Network: Genetic and Molecular Mechanisms of Common Cardiovascular Disorders: from Genes to Patients

Project: Genetics of Coronary Morphology

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Introduction

Myocardial infarction (MI) is both multifactorial in origin and phenotypically diverse. Multiple etiologic components and multiple morphological features of CAD make it likely that different causes lead to different disease patterns. Thus, in addition to differentiate factors leading to CAD, it is crucial to precisely specify distinct features of coronary morphology. Accordingly, we hypothesize intermediate phenotypes reflecting certain aspects of CAD facilitate the identification of distinct gene-phenotype relationships. Particularly, the phenotypic characterization of the coronary morphology may enable to specifically investigate whether distinct CAD patterns reflect distinct genetic causes. The importance of such substratification has recently been documented in the identification of the first stroke-related gene.[1]

In order to overcome the complexity of CAD in humans, we will also study the phenotype in animal models. In fact, man, mouse and rat share common features of cardiovascular calcification. Recently, we identified two significant QTLs (Dyscalc1 and Dyscalc2) linked to dystrophic cardiac calcification.[2,3] Moreover, we mapped a QTL suggestive for linkage with coronary calcification in a human region syntenic to Dyscalc2, providing a region of high priority to follow up in human studies.

Project Status

Studies in humans – heritability of coronary phenotypes

We retrospectively studied the coronary angiograms of 882 siblings with CAD from 401 families (Figure 1). These families were ascertained through index patients defined by MI before the age of 60 years and at least one sibling with MI or coronary revascularization procedures. Heritability calculations were performed using variance component analysis. Additionally, recurrence risks to siblings were analyzed.[4]

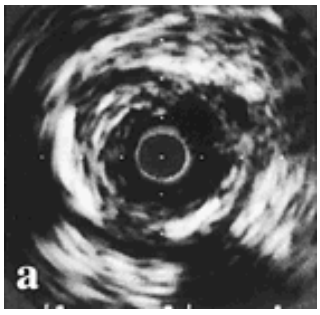

Fig 1: *Coronary calcification.*

Traditional cardiovascular risk factors and age at the first coronary event displayed significant heritable components. After adjustment for age and gender, significant heritabilities were identified for proximal stenoses, in particular left main disease (h^2=0.49±0.12; p=0.01), coronary calcification (h^2=0.51±0.17; p=0.001), and ectatic coronary lesions (h^2=0.52±0.07; p=0.001) (Figure 2).

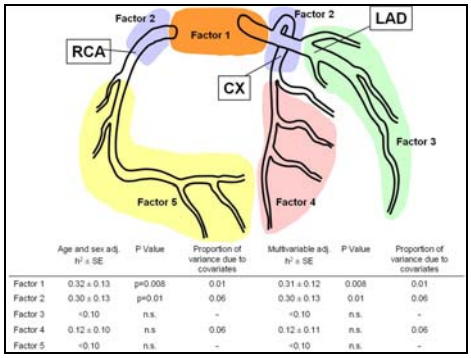

Fig 2: *Schematic overview of the factors resulting from principal component factor analysis with their heritability values.*

In contrast, no heritability was found for distal disease (h^2=0.05±0.19; n.s.), the pattern of coronary arterial blood supply or the number of diseased vessels (Fig. 2).

To confirm the heritability measures we analysed a second set of 1,500 coronary angiograms from MI families and tested for heritability of selected coronary phenotypes.

The analyses are ongoing; but preliminary results confirm the heritability measures in this second sample.

Animal studies – unravelling the Dyscalc1 locus

Dyscalc1 was mapped to a chromosomal region on mouse proximal chromosome 7. Since, Dyscalc1 was intensively investigated. We localized Dyscalc1 to a 10 cM chromosomal segment between D7Mit227 and D7Mit230 microsatellite markers. We confirmed the contribution of Dyscalc1 to dystrophic cardiac calcification (DCC) using a congenic line that carry the DCC-susceptible allele on a resistant genetic background.[5] We demonstrated that Dyscalc1 contribute not only to cardiac calcification but also to aorta calcification.[6] Furthermore, using bone marrow transplantation, we showed that calcium phosphate deposit was initiated in DCC-susceptible strains but influenced by infiltrating bone marrow cells.[7]

The aim of the ongoing project is to identify, among the 160 genes located in Dyscalc1 critical region, putative candidate genes representing the so far unknown Dyscalc1 gene(s).

