 2005	
17:00 – 18:00	*Registration* (Infodesk foyer lecture room)
20:00	*Welcome buffet / Get together* (foyer lecture room)

Saturday, December 3rd, 2005	
8:00	***Welcome Remarks*** **(lecture room)** Prof. Dr. C.R. Bartram (Dean of Med. Faculty, Heidelberg) Dr. Birgit Wetterauer (BMBF) Prof. Dr. H.A. Katus (Speaker and coordinator, Herz-Kreislauf-Netz)
8:15 – 9:30	***Overview talks*** **(lecture room)**
8:15	*Overview CV3 (Cardiomyopathies)* (Prof. Dr. H.A. Katus, Heidelberg)
8:30	*Overview CV1 (Hypertension and end organ damage)* (Prof. Dr. R. Kreutz, Berlin)
8:45	*Overview CV2 (CAD / MI)* (Prof. Dr. C. Hengstenberg, Regensburg)
9:00	*Overview CV4 (Heart failure)* (Prof. Dr. G. Hasenfuß, Göttingen)
9:15	*Overview Hemostaseology / Atherosclerosis* (Prof. Dr. W. Koenig, Ulm)
9:30 – 9:45	*Coffee break* (foyer lecture room)

9:30 – 9:45	*Coffee break* (foyer lecture room)
9:45 – 12.45	***Poster sessions*** **(Foyer lecture room)**
9:45	*Session CV1 / CV2*
10:45	*Session CV3*
11:45	*Session CV4 + Hemostaseology /Atherosclerosis*
12:45 – 13:45	*Lunch break* (buffet, foyer lecture room)
Afternoon schedule	
13:45 – 15:45	***Workshops*** **(Seminar rooms,** workshops each 2h)
	• Mutation screening (PD Dr. D. Weichenhan) • Mouse clinic (Dr. B. Ivandic) • 2D-Gelelectrophoresis (Prof. Dr. G. Hasenfuß / Dr. P. Schott)
15:45 – 16:00	*Coffee break*
16:00 – 18:00	***Workshops*** **(Seminar rooms,** workshops each 2h)
	• Zebra fish platform (Dr. W. Rottbauer) • Phenotype optimization by MRI (Dr. E. Giannitsis) • Genome wide association scans (Prof. Dr. L. Wojnowski, Dr. A. Pfeufer)
18:00	*Résumé / closing remarks* (lecture room) Prof. Dr. H.A. Katus
18:15	***End of meeting***

CV1
Hypertension and cardiovascular end organ damage

(Head: Prof. Dr. R. Kreutz, Berlin)

Comparative Genomics of Left Ventricular Hypertrophy and Dysfunction in Hypertension (Prof. Dr. R. Kreutz, Berlin)

Functional Genomics of Cardiac Damage in Hypertension
(Prof. Dr. M. Paul, Berlin)

Functional Genomics of Vascular Damage in Hypertension
(Prof. Dr. Th. Unger, Berlin)

Arterial hypertension is one of the most important risk factors for cardiovascular morbidity and mortality. Recent epidemiological analyses show that the prevalence of arterial hypertension increases dramatically in Germany and also world wide. The clinical importance of arterial hypertension is associated with the resulting cardiovascular damages. Experimental and clinical data reveal the genetic role in the manifestation and progression of cardiovascular damages following arterial hypertension. The main goal of the research project is the characterization of the genetic and molecular background of hypertensive end organ damage in the heart and arterial vessels.

By means of an integrated analytical approach the pathomechanisms of functional and structural organ damages are analyzed on the molecular level. Our analyses encompass experimental approaches in genetic modified models of mouse and rat. One main research topic is the genome wide association study in polygenetic animal models with left ventricular hypertrophy and dysfunction in the rat. New candidate genes for left ventricular damage in humans shall be identified by comparative genome analyses. These analyses where made possible by established network structures within topic CV1.3 which allow a wide spectrum of functional analyses of identified candidate genes. We carry out experimental analyses in cell culture and isolated organs as well as by in vivo studies involving Zebra fish, mouse and rat models. Additional clinical and functional analyses are performed in selected patients with hypertension and end organ damage. The data are confirmed by genetic analyses in larger population. Our research program shall contribute to the characterization of new target genes for diagnostics and therapy of hypertensive end organ damage.

CV2
Genetics of coronary artery disease and myocardial infarction

(Head: Prof. Dr. H. Schunkert, Lübeck)

Genome-wide Assessment of Siblings with Myocardial Infarction
(Prof. Dr. Ch. Hengstenberg, Regensburg)

Identification of Genes for Monogenic Forms of Myocardial Infarction
(PD Dr. J. Erdmann, Lübeck)

Genetics of Coronary Morphology (Prof. Dr. H. Schunkert, Lübeck)

Myocardial infarction (MI) is a multifactorial process. The genetic background of MI is unknown so far and scientists recently performed both, candidate gene analyses and genome-wide screenings in human populations. In the current analyses, gene variants relevant for the development of coronary artery diseases will be identified and characterized.

In the subproject CV2.1, a genome-wide analysis in families with increased incidence of MI revealed a locus on chromosome 14q32 suggesting the presence of a gene with a strong effect. The identification of this gene will be carried out using case-control studies using genetic variants (single nucleotide polymorphisms, SNPs). A refined analysis includes haplotypes, linkage disequilibrium testing and mapping, sequencing, and possibly functional analyses.

In subproject CV 2.2, chromosomal loci showing autosomal-dominant inheritance for MI will be analyzed in extended pedigrees. Candidate genes will be sequenced and functionally analyzed if they show a genetic variant.

In subproject CV2.3, the heritability of the distinct coronary morphology including the calcification and coronary ectatic lesions will be characterized. The preliminary data from coronary angiograms of sibling pairs and their extended families (n>1400) will be examined further for the characterization of the respective phenotypes. Genes being potentially involved in coronary calcification will be analyzed in more detail including a mouse model (vascular calcified mouse). In this model, chromosomal loci and candidate genes have been identified successfully.

CV3
Cardiomyopathies
(Head: Prof. Dr. H. A. Katus, Heidelberg)

Titin-mutations and new candidate genes in dilatative cardiomyophathy
(PD Dr. D. Weichenhan, Heidelberg)

Zebra fish mutants as model for cardiovascular diseases
(Dr. W. Rottbauer, Heidelberg)

Signal pathways in cardiovascular diseases (Dr. N. Frey, Heidelberg)

Genomic basis of variability of the cardiomyopathy phenotype
(Prof. Dr. H. A. Katus, Heidelberg)

Genetic modifiers of cardiomyopathy in the mouse model
(Dr. B. Ivandic, Heidelberg)

Net projects:

Population genetics of cardiovascular diseases (Prof. Dr. H. A. Katus, Heidelberg; Prof. Dr. H. Schunkert, Lübeck; PD Dr. S. Kääb, München)

Antisense-Oligonucleotide-mediated gene knock-out in Zebra fish embryo as model system for new cardiovascular genes (Dr. W. Rottbauer, Heidelberg)

Coordination office (Dr. T. Weis, Heidelberg)

Cardiomyopathies (CMPs) are the most frequent diseases of the human myocardium. CMPs lead to heart failure and sudden death and therefore contribute significantly to cardiovascular morbidity and mortality.

Dilated Cardiomyopathy (DCM) is responsible for 25% of all cases of heart failure. Symptomatic patients have a life expectancy of 5 years on average. Recent findings indicate that the individual genetic layout is of utmost importance in the etiology and clinical course of CMPs. In the last years a increasing number of genes has been identified which are involved in DCM. But even identical allelic configuration in family members often result in significantly different clinical phenotypes. On the other hand also different mutations in one protein lead to extremely different phenotypes indicating that protein-protein interactions also contribute to the disease phenotype. Moreover only little is known about the molecular pathways linking the (altered) gene to the clinical phenotype.

The scientific approach of the working groups led by Prof. Katus in Heidelberg in cooperation with Prof. Thierfelder (Berlin) focuses on a comprehensive understanding of CMPs from the genetic causes to the molecular pathways and modifier genes leading to the clinical phenotype. To accomplish this clinical approaches and sophisticated techniques in molecular genetics have been closely intertwined.

More than 30 known as well as new candidate genes for CMPs will be analyzed by a well established mutation screening platform based on the Denaturing Gradient Gel Electrophoresis technique (DGGE, Dr. D. Weichenhan). Currently about 530 analyses per day are performed corresponding to the screening of one patient per day regarding the whole gene set. In a complementary experimental approach novel DCM genes will be identified by characterizing new mutant Zebra fish lines with reduced cardiac contractility (Dr. W. Rottbauer). Presently more than 40 of these Zebra fish lines are available for these analyses. These mutations will also be tested in DCM families.

Yeast-two hybrid assays are performed to study the effect of mutations in DCM-relevant genes and to characterize protein-protein interactions and signal transduction pathways, respectively in detail (Dr. N. Frey). The function of newly identified interacting partners will be determined by in vivo overexpression and knock down studies in Zebra fish and mouse models, respectively (Dr. W. Rottbauer, Dr. B. Ivandic).

The entire Network is supported by three Net projects. The Zebra fish platform (Dr. W. Rottbauer) supports the functional characterization of up to 250 differentially regulated new genes by antisense-oligonucleotid mediated gene knock down in Zebra fish embryos.

The Net project “Population genetics of Cardiovascular diseases provides the whole network with population based data and biomaterial as well as selected disease phenotypes from the two German population genetics platforms popgen and KORA.

The Net project Coordination office was founded to support the speaker and coordinator and to coordinate all scientific and administrative activities (Dr. T. Weis).

CV4
Heart failure
(Head: Prof. Dr. G. Hasenfuß, Göttingen)

Genomic prediction of heart failure (Prof. Dr. G. Hasenfuß, Göttingen)

Genetic epidemiology of heart failure (Prof. Dr. H. Bickeböller, Göttingen)

Heart failure after Anthracycline cancer therapy
(Prof. Dr. L. Wojnowski, Mainz)

Heart failure is the most frequent cause of death in the western developed countries. It affects about 2% of the European population, its prevalence increases to about 10-15% among the elderly. More over, an additional 2% of the general European population is affected by either asymptomatic systolic (50%) or diastolic (50%) dysfunction. Either one can progress into symptomatic heart failure. In contrast to the cardiomyopathies (hypertrophic or dilated) and ischemic heart failure, where disease causing and/or susceptibility genes are known, no specific mutations are known to cause diastolic heart failure. To fill this gap, a cohort consisting of 1000 patients with isolated asymptomatic diastolic dysfunction has been collected and will be analyzed for mutations in genes affecting myocardial relaxation such as extracellular matrix proteins, titin and calcium regulating proteins (prospective study).
However, heart failure is a complex entity and not necessarily solely determined by genes but is more likely the result of interplay between environment and the genetic makeup of an individual. To address this complex interdependence, a cohort consisting of about 1500 anthrcycline treated patients with Non Hodgkin Lymphoma (German Non-Hodkin Lymphoma-B study) has been analyzed by a genome wide screen for single nucleotide polymorphisms (SNP) and anthracycline cardiotoxicity (ACT). Using this approach, we were able to discover significant associations between ACT and polymorphisms in the NADPH oxidase subunits as well as in the doxorubicin efflux transporter multi-drug resistance protein (MRP-1).
In summary, our project contributes significantly to the understanding of heart failure. In particular, our data will immediately be used to screen for patients with an increased risk for ACT before they receive anthracycline treatment and our data can be used to design new strategies to combat heart failure.

Hemostaseology / Atherosclerosis

(Head: Prof. Dr. W. Koenig, Ulm)

Inflammation processes in pathogenesis of type 2 diabetes and atherosclerosis (Prof. Dr. W. Koenig, Ulm)

Association studies and pharmacogenetics in disturbance of hemostasis (PD Dr. J. Oldenburg, Bonn)

Von Willebrand factor (Prof. Dr. R. Schneppenheim, Hamburg)

Atherosclerosis constitutes a chronic process of the arterial vessel wall which carries characteristic features of an inflammatory process, but is also accompanied during the early stages and in particular during acute ischaemic syndromes by a thrombotic component. Therefore, in the second funding period of the National Genome Research Network (NGFN-2) three research projects in the field of atherosclerosis and hemostasis have been integrated into the Herz-Kreislauf-Netz (Cardiovascular Diseases Network).

The project “Inflammatory processes in the pathogenesis of type 2 diabetes and atherosclerosis” led by Prof. Koenig focuses on the hypothesis of the contribution of gene polymorphisms involved in inflammatory pathways which might play an important role in the aetiology of atherosclerosis (coronary heart disease, CHD). For genotyping, two population-based cohorts and one case-control study are available which are phenotypically characterized in great detail.

The project “Association studies and pharmacogenetics in hemostatic disorders” led by Prof. Oldenburg explores the genetic variations of the plasmatic coagulation system, the fibrinolytic system, platelet proteins, and the vascular endothelium, which can cause rare monogenetic diseases of hemostasis as well as complex thromboembolic phenotypes. The goal of this project is to identify polymophism maps and gene haplotypes of the most important hemostatic factors. Association studies will be performed to reveal the contribution of identified haplotypes on the different phenotypes. A main focus represents the characterization of VKORC1 (Vitamin K Epoxid Reductase) which is the target of oral anticoagulation.

The project “Von Willebrand Factor in Haemorrhagic and Thrombotic Disorders” led by Prof. Schneppenheim deals with the genetic impact of disturbances in content or activity of the Von Willebrand Factor (VWF) which is the key protein in primary hemostasis. The activity of VWF is regulated by the metalloproteinase ADAMTS13. The project attempts to unravel the interaction between VWF and ADAMTS13 mutants and polymorphisms in bleeding or thrombotic disorders. For this purpose, genotyping of various SNPs will be performed in a cohort of female patients and controls from the CORA study, including 210 consecutive women with incident CHD and 230 population-based controls.

