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Review article

Genetics in congenital heart disease. Are we ready for it?

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A B S T R A C T

Genetics has rightly acquired an important place in almost all medical disciplines in recent years and this is certainly the case in the field of congenital cardiology. Not only has this led to greater insight into the pathophysiology of congenital heart defects but it also has a beneficial impact on patient management. Integration of clinical genetics in multidisciplinary centers of expertise for CHD is therefore a clear recommendation. Adult and pediatric cardiologists play a crucial role in the process of genetic evaluation of patients and families and should have be familiar with red flags for referral for further clinical genetic elaboration, counseling, and eventual testing. Some basic knowledge is also important for the correct interpretation of genetic testing results. In this review article, we provide a practical overview of what genetic evaluation entails, which type of genetic tests are possible today, and how this can be used in practice for the individual patient.

Genética en la cardiopatía congénita: ¿estamos preparados?

Palabras clave:
Cardiopatías congénitas
Consejo genético
Análisis genético

R E S U M E N

En los últimos años, la genética ha adquirido merecidamente un lugar importante en casi todas las disciplinas médicas, y este también es el caso en el campo de las cardiopatías congénitas. Esto no solo ha llevado a una mejor comprensión de la fisiopatología de los defectos cardiacos congénitos, sino que también conlleva un impacto positivo en el tratamiento del paciente. La integración de la genética clínica en centros acreditados para el abordaje de las cardiopatías congénitas es sin duda una recomendación clara. Los cardiólogos pediátricos y de adultos tienen un papel crucial en el proceso de evaluación genética de los pacientes y sus familias, por lo que deben conocer las señales de alerta que justifiquen un estudio genético más o menos elaborado, así como el asesoramiento y la realización de otras pruebas. Para la correcta interpretación de los resultados de las pruebas genéticas, es esencial disponer de algunos conocimientos básicos. En este documento de revisión se proporciona una visión general práctica de lo que implica la evaluación genética, qué tipo de pruebas genéticas son posibles hoy y cómo se aplican al paciente individual en la práctica clínica.

INTRODUCTION

Congenital heart defects (CHD) are among the most common birth defects, affecting approximately 1% of live born children.[1] Significant progress has been made in recent years in both the accuracy of clinical diagnosis and the treatment of CHD. This has increased survival and enhanced quality of life in a large proportion of affected patients, with adults with CHD now outnumbering children in many countries.[2] Along with this progress, the demand and search for possible explanations of the underlying cause of the heart defect has grown in recent years, both from patients and parents, as well as from the care providers involved. Recent technological advances spearheaded our understanding of the genetic basis of syndromic forms of CHD. In isolated CHD, however, knowledge of the molecular mechanisms is largely absent, although several lines of evidence indicate that genetics has an important contribution to make: the incidence of CHD affecting both twins is higher in monozygotic than in dizygotic twins,[3] the recurrence risk of CHD for siblings and offspring of patients with CHD is higher than in the general population,[4,5] and using high throughput technologies allows identification of a genetic anomaly in up to one third of patients with CHD, taking syndromic and nonsyndromic forms together.[6]

Knowledge of the underlying genetic cause(s) will help to fine tune personalized counseling and treatment options. It has already been established for various lesions that an underlying genetic defect influences the management and outcome of CHD. For example, patients with an atrial septal defect (ASD) due to *NKX2.5* pathogenic variants should be carefully monitored for arrhythmias.[7] Furthermore, especially in children, the underlying defect

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Abbreviations

ASD: atrial septal defect
BAV: bicuspid aortic valve
CHD: congenital heart defect
CNVs: copy number variations
DTC: direct-to-consumer

may indicate a risk for noncardiac complications such as neurodevelopmental delay, respiratory problems or renal dysfunction, requiring early intervention or follow-up to prevent or alleviate these manifestations.

Finally, knowledge of the genetic basis will affect counseling for the recurrence risk in siblings and offspring and may provide access to reproductive options using prenatal and preimplantation genetic diagnosis. Over the past decades, integration of epidemiological, clinical and genetic data have improved knowledge of CHD recurrence significantly. The prevailing hypothesis of CHD being inherited as a multifactorial trait was already challenged during the 1980s.[8] Rose et al.[9] observed a higher than expected occurrence of CHD based on multifactorial models in several families. Indeed, increased use and further refinement of imaging techniques led to the important observation that some (but not all) lesions belong to a broader phenotypic CHD spectrum that may occur in a familial context. Established examples are left-sided outflow defects: family members of children with hypoplastic left heart syndrome or severe left ventricular outflow tract (LVOT) obstruction were found to have a bicuspid aortic valve (BAV) at higher than expected rates.[10,11] In addition to the above, knowing the underlying cause may have a "therapeutic" effect, helping patients and their relatives to cope with and accept a rare disease.

Novel genetic techniques based on high throughput analyses, shorter turnaround times and at affordable costs have increased accessibility to genetic diagnosis. It is expected that this trend will continue at a rapid pace and genetics will provide answers in a growing number of cases.

Nevertheless, thorough genetic analysis comes with increasing challenges to interpretation of the results, and an ever-changing field of possibilities and drawbacks. This situation has prompted this review on the current status of genetic testing in the field of CHD.

BASICS OF GENETICS

Defining genetics

No testing without counseling!

When defining "genetics", it is important to make a distinction between the concepts of "genetic counseling" and "genetic testing". We emphasize from the outset that both concepts are inextricably linked—genetic testing must always be accompanied by correct counseling—but counseling will not result in testing in all cases.

According to the World Health Organization, genetic counseling is defined as "the process through which knowledge about the genetic aspects of illnesses is shared by trained professionals with those who are at an increased risk of either having a heritable disorder or of passing it on to their unborn offspring".

