

Research Report

2010

Preface

The year 2010 was a turbulent year – to say the least. It was overshadowed by the looming decision on the evaluation of the institute in 2009, which unfortunately turned out to be negative despite the proven scientific track record of many dedicated scientists and a great effort by all staff to take this hurdle.

Nevertheless, we continue to work on the many very good projects and strive to build a future for the undisputed research programs and its committed faculty and staff. I would like to take the opportunity to thank our *Scientific Advisory Board* for their critical SWOT analysis and input on how to develop future research programs within the frame of the university. I would also like to thank my colleagues from the *Executive Board* and the *Board of Trustees* as well as the Ministry of Innovation, Research and Education (MIWF) of the State North-Rhine-Westphalia as well as the Ministry of Health (BMG) for their continuous support in these difficult times.

Sincerely Yours,
Monika Stoll

Research Report 2010: Contents

Preface	2
Scientific Program 1 “Molecular Arteriosclerosis Research“	
Department “Cell Biology and Ultrastructure Research”	4
Workgroup “Signalling Controlled Mechanisms of Atherogenesis”	16
Workgroup “Lipid Metabolism & Metabolic Syndrome”	19
Scientific Program 2 “Genetic Predisposition for Cardiovascular Disease“	
Department “Molecular Genetic of Cardiovascular Disease”	23
Department “Genetic Epidemiology of Vascular Disorders”	30
Workgroup “Genetics of HDL Cholesterol and Molecular Diagnostics”	34
Publications	36
Imprint	37

Department

Cell Biology and Ultrastructure Research

Cellular lipid homeostasis regulated by autophagy

Lipid droplet accumulation is one of the hallmarks of atherosclerosis, the inflammatory process by which plaques develop in the arterial lumen which eventually lead to the potentially fatal complications of ischemic heart disease and stroke. Lipid droplets are not merely storage depots for superfluous intracellular lipids in times of hyperlipidemic stress, but metabolically active organelles involved in cellular homeostasis. Our concepts on the metabolic functions of lipid droplets have come from studies on lipid droplet-associated proteins. This realization has made the study of proteins, such as PAT family proteins, caveolins, and several others that are targeted to lipid droplets, an intriguing and rapidly developing area of intensive inquiry. Our existing understanding of the structure, protein organization, and biogenesis of the lipid droplet has relied heavily on microscopical techniques that lack resolution and the ability to preserve native cellular and protein composition. By revisiting the lipid droplet with freeze-fracture electron microscopy and the freeze-fracture replica immunocytochemistry labeling technique, new findings have emerged which challenge previous assumptions on where its associated proteins are localized within the cell and how these proteins targeted to lipid droplets, and on how lipid droplets form and grow. Our aim has been to present an integrated survey of a range of these new findings with a view to stimulating debate on their functional significance. We are, of course, mindful that morphology alone does not explain functional processes.



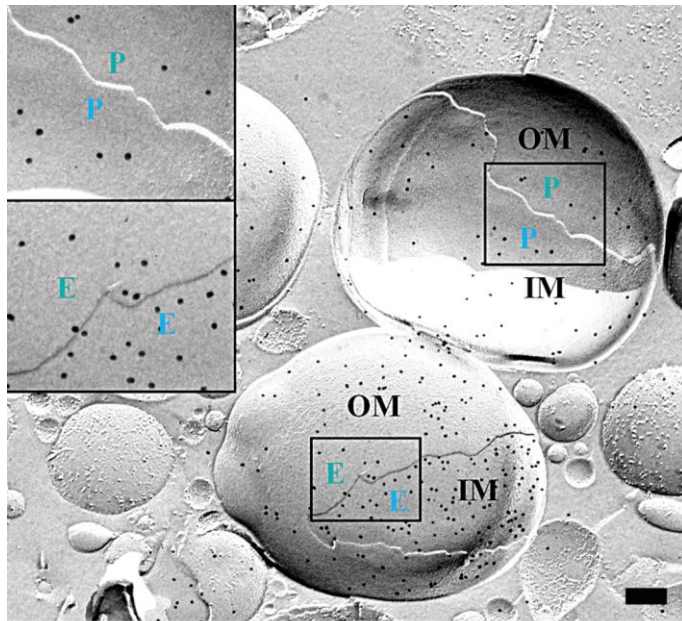
Univ.-Prof. Dr. rer. nat.
Horst Robenek (Director)

Equally, however, a knowledge of structure underpins and provides the framework for understanding function, and without that knowledge, functional assumptions may be led seriously astray. The unique advantages of FRIL have yielded new information on the structure, composition and protein organization of the lipid droplet. We know now that PAT family proteins in caveolins are not confined to the surface of the lipid droplet as previously believed, but pervade the lipid core. There is no irrefutable evidence for the widely held view that the lipid droplet is formed within the ER membrane bilayer; our findings that lipid droplets appear to develop enclosed by but external to specialized sites of the ER membrane bilayer that are enriched in adipophilin challenges the long held concept that they are formed within the ER membrane bilayer. PAT family proteins are not specific to the lipid droplet, but are widely present in the plasma membrane where, under conditions of lipid loading, they adopt a similar configuration to the specialized sites in the ER. The information that freeze-fracture immunocytochemistry provides is unique and further exploitation of the FRIL technique maybe expected as its scope and power become more widely appreciated.

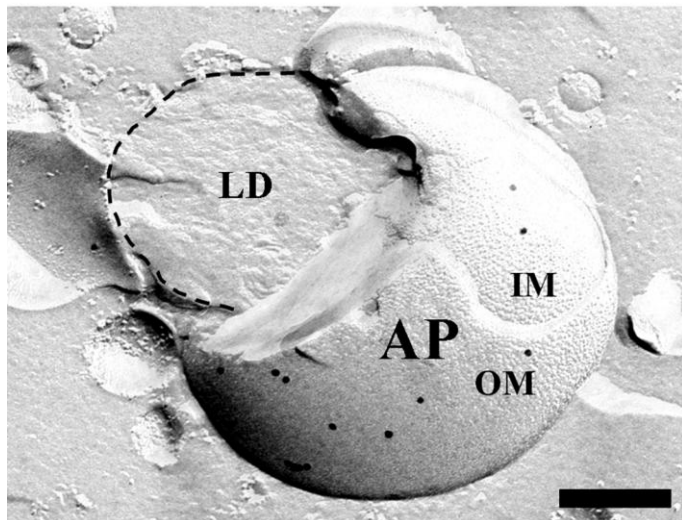
Thus, by using the FRIL technique we could show in collaboration with Proikas-Cezanne and coworkers (Tübingen) that autophagy plays an important role for the degradation of lipid droplets. Autophagy is an intracellular lysosomal bulk degradation process initiated by the generation of double-membraned autophagosomes that sequester cytoplasmic material and that fuse with lysosomes to autolysosomes for final degradation. Autophagosomal membranes were found to be predominantly composed of lipids with minimized protein content, as demonstrated by freeze-fracture electron microscopy. Proikas-Cezanne and coworkers identified the human WIPI gene family that is aberrantly expressed in a variety of human cancer types. They found that the WIPI protein family represents an ancient PI3P-binding β -propeller protein family that includes WIPI-1 and WIPI-2, both of which evolved from the yeast ancestral autophagy protein Atg18. They characterized WIPI-1 as a PI3P-effector protein functioning in the process of autophagy in human cancer cells. Upon the induction of autophagy and the generation of PI3P, both WIPI-1 and WIPI-2 specifically bind PI3P and localize at initial autophagosomal membranes that are positive for further autophagy proteins, such as LC3 and Atg16. To clarify the precise localization of WIPI-1 and WIPI-2 upon the induction of autophagy we conducted freeze-fracture immunoelectron microscopy because this method allows the employment of native, unfixed cells and, depending on the fracture, either of the two monolayers of a bilayer membrane is preserved. By FRIL we specifically detected membrane-bound WIPI-1, either endogenous WIPI-1 in G361 cells or GFP-WIPI-1 in stably transfected U2OS cells, in nutrient starved cells that undergo autophagy only. WIPI-1 specifically localized at autophagosomes, characterized by the unique lipid-rich, smooth double-membrane. Strikingly, WIPI-1 localized at both monolayers of both the outer and the inner autophagosomal membranes, and enriched in the inner autophagosomal membrane, as it was shown for PI3P in yeast autophagosomes. The calculated approximate diameter of WIPI-1 positive autophagosomes ranged between 0.5 – 2.5 μm with an average of about 1 μm , typical for autophagosomes.

In a number of WIPI-1 positive autophagosomes fractures displayed engulfed lipid droplets. In addition, upon autophagy induction WIPI-1 predominantly localized at the plasma membrane and the ER, particularly at the nuclear envelope close to simultaneously present WIPI-1 positive autophagosomes and tubular structures. Further, we also found WIPI-1 to localize at vesicles that lack lipid-richness and that are positive for LAMP-1, suggesting that these vesicles represent autolysosomes. Using stable U2OS cell lines that express either of the WIPI-2 isoforms WIPI-2B or WIPI-2D we found that WIPI-2 also localizes in all monolayers of double-membraned autophagosomes and at the plasma membrane specifically upon nutrient starvation. In contrast to WIPI-1, WIPI-2 did not localize predominantly to the ER/nuclear membrane although some WIPI-2D could be detected at the nuclear membrane. In addition, WIPI-2D was also found in membranes close to the Golgi cisternae. Here we provide first high-resolution imaging analyses which display both membranes of the double-membrane autophagosome. Thereby, the first detailed membranous localization of an autophagosomal protein (the WIPI proteins) is achieved, allowing us to distinguish between all monolayers of both the inner and outer autophagosomal membrane and to demonstrate that all monolayers harbor PI3P-bound WIPI proteins. Because we further identified specific membranes in which WIPI-1 and WIPI-2 accumulate upon autophagy induction, our data suggest that these preexisting membranes provide the source for WIPI-positive autophagosomes: the ER and the plasma membrane for WIPI-1 positive autophagosomes and the Golgi area and the plasma membrane for WIPI-2 positive autophagosomes.

These examples demonstrate that autophagosomes may be involved in cellular lipid homeostasis.



Freeze-fracture immuno-EM images of unfixed stable GFP-WIPI-1 U2OS cells (anti-GFP anti-serum/goat antirabbit 18 nm gold complexes) identified the localization of WIPI-1 on both the P- and E-monolayer of the inner (IM) and of the outer (OM) autophagosomal membrane.



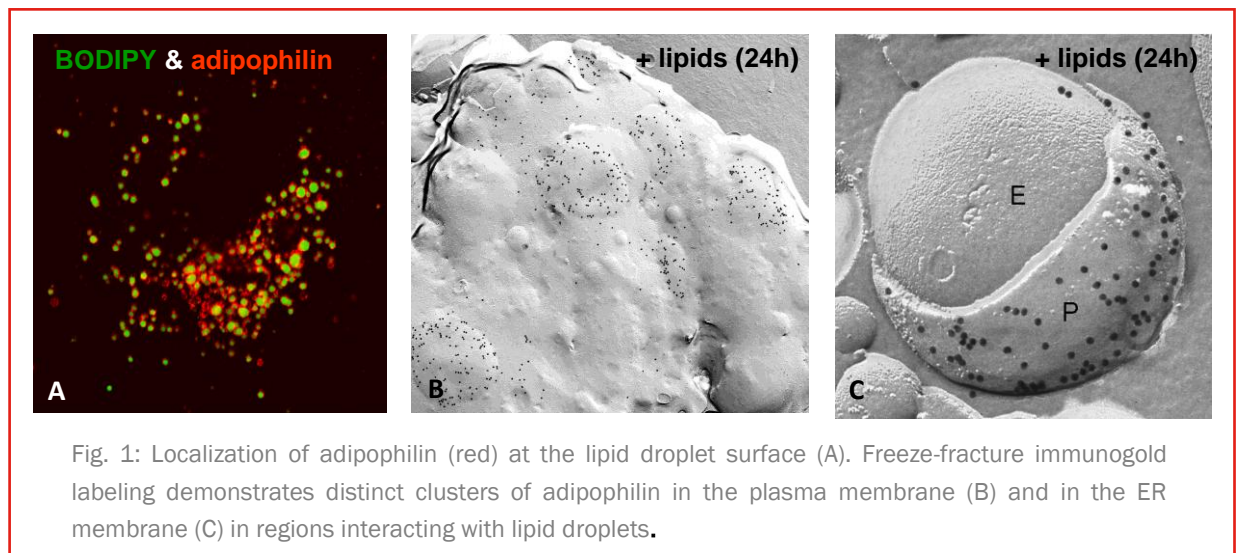
Engulfment of a lipid droplet (LD) by a WIPI-1-positive autophagosome (AP).

Perilipin substitutes adipophilin at the lipid droplet surface of macrophages

Lipid droplet accumulation in intimal macrophages (M Φ) is one of the hallmarks of atherosclerosis, the inflammatory process by which plaques develop in the arterial wall. Lipid droplets are composed of a hydrophobic core which is surrounded by a phospholipid monolayer. An important feature of the phospholipid monolayer is the presence of a series of characteristic proteins like the PAT protein family.

Our investigations are focused on adipophilin and perilipin, two members of the PAT protein family, which are probably involved in lipid droplet biogenesis, and lipid droplet stabilization. Adipophilin is the most abundant lipid droplet associated protein in human monocyte derived macrophages (HMDM), mouse peritoneal macrophages, or THP-1 M Φ (Fig. 1A). We also found adipophilin labeling in the plasma membrane and in the outer membrane of the

endoplasmic reticulum (ER) of THP-1 M Φ by using the high resolution freeze-fracture technique. Lipid incubation of THP-1 M Φ results in a specific clustering of adipophilin in specialized plasma membrane or ER domains, which are interacting with lipid droplets (Fig. 1B + C). Based on our localization studies it might be possible that adipophilin is involved in the biogenesis and/or growth of lipid droplets. Therefore, we investigated the expression pattern of adipophilin in the presence of lipids. Adipophilin expression is enhanced in the presence of lipids compared to unloaded control cells. The additional expression or suppression of adipophilin leads to increased or decreased amounts of lipid droplets in THP-1 M Φ . Additionally, we found enhanced or suppressed cholesterol levels of THP-1 M Φ transfected with EGFP-adipophilin or adipophilin siRNA.



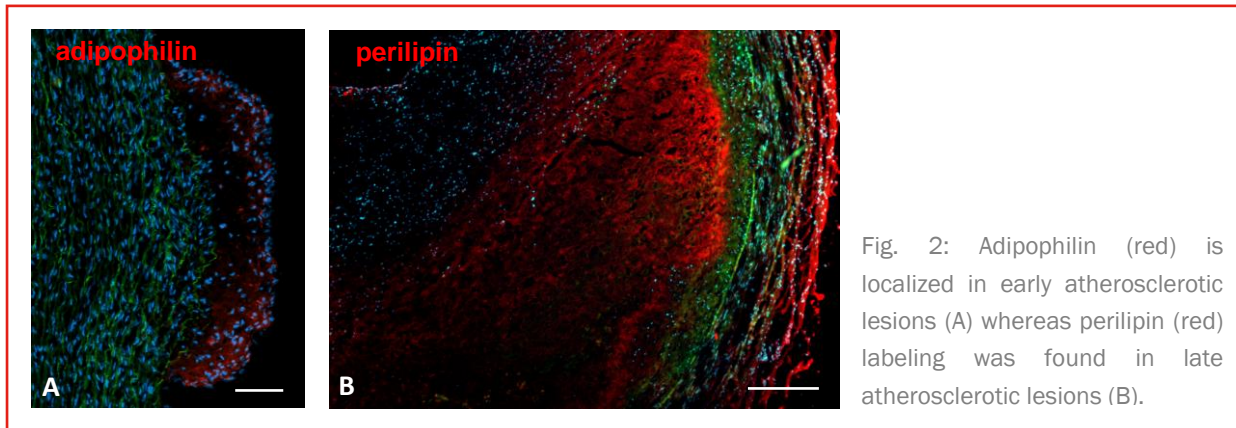


Fig. 2: Adipophilin (red) is localized in early atherosclerotic lesions (A) whereas perilipin (red) labeling was found in late atherosclerotic lesions (B).

