Genetics and Inheritance issues in congenital heart disease

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Citation for published version (APA):

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Chapter 1

Introduction and outline of this thesis
Introduction

Congenital heart disease (CHD) is among the most common birth defects, occurring in approximately 8 per 1,000 live births. Congenital heart disease comprises a wide range of cardiovascular malformations, from complex and critical defects presenting prenatally or in the newborn period, to mild defects that are not detected until adulthood. It leads to significant morbidity and mortality in children as well as adults. Due to improvements in cardiac surgery and medical care, the population of adult CHD patients is growing. Nowadays approximately 90% of CHD patients reach adulthood.

Most CHD occurs sporadically and in a non-syndromic fashion. In a subgroup of CHD a genetic origin can be demonstrated, which include chromosomal abnormalities, copy number variations (CNVs) and single gene defects. In the remaining cases there is significant heritability, which is currently largely unexplained. The majority of non-syndromic CHD is historically believed to be multifactorial in origin, i.e. multiple (unknown) genetic and environmental factors contributing to the CHD. In the last decades, many research projects have focused on the genetic causes of non-syndromic CHD and although significant progress has been made, the contributing genetic defects remain largely unknown. The traditional approaches for gene discovery, including positional cloning strategies, are hampered by the rarity of extended CHD families with clear Mendelian inheritance patterns, incomplete penetrance and variable expression of the genetic defects. Studies in animal models have elucidated many fundamental pathways involved in cardiac development leading to the identification of candidate genes. Although several genes involved in human CHD have been identified by screening these candidate genes in human patients, such candidate gene studies are hypothesis driven - genes that seem to be less obvious in heart development at first thus remain undiscovered. In addition, the non-coding regions of the genome have not been studied extensively. Moreover, due to the heterogeneous genetic and clinical nature of CHD, large cohorts of homogeneous CHD were difficult to realize in the past, hampering candidate gene studies or case-control studies of common variants leading to CHD susceptibility.

Despite these pitfalls, there has been progress in the identification of genes and signaling pathways that are involved in cardiovascular development. Due to new and evolving genetic analysis techniques, knowledge is currently expanding at an increasing pace. This introductory chapter provides an overview of non-syndromic and syndromic causes of CHD. In addition, the implications of CHD genetics for patients and family members are discussed.

Non-syndromic CHD

In the majority of patients, CHD occurs in a non-syndromic fashion. It happens mostly sporadically, although in some families a monogenetic inheritance pattern is present. A genetic cause can be demonstrated in only a small subset of individuals and families with non-syndromic CHD. In some patients, high-penetrance mutations in one of several genes that are known to be involved in heart development can be identified (see below). In addition, more recently de novo CNVs have been shown to contribute to several types of non-syndromic CHD, including tetralogy of Fallot (TOF), left-sided heart defects (bicuspid aortic valve (BAV), aortic coarctation) and several other types of
In some of these CNVs known CHD genes are located; e.g. \textit{GATA4}, which is located on 8p23.1, a region in which recurrent CNVs have been identified in CHD cases.\(^{6}\) It was estimated that 5\% to over 10\% of sporadic, non-syndromic CHD occurs because of a rare CNV. Penetrance is often incomplete.

Single gene defects and CNVs are found in only a minority of non-syndromic CHD, however. The majority of non-syndromic CHD is historically thought to be due to multifactorial inheritance. This implies that there is a cumulative effect of multiple variations in many different genes, each of which contributes only for a small part to a person’s susceptibility to CHD. These genetic susceptibility factors interact with each other as well as with environmental risk factors, which, if a certain threshold is reached, together lead to CHD. Several of such susceptibility variants with low penetrance have been identified through small-scale case-control studies, and recently the first genome-wide association studies (GWAS) in CHD have shown associations for some CHD types. Cordell et al. demonstrated two loci on 12q24 and 13q32 to be associated with TOF.\(^{8}\) In addition, a recent GWAS study of cases with VSD, ASDII, transposition of the great arteries (TGA), conotruncal malformations, and left-sided heart defects showed an association of single nucleotide polymorphisms in a region on chromosome 4p16 for ASD, but no significant associations were found for the other phenotypes studied.\(^{9}\) A GWAS study in a Han Chinese population identified two risk loci for CHD (including septal defects but also other CHD types) on 1p12 and 4q31.\(^{10}\) Odds ratios for CHD risk in these studies ranged from 1.2 to 1.4. It seems remarkable that no consistent loci were identified in these GWAS studies, however, differences in population ethnicity, patient characteristics and study design may explain these differences. Moreover, subcohorts with specific CHD types might have been too small to detect associations.