Genetic counseling in the setting of CHD was introduced more than half a century ago, whereby the most important setting was to inform parents of an affected child about their recurrence risk. Early studies already nicely demonstrated that informing parents in a dedicated counseling process had a beneficial effect.[12] More recent research has confirmed the beneficial effects of individualized genetic counseling sessions to the parents of children with CHD with regard to improving knowledge about the causes of CHD and enhancing psychosocial functioning, strongly recommending their inclusion in routine clinical practice.[13] With increasing numbers of adults with CHD, the indications for genetic counseling and testing have been expanded and "genetics" are listed as a requirement for adult CHD programs in the recently published American College of Cardiology/American Heart Association guidelines on adult CHD.[14]

Genetic counselors in CHD are graduate level trained health care professionals who have received training in both medical genetics and counseling with a particular focus on CHD. Genetic counselors will draw a 3-generation pedigree and collect all relevant clinical data from the proband and family members with special attention on miscarriages or neonatal deaths. Apart from their role in family history taking and in counseling patients and families about recurrence risk, risk for a specific syndrome and interpretation of results, genetic counselors may play an important role in triaging patients who should be referred for a complete genetic evaluation.[15]

Genetic elaboration of CHD requires a multidisciplinary approach in which, in addition to the (pediatric) cardiologist and genetic counselor, the clinical geneticist also plays a crucial role. Clinical geneticists are physicians who have undergone specific training in diagnostic evaluation, management, and genetic counseling. Training programs and certification are nation specific. Clinical geneticists will determine whether the heart defect is isolated or part of a syndrome, which is required to guide genetic testing and to determine the medical approach. Based on large epidemiological studies, syndromic cardiovascular malformations comprise at least 25% of all cardiovascular malformations.[4,16] Research in the setting of 22q11 deletion has already shown that cardiologists are less good at assessing syndromes and that clinical genetic evaluation is therefore desirable.[17] Once it has been determined whether or not a patient has a syndromic entity, the medical management of syndromic forms can also be better coordinated by a clinical geneticist in the context of a multidisciplinary team —patients with isolated CHD forms are of course best followed up by the (pediatric) cardiologist.[8]

Another important issue to take into account in the process of genetic counseling and testing is consent. It is essential for any genetic test that the patient (or his or her legal representative) is aware of the benefits and risks of such testing and gives written consent for the test. It is outside the scope of this article to discuss (important) aspects such as incidental findings and presymptomatic testing, but we would like to briefly address direct-to-consumer (DTC) testing.

In many countries, long gone are the days when genetic testing was confined to certified clinical genetic centers (laboratory-directed testing) for which strict rules apply for conducting clinical and molecular diagnostics. As a result of the technical progress in genetic testing on the one hand, and the increasing public demand on the other hand, significant growth has been observed in companies that offer testing known as DTC. These are tests in which samples (blood or saliva) are directly mailed to the laboratory, without preemptive counseling. DTC genetic tests may detect severe and highly penetrant monogenic disorders or genetic variants associated with increased susceptibility for common and complex diseases. There are concerns that variant interpretation from DTC testing may not always be correct. There have already been reports of cases of unnecessary treatment in healthy family members or false reassurance based on incorrect information.[18] One study showed that 40% of variants in a variety

of genes reported in DTC raw data were false positives.[19] This false information severely impacts patients and families in the first place but also overloads genetic counseling services who are consulted to clarify and rectify the results of tests ordered elsewhere.[20] It goes without saying that these issues create tension in the context of DTC genetic testing regarding the expectations and normative assessment of communication strategies.[21]

For these reasons, the European Society of Human Genetics has developed a policy on the advertising and provision of predictive genetic tests by such DTC companies.[22] We argue against the use of DTC testing in the CHD genetic testing context.

The technical (r)evolution of genetic testing

Evolution in cytogenetics

The seminal discovery of trisomy 21 as the genetic cause of Down syndrome in 1959[23] introduced genetic testing in CHD. Since then, novel methodologies and technical fine tuning have been instrumental in identifying the genetic cause of (mainly syndromic forms) of CHD. Classic karyotyping with G-banding has a rather low resolution of 3-5 Mb, and is currently only performed for specific indications, such as the confirmation of (mozaic) aneuploidies (Down syndrome, Turner syndrome, mosaic trisomy 8) and familial translocations.

Fluorescent *in situ* hybridization (FISH) makes use of a fluorescently labeled probe targeting specific genomic regions. It is used for the targeted detection of aberrations below karyotyping resolution, such as the 22q11.2 or 7q11.2 microdeletions in velocardiofacial syndrome and Williams-Beuren syndrome, respectively. A major breakthrough came with the introduction of array comparative genome hybridization (ArrayCGH),[24] also called chromosomal microarray analysis. Chromosomal microarray analysis competitively hybridizes shredded DNA of control and patient DNA, labeled with different fluorochromes on an array containing tens of thousands of molecular probes dispersed over the reference genome. Next, automatic reading of differences in color intensities detects genome-wide copy number variations (CNVs), ie, deletions or duplications, as small as 100 kb This technique is also referred to as "molecular karyotyping". Single nucleotide polymorphism (SNP) array is a similar test that uses SNPs to detect regions with a loss of heterozygosity. However, the huge amount of structural variability in the human genome has hampered straightforward interpretation of test results, especially in the years following the introduction of the test in the diagnostic setting. Initiatives such as the Database of Genomic Variants[25] have been detrimental in documenting normal variation while databases such as DECIPHER[26] have played a prominent role in identifying novel genomic structural defects as the basis of disease. Indeed, molecular karyotyping introduced the concept of "reversed genetics", a strategy whereby patients with the same genetic variant are compared to identify a genotype-phenotype correlation and delineate novel clinical entities, such as Koolen-de Vries syndrome.[27] The most frequently associated structural variants in CHD known to date are reported in more detail below. More recently, chromosomal microarray analysis is being replaced by methods based on low-coverage ("shallow") genome sequencing technologies (see below).