The influence of adipophilin on foam cell formation of M Φ led to the assumption that adipophilin may influence the formation of atherosclerotic lesions. Localization studies of adipophilin in human atherosclerotic lesions revealed adipophilin labeling mainly in early stages of atherogenesis. There, adipophilin co-localizes with intimal M Φ (Fig. 2A). However, late atherosclerotic lesions exhibit no adipophilin labeling.

Perilipin is the best characterized member of the PAT protein family. Alternative splicing of the perilipin gene leads to four perilipin isoforms (perilipin A-D). Perilipin A and B are expressed in adipocytes whereas perilipin C and D are found in steroidogenic cells. The occurrence of perilipin in M Φ is controversially discussed. HMDM and THP-1 M Φ are reported to express perilipin whereas others could not confirm these results. Our Western blot analyses using an anti-human “pan”-perilipin antibody directed against the N-terminus that is shared by all known perilipin isoforms, revealed perilipin A in HMDM and THP-1 M Φ . Immunofluorescence confocal microscopy of THP-1 M Φ after transfection with EGFP-perilipin A and incubation with acLDL or oleate confirmed that perilipin A is associated with lipid droplets. Additionally, the expression of EGFP-perilipin A leads to a replacement of adipophilin at the lipid droplet surface.

A unique characteristic of the perilipin protein sequence is the appearance of consensus motifs for phosphorylation of serine residues by cAMP-dependent protein kinase A (PKA). Human perilipin A includes five putative PKA consensus sites. Dephosphorylation of perilipin A stabilizes lipid droplets and inhibits lipolysis of the lipid droplet core by hormone sensitive lipase (HSL). Cyclic AMP-activated PKA phosphorylates lipid droplet associated perilipin A and cytoplasmic HSL. Phosphorylated perilipin A allows HSL to bind to lipid droplets and to hydrolyze the lipids of the lipid droplet core. We found that additional expression of EGFP-perilipin A enhanced the triglyceride content of THP-1 M Φ .

Perilipin expression in M Φ and its localization at the lipid droplet surface shows that perilipin is involved in atherogenesis. Our localization studies using an anti-human “pan”-perilipin antibody revealed perilipin labeling in late human atherosclerotic lesions co-localizing with M Φ (Fig. 2B) No labeling was found in early human atherosclerotic lesions.

Our results show that adipophilin and perilipin exhibit different functions during atherogenesis. We suppose that adipophilin supports the biogenesis and growth of lipid droplets in M Φ and therefore foam cell formation during early stages of atherogenesis. The substitution of adipophilin by perilipin in late atherosclerotic lesions is the first indication that perilipin might be able to stabilize lipid droplets of M Φ and therefore late atherosclerotic lesions.

Role of butyrophilin in secretion of lipid droplets

The unlimited uptake of lipids and their storage in form of lipid droplets induce foam cell formation of macrophages during atherogenesis. In this project, we are focused on the possibility of secretion of accumulated lipid droplets out of macrophages. Only one mechanism is known for secretion of lipid droplets: milk lipid droplets out of mammary epithelial cells for feeding the offspring. The surface of secreted milk lipid droplets are organized into a trilayer: an inner phospholipid monolayer derived from the lipid droplet and an outer phospholipid bilayer originating from the plasma membrane. The current assumption is that the secretion is mediated by a tripartite complex between the integral transmembrane protein of the plasma membrane butyrophilin (BTN), the soluble metabolic enzyme xanthine oxidoreductase (XOR) and the lipid droplet associated protein adipophilin (ADFP).

But our own studies with freeze-fracture replica immunolabeling have shown that BTN is detectable on the E-face of the bilayer of the plasma membrane and also on the P-face of the lipid droplet monolayer (Fig.1 A). Furthermore BTN is concentrated in the bilayer in a network of ridges that are tightly apposed to the monolayer (Fig. 1 A). However, ADFP is primarily associated in the lipid droplet monolayer and not arranged in a network of ridges (Fig. 1 B).

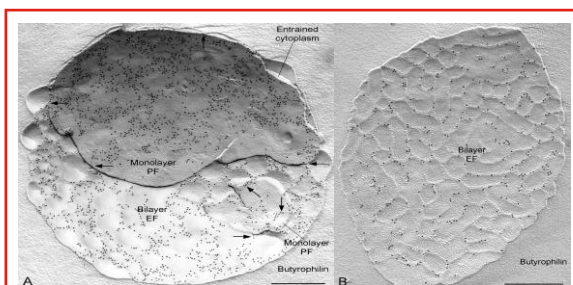
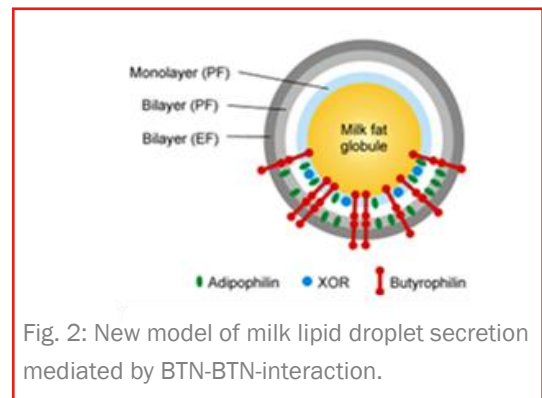


Fig. 1: Distribution and analysis of BTN. (A+B) Intensive labeling of BTN on the P-face (PF) of the monolayer and the E-face (EF) of the bilayer. (Bars, 0.5 μ m).

As a result of the localization of BTN in the plasma membrane and at the lipid droplet monolayer, we think an interaction of BTN and ADFP is not possible. Based on these results, we hypothesize a new model of milk lipid droplet secretion (Fig. 2): BTN induces exclusively the secretion of milk lipid droplets mediated by interaction of BTN in the monolayer of the lipid droplet and BTN in the plasma membrane of the mammary epithelial cell.



For a better understanding of the function of BTN and clarifying the mechanism of milk fat globule secretion the sequence of full length BTN (pEGFP-N-BTN wildtyp) and deletions were cloned into the pEGFP-C1-vector (Clontech Laboratories). The sequence of BTN is an integral transmembrane protein and exhibits an exoplasmatic domain, a medial transmembrane domain and a cytoplasmatic domain. The deletions contain consequently the following domains of the protein (Fig. 3).

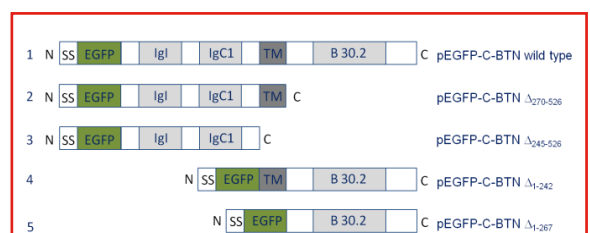
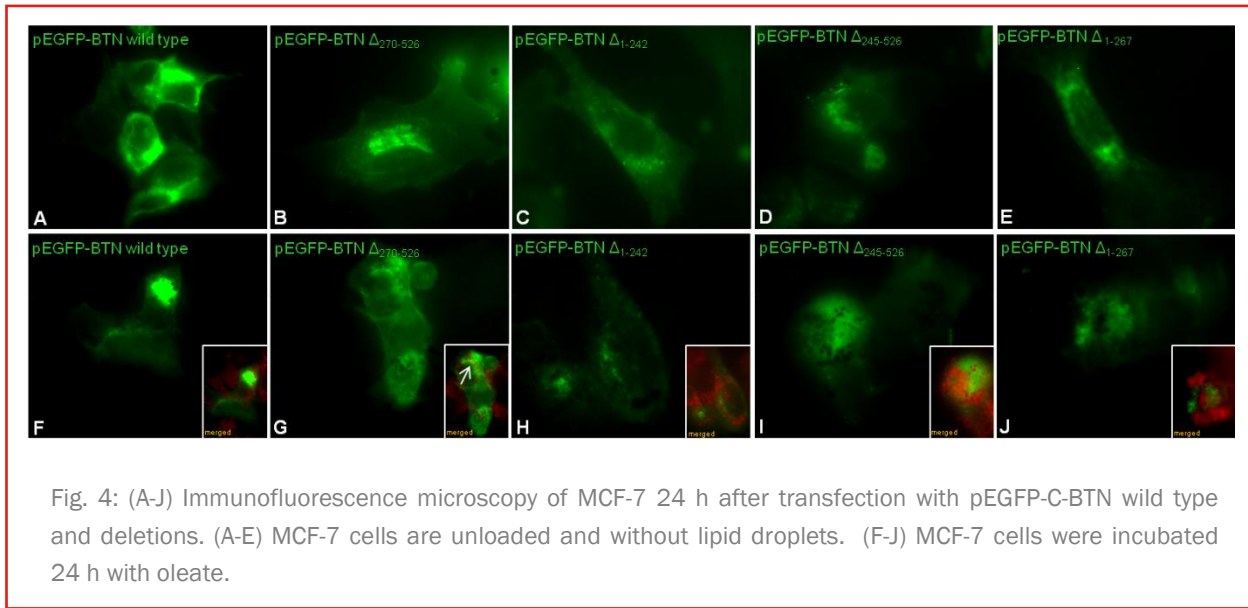
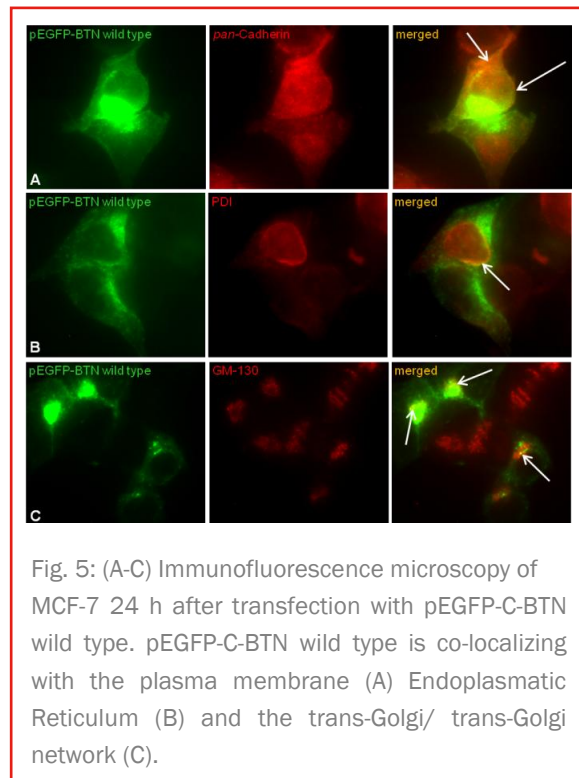


Fig. 3: Summary of recombinant vectors and numeric positions of amino acid residues of the deletions of BTN.



Transient transfection of MCF-7 cells (human breast cancer cell line) with pEGFP-C-BTN wild type and the truncated deletions offered first valuable clues to the expression of BTN in MCF-7 cells (Fig. 4). Fluorescence microscopy indicates significant differences between the dispensation of pEGFP-C-BTN wild type and deletions in oleate loaded (Fig. 4 F-J) and unloaded MCF-7 cells (Fig. 4 A-E). It seems like redistribution and concentration of BTN constructs within the cells to the point of the lipid droplets in oleate loaded MCF-7 cells (Fig. 4 F-J). But only pEGFP-C-BTN wild type (Fig. 4 F) and the deletions pEGFP-N-BTN $\Delta_{270-526}$ (Fig. 4 G) and pEGFP-C-BTN $\Delta_{245-526}$ (Fig. 4 I) are localizing potentially at lipid droplets (Fig. 4 F-H).

Furthermore, fluorescence microscopy shows fitting of pEGFP-C-BTN wild type with cell components in MCF-7 cells in different modes (Fig. 5 A-C). The full length protein is associated with the plasma membrane (Fig. 5 A, arrows). It also appears to be attached at the Endoplasmatic Reticulum (Fig. 5 B, arrows) or appeared to be associated with, or close to the trans-Golgi/trans-Golgi network (Fig. 5 C, arrows).

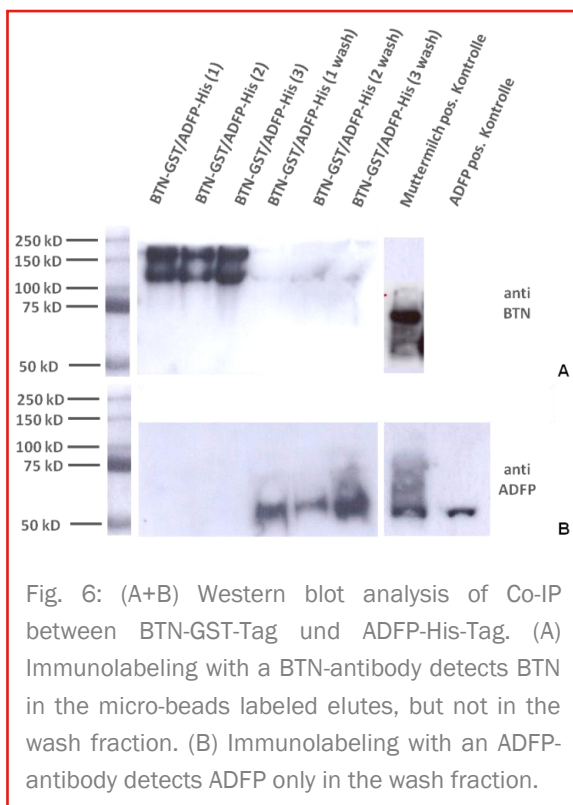


On the other hand we started to investigate the protein interaction of BTN with ADFP by co-immunoprecipitation (Co-IP). This method shall offer valuable clues to the interaction of these proteins. Therefore we incubated GST (Glutathion-S-Transferase) tagged recombinant BTN with recombinant His (poly-Histidin) tagged ADFP. After incubation the protein mix were labeled with anti-GST-Tag micro-beads and were eluted by anti-GST-Tag columns.

Western Blot analysis demonstrates detection of BTN with GST-Tag in the elute fraction (Fig. 6 A). The higher clique of both cliques may indicate an interaction of BTN with BTN itself.

In contrast ADFP with His-Tag is only located in the wash fraction and not in the labeled eluate (Fig. 6 B). Therefore ADFP is not interacting with BTN, but BTN interacts partial with BTN itself (Fig. 6). These results are supporting our new hypothesis of milk lipid droplet secretion.

Within this project, a facility should be found to antagonize the accumulation of lipid droplets in atherosclerotic lesions by lipid droplet secretion. Based on our current perceptions of lipid droplet secretion, we think BTN could be a possible new target to avoid lipid accumulation in atherogenesis. In the next step a macrophage cell culture model with stable BTN overexpression for supporting this model will be developed.

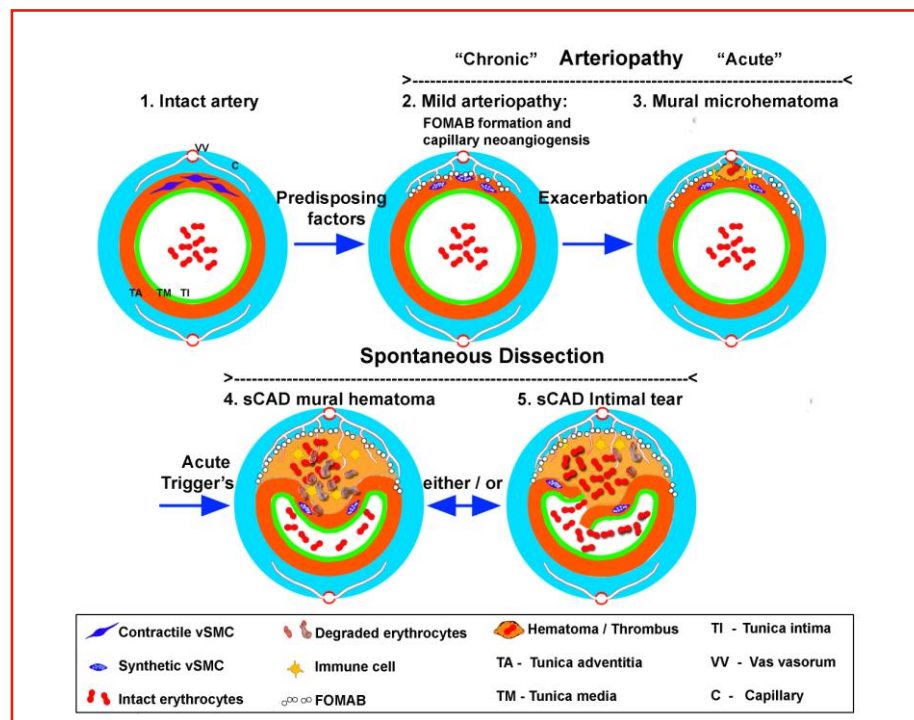


A clinical histopathological study of patients with spontaneous cervical artery dissection (sCAD)

In a long-term collaboration project with the Hospital of Neurology (UKM, Münster) investigations were performed in order to detect characteristic changes in patients with vascular occlusions of their brain supplying arteries. The studies were done with tissues obtained by biopsy from skin and from superficial temporal arteries of sCAD patients and, *post mortem* by autopsy for control, from individuals without any vascular diseases. Our histopathological

investigations should cast some light on the process of the formation of the so-called spontaneous cervical artery dissection (sCAD). Under various aspects our findings of sCAD-related changes were published in four publications in the recent years and accumulated now in a hypothesis (figure) of the progression of vascular damage leading to sCAD and stroke (Völker et al., in press).