Environmental factors that have been implied in CHD include maternal disease (e.g. diabetes mellitus, hypercholesterolemia, hyperphenylalaninemia), infectious agents (maternal Rubella), several medications (ACE inhibitors, retinoids) and substance abuse (alcohol, cocaine).\(^{11}\) Use of folic acid during pregnancy has been hypothesized to be protective against CHD in the child, and folate deficiency is suspected to be a CHD risk factor, however evidence for this association remains inconclusive.\(^{11}\) Some common variants in genes encoding enzymes involved in folic acid metabolism were shown to be ‘susceptibility alleles’ for CHD.\(^{12,13}\) A specific variation that has been investigated extensively, is the common variant c.667C>T of the \textit{MTHFR} gene, which in homozygous state is known to cause lower levels of plasma folate. Interestingly, the largest genetic study and meta-analysis performed so far showed no evidence for a relationship between several subtypes of CHD and the \textit{MTHFR} c.677C/T genotype (in CHD patients as well as mothers of CHD patients).\(^{14}\)

\textbf{Non-syndromic monogenetic CHD}

The proportion of non-syndromic CHD that has a monogenetic cause is not precisely known, but it is presumed to be small. Only few families demonstrate CHD with a clear Mendelian inheritance pattern. In these families, mostly autosomal dominant inheritance is seen. In some CHD families
as well as sporadic CHD, a pathogenic mutation underlying the defect can be identified. In these cases, there usually is extensive genetic as well as clinical heterogeneity; i.e. a particular type of CHD can be caused by mutations in different genes mutations, and mutations in one gene can lead to distinct types and severity of CHD, even within a family. Moreover, penetrance is often incomplete. The first reported single-gene mutation in humans with non-syndromic CHD was in the NKX2-5 gene, encoding a homeobox transcription factor.\textsuperscript{15} Since then, mutations in a substantial number of other genes have been identified, mostly by positional cloning or candidate gene approaches. Mutations have been found in several steps of the multiple pathways that contribute to heart development, including genes coding for extracellular receptor ligands, membrane receptors and transcription factors. Additionally, mutations in sarcomere protein genes and histone-modifying genes have been identified in patients with (familial) CHD.\textsuperscript{16} Table 1 provides an overview of a selection of the more well known genes involved in human CHD. A subset of these will be discussed in more detail below.

\textit{NKX2-5}

NKX2-5 belongs to the NK-2 family of homeodomain-containing transcription factors, which are conserved from flies to humans.\textsuperscript{17} Its role as a transcription regulator during early embryonic heart developmental has been known for many years. Mice haplo-insufficient for nkx2.5 show abnormalities of the (atrial) septum and valve development as well as hypoplasia of the cardiac conduction system, especially the AV-node.\textsuperscript{18,19} Analogue to these abnormalities in animal studies, in humans most NKX2-5 mutations have been reported in patients with (familial) atrial septal defects (ASD) and conduction disorders, mainly atrioventricular block.\textsuperscript{15,20,21} Although NKX2-5 mutations can also lead to other types of CHD, the proportion of patients carrying such a mutation is lower in these groups.\textsuperscript{20,21} The overall mutation detection rate in sporadic CHD is reported to be 2%.\textsuperscript{21} The mutations that have been identified are spread among the entire coding region of the gene, without genotype-phenotype correlation. Most mutations (were predicted to) lead to a truncated protein (haplo-insufficiency) or impaired protein function.

\textit{NOTCH1}

\textit{NOTCH1} encodes a large, single-pass transmembrane receptor that functions in a highly conserved cell-to-cell signaling system involved in multiple developmental processes.\textsuperscript{22,23} In mammals, there are four Notch receptors (1-4), which interact with their ligands (Delta-like 1, 3 and 4, and Jagged 1 and 2) that are expressed on the surface of adjacent cells.\textsuperscript{23} Notch1\textsuperscript{1,2} mice were shown to have impaired trabeculation and hypoplastic cardiac cushions. In 2005, Garg et al. performed a genome-wide linkage scan in a large family with bicuspid aortic valve, severe aortic calcification and other CHD, including TOF. A single locus was identified on chromosome 9q34-35 and subsequently \textit{NOTCH1} was identified as the causal gene.\textsuperscript{24} Following this paper, mutations in \textit{NOTCH1} were also identified in patients with other left ventricular outflow tract obstruction, including hypoplastic left heart syndrome and aortic coarctation.\textsuperscript{25} Functional studies of the mutations identified in
### Table 1. Genes in human non-syndromic CHD