Evolution in molecular genetics

The combined use of polymerase chain reaction and Sanger sequencing introduced the use of molecular analysis in the clinic. Nevertheless, analysis was expensive and time-consuming. Moreover, the identification of novel genes causing cardiovascular phenotypes was restricted to syndromic forms that could be investigated through linkage analysis in large families with dominant inheritance for the condition (eg, Noonan syndrome[28]), or in consanguineous families with recessive conditions (eg, Ellis-van Creveld[29]), while candidate gene approaches only sporadically identified a casual defect, often helped by the previous detection of a microdeletion encompassing the candidate gene (eg, CHARGE syndrome[30]). A second breakthrough came with the introduction of next-generation (or massively parallel) sequencing (NGS).[31] In brief, in NGS, DNA fragments of the region(s) of interest (either a specific panel of genes, the exome, ie, the coding regions of the DNA, or the genome, ie, the whole DNA sequence) are sequenced in parallel and the obtained "reads" are aligned to the reference sequence. The coverage at a certain genomic position refers to the number of times a base at a certain genomic position is independently sequenced. For a reliable interpretation, at least a coverage of 20x is necessary. Short read sequencing methods are primarily used in clinical laboratories because of their cost-effectiveness and low per-base error rate. The application of exome sequencing may than help to either analyze an extensive number of genes known to cause CHD and even to identify novel CHD candidate genes. However, short read lengths (50–500 bp) can produce misalignments and misassemblies in areas of high genome complexity, are unable to cover repeats reliably, and impair phasing of variants. Furthermore, the amplification process, which is indispensable in short read sequencing, creates an underrepresentation of bases in areas of high or low guanine-cytosine (GC) content.

Again, due to the huge variability in the human genome, variant interpretation is crucial, based on freely accessible databases and causality prediction with bioinformatic tools (see below). Similar to DECIPHER, databases such as GeneMatcher[32] arose to catalyze rare disease discovery by providing a platform to connect clinicians and researchers from around the world who share an interest in the same gene.

Third generation sequencing: evolution to a single genomic test?

Despite copy number analysis (with a resolution of at least 100 kb) and high performant sequence analysis using short read NGS, most of the structural variation is still missed. Structural variants comprising CNVs, inversions, and translocations make up to 10% of the genome and contribute greater diversity between 2 human genomes than any other form of genetic variation and may affect expression of genes.

In addition to large chromosomal defects such as translocations, inversions and especially CNVs, smaller cryptic structural variants (ranging from 50 bp to 50 kb) can also cause human diseases by affecting gene function or expression; eg, structural variants $>$ 20 kbp are up to 50-fold more likely to affect the expression of a gene compared with an SNV.

Third generation genome sequencing platforms use long read sequencing (LRS, $>$ 10 kb reads) and enable the elucidation of structural variations at a previously unparalleled resolution and overcome most of the shortcomings of short read sequencing.[33] This will eventually lead to a single analysis to cover most genomic DNA variation within the near future.

Third generation genome sequencing can be combined with other NGS approaches, such as transcriptome sequencing (library of expressed genes in a certain cell type) to identify variation at the level of expression and splicing. Nevertheless, the main hurdle will remain the interpretation of the variants, which often requires additional validation and which will remain the main burden in the diagnostic application of these techniques (see below).

Interpretation of genetic test results

Gene curation need

With the technical progress of genetic testing as described above, now enabling simultaneous testing of large numbers of genes, the temptation has been great to effectively set up more extensive panels for specific disorders. Commercial genetic laboratories in particular have taken this path and now offer panels for CHD that contain > 100 genes, for example.

However, some caution is advised in this trend. For multiple disorders, there is strong evidence that testing more genes inevitably leads to detection of more "variants of unknown significance" or VUSs, the interpretation of which is not easy and sometimes even risks creating unnecessary anxiety and discrimination in patients.[34,35] When selecting genes for inclusion in diagnostic panels or reporting from exome/genome sequencing, the clinical validity, ie, the strength of evidence that variation in that gene predisposes to the disease, needs to be carefully considered. A framework for semiquantitative assessment of gene-disease validity has been developed for many diseases (but not yet for CHD) by the Clinical Genome Resource, or ClinGen. In this framework, genes are classified into prespecified tiers based on the clinical, genetic and experimental evidence, along with discussion and consensus of clinical domain experts. These clinically validated genes can be used to prioritize genes for research and inform which genes should be included in disease panels.[36,37]

Variant curation need

The possibility of generating large amounts of genetic data through broad genetic screening has allowed rapid and more accurate genetic diagnosis. However, as already mentioned, each analysis yields multiple genetic variants (up to 50 000 variants per exome), which need appropriate interpretation. Distinguishing benign from pathogenic variants is essential for translating genetic results into clinical practice but remains challenging. Moreover, the number of VUSs is still too high. In 2015 the American College of Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) proposed a guideline to classify genetic variants for Mendelian disorders.[38] Classification is based on 28 criteria to finally classify a variant as benign (class 1), likely benign (class 2), variant of unknown significance (class 3), likely pathogenic (class 4) or pathogenic (class 5), where classes 2 and 4 provide greater than 90% certainty of a variant either being benign or disease-causing. The criteria include clinical findings, data retrieved from large human exome databases such as gnomAD and ExAC and data addressing the structural effect of a variant on the DNA/protein level.

Correct and detailed clinical findings are required, not only of the proband, but often also of family members. Cooperation with—and from—family members is therefore highly important, both in a diagnostic setting and in the further communication of the results. Further segregation of identified variants (both copy number variants and single nucleotide variants) requires verification in first-degree relatives, in which, in addition to DNA studies, clinical cardiovascular examination is also required to correctly assess the status of the individual. It is important to communicate this properly to the patient from the beginning of the counseling and testing process.