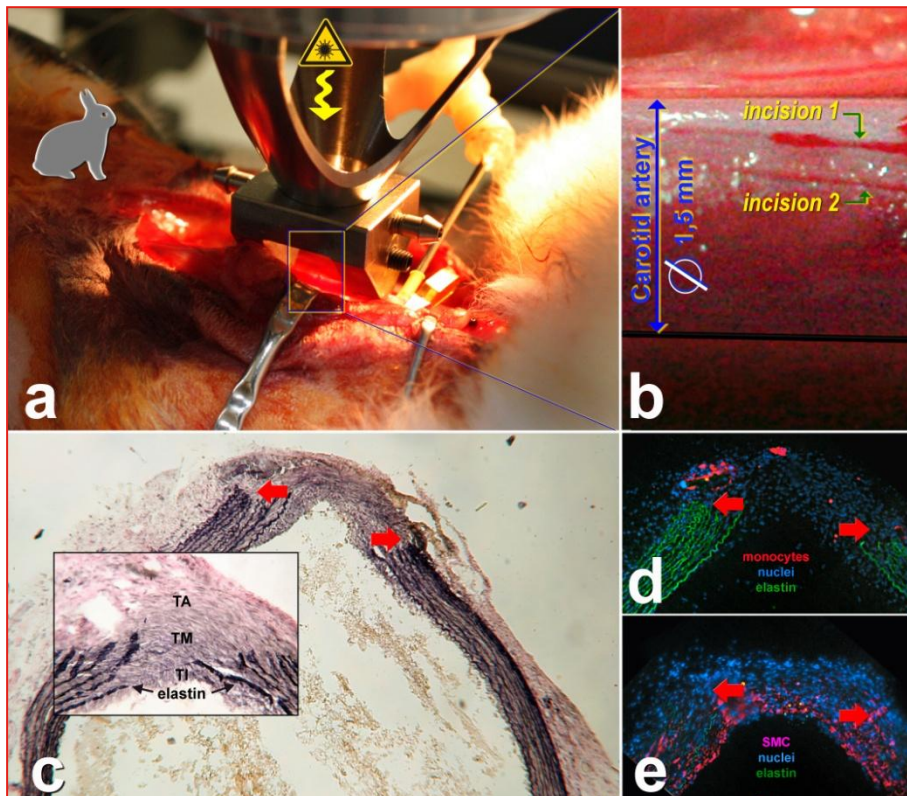
Superficial temporal artery biopsies suggest that sCAD is mainly a disease of the outer arterial layers, namely the arterial tunica adventitia (TA) and tunica media (TM) involving neoangiogenesis. Erythrocyte extravasation from the neoangiogenetic vessels and microhematoma formation along the medial-adventitial borderline led us to propose a pathogenetic model in which – based on a chronic angiopathy of the cervical arteries - sCAD is initiated by rupture of these neoangiogenetic vessels.



A new approach to vascular surgery by extra-luminal laser angioplasty (ELAN)

In collaboration with ELAN Vascular Technologies (P) Ltd. (Mumbai) and Rowiak GmbH (Hannover) a new technique is being developed at the LIFA laboratories in Münster for surgical treatment of vascular stenoses. In contrast to other methods of restoring blood flow in diseased arteries by intra-luminal angioplasty, e.g. balloon angioplasty or stent implantation, the ELAN group for the first time introduced a femto-second laser system for extra-luminal manipulation of such vessels *in vivo*. In our recent experiments standard rabbits were used. Their left carotid artery was exposed and several perivascular laser incisions were applied from outside

onto the vascular wall. For accurately controlling these laser incisions during the operation a computer tomography scanning unit was integrated in the laser system to obtain online three dimensional live images of the vascular wall and of the depth of incision. After a few weeks the animals were operated again to obtain the treated carotid segments for studying healing and widening of the vessel wall at the site of treatment. First results are illustrated in the figures below. Our experiments will be continued now with rabbits which develop atherosclerotic lesions.



a: In an anaesthetized rabbit the left carotid artery is exposed and positioned under the laser projection unit (square). **b:** Close-up photography of two laser incisions outside along the vascular wall. **c:** Three weeks after injury histological cross-sections of the treated area show interruption of the elastic layers (inset, elastin, red arrows), remodelling of the vascular tissue (TA, TM) and less intimal growth (TI). **d:** Inflammation and infiltration of monocytes (red) are modest. **e:** Newly formed smooth muscle cells (SMC, red) are present in the healing lesion.

Inflammatory remodelling of the normal and atherosclerotic vessel wall

Proinflammatory mediators play a key role in all aspects of vascular remodeling. In normal arteries these mediators regulate the structural integrity and stability of the vascular wall. During early atherogenesis they are involved in the processes of inflammatory infiltration, foam cell formation and lipid accumulation which lead to the formation of fatty streaks and at later stages to the formation of atherosclerotic lesions. Previous studies from our group point to a central role of granulocyte macrophage colony stimulating factor (GM-CSF) particularly in the processes of structural vascular remodeling. GM-CSF is a regulator of collagen and elastin both main structure elements vascular extracellular matrix (ECM). This was previously studied in genetic mouse models (GM-CSF-deficient mice) as well as in patients with vascular disease.

Our investigations on GM-CSF transgenic (GM-OE) mice revealed the not only GM-CSF-deficiency but also GM-CSF over-expression induces structural remodeling in the aorta and in cardiac arteries. Under GM-CSF over-expression a reduction and dysintegration of the elastic system was observed in the aorta as well as in cardiac arteries. This elastolysis is due to increased production of MMP-12. Further studies showed that not only elastolysis but also collagenolysis was increased. This increase was not due to increased expression of collagenolytic MMPs but to an activation of gelatinases (MMP-2 and -9) by MMP-12. Double-immunofluorescence studies revealed that in wild type mice MMP-12 was expressed only by few macrophages. In the heart of GM-CSF over-expressing mice the number of expressing cells was markedly increased. Expressing

cells were identified as macrophages and as smooth muscle cells. Endothelial cells did not stain for MMP-12. GM-CSF induction studies on cultured macrophages, smooth muscle cells and endothelial cells showed that this mediator stimulated MMP-12 expression by macrophages and smooth muscle cells. As indicated by the increased immunofluorescence for von Willebrand factor the vascularization was conspicuously increased in the heart of GM-CSF over-expressing mice.

Our studies on patients with thoracic aortic aneurysm and dissection revealed that in areas of exceeding matrix deterioration the levels of GM-CSF and MMPs in macrophages and smooth muscle cells were also increased (Weissen-Plenz et al. 2010).

Our data indicate that the imbalance of remodeling in the vascular ECM in GM-CSF over-expressing mice is due to the catabolic activation not only of macrophages but also of smooth muscle cells. Our data further suggest that in patients with inflammatory vascular disease the overstimulation of the GM-CSF-system may present the underlying trigger of adverse catabolic ECM remodeling, loss of functional texture (in the areas of persistent inflammation) and thus contribute to the destabilization of the aortic wall finally leading to dissection. Our studies further emphasize that a strictly regulated GM-CSF level is a prerequisite for a balanced metabolism of the vascular ECM and thus for the maintenance of vessel wall integrity and stability.

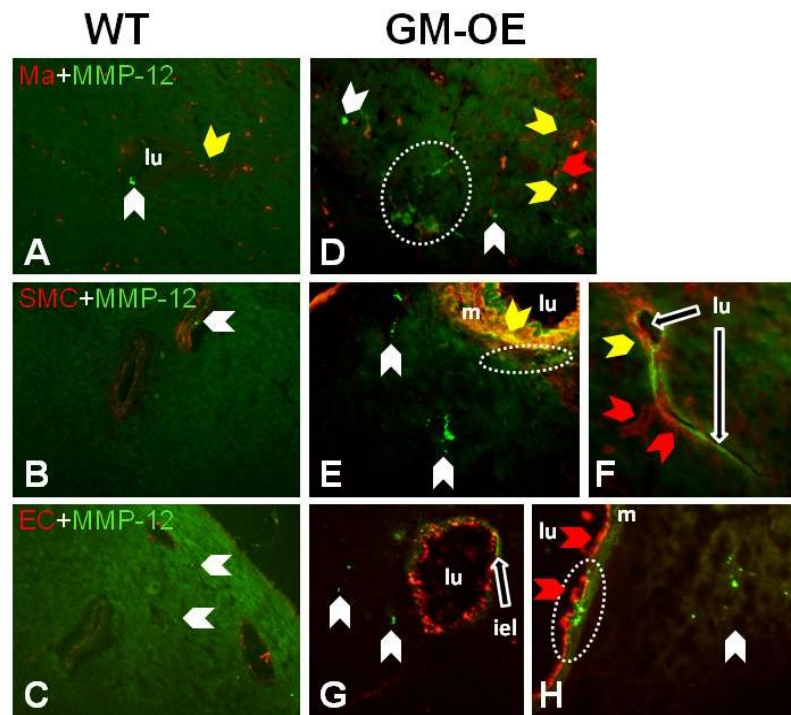


Fig. 1: Characterization of MMP-12-expressing cell types in the heart of GM-CSF over-expressing mice.

Immunofluorescence of MMP-12 as shown in the FITC channel (green, green arrows, open circles) in the myocardium and cardiac arteries of wild type (WT) mice (A-C) and (D-H) GM-CSF over-expressing (GM-OE) mice. Macrophages (Ma), smooth muscle cells (SMC) and endothelial cells (EC) were shown in the Rhodamin channel (red, red arrows). Cells double-labeled for MMP-12 and cell type specific antibodies appear yellow (yellow arrows). (A) In the heart of WT mice MMP-12 was expressed only by a subpopulation of Ma (yellow arrow). Neither SMC (B) nor EC (C) showed MMP-12 label. In the heart of GM-OE mice MMP-12 label was markedly increased. MMP-12 label was found in subpopulations of macrophages (D) and SMC (E&F) (yellow arrows). Endothelial cells did not express MMP-12 (G&H). m: media; lu: lumen; WT: wild type.

Work group

Signalling-controlled mechanisms of atherogenesis

N,N-Dimethylsphingosine – a pleiotropic arterioprotective signalling molecule

In previous studies we found that S1P is implicated as signalling molecule in the regulation of cell adhesion molecules in vascular endothelial cells (EC) and that vascular endothelial cells exposed to sphingosine 1-phosphate (1-10 μ M) in the absence of other stimuli promote the expression of E-selectin mRNA and protein and the adhesion of THP-1 monocytes in a strictly dose-dependent fashion. In this report we investigated the S1P-antagonistic action of N,N-dimethyl-D-erythro-sphingosine (DMS). Both S1P and DMS are secondary metabolites of sphingomyeline and derivatives of sphingosine, which is intracellular, converted to different signal molecules either by phosphorylation to S1P or by methylation to DMS.

1. DMS attenuates the TNF-alpha-stimulated expression of E-selectin

Cultured EC express E-selectin protein in response to TNF-alpha (10 ng/mL) stimulation. After pretreatment with S1P transcription and translation of E-selectin is attenuated by about 50% ($p < 0.05$) versus control cells. A similar repression effect is shown by DMS known as a secondary metabolite of sphingomyeline degradation. The suppressor effect of DMS and S1P is completely reverted by the phosphatidylinositol 3-kinase inhibitor LY294002 (Fig. 1), indicating that a signalling cascade initiated by the G-protein coupled receptor and including the PI-3K is involved in this



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process. However, whether DMS is a direct ligand of S1P_{1,3} receptor or becomes a ligand by chemical modification like the FTY720 immune modulator that has to be phosphorylated by sphingosine kinase-2 before acting as a receptor, needs further clarification. The effect of DMS is concentration-dependent (Fig. 2).

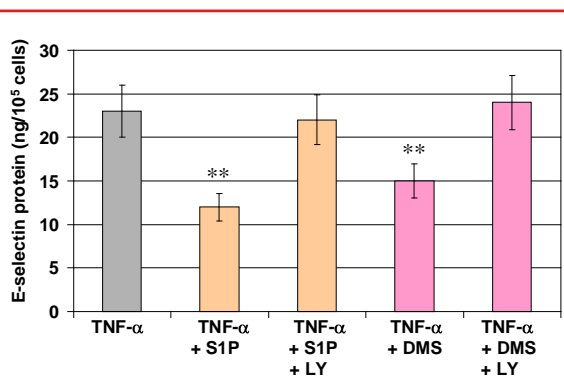
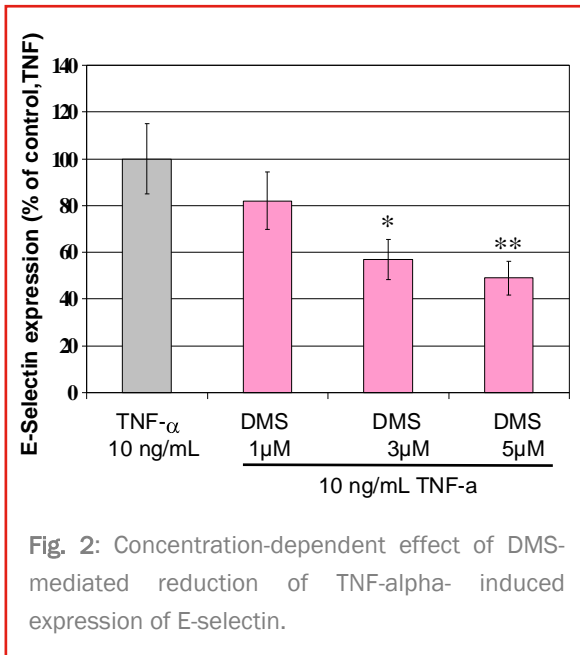


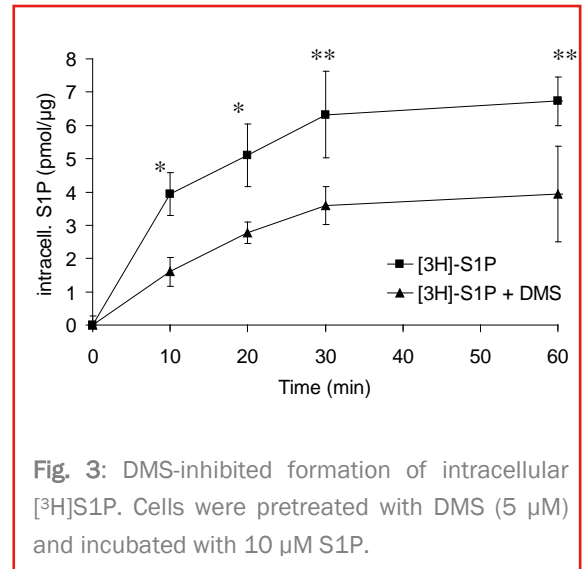
Fig. 1: S1P and DMS attenuate the TNF-alpha induced expression of E-selectin. The effect is completely reverted by LY294002.



2. DMS controls the translocation of exogenous in endogenous S1P

The uptake pathways of S1P by EC was investigated using [3 H]sphingosine 1-phosphate ([3 H]S1P). When confluent cells were incubated with 10 μ M S1P containing 300,000 dpm [3 H]S1P (in the following [3 H]S1P) the intracellular [3 H]S1P increases to a saturation-like level of about 6 pmol/ μ g cell protein. After pretreatment of the cells with 5 μ M DMS a retarded intracellular increase and a lower level of [3 H]S1P was determined (Fig. 3). This DMS effect is considered to be a result of the reduced rephosphorylation of [3 H]sphingosine that enters the cell predominantly after extracellular dephosphorylation of [3 H]S1P as [3 H]sphingosine. The highly lipophilic [3 H]sphingosine passes the cell membrane without any carrier support and is intracellularly used for synthesis of ceramide or rephosphorylated by the sphingosine kinase. The sphingosine kinase, however, is inhibited by DMS resulting in a reduced intracellular [3 H]S1P concentration.