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<tr>
<th>Gene</th>
<th>Locus</th>
<th>Protein</th>
<th>CHD</th>
<th>Associated syndrome</th>
<th>References</th>
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</table>

**Extracellular ligands**
- BMP2: Bone morphogenetic protein receptor
- CFC1: Cysteine-rich protein
- CRELD1: Cysteine-rich protein with domains
- NOTCH1: NOTCH1
- TDP4F: Tetraspanin-derived growth factor
- GDF1: Growth differentiation factor
- JAG1: Jagged-1
- LEFTY2: Left-right determination factor
- VEGF: Vascular endothelial growth factor
- GAP4: Catenin
- SMAD6: MAD-related protein
- TAF2: TAF-1-binding protein
- TBC1: Alpha-cardiac actin
- ACTC1: Actin
- ELN: Elastin
- MYH6: Myosin heavy chain
- MYH4: Myosin heavy chain
- MYH11: Myosin heavy chain
- MYBPC3: Myosin binding protein

**Signaling molecules**
- BMP2: Bone morphogenetic protein receptor
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- MYH6: Myosin heavy chain
- MYH4: Myosin heavy chain
- MYH11: Myosin heavy chain
- MYBPC3: Myosin binding protein

**Structural molecules**
- BMP2: Bone morphogenetic protein receptor
- CFC1: Cysteine-rich protein
- CRELD1: Cysteine-rich protein with domains
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- GDF1: Growth differentiation factor
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- TBC1: Alpha-cardiac actin
- ACTC1: Actin
- ELN: Elastin
- MYH6: Myosin heavy chain
- MYH4: Myosin heavy chain
- MYH11: Myosin heavy chain
- MYBPC3: Myosin binding protein

**Gap junctions**
- BMP2: Bone morphogenetic protein receptor
- CFC1: Cysteine-rich protein
- CRELD1: Cysteine-rich protein with domains
- NOTCH1: NOTCH1
- TDP4F: Tetraspanin-derived growth factor
- GDF1: Growth differentiation factor
- JAG1: Jagged-1
- LEFTY2: Left-right determination factor
- VEGF: Vascular endothelial growth factor
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- SMAD6: MAD-related protein
- TAF2: TAF-1-binding protein
- TBC1: Alpha-cardiac actin
- ACTC1: Actin
- ELN: Elastin
- MYH6: Myosin heavy chain
- MYH4: Myosin heavy chain
- MYH11: Myosin heavy chain
- MYBPC3: Myosin binding protein

**Peripheral pulmonary hypoplasia**
- BMP2: Bone morphogenetic protein receptor
- CFC1: Cysteine-rich protein
- CRELD1: Cysteine-rich protein with domains
- NOTCH1: NOTCH1
- TDP4F: Tetraspanin-derived growth factor
- GDF1: Growth differentiation factor
- JAG1: Jagged-1
- LEFTY2: Left-right determination factor
- VEGF: Vascular endothelial growth factor
- GAP4: Catenin
- SMAD6: MAD-related protein
- TAF2: TAF-1-binding protein
- TBC1: Alpha-cardiac actin
- ACTC1: Actin
- ELN: Elastin
- MYH6: Myosin heavy chain
- MYH4: Myosin heavy chain
- MYH11: Myosin heavy chain
- MYBPC3: Myosin binding protein

**Other conditions**
- CHD: Congenital heart disease
- TAPVR: Total anomalous pulmonary venous return
- AVSD: Atrial septal defect
- VSD: Ventricular septal defect
- DORV: Double outlet right ventricle
- DORV: Double outlet right ventricle
- TOF: Tetralogy of Fallot
- PAH: Pulmonary arterial hypertension
- PDA: Patent ductus arteriosus
- EA: Ebstein anomaly
- AD: Autosomal dominant
human patients with CHD showed a reduction of the amount of receptor at the cell surface as well as reduced binding of ligands to the receptor.\textsuperscript{25,26} Mutations in other components of the Notch signaling pathway can also lead to syndromic and non-syndromic CHD. Mutations in \textit{JAG1}, encoding one of the Notch receptor ligands, cause Alagille syndrome characterized by liver disease, dysmorphic features, vertebral abnormalities and CHD, specifically TOF. \textit{JAG1} mutations have also been identified in patients with non-syndromic CHD.\textsuperscript{27} In some patients with Alagille syndrome, mutations in \textit{NOTCH2} are found.\textsuperscript{28}

