Genetics and inheritance issues in congenital heart disease
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Chapter 11

Summary and future perspectives
Summary

Congenital heart disease (CHD) is among the most common birth defects, occurring in approximately 8 per 1,000 live births. It leads to significant morbidity and mortality in children as well as adults. Due to improvements in cardiac surgery and medical care, nowadays approximately 90% of CHD patients reach adulthood. Although the causes of CHD are largely unknown, there has been significant progress in the identification of genes and signaling pathways that are involved in cardiovascular development and CHD. The increasing knowledge enables physicians caring for CHD patients to inform patients about the origin of their CHD, the inheritance pattern and possible risk for extracardiac disease. This thesis focuses on the genetics of non-syndromic and syndromic CHD, the implications it has for (adult) patients as well as the adult CHD patients' perspective on inheritance issues.

In Chapter 1, the current knowledge about genetic causes of CHD is summarized. An overview of causes of common syndromic and non-syndromic CHD is given. Additionally, implications for genetic counseling of CHD patients are discussed, mainly focused on the adult with CHD.

Part I of this thesis comprises studies searching for the genetic causes of non-syndromic CHD, using different strategies. In Chapter 2, traditional genome-wide linkage analysis was used to unravel the genetic cause of a unique autosomal dominant phenotype in a large four-generation family. Affected individuals presented with CHD (including atrial septal defect type I and II, tetralogy of Fallot and persistent left superior vena cava) and/or low atrial rhythm. This phenotype seems to represent a mild or variant expression of left atrial isomerism or a developmental defect of the sinus node and surrounding tissue. A 39-Mb locus at chromosome 9q was identified that co-segregated with this autosomal dominant disorder. By means of direct sequencing we analyzed several candidate genes within this locus, but no mutations were identified. The causal defect has not been identified yet. Chapter 3 describes the comprehensive genetic analysis of a cohort of unrelated probands with Ebstein anomaly. Using next generation sequencing and direct DNA sequencing, we found a disease-associated mutation in MYH7 in 8%. MYH7 encodes the sarcomere protein beta-myosin heavy chain. Mutation positive probands and family members showed left ventricular noncompaction as well as various CHD, including Ebstein anomaly and ventricular septal defect. The penetrance of the left ventricular noncompaction was high, whereas there was significant pleiotropy and reduced penetrance regarding the observed CHD. With this study we showed that there is an association between Ebstein anomaly with left ventricular noncompaction and mutations in MYH7.

Chapter 4 focuses on the possible role of sodium channels in CHD. The SCN5A gene encodes the alpha-subunit of the cardiac sodium channel. Mutations in SCN5A underlie several arrhythmia syndromes, including Brugada syndrome and Long QT syndrome. In animal studies, deficiency of scn5a was shown to lead to developmental abnormalities of the heart, seemingly due to a non-electrogenic function of the channel. We explored the role of SCN5A mutations in human
CHD. In the first part of this study, we determined the proportion of CHD in a consecutive cohort of SCN5A loss-of-function mutation carriers and in a cohort of gain-of-function mutation carriers. The proportion of CHD in the loss-of-function mutation cohort was 3.1%, which was significantly higher than reported in the general population. Observed CHD were mainly septal defects. There were no CHD in the gain-of-function mutation cohort. In the second part of this study, we sequenced SCN5A in a cohort of patients with a septal defect and conduction disease and identified a (possibly) pathogenic mutation in 3.7% of patients. Our results suggest a role for SCN5A loss-of-function mutations in CHD in humans, particularly septal defects.

Part II of this thesis describes studies focuses on CHD in association with other (extracardiac) abnormalities, including syndromic as well as non-syndromic CHD. In Chapter 5, we showed that 22q11.2 deletion syndrome is highly under-recognized in adults with TOF or pulmonary atresia and ventricular septal defect. We investigated patients with these CHD types registered in CONCOR, a nationwide registry and DNA-bank for adult patients with CHD. Using Multiplex ligation dependent probe amplification, we identified a 22q11.2 deletion in 6.5% of tetralogy of Fallot patients and 16.5% of pulmonary atresia with ventricular septal defect patients. More than half of the 22q11.2 deletions were unknown before this study; these patients had thus not been diagnosed with the syndrome. As the syndrome may have important clinical and reproductive implications, we propose that a diagnostic test should be considered in all adult patients with tetralogy of Fallot and pulmonary atresia with ventricular septal defect.