The S1P concentration-reducing effect of DMS was confirmed by HPLC measurements of the intracellular S1P levels. Under steady state conditions EC maintain a S1P level of about 20 ng sphingosine 1-phosphate/ mg cell protein. This is elevated transiently by 10 ng/mL TNF-alpha to about 40 ng and reduced to basal levels by stimulation with a combination of 10 ng/mL TNF-alpha and 5 μ M DMS.



3. DMS reduces the S1P-induced phosphorylation of the p65 subunit of NF-kappaB

Since a nuclear translocation of NF-kappaB including its subunit p65 is required for complete expression of E-selectin a positive correlation between the expression of E-selectin protein and the phosphorylation of NF-kappaB could be postulated. In cells not stimulated by TNF-alpha 10 μ M S1P increases the phosphorylation by about 100% of control and a further increase was achieved in the presence of LY294002. In contrast, DMS and the synthetic sphingosine kinase inhibitor depressed the S1P effect to 140% and 120% of control respectively. Accordingly, the expression of E-selectin shows a positive correlation to the NF-kappaB phosphorylation (Fig. 4).

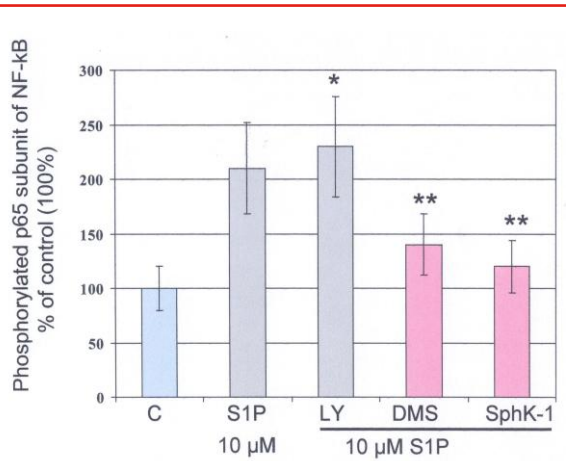


Fig. 4: Alteration of S1P-stimulated phosphorylation of the p65 subunit of NF-kappaB by various modulators (DMS, SphK-I, LY 294002).

4. Conclusion and perspectives

Taken together, the antagonistic action of DMS and S1P provides a new principal aspect of atherogenesis. The counterbalance of dimethylsphingosine and sphingosine 1-phosphate drives early development of arteriosclerosis into opposite directions and based on our data we assume that counteracting of the agonist S1P and the antagonist DMS represents a primary fine-tuning system which decides about the further development either directed to the formation of an arteriosclerotic plaque or to protection of the arterial wall and maintaining physiological conditions. In addition, the antagonistic action of S1P and DMS can be regarded as a paradigm for pro-inflammatory and anti-inflammatory processes.

The inhibitory effect of DMS on the S1P-induced NF-kappaB nuclear translocation was confirmed by immunofluorescence analysis of the p65 subunit of NF-kappaB in cells without TNF-alpha stimulation.

Thus, we provide evidence that a regulatory role of LY294002 on the one hand and DMS on the other hand is consistent with the variation of the S1P induced E-selectin expression and the binding of THP-1 monocytes to EC.

Work group

Lipid Metabolism and Metabolic Syndrome

Research focus of the group

In the past few years, the work group took an active part in several internationally recognized studies related to the identification of common and rare genetic variants which influence the risk of cardiovascular diseases. The group participated in genome-wide association studies and genome-wide quantitative trait (QTL) analyses of coronary heart disease and myocardial infarction (Nat. Genet., in press), Diabetes and blood glucose level (Nat. Genet. 2010), hypertension and blood pressure (Nat. Genet. 2009), blood lipid and lipoprotein (a) level (NEJM 2009) and biomarker level (i.e. homocysteine, a known cardiovascular risk factor) (Blood 2009 and Cir. Cardiovasc. Genet. 2009). All these activities were performed in the framework of large international consortia. The consortia approach made it possible to study exceedingly large case-control cohorts, which was necessary to warrant a sufficiently high statistical power to detect the moderate effects on disease risk which were expected to result from any single genetic variant. The group leader is a member of several international consortia aimed at the identification and characterization of genetic variants related to cardiovascular disease risk (i.e. the Precocious Coronary Artery Disease (PROCARDIS) consortium, the Global Blood Pressure Genetics (BPgen) consortium, the Meta-analysis of Glucose And Insulin-related Traits (MAGIC) consortium) and the Coronary Artery Disease Genetics Consortium Discovery Study (C4D Study).

The results of all of these investigations are aimed at a better understanding of the mechanisms contributing to cardiovascular diseases. Moreover, an important goal of our work is to contribute to the development of



Group leader:
Prof. Dr. rer. nat.
Udo Seedorf

improved risk assessment tools to identify patients at risk of cardiovascular diseases. Current risk prediction tools rely on the classical cardiovascular risk factors, such as age, sex, blood pressure, HDL- and LDL cholesterol, Diabetes status and/or family history of cardiovascular diseases. Since risk prediction algorithms based on these classical risk factors alone are not very specific, an important medium-term goal of our work is to identify a limited number of relevant genetic markers to be integrated into optimized risk prediction tools. We hope that this strategy is going to lead to improved patient care (i.e. through personalized medicine, taking advantage from genetic testing which may contribute to predict the cardiovascular event risk more specifically than is possible today). A strong bioinformatic and technical support is essential for reaching our goals. Therefore, our success depends importantly on collaborating closely with the Department of Genetic Epidemiology of Vascular Diseases (Prof. Monika Stoll) and the Institute's Core Facility.

Progress report 2010

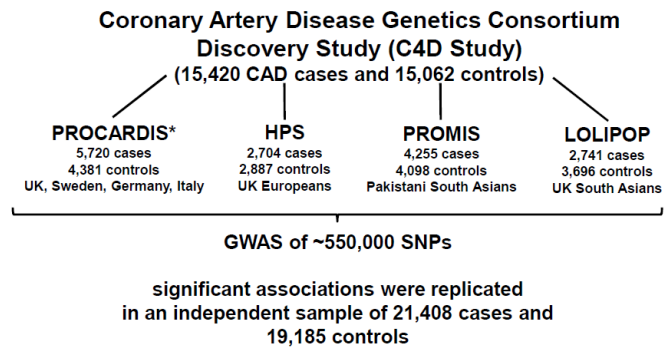
In 2010, progress was obtained in the following areas:

1. In the framework of the MAGIC study, we provided glucose-related phenotype data on ~900 PROCARDIS participants including 265 German control subjects with genome-wide association data for a study to identify novel glycaemic loci. The meta-analyses comprised 21 genome-wide association studies informative for fasting glucose, fasting insulin and indices of β -cell function (HOMA-B) and insulin resistance (HOMA-IR) in a total of 46,186 non-diabetic participants. Follow-up of 25 loci in up to 76,558 additional subjects led to the identification of 16 loci associated with FG/HOMA-B and two associated with FI/HOMA-IR. These included nine new loci influencing fasting glucose level (in or near ADCY5, MADD, ADRA2A, CRY2, FADS1, GLIS3, SLC2A2, PROX1 and FAM148B) and one influencing FI/HOMA-IR (near IGF1). In addition, association of ADCY5, PROX1, GCK, GCKR and DGKB/TMEM195 with type 2 diabetes risk could be demonstrated. Within these loci, likely biological candidate genes influence signal transduction, cell proliferation, development, glucose-sensing and circadian regulation. The study was published in *Nat. Genet.* 2010;42:105-116.
2. In the framework of the BPgen and PROCARDIS consortia, we provided phenotype and genotype data of ~1,050 PROCARDIS study participants for the Japanese Millennium Genome Project which were used to replicate initial findings indicating that common variants in the ATP2B1 gene were associated with susceptibility to hypertension. The study was published in *Hypertension* 2010;56:973-980.
3. In the framework of the BPgen and PROCARDIS consortia, we collaborated with the Cohort for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium in a genome-wide association study to identify genetic variants associated with retinal vascular caliber. There is increasing evidence

that the microcirculation plays an important role in the pathogenesis of cardiovascular diseases. It is assumed that changes in retinal vascular caliber reflect early microvascular disease and predict incident cardiovascular events. The study comprised data from four population-based discovery cohorts with a total of 15,358 unrelated Caucasian individuals, who were members of the CHARGE consortium, and replication data in four independent Caucasian cohorts ($n = 6,652$). All participants had retinal photography and retinal arteriolar and venular caliber measured from computer software. In the discovery cohorts, 179 single nucleotide polymorphisms (SNP) spread across five loci were significantly associated with retinal venular caliber, but none showed association with arteriolar caliber. Collectively, these five loci explained 1.0%–3.2% of the variation in retinal venular caliber. Four out of these five loci were confirmed in independent replication samples. In the combined analyses, the top SNPs at each locus were: rs2287921 (19q13, within the RASIP1 locus), rs225717 (6q24, adjacent to the VTA1 and NMBR loci), rs10774625 (12q24, in the region of ATXN2,SH2B3 and PTPN11 loci), and rs17421627 (5q14, adjacent to the MEF2C locus). Based on phenotype and genotype data coming, amongst others, from our group and the PROCARDIS study, it could be demonstrated that locus 12q24 was also associated with coronary heart disease and hypertension (published in *PLoS Genet.* 2010;6: e1001184).

4. In preparation of a genome-wide QTL analysis to identify genes which influence lipoprotein (a) (Lp(a), a cardiovascular risk factor), we measured Lp(a) levels in 1,000 healthy control subjects and 1,000 coronary heart disease cases and determined their apolipoprotein (a) isoforms by a Western-blot technique. The data should be used in a future GWAS study aimed at identifying common variants located outside of the LPA locus on chromosome 6q27 which influence Lp(a) levels.

C4D: a genome-wide association study on coronary artery disease in Europeans and South Asians



*LIFA, Münster, Germany; Karolinska, Stockholm, Sweden; University of Oxford, UK; Mario Negri, Milan, Italy

Fig. 1: Study design, sample origin and sample sizes underlying the C4D Study.

5. The group collaborated in the framework of the Coronary Artery Disease Genetics Consortium Discovery Study (C4D Study, see Fig. 1 for a description of the study samples and study design of the C4D study) to identify common genetic variants associated with coronary artery disease in Europeans and South Asians. The specific contributions of our group to this study included, amongst others, collecting German cases and controls to be incorporated in the study, performing biochemical lipid phenotyping and clinical chemistry analyses of the entire PROCARDIS cohort as part of the C4D study (5,720 cases and 4,381 controls) and incorporating functional plausibility considerations for the large fraction of lipid metabolism-related genes which were found associated with coronary artery disease in this study. The C4D meta-analysis involved genotyping of ~575,000 SNPs in a discovery sample, comprising 15,420 coronary artery disease

cases (8,424 Europeans and 6,996 South Asians) and 15,062 controls. The data provided little evidence for ancestry-specific associations, which supported the use of combined analyses of the data sets from ethnically diverse origin. A total of 19 loci showed significant associations with CAD in an independent replication sample of 21,408 cases and 19,185 controls, and also achieved genome-wide significance in the combined discovery and replication analysis (Fig. 2). Amongst these 19 loci were 5 novel loci: LIPA on 10q23, PDGFD on 11q22, ADAMTS7- MORF4L1 on 15q25, a gene rich locus on 7q22, and KIAA1462 on 10p11. Interestingly, 7 out of these 19 loci had an implicated role in lipid metabolism. The overwhelming majority of these loci were associated with LDL cholesterol, but none with HDL cholesterol (Fig. 3).

A Large Number of SNPs Determines a Small Fraction of Coronary Risk

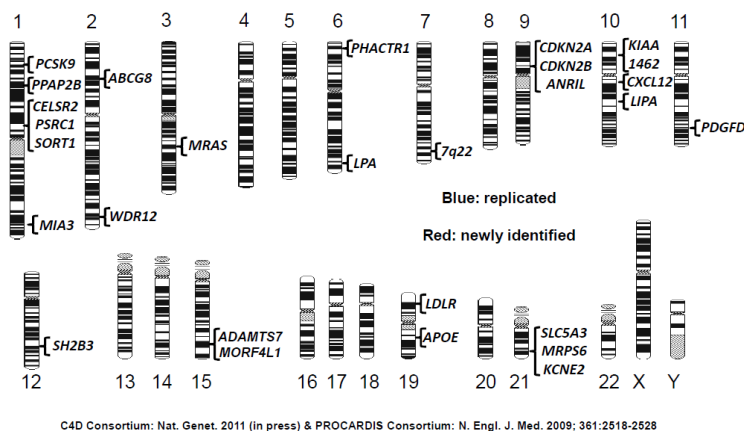


Fig. 2: Chromosomal localization of SNPs linked to 19 loci associated with early onset coronary artery disease in the C4D study (Nat. Genet., in press).

7 out of the 19 Loci Identified or Replicated in C4D / PROCARDIS have a Role in Lipid Metabolism

Locus	Gene	Protein	Effect on
1p13	SORT1	Sortilin	LDL-C level
1p34	PCSK9	Proprotein convertase, subtilisin/kexin type 9	LDL-C level
2p21	ABCG8	ABC transporter G8	LDL-C level
6q27	LPA	Apolipoprotein (a)	Lipoprotein(a) level
10q23	LIPA	Lysosomal acid lipase	Cholesterol ester & triglyceride metabolism
19p13	LDLR	LDL receptor	LDL-C level
19q13	APOE	Apolipoprotein E	LDL-C level

C4D Consortium: Nat. Genet. 2011 (in press) & PROCARDIS Consortium: N. Engl. J. Med. 2009; 361:2518-2528

Fig. 3: Seven lipid metabolism-related genes implicated to be associated with coronary artery event risk in the C4D study and their QTL effect (Nat. Genet., in press).

6. In a study performed in collaboration with the Department of Genetic Epidemiology of Cardiovascular Diseases (Prof. Stoll), the Institute of Epidemiology (Prof. Berger) and the Neurology Department (Prof. Ringelstein and Prof. Kuhlenbäumer) of the University of Münster, we could show association of two apolipoprotein(a) gene (LPA) SNPs (rs3798220 and rs10455872) with certain subtypes of stroke in a German case-control study of stroke. The study included 625 large atherosclerotic, 585 cardioembolic, 295 lacunar stroke cases and 974 healthy controls. High Lp(a) level is an emerging risk factor for coronary artery disease and we had shown earlier that the two common LPA variants rs3798220 and rs10455872 were strongly associated with Lp(a) level and the

risk of coronary artery disease. The results of our present study demonstrated that the variant rs10455872 was also associated with ischemic stroke (age and sex adjusted odds ratio of 1.66 and a 95% confidence interval (CI) of 1.22-2.25). Differentiation of the stroke type according to the classification scheme proposed by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) revealed that the SNP associated strongly with lacunar stroke (TOAST-3; OR: 2.37; 95% CI: 1.55-3.63), but only quite moderately with large artery atherosclerotic stroke (TOAST-1; OR: 1.57; 95% CI: 1.09-2.27) or cardioembolic stroke (TOAST-2; OR: 1.54; 95% CI: 1.01-2.27). The rarer variant rs3798220 (MAF 1.5%) associated exclusively with lacunar stroke (OR: 2.31; 95%CI: 1.08-4.91).

