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Genetic counselling and testing in adults with congenital heart disease: A consensus document of the ESC Working Group of Grown-Up Congenital Heart Disease, the ESC Working Group on Aorta and Peripheral Vascular Disease and the European Society of Human Genetics

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Abstract

Thanks to a better knowledge of the genetic causes of many diseases and an improvement in genetic testing techniques, genetics has gained an important role in the multidisciplinary approach to diagnosis and management of congenital heart disease and aortic pathology. With the introduction of strategies for precision medicine, it is expected that this will only increase further in the future. Because basic knowledge of the indications, the opportunities as well as the limitations of genetic testing is essential for correct application in clinical practice, this consensus document aims to give guidance to care-providers involved in the follow-up of adults with congenital heart defects and/or with hereditary aortic disease. This paper is the result of a collaboration between the ESC Working Group of Grown-Up Congenital Heart Disease, the ESC Working Group on Aorta and Peripheral Vascular Disease and the European Society of Human Genetics. Throughout the document, the importance of correct counseling in the process of genetic testing is emphasized, indications and timing for genetic studies are discussed as well as the technical modalities of genetic testing. Finally, the most important genetic diseases in adult congenital heart disease and aortic pathology are also discussed.

Keywords

Genetic testing, genetic counseling, adult congenital heart disease, heritable thoracic aortic disease

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Introduction

The need for a multidisciplinary approach for the follow-up of patients with adult congenital heart disease (ACHD) has been recognised for some decades and now also includes genetics,[1] reflecting the increasing clinical applications.

The historical argument that genetic testing is costly and time-consuming is no longer valid and, thanks to spectacular technical progress, we are now able to sequence the complete genome for a reasonable price. The downside of the coin, however, is that genetic testing results are not always straightforward to interpret and this has now become the bottleneck of genetic testing. Essential in both defining an indication and in the interpretation of genetic testing in the clinic is appropriate genetic counselling – both before and after possible testing. To estimate which patients are eligible for genetic counselling/testing, some basic knowledge of the current insights and possibilities is desirable. With this article we wish to provide guidance for care-providers specifically involved in the care of adult patients with congenital (hereditary) cardiovascular defects.

Because many ACHD centres also follow patients with aortopathies, two major categories of patients are discussed: (a) patients with structural congenital heart disease (CHD); (b) patients with heritable thoracic aortic disease (HTAD). In these disease groups, both syndromic and non-syndromic entities exist, and it is important to acknowledge some differences in diagnosis and management between these entities – also with regards to genetic counselling and testing. On the other hand, insights gained in recent years indicate that these boundaries between syndromic and non-syndromic forms are gradually fading. In clinical practice, a genetic aetiology is more commonly searched for – and identified in syndromic forms than in the non-syndromic forms of CHD and HTAD. Consequently, genetic counselling has become part of standard care for syndromic CHD and HTAD in most centres. In non-syndromic presentations, this is often not (yet) the case. However, largely thanks to knowledge that was gathered through the study of genetics of syndromic entities, to a better knowledge of genes involved in cardiovascular development, and to translational research, better insights into the genetic architecture of isolated CHD and HTAD are now provided, and genetic counselling can also be offered in selected cases.

A graphical overview of the data presented is provided in Figure 1.

Rationale for genetic counselling and testing in ACHD

Evidence in support of a major genetic contribution in CHD includes increased recurrence risk in first-degree relatives, greater concordance of CHD in monozygotic compared to dizygotic twins and a higher rate of CHD in families with consanguinity.[2,3]

Advances in genetic sequencing technology suggest that up to one-third of CHD cases may be explained by a genetic cause,[4] although this number is probably an overestimation of genetic diagnosis in the non-syndromic group and an underestimation in the syndromic group. Aneuploidies have first been linked to syndromic CHD (e.g. Down syndrome) more than half a century ago and explain ±10% of CHD cases.[5] An excess burden of rare, often de novo, copy number variants (CNVs) has been observed both in syndromic (e.g. most 22q11 deletions) and non-syndromic CHD, explaining an estimated 15–20% and 5% of patients, respectively.[6,7]

Single nucleotide variants (SNVs – mostly de novo) in several hundreds of genes account for about 10% of CHD.[4] Owing to inherent limitations of the available testing methods and difficult interpretation of the results (see below), current approximations might still underestimate the true contribution of CNVs and SNVs to the aetiology of CHD, and a significant proportion of CHD heritability still remains unexplained.

In HTAD, several recent studies indicate the presence of a causal variant in known genes in up to 20% of non-syndromic cases.

Understanding the underlying genetic causes of CHD is important for patients and families with regards to clinical management and family planning for a number of reasons:

1. Confirm diagnosis

For ACHD patients, knowledge about the genetic origin and inheritance of their CHD or HTAD is important because this information may reduce uncertainty and provide reassurance.[8,9]

In both CHD and HTAD, it is important to recognise that variants in genes that are traditionally associated with syndromic forms can also occur in patients with isolated cardiovascular features. Patients and families need to be well informed about this clinical variability and, in particular, also about the unpredictability of the phenotype in other family members. Also, a negative genetic test would not definitively exclude a recurrence risk, as heritability is so far incompletely explained for both conditions.

Regarding the wide genetic architecture of CHD, the testing approach should be guided by the presence of cardiac and extracardiac features (syndromic or non-syndromic CHD), the number of affected relatives, and the accessibility to the parents.