If an abnormal test result is confirmed, it is also recommended to check the result further with additional family members. Caregivers are not permitted by law to contact family members; contact must go through the patient (or his or her representative) and it is also important to communicate this correctly to the patient when discussing the results.

In addition to clinical criteria in patients and family members, the molecular characteristics of the variants are also taken into account. Computational analysis of a variant with modeling of the expected effects of the gene or variant on protein structures and function can provide supporting evidence to establish pathogenicity. Despite careful interpretation and international initiatives for data curation, the clinical consequences for many variants obtained through in-depth molecular analysis remain unknown. Several tools, including transcriptome, proteomic, metabolomic, lipidomic and methylomic analysis, may help to identify the molecular consequences. However, the respective (multi)omic signatures are unknown for most genes defective in CHD. In addition, some genes may only be relevant during cardiac development and postnatal testing on other tissues might be irrelevant. Also, animal modeling for specific diseases is time-consuming, expensive, and impossible in the clinical setting. However, direct mutagenesis using the CRISPR_Cas9 techniques is becoming increasingly efficient and may eventually be helpful in the interpretation of genomic variants.

Although the ACMG/AMP guidelines definitely introduced major improvements in the interpretation of genetic variants, they often still leave room for subjective interpretation and therefore several groups have proposed more gene specific classifications.[39]

Since the publication of the guidelines, several tools have been developed to aid the interpretation of genetic variants (table 1). Moreover, several online repositories are also available to consult variants which have been previously classified by other laboratories (table 1). Caution, however, should be exercised when consulting these repositories since variant interpretation remains subjective and is not always performed by experienced groups. Clinvar, one of these archives, not only provides an interpretation of a specific variant, it also provides a level of review supporting the assertion of clinical significance.

Retesting

Genetics is a dynamic and rapidly evolving field. For many clinical phenotypes, new genomic data are regularly discovered and test findings issued today may be outdated tomorrow. Therefore, regular and careful reconsideration of genetic counseling and testing should take place, especially in those individuals/families with a high level of suspicion but without identification of a genetic defect. In the same line, genetic variant interpretation can change over time and previously found genetic variants (especially VUSs) should be regularly reassessed in light of new published data.[57]

Again, and even more so than in the initial counseling process, retesting and communication of altered test results should be undertaken with care to ensure that the results are correctly interpreted by patients and their families.[58]

THE SPECTRUM OF GENETIC DEFECTS IN CHD

Up to 25% to 30% of patients with CHD have other associated extracardiac manifestations.[59] The association of CHD in several chromosomal aneuploidies and CNVs such as Down syndrome, Turner syndrome and 22q11 deletion syndrome has been well-stablished. Other CNVs and single gene variations have also shown high penetrance of CHD. For the other nonsyndromic CHD, several genes showing Mendelian inheritance (mostly autosomal dominant but in some cases also autosomal recessive) have been

Table 1
Overview of freely available online tools for classification and interpretation of genetic variants

Tools for the classification of genetic variants	
Tool	Description
Clingen Pathogenicity Calculator[40,41]	Based on ACMG/AMP guidelines and further expert input Need for manual data entry Registration needed Option to directly submit to Clinvar
Varsome[42,43]	Based on ACMG/AMP guidelines, no expert input? but extra bioinformatic support Automated variant interpretation Option of manual modification of the classification
Intervar[44,45]	Based on ACMG/AMP guidelines, no expert input? Automated variant interpretation Option of manual modification of the classification
Franklin[46]	Based on ACMG/AMP guidelines, no expert input? Automated variant interpretation Option of manual modification of the classification
Cardioclassifier[47,48]	Only for cardiovascular diseases Based on ACMG/AMP guidelines and some specific expert knowledge Automated variant interpretation, no option of manual modification of the classification Free registration needed
Online repositories	
Clinvar[49,50]	Partner of ClinGen Classification is reviewed by experts and assigned a reviewed status Regular update
Leiden Open Variation Database[51,52]	Partner in the Human Variome Project Regular update of classified variants
Universal Mutation Database[53,54]	Partner in the Human Genome Variation Society Data restricted to certain locus-specific variants
Human Gene Mutation Database[55,56]	Free registration needed Repository of main published data on a certain variant rather than an interpretation archive Regular update

ACMG/AMP, American College for Medical Genetics/Association for Molecular Pathology.

identified. Of note, some of these genes might be involved in both syndromic and nonsyndromic cases. Table 2 and table 3 provide a summary of several forms of CHD associated with genetic disorders, as well as the most relevant clinical manifestations of the most frequent syndromes. Overall, many genes involve transcription factors, signaling pathways, or chromatin remodelers. Hence, the dosage and alteration of gene expression is a likely relevant mechanism in CHD. Therefore, other mechanisms, including structural variants, might currently be underdiagnosed in CHD. Also, altered gene dosage at critical developmental stages offers a window for environmental factors to interfere with cardiac development. Finally, somatic mosaicism in cardiac progenitor cells is still under debate.

Gene identification in isolated CHD has been hampered by several factors: first, defects in different genes may result in similar phenotypes, and different phenotypes may result from defects in the same gene. Second, especially in sporadic cases, the CHD may have a multifactorial cause. Despite high throughput molecular screening, unraveling multifactorial disease is still in its infancy. Recent advances in polygenic risk scores have been proposed for familial cancer and cardiomyopathies. It is to be expected that this will also hold true for CHD.

PRACTICAL APPLICATIONS OF GENETIC EVALUATION IN CHD

Along with increasing knowledge, the clinical impact of genetic testing in CHD shows continuous expansion. However, to incorporate genetic testing as part of the standard care of patients with CHD, several considerations deserve attention.