Department

Molecular Genetics of Cardiovascular Disease

General Strategy

Non-hypothesis driven genome-wide approaches to identify genomic loci associated with complex disease phenotypes often fail to disclose a specific functional effect of the detected variant(s), since they may only serve as markers in linkage disequilibrium with the causal functional variant. Consequently, a reliable molecular profiling of genetic variation and a better knowledge of the molecular/functional structure of the locus of interest is mandatory. Appropriate large-scale and well-phenotyped populations are available in the department of “Molecular Genetics of Cardiovascular Disease”. Together with our French (F Cambien, INSERM UMRS 937; Université Pierre et Marie Curie, UPMC, Paris 6; Tregouet et al, Diabetes 2009; Morange et al, Am J Hum Genet 2010), Belgian/Dutch (JA Staessen, University of Leuven, and University of Maastricht) and Italian (O Parodi, University of Milan, GISSI-Prevenzione and cardiac resynchronization therapy patients in the EU-FP7-ICT-2007-2, VPH2-Virtual Pathological Heart of the Virtual Physiological Human) collaborators, we have access to study populations at-large (<http://www.genecanvas.org>). We recently joined The International Consortium for Blood Pressure Genome-wide Association Studies (ICBP) and genotyped 4000 individuals of the EPOGH Study for BP loci (Ehret et al, Nature. 2nd revision). We also collaborate with local partners with respect to recruitment and in-depth-phenotyping of population-based individuals and families (E Brand, University Clinic Münster, 6th framework programme life sciences, genomics and biotechnology for health of the EU, enlarging the EPOGH cohort), CAD patients with renal disease (E Brand/HJ Pavenstädt/G Breithardt, CAD-REF registry), CAD/stroke patients (U Keil, Institute of Epidemiology and Social Medicine,



Univ.-Prof. Dr. Dr. med.
Stefan-Martin Brand
(Director)

University of Münster, EUROASPIREIII, Kotseva et al, Lancet 2009, Kotseva et al, Eur J Cardiovasc Prev Rehabil 2009; Kotseva et al, Eur J Cardiovasc Prev Rehabil 2010) as well as cardiovascular patients (P Baumgart, Clemenshospital Münster; P Kleine-Katthöfer, St. Franziskus-Hospital, Münster, MolProMD, Dördelmann et al, J Biol Chem 2008).

“Classical” candidate genes are selected from cardiovascular pathophysiology (cell adhesion molecules/respective ligands, signal transduction cascades, transcription factors, pro-inflammatory molecules, etc.) or via genome-wide association (GWA) or expression (GWE) studies (replicated loci from publicly available resources and personal communication from collaborators; Tregouet et al, Diabetes 2008; Morange et al, Am J Hum Genet 2010).

BMBF-funded:

Cardomics (Integrated Genomics and Coronary Artery Disease). Subproject: Functional characterization of pathways linking genes/variants identified through GWA and GWE to cardiovascular diseases.

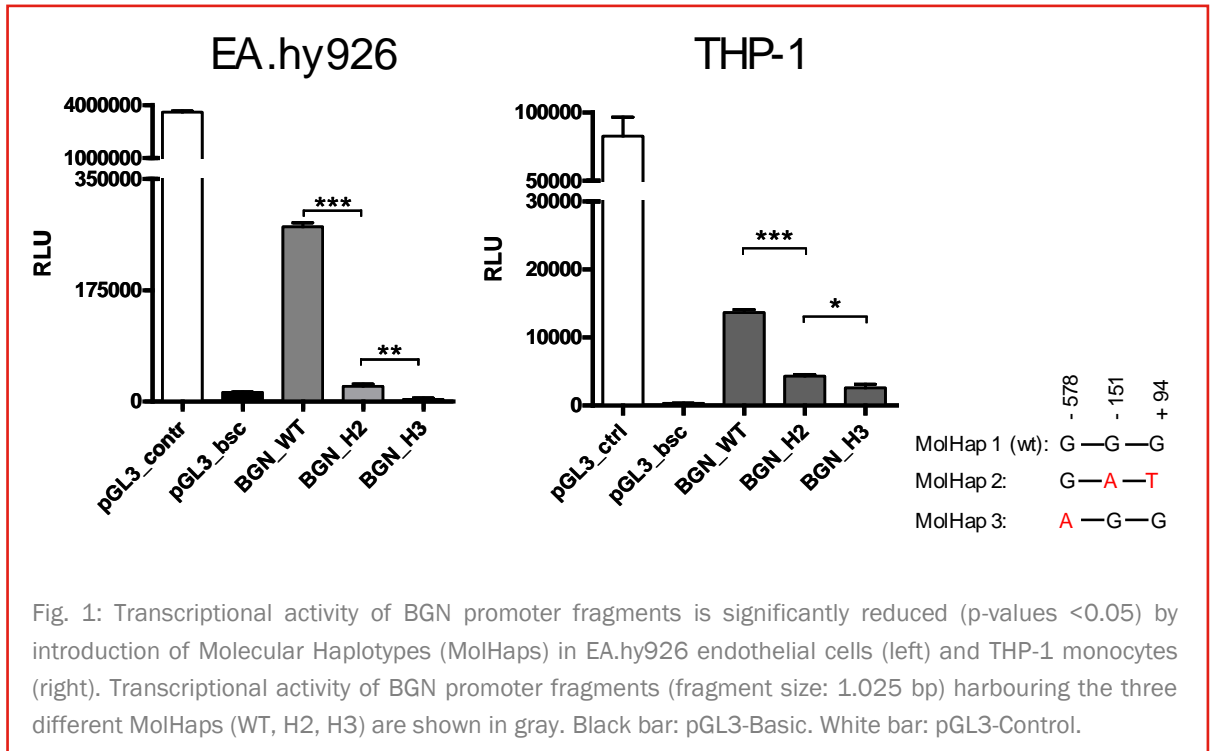
The principle aim of the investigation is characterizing the functional role of selected, relevant genes and variants in CAD, exemplified by ARHGEF3, a Rho GEF. The analyses of variant expression levels, transcript and protein half-lives and the identification of a putatively altered recruitment, quantitatively and qualitatively, of downstream partners will allow for a transition and integration of genetic data into (patho-) physiological pathways and functional networks. ARHGEF3 is located on the minus strand of chromosome 3. There are no reports on its transcriptional activity or interference with expression of ARHGEF3. To detect endogenously expressing (at the transcript level) cells and cell lines for further analyses we used the following cells: Ea.Hy926, MCF7, SaOs2, U2O, U937, THP1, HEK293T, HEC-1B, HepG2, HUVEC, HAoEC. We detected, by semi-quantitative PCR, general ARHGEF3 transcript expression in MCF7, SaOs2, U2Os, HEC-1B, and EaHy926 cells, and in primary cells HAoEC and HUVEC. In primary human monocytes, no ARHGEF3 expression was detected, congruent with the literature. Conversely, in U937 and THP1 monocytic cell lines noticeable ARHGEF3 transcript content was detected, but no endogenous transcripts in HEK293T cells. Isoform NM_1128615 was detected in MCF7, U2Os and in HEC-1B cells, and to a minor extend in SaOs2 cells but not in HAoEC cells. Isoform NM_019555 was present in HUVEC and HepG2 cells and very faintly in monocytic THP1 and U937, as well as Ea.Hy926 cells. Isoform NM_1128616 was positively tested for in HEC-1B, Ea.Hy926 and U2Os cells. We detected endogenous expression of all three Ref. Seq. transcripts in different cell lines, as well as a negative control (HEK293T), and proceed a) to the identification of putative novel splice sites and b) to preparation of stably expressing cells lines for ARHGEF3-tandem tagged expression vectors.

Other selected projects

RhoA

RhoA and TCTA are in close vicinity to each other in divergent orientation (RhoA on the (-), TCTA on the (+) strand, separated by 112 bp of 5'-flanking region. By this back-to-back constellation, gene regulatory regions of the one gene reside within intron and exon regions of the other, and vice versa. Transcription of the one gene disables, by opening of the helix, transcription factor binding required for transcription of the other, yet both single-copy genes are ubiquitously expressed at even tissue and differentiation-specific transcript.

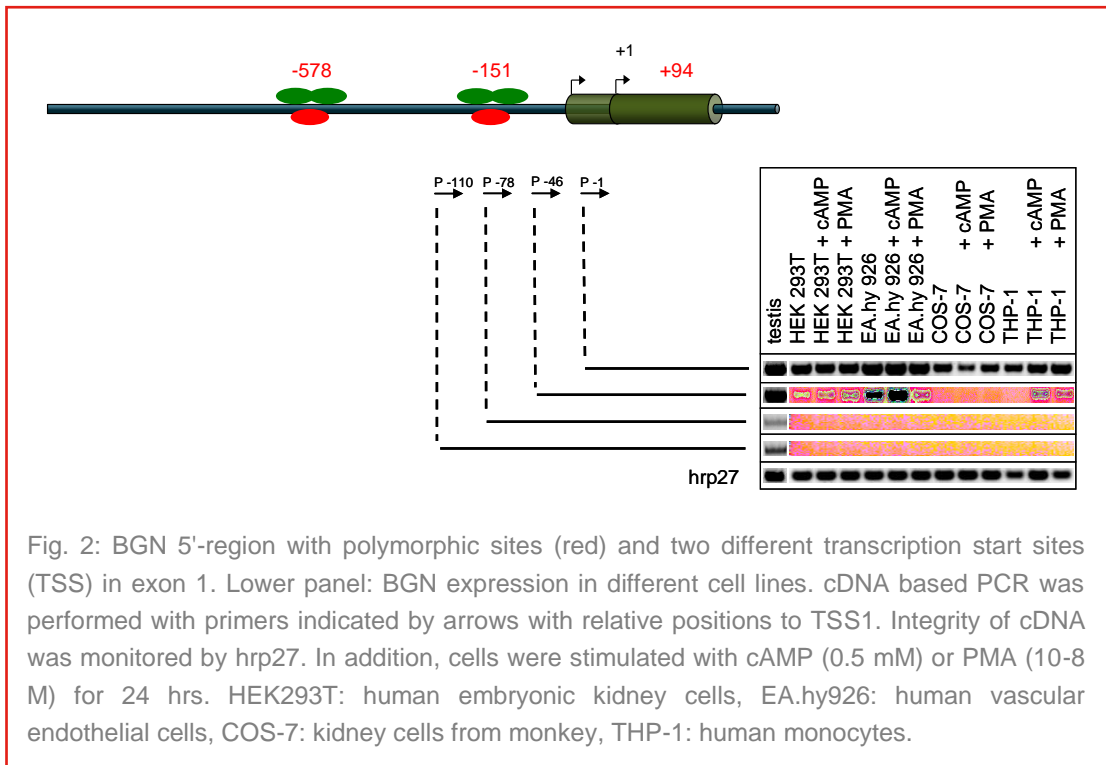
RhoA is a small RhoGTPase and exerts a plethora of cellular functions, among which differentiation of smooth muscle cells, especially vascular (through transcription factors SRF, MRTF-A and -B), vasomotoric activity (RhoK, MLC and myosin phosphatase) and is involved in inflammatory processes by activation of NFkB and expression of cytokines and endothelial adhesion molecules. TCTA transcripts are ubiquitously expressed, with slightly elevated levels in kidney. Data on immune-reactive TCTA protein is lacking. First, serial deletions of both 5'-flanking regions were analyzed by reporter gene assays in the vascular endothelial cell line EA.hy926 and showed that the 112 bp mutual promoter region, in which the core assembly site of the basal transcription apparatus is altered by a common functional SNP (-112T>C [rs940045]), disturbing one of two functional TATA-boxes is a bidirectional promoter. Especially RhoA depends on an unaltered nucleotide composition in the promoter region. By sequencing of 1600 bp of both RhoA and TCTA 5'-flanking regions in 60 patients of the MolProMD study two variants (-923G>A [rs6779524]; -1338T>C [rs6784820]) were confirmed in the 5'-flanking region of RhoA and three variants, one of which is novel (-1112C>T [rs4855877]; -1216G>A [rs73088137]; -619G>A) in the 5'-flanking region of TCTA. To analyze the dual promoter in its natural composition simultaneously within the same cell, a reporter gene vector was constructed, in which the natural RhoA/TCTA tandem promoter is linked to two non-interfering fluorescent proteins (EGFP, (-) strand, ds-Red (+) strand). All data have been reported in the PhD thesis of Dr. rer. nat. Bianca Schröer (magna cum laude, December 2010).



LTC4S

LTC4S is a key component of the inflammatory 5'-lipoygenase pathway and nearly exclusively expressed in cells of the myeloid lineage such as macrophages within arteriosclerotic lesions. In all other tissues, its enzymatic function is executed by other glutathione S-transferases. We were able to show by 5'-serial deletion reporter gene assays (in two individual human monocytic cell systems, THP1 and U937), which part of the 5'-flanking region is responsible for expression in monocytes and hence serves as promoter, and more specifically, cell-type specific cis-regulatory regions could be located. By sequencing of genomic DNA from a patient cohort (MolProMD) with a cardiovascular disease phenotype (primary hypertension, myocardial infarction) we detected four single nucleotide polymorphisms (SNP), two of which were novel (-1783G>A, -1412C>T, -1008G>A (rs60446982), -380A>C (rs730012)). These polymorphisms compose six natural molecular haplotypes with significantly different expression activities in THP1 and U937 cells. With this new knowledge of the sufficiently active LTC4S promoter region in macrophages, a first base for a future

pharmacological approach and with knowledge of the natural molecular haplotypes the basis for a personalized adaptation is set. All data are reported in the PhD thesis of Dipl. Biol. Friederike Bruns (scheduled for spring 2011)



BGN

The extracellular matrix proteoglycan biglycan (BGN) is involved in CVD pathophysiology. It mediates the subendothelial retention of atherogenic apoB-containing lipoproteins, exerts pro-inflammatory effects and mediates remodelling after MI. BGN-deficient mice show a significant higher mortality rate. Since little is known about the structural architecture or functional aspects of the BGN promoter region, we reanalyzed putative transcription start sites. By direct sequencing of 1125 bp of the BGN 5'-flanking region in 57 patients with CVD, positions of genetic variants were identified. Determination of molecular haplotypes (MolHaps) was achieved by subcloning and resequencing. We performed molecular and functional profiling assays using reporter gene experiments, band shift assays, chromatin immunoprecipitation (ChIP) assays, and co-expression analyses in human vascular endothelial cells (EA.hy926) and monocytes (THP-1). As a result, MolHaps2 and 3 significantly reduced the

after MI compared to wild type mice. The human *BGN* gene consists of 8 exons, 7 introns, and is transcribed into nine transcripts. The *BGN* 5'-flanking region lacks both a TATA or CAAT box and is suggested to drive transcription from various GC-elements.

transcriptional activity of BGN promoter fragments in human endothelial cells and monocytes (Fig. 1). We were able to show that alternative TSS are used, depending on cell line and stimulation (Fig. 2) and that polymorphic positions G-578A, G-151A and G+94T reside within cis-active promoter elements (Fig. 3). We propose a transcriptional module composed of activating transcription factor SP1 and the AP-1 transcription factor complex under the control of cell type-specific ETS-factors at the polymorphic position G-578A (Fig. 4). All data have been reported in the PhD thesis of Dr. rer. nat. Boris Schmitz (magna cum laude, December 2010)

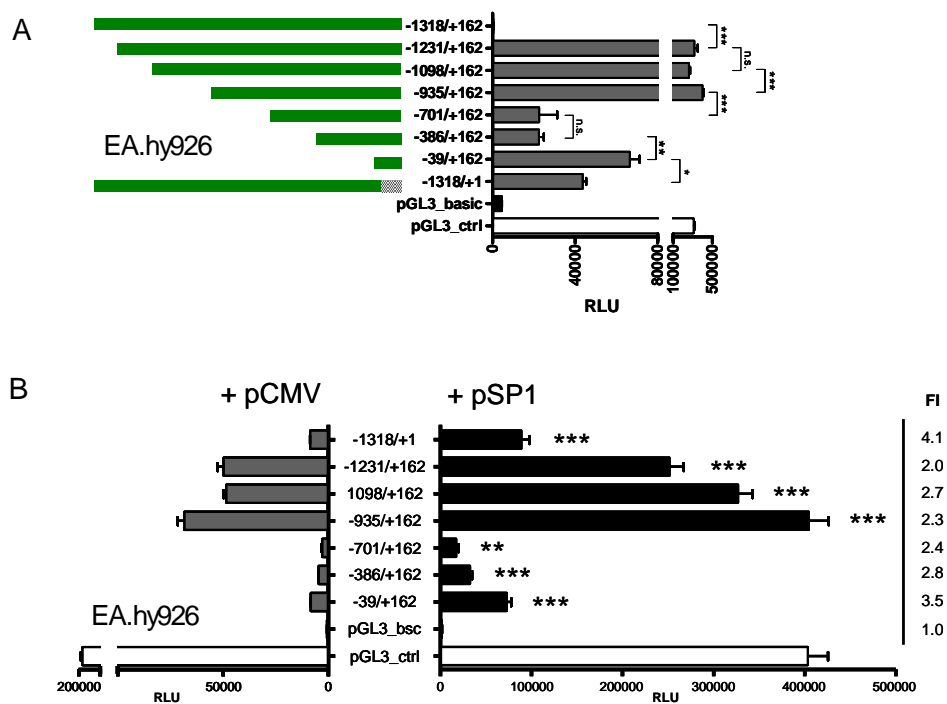


Fig. 3: A. Transient transfection of BGN promoter fragments in EA.hy926 cells. A portion with substantial transcriptional activity resides between position -1231 and -935. Basal transcriptional activity is located in the 5'-UTR. **Left:** Lengths of promoter fragments including 5'-UTR (green bars); the truncated fragment without, 5'-UTR is marked by a dashed box. **Right:** Transcriptional activity of promoter fragments with corresponding lengths in bp.