2. Guide management

Confirmation of a diagnosis by genetic testing allows identification of individuals at increased risk for co-morbidities, such as peripheral arterial aneurysms in some

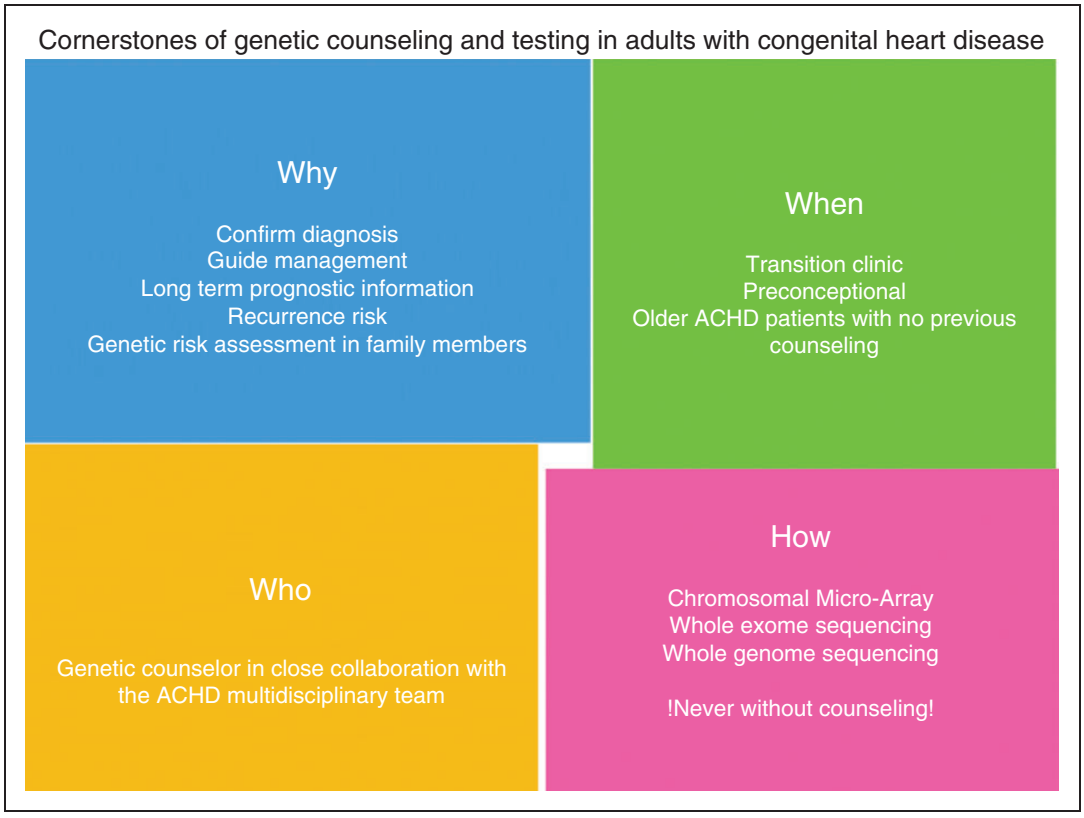

Figure 1. Schematic illustration of the cornerstones of genetic counselling and testing in adults with congenital heart disease. ACHD: adult congenital heart disease.

cases of HTAD, heart failure,[10,11] arrhythmias[12] or neurodevelopmental disorders,[13] who will benefit from early screening and intervention. Ultimately, the objective of genetic diagnosis would be to provide optimal medical management, including targeted curative therapies in the future, as demonstrated in animal experiments of in-vivo pharmacological activation of Wnt signalling in atrioventricular septal defect (AVSD).[14]

3. Long-term prognostic information

Post-operative mortality is increased in patients with 22q11.2 deletion due to the added complexity of cardiac lesions and extra-cardiac co-morbidities.[15]

Genes implicated in Rasopathy syndromes (Noonan, Noonan with multiple lentigines, cardiofaciocutaneous syndrome and Costello syndromes) are associated with ventricular hypertrophy in later development.[15] Thus, the genetic environment modulates the risk for systolic or diastolic dysfunction and heart failure and provides a basis to follow up accordingly.

4. Recurrence risk

As an increasing proportion of the CHD population reaches the reproductive age, recurrence risk has become of paramount importance. Although transmission risk can be easily predicted for those disorders with a Mendelian inheritance pattern, these forms represent a minority and the underlying gene defect may remain unknown in a substantial amount of cases, even after thorough screening of the most confident CHD/HTAD genes. In addition, the aspects of variable clinical expression and reduced penetrance in many of these disorders should be adequately addressed during the counselling process.

In the absence of a clear genetic diagnosis, recurrence risk can be estimated with caution from epidemiological studies and an overall estimate of sibling recurrence risk for CHD is 2.7% in one large series.[16] Of note, the absence of a positive familial history (or the absence of CHD in the parents) would not preclude a recurrence risk for a given patient, regarding the rate of de novo mutations. One should also consider the higher rate of miscarriage and variable disease expression in CHD, so that the real recurrence risk can reach up to 50%.