\textbf{MYH6}

In addition to mutations in transcription factors and receptor/ligand molecules, mutations in structural proteins can also lead to CHD. An example is the alpha-myosin heavy chain, a cardiac sarcomeric protein, which is encoded by \textit{MYH6}. It is expressed at high levels in the developing atria, and studies in animal models showed that deficiency of \textit{MYH6} during heart development leads to disturbed cardiogenesis and subsequent heart malformations.\textsuperscript{29,30} In 2005, a missense mutation in \textit{MYH6} was shown to cause an autosomal dominant form of ASD with incomplete penetrance.\textsuperscript{29} More recently, mutations were also identified in small proportions of patients with other CHD, among which tricuspid atresia, VSD and pulmonary stenosis.\textsuperscript{31,32} These mutations were shown to lead to disturbance of the assembly of normal myofibrils.\textsuperscript{31} Like mutations in other sarcomeric protein genes, including \textit{MYH7} and \textit{MYBPC3}, mutations in \textit{MYH6} have also been identified in patients with hypertrophic and dilated cardiomyopathy.\textsuperscript{33}

\textbf{Syndromic CHD}

There are numerous syndromes in which CHD may occur, and many of these are associated with specific CHD types. These syndromes can be caused by chromosomal abnormalities, including aneuploidies and structural aberrations. Moreover, mutations in genes involved in pathways that are important in the development of multiple organ systems can lead to syndromic CHD. Interestingly, mutations in some of the genes involved in syndromic CHD have also been identified in non-syndromic CHD patients, including \textit{TBX1}, \textit{TFAP2B} and \textit{JAG1}.\textsuperscript{27,34-36} Table 2 provides an overview of some well-known syndromic forms of CHD. A few of these are discussed in more detail below.

\textit{Turner syndrome}

Turner syndrome is caused by complete or partial monosomy for the X chromosome in all or part of the cells. It occurs in 1 in 2500 female live births. However, the majority of Turner syndrome conceptions do not survive until term. The main clinical features include fetal lymphedema, webbed neck, short stature, gonadal dysgenesis and usually a normal intelligence, with nonverbal learning disability. CHD is present in 20-40\% of patients.\textsuperscript{37,38} However, in Turner syndrome fetuses with cystic hygroma, who include the more severely affected patients with a high mortality in utero, the prevalence of CHD is much higher. CHD in Turner syndrome mostly comprises left-sided heart defects, including bicuspid aortic valve and aortic coarctation. Moreover,
patients are at increased risk of aortic aneurysm and dissection. An association between karyotype and CHD has been reported; frequency of CHD is higher in patients with 45,X than in those with structural abnormalities of the X-chromosome (including X-chromosome deletions, ring chromosome X and isochromosome X), though a higher prevalence of bicuspid aortic valve in patients with ring chromosome X was also reported. The genetic mechanisms that are implicated in CHD in Turner syndrome are unknown.

22q11.2 microdeletion syndrome

The incidence of the 22q11.2 microdeletion syndrome is estimated to be at least one in 4,000, making it the most common microdeletion syndrome in humans. Most patients have a specific 3 Mb deletion, but atypical deletions are also found. In past and present times, several diagnostic terms have been assigned to the combination of features associated with microdeletion 22q11.2, including velocardiofacial syndrome, DiGeorge syndrome and CATCH22. These terms represent varying manifestations of the same genetic entity. The features associated with 22q11.2 microdeletion syndrome include CHD, cleft palate, velocpharyngeal insufficiency with hypernasal speech, hypocalcaemia, psychiatric disturbances, (mild) dysmorphic facial features and mild to moderate mental retardation (reviewed in Kobrynski and Sullivan). The presence and severity of clinical features is highly variable, however. This can make it difficult to recognize the syndrome in mildly affected patients, especially in adults. Three quarter of patients have CHD which typically constitute conotruncal malformations such as interrupted aortic arch type B, truncus arteriosus communis, TOF and pulmonary atresia (PA) with ventricular septal defect (VSD). The frequency of 22q11.2 deletion in children with these particular heart defects ranges from about 10% (TOF) to up to 50% (interrupted aortic arch type B), and screening for the deletion is therefore warranted in all patients with these CHD.