B. Transcription factor SP1 activates BGN transcriptional activity in EA.hy926 co-expression experiments. Right: Each constructs' relative activity over the empty shuttle vector pGL3-Basic was calculated in the absence or presence of overexpressed transcription factor SP1 and expressed as fold induction (FI). Left: The empty vector pCMV served as mock control. n.s.: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

probe GCTGCCAGGGGGGCCGGGAAGCCTGCCCCCT
 mutated probe GCTGCCATGGTGGCCGGGAAGCCAGCACCCCT

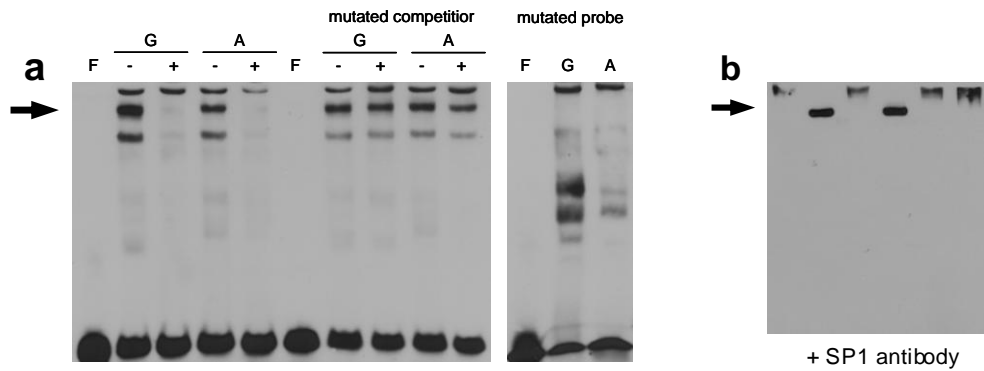
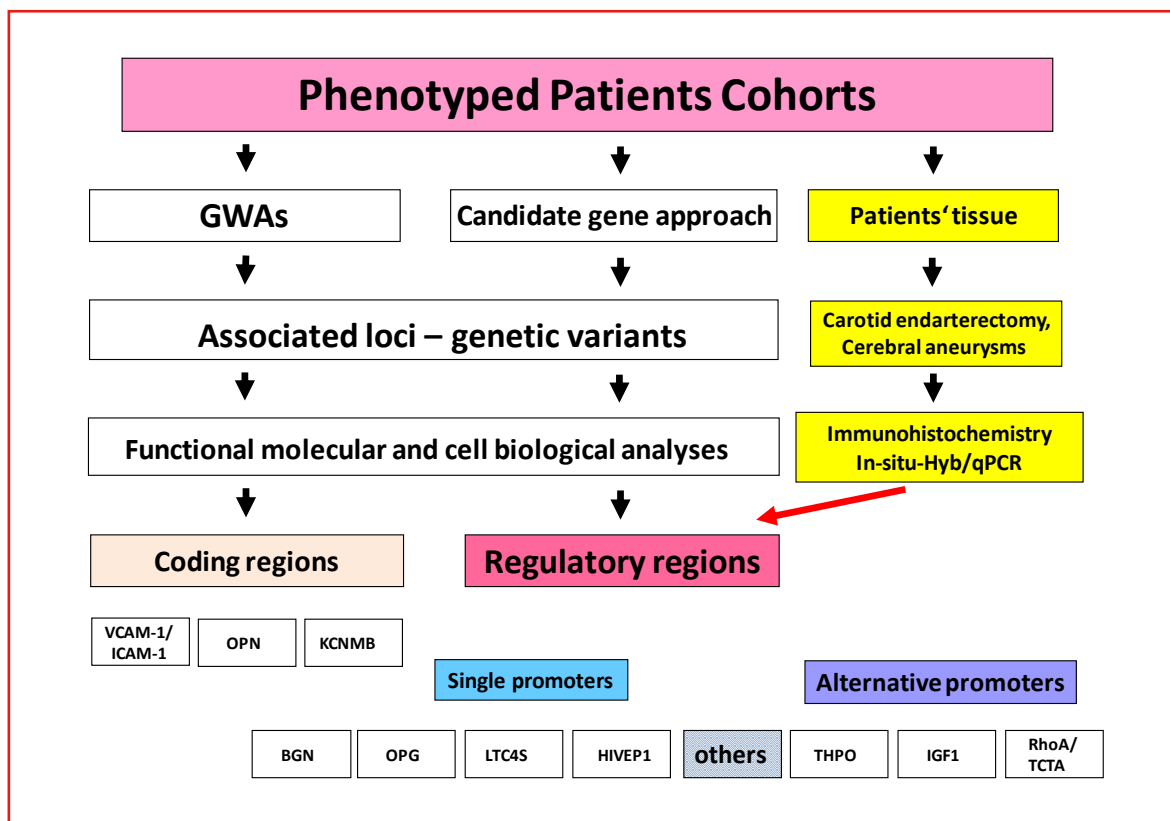


Fig. 4: Sequence-specific binding of transcription factor SP1 at position G-151A in electrophoretic mobility shift assays (EMSA) using EA.hy926 nuclear extracts.

a: EMSA experiments were performed using biotinylated probes (31 bp) harboring the G (wt) or A allele and EA.hy926 nuclear extracts. F: free probe, (-): 40 fmol probe and nuclear extract, (+): 8 pmol specific competitor. Two specific signals were detected for the G (wt) and A allele. No competition of signals was observed using the mutated competitor (sequence as depicted above). Use of the mutated probe resulted in differential binding patterns.

b: EMSA detected with specific SP1 antibody after blotting. Ø: Lanes without probe served as negative control. M: mutated oligonucleotide.



Department

Genetic Epidemiology of Vascular Disorders

The department Genetic epidemiology of vascular disorders investigates the complex interaction between (classical) risk factors, genetic variance and genomic architecture on the development and progression of common complex diseases. Within these, the spectrum of cardiovascular diseases ranges from thromboembolic events in children, through classical arteriosclerotic diseases, such as coronary artery disease, myocardial infarction and stroke, to cardiac diseases such as hypertensive left ventricular hypertrophy (LVH) and contractile dysfunction (cardiomyopathies, (DCM)). In particular, we are interested in the investigation of genetic factors in the context of systems biology, which integrate genetic, genomic and phenotypic components and thus allow for a deeper insight into the complex interaction of multiple factors on different levels.

The research combines both aspects, genetics of cardiovascular diseases and chronic inflammation, and integrates gene identification and characterization in model systems with genetic epidemiological studies in human study populations and clinical relevance.

After an extraordinarily successful year 2009, with publications in highly renowned scientific journals such as *Nature Genetics*, *Nature Medicine*, *Blood* and *Genome Research*, the year 2010 was under the premise of data generation, grant writing and the initiation of new research agendas, which will start to come to fruition in 2011.



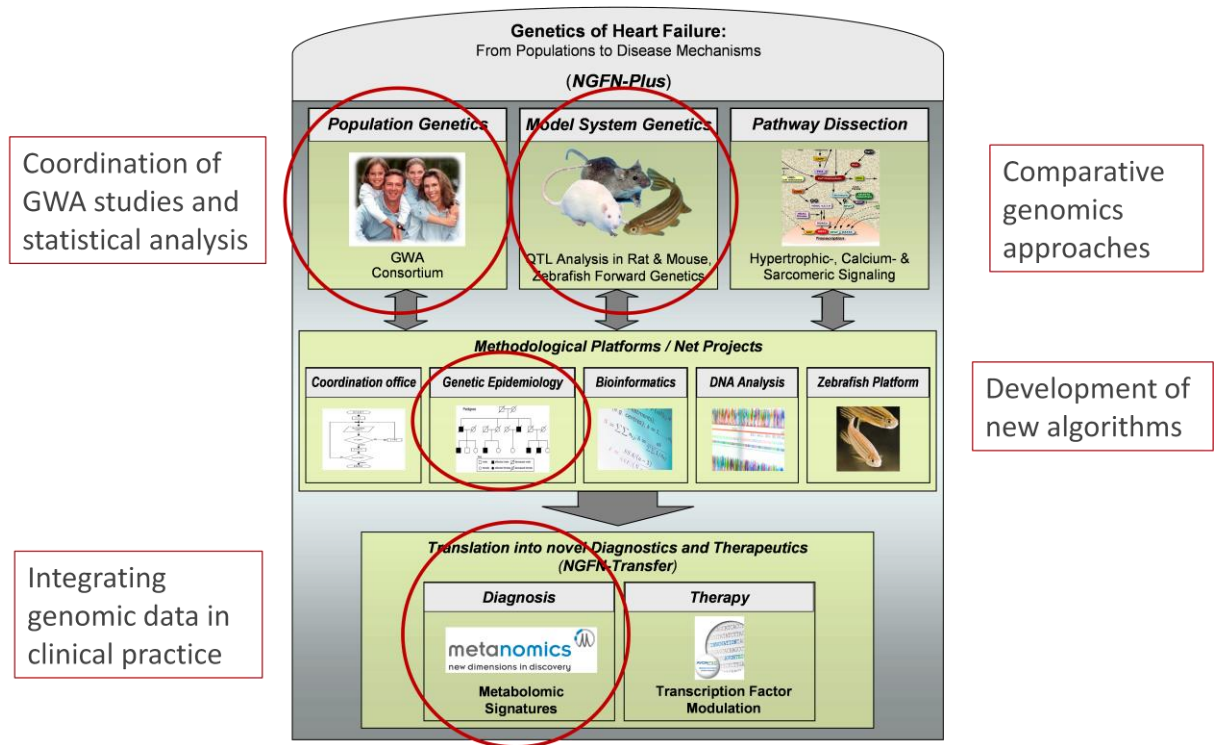
Univ.-Prof. Dr. sc. hum.
Monika Stoll
(Chairperson)

Genome Wide Association Studies (GWAS) for cardiovascular diseases

Within the frame of the National Genome Research Network (NGFN_{plus}), our group continues to support as core project genetic epidemiology of heart diseases the associated research groups, and has continuously been funded through the since 2001. In 2010, we have successfully in applied for funding for the final two years of NGFN through the Federal Ministry of Science and Education (BMBF) and will receive funding until mid 2013, when the program expires.

Within NGFN_{plus}, our group plays a central role in the design and the statistical analysis of GWAS for cardiovascular diseases. In addition, we are developing novel statistical algorithms for the analysis of common, complex diseases, with the aim to improve the prediction of clinical risk algorithms through the inclusion of genetic factors.

What we do...



In 2010, the analyses of two GWAS continued to be in the center of our NGFN_{plus} activities, including the replication of SNPs or genome wide significance ($p < 10^{-6}$) from the initial GWAS for LVH and DCM, respectively. These analyses further reduced the number of relevant susceptibility SNPs located on human chromosome 6, where a combined p-value for screening and replication studies of $p = 10^{-11}$, and thus, replicated genome-wide significance could be achieved using more than 3000 DCM cases and 7000 controls. The underlying genes were forwarded to functional studies in genetic model organisms e.g. zebrafish or rodents, and are currently being investigated concerning their functional relevance in our collaborators groups in Berlin, Heidelberg, Kiel and Ulm. In a second GWAS we investigated the genetic basis of hypertensive left ventricular hypertrophy (LVH) together with our cooperation partners Reinhold Kreutz at Charité Berlin and Norbert Hübner at the Max-Delbrueck-Center of Molecular Medicine in Berlin-Buch.

This analysis led to the identification of multiple replicated SNPs on human chromosome 11, which are also linked to LVH in a rat model of hypertensive end-organ damage, and sparked a number of functional studies in model organisms, which are currently underway. Currently, we are focusing on two positional candidate genes, which might contribute to the pathogenesis of LVH. The corresponding manuscripts for both, DCM and LVH GWAS, are currently in preparation.

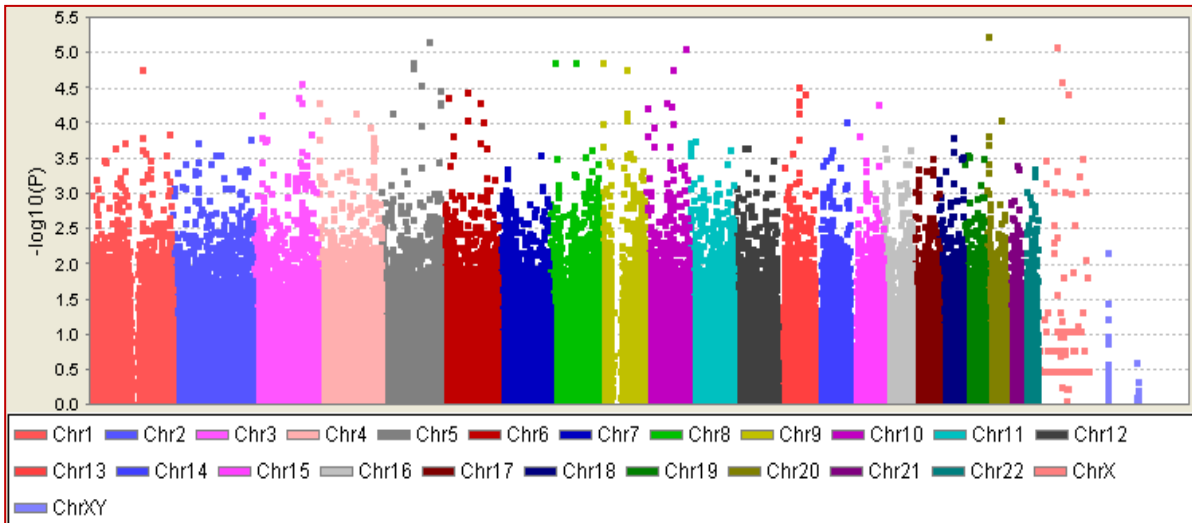


Fig.2: Manhattan plot for our GWAS for pediatric stroke. 4 SNPs exceeding the commonly accepted threshold of $p < 10^{-6}$ could be identified, plus an additional ~40 SNPs suggestive of association ($p < 10^{-5}$), highlighted proteolysis, cell-matrix adhesion, integrin-mediated signal transduction and platelet activation as key biological pathways in the pathogenesis of pediatric stroke.

Pediatric thromboembolism and stroke

In cooperation with Ulrike Nowak-Göttl (formerly UKM, now UK-SH) we investigate the classical and genetic risk factors for pediatric stroke and thromboembolism. The genetic-epidemiological investigation of risk factors for repeated thrombotic events in children were published in renowned journals in recent years (Kenet et al. *Lancet Neurology* 2007; Young et al. *Circulation* 2008, Nowak-Göttl et al. *Environmental Health Perspectives* 2008, Nowak-Göttl et al. *Blood* 2009). As cooperation partner of Ulrike Nowak-Göttl we successfully applied for funding at the Münster IZKF (Interdisciplinary Center for Clinical Research) to investigate the genetic basis of pediatric stroke in more detail, and the corresponding GWAS was completed in 2010. This analysis identified a number of interesting susceptibility genes for pediatric stroke at the interface between the vascular wall and coagulation i.e. five genes of the ADAMTS gene family of metalloproteinases and multiple genes involved in integrin-mediated cellular signaling. In addition, we initiated a GWAS for pediatric venous thrombosis using the 660W Illumina Infinium SNP arrays, which is currently underway and will likely be completed in the middle of 2011.