In addition to the cardiac phenotype, the extracardiac phenotype and family history are essential in guiding genetic investigation. If genetic testing or the pedigree is not informative, estimates are largely based on observations in the offspring of large groups of CHD patients.[16] Recurrence rates are lesion-specific,

generally higher when the mother is the affected parent and greater in offspring than siblings.[3,16,17]

A nationwide Danish cohort study showed that heterotaxy, atrio-ventricular septal defect and left-sided outflow tract obstructive lesions had a higher recurrence risk.[3]

5. Genetic risk assessment in family members

Determining the underlying genetic pattern is important to evaluate if there might be other family members for whom genetic testing or screening would be appropriate. There might be a rationale to screen asymptomatic family members for conditions that include CHD as one aspect of a pleiotropic phenotype.[9] Based on the recurrence risk and discordant phenotype in first-degree relatives of patients with left-sided obstructive lesions, echocardiographic screening to detect silent defects may also be recommended for family members.[8]

Timing for genetic counselling and indications for genetic testing in ACHD

Transition clinic counselling

Late recognition of a genetic condition is not uncommon, particularly in patients with absent, subtle or late-onset extra-cardiac features.

The approach to the young ACHD patient in transition care should include the recognition of a suggestive clinical phenotype based on associated non-cardiac features.[18] A detailed assessment of medical history of other family members that spans at least three generations may be essential. Consanguinity should be documented.[9,18]

Genetic testing is recommended in any adolescent not previously tested that presents with:

- A phenotype of a recognisable chromosomal syndrome or with a congenital heart defect combined with
  - facial dysmorphism
  - skeletal defects
  - visceral organ malformations
- Growth delay
- Developmental delay or learning disorders, behavioural or psychiatric disorders.
- Family history with one or more first-degree relatives with CHD or multiple miscarriages and/or siblings with birth defects.

Given the already mentioned lesion specific recurrence risk of CHD[17] some entities should raise more suspicion, including tetralogy of Fallot, interrupted aortic arch, truncus arteriosus, ventricular septal defect (VSD) with ascending aortic aneurysm, anomalous branch pulmonary arteries.

A graphical illustration of the genetic counselling- and testing process in CHD is provided in Figure 2. Indications for genetic counselling and testing in HTAD are mentioned below.

The timing of genetic counselling must balance the needs of the patient with the developmental status and should be individualised. Sexual and contraceptive practices should be explored and the importance of planning pregnancies needs to be discussed.[8,18,19] Information to give during genetic counselling includes the probability of a genetic origin, the risk of transmission within the family, and explanation about the clinical manifestations and natural history of the disease.

Preconceptional counselling

Although reproductive fitness is impaired in some syndromic forms of CHD, limiting transmission of large-effect mutations, there is currently an increasing number of patients entering reproductive age and reproductive decision-making assumes greater importance in adulthood.

Preconceptional counselling regarding reproductive choices and recurrence risk may be a complex process that takes into account phenotype and family history. It also requires exploring the patient's perception of risk and motivations and the prognosis for the individual patient.[8]

The optimal time for evaluation of genetic risk, genetic counselling and discussion of the availability of prenatal testing is before pregnancy. However, having genetic counselling and testing during pregnancy might still be helpful.[19] In case of a complex disorder with poor prognosis, termination of pregnancy can be discussed.

A prenatal diagnostic test can be performed to identify known or new chromosomal or other genetic abnormalities, although these procedures carry a foetal loss rate of 0.3–0.5%. If the gene defect is known, preimplantation diagnostic testing and screening can be used to identify embryos with specific genetic abnormalities prior to transfer.[20] Foetal echocardiography at 18–22 weeks of gestation is routinely used to exclude major cardiac defects.

Older ACHD patients (with no or very limited testing in the past)

The majority of adults with CHD have not had counselling even though some of them were found to harbour a genetic variation in childhood. This issue should be revisited in all adults with CHD in whom a genetic aetiology is suspected.

There may be benefit from retesting patients with a negative genetic result as a child. Using next generation

