SELECTED SCIENTIFIC PROJECTS (SINCE 2006)

Project 2013 – 2017

Applicant and principal investigator (PI)
Mátyás G

Project title
Pharmacological therapy for Ehlers-Danlos syndrome vascular type (EDS IV) – Evaluation of substances to increase the aortic stability in mouse models

Summary
Aortic dilatation/dissection (AD) is a life-threatening condition associated with considerable morbidity and mortality rates. AD can occur spontaneously due to cardiovascular risk factors (e.g. hypertension) and/or in association with rare genetic disorders, such as familial thoracic aortic aneurysms leading to type A dissections (TAAD), Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), and the vascular type of Ehlers-Danlos syndrome (EDS IV). EDS IV is an autosomal dominant connective tissue disorder with a prevalence of 1-2 in 100,000 individuals. It is characterized by thin translucent skin, easy bruising, and typical facial features as well as by fragile walls of hollow organs and larger arteries, which leads to an increased risk for rupture. Consequently, the most severe complication is the increased risk for dissections and subsequent ruptures of the aorta and large arteries, leading to sudden death.

EDS IV is caused by mutations in the gene COL3A1, which encodes the alpha 1 chain of type III collagen, a fibrillar collagen. It is expressed in walls of hollow organs, such as large blood vessels, bladder, bowel, and uterus. In the majority of EDS IV cases missense mutations leading to glycine substitutions can be found, but other missense or splice site mutations as well as small deletions and insertions have also been detected. Only few nonsense mutations leading to functional COL3A1 haploinsufficiency have been reported and the first case of true COL3A1 haploinsufficiency has recently been described by our group. Haploinsufficiency means that only one of the two alleles leads to a protein and thus the total amount of protein is reduced. To date, there is no targeted therapy for EDS IV patients available; only disease management and treatment of symptoms. The advantage of true haploinsufficiency is that there is “only” a lack of healthy/normal protein, but no truncated/mutated protein. This makes targeted therapy easier as “only” the amount of protein has to be increased. Furthermore, for EDS IV only two mouse models exist, which both have a knockout allele of Col3a1 and thus in the heterozygous state display true Col3a1 haploinsufficiency. This means that at the moment animal experiments for EDS IV can only be done with this gene defect, matching the aim of our proposed research work perfectly.

The primary goal of our project is to use these two mouse models in order to find a pharmacological therapy for true COL3A1 haploinsufficiency in EDS IV by increasing the total amount of type III collagen and therefore improve the mechanical stability of the aorta as well as reduce the risk for aortic ruptures. In a second step, we will evaluate if and how the best substance(s) and acquired knowledge can be applied to further cases of EDS IV and related aortic disorders as well, with the goal to be able to help as many patients as possible. In our animal experiments, we will mainly use a recently described mouse model, which has true haploinsufficiency of Col3a1 due to a spontaneous deletion and leads to an increased mortality rate due to aortic dissection in heterozygous animals, like in humans. These mice with true Col3a1 haploinsufficiency will be treated with ten candidate substances, most of which are already approved for human use for other purposes, with the goal to find substance(s) that will increase the mechanical stability of the aortic wall and therefore reduce mortality due to aortic dissection. The best substance is expected to represent a therapeutic approach for EDS IV patients with true COL3A1 haploinsufficiency as well as for patients with different types of COL3A1 mutations.

Support
- Gebauer Stiftung
- Isaac Dreyfus-Bernheim Stiftung
- Wolfermann-Nägeli-Stiftung
Project 2011 – 2016

Applicant and principal investigator (PI)
Mátúias G

Project title
Molecular basis of Marfan syndrome and related aortic disorders:
Whole exome and whole genome sequencing of rare diseases

Summary
Marfan syndrome (MFS) is one of the most prevalent rare connective tissue disorders, affecting 1-3:10'000 individuals. MFS is characterized by skeletal, ocular, and cardiovascular manifestations and exhibits a broad range of severity. The phenotypic spectrum of MFS overlaps with many rare monogenic aortic disorders characterized by life-threatening aortic dilatation/dissection (AD), which affect(s) millions of people worldwide. Only a limited part of genes mutated in AD/MFS is known. The main aim of the research project is therefore to detect novel genes and genetic modifiers implicated in the pathogenesis of AD/MFS. This aim will be achieved by using whole exome (WES) and, most recently, whole genome (WGS) sequencing, after comprehensive evaluation of these most powerful next generation sequencing (NGS) technologies (s. our evaluation by Meienberg et al. 2015, Nucl Acids Res 43:e76). In addition to qualitative analyses, which can detect point mutations and small insertions/deletions, WES/WGS data will also be evaluated/used for quantitative sequence analysis in order to detect large insertions and deletions (CNVs, copy number variations). The outcome of this research project will provide important new insights into the molecular basis of genetically heterogeneous aortic disorders. This knowledge allows not only improved diagnostics, surgical decision, and targeted clinical management in AD/MFS patients but it will also enable genetic counseling and early (preimplantation, prenatal, and presymptomatic) molecular diagnosis in affected families. Furthermore, our research is expected to have a widespread impact by serving as a model for any other monogenic disorder with locus heterogeneity and partly unknown molecular basis.