GWAS for myocardial infarction (The CARDIOGRAM Consortium)

In the framework of the CARDIOGRAM Consortium, we participated in a meta-analysis of multiple GWAS for early onset myocardial infarction involving more than 100.000 affected individuals and healthy controls from around the world. This GWA study identified an additional thirteen genetic variants which were significantly associated with early-onset myocardial infarction. The manuscript is *in press* in the renowned scientific journal *Nature Genetics* and will be published in early 2011. An additional publication on refined risk algorithms for the prediction of cardiovascular events including these genetic risk variants are currently *in revision*. These results were the basis for our successful grant application in cooperation with Albert Sickmann (ISAS, Dortmund) and Thomas Scheffold (Cardiac Research, Dortmund) in the framework of the Bio.NRW EFRE program, which will likely start to be funded through the State Ministry of Science, Innovation, and Research (MWIF) by January 2011. This project aims at the identification of novel biomarkers for the prevention of arteriosclerosis and its associated diseases and focuses on the identification of such markers through a combination of genomics and proteomics in families with an increased burden of arteriosclerosis.

GWAS for Heparin-induced thrombocytopenia (HIT)

Heparin-induced thrombocytopenia (HIT) is the development of thrombocytopenia (a low platelet count), due to the administration of various forms of heparin, an anticoagulant. HIT predisposes to thrombosis and the abnormal formation of blood clots inside a blood vessel. The treatment of HIT requires both protection from thrombosis and choice of an agent that will not reduce the platelet count further. Pharmacogenetic studies can help to elucidate the underlying mechanisms and may aid to develop diagnostic markers to predict this severe adverse drug reaction in advance. In collaboration with our partner Reinhold Kreutz (Charité Berlin) we performed a GWAS for HIT in 96 individuals affected by HIT following the administration of heparin and 96 age- and sex-matched individuals without this adverse drug reaction using the 370CNV Infinium bead arrays. The screening experiment identified two genomic loci, located on chromosome 5 and 18, which were significantly associated with the development of HIT at genome wide significance ($p < 10^{-6}$). The respective SNPS were successfully replicated in additional 96 individuals affected by HIT, and 192 additional age- and sex-matched controls. These genetic markers are likely to improve our ability to predict the occurrence of HIT, and may thus lead to direct clinical benefits. The accompanying manuscript is currently in preparation.

Core facility for high-throughput genetics and genomics

The Core facility for high-throughput genetics and genomics which is affiliated with our department was upgraded in 2010 to maintain the highest technical standard available in genomic research and remain competitive in this research arena. We upgraded the existing iScan platform to the novel HiScan including a next-generation sequencing module (HiScanSQ), and are now ready to offer massive parallel sequencing in addition to our current portfolio including GWAS, whole genome expression and methylation studies, as well as custom genotyping

services. The platform was used by many internal and external cooperation partners in the past year and has sparked many new research projects and collaborations. In house, the group of Stephan Rust successfully applied homozygosity mapping to identify a genomic region contributing to a severe metabolic phenotype in children, which is currently being followed up by next generation sequencing to identify the underlying genetic architecture through whole exome sequencing. As described in the paragraph relating to pediatric thromboembolism and stroke, a GWAS involving 370CNV and 660W Illumina Infinium chips was performed for pediatric stroke and thromboembolism, partially financed by the IZKF Münster through our collaboration with Ulrike Nowak-Göttl. The Core platform also supported the research of many research groups at the Medical Faculty of the WWU e.g. research on methylation patterns in small cell lung cancer by the group of Carsten Müller-Tidow (Oncology) or genome wide expression profiles for cardiovascular traits for the group of Frank Ulrich Müller (Pharmacology). With the establishment of the next generation sequencing platform, our Core Unit becomes increasingly popular as supporting platform for joint grant applications with groups at the WWU from both, the Faculties of Biology and Medicine, and will continue to be a research engine for state-of-the-art genomic research in Muenster.

Personnel

Astrid Farwick, post-doctoral fellow in our group received an award by the Deutsche Gesellschaft für Ophthalmologie (DOG) for her PHD thesis on the genetic predisposition to age-related macula degeneration (AMD) including inflammatory and classical cardiovascular risk factors. Anika Sietmann, graduate student in our group received her PhD on the genetic basis of left ventricular hypertrophy in early 2010. Jan Vollert, bachelor student from the FH Gelsenkirchen successfully finished his bachelor thesis and continues to assist our group in implementing new bioinformatics tools for genomics.

Work Group

Genetics of HDL Cholesterol and Molecular Diagnostics

Research focus of the group

Our major interest is in genes controlling cholesterol metabolism. In the recent year we added SNP-chip-technology and next generation sequencing to discover the underlying genes of traits affecting HDL- and LDL-cholesterol metabolism. These techniques were also employed in molecular diagnosis of other diseases e.g. congenital disorders of glycosylation.

Next generation sequencing of the exome in a family to discover a new HDL locus

In a family where two independent inherited variants were causing low HDL, one variant had been earlier identified as a deletion in ApoA1, the major structural protein of HDL. Regarding the other variant, known candidate genes and most of the genome had been excluded by genome wide linkage analysis. However, the remaining genomic regions were too large to immediately delineate hot candidates. Therefore, we started next generation sequencing in Oct. 2010 and decided to sequence the whole exome of the index patient carrying both, the ApoA1 defect and the new unknown defect, as well as the exome of his son, who inherited only the unknown defect. Results of the combined analysis are expected in 2011 and will provide the basis for characterization of a new HDL-affecting gene.

Further research on the function of the ABCA1

In the meantime we have completed our arsenal of natural and artificial variants of ABCA1 to study and discriminate the effects of different variants that show defects in one or either of the two ATP-binding cassettes of ABCA1. Since one of our patients had a



Group leader:
Dr. rer. nat.
Stephan Rust

cassettes of ABCA1. Since one of our patients had a N935S variant affecting just one of the cassettes and had no atherosclerosis but almost no HDL, there seems to be some residual function that we want to analyze in detail. This function has no positive effect on HDL, but nevertheless does protect from atherosclerosis. The combination of these analyses and of understanding the input from other new players (see above) will allow us to determine which points are essentials in HDL-metabolism and provide a knowledge based target for therapy.

Molecular diagnostics

In 2010 we extended molecular diagnosis to discover the molecular basis of diseases where a direct candidate for sequencing by standard Sanger sequencing could not be obtained by analysing the phenotype or where all known candidates were already checked. This is the typical situation for rare diseases that we analyze in cooperation with Prof. T. Marquardt, childrens hospital of the University clinics Münster. In many cases, the affecteds are offspring of a consanguineous marriage and we can assume a recessive inheritance with homozygosity at the

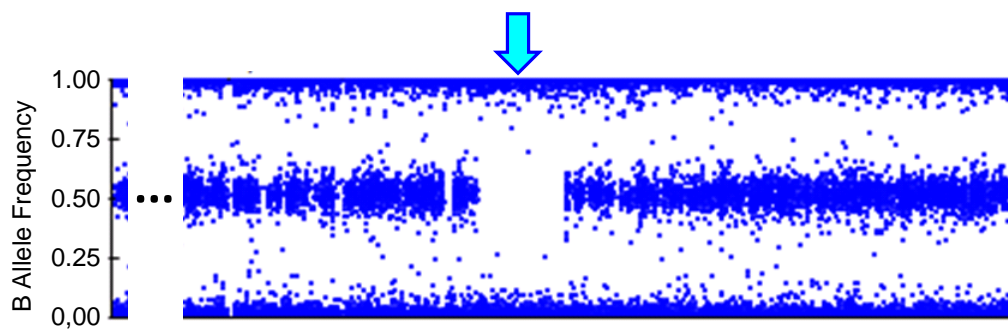


Fig. 1: Homozygosity mapping with an Illumina 1M Duo SNP chip (typical detail, taken from chromosome 10q). Each blue dot represents the SNP-genotype at one SNP-locus with two alleles A and B: AA (dot at the lower limit), AB (middle range), BB (top signal B). The homozygous region, where no dots in the middle range occur but only dots indicating homozygosity for AA or BB, is highlighted by an arrow.

disease locus and within close vicinity of the defect. Thus, we applied SNP chip based homozygosity mapping to identify regions where affected sibs shared a homozygous region, while unaffected sibs did not show homozygosity regarding the respective region. A typical example is illustrated in figure 1 representing analysis results of a family with an recessively inherited congenital disorder of glycosylation (CDG).

The total candidate region is essentially reduced compared to the whole genome (this case in total 62 Mbp from 6 different chromosomes versus 3 Gbp whole genome). Extracting all genes from these candidate regions provided a candidate list of about 1100 entries. Careful inspection of the list revealed roughly ten candidates for which an involvement in the glycosylation process seem to be possible. Finally a defect was identified in a gene that is part of the translocation machinery in the rough endoplasmatic reticulum and obviously is needed for correct transfer of the preformed sugar-chain by the oligosaccharyl-transferase complex to proteins during their synthesis and entry into the ER. The defect affects the ATG-start codon. From literature it is known that some residual translation is possible from some mutated start codons and this seems also to be the case for the current defect. Such a defect in the translocon has not been described before and the gene has not been discussed as a player in CDGs before. Since total enzyme defects in the glycosylation cascade typically

are not compatible with life, a small leakage to residual functions is expected. Further characterization of the defect is in progress.

We discovered the underlying defect by homozygosity analysis followed by classical sequencing in 3 diseases. However, in some families the candidate regions are still to large to delineate a manageable number of sufficiently likely candidates. Therefore we initiated whole exome sequencing. From the current pipeline in next generation sequencing we expect one HDL-gene, one LDL-lowering defect (a potential drug target for cholesterol lowering) and another gene involved in cholesterol deposition in the lung (discovered in two sibs, one of them finally died with confirmed diagnosis "Schweinegrippe"). Furthermore the sequencing procedure was started for a gene involved in absorption of fatsoluble vitamins and additional cases of unresolved CDGs. The analysis results are expected early in 2011.

PROCARDIS

As part of the EU-PROCARDIS consortium the group participates in the investigation of the genetic basis of precocious arteriosclerosis. In an extended study recently "A genome-wide association study in Europeans and South Asians identifies five novel loci for coronary artery disease" was submitted and is now accepted for publication in Nature Genetics (to be published in 2011).

Publications 2010

The ARF-like GTPase ARFRP1 is essential for lipid droplet growth and is involved in the regulation of lipolysis

Hommel A, Hesse D, Völker W, Jaschke A, Moser M, Engel T, Blüher M, Zahn C, Chadt A, Ruschke K, Vogel H, Kluge R, Robenek H, Joost HG, Schürmann A.

Mol Cell Biol. 2010;30:1231-42

Conversion of glycerol to poly(3-hydroxypropionate) in recombinant *Escherichia coli*

Andreessen B, Lange AB, Robenek H, Steinbüchel A.

Appl Environ Microbiol. 2010;76:622-6

Cell surface analysis of the lipid-discharging obligate hydrocarbonoclastic species of the genus *Alcanivorax*

Lange AB, Tenberge KB, Robenek H, Steinbüchel A.

Eur J Lipid Sci Technol. 2010;112:681-91

Induction of fatty acid synthesis is a key requirement for phagocytic differentiation of human monocytes

Ecker J, Liebisch G, Englmaier M, Grandl M, Robenek H, Schmitz G.

Proc Natl Acad Sci U S A. 2010;107:7817-22

Aortic dissection associated with Cogan's syndrome: deleterious loss of vascular structural integrity is associated with overstimulation in macrophages and smooth muscle cells

Weissen-Plenz G, Sezer O, Vahlhaus C, Robenek H, Hoffmeier A, Tjan TD, Scheld HH, Sindermann JR.

J Cardiothorac Surg. 2010;5:66-70

Damage of guinea pig heart and arteries by a trioleate-enriched diet and of cultured cardiomyocytes by oleic acid

Kriegelstein J, Kewitz T, Kirchhefer U, Hofnagel O, Weissen-Plenz G, Reinbold M, Klumpp S.

PLoS One. 2010;5:e9561

Endocytotic segregation of gliadin peptide 31-49 in enterocytes

Zimmer KP, Fischer I, Mothes T, Weissen-Plenz G, Schmitz M, Wieser H, Büning J, Lerch MM, Ciclitira PC, Weber P, Naim HY.

Gut. 2010;59:300-10

Sphingosine 1-phosphate (S1P) induces expression of E-selectin and adhesion of monocytes via intracellular signalling pathways in vascular endothelial cells

Weis T, Völker W, Holtwick R, Al Chahaf M, Schmidt A.

Eur J Cell Biol. 2010;89:733-41

Research update for articles published in EJCI in 2008

Schmidt A.

Eur J Clin Invest. 2010;40:770-89

Segmental mediolytic arteriopathy (SMA) of central, visceral and peripheral vessels

Schönefeld E, Völker W, Torsello G.

Dtsch Med Wochenschr. 2010;135:745-9.

Topography of lipid droplet-associated proteins: insights from freeze-fracture replica immunogold labeling

Robenek H, Buers I, Robenek MJ, Hofnagel O, Rübél A, Troyer D, Severs NJ.

J Lipids. 2011; Article ID 409371

Apolipoprotein E (ApoE) induces anti-inflammatory phenotype in macrophages

Baitsch D, Bock HH, Engel T, Telgmann R, Müller-Tidow C, Varga G, Bot M, Herz J, Robenek H, von Eckardstein A, Nofer JR.

Arterioscler Thromb Vasc Biol. 2011; in press

Peptide translocation of transporter associated with antigen processing expressed in *Pichia pastoris* is modulated by lipids

Schölz C, Parcej D, Robenek H, Urbatsch I, Tampe R.

J Biol Chem. 2010; in press

Freeze-fracture replica immunolabeling reveals human WIPI-1 and WIPI-2 as membrane proteins of autophagosomes

Proikas-Cezanne T, Robenek H.

J Cell Mol Med. 2010; in press

Regulatory role of proteinase-activated receptors-1 and -2 in experimentally induced skin fibrosis and scleroderma

Cevikbas F, Seeliger A, Fastrich M, Robenek H, Homey B, Phillips C, Steinhoff M.

Am J Pathol. 2010; submitted

SR-PSOX at sites predisposed to atherosclerotic lesion formation mediates monocyte-endothelial cell adhesion

Hofnagel O, Buers I, Engel T, Robenek H.

Atherosclerosis. 2010; under revision

NPP1 promotes atherosclerosis but suppresses intimal atherosclerotic plaque calcification in apoE knockout mice

Nitschke Y, Weissen-Plenz G, Terkeltaub R, Rutsch F.

J Cell Mol Med. 2011; in press

Optimized protocol for efficient non-viral transfection of premature THP-1 macrophages

Maeß M, Buers I, Robenek, Lorkowski S.

CSH Protocols. 2010; in press

The outer arterial wall layers are primarily affected in patients with spontaneous cervical artery dissection

Völker W, Dittrich R, Grewe S, Nassenstein I, Csiba L, Herczeg L, Szilvia V, Robenek H, Kühlenbäumer G, Ringelstein EB.

Neurology. 2010; in press

Lipid droplet-associated proteins: an emerging role in atherogenesis

Buers I, Rübél A, Severs NJ, Robenek H

Histol Histopathol. 2011;26:631-42

Follicular fluid high density lipoprotein (FF-HDL)-associated sphingosine 1-phosphat (S1P) promotes human granulosa lutein cell migration via S1P receptor type 3 (S1P3) and small G protein Rac1

Becker S, von Otte S, Diedrich K, Robenek H, Nofer JR.

Biol Reprod. 2010; 84:604-12

Native high-density lipoproteins inhibit platelet activation via scavenger receptor

Brodde MF, Korporaal SJ, Herminghaus G, van Berkel TJ, Tietge UJ, Robenek H, van Eck M, Kehrel BE, Nofer JR.

