Genetics in cardiovascular diseases

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ABSTRACT

Cardiovascular diseases (CVDs) are a wide group of disorders affecting the heart and blood vessels, including coronary artery, valve, pericardial, conduction system, myocardial and vascular diseases, either congenital or acquired, which can be also heritable. The advent of next generation sequencing (NGS) was accompanied by quick advances in understanding the genetic basis of human diseases, prompting translation of genetics to the clinic. Precision medicine is based on these findings and on the role of genetic testing to improve the diagnosis, to identify individuals with previously unrecognized disease and family members at risk of future disease development which require longitudinal follow-up. However, the probabilistic nature of genetic testing and the subjectivity of genetic variants classification weighted on current evidence, making this powerful clinical tool difficult to be applied in precision diagnostics and therapeutics. Here, we reviewed systematically the genetic basis of CVDs with special emphasis on the current role of NGS in clinical diagnosis and risk assessment, underlying the need of multidisciplinary cardio-genetic referral centers.

Introduction

The dawn of next-generation sequencing (NGS) technology leads to the storage of massive genome sequence data of patients with rare monogenetic diseases as well as complex disorders, paving the way for personalized medicine. Mendelian cardiovascular diseases (CVDs) include familial hypercholesterolemia, cardiomyopathies, primary arrhythmias syndromes, thoracic aortic aneurysms and dissections and some congenital heart diseases (CHD). The scientific community consensus attributes to genetic testing a leading role for the disease management and genetic counselling to identify asymptomatic family members at risk of developing some diseases.[1] As such here, we summarize the current findings and applications of NGS in cardiovascular medicine.

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Coronary artery disease

Atherosclerotic coronary artery disease (CAD) is the leading cause of mortality in adults (older than 35 years) worldwide. The risk of developing CAD is modulated by an interplay between genetic and lifestyle factors.[2] Clinical observations dating back to the '50s support the notion that 50% of fatal CAD is heritable.[3] Since 2007, genetic association studies identified about 60 genetic loci linked to CAD, as to prove that the genetics of CAD largely derive from the cumulative effect of multiple common risk alleles.[4-6] Among the risk factors predisposing to CAD, familial hypercholesterolemia (FH) is the most commonly encountered genetic condition causing high levels of low-density lipoprotein (LDL). Advances in molecular genetics revealed that FH is more common and complex than previously thought, with the estimated prevalence of heterozygous FH of 1:250[7] and homozygous FH up to 1:300,000.[8] LDL has manifold deleterious effects on vascular function, including normal

arterial response to vasodilatatory stimuli, vascular inflammation through multiple mechanisms, and internalization by arterial wall macrophages when LDL particles become oxidized. When overloaded with cholesterol, arterial wall macrophages become foam cells, which are components of the atherosclerotic plaques that can eventually occlude arteries, leading to tissue ischemia. At least 9 different genes have been linked to FH harboring thousands of causative variants, among which LDL-receptor-*LDLR*, apolipoprotein B-*APOB*[9,10] and microsomal triglyceride transfer-*PCSK9*,[11] accounting for >80%, 5-10% and ~1% of FH characterized cases with monogenic basis, respectively (Table 1). Variant types include large-scale DNA copy number variations (CNVs, about 10%),[12] nonsense mutations within the coding region, missense mutations altering a single amino acid residue, small insertions or deletions (frameshifts) within or near the coding sequence and splicing site mutations occurring at the intron-exon boundaries.

Valvular heart disease

Valvular heart disease encompasses both congenital and acquired conditions increasing significantly morbidity and mortality worldwide.[13] Understanding of the mechanism underlying cardiac valve development led to the identification of several genetic etiologies for valvular disease.

Bicuspid aortic valve (BAV) is a congenital valvular defect that affects about 1-2% of the general population.[14,15] BAV has an autosomal dominant inheritance with reduced penetrance and variable expressivity. BAV has been described as an isolated trait or associated with syndromic conditions [*e.g.*, Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS) and Turner syndrome]. Complications of BAV may lead to aortic valve stenosis and regurgitation, infective endocarditis, ascending aortic aneurysms and dissection.[16-18] The first non-syndromic BAV genetic etiology was identified in *NOTCH1*[15] segregating as autosomal dominant disease in all affected family members. *NOTCH1* haploinsufficiency is the main cause found in ~4% of BAV patients.[19-21] Recently, *GATA4/5* has been linked to aortic valve morphogenesis and endocardial cell differentiation,[22-25] reduced *UFD1L* gene expression[26] and involvement of a locus containing *AXIN1/PDIA2*[27] have shed light on the complex landscape of BAV aided by sequencing technologies advancement (Table 2).