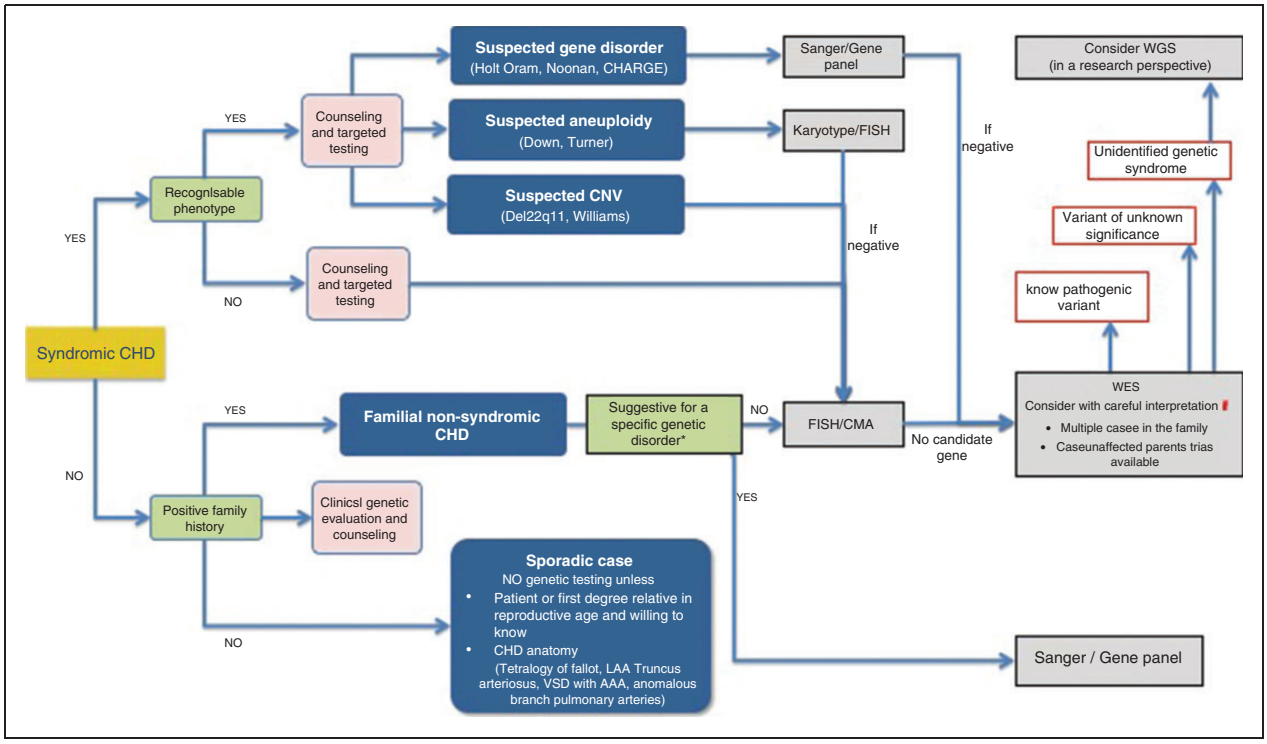

Figure 2. Graphical illustration of the genetic counselling-and testing process in congenital heart disease (CHD).
AAA: ascending aortic aneurysm; AV: atrio-ventricular; CMA: chromosmal micro-array; CNV: copy number variant; ELN: elastin; FISH: fluoresence in situ hybridisation; IAA: interrupted aortic arch; VSD: ventricular septal defect; WES: whole exome sequencing; WGS: whole genome sequencing.
*e.g. ELN for familial supravalvular aortic stenosis; TFAP2B for familial patent ductus arteriosus, NKX2.5 for familial atrial/ventricular septal defect with AV block.

sequencing (NGS) technology and data processing software, new CHD causative genes will be more easily discovered (see Methods section).

Who should perform genetic counselling and testing in ACHD

With the increasing complexity of genetic test results, ensuring that individuals are well informed about the process and consequences of genetic testing prior to testing is a challenging but crucial task. Close collaboration between cardiologists and clinical geneticists is of great importance for defining the clinical phenotype and correct interpretation. The test results should be conveyed by a trained genetic counsellor, skilled in delivering complex information to the proband and his/her family. Therefore cardiac genetic counsellors should be part of the multidisciplinary team of all ACHD clinics.

Genetic counsellors are mostly employed by medical genetic facilities that are located in tertiary centres offering combined clinical and laboratory genetic services. Often, they also provide support and expertise to a number of peripheral hospitals located in urban areas.

All genetic tests take place in medical genetic facilities that must be certified and adhere to the healthcare rules and regulations that are necessary to ensure patient safety. Results are kept confidential according to the confidentiality laws.

These certified medical genetic facilities are challenged by an increasing number of commercial laboratories offering direct-to-consumer (DTC) genetic tests providing 'predictive health information'. These are tests where the customer samples blood or DNA at home and mails the sample to the laboratory. The DTC genetic tests may detect severe and highly penetrant monogenic disorders or genetic variants associated with increased susceptibility for common and complex diseases. Results are provided directly to the customer by mail or Internet without a physician order or interpretation.

There are some major concerns regarding these tests and therefore the European Society of Human Genetics has developed a policy on advertising and provision of predictive genetic tests by such DTC companies.[21] We argue against the use of DTC testing in the CHD genetic testing context.

Methods for variant detection and interpretation

The advent of NGS technologies has significantly pushed CHD gene discovery forward. As mentioned above, non-negligible numbers of CHD cases can be explained by either aneuploidy, CNV or an inherited or de novo point mutation in one of the known genes,[4] each of which requires specific detection methods.

Chromosomal microarray (CMA)

CMA is nowadays used as the first-tier test especially – but not exclusively – in the context of syndromic CHD to detect aneuploidies as well as CNVs ($\geq$25 kb).[22,23] Balanced inversions and translocations cannot be detected by this technique. CMA-based analysis of chromosomal anomalies is performed using either Single Nucleotide Polymorphism (SNP) genotyping (SNP array), array-based comparative genomic hybridization (aCGH) or a combination of both. CMA platforms may differ between laboratories, but their design, manufacture and implementation should adhere to the guidelines of the American College of Medical Genetics and Genomics.[24] While CMA can also be worthwhile in sporadic but very severe non-syndromic cases, there is currently no strong indication for CMA in non-syndromic CHD families or isolated cases with mild-to-moderate disease who only come to medical attention in adulthood.