Differential gene expression

On the basis of differential gene expression patterns, which were determined in various tissues from resistant C57BL/6 and susceptible C3H/He mice, we identified putative candidate genes in the Dyscalc1 critical region. Specific primer pairs for each gene of the 160 genes located in Dyscalc1 interval were designed and polymerase chain reaction (PCR) has been performed for detecting the gene expression in different organ tissue. Genes that were expressed in myocardial tissue have been further investigated using Real-time RT-PCR (Figure 3).

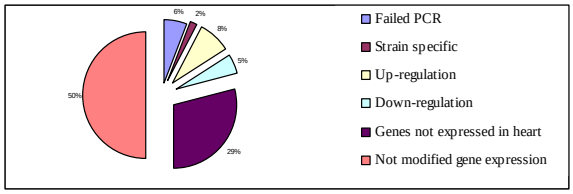

Fig 3: *Differential gene expression analysis using real-time RT-PCR for genes located in the Dycsalc1 critical region.*

Sequence analyses of putative candidate genes are ongoing and experiments are underway to further examine the biological functions of genetic variants in these candidate genes in mice and humans.

In-silico mapping of Dyscalc1 critical region
In a complementary approach we further tried to narrow down the Dyscalc1 critical region using "in silico"-mapping.[8]

Analyzing recombination among eight laboratory strains within the Dyscalc1 segment further narrowed down this region to a 1-Mb segment within an "in silico" QTL peak with a significant R value of 1.8 (Figure 4). These results demonstrate that "in silico"-mapping is a powerful tool that could be used to confirm fine mapping results of classical QTL analysis.

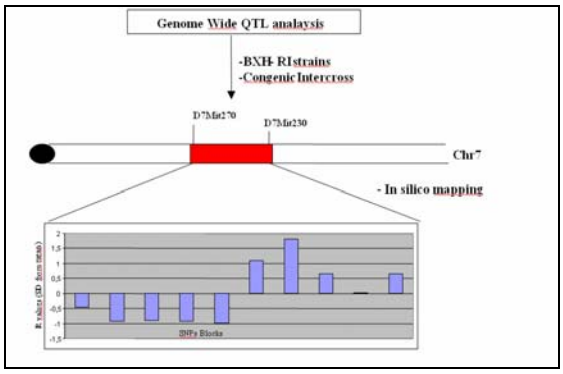

Fig 4: *Schematic presentation of the "in silico" mapping approach and results.*

Human Studies – Identification of genetic variants in candidate genes and association studies
Until now, we sequenced five candidate genes (5´UTR, 3´UTR, promoter, and coding exons) within the Dyscalc1 critical region in a subgroup of 48 patients with different degrees of coronary calcification.

The frequency of variants leading to amino acid substitutions will be further determined in larger sets of patients with different coronary phenotypes and controls. Our aim is to identify genetic variants associated with specific coronary phenotypes.

Lit.: ***1.*** *Gretarsdottir S et al. (2003) The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet. 35:131-8.* ***2.*** *Ivandic BT et al. (1996) A locus on chromosome 7 determines myocardial cell necrosis and calcification (dystrophic cardiac calcinosis) in mice. Proc Natl Acad Sci U S A. 93:5483-8.* ***3.*** *Ivandic BT et al. (2001) New Dyscalc loci for myocardial cell necrosis and calcification (dystrophic cardiac calcinosis) in mice. Physiol. Genomics 6:137-44.* ***4.*** *Fischer M. et al. (2005) Distinct heritable patterns of angiographic coronary artery disease in families with myocardial infarction. Circulation 111, 855-862.* ***5.*** *Aherrahrou et al. (2004) A locus on chromosome 7 determines dramatic up-regulation of osteopontin in dystrophic cardiac calcification in mice. Am J Pathol. 164:1379-87.* ***6.*** *Kazmarek et al. (2005) Reduced cardiac calcinosis in DCC-susceptible congenic mice reconstituted with DCC-resistant bone marrow cells. Poster presentation; European Society of Cardiology, Stockholm, Sweden.* ***7.*** *Doehring et al. (2005) Dyscalc1 determines aortic calcification in mice. Poster presentation; European Society of Cardiology, Stockholm, Sweden.* ***8.*** *Grupe A et al. (2001) In silico mapping of complex disease-related traits in mice Science. 292(5523): 1915-8.*