Chapter 6 focuses on the morphology of the bicuspid aortic valve (BAV) in fetuses with Turner syndrome (monosomy X). We studied 37 post-mortem heart specimens of Turner syndrome fetuses and concluded that the vast majority shows abnormal aortic valve morphology. BAV was present in 75%. Different BAV morphology types were present; both BAV with fusion of the right and left coronary leaflets (Type 1 BAV) and BAV with fusion of the right coronary and noncoronary leaflets (Type 2 BAV) were encountered. Type 2 BAV seemed to be overrepresented in our fetal Turner syndrome population as compared to reports in adult Turner syndrome patients; this might imply a worse prognosis of Type 2 BAV than Type 1 BAV, possibly due to associations with additional cardiovascular abnormalities.

In Chapter 7, we describe clinical and functional studies of the p.A119S variant in NKX2-5. Mutations in NKX2-5 are a well-known cause of human CHD; more recently mutations in this gene were also identified in patients with thyroid dysgenesis. The p.A119S variant had been described as causal for thyroid dysgenesis; we encountered this variant in two probands with CHD. In this study, we showed that p.A119S is a rare variant that behaves equal to wildtype NKX2-5 and does not cause CHD or thyroid dysgenesis.

Chapter 8 focuses on the previously suggested association between CHD and neuroblastoma, with conflicting evidence in previous literature. We assessed this association by conducting an echocardiographic study in a large single center cohort of consecutive neuroblastoma patients. We did not find a higher prevalence of CHD compared to the control groups and conclude that,
based on the results of our study as well as an extensive systematic review of the existing literature, clear evidence of an association between neuroblastoma and CHD is lacking.

**Part III** includes studies on the role of the clinical geneticist and the adult patients’ perspective on genetics and inheritance of CHD. **Chapter 9** describes the results of a questionnaire study in almost 500 adults with CHD. The majority of adult CHD patients was shown to lack knowledge about inheritance issues and over 40% desires more information about this topic. Almost half of patients has concerns regarding inheritance issues. A dedicated counseling program for adults with CHD may address these topics and thus improve patient care.

**Chapter 10** regards adult CHD patients who consulted a clinical geneticist. We performed a retrospective study and showed that an etiological diagnosis other than the most common ‘multifactorial’ CHD is provided in more than a quarter of these patients. Moreover, patients value the clinical genetics consultation as positive. These latter two studies demonstrate the added value of the clinical geneticist in the care for adults with CHD.

Finally, **Chapter 11** discusses future perspectives regarding research and clinical care in the field of CHD genetics.

**Future perspectives**

**Research perspectives**

Although significant progress has been made in the identification of genes and pathways involved in cardiogenesis and (human) CHD, the majority of CHD is unexplained so far. As yet, it remains a challenge to identify additional genes, pathways and mechanisms that are involved in human CHD. However, new genetic analysis techniques are radically changing our way of investigating CHD causes. Classical approaches for gene identification, such as positional cloning strategies, have proven to be difficult to realize due to the lack of extended CHD families with Mendelian inheritance. They will be complemented or replaced by contemporary strategies, which have already yielded promising results.

It is increasingly recognized that copy number variations (CNVs) underlie CHD in a subset of patients.\textsuperscript{1-3} The identification of recurrent CNVs provides opportunities for the discovery of new genes and pathways involved in human CHD by recognition of candidate genes within these regions. Moreover, newer approaches using next generation sequencing can identify large numbers of variations per exome or genome. The decreasing costs of such technologies make that these are becoming available at lower thresholds, and these strategies may yield exciting new insights. Such studies bring huge amounts of data, however, and we still have to learn a lot about its analysis and interpretation. From these data, CHD causing mutations have to be identified by focusing on rare variants that occur in genes involved in heart development and are predicted to have deleterious consequences on protein functions. Moreover, whole genome sequencing can
also address the large component of the genome that does not code for proteins, but is nonetheless biologically functional. For example, a homozygous mutation in a cardiac enhancer of \textit{TBX5} (located 90 kb downstream) was identified in a CHD patient.\textsuperscript{4} The potential role of such non-coding variants in CHD has not been explored extensively but should undoubtedly be subject of future studies. New data from next generation sequencing studies have to be combined with data from systems biology approaches, by constructing molecular networks and thus elucidating pathways and interaction of molecules involved in heart development.\textsuperscript{5} For example, a recent bioinformatics study of rare CNVs identified in 2500 human CHD cases found that the genes in these CNVs were significantly enriched for involvement in the Wnt signaling pathway, thereby providing the first evidence that this pathway is involved in human CHD.\textsuperscript{2} Additional advancements and opportunities for further research in CHD may arise from initiatives such as CONCOR (CONgenital CORvitia), CHD-GENES (Congenital Heart Disease Genetic Network Study) and CHeartED (Congenital Heart and Environment/Epidemiological Database).\textsuperscript{6-8} These initiatives provide large collections of homogeneous clinical CHD cases, with extensive clinical data as well as DNA and sometimes tissue of affected patients. Such large collections, particularly for the rarer malformations, were lacking in the past and therefore hampered genetic studies. Now these large collections are available, genetic studies such as genome-wide association studies (GWAS) become possible. This likely increases our knowledge about common genetic variations influencing the risk of CHD. Although GWAS have been performed widely in various complex diseases, only few were done in CHD phenotypes to date. These few studies provided evidence that common variants contribute to at least TOF and septal defects.\textsuperscript{9-11} Further GWAS studies will surely follow, in different subtypes of CHD.