Whole exome sequencing (WES)

Although molecular diagnostic laboratories still use targeted sequencing of CHD gene panels to detect single nucleotide variants, WES is becoming the mainstay of single nucleotide variation discovery. A combined approach that superimposes virtual panels focusing on genes previously associated with CHD on WES data is also commonly used.[25–27] Although still more expensive than gene panel sequencing, WES has the advantage that it is more unbiased and allows instant data re-analysis upon the identification of novel CHD-linked genes.[13,28] After an initial study in 2013 in 362 CHD patients,[28] two recent research WES studies in 2871 and 1891 CHD probands have been performed,[26,29] from which several important lessons can be learned. Firstly, inherited and de novo point mutations in the currently known CHD genes explain $\pm$10% of patients, with de novo mutations having the largest contribution.[26,29] Secondly, recessive inheritance of CHD is underrated and should be considered, especially in populations with high consanguinity.[29] Of note, Jin et al. also reported that recessive disease-causing alleles in established autosomal dominant CHD genes exist, resulting in more severe clinical phenotypes. Thirdly, de novo point mutations are highly enriched in syndromic CHD, whereas inherited protein-truncating variants in heart-related genes are more common in non-syndromic CHD.[26,29] Finally, mutations in a single gene lead to a wide spectrum of CHD phenotypes.[26,29] As such, de novo WES analysis in case-unaffected parents trios is strongly recommended for sporadic syndromic CHD, but not for relatively mild non-syndromic adult phenotypes. With respect to familial CHD, WES is encouraged if the DNA of multiple affected family members is available to allow shared variant analysis.

Whole genome sequencing (WGS)

WGS is a one-time experiment allowing genome-wide interrogation of single nucleotide variation, CNVs and balanced/unbalanced structural variants. Although CMA and WES are currently still faster, cheaper and less challenging to interpret from a bioinformatics and biological point of view, they are expected to be replaced by WGS as the frontline genetic CHD test soon.

To date, only one published research study has applied WGS to the CHD field in neonatal/paediatric patients.[30] Damaging variants and variants of unknown significance were identified in 35% of patients.

For now, we suggest that trio WGS can be considered in selected syndromic or severe isolated CHD cases where no pathogenic variant was found using CMA and WES.

Importantly, genetic testing in the setting of diagnosis is to be differentiated from testing in a research context. The set of genes in a diagnostic setting needs to be restricted to genes with a proven gene-disease association. A recommendation for designing gene panels for HTAD in these various settings has been developed.[31]

Inherent to more extended genetic screening using WES and WGS is the detection of more variants of unknown significance and of variants in genes with an unlikely association with the phenotype. We do not recommend reporting these results back to the patients on a routine basis. Careful variant classification according to American College of Medical Genetics and Genomics (ACMG) guidelines is mandatory, and genetic counselling plays a central role in validating variants through familial segregation analysis, as well as in discussing the limits of risk stratification for the relatives.

The genetic counselling should include discussions on potential re-analysis and even re-testing after predefined intervals in gene-elusive patients with a high suspicion of genetic disease are necessary.

Specific disorders

Structural congenital heart defects

Cardiac development is a complex and coordinated process involving cells from different origins of the

embryo called the 'heart fields'.[32,33] The proliferation, differentiation and migration of the cardiac progenitors is tightly controlled by a complex gene regulatory network, and perturbations can lead to CHD.

Syndromic forms of CHD include Down syndrome, Turner syndrome, velocardiofacial (DiGeorge) syndrome and Williams-Beuren syndrome among others (Table 1).

Historically, aneuploidies were the first genetic defects identified in CHD. Trisomy 21 (Down syndrome) is the most common human aneuploidy (1/600 live births; estimated prevalence 1/1000) and is associated with a 40–50% prevalence of CHD, including tetralogy of Fallot, AVSD, atrial septal defect (ASD), VSD, bicuspid aortic valve and persistent ductus arteriosus (PDA).

Turner syndrome, a complete or partial X-chromosome monosomy, occurs in around 1/2000 female births. Characteristic clinical features include short stature, premature ovarian failure and lymphoedema. Cardiac defects occur in up to 50% of women with Turner syndrome and include mainly bicuspid aortic valve, aortic coarctation and abnormal pulmonary venous return. Aortic dilatation, entailing a risk for aortic dissection is present in 22% of Turner women and is associated with age, hypertension, bicuspid aortic valve (BAV), XO karyotype and growth hormone treatment.[34–37]

The 22q11.2 deletion (historically also described as DiGeorge syndrome, velocardiofacial syndrome) is the most common microdeletion syndrome in humans, occurring in up to 1/5950 live births.[38,39] One of the main genes in the 22q11.2 region is TBX1, a transcription factor controlling second heart field development.[40] Cardiac defects, present in 60–75% of patients include tetralogy of Fallot, interrupted aortic arch, right aortic arch, VSD and truncus arteriosus. Associated features include neuropsychological disorders, facial traits (tubular nose, cleft palate) and thymic and parathyroid disorders. Interestingly, the expressivity of this microdeletion syndrome can be extremely variable ranging from syndromic presentations to isolated CHD. In a prospective screen, up to 18% of tetralogy of Fallot patients presented with a 22q11.2 deletion.[41,42]

Another CNV syndrome is Williams-Beuren syndrome (del 7q11.23), occurring in 1/10000 births. Supravalvular aortic and/or pulmonary stenosis are the main cardiovascular features and are associated with growth deficiency, a hyper-sociable personality, mild cognitive disorders and skeletal dysmorphism.[43,44] The cardiac phenotype of Williams-Beuren syndrome is related to elastin (ELN) gene haploinsufficiency.