First, genetic testing should definitely be considered in specific subgroups of patients: those with syndromic features and those with multiple affected family members are most likely to be affected by an underlying genetic problem. Second, genetic testing and counseling should be tailored to the individual patient. The selection of the genetic test, as well as the appropriate timing, should be based on an individual basis. Third, when a genetic cause of CHD is identified, this should be accompanied by counseling to discuss appropriate management of the patient and his/her family and—when indicated—the recurrence risk.

Last, but definitely not least, genetic testing needs to take psychological, social and cultural aspects into account. The decision to proceed with such testing should be a well-informed decision in which the patient has the final word.

Some of these issues are discussed in more detail below and are illustrated in figure 1.

Who should be referred for genetic evaluation?

A first situation in which genetic testing should be considered, is in case of other extracardiac abnormalities, suggesting a syndromic entity. To correctly identify children and adults in this group, an accurate review of the medical history and extensive phenotypic characterization by a clinical geneticist is essential. Moreover, phenotypical screening of first degree family members can be necessary to identify syndromic features. Clinical manifestations that should trigger suspicion of an underlying syndromic problem are intellectual disability or sensory deficits, the presence of dysmorphic features and/or small stature, and the association of other congenital or endocrine disorders.[15,60]

A second situation in which genetic evaluation should be considered is when multiple family members are affected. Familial forms of CHD represent a small number of all CHDs, as reflected in a

Table 2
Overview of the different genes and syndromes associated with congenital heart defects

Congenital heart defect	Associated genes (nonsyndromic forms)	Associated syndromes
(T)APVR		Turner syndrome (*monosomy X*) Kabuki syndrome. (*MLL2, KDM6A*)
ASD	*MYH6, ACTC1, GATA4, TBX20, TLL1, NKX2.5, CITED2, GATA6, TBX5*	Down syndrome (*trisomy 21*) 1p36 deletion syndrome Holt-Oram syndrome (*TBX5*) Ellis-van Creveld syndrome (*EVC**) Kabuki syndrome (*MLL2, KDM6A*) Rasopathies (*PTPN11, HRAS*)
VSD	*GATA4, NKX2.5, CITED2, TBX5, ETS1*	Down syndrome 22q11deletion syndrome 1p36 deletion syndrome Jacobsen syndrome (11q terminal del) Holt-Oram syndrome (*TBX5*) Kabuki syndrome (*MLL2, KDM6A*) Ellis-Van Creveld syndrome (*EVC**) Rasopathies (*PTPN11, HRAS*)
AVSD	*GJA1**, *GATA6, GATA4, CRELD1, NR2F2*	Down syndrome (*trisomy 21*)
HLHS	*GJA1**, *NKX2.5*	Turner syndrome (*monosomy X*) Jacobsen syndrome (11q terminal del) Adams-Oliver syndrome (*NOTCH1*) Kabuki syndrome (*MLL2, KDM6A*) CHARGE syndrome (*CHD7*) 22q11.2 deletion
TOF	*NKX2.5, GATA4, GATA6, TBX1, JAG1, ZFPM2*	Down syndrome (*trisomy 21*) 22q11 deletion syndrome 1p36 deletion syndrome CHARGE syndrome (*CHD7*) Kabuki syndrome (*MLL2, KDM6A*) Alagille syndrome (*JAG1, NOTCH2*) Myhre syndrome (*SMAD4*)
TGA		22q11 deletion syndrome Jacobsen syndrome (11q terminal del) MED13L related intellectual disability
Truncus arteriosus	*NKX 2.5, NKX2.6, GATA6, TBX1, ACTA2, R187*	22q11 deletion syndrome CHARGE syndrome (*CHD7*)
Aortic coarctation/interrupted aortic arch	*NKX 2.5, NKX2.6, GATA6, TBX1*	Turner syndrome (*monosomy X*) 22q11 deletion syndrome CHARGE syndrome (*CHD7*) Myhre syndrome (*SMAD4*)
Aortic valve anomalies	*NOTCH1, SMAD6, ROBO4*	Turner syndrome (*monosomy X*) Jacobsen syndrome (11q terminal del) Adams-Oliver syndrome (*NOTCH1*) Kabuki syndrome (*MLL2, KDM6A*)
Ebstein anomaly	*GATA4, NKX2.5, MYH7*	1p36 deletion syndrome CHARGE syndrome (*CHD7*) VACTERL association Kabuki syndrome (*MLL2, KDM6A*) Holt-Oram syndrome (*TBX5*) Cornelia de Lange syndrome
Pulmonary valve anomaly	*GATA4*	Rasopathies (*PTPN11, HRAS*)
Supravalvular aortic and pulmonary stenosis	*ELN*	Williams-Beuren syndrome (*deletion 7q11.2*) Alagille syndrome (*JAG1, NOTCH2*)
PDA	*PRDM6, ACTA2, R187, MYH11*	Char syndrome (*TFAP2B*) 1p36 deletion syndrome

ASD, atrial septal defect, AVSD, atrioventricular septal defect; HLHS, hypoplastic left heart syndrome; PDA, persistent ductus arteriosus; (T)APVR, (total) abnormal pulmonary venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
* Autosomal recessive inheritance.

Danish population study where only 2.2% of the CHD were familial.[4] Nevertheless, high diagnostic yields can be achieved in some families as shown by cases of familial supravalvular aortic stenosis in which genetic involvement can be found in 85% of families.[61]

Genetic testing may also be considered in neonates and infants with CHD. Genetic factors are important determinants of neurodevelopment and extracardiac lesions in children with CHD.[62,63] Knowledge of this genetic predisposition might improve the long-term outcome of these children.