There are several other interesting fields related to CHD origin that require further study. One of these is the biological impact of gene-environment interactions, such as interactions between variants in genes involved in glucose metabolism and nutrition during pregnancy. Other fields that need further exploration are epigenetic mechanisms, since chromatin-remodeling and histone-modifying factors regulating gene expression to control cardiovascular development have already been identified.\textsuperscript{12,13} Moreover, recently de novo mutations in histone-modifying genes were identified in CHD patients.\textsuperscript{14} The role of microRNAs also warrants further study, as animal studies suggested that deficiency of certain microRNAs leads to decreased levels of cardiac transcription factors\textsuperscript{15} and to CHD. In addition, studies in heart tissue of human CHD patients showed microRNA dysregulation.\textsuperscript{16}

The relative importance of genetic factors is increasingly recognized in the origin of CHD. Due to rapid molecular advances, the large group of CHD patients historically assigned to have a ‘multifactorial’ origin might prove to be much more genetically heterogeneous. It will remain a major challenge to combine the knowledge of the different fields of investigation and to answer the question of how genetic, epigenetic and environmental factors interact with each other within individual patients to eventually lead to CHD.
Clinical implications

The increase in availability of next generation sequencing facilities in a research setting as well as the growing knowledge about genetic mechanisms will undoubtedly augment our knowledge about heart development and the origins of CHD. These innovations can also be expected to change clinical care for CHD patients in the (near) future. The above mentioned next generation sequencing facilities are not only used in a research setting but are also becoming more and more available in daily clinical practice at decreasing costs. Thus, panels of candidate genes or even total exomes or whole genomes may be analyzed in individual syndromic or non-syndromic CHD patients in a clinical setting. The goal of such analysis would be to identify mutations and variants contributing to CHD in that particular patient. However, clinical utility and relevance to patient management will need to be demonstrated before testing will become routinely available and implemented in care for CHD patients. It is likely that only a small subset of patients have CHD caused by single (or even a few) mutations. Consequently, next generation sequencing in an individual patient will probably identify many rare variants of which the isolated or combined significance cannot easily be predicted. However, as we increasingly gain experience with next generation sequencing in clinical care, it is likely that we will be able to inform more patients and their families about the causes, inheritance and recurrence risk of CHD in their particular situation. This not only helps patients who simply ‘need to know’ the cause of their CHD; it may also influence reproductive choices of CHD patients and their relatives. In combination with continuously improving screening and diagnostic possibilities before and during a pregnancy, such as prenatal- and pre-implantation genetic diagnosis and advanced fetal echocardiography, patients will have more influence on the risk of having a child affected with CHD and/or associated abnormalities.

Another opportunity for improvements in patient care could come from future studies on gene-environment interactions. It would be a major achievement if we could identify patients who are particularly susceptible for developing CHD in certain environmental settings (for example, the use of certain drugs during pregnancy could lead to CHD in patients with a certain genetic predisposition). This would enable personalized measures to decrease the risk of CHD in offspring. For the longer term, one may speculate that increasing insights in mechanisms of heart development will lead to future therapeutic measures for attenuation of CHD, such as intra-uterine molecular interventions including supplementation of deficient molecules or epigenetic manipulations.

The ultimate challenge for the coming years is to find ways to analyze and interpret the large amount of data generated by renewed technologies, to apply this knowledge to individual patients and to develop strategies for prevention, treatment or repair of CHD.
Reference List

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