In over 90% of patients the deletion has arisen de novo, whereas in the remaining patients it is inherited from one of the parents. Individuals with 22q11.2 microdeletion syndrome have a 50% chance of transmitting the deletion to their offspring. The 22q11.2 locus encompasses the TBX1 gene, encoding a transcription factor of the T-box family. Mice with one or two disrupted copies of TBX1 were shown to have CHD that are similar to those in 22q11.2 microdeletion syndrome, implying that haplo-insufficiency of TBX1 is a major cause of heart defects in patients with a 22q11.2 deletion. Indeed, mutations in TBX1 have also been identified in human patients with CHD, with and without the other abnormalities seen in 22q11.2 microdeletion syndrome. More recently, CRKL was also identified as a possible CHD candidate gene residing in the 22q11 locus.
### Table 2. Syndromic forms of CHD

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Genetic basis</th>
<th>Inheritance pattern</th>
<th>Cardiac disease</th>
<th>Extracardiac manifestations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aneuploidies</strong></td>
<td></td>
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<tr>
<td>Down syndrome</td>
<td>Trisomy 21</td>
<td>Sporadic/chromosomal</td>
<td>AVSD</td>
<td>Intellectual disability, distinct facial features, hearing loss, early onset dementia</td>
<td>78</td>
</tr>
<tr>
<td>Turner syndrome</td>
<td>Monosomy X</td>
<td>Sporadic/chromosomal</td>
<td>BAV, CoA, HLH, ascending aorta aneurysm</td>
<td>Lymphedema, short stature, gonadal dysgenesis, learning disability</td>
<td>78</td>
</tr>
<tr>
<td>Patau syndrome</td>
<td>Trisomy 13</td>
<td>Sporadic/chromosomal</td>
<td>ASD, VSD, polyvalvular disease</td>
<td>Growth retardation, orofacial cleft, microphthalmia, central nervous system abnormalities, polydactyly</td>
<td>78</td>
</tr>
<tr>
<td>Edward syndrome</td>
<td>Trisomy 18</td>
<td>Sporadic/chromosomal</td>
<td>ASD, VSD, polyvalvular disease</td>
<td>Growth retardation, clenched fingers, distinct facial features, central nervous system abnormalities</td>
<td>78</td>
</tr>
<tr>
<td><strong>Structural chromosomal abnormalities</strong></td>
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<tr>
<td>22q11.2 Microdeletion syndrome</td>
<td>Deletion 22q11.2 (TBX1)</td>
<td>AD (de novo &gt;90%)</td>
<td>IAA type B, TOF, PA/VSD, aortic arch abnormalities</td>
<td>Intellectual disability, velopharyngeal cleft/insufficiency, psychiatric disorders, thymic hypoplasia, hypocalcemia, mild facial dysmorphism</td>
<td>40,41,130</td>
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<tr>
<td>Williams syndrome</td>
<td>Deletion 7q11.23 (ELN)</td>
<td>AD (mostly de novo)</td>
<td>SVAS, PAS</td>
<td>Intellectual disability, multiple arterial stenosis, outgoing personality, distinct ‘elfin’ face, hypercalcemia</td>
<td>55,131</td>
</tr>
<tr>
<td>Wolf-Hirschhorn syndrome</td>
<td>Deletion 4p16.3</td>
<td>AD (de novo)</td>
<td>ASD, PS, VSD, TOF</td>
<td>Intellectual disability, growth retardation, central nervous system abnormalities, skeletal abnormalities, ‘Greek helmet’ face</td>
<td>132</td>
</tr>
<tr>
<td>Cri-Du-Chat syndrome</td>
<td>Deletion 5p15.2</td>
<td>AD (de novo)</td>
<td>VSD, ASD, TOF</td>
<td>Severe intellectual disability, high-pitched cry, microcephaly, micrognathia, facial dysmorphism</td>
<td>133,134</td>
</tr>
<tr>
<td>1p36 Deletion syndrome</td>
<td>Deletion 1p36</td>
<td>AD (de novo)</td>
<td>PDA, NCCM</td>
<td>Intellectual disability, microcephaly, eye abnormalities, central nervous system abnormalities, distinct facial features</td>
<td>135</td>
</tr>
<tr>
<td>Disorder</td>
<td>Genomic Abnormality</td>
<td>Mode of Inheritance</td>
<td>Clinical Features</td>
<td></td>
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<tr>
<td>Noonan syndrome and other syndromes</td>
<td>Single gene defect</td>
<td>AD</td>
<td>Short stature, distinct facial features, pectus deformity, webbed neck</td>
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<tr>
<td></td>
<td>PTPN11, KRAS, SOD1,</td>
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<td></td>
<td>Braf, Mek1, Hras,</td>
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<td>Nras, Schoc2, Cbl</td>
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<tr>
<td>Holt-Oram syndrome</td>
<td>Single gene defect</td>
<td>AD</td>
<td>Radial ray defects upper limbs</td>
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<tr>
<td></td>
<td>Tbx5</td>
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<tr>
<td>Alagille syndrome</td>
<td>Single gene defect</td>
<td>AD</td>
<td>Intrahepatic bile duct paucity, skeletal abnormalities, posterior embryotoxon,</td>
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<td></td>
<td>Jag1, Notch2</td>
<td></td>
<td>distinct facial features</td>
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<tr>
<td>CHARGE syndrome</td>
<td>Single gene defect</td>
<td>AD</td>
<td>Coloboma, choanal atresia, retarded growth and development, genital abnormalities,</td>
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<tr>
<td></td>
<td>Chd7</td>
<td></td>
<td>ear anomalies</td>
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<tr>
<td>Char syndrome</td>
<td>Single gene defect</td>
<td>AD</td>
<td>Distinct facial features, fifth finger anomalies</td>
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<tr>
<td></td>
<td>Tafap2b</td>
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<tr>
<td>Kabuki syndrome</td>
<td>Single gene defect</td>
<td>AD</td>
<td>Distinct facial features, skeletal anomalies, fetal finger pads, intellectual</td>
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<tr>
<td></td>
<td>Mll2</td>
<td></td>
<td>disability, growth deficiency</td>
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</tbody>
</table>