Support
- COFRA Foundation
- Dr. med. Arthur und Estella Hirzel-Callegari Stiftung
- Ebnet-Stiftung
- Foundation for People with Rare Diseases
- Functional Genomics Center Zurich
- Gebauer Stiftung
- G & J Bangerter-Rhyner-Stiftung
- Stiftung FERNE HORIZONTE
Project 2008 – 2011

Applicant and principal investigator (PI)
Mátéás G

Project title
Molecular basis of Marfan syndrome: In silico, in vitro and candidate gene analyses

Summary
Mutations in the \textit{FBN1} gene cause Marfan syndrome (MFS), an autosomal dominant connective tissue disorder. Recently, heterozygous mutations in the \textit{TGFBR1} and \textit{TGFBR2} genes have been reported in MFS type 2 (MFS2) and type 3 (Loeys-Dietz aortic aneurysm syndrome, LDS) as well as in familial thoracic aortic aneurysms and dissections (TAAD). The knowledge about the presence of mutations is important for presymptomatic diagnosis and genetic counseling. However, a critical issue is to identify whether a given sequence variant is a harmless polymorphism or a disease-causing mutation. For this reason, the \textit{first part} of the present project focuses on the evaluation and application of computational tools to assess the pathogenic impact of \textit{FBN1} sequence variants. In the case of unclear or confusing computational predictions, \textit{in vitro} tests and cosegregation analyses will also be performed.

In patients with suspected MFS, mutation analyses have failed to detect \textit{FBN1}, \textit{TGFBR1} or \textit{TGFBR2} involvement in at least 10%-30% of cases, suggesting that the disease-causing mutation occurs in a different gene. The aim of the \textit{second part} of the present project will therefore be to identify novel genes involved in MFS by analyzing candidate genes and screening for DNA copy number variation using high-resolution SNP arrays.

The results of this project will provide the basis for improved molecular diagnostics and a better understanding of the molecular pathogenesis and phenotypic variability of MFS and related disorders, which affect at least half a million people worldwide.

Support
- Foundation for People with Rare Diseases
- Functional Genomics Center Zurich
- Jubiläumsstiftung Swiss Life
- Swiss National Science Foundation
Project 2007

Applicant and principal investigator (PI)
Mátyás G

Project title
High-resolution DNA copy number analysis in Marfan syndrome-related disorders using GeneChip Mapping arrays

Summary
Mutations in the \textit{FBN1} gene cause Marfan syndrome (MFS), an autosomal dominant connective tissue disorder. Recently, heterozygous mutations in the \textit{TGFBR1} and \textit{TGFBR2} genes have been reported in MFS type 2 (MFS2) and type 3 (Loeys-Dietz aortic aneurysm syndrome, LDS) as well as in familial thoracic aortic aneurysms and dissections (TAAD). Very recently, we have found that MFS-like phenotypes are caused not only by single-base substitutions (point mutations) but are also associated with large rearrangements of these genes not detectable by standard PCR-based screening assays. In addition, increasing evidences suggest that MFS-related disorders are caused by mutations in genes not yet associated with MFS-related clinical phenotypes.

By using the GeneChip Human Mapping 500K arrays, the study aims (1) to narrow down the breakpoints of large deletions recently identified by Multiplex Ligation-dependent Probe Amplification (MLPA) technique, and (2) to identify genes not yet associated with MFS-related disorders by detecting DNA copy number variations in the human genome.

The outcomes of the present project contribute to the better understanding of the molecular pathogenesis of MFS-related disorders and fulfil the current needs of molecular diagnostics, enabling presymptomatic diagnosis and genetic counselling in the affected families.

Support
- Functional Genomics Center Zurich
- Swiss Heart Foundation
Project 2006 – 2007

Applicant and principal investigator (PI)
Mátyás G

Project title
Molecular basis of Marfan syndrome: investigation of exonic mutations affecting splicing and large deletions in the \textit{FBN1} gene

Summary
Mutations in the human \textit{FBN1} gene cause Marfan syndrome (MFS), an autosomal dominant connective tissue disorder. Knowledge about \textit{FBN1} mutations is important for early diagnosis, management, and genetic counseling of MFS patients. However, a critical issue is to identify whether a given nucleotide change is a harmless polymorphism or a disease-causing mutation. While nonsense and known missense mutations suggest a causative effect, the pathogenic consequence of sporadic cases of silent and novel missense mutations can only be determined experimentally (e.g. mRNA, protein, and familial cosegregation analyses). For this reason, the first part of the present project should focus on pre-mRNA processing where the effect of exonic nucleotide changes can be functionally defined. Based on a large collection of exonic \textit{FBN1} mutations we will assess how and how often missense and silent mutations influence the normal splicing of the \textit{FBN1} gene. Using a transfection assay, the effect of specific splicing factors will also be investigated.

Previous studies have revealed that approximately 70% of patients who fulfilled the clinical criteria of MFS have mutations in the \textit{FBN1} gene, suggesting the presence of a second Marfan locus (MFS2) and/or large deletions not detectable by currently used PCR-based assays. This leaves about 30% of MFS patients without any molecular explanation for the clinical diagnosis, prohibiting prenatal and presymptomatic diagnostics in the respective family. The aim of the second part of the present project will therefore be to develop a suitable screening method for the detection of whole-exon deletions/insertions in the \textit{FBN1} genomic DNA. Using this method, the frequency of large deletions should be assessed in DNA samples from patients in whom previous PCR-based sequencing revealed no \textit{FBN1} mutations.

The outcomes of the present project would fulfill the urgent needs of molecular diagnostics and broaden our knowledge of the molecular pathogenesis of MFS, which affects at least half a million people worldwide.

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- Research Funding of the University of Zurich
- Swiss Heart Foundation