CHD caused by single gene mutations can be either familial (with autosomal dominant or recessive inheritance pattern), or – more frequently – present in sporadic cases, related to de novo mutations or to low penetrance. Although many genes have been associated with cardiac development in animal models, caution is warranted regarding the translation of animal data to humans. Further studies are required to validate their role in human CHD, by showing a significant variant burden in patients with CHD compared to controls. Diagnostic gene panels should be curated from CHD candidate genes. As the number of novel genes for (non-)syndromic CHD is rapidly increasing, diagnostic genetic testing by clinical WES or WGS is preferred over targeted gene panels, preferably with multiple affected cases in familial CHD, or in trio for sporadic syndromic CHD, given the high rate of de novo mutations in the latter. Here, we describe the main genes involved in human CHD, and for which clinical data are available.

A first class of CHD-related genes are cardiac transcription factors (see Table 2). NKX2-5 pathogenic variants lead to several structural CHDs, including ASD, VSD, Ebstein anomaly and tetralogy of Fallot.[45,46] TBX5 is responsible for Holt-Oram syndrome associating limb- and cardiac defects, including VSD, ASD, AVSD, conduction defects and hypoplastic left ventricle.[47] At the molecular level, TBX5 and NKX2-5 cooperate together to transactivate other cardiac developmental genes, explaining the wide diversity of cardiac defects associated with those genes, but also the phenotypical overlap between them.[46,47] Importantly, NKX2-5 and TBX5 pathogenic variants are both frequently associated with conduction disorders leading to premature atrio-ventricular (AV)-block. GATA4 pathogenic variants lead to ASD, VSD, AVSD and pulmonary stenosis.[48] As already mentioned, TBX1 SNVs give rise to similar CHDs as in 22q11 deletion syndrome.[40]

A second important pathway is the Rasopathy-Mitogen-Activated Protein Kinase (RAS-MAPK) signalling pathway, which is clinically linked to Noonan syndrome and related disorders. Noonan syndrome is an autosomal dominant disorder associating CHD (mainly pulmonary stenosis), facial dysmorphism (hypertelorism, ptosis, macrocephaly) and neurodevelopmental disease with frequent growth retardation.[49] Noonan syndrome can also be associated with hypertrophic cardiomyopathy (HCM), especially also in older age. The syndrome is most commonly caused by pathogenic variants in PTPN11 (50% of the cases), or in other genes involved in the RAS-MAPK signalling pathway including SOS1, RAF1, RIT1, KRAS, SHOC2, NRAS, SOS2 (Table 1).

The Notch signalling pathway is the third key signalling pathway during cardiac development, with functions that control neural crest cell proliferation and differentiation, auriculo-ventricular patterning, as well as left/right patterning.[50] Pathogenic variants in the Notch-ligand JAGGED1 (90%) and the NOTCH2 receptor (2%) are associated with the Alagille

Table 1. Main syndromes associated with congenital heart disease (CHD) (with an estimated prevalence >1/15000).

Syndrome name	Genetic defect	Prevalence	CHD frequency	Main cardiovascular features	Extra-cardiac features	Surveillance – points of care
Down	**Trisomy 21**	1/1000	40–50%	ASD, VSD, AVSD, PDA	**Short stature**, **facial traits**, **neurodevelopmental delay**, immune disorders, hypothyroidy, allantoid instability	Regular check of thyroid function tests, obstructive sleep apnoea, auditive tests, blood malignancy
Klinefelter	**XXY**	1/1000	50%	PDA, ASD	Gynaecomastia, hypogonadism	Androgen replacement therapy
Turner	**XO**	1/2000	35%	BAV, CoA, bovine aorta, elongated aortic arch, partial abnormal pulmonary venous return, ASD, VSD, coronary anomalies	**Short stature, webbed neck**, bowed arms, **ovarian** and thyroid disorders, lymphoedema, premature osteoporosis	HTN and cardiovascular risk factors, growth hormone, oestrogen supplementation, ovarian reserve sparing, careful aortic evaluation and follow-up (pregnancy)
Noonan	**PTN11, KRAS, SOS1, SOS2, RAF1, BRAF, MEK1, CBL, NRAS, NF1, MAP2K1, MAP2K2, RIT1, SHOC2**	1/1000-1/2500	50–80%	PS, ASD, VSD, AVSD, PDA, HCM	Hypertelorism, **antimongoloid palpebral cleft**, low set ears, cryptorchidia, mild neurodevelommental delay, pectus excavatum, widely spaced nipples, growth hormone deficiency/ resistance, coagulation disorders	Careful coagulation evaluation, ophthalmologic and audiological screen, developmental assessment. If no cardiac defect, repeat cardiac evaluation every 5 years.
Edwards	**Trisomy 18**	1/5000	>90%	ASD, VSD, PDA, valvular	Hypertonia, microcephalia, hypertelorism, abnormal ears, **clenched fingers**, growth retardation	
DiGeorge	**22q11 del (TBX1)**	1/6000	60–75%	ToF, ASD, VSD, BAV, CoA, interrupted aortic arch, truncus arteriosus	Thymus and parathyroid hypo-aplasia (hypocalcaemia), cleft palate, facial traits, neurodevelopmental delay, **psychological disorders**	Use of de-leukocytised blood products, careful operative management, check calcium level
CHARGE	**CHD7, SEMA3E**	1/8500-10000	80%	Conotruncal defects, ASD, VSD	Neurodevelopmental delay, autistic traits, dysmorphic facies (**coloboma**), palate cleft, **choanal atresia**, abnormal ears	Psychomotor development related to sensory functions
Williams-Beuren	**del 7q11.23**	1/10000	75%	Supravalvular AS, supravalvular PS	**Hypersociable** behaviour, **music**, facial features, hypercalcaemia, renal artery stenosis and nephrocalcinosis	Careful arterial evaluation (renal, coronary), risk factor, careful operative management
Heterotaxia	**NODAL/LEFTY2, ZIC3, ACVR2B, GDF1, CFC1**	1/15000	>90%	TGA, ASD, VSD, TAPVR, DORV, PS, TTF, congenital AV block	Partial or complete situs inversus, asplenia, polysplenia, liver and renal malformation	Left atrial isomerism frequently without intracardiac structural defect
Smith-Magenis	**Del 17p11.2**	1/15000	<45%	ASD, VSD, MVP, PS, PA	Intellectual disability, square-shaped face, sleep disorders	