In addition to the indications mentioned above, adult patients with CHD and an active desire to have children (both men and women) are best referred for genetic counseling and possible testing. The advent of new preconceptional and prenatal techniques allows the preclusion of transmission of the CHD to the next generation. If there is an unknown underlying genetic disorder,

Table 3
Overview of the main systemic and cardiovascular features of genetic syndromes associated with CHD

Syndrome	Molecular diagnosis	Main systemic features	Main cardiovascular features
Chromosomal aneuploidy			
Down syndrome	Trisomy 21 Translocation of chromosome 21 Mozaicism	Characteristic facial features Intellectual disability Hypotonia, short stature Gastrointestinal atresia	Frequent: AVSD, VSD, ASD Other associated defects: PDA, TOF
Turner syndrome	Monosomy X mozaicism	Webbed neck and low posterior hairline Lymphedema Short stature, barrel chest Delayed puberty Infertility Hearing loss, ENT problems Liver disease	Frequent: BAV, CoA, ascending aortic dilatation Other associated defects: (T)APVR, HLHS
CNVs			
22q11 syndrome	Deletion 22q11.2	Characteristic facial features, nasal speech Palatal abnormalities and feeding problems Learning difficulties Immunodeficiency Hypocalcaemia	Frequent: TOF, truncus arteriosus, VSD, IAA Other associated defects: TGA, ASD, TOF, HLHS
Williams-Beuren syndrome	Deletion 7q11.23	Dysmorphic features "Social personality", psychiatric problems Endocrine abnormalities Skeletal and connective tissue anomalies	Frequent: SVAS Other associated defects: SPVS, PPS
Jacobsen syndrome	Deletion 11q23 terminal	Dysmorphic features Growth retardation and intellectual disability Thrombopenia and platelet dysfunction Immunodeficiency	Frequent: VSD, mitral valve anomalies, BAV, HLHS Other associated defects: TGA, AVSD, PVS,PDA
1p36 deletion syndrome	Deletion 1p36	Dysmorphic features Intellectual disability Structural brain anomalies Vision and hearing loss Obesity	Frequent: ASD, VSD, Ebstein, PDA, TOF Other associated defects: LVNC, DCMP
8p23.1 deletion syndrome	Deletion 8p23.1	Diaphragmatic hernia	ASD, cor triatriatum, VSD, TOF
Single gene variation			
Alagille syndrome	*JAG-1, NOTCH-2*	Characteristic facial features Cholestasis Posterior embryotoxon Butterfly vertebrae	Frequent: PPS, SVAS Other associated defects: ASD, VSD, TOF
Holt-Oram syndrome	*TBX5*	Radial ray defects	Frequent: ASD (monoatrium), conduction anomalies
Char syndrome	*TFAP2B*	Characteristic facial features Aplasia/hypoplasia mid-phalanx of fifth finger Mild ID	Frequent: PDA
Ellis-Van Creveld syndrome	*EVC*[*]	Characteristic facial features Rhizomelic short stature polydactyly Nail hypoplasia Dental abnormalities Oral frenula	Frequent: ASD (monoatrium) Other associated defects: mitral and tricuspid anomalies, PDA, VSD, HLHS
Adams-Oliver syndrome	*ARHGAP31* *DOCK6*[*] *RBPJ* *DLL4* *NOTCH1* (mostly associated with CHD)	Skin and scalp defects Limb anomalies: syndactyly, polydactyly, short distal phalanx Microcephaly and developmental disorder in 1/3	Frequent: parachute MV, BAV, AS, CoA, HLHS Other associated defects: TOF, ASD, VSD, truncus arteriosus
Kabuki syndrome	*MLL2* *KDM6A*	Characteristic facial features Intellectual disability Skeletal anomalies: scoliosis, hip dysplasia, vertebral anomalies Urogenital anomalies	Frequent: BAV, CoA, HLHS, ASD, VSD Other associated defects: PAPVR, TOF, PVS, mitral valve anomalies
CHARGE syndrome	*CHD7*	Coloboma Choanal atresia Growth retardation and intellectual disability Urogenital anomalies Ear anomalies and hearing loss Cranial nerve palsy	Frequent: TOF, IAA, truncus arteriosus Other associated defects: vascular rings, AVSD, VSD, PDA

Table 3 *(Continued)*
Overview of the main systemic and cardiovascular features of genetic syndromes associated with CHD

Syndrome	Molecular diagnosis	Main systemic features	Main cardiovascular features
Noonan syndrome	*PTPN11* (associated with PVS) SOS1 (associated with ASD) RAF1 (associated with HCMP) Other: *RIT1, KRAS, SHOC2, NRAS, SOS2 and BRAF*	Characteristic facial features, webbed neck Short stature and pubertal delay Hypothyroidism Hematologic anomalies and malignancies Lymphedema	Frequent: HCMP, PVS Other associated defects: mitral valve defect, ASD, CoA, TOF
Costello syndrome	*HRAS*	Coarse facial features Deep creases of palms and soles Hypotonia, feeding problems Higher risk for malignancies Intellectual disability	Frequent: HCMP, PVS Other associated defects: mitral valve defect, ASD, CoA, TOF
Leopard syndrome	*PTPN11* *RAF1* *BRAF*	Multiple lentigines of the face, back and upper trunk Characteristic facial features	Frequent: HCMP, PVS Other associated defects: mitral valve defect, ASD, CoA, TOF
Myhre syndrome		Characteristic facial features Mild intellectual disability Autism Short stature Thick skin Joint contractures Cataract	Aortic stenosis, TOF

ASD, atrial septal defect; AVSD, atrioventricular septal defect; BAV, bicuspid aortic valve; CHD, congenital heart defects; CoA, coarctation of the aorta; DCMP, dilated cardiomyopathy; HCMP, hypertrophic cardiomyopathy; HLHS, hypoplastic left heart syndrome; IAA, interrupted aortic arch; LVNC, left ventricular non compaction; PAPVR, partially abnormal pulmonary venous return; PDA, persistent ductus arteriosus; PVS, pulmonary valve stenosis; SVAS, supravalvular aortic stenosis; (T)APVR, (total) abnormal pulmonary venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.
* Autosomal recessive inheritance.

genetic counseling is still very important to estimate the recurrence risk.