Poster no. 1

Rat chromosome 19 of the SHR rat protects against salt-sensitive hypertension and cardiovascular hypertrophy in the Dahl SS rat

A. Schulz[1], N. Wendt[1], M. Wehland[1], J. Weiss[1], Anja-Kristin Siegel[1], M. Stoll[2], R. Kreutz[1]

[1]Institut for clinice Pharmacology und Toxicology Charité Universitäty Medicine Berlin, [2]Institute for Arteriosclerosis Research, Universitäty Münster

Recently, we have identified a quantitative trait locus linked to cardiovascular hypertrophy in a salt-fed F2-population derived from salt-sensitive Dahl (SS) and spontaneously hypertensive (SHR) rats. This study sought to test the relevance of rat chromosome 19 (RNO19) for hypertensive target organ damage in SS. We therefore generated a speed consomic stain in which RNO 19 from SHR was introgressed into the SS genetic background, thus creating the SS-19SHR strain.

Male animals (n=14-21) were studied either under a normal (0.2% NaCl) or a high salt diet (4% NaCl) beginning at 6 weeks of age for 8 weeks, respectively. All measurements were performed at the age of 14 weeks. Systolic blood pressure (SBP) measurements were performed on three consecutive days. SBP in the salt-fed SS-19SHR was significantly lower compared to the SS rats (186±30 vs. 213 ± 14 mmHg, $p<0.0001$). More importantly, the relative weight of the left ventricle in salt-fed SS-19SHR (2.50 ± 0.28 mg/g) was similar to that of SHR (2.61 ± 0.23 mg/g) and significantly reduced compared to SS (3.1 ± 0.5 mg/g, $p<0.00001$). In addition, the relative weight of the aorta was also significantly lower in salt-fed SS-19SHR rats (1.41 ± 0.24 mg/mm) compared to SS (1.65 ± 0.2 mg/mm, $p= 0.0046$). These results show that there is at least one genetic locus in the genome of the SHR rat on chromosome 19 that has a powerful protective effect on salt-sensitive hypertension and progression of cardiovascular hypertrophy in the SS rat.

Poster no. 2

Identification of genetic factors contributing to heart failure in SHHF rats

Judith Fischer[1], Jan Monti[2], Herbert Schulz[1], Rainer Dietz[2], Norbert Hubner[1]

[1]Max-Delbrück-Center for Molecular Medicine, Berlin-Buch , [2]Franz-Volhard Clinic, Charité-Universitätsmedizin Berlin,

Heart failure is a quantitative trait with genetic factors contributing to the disease in humans and rats.

Our goal is to identify quantitative trait loci (QTL) and the underlying molecular mechanisms contributing to heart failure. We use spontaneously hypertensive heart failure (SHHF) rats as a model of the human disease. To identify QTL related to heart failure we established two intercross populations derived from SHHF and WKY rats and from SHHF rats and SHRSP rats.

The two different crosses were established to identify genetic loci contributing specifically to heart failure. Carrying out a cosegregation analysis in F2 animals bred from SHHF and SHRSP, two equally hypertensive strains, and in F2 animals bred from SHHF and WKY, a normotensive strain, allows us to test for the presence of genes that are directly linked to heart failure, removing blood pressure as a trait influencing variable.

Cardiac function was determined in parental, F1, F2 (WKY x SHHF; n=200) and F2 (SHRSP x SHHF; n=220) animals by in-vivo hemodynamic measurements assessing 30 cardiac parameters including end systolic and diastolic pressure, ejection fraction, cardiac output and left ventricular volume. We also measured B-type natriuretic peptide (BNP) in plasma and a range of morphometric parameters. Furthermore, we performed a genome screen in all 420 F2 animals with microsatellite markers and SNPs.

Linkage analysis was performed to extract the maximum information from two crosses with divergent genetic background using a combined-cross analysis, which improves the power and resolution of QTL mapping by utilizing the combined information of both crosses. With a combined-cross analysis one is able to identify shared and cross –specific OTL.

This analysis revealed significant QTL for a number of hemodynamic parameters on several chromosomes which form clusters indicating their physiological relatedness. Interestingly, these QTL clusters are largely distinct according to volume or pressure parameters. Linkage of plasma BNP levels in F2 rats co-localizes with a QTL for end diastolic pressure.

Poster no. 3

Coronary vessel formation in the heart requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1

Nicole Wagner[1], Kay-Dietrich Wagner[1], Karin Kirschner[2], Bernd J. Thiele[2], Benno Nafz[2], Andreas Schedl[1], Holger Scholz[2]

[1]INSERM U636, Centre de Biochimie, Faculté des Sciences, Nice, France [2]Johannes-Müller-Institut for Physiology, Charité – University of Medicine Berlin

The formation of intramyocardial blood vessels is critical for normal heart development and tissue repair after infarction. We report here expression of the Wilms' tumor gene-1, Wt1, in coronary vessels, which could contribute to the defective cardiac vascularization in Wt1-/- mice. Furthermore, the high affinity neurotrophin receptor TrkB, which is expressed in the epicardium and subepicardial blood vessels, was nearly absent from Wt1-deficient hearts. Activation of Wt1 in an inducible cell line significantly enhanced TrkB expression. The promoter of NTRK2, the gene encoding TrkB, was stimulated approximately 10-fold by transient co-transfection of a Wt1 expression construct. The critical DNA binding site for activation of the NTRK2 promoter by Wt1 was delineated by DNaseI footprint analysis and electrophoretic mobility shift assay. Transgenic experiments revealed that the identified Wt1 consensus motif in the NTRK2 promoter was necessary to direct expression of a reporter gene to the epicardium and the developing vasculature of embryonic mouse hearts. Finally, mice with a disrupted Ntrk2 gene lacked a significant proportion of their intramyocardial blood vessels. These findings demonstrate that transcriptional activation of the TrkB neurotrophin receptor gene by the Wilms' tumor suppressor Wt1 is a crucial mechanism for normal vascularization of the developing heart. Together with our previous results (FASEB J. 16: 1117-1119, 2002; FASEB J. 17: 1364-1366, 2003) the present data testify that the Wt1 transcription factor plays an essential role during myocardial blood vessel formation in the developing heart and after regional tissue ischemia. Future studies will be aimed at identifying relevant downstream target genes of Wt1 during myocardial vasculogenesis.

Poster no. 4

Functional genomics of cardiac damage in hypertension

D.Müller[1], F.Luft[2], M.Bader[1], B.Nafz[2], H.Scholz[2], B. Thiele[2]

[1]MDC Berlin [2]Charite University Medicine Berlin

Topic 1: Role of circulating and local aldosterone in hypertensive cardiac damage.
The role of aldosterone (Ald) in angiotensin (Ang) II-induced heart and kidney damage in a rat model overexpressing the human renin and angiotensinogen genes was examined. The rats were adrenalectomized or treated with FAD 286, an Ald synthase inhibitor. We found that Ald plays a major role in Ang II-induced organ damage and that Ald in the heart is largely if not entirely blood-borne rather than locally produced.

Topic 2: Cardioprotective mechansims of kinins in transgenic rats. Hypertension resulting in left ventricular hypertrophy and fibrosis can lead to cardiac dysfunction. We generated a transgenic rat model overexpressing the bradykinin B2 receptors specifically in the cardiomyocytes (TGR(MLCB2)) to investigate the role of the kallikrein-kinin system (KKS) in cardiac hypertrophy and fibrosis. Left ventricular pressure was increased in isolated hearts from TGR(MLCB2) compared with Sprague-Dawley (SD) control rats leading to increased blood pressure. To initiate cardiac hypertrophy, SD and TGR(MLCB2) rats were submitted to an interkidney aortic constriction. Left ventricular pressure increased further and more pronounced in TGR(MLCB2) rats by this treatment but cardiac hypertrophy developped equally to SD control rats. This confirms a role of the KKS in cardiac hypertrophy and supports the possibility to use of the system as drug target.

Topic 3: Functional genomics of blood pressure variability on cardiac damage.
Formation of intramyocardial blood vessels is critical for heart development and tissue repair after blood pressure variability induced transient ischemia. We report here expression of the Wilms' tumor gene-1, *Wt1*, in coronary vessels. Activation of Wt1 significantly enhanced TrkB expression. Transgenic experiments revealed that the identified Wt1 consensus motif in the *NTRK2* promoter was necessary to direct expression of a reporter gene to the epicardium and the developing vasculature of embryonic mouse hearts. Thus, we were able to show that transcriptional activation of the *TrkB* gene by Wt1 plays an essential role during myocardial blood vessel formation in the developing heart and after regional, transient ischemia.

Poster no. 5

Regulation of the proteolytic balance in human aortic aneurysms

Kaschina E[1], Doerfel N[1], Scholz H[2], Schmidt S[3], Sommerfeld M[1], Vosgerau U[1] , Unger Th[1]

[1]Center for Cardiovascular Research (CCR) / Institut für Pharmacology und Toxicology, Campus Charité Mitte, Charité – University Medicine Berlin [2]Vascular Center Berlin [3]Institute of Forensic Medicine, CCM, Charité – University Medicine Berlin

Abdominal aortic aneurysm is a complex vascular disorder which carries a significant mortality. We have recently shown the development of aortic aneurysms in rats lacking high molecular weight kininogen (HMWK), a natural inhibitor of cystein proteases. In this study we further investigated the role of protease-antiprotease cascades (cathepsins, kininogens, MMPs, TIMPs) in the elastolysis and apoptosis of the aorta.

We examined aneurysmatic abdominal aortic tissue from patients undergoing surgery and healthy aortic tissue (n=12 in each group) using Western blot protein analysis, immunohistochemistry and real-time PCR. Protein analysis demonstrated an up-regulation of the active forms of cathepsins D, L, H and pro-MMP2, MMP3, MMP9, pro-MMP12 in the aneurysmal- as compared to healthy aortic tissue. Consistently with the MMP up-regulation, the active form of TIMP-4 was downregulated. Cathepsin D, a lysosomal enzyme known to generate angiotensin I, was widespread in all layers of the aorta and co-localized with inflammatory infiltrates in the adventitia. Active vascular chymase, a protease involved in the generation of angiotensin II, as well as angiotensin AT1 receptors were also strongly up-regulated (3.2- and 3.0-fold, respectively) compared with healthy aorta.

On the other hand, a cystein proteases inhibitor, HMWK, was down-regulated (2-fold) in the human aneurysmatic aortic tissue, co-localizing with neutrophil elastase. The protective role of HMWK was also confirmed in cell culture experiments. In primary rat and human VSMC, single chain high molecular weight kininogen (10-40nM) prevented cytokine (IL1-α, TNF-α, IFN-g) and Fas Ligand - induced apoptosis as determined by protein expression of cleaved caspase–3 and by measuring of the apoptosis index after nuclear staining with Hoechst 33342. Moreover, two-chain high molecular weight kininogen (HKa) 1μM decreased MMP-2 and MMP-9 expression (29 % and 32% respectively) induced by IL-1α in VSMC.

Together these data demonstrate that activation of cystein proteases, MMPs and proteolytic components of the renin angiotensin sytem, cathepsin D and chymase, as well as up-regulation of the AT1 receptors are involved in the pathogenesis of aortic aneurysm. Down-regulation of the anti-proteolytic mediators (HMWK and TIMP-4) contribute to aneurysm formation.

Poster no. 6

Differential protein expression in human abdominal aorta: aneurysmal disease versus atherosclerosis.

Kaschina E[1], Scholz H[2], Schmidt S[3], Maercker C[4], Unger Th[1]

[1]Center for Cardiovascular Research (CCR) / Institut for Pharmacology und Toxicology, Campus Charité Mitte, Charité – University Medicine Berlin [2]Vascular Center Berlin [3]Institute of Forensic Medicine, CCM,Charité – University Medicine Berlin [4]RZPD, German Ressource Center of Genom Research at DKFZ, Heidelberg

Objective: Abdominal aortic aneurysm (AAA) is a common vascular disorder frequently associated with long-term history of arterial hypertension and atherosclerosis. Changes in protein expression that underlie the development of AAA versus atherosclerosis are poorly defined. The present study focused on molecules involved in the transition from atherosclerotic lesions to aneurysm formation.

Design and Methods: We compared AAA tissue from patients undergoing surgery with atherosclerotic aortic tissue using protein microarray technology, Western blot analysis and immunohistochemistry. Proteins were isolated from human aortic specimens (eight in each group). An Antibody Array was overlayed with Cy3- and Cy5-labeled protein samples. After laser scanning of the two colors on the same array and computer analysis, the ratio (r) between the protein levels in atherosclerotic and AAA tissue was calculated.

Results: Of 512 proteins studied in protein microarray, 20 showed significantly different expression in AAA as compared with atherosclerotic tissue. Differences were found in the expression of proteins involved in the actin regulation, inflammation as well as in nuclear factors. Eight of the twelve most highly expressed proteins were actin regulatory proteins (casein kinase (r=1.7), LSP-1 (r=1.63), laminin beta 3 (r=1.54), MST3 (r=1.53), p70 (r=1.38)). Five of eight low-expression proteins regulate actin reorganisation and participate in cell regeneration (dematin (r=0.47), p140mDia (r=0.65), EG5 (r=0.68), nexilin (r=0.8). Of these, LSP-1 and demantin showed the most significant regulation in Western blot analysis. Inflammatory monocytic chemoattractant protein (r=1.44) and cytokine MCP1 (r=1.43) trended toward up-regulation in AAA. Among the transcription factors, most highly expressed in AAA was HSF4 (r=1.58).

Conclusion: Our data strongly suggest that an imbalance in actin regulatory proteins and activation of inflammatory factors are instrumental in promoting the transition from atherosclerotic lesions to aneurysm formation in the human aorta.