Supravalvular aortic stenosis (SVAS) can be either associated with Williams-Beuren syndrome (characteristic face, behavioral disorders and hypercalcemia) or isolated. The estimated incidence is approximately 1 out of 25,000 births and the mean prevalence in the general population is 1/7500.

Clinical presentation often consists of a systolic murmur that prompts a cardiological screening with ventricular hypertrophy. A progressive *hourglass* narrowing of the aorta and/or pulmonary artery lumen, is typically detected by echocardiography.

The disease is either sporadic or familial. If familial, it is transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity. SVAS is caused by a deletion in the elastin-*ELN gene*, which is located on chromosome 7q11.23.[28]

Mitral valve prolapse (MVP) is considered the most common degenerative valvular heart defect in the general population (2%-3%), characterized by abnormal atrial displacement of the MV leaflets during systole.[29] Genetic basis of MVP syndromic forms are found in MFS which lay mainly on fibrillin1-*FBN1*. To date the only gene linked to non-syndromic MVP in humans is filamin A-*FLNA*, an intracellular actin-binding filamentous protein with numerous roles in cell migration, scaffolding functions and signaling.[30,31] However, the disease seems more in keeping with a congenital valvular dystrophy than with the classical MVP.[32] Finally, murine models with non-syndromic MVP made evident the association of abnormal myxomatous phenotype also with Adams9 and Dchs1 haploinsufficiency.[33]

However, genetic test is not routinely performed for diagnostic purposes in valvular heart disease, with the exception of syndromic forms.

Table 1. Familial hypercholesterolemia.

Gene	Frequency (%)
LDLR	80-85
APOB	5-10
PCSK9	~1

Table 2. Bicuspid aortic valve.

	Gene
Non-syndromic bicuspid aortic valve	*NOTCH1* *GATA5* *GATA4* *ACTA2* *UFD1L* *AXIN1* *ENG* *EGFR* *SMAD6*
Syndromic bicuspid aortic valve	*FBN1* *TGFBR1/2* *ACTA2* *KCNJ2* *ELN* *HOXA1* *CLO3A1* *45,X karyotype*

Aortic disease

Thoracic (TAA) and abdominal (AAA) aortic aneurysms might exhibit a genetic component in their etiopathogenesis. The prevalence rate of AAA for men > 65 years old ranges from 1.7% to 7.2%. Even though various environmental factors have been implicated, a genetic diathesis has been advanced, with family history being one of the strongest risk factors.[34] TAA and thoracic aortic aneurysm dissection (TAAD) are known to be associated with inherited connective tissue disorders, such as MFS, LDS and vascular Ehlers-Danlos syndrome (EDS). TAA and TAAD are less common, with an incidence of 10.4 per 100,000 person/year and 2.2 per 100,000 person/year, respectively.[35] Research studies in these conditions demonstrated that the genetic component is even stronger, with 15% of patients having a positive family history and exhibiting mostly an autosomal dominant pattern of inheritance with high penetrance.[36]

MFS is a common autosomal dominant disorder (1:2000-1:10,000) with a variety of phenotypes and is known to be associated with mitral valve disease, TAA and TAAD. Aortic dilatation in MFS syndrome occurs at the sinuses and the tubular portion of the ascending aorta. In 1991 *FBN1* was identified as the causative gene which encodes for an extracellular matrix glycoprotein abundantly present in the suspensory ligament of the lens, the periosteum of bone, and the aortic media.[37] To date more than 1500 distinct *FBN1* mutations have been described.[38] Individual families may have their own private mutation, however family members sharing the same mutation often display a heterogeneous phenotype. Interestingly, 25% of MFS are caused by *de novo* mutations.[39] Mutations of the *FBN1* gene may directly affect the structure of the extracellular matrix but they may also have an effect on the transforming growth factor (TGF) β-binding protein complexes, leading to uncontrolled release of TGF-β, which has been associated with aneurysm formation. This is an important discovery, as some of the most recent studies on sporadic TAA have also focused on TGF-β signaling pathways, suggesting that syndromic and non-syndromic TAA may share, to an extent, a common genetic background.[40]

LDS is a rare autosomal dominant syndromic disorder (unknown incidence) combining the triad of arterial tortuosity and aneurysms throughout the arterial tree, hypertelorism and bifid uvula.[41] It is characterized by variable expression and aggressive TAAs which can grow 10 times faster than those of MFS.[42] LDS has been associated with mutation in TGF-β receptor-*TGFBR*, TGF-β downstream effector *SMAD3*, TGF-β2 ligand-*TGFB2*, and TGFβ 3 ligand-*TGFB3*.[39,43]

Vascular type of EDS is a very rare autosomal dominant disorder (<1:1,000,000) characterized by the risk of spontaneous intestinal, uterine, and arterial rupture as well as joint and cutaneous manifestations.[44] The culprit gene *COL3A1* encodes for type III procollagen, a component of skin, vessel wall, and hollow organs.[45,46] The defect results in friable aortic tissue with tears along the aorta and its branches, leading to rupture and dissection often without previous aneurysm and high surgical mortality.