As already mentioned, adult patients with CHD who underwent genetic evaluation with older techniques might benefit from a reevaluation and retesting.

Tailoring genetic testing

Selecting the most appropriate genetic test for each individual CHD patient is very important and requires close collaboration between clinical and molecular geneticists. It has been shown that a careful pretest review by a genetic counselor in consultation may reduce the proportion of inappropriate tests by 26%.[64] The choice of technique during genetic diagnosis is highly dependent on the clinical presentation and family history. Figure 1 shows a flowchart on how to tailor these techniques to a specific patient.

Actionability of genetic findings

When referring a patient for genetic counseling and testing, the key question is always whether identification of a genetic defect can be of benefit to the patient or his/her family. In this respect, the following arguments can be taken into account:

Improved management

Knowledge of an underlying genetic problem can be important to diagnose and improve management of extracardiac complications in children and adults with CHD. For example, patients with 22q11 deletion may have decreased T cell immunity and therefore be at risk of severe infectious diseases; patients with Alagille syndrome can suffer from ophthalmologic and liver complications; children with Noonan syndrome have short stature and may benefit from growth hormone therapy.

Some genetic defects have been associated with an increased risk of other cardiovascular complications. A known example is the association between ASD and conduction disorders in those patients carrying a pathogenic variant in *NKX2.5*. These patients are more likely to develop atrioventricular block, ventricular dysfunction, and sudden cardiac death.[65] Various types of CHD have been associated with genes causing familial cardiomyopathy. Some examples are the *ACTC1*, *MYH6*, and *MYBPC3* genes.[66–68]

Recurrence risk and implications for other family members

When discussing recurrence risk, knowledge of an underlying genetic entity is essential. Risk estimates will also vary according to the setting of siblings or offspring and, in some cases, risks differ for fathers and mothers. If there is a known genetic disorder, recurrence risk in a sibling will greatly depend on the type of inheritance and on the de novo character of the anomaly. For those genetic disorders with an autosomal recessive pattern, the recurrence risk is 25%. For those with an autosomal dominant pattern, the recurrence risk will be 50% if one of the parents is affected and up to 1% in case of a *de novo* variation.[69] For adult patients with CHD with a known genetic condition, the recurrence risk for the offspring will be 50% if the disorder is autosomal dominant. For those patients with autosomal recessive anomalies, the recurrence risk for children is similar to that in the general population, but each child will be carrier of 1 allele with the anomaly. If no underlying genetic anomaly is found, the recurrence risk is still higher than in the general population. Considering all CHD together, the recurrence risk for siblings is estimated at 2.1%