Poster no. 7

Genome-wide Assessment of Siblings with Myocardial Infarction

Sédlacek, K., Stark, K., Fischer, M., Bäßler, A., Reinhard, W., Hengstenberg, C.

Clinic and Policlinic for Internal Medicine II - University Hospital Regensburg

Myocardial infarction (MI) and coronary artery disease (CAD) are the most frequent causes of death in Western societies. Besides known risk factors, epidemiological studies documented a strong genetic component in the etiology of MI. We are aiming at finding these genetic risk factors using a family-based strategy and a genome-wide screening in a large set of affected families. Furthermore, within chromosomal regions that are linked to the disease, we intend to identify those genes that are relevant for the development of MI, using case-control association studies.Recently, we performed a systematic genome-wide search in 1,406 individuals from our first MI family set (corresponding to 513 patient from different families with 618 affected sibs) using microsatellite markers. A region on chromosome 14 was identified with a significant LOD score of 3.9 for MI (genome-wide $p<0.05$). Moreover, a second MI family set was identified and subjected to a second genome-wide screening (n=2,394 individuals).Based on these findings, further characterization of the chromosome 14q32 region is under way. In our ongoing project, a close collaboration with the GSF-Gene Mapping Center (SMP-DNA) has been successfully established, based on the ILLUMINA technology for high throughput genotyping. MI case-control populations are investigated in association studies using SNPs and SNP haplotypes localized in the chromosome 14q32 region. By genotyping a large number of SNPs in affected MI patients (Regensburger Herzinfarkt-Familienstudie) as well as in healthy control subjects (married-in spouses from above and KORA S2000 DNA, obtained from the SMP-EPI at the GSF-Institute for Epidemiology), we intend to identify genetic variants responsible for the increased risk of myocardial infarction and atherosclerosis underlying the chromosome 14q32 linkage signal.

Poster no. 8

Genetic analysis in extended families with myocardial infarction showing an autosomal - dominant mode of inheritance

A. Götz[1], B. Mayer[1], I. R. König[1], W. Lieb[1], C. Hengstenberg[2], H. Schunkert[1], J. Erdmann[1]

[1]Universitätsklinikum Schleswig-Holstein, Campus Lübeck, [2] Universitätsklinikum Regensburg

In rare cases, coronary artery disease (CAD) and myocardial infarction (MI) appear to be inherited in an autosomal - dominant mode. In recent years a total of 19 multiplex families consisting of maximally 22 MI patients per family were identified by systematic large scale screening.
We aim to identify the underlying genetic defect in these families showing an autosomal-dominant inheritance pattern of MI.

Power simulations using the SLINK software package and applying several models of inheritance were carried out. Analyses consistently revealed the highest theoretical LOD scores under the assumption of an autosomal-dominant inheritance. In addition, we evaluated heritability of the binary phenotype MI and additional quantitative phenotypes (body constitution: height, weight, and body mass index (BMI), systolic and diastolic blood pressure and lipids: cholesterol, LDL, HDL, and triglycerides) in 522 (therein 117 patients with MI) participants from 19 families. Heritability estimates in the narrow sense were obtained by a variance-component analysis using SOLAR. Corresponding 95% confidence intervals and p-values were based on the one-step jack-knife approximation to the robust estimator of variance and were estimated in a script language. Not surprisingly, heritability value for MI was particularly strong (75.3%; 95%CI [73.1,77.5]; $p<0.01$), due to the selection criteria for our families of at least three affected living MI siblings and at least one affected living first cousin. To detect new candidate regions, we performed model-based and model-free linkage analyses in these families. Methodologically, we use Lander-Green as well as Markov chain Monte Carlo algorithm. We will present results of our whole genome linkage study.

In conclusion, the simulation data obtained with SLINK, the heritability estimate of 75.3% for MI and preliminary results of our model-based and model-free linkage analyses, support our hypothesis that this collection of 19 extended families will enable us to identify genetic defects leading to MI.

Poster no. 9

Comprehensive mutation screening in known and novel candidate genes for dilated cardiomyopathy

Raphael Zeller, Boris Ivandic, Philipp Ehlermann, Oliver Mücke, Hugo A. Katus, Dieter Weichenhan

Internal Medicine III - Cardiology, Universitäty Hospital Heidelberg,

Dilated (DCM) and hypertrophic (HCM) cardiomyopathy are diseases of the heart muscle and major causes of morbidity and mortality. Familial aggregation is observed in a significant percentage of cases, mostly inherited in an autosomally dominant fashion. Many genetic loci and more than 20 disease genes have been identified; in clinical practice, however, the genetic causes are only very rarely disclosed and used for prognosis of the disease. Moreover, the unusually high genetic heterogeneity, particularly of DCM, renders the search for genetic causes a daunting task. Counselling patients and their relatives requires, however, extensive and reliable mutation screening which may also provide valuable information on novel disease pathways and the functional relevance of protein domains and interaction sites. We have established Denaturing Gradient Gel Electrophoresis (DGGE) for comprehensive mutation screening in the coding exons of 11 known and 16 putative candidate genes for DCM and HCM. From 334 coding exons, we presently analyse 314 (94%) by DGGE. Identified variants are confirmed by resequencing. Screening of 19 DCM and 29 HCM patients for mutations in genes MYBPC3, MYH7 and TNNT2, known candidates for both DCM and HCM, revealed 14 mutations (11 in HCM, 3 in DCM patients) of which 5 were novel. Screening of the DCM patients alone for mutations in the remaining 24 genes identified 3 novel mutations of which 2 resided in new candidate genes. A screening by-product was the identification of 94 different polymorphisms; 24 polymorphisms were novel and may be useful in disease association studies. In several cases, we observed clear segregation of the mutation with the disease. These data underscore the causative nature of the mutation and may be of particular value in counselling affected families. Regular clinical follow-up of patients will enable us to correlate the time course of the disease with a particular genetic defect and, thus, provide novel insights in the prognosis of the disease. Novel mutations and candidate genes are further characterized in functional assays and computer modelling.

Poster no. 10

Functional in vivo Evaluation of Novel Cardiovascular Genes by Antisense Oligonucleotide-Mediated Gene Knock-down in Zebrafish Embryos

Georgia Wessels, Sabine Marquart, Jörg Rauch, Steffen Just, David Hassel, Garnet Bendig, Nicole Trano, Alexander Grundt, Tillman Dahme, Inken Huttner, Alexander Hess, Britta Vogel, Wolfgang Rottbauer

Internal Medicine III – Cardiology, Universitätsklinikum Heidelberg

During NGFN-1, numerous expression studies on heart tissue from patients and animal models with different cardiovascular diseases revealed several hundred differentially regulated novel genes. Their *in vivo* role in cardiovascular function and disease development now needs to be evaluated systematically to help reconstructing disease specific molecular pathways. Mouse gene knock-out strategies, however, are time consuming and require huge financial resources. Recently, injection of Morpholino-modified antisense oligonucleotides (Morpholinos) evolved as a fast and inexpensive method to study cardiovascular gene loss-of function phenotypes in the living zebrafish. The injected Morpholinos, reliable inhibit translation of the targeted gene for at least 96 hours. The transparency of zebrafish embryos facilitates the assessment of its cardiovascular function by light microscopy, with quantitation possible by direct hemodynamic measurements.

We established now a knock-down platform which aims to characterize more than 200 loss-of function phenotypes in the zebrafish. To do so, we first established in cooperation with Dr. Merk (IBE, Munich) a web-based database that allows for proper storage and accessibility of the acquired datasets. According to our aims, we evaluated so far more than 50 novel genes that were found to be differentially regulated in various cardiovascular diseases for their function in the zebrafish cardiovascular system by antisense mediated gene-knock-down. To do so, corresponding ZF orthologous genes were identified, Morpholinos against these genes designed and into 1-8-cell-stage embryos injected. The effect of the gene knockdown on morphology and function of the cardiovascular system was characterized by digital video microscopy. Fractional shortening of both chambers as well as blood velocities were measured and electrocardiograms (ECGs) recorded when cardiac arrhythmias were found to be induced. Heart morphology was evaluated by light microscopy and digital imaging as well as structural and ultrastructural analyses performed. Timing and location of targeted gene expression using gene-specific antisense RNA probes was determined. Some examples of these knock-down studies will be presented.

The results of these experiments will reveal new gene functions and genetic pathways underlying cardiovascular diseases and will guide the design of proper transgenic animal models for further characterization of the gene's functions in the mammalian heart.

Poster no. 11

Calsarcin-1 knock-out mice display exacerbated cardiomyopathy in response to pressure overload and chronic calcineurin activation

D. Frank[1], T. Barrientos[1], J. Shelton[4], Christian Kuhn[3], H. Weiler[3], J.A. Hill[2], H.A. Katus[1], E.N. Olson[1], N. Frey[1]

Dept. of Medicine III, University of Heidelberg, [1]Dept. of Molecular Biology, [2]Dept. of Cardiology, [3]Blood Research Institute, The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin [4]Dept. of Pathology, UT Southwestern Medical Center, Dallas, Texas;

Signalling by the calcium-dependent phosphatase calcineurin profoundly influences growth and gene expression of cardiac and skeletal muscle. Calcineurin binds to calsarcins, a family of muscle-specific proteins of the sarcomeric Z-disc, a focal point in the pathogenesis of human cardiomyopathies. We show that calsarcin-1 negatively modulates the functions of calcineurin, such that calcineurin signalling is enhanced in striated muscles of calsarcin-1 null mice. As a consequence of inappropriate calcineurin activation, calsarcin-1 mutant mice display an excess of slow skeletal muscle fibers. The absence of calsarcin-1 also activates a hypertrophic gene program, despite the absence of hypertrophy, and enhances the cardiac growth response to pressure overload. In contrast, cardiac adaptation to other hypertrophic stimuli, such as chronic catecholamine stimulation or exercise, is not affected. These findings reveal important roles for calsarcins as modulators of calcineurin signalling and the transmission of a specific subset of stress signals leading to cardiac remodelling in vivo.

Poster no. 12

Myozap, a novel cardiac-enriched protein, is localized to the intercalated disc and interacts with Zo-1

Claudia Rohr[1], Thalia Seeger[1], Christine Grund[2], Werner Franke[2], Hugo A Katus[1], Eric N Olson[3], Norbert Frey[1]

[1]Dept. of Medicine III, University Heidelberg, [2]German Cancer Research Center (DKFZ), Heidelberg, [3]Dept. of Molecular Biology, UT Southwestern Medical Center, Dallas, TX

Utilizing a bioinformatics approach to identify novel cardiac-specific proteins, we identified and cloned a novel gene, termed Myozap (myocardium-enriched Zo-associated protein). In adult tissues, Myozap expression is restricted to the heart. In contrast, Myozap is expressed in several embryonic tissues and is first detected in the heart at embryonic day 8.0. To begin to determine the function of Myozap, we performed a yeast two-hybrid screen for Myozap-interacting proteins. From this screen we identified four independent clones encoding Zo-1, a protein associated with tight junctions. Interestingly, in cardiac tissue Zo-1 is localized to the intercalated disc, a specialized structure that provides the basis for structural and functional coupling of individual cardiomyocytes. Moreover, Zo-1 directly interacts with connexin-43, the principal component of gap junctions in the myocardium. Immunostaining of cardiac tissue and cultured cardiomyocytes with specific antisera showed that Myozap is highly enriched at intercalated discs as well, where it colocalizes with Zo-1. Since alterations of intercalated disc proteins, such as cadherins and connexin-43, have been associated with the structural and electrical perturbations of the heart, we speculate that Myozap might also play a role in the pathogenesis of cardiomyopathies.

Poster no. 13

Contribution of Genetic Variation in Modifier Genes to Cardiomyopathy Phenotypes

Ivandic B[1], Friedrichs F[2], Zugck C[1], El-Mokhtari NE[3], Fritschka S[4], Stoll M[2], Regitz-Zagrosek V[4], Schreiber S[3], Krawczak M[3], Katus HA [1]

[1]Internal Medicine III – Cardiology, Universitätsklinik Heidelberg [2]Leibniz Institut für Arteriosklerosеforschung, Universität Muenster [3]PopGen, Universität Kiel [4]Deutsches Herzzentrum Berlin, Charité Universitätsklinikum

Age of onset, progression and expressivity of the cardiomyopathy (DCM) phenotype may vary considerably among family members carrying identical cardiomyopathy mutations. Common genetic variation in modifier genes and ethnic background contribute to the observed variability. Since inflammation is linked to the progression of contractile dysfunction, we genotyped a well characterized cohort of 792 patients with dilative and ischemic cardiomyopathies as well as 721 controls for a variety of genes involved in inflammation. These studies revealed a significant association of contractile dysfunction with polymorphisms in IL1, IL6, IL8, IL10 and members of other pathways, e.g. the Toll-like receptor signalling pathway. The goal of this project is to confirm and extend these findings by typing additional SNPs in genes linked to inflammation in an extended cohort of 1500 DCM patients and 2000 controls.

So far, we analysed 3 DCM groups of cases/controls (n=764/716; n=866/1788; n=184/552) with 1998 cases and 3056 controls altogether (the third group of DCM patients was recruited in collaboration with the PopGen platform; Prof. Dr. Schreiber, Prof. Dr. Krawczak, University of Kiel). Many of the previous associations remained significant in 2 of these 3 cohorts (please find detailed results on IL6 in the poster presentation “Epistatic interaction in variants of the interleukin 6 receptor (IL6R) and its signal transducer (IL6ST) confers susceptibility to DCM“ from Friedrichs and colleagues). The extensive characterisation of the haplotype structure of associated genes has been initiated now. This analysis includes SNPs identified in NGFN-1, and additional SNPs characterized in collaboration with the NGFN network of environmental diseases (studies of inflammatory bowel disease). Finally, molecular and cell biological studies will ensue to understand the functional consequences of the relevant polymorphisms. This project is an excellent example of successful collaborations with members from NGFN elements GEM, SMP-DNA, and research groups at several universities.