CHD, congenital heart disease; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CoA, aortic coarctation; HLH, hypoplastic left heart; ASD, atrial septal defect; VSD, ventricular septal defect; IAA, interrupted aortic arch; TOF, tetralogy of Fallot; PA, pulmonary atresia; SVAS, supravalsar aortic stenosis; PAS, pulmonary artery stenosis; PS, pulmonary stenosis; NCCM, noncompaction cardiomyopathy; HCM, hypertrophic cardiomyopathy; PPH, peripheral pulmonary hypoplasia; PDA, patent ductus arteriosus; AD, autosomal dominant.
**Down syndrome**

Down syndrome, caused by an extra copy of chromosome 21, is the most common chromosomal abnormality among live born infants and is the most frequent cause of intellectual disability. In over 95% of patients, there is a free copy of chromosome 21 in all cells and in most of the remaining patients, a Robertsonian translocation involving chromosome 21 and another acrocentric chromosome leads to the Down syndrome phenotype. The syndrome is characterized by well-defined phenotypic features including characteristic facial features, growth retardation, hypotonia and congenital malformations. About 50% of patients have CHD, most frequently atrioventricular septal defect (AVSD) and VSD. A CHD “critical region” on chromosome 21 as well as several candidate genes have been proposed, but the genetic basis and pathogenesis of CHD in Down syndrome remain largely unknown. Mutations in \textit{ALK2} and \textit{CRELD1} have been identified in some Down syndrome patients with AVSD.

**Williams syndrome**

Williams syndrome is caused by a heterozygous deletion of circa 1.5 - 2 Mb on chromosome 7q11.23. Apart from CHD, which is present in 80% of patients, clinical features include intellectual disability, characteristic facial features (‘elfin facies’), hypercalcemia, connective tissue abnormalities and a friendly and outgoing personality. The most typical CHD in Williams syndrome comprise supravalvar aortic stenosis (SVAS) and peripheral pulmonary artery stenosis, although other CHD have also been described. SVAS is related to the absence of one copy of the Elastin (\textit{ELN}) gene which is located in the deleted locus. Mutations in \textit{ELN} have been identified in patients with non-syndromic SVAS and associated arteriopathies, although some individuals were described to have a “Williams-like” mouth or connective tissue abnormalities such as inguinal hernias.