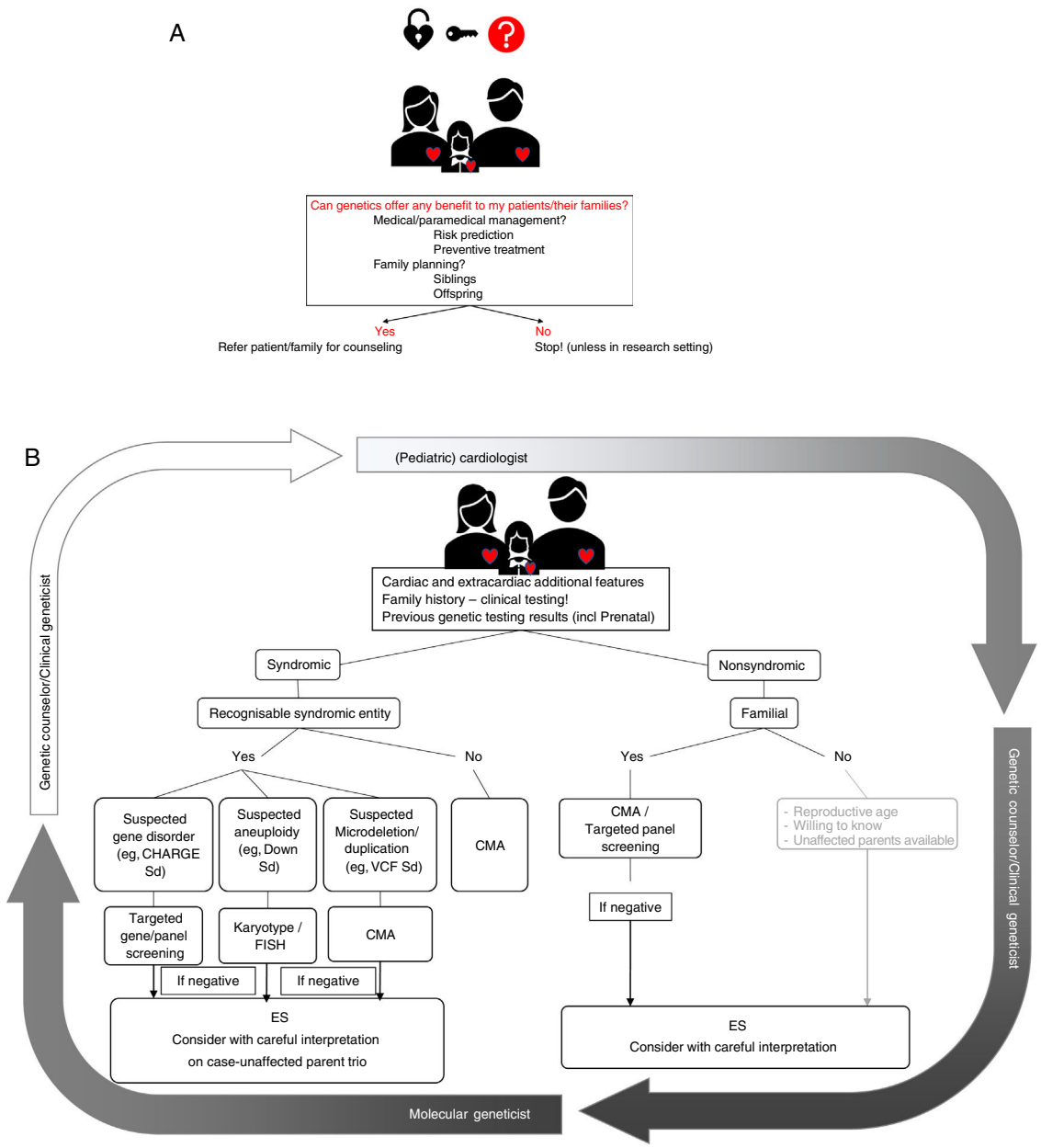

Figure 1. The process of genetic evaluation in congenital heart disease A: carefully weigh the possible benefit of genetic assessment. B: algorithm for clinical/molecular testing. In a first step, (pediatric) cardiologists in collaboration with clinical geneticists and genetic counselors will check for additional cardiac and clinical features to rule out syndromic entities. Based on this, subsequent appropriate genetic testing will be set up in a stepwise approach. Exome sequencing is a final step that needs careful consideration and interpretation. Results will be relayed to the clinician and eventually to patients with appropriate counseling. CMA, chromosomal microarray analysis; ES, exome sequencing; FISH, fluorescent *in situ* hybridization; Sd, syndrome; VCF, velocardiofacial syndrome.

and for the offspring at 4.4%, with women in general having a higher recurrence rate than men.[70] Table 4 summarizes the known epidemiological recurrence risk for the most common CHD in the absence of a known underlying genetic cause. Some lesions such as heterotaxia, right ventricular outflow track obstruction and atrioventricular septal defect, present higher familial clustering. The recurrence risk is 80-fold to 25-fold higher than in the general population.[4]

Another important aspect in terms of counseling family members is the need for further clinical assessment. For some lesions, such as LVOT obstruction, there is known variation in the clinical spectrum ranging from an asymptomatic BAV to a severe hypoplastic left heart syndrome.[75] In one study, the relative risk of having a BAV in a parent or a sibling of a patient with a LVOT obstructive lesion was 5.05 (95% confidence interval, 2.2-11.7).[11] This high incidence together with the fact that many of the complications of left-sided lesions are treatable or preventable, led to the rationale that first degree family members of patients with LVOT obstruction should undergo echocardiographic assessment.[76] Currently no systematic screening of family members is recommended for other forms of nonsyndromic CHD.

Prenatal diagnosis and fetal screening

Prenatal diagnosis is possible whenever a genetic cause of the CHD has been identified. In this case, transmission to the next generation can be prevented through preimplantation diagnosis, a

Table 4
Recurrence risk of CHD in siblings and offspring of patients with nonsyndromic CHD without a known molecular defect[5,70–74]

Lesion	Siblings, %	Offspring, %	
		Father affected	Mother affected
(T)APVR	NR	3.7	
ASD	1.7-3	1.5-5.7	4-6
VSD	1.6-3.8	2.9-7.5	2.9-7.1
AVSD	3-6.5	1-4.5	11.5-14
Left-sided obstruction	1.25-11	5.9-7.4	5.9-14.3
TOF	2.5-6.5	1.5-3.8	2.5-18.2
TGA	1-3	1.5	
Truncus arteriosus	5-9.5	NR	
Ebstein anomaly	13.3	NR	6
Pulmonary valve anomaly	5.4	2-3.5	4-6.5
PDA	3	2-2.5	3.5-4

ASD, atrial septal defect; AVSD, atrioventricular septal defect; CHD, congenital heart defects; NR, not reported; PDA, persistent ductus arteriosus; (T)APVR, (total) abnormal pulmonary venous return; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

technique based on in vitro fertilization, which occurs before pregnancy and selects the unaffected embryos.

An alternative is prenatal testing, which conventionally implies chorionic villus or amniotic fluid sampling in the early stages of pregnancy. In more recent years, noninvasive prenatal testing (NIPT) has emerged as a noninvasive alternative for prenatal testing. NIPT was primarily developed to detect trisomy 21 in the fetus early in pregnancy with high specificity (> 99%) and sensitivity (> 99%) in the absence of fetal anomalies. During pregnancy, cells from the placenta (containing fetal DNA) lyse into the maternal circulation. The test is based on the relative number of reads that map to a certain chromosome in maternal plasma (cell free DNA). Hence, the test can detect other aneuploidies such as trisomy 13 and 18, Klinefelter (47,XXY), Turner (45,X0), or triple X (47,XXX) syndrome, albeit with a somewhat lower sensitivity and specificity. Technological fine tuning will eventually render NIPT suitable to detect *de novo* variants (both CNVs and SNVs)[77,78] and targeted testing of inherited variants.[79] It goes without saying that broad scale application of NIPT requires careful consideration of ethical issues. In both instances of prenatal testing, termination of pregnancy may be considered if the results of the test are abnormal test.

Some couples may choose not to undergo prenatal diagnosis, in which case, the possibility of genetic screening in the newborn should be discussed. If no underlying genetic cause of the CHD has been identified or no prenatal testing has been performed, fetal echocardiography is recommended. This should be performed in a specialized center at 18 to 20 weeks of pregnancy.[80]

CONCLUSION

Genetic evaluation of patients with CHD is being conducted on an increasingly large scale and will in many cases undoubtedly help us to optimize (para)medical management in individual patients and their families. Moreover, knowledge of genetics further helps us to understand the underlying pathophysiology of these conditions, which will certainly contribute in the long-term to developing more targeted treatments.

Genetic evaluation should be done correctly in each patient/family with careful counseling prior to testing as well as upon communication of any results. Implementation of a correct strategy has already clearly demonstrated that this leads to more efficient testing, greater patient satisfaction, and more correct medical management

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CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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