Poster no. 14

Identification of Genetic Modifiers of Cardiomyopathy in Mice

Ivandic B[1], Schoensiegel F[1], Schrewe A [1,2], Frank D[1], Müller O[1], Frey N[1], Hrabé de Angelis M[2], Katus HA[1]

[1]Internal Medicine III – Cardiology, Universitätsklinik Heidelberg [2]German Mouse Clinic, GSF Forschungszentrum, Neuherberg

Systematic investigation of the influence of genetic modifiers on cardiomyopathy phenotypes is difficult in humans, because environmental factors cannot be controlled and genetic variation is large. In inbred mouse models, however, environmental and genetic complexity can be controlled. We apply modern breeding protocols to create congenic mutant mouse models, which will be examined to identify novel disease-specific and genetic modifier pathways.

At present, we generate 12 novel congenic mutant strains transferring the mutant alleles from 3 established cardiomyopathy models (Mlp -/-, Sgcd -/-, and Myoz2 -/- on mixed C57BL/6 x 129 backgrounds) onto 4 inbred acceptor strain (C57BL/6, C3H/He, FVB/N, 129S1/SvImJ). Transfer of alleles overexpressing calcineurin failed, because these transgenic mice exhibited a very high mortality (average survival 8 weeks; range 2-20 weeks of age). Similarly, the use of BALB/c as inbred acceptor strain was abandoned, because this strain produced only small litters and exhibited a relatively short life span of 6 months. In collaboration with the German Mouse Clinic (Prof. M. Hrabé de Angelis, SMP Models) we will characterize cardiac pathology (histology) and function (ECG, echocardiography) in all congenic mutants to identify the acceptor strain backgrounds, which determine high and low disease penetrance. Then, myocardial gene expression (Affymetrix technology) will be compared in strains, which share a mutant allele, but exhibit opposite extremes of disease penetrance in order to dissect pathways associated with the disease-causing allele and the influence of modifier genes. Results of this work may provide novel targets for drugs improving contractile dysfunction in cardiomyopathy patients. This project is an excellent example of successful collaborations with members of NGFN elements SMP Models, partners at Heidelberg and research groups at several international universities.

Poster no. 15

Epistatic interaction in variants of the interleukin 6 receptor (IL6R) and its signal transducer (IL6ST) confers susceptibility to DCM

F. Friedrichs[1,2], B. Ivandic[2], M. Müller-Bardorff[3], Ch. Zugck[2], V. Regitz-Zagrosek[4,5], R. Hetzer[5], S. Schreiber[6], N. Wedemeyer[6], N. El Mokhtari[6], M. Stoll[1], H. Katus[2]

[1]Leibniz-Institute for Arteriosclerosis Research, Münster, [2]Internal Medicine III -Cardiology, University Heidelberg, [3]Cardiology, University Clinic Schleswig-Holstein, Campus Lübeck, [4]Center for Cardiovascular Research, Charité, Berlin, [5]German Heart Institute (DHZB), Berlin, [6]University Clinic Schleswig-Holstein, Campus Kiel

Dilated cardiomyopathy (DCM) is a disorder characterized by cardiac dilation and reduced systolic function. Genetic studies on familial DCM have identified a multitude of DCM loci and underlying disease mutations in genes encoding for structural myocyte elements. However, clinical and experimental studies point towards an involvement of modifier genes that significantly influence the onset and the progression of DCM. We studied the role of a 'proinflammatory' genetic background (81 SNPs located in 35 genes) in the development and progression of DCM in a clinically well characterized cohort of 410 DCM patients and 722 healthy controls.

Genetic epidemiological studies using logistic regression models revealed that the interleukin 6 (IL6) pathway is potentially disturbed in patients with DCM. We found association in two connected proteins of this pathway, namely negative association of a single nucleotide polymorphism in the interleukin 6 receptor (IL6R) gene (odds ratio (OR) = 0.617, 95 % confidence interval (CI) = 0.410-0.927, p=0.02, recessive inheritance model) and a trend to positive association of a polymorphism in the coupled signal transducer, IL6ST (OR=1.627, 95 % CI = 0.988-2.678, p=0.056, additive inheritance model). Gene-gene interaction studies revealed a significant interaction between both genes. In the absence or the presence of just one copy of the IL6R variant the odds ratio for the IL6ST variant is 2.193 (95 % CI = 1.247-3.856, p=0.006) while in the presence of two copies of the IL6R variant the IL6ST polymorphism is no longer risk associated (OR=0.497, 95 % CI = 0.149-1.654, p=0.254). Thus, the status of the IL6R variant is an effect modifier of the association between the IL6ST variant and DCM (p=0.028 as assessed by a logistic regression interaction model with product variable). The association of SNPs in the IL6R and IL6ST genes was replicated in an independent study sample consisting of 725 DCM patients and 1786 controls (IL6R OR=0.78, 95% CI 0.60-1.01, IL6ST OR=1.33, 95% CI=0.99-1.79). All odds ratios are adjusted for age and gender. We conclude that genetic variation in the IL6 pathway is associated with DCM. Furthermore, there is an interaction of variants within the IL6R and IL6ST genes as the presence of the IL6R variant suppresses the effect of the IL6ST variant. Our study highlights the complex nature of DCM and the necessity to evaluate gene-gene interactions in the context of genetic association studies of polygenic diseases.

Poster no. 16

Coordination Office

T. Weis[1], S. Kääb[2]

[1]Dept. of Medicine III, University Heidelberg; [2]LMU München;

The initial funding period of the National Genome Research Network (NGFN) had been focused on site specific funding of genomic research. In the second phase of funding (NGFN-2) the network character should be more emphasized. So it became possible to establish central coordinating structures within the subnets of NGFN-2. Experience from other research networks had shown that the existence of a central coordinating facility was often correlated with a more effective and successful work of the network as a whole. The Herz-Kreislauf-Netz has jumped at this chance and established a coordinating office as a defined subproject in Heidelberg which is the site of the speaker and coordinator Prof. Dr. Hugo A. Katus. A scientific coordinator and CEO and a secretary where hired and the coordinating office was equipped and founded in fall 2004. The work package of the coordinating office had already be defined in the NGFN-2 application of NGFN-2:

- build up a effective communication structure
- maintain the information flow between the subprojects
- keep close contact to the funding organisation (BMBF, DLR) and NGFN-project management
- organize scientific meetings and workshops
- develop and negotiate cooperation and consortial contracts
- establish a special website for scientists and patients
- maintain and optimize the Task force data base
- develop a data safety concept for the data base
- take care for all kinds of public relation
- develop a corporate identity and corporate design structure
- build up contacts to international partners
- coordination of the reports to the funding organizations
- controlling of the financial ressources
- support the implementation and harmonization of SOP`s
- develop and maintain a professional website for the Herz-Kreislauf-Netz
- coordinate scientific evaluation of the network

After the funding of NGFN-2 has been finished the coordinating office shall help to coordinate consecutive applications for national (NGFN-3) or international (EU-application in framework 7) research projects in functional cardiovascular genomics.

Poster no. 17

Population Genetics of Cardiovascular Diseases

T. Weis[1], S. Kääb[2], H. Schunkert[3], J. Erdmann[3], H.-E. Wichmann[4], H.A. Katus[1]

[1]Dept. of Medicine III, University Heidelberg; [2]Dep. Med. 1, LMU Munich; [3]Univ. Hosp. Schleswig Holstein (Lübeck); [4]GSF, Neuherberg

Cardiovascular diseases are the predominant causes of death in the western countries. We all know that the well known risk factors like smoking, obesity, hypercholesterinaemia, diabetes etc. can promote cardiovascular diseases. But the last years of research revealed that the individual genetic layout can be of major importance for the development and progress of cardiovascular diseases. Most cardiovascular diseases are influenced by a large number of different genes. Therefore the identification of relevant genes or SNP`s is very difficult and requires a meticulous phenotyping and examination of relatives. New candidate genes must be identified by whole genome analyses, which is cost- and time-consuming. If there are no affected families association studies must be performed which require large numbers of index patients and control individuals from the normal population to compare genetic markers or SNP`s. All research sites have their portfolio of man patient samples. But often special phenotypes or age and gender matched subcohorts are needed. Beside this even a University hospital normally does not have access to controls from the normal population.

Against this background the net project “Population genetics has been established to provide the researchers with population based data and biomaterials of two large German population genetic platforms: KORA (GSF, Neuherberg, Germany) and popgen (Univ. of Schleswig-Holstein, Campus Kiel). These platforms have collected data and biomaterial (mainly blood / DNA) from many thousand of patients and controls. KORA is a research platform for population based health research in the fields of epidemiology, health economics and health care. KORA is based on the Augsburg center of the WHO MONICA project on trends and determinants of cardiovascular diseases which started 20 years ago. KORA includes 4 survey populations with about 18,000 participants popgen is a relatively new population genetics platform located in Schleswig-Holstein (northern Germany). popgen intends to recruite all patients from one of 12 selected phenotypes in a precisely defined and circumscribed geografical area with a low fluctuation of the local population.” Additionally some thousand controls from the normal population will be recruited via the local registration offices.

Poster no. 18

The Kiel SNPlex genotyping Platform

Peter Jagiello[1], Nour Eddine El Mokhtari[2], Stefan Schreiber[1]

[1]Institute for Clinical Molecular Biology, University Kiel, [2]Clinic for Cardiology, University Kiel,

Fine-mapping technologies are essential for detailed analyses for lead region follow-ups or candidate gene studies using tagging SNPs. Ideally, those approaches fulfil at least three main criteria: fast, flexible in design as well as handling and - not worth mentioning - cost-effective. Therefore, the Kiel centre uses the SNPlex technology developed by Applied Biosystems. SNPlex provides a versatile genotyping approach for intermediate throughput with competitive pricing for the relevant applications. Depending on the amount of SNPs needed, SNPlex can be designed at a 48 or 96 multiplex level. This technology allows the fast analyses of well suited subsets of markers for in-depth investigation of association leads or e.g. tagging SNP approaches with exceptional accuracy and reproducibility in cohorts of comparatively large amounts of individuals (from 2000-5000). The consumable costs per genotype range from 6-8 cents. However, SNPlex is based on a highly multiplex technology, which includes some restrictions in study design. Approximately 80 percent of markers in non-repetitive regions are expected to be suitable for SNPlex assays. In case of essential markers, which failed the SNPlex design such as functional SNPs, alternative techniques can be used comprising TaqMan© (Applied Biosystems) as the first-line fallback option or direct high-throughput sequencing. In comparison with SNPlex, both technologies are relatively expensive. In this instance, the Kiel centre uses the SNPlex technology presenting with multiple advantages. So far, the Kiel platform has generated >80 million SNPlex genotypes in 2005.

Poster no. 19

VEGF-PLCg1 pathway controls cardiac contractility in the embryonic heart

Just, Steffen[1], Wessels, Georgia[1], Trano, Nicole[1], Katus, Hugo A.[1], Fishman, Mark C.[2], Rottbauer, Wolfgang[1]

[1]Dept. of Medicine III, University Heidelberg, [2]Novartis Institutes for BioMedical Research, Cambridge, US

Biomechanical stress induces augmented generation and secretion of Vascular Endothelial Growth Factor (VEGF) in cardiomyocytes. If the cardiac VEGF pathway acts only in a paracrine manner - by affecting cardiac blood vessels - or if it acts on cardiomyocytes in a direct autocrine fashion, awaits to be shown. We recently isolated the zebrafish mutation dead beat (dedm582) in a large-scale ENU-mutagenesis screen for recessive lethal mutations that perturb cardiac function. ded mutant embryos display progressive, ventricle-specific reduction of cardiac contractility although the myofibrillar structures are unaffected. We found through positional cloning that this defect is due to a nonsense-mutation in the phospholipase C g1 (plcg1) gene. PLCg1 is known to be essential for the transduction of VEGF signals in blood vessel development (vasculogenesis and angiogenesis). Here we show that PLCg1 deficient ded embryos do not only have a cardiac phenotype but also an impaired vascular development.By means of modified antisense oligonucleotides mediated gene knock-down we confirmed that VEGF acts independently and cell-autonomously in vascular development and the maintenance of ventricular contractility. The abrogation of VEGF leads to a cardiac phenotype similar to the ded phenotype. The VEGF signals responsible for ventricular contractility are mediated by the VEGF receptor VEGFR-1 (FLT-1). We identified flt-1 by searching the zebrafish genome and located the gene on zebrafish linkage group 24 by radiation hybrid mapping. Gene knock-down studies substantiate the essential role of FLT-1 to maintain ventricular contractility. In summary: Here we demonstrate for the first time that VEGF by acting through the tyrosine kinase receptor FLT-1 and consecutive activation of PLCg1 regulates the contractility of ventricular cardiomyocytes in a cell-autonomous and probably autocrine manner by modulating calcium transients. Targeting and manipulating the VEGF-PLCg1 pathway could open up new ways for medical treatment of human heart disease (cardiomyopathy, cardiac insufficiency).