AF: atrial fibrillation; AS: aortic valve stenosis; ASD: atrial septal defect; AVSD: atrio-ventricular septal defect; BAV: bicuspid aortic valve; CoA: aortic coarctation; DORV: double outlet right ventricle; HCM: hypertrophic cardiomyopathy; MVP: mitral valve prolapse; PA: pulmonary atresia; PDA: persistent ductus arteriosus; PS: pulmonary valve stenosis; TAPVR: total anomalous pulmonary venous return; TGA: transposition of the great arteries; ToF: tetralogy of Fallot; VSD: ventricular septal defect.

Table 2. List of the genes involved in heritable thoracic aortic disease (HTAD) categorised according to their main functional class.

Gene name	Syndrome name (if applicable)	Main cardiovascular features	Additional clinical features indicative for a syndromic entity
HTAD related to genes encoding components of the extracellular matrix			
FBN1	Marfan syndrome	**Sinus of valsalva aneurysm**, aortic dissection, mitral valve prolapse, main pulmonary artery dilatation, left ventricular dysfunction	**Lens luxation**, skeletal features (arachnodactylia, pectus deformity, scoliosis, flat feet, increased arm span, dolichocephalia), dural ectasia, striae
COL3A1	Vascular Ehlers Danlos Syndrome	**Arterial rupture and dissection without preceding dilatation/aneurysm**	**Gastro-intestinal rupture, thin and translucent skin**, dystrophic scars, facial characteristics (Madonna face, thin lips, deep set eyes), club feet, uterine rupture
LOX		Fusiform aneurysm of aortic root and ascending aorta	Scoliosis, joint hypermobility, skin striae
MFAP5[a]		MFS features	
BGN[a]	Meester Loeys syndrome	Early-onset ascending aortic aneurysm and dissection	Facial dysmorphism pectus deformities, joint hypermobility or contractures, skin striae
HTAD related to genes encoding components of the TGFβ pathway			
TGFBR1	Loeys Dietz Syndrome	**Sinus of valsalva aneurysm**, aortic dissection, arterial aneurysms and dissections, **arterial tortuosity**, patent ductus arteriosus, atrial septal defect, bicuspid aortic valve	**Bifid uvula/cleft palate, hypertelorism**, pectus abnormalities, scoliosis, club feet
TGFBR2			
SMAD2			
TGFβ3			
SMAD3	Aneurysms osteoarthritis syndrome		Osteoarthritis
TGFβ2			
HTAD related to genes encoding proteins involved in the contractile apparatus of vascular smooth muscle cells			
ACTA2	Multisystemic SMC dysfunction syndrome	**Ascending aortic aneurysm**, aortic dissection, **patent ductus arteriosus**, aortic coarctation, aortopulmonary window, pulmonary arterial hypertension	**Congenital mydriasis**, malrotation of the gut, Moya-Moya like disease, periventricular white matter hyperintensities
MYLK			
PRKG1			
MYH11			
LMOD1			

SMC: Smooth Muscle Cell; TGFB: Transforming Growth Factor Beta Receptor Beta. Typical cardiovascular and clinical features are listed in bold. Genes have been curated for the gene-disease association within the Clinical Genome (ClinGen) resource. Syndrome names are listed when applicable and are used when syndromic features are present (all these genes can be responsible for non-syndromic HTAD). Main cardiovascular and extra-cardiac features are listed. [a]Recent genes for which the association is not yet confirmed.

syndrome, associating cholestatic liver disease, abnormal kidney development and CHD with tetralogy of Fallot and peripheral pulmonary stenosis being the most frequent defects.[51,52] NOTCH1 pathogenic variants are associated with aortic coarctation, VSD, hypoplastic left heart syndromes, rare instances of familial BAV and tetralogy of Fallot.[53–55]

Finally, chromatin modifiers are key players in cardiac development, by modulating gene expression. This same tight regulation of gene expression is required for brain and other organ development, making pathogenic variants of chromatin modifiers a frequent cause of syndromic CHD. Among those, KMT2A and KDM6A pathogenic variants are associated with Kabuki syndrome involving the brain, cardiac and urogenital systems.[56] Heart defects range from simple lesions such as aortic coarctation (the most frequent), ASD, VSD, PDA to complex CHD including tetralogy of Fallot and univentricular heart. Sotos syndrome (NSD1 – developmental delay with facial dysmorphism – ASD, VSD), CHARGE syndrome (CHD7 - coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies with developmental delay and autistic features) are other examples of syndromes caused by abnormal chromatin regulation.