Atherosclerosis. 2011;215:374-82

TGF α associated MUC2 and MUC3 expression of the gastric epithelium in Ménétrier's disease during remission of ulcerative colitis

Zimmer KP, Weissen-Plenz G, Scholand KA, Herbst H, Naim HY.. Gut 2011; in press

Failure of rescue: In advanced atherosclerotic lesions calcification positively correlated with NPP1 Expression

Nitschke Y, Hartmann S, Torsello G, Horstmann R, Seifarth H, Weissen-Plenz G, Rutsch F. J Cell Mol Med. 2010; in press

Staphylococcus aureus phenotype Switching: an effective bacterial strategy to escape host immune response and establish a chronic infection

Tuchscher L, Medina E, Hussain M, Völker W, Heitmann V, Niemann S, Holzinger D, Roth J, Proctor RA, Becker K, Peters G, Löffler B. EMBO Mol Med. 2011; in press

New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk

Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, Lindgren CM, Mägi R, Morris AP, Randall J, Johnson T, Elliott P, Rybin D, Thorleifsson G, Steinthorsdottir V, Henneman P, Grallert H, Dehghan A, Hottenga JJ, Franklin CS, Navarro P, Song K, Goel A, Perry JR, Egan JM, Lajunen T, Grarup N, Sparsø T, Doney A, Voight BF, Stringham HM, Li M, Kanoni S, Shrader P, Cavalcanti-Proença C, Kumari M, Qi L, Timpson NJ, Gieger C, Zaba C, Rocheleau G, Ingelsson E, An P, O'Connell J, Luan J, Elliott A, McCarroll SA, Payne F, Roccascocca RM, Pattou F, Sethupathy P, Ardlie K, Ariyurek Y, Balkau B, Barter P, Beilby JP, Ben-Shlomo Y, Benediktsson R, Bennett AJ, Bergmann S, Bochud M, Boerwinkle E, Bonnefond A, Bonnycastle LL, Borch-Johnsen K, Böttcher U, Brunner E, Bumpstead SJ, Charpentier G, Chen YD, Chines P, Clarke R, Coin LJ, Cooper MN, Cornelis M, Crawford G, Crisponi L, Day IN, de Geus EJ, Delplanque J, Dina C, Erdos MR, Fedson AC, Fischer-Rosinsky A, Forouhi NG, Fox CS, Frants R, Franzosi MG, Galan P, Goodarzi MO, Graessler J, Groves CJ, Grundy S, Gwilliam R, Gyllenstein U, Hadjadj S, Hallmans G, Hammond N, Han X, Hartikainen AL, Hassanal N, Hayward C, Heath SC, Hercberg S, Herder C, Hicks AA, Hillman DR, Hingorani AD, Hofman A, Hui J, Hung J, Isomaa B, Johnson PR, Jørgensen T, Julia A, Kaakinen M, Kaprio J, Kesaniemi YA, Kivimäki M, Knight B, Koskinen S, Kovacs P, Kyvik KO, Lathrop GM, Lawlor DA, Le Bacquer O, Lecoquer C, Li Y, Lyssenko V, Mahley R, Mangino M, Manning AK, Martínez-Larrad MT, McAteer JB, McCulloch LJ, McPherson R, Meisinger C, Melzer D, Meyre D, Mitchell BD, Morken MA, Mukherjee S, Naitza S, Narisu N, Neville MJ, Oostra BA, Orrù M, Pakyz R, Palmer CN, Paoalisso G, Pattaro C, Pearson D, Peden JF, Pedersen NL, Perola M, Pfeiffer AF, Pichler I, Polasek O, Posthuma D, Potter SC, Pouta A, Province MA, Psaty BM, Rathmann W, Rayner NW, Rice K, Ripatti S, Rivadeneira F, Roden M, Rolandsson O, Sandbaek A, Sandhu M, Sanna S, Sayer AA, Scheet P, Scott LJ, Seedorf U, Sharp SJ, Shields B, Sigurdsson G, Sijbrands EJ, Silveira A, Simpson L, Singleton A, Smith NL, Sovio U, Swift A, Syddall H, Syvänen AC, Tanaka T, Thorand B, Tichet J, Tönjes A, Tuomi T, Uitterlinden AG, van Dijk KW, van Hoek M, Varma D, Visvikis-Siest S, Vitart V, Vogelzangs N, Waeber G, Wagner PJ, Walley A, Walters GB, Ward KL, Watkins H, Weedon MN, Wild SH, Willemsen G, Witteman JC, Yarnell JW, Zeggini E, Zelenika D, Zethelius B, Zhai G, Zhao JH, Zillikens MC; DIAGRAM Consortium; GIANT Consortium; Global BPgen Consortium, Borecki IB, Loos RJ, Meneton P, Magnusson PK, Nathan DM, Williams GH, Hattersley AT, Silander K, Salomaa V, Smith GD, Bornstein SR, Schwarz P, Spranger J, Karpe F, Shuldiner AR, Cooper C, Dedoussis GV, Serrano-Rios M, Morris AD, Lind L, Palmer LJ, Hu FB, Franks PW, Ebrahim S, Marmot M, Kao WH, Pankow JS, Sampson MJ, Kuusisto J, Laakso M, Hansen T, Pedersen O, Pramstaller PP, Wichmann HE, Illig T, Rudan I, Wright AF, Stumvoll M, Campbell H, Wilson JF; Anders Hamsten on behalf of Procardis Consortium; the MAGIC investigators,

Bergman RN, Buchanan TA, Collins FS, Mohlke KL, Tuomilehto J, Valle TT, Altshuler D, Rotter JI, Siscovick DS, Penninx BW, Boomsma DI, Deloukas P, Spector TD, Frayling TM, Ferrucci L, Kong A, Thorsteinsdottir U, Stefansson K, van Duijn CM, Aulchenko YS, Cao A, Scuteri A, Schlessinger D, Uda M, Ruokonen A, Jarvelin MR, Waterworth DM, Vollenweider P, Peltonen L, Mooser V, Abecasis GR, Wareham NJ, Sladek R, Froguel P, Watanabe RM, Meigs JB, Groop L, Boehnke M, McCarthy MI, Florez JC, Barroso I. Nat Genet. 2010;42:105-116

Common variants in the ATP2B1 gene are associated with susceptibility to hypertension: the Japanese Millennium Genome Project

Tabara Y, Kohara K, Kita Y, Hirawa N, Katsuya T, Ohkubo T, Hiura Y, Tajima A, Morisaki T, Miyata T, Nakayama T, Takashima N, Nakura J, Kawamoto R, Takahashi N, Hata A, Soma M, Imai Y, Kokubo Y, Okamura T, Tomoike H, Iwai N, Ogiwara T, Inoue I, Tokunaga K, Johnson T, Caulfield M, Munroe P, Seedorf U as member of Global Blood Pressure Genetics Consortium, Umemura S, Ueshima H, Miki T. Hypertension 2010;56:973-80

Four novel Loci (19q13, 6q24, 12q24, and 5q14) influence of the microcirculation in vivo

Ikram MK, Xueling S, Jensen RA, Cotch MF, Hewitt AW, Ikram MA, Wang JJ, Klein R, Klein BE, Breteler MM, Cheung N, Liew G, Mitchell P, Uitterlinden AG, Rivadeneira F, Hofman A, de Jong PT, van Duijn CM, Kao L, Cheng CY, Smith AV, Glazer NL, Lumley T, McKnight B, Psaty BM, Jonasson F, Eiriksdottir G, Aspelund T; Seedorf U as member of Global BPgen Consortium, Harris TB, Launer LJ, Taylor KD, Li X, Iyengar SK, Xi Q, Sivakumaran TA, Mackey DA, Macgregor S, Martin NG, Young TL, Bis JC, Wiggins KL, Heckbert SR, Hammond CJ, Andrew T, Fahy S, Attia J, Holliday EG, Scott RJ, Islam FM, Rotter JI, McAuley AK, Boerwinkle E, Tai ES, Gudnason V, Siscovick DS, Vingerling JR, Wong TY. PLoS Genet. 2010;6:e1001184

A genome-wide association study in Europeans and South Asians reveals five novel loci for coronary artery disease.

Peden JF, Hopewell JC, Saleheen D, Chambers JC, Hager J, Soranzo N, Collins R, Danesh J, Elliott P, Farrall M, Stirrups K, Zhang W, Hamsten A, Parish S, Lathrop M, Watkins H, Clarke R, Deloukas P, Kooner JS, Goel A, Ongen H, Strawbridge RJ, Heath S, Mälarstig A, Helgadottir A, Ohrvik J, Murtaza M, Potter S, Hunt SE, Delepine M, Jalilzadeh S, Axelsson T, Syvänen AC, William R, Bumpstead S, Gray E, Edkins S, Folkersen L, Kyriakou T, Franco-Cereceda A, Gabrielsen A, Seedorf U, MUTHER consortium, Eriksson P, Offer A, Bowman L, Sleight P, Armitage J, Peto R, Abecasis G, Ahmed N, Caulfield M, Donnelly P, Froguel P, Kooner AS, McCarthy MI, Samani NJ, Scott J, Sehmi J, Silveira A, Hellénus ML, van 't Hooft FM, Olsson G, Rust S, Assmann G, Barlera S, Tognoni G, Franzosi MG, Linksted P, Green FR, Rasheed A, Zaidi M, Shah N, Samuel M, Mallick NH, Azhar M, Zaman KS, Samad A, Ishaq M, Gardezi AR, Memon FR, Frossard PM, Spector T, Peltonen L, Nieminen MS, Sinisalo J, Salomaa V, Ripatti S, Bennett D, Leander K, Gigante B, de Faire U, Pietri S, Gori F, Marchionni R, Sivapalaratnam S, Kastelein JJP, Theodoraki EV, Dedoussis GV, Engert JC, Yusuf S, Anand SS. Nat Genet. 2011; in press

A follow-up study of a genome-wide association scan identifies a new venous thromboembolism susceptibility locus on chromosome 6p24.1 - A possible link with arterial thrombosis

Morange PE, Bezemer I, Saut N, Bare L, Burgos G, Brocheton J, Durand H, Schved JF, Pernod G, Galan P, Drouot L, Biron-Andreani C, Zelenika D, Germain M, Nicaud V, Heath S, Ninio E, Delluc A, Münzel T, Zeller T, Brand-Herrmann SM, Alessi MC, Tiret L, Lathrop M, Cambien F, Blankenberg S, Rosendaal FR, Emmerich J, Tréguët DA. Am J Hum Genet. 2010;86:592-5

Management of cardiovascular risk factors in asymptomatic high-risk patients in general practice: cross-sectional survey in 12 European countries

Kotseva K, Wood D, De Backer G, De Bacquer D, Pyörälä K, Reiner Z, Keil U; EUROASPIRE Study Group, . Pyörälä K, De Backer G, Keil U, Ambrosio GB, Cokkinos D, Deckers JW, Dzerve V, Fraz Z, Gaita D, Gotcheva N, Graham I, Kotseva K, Laucevicius A, Lehto S, McGregor K, Nicolaidis P, Oganov R, Ostör E, Pajak A, Reiner Z, Simon J, Tokgözoğlu L, De Velasco J, Wood D, Wood D, Kotseva K, Jennings C, Xenikaki D, Winnicki J, De Backer G, De Bacquer D, Manini M, Bramley C, Boule C, Taylor C, McGregor K, Sundvall J, Lund L, De Sutter J, Piessens V, Van den Abbeele H, Dierickx E, Muylaert P, Present L, Braeckman M, Bruggeman H, Debackere P, Heytens S, Deleu K, Behaeghe P, Van De Wiele J, Van Aelst F, Taragola H, Spenninck E, Verkinderen B, Van Baeveghem S, De Waele B, De Waele C, Calis B, Schiettecatte V, Vermaercke C, Willems S, Van Imschoot K, De Vriese M, Maudens P, Maudens F, Gotcheva N, Georgiev B, Kastamanov R, Toneva K, Pavlov T, Stoev I, Alexandrov A, Miladinov A, Valterova A, Reiner Z, Marković BB, Repalust NV, Nekić VC, Soldo D, Petricek G, Adzic ZO, Marijic G, Lehto S, Saloranta P, Savolainen J, Helminen EE, Keil U, Heidrich J, Prügger C, Wellmann J, Neiteler G, Kalic M, Siebert E, Brand-Herrmann SM, Telgmann R, Barth F, Berger U, Briefs HJ, Degener L, Friedewald W, Heinemann-Vechtel O, Hüning U, Kalbfleisch C, Krösmann M, Neugebauer U, Noack KH, Richter-Millers B, Schuster A, Wahle K, Ambrosio GB, Vanuzzo D, Mirolo R, Pilotto L, Zamaro G, Adinolfi V, Da Porto M, Gubiani M, Canciani L, Dzerve V, Hansone S, Gozite A, Liepa L, Zeze I, Putane L, Ecina V, Ajja Rubine I, Rozkova N, Bricina N, Erglis A, Pajak A, Kawecka-Jaszcz K, Wolfshaut-Wolak R, Jankowski P, Windak A, Krzysztoń J, Makowiec-Dyrda M, Giza B, Sadek-Ratajczak J, Sobalski T, Korman T, Gaita D, Pop M, Buzulica C, Cicala C, Nicoara M, Zarici I, Iurciuc M, Iurciuc S, Roman C, Gavruta M, Breteanu L, Avram CA, Sarau CA, Avram C, Craciun L, Frasc Z, Frasc-Stefan T, Dovc M, Celan-Lucu B, Kralj S, Jurkovic G, Fonda A, Bercic MM, De Velasco J, Maiques A, Mendez G, Buigues C, Montero N, Amoraga A, Bonet Y, Cuevas R, Torres Y, Wood D, De Vries H, Koul B, Fellowes D, Sleight C, Purwar R, Harrison M, Saini S, Walton I, Thomas S, Igbanoi J, Bello F, Elgamel V, Kudyba M. *EUROASPIRE III*. *Eur J Cardiovasc Prev Rehabil*. 2010;17:530-40

Genetic Variants in the C-Reactive Protein Gene Are Associated with Microangiopathic Ischemic Stroke

Kuhlenbaeumer G, Hüge A, Berger K, Kessler C, Voelzke H, Funke H, Stoegbauer F, Stoll M, Ringelstein EB. *Cerebrovasc Dis*. 2010;30:476-82

Genetic TPH2 variants and the susceptibility for migraine: association of a TPH2 haplotype with migraine without aura

Jung A, Hüge A, Kuhlenbäumer G, Kempt S, Seehafer T, Evers S, Berger K, Marziniak M. *J Neural Transm*. 2010;117:1253-60

Lack of association between the Trp719Arg polymorphism in kinesin-like protein-6 and coronary artery disease in 19 case-control studies

Assimes TL, Hólm H, Kathiresan S, Reilly MP, Thorleifsson G, Voight BF, Erdmann J, Willenborg C, Vaidya D, Xie C, Patterson CC, Morgan TM, Burnett MS, Li M, Hlatky MA, Knowles JW, Thompson JR, Absher D, Iribarren C, Go A, Fortmann SP, Sidney S, Risch N, Tang H, Myers RM, Berger K, Stoll M, Shah SH, Thorgeirsson G, Andersen K, Havulinna AS, Herrera JE, Faraday N, Kim Y, Kral BG, Mathias RA, Ruczinski I, Sukitipat B, Wilson AF, Yanek LR, Becker LC, Linsel-Nitschke P, Lieb W, König IR, Hengstenberg C, Fischer M, Stark K, Reinhard W, Winogradow J, Grassl M, Grosshennig A, Preuss M, Schreiber S, Wichmann HE, Meisinger C, Yee J, Friedlander Y, Do R, Meigs JB, Williams G, Nathan DM, MacRae CA, Qu L, Wilensky RL, Matthai WH Jr, Qasim AN, Hakonarson H, Pichard AD, Kent KM, Satler L, Lindsay JM, Waksman R, Knouff CW, Waterworth DM, Walker MC, Mooser VE, Marrugat J, Lucas G, Subirana I, Sala J, Ramos R, Martinelli N, Olivieri O, Trabetti E, Malerba G, Pignatti PF, Guiducci C, Mirel D, Parkin M, Hirschhorn JN, Asselta R, Duga S, Musunuru K, Daly MJ, Purcell S, Eifert S, Braund PS, Wright BJ, Balmforth AJ, Ball SG; Myocardial Infarction Genetics Consortium; Wellcome Trust Case Control Consortium; Cardiogenics, Ouwehand WH, Deloukas P, Scholz M, Cambien F, Hüge A, Scheffold T, Salomaa V, Girelli D, Granger CB, Peltonen L, McKeown PP, Altshuler D, Melander O, Devaney JM, Epstein SE, Rader DJ, Elosua R, Engert JC, Anand SS, Hall AS, Ziegler A, O'Donnell CJ, Spertus JA, Siscovick D, Schwartz SM, Becker D, Thorsteinsdottir U, Stefansson K, Schunkert H, Samani NJ, Quertermous T. *J Am Coll Cardiol*. 2010;56:1552-63

The identification of phosducin as a novel candidate gene for hypertension and its role in sympathetic activation

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