Poster no. 20

Genomic predictors of heart failure following therapies with anthracyclines

Leszek Wojnowski[1,9], Bettina Kulle[2], Markus Schirmer[1], Gregor Schlüter[3], Albrecht Schmidt[4], Stefan Vonhof[4], Heike Bickeböller[2], Mohammad Reza Toliat[6], Eun-Kyung Suk[6], Mladen Tzvetkov[1], Anke Kruger[9], Silvia Seifert[3], Marita Kloess[7], Heidi Hahn[3], Markus Loeffler[7], Peter Nürnberg[6], Michael Pfreundschuh[8], Lorenz Trümper[5], Jürgen Brockmöller[1], Gerd Hasenfuss[4]

[1]Clinical Pharmacology, [2]Genetic Epidemiology, [3]Human Genetics, [4]Cardiology and Pneumonology, [5]Haematology and Oncology, Georg August University, Göttingen, [6]Gene Mapping Center, Max-Delbrück-Centre, Berlin, [7]Institute for Medical Informatics, Statistics and Epidemiology (IMISE), Leipzig, [8]Department of Internal Medicine I, University Saarland, Homburg, [9]Department of Pharmacology, University Mainz,

A significant number of cancer patients treated with anthracyclines like doxorubicin or daunorubicin develop cardiotoxicity (ACT), mainly presenting as arrhythmias (acute ACT) or congestive heart failure (chronic ACT). There are no data on pharmacogenomic predictors of ACT. We genotyped participants of the German Non-Hodgkin Lymphoma (NHL-B) study who were followed up for the development of heart failure for a median of more than 3 years. SNPs were selected from 82 genes with conceivable relevance to ACT.

Out of 1697 patients, 55 developed an acute and 54 a chronic ACT (cumulative incidence of either form 3.2%). We detected 5 significant associations with polymorphisms of the NAD(P)H oxidase and doxorubicin efflux transporters. Chronic ACT was associated with a variant of the NAD(P)H oxidase subunit NCF4 (rs1883112, -212A>G, OR: 2.5, 95% CI: 1.3 - 5.0). Acute ACT was associated with the His72Tyr polymorphism in the p22phox subunit (rs4673, OR: 2.0, 95% CI: 1.0 - 3.9) and with the variant 7508T>A (rs13058338, OR: 2.6, 95% CI: 1.3 – 5.1) of the RAC2 subunit of the same enzyme which is the main source of superoxide production in the cell. In agreement with these results, mice deficient in the NAD(P)H oxidase activity, unlike wild-type mice, were resistant to chronic doxorubicin treatment. Preliminary data indicate that doxorubicin may enhance the superoxide production by the NAD(P) oxidase. In addition, acute ACT was associated with the Gly671Val variant of the doxorubicin efflux transporter MRP1 (multi drug resistance-associated protein 1) OR: 3.6, 95% CI: 1.6 – 8.4) and with the Val1188Glu-Cys1515Tyr (rs8187694-rs8187710) haplotype of the functionally similar MRP2 (OR: 2.3, 95% CI: 1.0 – 5.4). Polymorphisms in adrenergic receptors previously demonstrated to be predictive of heart failure were not associated with ACT. In conclusion, genetic variants in doxorubicin transport and free radical metabolism may modulate the individual risk to develop ACT.

Poster no. 21

MLP – a determintant of the phenotype of Myosin binding protein C deficiency

Ralph Knöll[1], Lucie Carrier[2], Torbjorn Holm[3], Martin Braach[1], John Ross[4], Albrecht Schmidt[1], Harald Kögler[1], Gerd Hasenfuss[1], Ken Chien[4,5]

[1]Georg August Universität Göttingen [2]Universitätsklinikum Hamburg Eppendorf [3]Universitätsklinikum Oslo, Norwegen [4]University of California at San Diego, USA [5]Harvard Medical School, USA

Myosin binding protein C (MyBPC) mutations represent a major cause of familial hypertrophic cardiomyopathy. In addition, Muscle LIM protein (MLP) is known as part of a macromolecular biomechanical stress sensor complex and as such is implicated in hypertrophy signaling pathways. MLP mutations, similar to MybPC, have also been found to be the cause of different forms of cardiomyopathy.

A yeast two hybrid screening revealed an interaction between MLP and MyBPC. To gain more insight into underlying molecular mechanisms we generated MyBPC deficient (MyBPC-/-) as well as MyBPC and MLP double deficient (MyBPC-/-/MLP-/-) mice.

Papillary muscle contractile function of MyBPC-/-/MLP+/- mice was significantly impaired in comparison to MyBPC-/-/MLP-/- littermates: Upon raising the stimulation rate from 4 to 10 Hz, force development slightly increased in MyBPC-/-/MLP-/- (+9 ± 9%; n=6) but decreased in MyBPC-/-/MLP+/- mice (-19 ± 8%; n=11; p=0.03). b-adrenergic stimulation with a saturating concentration of isoprenaline (1 µmol/L) revealed, however, that the maximum inotropic reserve was similar in both groups: Force development increased in MyBPC-/-/MLP-/- preparations by 122 ± 23%; n=6) while the increase was 126 ± 34% in MyBPC-/-/MLP+/- mice (n=11; n.s.). mCIP mRNA expression (a marker of calcineurin activity) was significantly increased in the MyBPC-/-/MLP+/+ versus MyBPC-/+MLP+/+ and MyBPC+/+/MLP+/+ mice, but was found to be decreased in the MyBPC-/-/MLP-/-. Apoptosis rates were not different but Masson Trichrome and Picro Sirius Red staining revealed an increase in fibrosis in the MyBPC-/-/MLP-/- mice. MyBPC-/- and MyBPC-/-/MLP+/- mice develop hypertrophy followed by severe defects in myocardial function, whereas MyBPC-/-/MLP-/- mice present with significantly improved cardiac performance. Our data are compatible with the function of MLP as a mechanosensor and signal integrator, whose presence acts as a pivotal genetic modifier of the cardiomyopathic phenotype in the setting of MyBPC deficiency.

Poster no. 22

CRP Gene Polymorphisms, CRP Plasma Levels and Incident Coronary Heart Disease (CHD): MONICA/KORA Augsburg Cohort Study, 1984-2002 Regulation of the proteolytic balance in human aortic aneurysms

Wolfgang Koenig[1], Melanie Kolz[2], Natalie Khuseyinova[1], Jens Baumert[2], Norbert Klopp[2], Barbara Thorand[2] , Thomas Illig[2]

[1]Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, [2]GSF National Research Centre for Environment and Health, Institute of Epidemiology, Neuherberg

C-reactive protein (CRP) has emerged as an independent predictor of CHD. Recently, studies suggested that CRP concentration is modulated by genetic factors, thereby fo-cusing on several single nucleotide polymorphisms (SNPs) within the CRP gene. How-ever, epidemiologic data are sparse and remain somewhat controversial. Therefore, we systematically investigated in a large prospective cohort, whether various SNPs which cover the whole region of CRP gene contribute to differences in CRP concentrations in circulation and whether this genetic predisposition could account for increased risk of future CHD events. Methods and Results: A case-cohort study was conducted in initial-ly healthy, middle-aged men and women based on data from the MONICA/KORA Augsburg studies collected between 1984 and 2002 (mean follow-up time 11.0 years). Concentrations of CRP were measured in stored serum samples of 292 case subjects with incident CHD (non-fatal and fatal myocardial infarction and coronary death) and 1606 non-case subjects. Genotyping was performed on the Sequenom MALDI-TOF MS system. The CRP concentration was significantly higher in cases than in non-cases (geometric mean 2.6 vs 1.4 mg/L; p-value <0.001). We analysed four SNPs: two SNPs in the CRP promoter region (1. position -390, C/T/A, tri-allelic, rs3091244; 2. -717, C/T, rs2794521), one intronic (T/A, rs1417938) and one exonic (1059 G/C, rs 1800947). In crude and in multivariable analyses no consistent association was found between vari-ous SNPs within the CRP gene and incident CHD. However, CRP baseline concen-trations in the ramdomly drawn subcohort (n=1,695) were significantly modulated by three of the four analysed DNA variants (rs3091244, rs1417938, rs1800947) e.g. for the intronic SNP (rs1417938) CRP plasma levels were 1.32 vs 1.52 vs 2.00 mg/L for TT (n=782) vs TA (n=756) vs AA (n=157) genotype carriers, respectively; p for trend <0.0001); whereas -717 C/T SNP (rs2794521) in the CRP promoter had no impact on CRP concentration. Conclusions: These data are in support of CRP as a marker of CHD and the causal modulation of SNPs within the gene on their plasma concen-trations but do no lend support to a causal role of CRP in the pathophysiology of CHD.

Poster no. 23

Lp-PLA2 gene polymorphisms, plasma levels of Lp-PLA2 and risk of coronary heart disease: Results from a large case-control study

Natalie Khuseyinova[1], Dietrich Rothenbacher[2], Michael M. Hoffmann[3], Hermann Brenner[2], Wolfgang Koenig[1]

[1]Department of Internal Medicine II, Cardiology, University of Ulm Medical Center, [2]Department of Epidemiology, German Centre for Research on Ageing at the University of Heidelberg, [3] Department of Clinical Chemistry, University of Freiburg

Background: Lipoprotein-associated phospholipase A2 has recently been implicated as an emerging risk marker for coronary heart disease (CHD). It has also been demonstrated that genetic variants within the Lp-PLA2 gene might influence Lp-PLA2 plasma concentration. However, so far published data remain controversial and are very scarce. We sought to determine various Lp-PLA2 single nucleotide polymorphisms (SNPs) within the Lp-PLA2 gene (PLA2G7) in a large case-control study. Furthermore, their association with stable CHD as well as the effect of PLA2G7 SNPs on Lp-PLA2 plasma concentration has also been assessed.Methods and Results: A large case-control study, comprising 312 patients with angiographically proven stable CHD and 477 age- and gender-matched healthy blood donors served as the study base. We investigated the distribution of various PLA2G7 SNPs in the coding region (Arg92His in Exon 4 (rs1805017); Ile198Thr in Exon 7 (rs1805018), Ala379Val in Exon 11, (rs1051931), as well as two SNPs in the promoter region at positions 2061 A/G (rs10948301), 3015 T/C (rs1421378) relative to AF027357. PLA2G7 genotypes were determined by RFLP-PCR. In addition, Lp-PLA2 plasma mass (diaDexus PLAC test) and activity (as measured by 2 different assays: Hybrid Immuno Capture (HIC) and colorimetric activity method (CAM)) were assessed. We found no association between the various polymorphisms and increased risk for CHD after adjustment for age, gender, BMI, duration of school education, smoked pack years, alcohol consumption, history of hypertension and diabetes. Moreover, there was no consistent association between Lp-PLA2 gene variants and multivariate-adjusted mean values of Lp-PLA2 in plasma: only the common allele coding for Arg 92 showed a slight decrease in Lp-PLA2 mass (249.6 vs 269.3 vs 271.4 for Arg/Arg, Arg/His and His/His respectively; p=0.04), with no association for Lp-PLA2 activity. Conclusions: In this large case-control study, no consistent association between variants in the Lp-PLA2 gene and plasma levels of Lp-PLA2 mass and activity as well as the presence of CHD were found.

Poster no. 24

VKORC1 Haplotypes and their impact on oral anticoagulation

Matthias Watzka[1,2], Christof Geisen[2], Katja Sittinger[2], Michael Steffens[3], Laurynas Daugela[2], Erhard Seifried[2], Clemens R. Müller[4], Thomas F. Wienker[3], Johannes Oldenburg[1]

[1]Institute for Experimental Haematology and Transfusion Medicine, University of Bonn, [2]Institute of Transfusion Medicine and Immunohaematology, University of Frankfurt/Main, [3]Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, [4]Institute of Human Genetics, Biocentre, University Würzburg,

Introduction: Coumarins target blood coagulation via inhibition of the vitamin K epoxide reductase complex (VKOR) This complex recycles vitamin K epoxid to vitamin K hydroquinone, an essential cofactor for the post-tarnslational gamma-carboxylation of several blood coagulation factors. Shortly after cloning of the VKORC1 gene allelic variants were shown to have an substatial impact on VKORC1 mRNA transcription rate, influencing warfarin dosage, the worldwide most commonly prescribed oral anticoagulant. Methods: In order to elucidate the role of VCORC1 sequence variants in warfarin sensitivity, we established a complete SNP map of VKORC1 and CYP2C9 gene loci in 200 blood donors from Western Germany. Data were compared to those from patient cohorts with partial warfarin resistance and warfarin sensitivity. Results: We identified 28 SNPs within the VKORC1 genomic sequence. Six of these formed three main haplotypes (VKORC1*2: 42%, VKORC1*3: 38%, and VKORC1*4: 20%), which were in complete linkage disequilibrium. These haplotypes were further subdivided by additional polymorphisms. The three main haplotypes cover more than 99% of the genetic variability of the VKORC1 gene in Europeans. In a cohort of 50 consecutive patients without mutations in the VKORC1 coding region who presented with phenotypes of either increased coumarin sensitivity or partial coumarin resistance we found a strong association of the VKORC1 haplotypes and these phenotypes. Thirteen of fourteen patients (93%) with increased coumarin sensitivity but none of the patients with partial coumarin resistance were found to be homozygous for VKORC1*2. Vice versa non VKORC1*2 haplotypes were found homozygous in 31 patients (86%) with partial coumarin resistance but in none of the patients with increased coumarin sensitivity. The association of VKORC1 haplotypes and coumarin dose phenotypes was highly significant between the two cohorts and also when compared to the blood donor controls. The defective CYP2C9 alleles *2 and *3, known to substantially influence warfarin metabolism were found in 43% of the coumarin sensitive and 19% of the partial coumarin resistant patients which differed not significantly.

Poster no. 25

Establishment of a Cardiovascular Screen in the German Mouse Clinic

Anja Schrewe[1,3], Boris Ivandic[1], Paulus Kirchhof[2], Joerg Stypmann[2], Valerie Gailus-Durner[3], Martin Hrabé de Angelis[3] , Hugo Katus[1]

[1]Dept of Medicine III, Div of Cardiology, University Heidelberg, [2]Dept of Medicine C, Div of Cardiology, University of Muenster, [3]Institute of Experimental Genetics, GSF National Research Center

The German Mouse Clinic (www.mouseclinic.de) is a national core facility for the comprehensive phenotyping of mice. It is funded by the German National Genome Research Network (www.ngfn.de). Recently, non-invasive cardiovascular phenotyping methods (primary screen) have been established newly to complement the already extensive phenotyping program.