**Holt-Oram syndrome**

Holt-Oram syndrome is an autosomal dominant ‘heart-hand syndrome’, occurring in about 1 in 100,000 individuals. Affected individuals show radial ray malformations, including an abnormal carpal bone and absent, hypoplastic, or triphalangeal thumbs with or without radial dysplasia. The limb abnormalities may be symmetrical or asymmetrical. Three quarter of patients have CHD, most commonly ostium secundum ASD and VSD, as well as progressive conduction disease. Although rare, other CHD have also been reported. Lower limb defects or other extracardiac malformations or disease (including intellectual disability) do not belong to Holt-Oram syndrome, thus if these are present Holt-Oram syndrome is unlikely.

In 1997, \textit{TBX5} was identified as the causal gene in Holt-Oram syndrome. \textit{TBX5} functions as a transcription factor that has an important role in cardiac growth and development (especially in cardiac septation), in the development of a cardiac conduction system, and in forelimb specification and outgrowth. \textit{TBX5} can interact with other transcription factors including \textit{NKX2-5} and \textit{GATA4}. A pathogenic mutation in \textit{TBX5} can be identified in over 70% of patients, mostly but not always through the mechanism of \textit{TBX5} haplo-insufficiency. About 85% of affected individuals
have been reported to have Holt-Oram syndrome as the result of a de novo mutation. In a small number of patients with Holt-Oram syndrome, mutations in SALL4 have been demonstrated.\textsuperscript{71}

CHD recurrence risks

In only a minority of CHD patients, it is possible to provide a CHD recurrence risk based on known Mendelian inheritance in a family or on figures related to chromosomal abnormalities. When a (genetic) cause is not identified in an individual non-syndromic patient, empirical estimates have to be used to provide recurrence risk numbers. Several studies have shown that there is an increased risk of CHD for relatives of CHD patients, mostly in the order of magnitude of 2 to 15\% for first-degree relatives of a sporadic patient.\textsuperscript{72-74} A recent population-based study in Denmark demonstrated that the relative risk for all types of CHD taken together is 3.2 among first degree relatives and 1.8 among second degree relatives.\textsuperscript{75} Risk numbers vary significantly between different types of CHD, however. For example, the heritability of heterotaxy as well as BAV and other left ventricular outflow tract lesions is estimated to be much higher.\textsuperscript{75-77} Within families, the relative risk is highest for same-type CHD, but the risk is also increased for dissimilar types.\textsuperscript{75} If more than one family member is affected, the recurrence risk for other relatives increases. Moreover, the risk for offspring of women with CHD is higher than for offspring of men with CHD\textsuperscript{72,73}; generally the risk for offspring of female CHD patients is estimated to be about 5-6\%, whereas the risk for offspring of male CHD patients is about 2-3\%. The reasons for this gender difference are unknown; imprinting mechanisms or maternal and environmental factors may play a role. Sibling recurrence risks are generally stated to be 2-3\%. If two siblings are affected, the recurrence risk increases to 10\%.\textsuperscript{78}

Genetic counseling in adult CHD

The population of adult CHD patients is growing, and an increasing number of patients will have children. These patients may have questions regarding the origin and inheritance of their disease and the recurrence risk in (future) offspring. For the individual patient, knowledge about the underlying (genetic) origin of CHD is important because 1) the patient and his or her offspring may be at risk for extracardiac disease (in syndromic cases); 2) an individualized recurrence risk for offspring based on the underlying cause can be established; 3) there may be other relatives for whom genetic or cardiologic examination may be appropriate.\textsuperscript{76,80} The majority of adults with CHD has not had genetic testing or counseling, and those who did in childhood were probably too young to participate in the counseling process. In general, the knowledge of adult CHD patients about inheritance issues, including pregnancy-related topics and recurrence risk in offspring, has been shown to be low.\textsuperscript{81-84} Clinical genetic consultation can aid in increasing awareness and knowledge about such issues. It may help patients to understand the genetic basis of their CHD (and extracardiac disease), and the implications for family members including their offspring.

By means of a detailed medical history of cardiac and extracardiac disease, extensive family history and physical examination aimed at dysmorphic features the clinical geneticist can gather
information to establish a strategy for genetic testing and come to an etiological diagnosis in an individual patient. Subsequently, recurrence risks targeted to the patient’s specific situation can be provided as accurate as possible. In addition to the establishment of an etiological diagnosis and the estimation of a recurrence risk, genetic counseling involves education about management (of cardiac and extracardiac manifestations), inheritance issues and familial implications. Reproductive options, including prenatal ultrasound investigations, prenatal diagnosis and pre-implantation diagnosis, may also be discussed. Moreover, the psychological implications of the diagnosis and recurrence risk are addressed.