Heritable Thoracic Aortic Disease (HTAD)

HTAD comprises a range of disorders defined by the occurrence of aortic disease (aneurysm/dissection) mainly in the ascending aorta. The disorder may be limited to aortic disease (non-syndromic HTAD) or also include extra-aortic features (syndromic HTAD).[57] An estimated 20% of patients with non-syndromic thoracic aortic disorders have a family history of the disease, which indicates a significant genetic component.[58]

Careful multidisciplinary clinical evaluation of the proband should be undertaken to help identify specific syndromes. Detailed family history and clinical assessment of first-degree relatives are required to differentiate familial and sporadic cases.

Genetic testing in HTAD is paramount to allow for effective family screening, since the phenotype is very variable, even within families, with incomplete penetrance in some cases. Testing is also important to determine the optimal care for each patient: aortic risk appears to depend both on the affected gene and the particular phenotype of the patient.[59]

For example, smaller women with severe extra aortic features carrying a Transforming Growth Factor Beta Receptor (TGFBR) 2 pathogenic variant are at increased risk for dissection and should be referred earlier for surgical intervention.[60]

The genetic basis of non-syndromic Thoracic Aortic Disease (TAD) is more complex and genetic analysis identifies a pathogenic variant in only up to 20% of patients. However, pathogenic variants in these genes imply a wide spectrum of risk as well as possibly including different extra-aortic vascular manifestations. This last observation implies a controversial approximation between syndromic and non-syndromic entities.[61]

No formal guidelines or criteria are available to select patients in whom genetic testing should be undertaken in HTAD. A consensus, based on expert opinion within the HTAD Rare Disease Group of VASCERN includes the following criteria: genetic testing may be considered after proper counselling and evaluation when at least two members of a family present HTAD or in isolated cases when (a) children (<18 years) present with aortic dissection or an aortic root diameter Z-score ≥3 or (b) adults present with aortic dissection or an aortic root diameter Z-score >3.5 or with a Z-score between 2.5–3.5 and <60 years or >60 years, and no arterial hypertension.

Pathogenic variants in over 30 genes have been reported for HTAD,[31] although the level of gene-disease association is not equally strong for each of these genes and for each disease entity. Also, variants of unknown significance in these same genes may at least partly account for a risk of thoracic aortic aneurysms/dissections.[62] With the current knowledge available, we do however not recommend reporting of such variants of unknown significance back to patients and families.

Stronger gene-disease associations exist for the syndromic forms, for example for Marfan syndrome, where FBN1 pathogenic variants are identified in >95% of cases. A major effort to semi-quantitatively assess an association for non-syndromic HTAD has been done using the Clinical Genome Resource (ClinGen) framework.[31] The final list of genes with a 'definitive' or 'strong' association was reduced to 11 and, in a diagnostic setting, genetic analysis for non-syndromic thoracic aortic aneurysm/dissection should at least include these genes.

The majority of HTAD genes can be categorised into three groups of genes, which encode proteins involved in (a) vascular smooth muscle cell contraction and adhesion to the (b) extracellular matrix or (c) TGF-β signalling pathway. The major genes with their respective syndromic entities, if applicable, are shown in Table 2. Research in humans and animal models has revealed a close interaction between these three categories. Pathogenic variants in genes encoding extracellular matrix components will lead to an abnormal and fragile extracellular matrix, thereby facilitating aortic dilation and rupture. Loss-of-function pathogenic variants in genes encoding proteins of the TGF-β pathway probably alter the reparation path, in part mediated by the TGF-β pathway. Pathogenic variants in genes encoding proteins of the contractile

apparatus of smooth muscle cells may alter tensegrity, i.e. transmission and perception of forces within the aortic wall by smooth muscle cells, which in response cannot adequately assure homeostasis of the extracellular matrix.

Special mention must be made of the association between the presence of BAV and thoracic aortic aneurysm. BAV is present in 1–2% of the general population, with a 3/1 male predominance. BAV may be associated with TAA located either at the level of the sinuses of Valsalva, or (more commonly) at the tubular part of the ascending aorta. This association is by no means systematic (>75% of cases), and its explanation is unclear.[63] Both intrinsic aortic wall abnormalities due to genetic variation and wall alteration secondary to flow mechanics in the ascending aorta have been proposed as possible causes. Familial occurrence of BAV has clearly been established with rates of 5–10% in first-degree relatives in various studies. Interestingly, the incidence of TAA in first-degree relatives is even higher in family members with TAV or BAV.[64] The genetic basis of BAV is unclear. Rare pathogenic variants have been identified in a number of genes (SMAD6, NOTCH1, ROBO4, TBX20); however, these variants account for <5% of all BAV/TAA cases.[65,66]

Echocardiographic screening in first-degree relatives of BAV patients is recommended and may be appropriate, particularly in boys, athletes and if hypertension is present. Genetic screening may be considered in familial cases with associated TAA.

Author contribution

JDB, WB and JRH contributed to the conception and design of the work. All authors were assigned to specific subtopics and drafted the manuscript accordingly. All critically revised the manuscript and gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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