Various collaborators contribute genetically manipulated mice (knock-out, transgenic, ENU, gene trap, recombinant inbred) to identify and phenotype novel mouse models for human diseases.For the primary cardiovascular screen we have established echocardiography (Vevo660, Visualsonics Inc.) and digitized ECG (EMKA Inc.) under isoflurane sedation as well as blood pressure measurements (tail cuff).

Interesting phenotypes, identified as a results of the primary screen, are further characterized in secondary and tertiary screens offered by the Cardiovascular Disease Network (www.herz-kreislauf-netz.de) and the Muenster Mouse Clinic. The methods of the primary screen will be adapted to standards used in the EUMORPHIA and NIH NHLBI programs to allow comparability.

Poster no. 26

Data base Herz-Kreislauf-Netz

T. Weis[1], M. Hinterseer[2], S. Merk[3], M. Dugas[3], R. Knöll[4], N. El Mokhtari[5], B. Ivandic[1], C. Zugck[1], S. Kääb[2], B. Mayer[6], C. Lawerenz[7], H. Schunkert[6], G. Hasenfuss[4], C. Hengstenberg[8], H.-E. Wichmann[9], H.A. Katus[1]

[1]Internal Medicine III, University Heidelberg; [2]Dep. Med. 1, LMU Munich; [3]IBE, LMU Munich; [4]Heart Center Univ. Hosp. Göttingen; [5]Univ. Hosp. Schlesw. Holst (Kiel); [6]Univ. Hosp. Schlesw. Holstein (Lübeck); [7]DKFZ Heidelberg; [8]Univ. Hosp. Regensburg; [9]GSF, Neuherberg

Genetic epidemiology and association studies are powerful tools in the analysis of the genetic background of cardiovascular diseases. Studies often require large numbers of patients which are matched to specific criteria (age, gender, risk factors, additional diseases etc.). Even University hospitals have only limited portfolios of patient data or biomaterial to perform all studies which may be of interest. Against this background the Herz-Kreislauf-Netz (Cardiovascular Diseases Network) decided to facilitate the mutual sharing of patient data and DNA between the network partners. So a joint data base has been established to represent the local data bases of the partners. Because of data safety reasons and easy handling the data base should not represent a 1:1 copy of all local data bases. The intention was to give a short but highly significant overview about cardiovascular phenotypes, patient data and information about biomaterial of the different data collections in the network. The data base is hosted at the IBE Munich and contains a minimal data set of about 45 parameters per patient and has been closely harmonized with the NGFN core data set managed by Chris Lawerenz (DKFZ, Heidelberg). The web-access to the data base is password restricted and limited to the project partners. The data transfer is protected by a SSL algorithm. All patient data will be pseudonymized by a secure two step procedure. The second pseudonymization step will probably performed by the TMF PID generator, which will provide state of the art automatic pseudonymization. Interested partners can easyly identify patients with special phenotypes by a search mask. The interested party cooperates with the data owner to exchange patient data and / or biomaterial. All partners will sign a cooperation contract to ensure that informed consent forms will comply with data safety regulations. Moreover a data safety concept is in progress and will be closely attuned with the TMF e.V. (Berlin) and the data safety representative of Baden-Württemberg, which is the site of the network coordinator. In the future we hope to represent many thousands of patients in the data base of our network. A NGFN wide copy of this data base concept is planned or in progress, respectively. This concept of a data base ("data exhibition") will help to optimize the utilization of data and biomaterial in the Herz-Kreislauf-Netz and to perform many additional scientific studies.

Poster no. 27

Protein arrays for antibody screenings and expression studies

Andreas Hörlein[1], Heidrun Ridinger[1], Karl-Friedrich Becker[2], Christiane Rutenberg[1], Christine Schmitt[1], Christian Maercker[1,3]

[1]RZPD German Ressour Center of Genomresearch, [2]Institut for Pathology, TU München. [3]University of Applied Sciences

Within NGFNII, the focus of RZPD's screening service is on applications for antibody and protein expression arrays. Currently we run different feasibility studies to extend our service portfolio. In addition to serum screening and antibody characterization, which has been established during the last year, we are now working on a new approach on RZPD's protein expression arrays. These arrays are constructed from expression-verified cDNA clones from certain tissues. Each array consists of up to 27,648 proteins, which are printed in duplicates (total up to 55,296 protein spots) onto 22 cm x 22 cm PVDF membranes. The protein expression arrays allow researchers a time-, cost-, and material-efficient functional analysis regarding target protein identification, using antibodies and sera, functional assays, e.g. phophorylation, ribosylation, methylation, identification of DNA/RNA binding proteins, and identification of protein-protein interactions. In a recent study, the Human Fetal Brain Protein Expression Array was overlayed with cerebrospinal fluid (CSF) from depression patients (Turck) to search for autoantibodies which might lead to potential targets for diagnostics and therapeutic applications.

Another issue is spotting of customized antibody sets, because commercial arrays very often depend on certain antibody suppliers and therefore are limited in complexity and choice of model organisms. Based on transcriptome analyses and immunohistochemistry studies in a rat model on heart hypertrophy, it was possible to select antibodies specific for proteins involved in extracellular matrix remodeling, spot them on glass slides (Poustka), and label protein extracts with Cy3 and Cy5 for protein expression profiling. The results of the experiments allowed us to confirm RNA results on protein level, which might be stimulating for other users working on similar projects. As complementary assay we are also planning the reverse assay, that is spotting of proteins extracted from formalin fixed tissue and detection with individual antibodies. Within our ongoing work on the BD500 antibody array we applied a low sample protocol with protein lysates isolated from formalin fixed tissue slides. And indeed we were able to detect expression differences between normal and tumor tissue with as little as 50µg whole-protein lysate. Therefore, this is a very attractive method not only for pathologists, but also for all scientists working with microdissections.

Poster no. 28

Resequencing platform for analysis of candidate genes for complex diseases

Bernd Timmermann[1], Ralf Sudbrak[1], Richard Reinhardt[1], StefanTaudien[2], Klaus Huse[2], Matthias Platzer[2]

[1]Max Planck Institute for Molecular Genetics, Berlin, [2]Leibniz Institute for Age Research - Fritz Lipmann Institute, Jena

The identification of genes predisposing to human diseases is important to understand the molecular basis of the disease and individually different drug response, and will establish new routes to diagnosis and therapeutic advances of immense medical benefit. A key step in all strategies for disease gene identification is the comparative sequence analysis of candidate genes in patients and controls to identify those specific sequence variations (SNPs) associated with complex disease. We therefore have implemented a technology service platform for candidate gene sequence analysis, that integrates high throughput (HT) resequencing and bioinformatic techniques (programs to predict haplotypes and LD structures).The need for such a service infrastructure has already been expressed by a number of researchers with a strong interest in candidate gene sequencing and gene-based haplotype analysis from the various clinical networks.

The coordination of the various sequencing requests from the network partners will be performed through our WEB site (http://www.resequencing.mpg.de/). This information is accessible to all NGFN-partners and it should be obvious to all, what will be sequenced, what has been sequenced so far and who is the responsible person for the specific project. The coordinator will define and control the quality standards for the templates to be worked on, control the scheduling of the sequencing process, be the person in charge for questions from the network partners and has the decision about what will be sequenced, in the case of any template quality problems. Costs for comparative candidate gene sequencing are highly depending on the gene specific exon / intron structure, template quality etc. and can be calculated on an individual basis only.

Poster no. 29

Case-cohort design – a suitable method to evaluate genotypecharacteristics and associations in the presence of a restricted budget

Jens J. Baumert[1], Barbara Thorand[1], Andrea Schneider[1], Hannelore Löwel[1], Wolfgang Koenig[2]

[1]GSF - National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg,
[2] Department of Internal Medicine II, Cardiology, University of Ulm Medical Center, Ulm,

Context: In a cohort study, it is often not feasible to measure expensive genotype parameters in the full cohort due to financial or personal restrictions. In this situation, a case-cohort design is regarded as a suitable study design to evaluate characteristics of the full cohort and associations to follow-up events.

Objective: We aimed to compare results concerning the distribution of socio-demographic variables and cardiovascular risk factors and associations on the prediction of incident coronary events using the “classical” cohort design and the case-cohort design with different weighting schemes.
Methods: Data were derived from three population based MONICA/KORA Augsburg surveys which included 9,300 participants aged 35 to 74 years with available serum samples and without a history of a myocardial infarction. Participants were followed up for an average of 11.0 years. For the case-cohort design, a subcohort of 2,163 participants was randomly drawn stratified by sex and survey with different sampling fractions. Different weighting schemes were used to estimate the distribution of basic characteristics in the full cohort. Cox proportional hazards models were applied to estimate relative risks for a coronary event with different correction methods for the case-cohort design.

Results: The distributions of basic variables were very close to each other using different weighting schemes. Moreover, the hazard ratios for the incidence of a coronary event estimated by Cox proportional hazards models in a case-cohort design with different weighting schemes were similar for various risk factors varying only by a small percentage. Regarding estimated results for the full cohort, the hazard ratios of various variables revealed to be of similar magnitude.

Conclusions: In the presence of data collection restrictions, the case-cohort design is a suitable study design to estimate the distribution of various variables and their relative risks on the prediction of follow-up events in the full cohort.

Poster no. 30

Automated yeast-two hybrid screening: From single screens to PROTEOMES

F. Schwarz[1], M. Schick[1], J. Haas[2], B. Korn[1], Manfred Koegl[1]

[1]RZPD German Ressource Center of Genome Research, Heidelberg
[2]Max-von-Pettenkofer Institut, LMU München

Large scale protein interaction networks play an increasingly important role in proteomic research. Application of the yeast two-hybrid method has already allowed the analysis of the proteomes of several model organisms. We have streamlined and automated the method to dramatically increase its throughput and reliability. In a pilot project focussing on nuclear receptors as a family of drug targets, we have performed 425 screens yielding 6425 interacting fragments that form 1613 interaction pairs (Albers et al., Mol Cell Proteomics 2005, 4:205-213). Statistic analysis has allowed to select a subset of high-confidence interactions containing 61% of validated interactions. Methods to reproduce yeast two-hybrid based protein interactions on protein microarrays are under development in our laboratory. In a current project, the complete set of viral proteins of Varicella Zoster Virus, the causative agent of chicken pocks and shingles, is analysed for interactions with host cell protein. In this project, we will perform close to 300 screens with 96 different bait proteins. The automated system has now been opened for collaborations and service projects with the aim to generate a freely accessible and comprehensive protein interaction map of the human proteome (contact: koegl@rzpd.de).

Poster no. 31

The VLDL-Receptor - a link between heart and adipose tissue. Initial results utilising the database of the „Marburger Präventions-Allianz“

M. Soufi, A.M. Sattler, V. Ruppert, B. Kurt, M. Herzum, B. Maisch, J.R. Schaefer

Dr. R. Pohl Stiftungsprofessur “Präventive Kardiologie”, Zentrum Innere Medizin, Uniklinikum Giessen-Marburg, Standort Marburg

Coronary artery disease (CAD) is the leading cause of death in the western population. The metabolic syndrome is a crucial CAD risk factor. One new candidate gene that is poorly studied for its potential in causing obesity and CHD is the very low density lipoprotein (VLDLR) receptor. The VLDLR expression is limited to fatty acid active tissues (adipose, heart, muscle). Studies with VLDLR knockout mice under high fat diet showed impaired fatty acids uptake and cellular triglyceride storage in adipose tissue. From our understanding fatty acid metabolism is a crucial step in atherogenesis and we hypothesize that the need of fatty acids for energy supply of the heart is one step in developing CAD (Atherosclerosis. 2005; 180(2):417-8). Our data suggest an important role of the VLDL receptor in the development of obesity and CAD. So far only 2 mutations affecting the VLDLR-structure were reported. To evaluate the influence of putative VLDLR mutations on CAD and obesity, we screened the complete VLDLR gene in 129 preselected subjects (BMI < 25; triglycerides >150mg/dl) from the study cohort of the „Marburger Präventions-Allianz“, which is a multimodular concept for the prevention of CAD at the University-clinic of Marburg and now consists of a database of more than 5500 patients. DGGE - screening of so far 129 patients with hypertriglyceridemia detected a total of 6 mutations, 4 of which were new and so far not reported in the Ensembl database. The mutations were located within highly conserved domains of the VLDLR. In addition screening of 135 subjects from a normolipidemic control group identified only a single VLDLR mutation. Interestingly most of these patients were diabetics and suffered from CAD. To study the role of the detected mutations on impaired VLDLR function, we transfected cells with expression plasmids bearing the mutations. First results from transfection experiments confirmed a strongly reduced uptake of DiI-labelled VLDL by the mutated receptors, indicating a functional consequence of our newly discoveredVLDLR- mutations. To clarify the frequency and the clinical impact of the novel detected VLDLR mutations, we are currently under way to examine a larger study group of 2500 subjects of the “Marburg Präventions Allianz”.These data will provide information on the underlying mechanisms interfering with obesity and its related disorders in man.