As CHD is not uncommon and mostly multifactorial in origin with general recurrence risks applying, at current times it seems not eligible to counsel all adult CHD patients. It is however important to identify the subgroup of patients that is especially likely to benefit from genetic counseling. These patients are summed in Table 3. Physicians caring for adult CHD patients should actively identify those patients and refer them at a low threshold.

**Table 3.** Adult CHD patients who may benefit from clinical genetic testing and counseling

- Patients desiring having children (preconceptional counseling)
- Patients with extracardiac abnormalities/disease
  - Congenital malformations
  - Intellectual disability
  - Dysmorphic features
  - Multisystem involvement (e.g. endocrinologic, hematologic, immunologic or sensorineural disorders)
  - Psychiatric disorders
- Patients with CHD with high risk of 22q11.2 deletion (IAA, TA, TOF, PA with VSD, AAA)
- Patients with a family history of CHD
- Any other patient who is interested in learning about the origin and inheritance of their CHD

CHD, congenital heart disease; IAA, interrupted aortic arch; TA, truncus arteriosus; TOF, tetralogy of Fallot; PA, pulmonary atresia; VSD, ventricular septal defect; AAA, aortic arch anomaly.
Outline of this thesis

This thesis focuses on the genetics of non-syndromic and syndromic CHD, the implications it has for (adult) CHD patients and the adult CHD patients’ perspective on inheritance issues. It presents different types of studies, including clinical and genetic studies in families with multiple affected individuals with CHD and in patient cohorts with specific CHD subtypes, a morphologic study of post-mortem hearts, as well as questionnaire studies.

In Part I of this thesis, clinical and genetic studies in non-syndromic CHD are presented. These studies were aimed to the identification of genes that are implied in human CHD. Chapter 2 describes the linkage analysis and subsequent identification of a disease locus in a large family with a novel phenotype, which resembles a mild form of left atrial isomerism. Chapter 3 presents the analysis of the sarcomere protein gene MYH7 in a cohort of patients with Ebstein anomaly. In Chapter 4, the role of mutations in the cardiac sodium channel gene SCN5A in CHD is explored; on the one hand, a cohort of patients with a septal defect and conduction disease was analyzed for SCN5A mutations. On the other hand, a cohort of SCN5A mutation carriers was evaluated for the frequency of CHD.

Part II focuses on CHD in association with other (extracardiac) abnormalities, including syndromic CHD. Chapter 5 is dedicated to the 22q11.2 deletion syndrome. Although it is currently common practice to test children with specific CHD types for the presence of a 22q11.2 deletion, this has not routinely been performed by patients who have already reached adult age. We therefore evaluated the prevalence of 22q11.2 microdeletion syndrome in adults with TOF and pulmonary atresia with VSD, which is described in chapter 5. In Chapter 6, we focus on CHD in Turner syndrome; in this syndrome left-sided heart defects including aortic valve abnormalities are especially common. We specifically looked at the morphology of the (bicuspid) aortic valve early in development in a selected group of TS patients with adverse outcome, namely in post-mortem heart specimens of Turner syndrome fetuses. Also in the absence of a recognized syndromic etiology, CHD is associated with several extracardiac malformations or disease. As common developmental pathways may underlie these associations, genes implied associated abnormalities may also be implied in cardiac development and CHD, and vice versa. One of the genes that are already well-established in CHD is NKX2-5. More recently, NKX2-5 was also implicated in human thyroid dysgenesis. In Chapter 7 we studied a specific variant in NKX2-5, which we encountered in two unrelated probands with CHD and which had been identified before in a patient with thyroid dysgenesis. Chapter 8 focuses on childhood cancer: we evaluated whether CHD is more frequent in children with neuroblastoma, as was previously reported.

Whereas Part I and II mainly focus on genetic causes and pathogenetic mechanisms leading to CHD, Part III appraises the clinical implications and patients’ perspectives on genetics and inheritance.
of CHD. **Chapter 9** reports on the information adult CHD recalled to have received about the inheritance of their CHD, patients’ knowledge about inheritance and their concerns in this regard. **Chapter 10** focuses on adults with CHD who consulted a clinical geneticist. We report on the etiologic diagnoses that are made by the geneticist in these patients. Additionally, patient satisfaction with genetic counseling and reproductive choices are presented.

Finally, in **Chapter 11**, the major findings presented in this thesis are summarized and future perspectives for research and clinical care are discussed.
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