Poster no. 32

Use of gene expression profiles in blood leukocytes to predict essential hypertension

Svenja Debey[1], Thomas Zander[1], Rudolf J. Wiesner[2], Joachim L. Schultze[1]

[1]Internal Medicine I, Molecular Tumorbiology and Tumorimmunology, University of Cologne [2]Institute of Vegetative Physiology, University of Cologne

Hypertension is a complex multifactorial disorder which is influenced by genetic, environmental and demographic factors and is one of the most important risk factors for cardiovascular diseases including coronary artery disease, stroke, heart failure, renal and peripheral artery disease. To date no reliable phenotypic markers are identified that might help to diagnose hypertension or to monitor anti-hypertensive treatment outcome.Over the past years gene expression profiling of peripheral blood mononuclear cells (PBMC) has become a powerful tool for molecular diagnosis and classification of cancer diseases, as well as for prediction of clinical outcome and survival of cancer patients.Since changes in the expression profile of blood leukocytes in hypertensive patients has described earlier, we hypothesized that those gene expression signatures might be used to predict hypertension. Therefore, we performed microarray analysis in a cohort of 10 normotensive healthy individuals and 10 untreated hypertensive patients using PBMC. Combining gene expression patterns and supervised class prediction algorithms we identified a set of marker genes for hypertensive and normotensive individuals. These marker genes could be used to generate classifiers, resulting in very robust and accurate prediction of samples with unknown class assignment as tested with different algorithms e.g. the split-sample method and different cross-validation models. Our findings strongly support the use of such technologies to enhance treatment monitoring and optimization of anti-hypertensive therapies.

Poster no. 33

Complex association analysis in human hypertension

Bernd Timmermann, Richard Reinhardt

Max Planck Institute for Molecular Genetics, Berlin

Although rare monogenic forms of blood pressure dysregulation exist, hypertension mostly arises as a complex quantitative trait, where multiple genetic and environmental factors interact to produce the final phenotype. To date, geneticists have failed to identify common genes with large effects on human hypertension. It is known that two unrelated people are identical for approximately 99.9% of their DNA sequence and that the remaining 0.1% contains the genetic variation influencing how individuals differ in their risk of disease. The discovery of the DNA sequence variants that contribute to common disease risk offers one of the best opportunities for understanding the complex cause of disease in humans. Although each SNP can be analysed independently, it is much more informative to analyse SNPs in a region of interest simultaneously. Alleles of SNPs that are close together (e.g. SNPs within the same gene) tend to be inherited together, a phenomenon called linkage disequilibrium (LD). A region of SNPs with a high LD to each other is called a 'haplotype block'. The International HapMap project was launched in 2001 by the International HapMap Consortium with the goal of developing a haplotype map of the human genome. Phase I of the project is now finished and the haplotype map is now publicly available (http://www.hapmap.org) and, for each gene or chromosome region, includes a set of strongly associated SNPs, the haplotypes in the selected region and the SNPs that tag them, providing a tool for association studies, both on candidate genes and in whole genome studies.The definition of haplotype blocks is always dependent on marker density and, for some genomic regions or in certain populations, high frequency SNPs or indeed clear block boundaries might not exist. An approach using haplotype blocks and tag SNPs for association studies could not be particularly powerful for diseases caused by rare variants, even if relatively rare variants can potentially be discovered by many genotyping methods. On this reason we use resequencing in large populations as tool for the detection of the hole genetic variability of a certain genomic region (e.g. candidate genes for complex diseases). We will present data of our resequencing projects in comparison to the corresponding HapMap-related data. Our presentation will show examples of bioinformatics techniques to analyze complex haplotype-phenotype relationships.

Poster no. 34

Insights from the Distribution of Genome-wide identified SNP-QTLs for Cardiac Repolarization (QT-interval) and their Interaction Analysis in Men and Women

Arne Pfeufer[1], Dan E. Arking[2], Shapour Jalilzadeh[1], Mahmut Akyol[1], Wendy Post[2], W.H. Linda Kao[2], Morna Ikeda[2], Shin Lin[2], David J. Cutler[2], Siegfried Perz[3], H. Erich Wichmann[4], Eduardo Marban[2], Peter M. Spooner[2], Aravinda Chakravarti[2], Thomas Meitinger[1], Stefan Kaab[5]

[1]Institute of Human Genetics, Technical University Munich and GSF National Research Center, [2]Johns Hopkins Medical Institutes, Baltimore, MD; [3]Institute of Medical Informatics, [4]Institute of Epidemiology, GSF National Research Center, [5]Dept of Medicine I, University of Munich,

Aim: In order to identify quantitative trait loci (QTLs) of cardiac repolarization (QT-interval) we performed a genome-wide SNP LD-mapping study using Affymetrix 100k arrays. In the general population QT is normally distributed, has a moderate heritability (>0.3), and - if extremely shortened or prolonged predisposes to an increased risk of sudden cardiac death.Corrected QT interval (for heart rate (0.743 explained variance), age (0.047) and gender (0.011)) was called QTc_RAS.

Method: From the population-based KORA S4 survey (including n=2,171 women) we selected women from each extreme of the QTc_RAS distribution (<7.5th and >92.5th percentile) and successfully genotyped them for 88.500 SNPs with CR >0.85 and MAF >0.025 (screening, phase I). Only women were initially selected to avoid the interaction of QTLs with gender as a confounder. Of the ~160 individuals from each extreme of QTc_RAS (385.7±7.7ms vs. 444.8±3.6 ms), 103 could be successfully genotyped. In phase II, the most significant genome-wide SNPs ($p<10^{-4}$) were genotyped in an additional 200 females from each extreme. In phase III, SNPs that were significant at ($p<5*10^{-3}$) in the combined phase I and phase II datasets where then screened in all 4,000 individuals of the KORA S4 survey. In addition we are currently genoty-ping 200 men in a similar manner as a male phase I and expanding the total dataset to n=12.000 individuals from the combined KORA surveys S1, S2 and S4.

Analytical Strategy: Given the small effect sizes of all the QTLs for QT observed we aimed at optimizing our analytical strategies: To screen phase I datasets of females and males we compared eight different test statistics in order to select loci for confirmation in larger samples.

Results: The distribution of QTLs between males and females differed significantly. Observed QTL's effects were generally weak and the weak sizes of QTL's effects made multiple locus interaction analysis heavily depended on an enlarged dataset (n=12.000). As the process of unraveling the genuine genetic architecture of human QTLs and their interactions with nongenetic covariates and with themselves is ongoing, we are convinced that our future better understanding of human QTL's properties will enable us to devise more time and cost efficient analytical strategies to screen and confirm the entire spectrum of QTLs for any quantitative trait to reasonable levels of statistical and scientific certainty.

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+39 382 503 829
+39 382 525 866
e.arbustini@smatteo.pv.it

McKenna William Prof. Dr.
Clinical Director
The Heart Hospital
16-18 Westmoreland Street
London W1G 8PH
Great Britain
+44 20 7573 8841
w.mckenna@ucl.ac.uk

Samani Nilesh J. Prof. Dr.
Department of Cardiovascular SciencesCardiology GroupClinical Sciences WingGlenfield Hospital
Groby Road
Leicester LE3 9QP
Great Britain
+44 116 256 3021
+44 116 287 5792
njs@le.ac.uk

Wilde Arthur Prof. Dr.
Department of Clinical and Experimental Cardiology, AMC Amsterdam
PO BOX 22700,
1100 DE Amsterdam,
Netherland
m.kraaijenhof@AMC.UVA.NL

List of participants
in alphabetical order

Bader Michael Prof. Dr.
Max Delbrück Center for
Molecular Medicine (MDC)
Robert-Rössle-Str.10
13125 Berlin
+49 30 9406 2193
+49 30 9406 2110
mbader@mdc-berlin.de

Bartram Claus R**.** Prof. Dr.
Dekan der Medizinischen
Fakultät Heidelberg
Universitätsklinikum Heidelberg
Im Neuenheimer Feld 366
69120 Heidelberg
+49 6221 56 5151
+49 6221 56 5155
Cr.Bartram@med.uni-heidelberg.de

Baumert Jens
GSF Research Center
Ingolstädter Landstr. 1
85764 Neuherberg
+49 89 3187 4234
+49 89 3187 3667
baumert@gsf.de

Debey Svenja Dr.
Molecular Tumor Biology and
Tumor Immunology
University of Cologne
Kerpenerstr 62
50924 Köln
+49 221 478 3662
+49 221 478 3647
Svenja.Debey@uk-koeln.de

Deng Shiwei
Institut für Pharmakologie
Johannes-Gutenberg Universität
Obere Zahlbacher St. 67
55101 Mainz
+49 6131 39 33260
+49 6131 39 33449
deng@uni-mainz.de

Eggle Daniela
Molecular Tumor Biology and
Tumor Immunology
Internal Medicine 1
University of Cologne
Kerpener Str. 62 / Haus 16
50924 Köln
+49 221 478 3662
+49 221 478 86059
deggle@uni-koeln.de

Eichele Gregor Prof. Dr.
MPI Für Experimentelle Endokrinologie
Feodor-Lynen-Str. 7
730625 Hannover
+49 511 5359 100
+49 511 5359 186
gregor.eichele@mpihan.mpg.de

El Mokhtari Nour Eddine Dr.
Universitätsklinikum
Schleswig-Holstein
Institute for Clinical Molecular Biology
Schittenhelmstrasse 12
24105 Kiel
+49 431 597 1440
+49 431 597 1470
elmokhtari@cardio.uni-kiel.de

Erdmann Jeanette PD Dr.
UK-SH, Campus Lübeck
Medizinische Klinik II
Ratzeburger Allee 160
23552 Lübeck
+49 451 500 4857
+49 451 500 6437
jeaberlin@versanet.de

Fischer Judith
Max-Delbrück-Centrum
für Molekulare Medizin (MDC)
Robert-Rössle-Str. 10
13125 Berlin
+49 30 9406 2531
+49 30 9406 2110
j.fischer@mdc-berlin.de

Frank Derk Dr.
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 410
69120 Heidelberg
+49 6221 56 39830
+49 6221 56 4866
derk.frank@med.uni-heidelberg.de

Frey Norbert Dr.
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 410
69120 Heidelberg
+49 6221 56 38685
+49 6221 56 4866
norbert.frey@med.uni-heidelberg.de

Friedrichs Frauke
Leibniz-Institute for
Arteriosclerosis Research
Domagkstr. 3
48149 Münster
+49 251 83 56258
+49 251 83 56205
frafried@uni-muenster.de

Gödtel-Armbrust Ute
Institut für Pharmakologie
Johannes-Gutenberg Universität
Obere Zahlbacherstr. 67
55131 Mainz
+49 6131 3933 173
+49 6131 3933 449
goedtela@uni-mainz.de

Hasenfuss Gerd Prof. Dr.
Georg August Universität
Herzzentrum Göttingen
Abt. Kardioloie und Pneumologie
Robert Koch Str. 8
37099 Göttingen
+49 551 396 351
+49 551 391 6389
hasenfus@med.uni-goettingen

Hassel David
Otto-Meyerhof-Zentrum
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 4835
+49 6221 56 4866
david.hassel@med.uni-heidelberg.de

Heiß Rolf Dr.
Friedrich-Wilhelm Str. 31
76356 Weingarten

Hengstenberg Christian Prof. Dr.
Klinikum der
Universität Regensburg
Innere Medizin II
Franz-Josef-Strauss-Allee 11
93053 Regensburg
+49 941 944 7258
+49 941 944 7235
christian.hengstenberg@
klinik.uni-r.de

Hübner Norbert Prof. Dr.
Max-Delbrück-Centrum
für Molekulare Medizin (MDC)
Robert-Rössle-Str. 10
13125 Berlin
+49 30 9406 2530
+49 30 9406 2110
nhuebner@mdc-berlin.de

Ivandic Boris Dr.
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 410
69120 Heidelberg
+49 6221 56 39119
+49 6221 56 4866
boris.ivandic@med.uni-heidelberg.de

Jagiello Peter Dr.
Universitätsklinikum
Schleswig-Holstein
Institute for Clinical Molecular Biology
Schittenhelmstr. 12
24105 Kiel
+49 431 597 3882
+49 431 597 1842
p.jagiello@ikmb.uni-kiel.de

Janitz Michal Dr.
Max Planck Institute for
Molecular Genetics
Fabeckstrasse 60-62
14195 Berlin
+49 30 8413 1463
+49 30 8413 1462
janitz@molgen.mpg.de

Janke Daniel
Institut für Pharmakologie
Johannes-Gutenberg Universität
Obere Zahlbacherstr.67
55101 Mainz
+49 6131 39 30180
+49 6131 39 33449
langt@uni-mainz.de

Junker Ralf
Deutsches Zentrum für
Luft- und Raumfahrt e.V. (DLR)
Heinrich-Konen-Str. 1
53227 Bonn
+49 228 3821 273
+49 228 3821 257
ralf.junker@dlr.de

Just Steffen
Otto-Meyerhof-Zentrum
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 8131
+49 6221 56 4866
steffen.just@med.uni-heidelberg.de

Kääb Stefan PD Dr.
Ludwig-Maximilians-Universität München,
Medizinische Klinik u. Poliklinik I,
Großhadern
Marchioninistr. 15
81377 München
+49 89 7095 3049
+49 89 7095 6076
stefan.kaab@med.uni-muenchen.de

Kaschina Elena Dr.
Center for Cardiovascular Research
(CCR)/Institut für Pharmakologie
und Toxikologie,
Charité-Berlin
Hessische Strasse 3-4
10115 Berlin
+49 30 4505 25024
+49 30 4505 25901
elena.kaschina@charite.de

Kassner Nina
Institut für Pharmakologie,
Johannes-Gutenberg Universität
Obere Zahlbacher Str. 67
55101 Mainz
+49 6131 3933 260
+49 6131 3933 449
kassner@uni-mainz.de

Katus Hugo A. Prof. Dr.
Universitätsklinik Heidelberg
Ärtzl. Direktor
Innere Medizin III
Im Neuenheimer Feld 410
69120 Heidelberg
+49 6221 56 8670
+49 6221 56 5516
sekretariat_katus@med.
uni-heidelberg.de

Khuseyinova Natalie Dr.
Department of Internal Medicine II -
Cardiology , Medical Center
University of Ulm
Robert-Koch Str. 8
89081 Ulm
+49 731 500 24431
+49 731 500 31168
natalie.khuseyinova@medizin.uni-ulm.de

Knöll Ralph Prof. Dr.
Georg August Universität
Herzzentrum Göttingen
Abt. Kardioloie und Pneumologie
Robert Koch Str. 40
37099 Göttingen
+49 551 39 5316
+49 551 39 13592
rknoell@med.uni-goettingen.de

Koegl Manfred Dr.
RZPD German Resource Center
for Genome Research
Im Neuenheimer Feld 515
69120 Heidelberg
+49 6221 4247 10
+49 6221 4247 04
koegl@rzpd.de

Koenig Wolfgang Prof. Dr.
Department of Internal Medicine II - Cardiology , Medical Center
University of Ulm
Robert Koch Str, 8
89081 Ulm
+49 731 24441
+49 731 33872
wolfgang.koenig@medizin.uni-ulm.de

Kreutz Reinhold Prof. Dr.
Department of Clinical Pharmacology
Charite University Medicine Berlin
Hindenburgdamm 30
12200 Berlin
+49 30 8445 2279
+49 30 8445 4482
reinhold.kreutz@charite.de

Kruger Anke Dr.
Institut für Pharmakologie
Johannes-Gutenberg Universität
Obere Zahlbacher Str. 67
55101 Mainz
+49 6131 3933 180
+49 6131 3933 449
kruger@uni-mainz.de

Krüger Olaf Dr.
NGFN Projektmanagement
Projektträger im DLR
Heinrich-Konen-Straße 1
53227 Bonn-Oberkassel
+49 228 3821 335
+49 228 3821 332
olaf.krueger.1@dlr.de

Lang Thomas Dr.
Institut für Pharmakologie
Johannes-Gutenberg Universität
Obere Zahlbacherstr.67
55101 Mainz
+49 6131 39 30180
+49 6131 39 33449
langt@uni-mainz.de

Maercker Christian Prof. Dr.
RZPD Deutsches Ressourcenzentrum für Genomforschung
Im Neuenheimer Feld 515
69120 Heidelberg
+49 6221 4247 41
+49 6221 4247 04
maercker@rzpd.de

Malzahn Dörthe Dr.
University of Goettingen,
Dep. of Genetic Epidemiology
Humboldtallee 32
37073 Goettingen
+49 551 39 1433
+49 551 39 14094
dmalzah@gwdg.de

Marquart Sabine
Otto-Meyerhof-Zentrum
Uniklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 4835
+49 6221 56 4866
sabine.marquart@med.
uni-heidelberg.de

Müller Dominik PD Dr.
Max-Delbrück Center
für Molekulare Medizin (MDC)
Wiltbergstr. 50
13125 Berlin
+49 30 9406 4581
+49 30 9406 4513
mueller@fvk-berlin.de

Nafz Benno PD Dr.
Charité Universitätsmedizin
Centrum für Physiologie
Tucholskystr. 2
10117 Berlin
+49 30 450 528 219
+49 30 450 528 914
b.nafz@gmx.de

Oldenburg Johannes Prof. Dr.
Institut für Experimentelle Hämatologie
und Transfusionsmedizin
Universitätsklinikum Bonn
Sigmund-Freud-Str. 25
53105 Bonn
+49 228 287 5175
+49 228 287 4783
johannes.oldenburg@ukb.uni-bonn.de

Paul Martin Prof. Dr.
Department of Clinical Pharmacology,
Charite University Medicine Berlin
Hindenburgdamm 30
12200 Berlin
+49 30 8445 1701
+49 30 8445 1762
martin.paul@charite.de

Pfeufer Arne Dr.
TU Munich
Inst. Humangenetik
Trogerstr. 32
81371 München
+49 89 3187 3545
+49 89 3187 3545
arne.pfeufer@web.de

Rauch Jörg Dr.
Otto-Meyerhof-Zentrum
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 8131
+49 6221 56 4866
gerd-joerg.rauch@med.
uni-heidelberg.de

Reinhardt Richard Dr.
MPI for Molecular Genetics
Ihnestr. 63-73
14195 Berlin
+49 30 8413 1226
+49 30 8413 1365
rr@molgen.mpg.de

Rohr Claudia
Otto-Meyerhof-Zentrum
Uniklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 4535
+49 6221 56 4866
claudia.rohr@med.uni-heidelberg.de

Rottbauer Wolfgang Dr.
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 410
69120 Heidelberg
+49 6221 56 4697
+49 6221 56 4866
wolfgang.rottbauer@med.
uni-heidelberg.de

Ruiz Patricia Prof. Dr.
Center for Cardiovascular Research,
Charité Universitätsmedizin &
Max-Planck Institute for Molecular
Genetics, Dept. Vertebrate Genomics
Hessische Str. 3-4
10115 Berlin
+49 30 450 578 744
+49 30 450 525 901
ruiz@molgen.mpg.de

Schäfer Jürgen Prof. Dr.
Klinikum der Philipps-Universität
Innere Medizin-Kardiologie
Baldingerstr. 1
35033 Marburg
+49 6421 28 63578
+49 6421 28 68954
juergen.schaefer@mailer.uni-marburg.de

Schoensiegel Frank Dr.
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 1505
+49 6221 56 4866
frank.schoensiegel@med.uni-
heidelberg.de

Schultze Joachim L. Prof. Dr.
Molecular Tumor Biology
and Tumor Immunology
Internal Medicine 1
University of Cologne
Kerpener Str. 62 / Haus 16
50924 Köln
+49 221 478 3662
+49 221 478 86059
schultze@uni-koeln.de

Seeger Thalia
Otto-Meyerhof-Zentrum
Uniklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 1505
+49 6221 56 4866
thalia.seeger@med.uni-heidelberg.de

Soufi Muhidien Dr.
Klinikum der Philipps-Universität
Innere Medizin-Kardiologie
Baldingerstr. 1
35033 Marburg
+49 6421 28 63578
+49 6421 28 68954

Stark Klaus Dr.
Klinikum der
Universität Regensburg
Innere Medizin II
Franz-Josef-Strauss-Allee 11
93053 Regensburg
+49 941 944 7340
+49 941 944 7341
klaus.stark@klinik.uni-regensburg.de

Stoll Monika Prof. Dr.
Leibniz-Institute for
Arteriosclerosis Research
Domagkstr. 3
48149 Münster
+49 251 83 57206
+49 251 83 56205
mstoll@uni-muenster.de

Thierfelder Ludwig Prof. Dr.
Max-Delbrück Center
für Molekulare Medizin (MDC)
Robert-Rössle Str. 10
13092 Berlin
+49 30 9417 2398
+49 30 9417 2540
lthier@mdc-berlin.de

Trano Nicole
Otto-Meyerhof-Zentrum
Uniklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 2457
+49 6221 56 4866
nicole.trano@med.uni-heidelberg.de

Unger Thomas Prof. Dr.
Center for Cardiovascular Research
(CCR)/Institut für Pharmakologie
und Toxikologie
Charité-Berlin
Hessische Srasse 3-4
10115 Berlin
+49 30 4505 25001
+49 30 4505 25901
thomas.unger@charite.de

Watzka Matthias Dr.
Institut für Transfusionsmedizin und
Immunhämatologie
Universitätsklinikum Frankfurt
Sandhofstr. 1
60528 Frankfurt
+49 228 287 6719
+49 228 287 4320
m_watzka@web.de

Weichenhan Dieter PD Dr.
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 1505
+49 6221 56 4866
dieter.weichenhan@med.uni-heidelberg.de

Weis Tanja Dr.
Universitätsklinik Heidelberg
Innere Medizin III Koordinationsbüro
Herz-Kreislauf-Netz
Im Neuenheimer Feld 410
69120 Heidelberg
+49 6221 56 38064
+49 6221 56 7157
tanja.weis@med.uni-heidelberg.de

Wessels Georgia Dr.
Otto-Meyerhof-Zentrum
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 39874
+49 6221 56 4866
georgia.wessels@med.
uni-heidelberg.de

Wetterauer Birgit Dr.
BMBF
Hannoversche Str. 28-30
10115 Berlin
+49 1888 57 5168
+49 1888 578 5168
birgit.wetterauer@bmbf-bund.de

Wichmann H.-Erich Prof. Dr. Dr.
GSF Genetische Epidemiologie
Institut für Epidemiologie
Ingolstädter Landstraße 1
85764 Neuherberg
+49 89 3187 4066
+49 89 3187 4499
wichmann@gsf.de

Witsch von Matthias Dr.
Projektträger im DLR
Heinrich-Konen-Str. 1
53227 Bonn
+49 228 3821 209
+49 228 3821 257
matthias.vonwitsch@dlr.de

Wojnowski Leszek Prof. Dr.
Institut für Pharmakologie
Johannes-Gutenberg Universität
Obere Zahlbacher Str. 67
55101 Mainz
+49 6131 39 33460
+49 941 5992 36727
wojnowski@uni-mainz.de

Zeller Raphael
Otto-Meyerhof-Zentrum
Universitätsklinik Heidelberg
Innere Medizin III
Im Neuenheimer Feld 350
69120 Heidelberg
+49 6221 56 7395
+49 6221 56 4866
raphael.zeller@med.uni-heidelberg.de

Sponsors of the meeting
in alphabetical order

ABgene
Wenden Straße 23
20097 Hamburg
www.abgene.com

Affymetrix UK Ltd.
Voyager, Mercury Park,
Wycombe Lane, Wooburn Green
High Wycombe HP10 0HH
United Kingdom
www.affymetrix.com

Applied Biosystems
Applera Deutschland GmbH
Frankfurter Str. 129 B
64293 Darmstadt
www.appliedbiosystems.com

Eurogentec
Liege Science Park
Rue Bois Saint Jean 5
B-4102 Seraing
www.eurogentec.be

febit biotech GmbH
Im Neuenheimer Feld 517
69120 Heidelberg
www.febit.de

Lilly Deutschland GmbH
Critical Care Europe
Saalburgstr. 153
61350 Bad Homburg
www.lilly-pharma.de

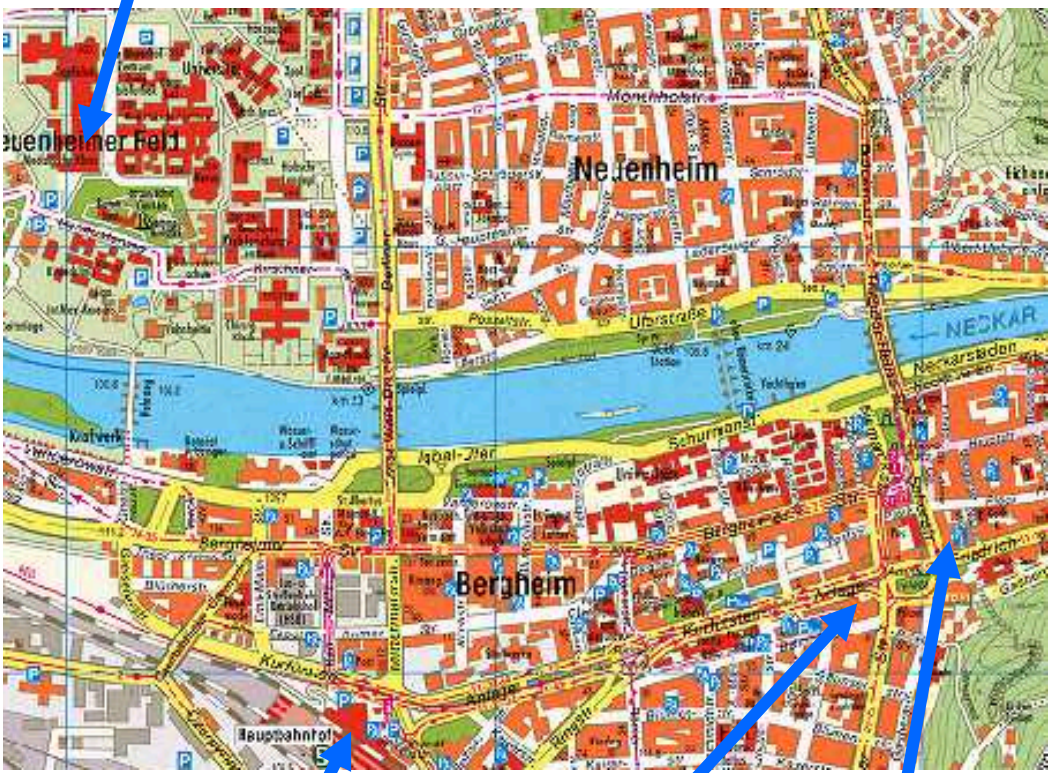
Ingeny International
Amundsenweg 71
4462 GP Goes
Netherland
www.ingeny.com

MSD SHARP & DOHME GMBH
Lindenplatz 1
85540 Haar
www.msd.de

Sanofi-Aventis
Deutschland GmbH
Potsdamer Straße 8
10785 Berlin
www.sanofi-aventis.de

Map of City of Heidelberg

Medical Clinic
(Krehl Klinik)



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Crowne Plaza

Hotel
Europäischer Hof

Map of University Hospital

By train: Take the bus no. 33 from the train station`s forecourt (direction “Schwimmbad“) until “Medizinische Klinik“

By airplane: Take the train from Frankfurt airport to Heidelberg railway station and then the bus no. 33 (see above)

Alternatively take the shuttle from Frankfurt airport to the Crowne Plaza hotel in Heidelberg and then take a taxi to the Medical clinic.

By car: From motorway A6 until Mannheim intersection to A656, from A5 until Heidelberg intersection to A656 (direction Heidelberg). University Hospital or Zoo is signposted.

Notes

Notes

Contakt und Information: Dr. Tanja Weis
Coordination Office Herz-Kreislauf-Netz
in the National Genome Research Network

Phone: +49 6221 56 38064
Fax: +49 6221 56 7157
herz.kreislaufnetz@med.uni-heidelberg.de
www.herz-kreislauf-netz.de