Familial TAAD (FTAAD) represents a group of non-syndromic disorders characterized by isolated TAAs, without associated systemic features. FTAAD shows an autosomal dominant transmission with great clinical variability and low penetrance. Recently, mutations in genes usually associated with syndromic forms have been reported in FTAAD patients such as *MYH11, ACTA2, MYLK, TGFB2, PRKG1*.[47,48] However few data are already available on this new molecular entities and further studies are required (Table 3).

Cardiomyopathies

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy, defined by the presence of asymmetric left ventricular hypertrophy (LVH) occurring in the absence of known secondary cause, such as hypertension or aortic stenosis, in conjunction with normal global cardiac systolic function and impaired relaxations.[49] Prevalence is estimated in young adults as about 1 out of 500, with much lower rates in patients <25 years of age.[50] Phenotypic expression of cardiac hypertrophy is age-dependent, and accelerates during puberty and adolescence, typically manifesting by the 3rd and 4th decade of life.

LVH is commonly concentric, involving the interventricular septum, posterior and lateral walls. In about 30-40% of the patients, hypertrophy predominantly involves the interventricular septum, leading to asymmetric septal hypertrophy. HCM involving predominantly the cardiac apex is present only in a minority of cases. Histologically, cardiac myocyte disarray is the hallmark

Table 3. Aortopathies.

	Gene
Marfan syndrome	*FBN1*
Loeys-Dietz syndrome	*TGFBR* *SMAD3* *TGFB2* *TGFB3*
Ehlers-Danlos syndrome	*COL3A1*
Familial thoracic aortic aneurysm dissection	*MYH11* *ACTA2* *MYLK* *TGFB2* *PRKG1*

of HCM and typically occurs in conjunction with myocyte hypertrophy and interstitial fibrosis. Myocyte disarray in HCM typically involves >20% of the myocardium and is more prominent in the septum. LVH is defined as 13 mm or greater left ventricular wall thickness in adults and a z-score >2 in children.[51]

The cardinal symptoms are palpitations, pre-syncope, syncope due to ventricular arrhythmias at risk of sudden cardiac death (SCD). The second set of symptoms, which include dyspnea, orthopnea and peripheral edema, are related to diastolic dysfunction and heart failure with preserved ejection fraction. Systolic dysfunction usually occurs at later ages.

HCM exhibits an autosomal dominant mode of inheritance even though sporadic forms have been found in about one third of the cases.[52] Disease-causing mutations are detected mainly in genes encoding sarcomeric proteins (Table 4). The first missense mutation identified was located on the β-myosin heavy chain-*MYH7* gene[53] and up to date in 75% of cases an identifiable pathogenic variant is found in myosin binding protein c-*MYBPC3*[54] and *MYH7*. Less than 10% of cases carry variants in other genes encoding for the regulatory/essential light chains of the thick filaments (myosin light chain 2-*MYL2*; myosin light chain 3-*MYL3*)[55] and for sarcomere thin filaments proteins (troponin T-*TNNT2*; troponin I-*TNNI*3, troponin C-*TNNC1*, α tropomyosin-*TPM1*, α actin-*ACTC1*).[56-61] Multiple sarcomeric protein mutations are present in up to 5% of individuals and tend to have a more severe phenotype with earlier onset. Mutations in non-sarcomeric genes could also cause primary cardiac hypertrophy resembling HCM caused by sarcomeric mutations, but the pathophysiology in such conditions is different as such are considered HCM phenocopies.[62]

Approximately 5% of adults and children with unexplained LVH are *secondary HCM*, caused by metabolic disorder, mitochondrial diseases, syndromic and neuromuscular diseases.[63] Many of these conditions are hereditary, mostly displaying as autosomal recessive trait but also X-linked. Cardiac assessment should be an integral part of managing patients with these multisystem diseases, to avoid confusion leading to erroneous diagnoses of a primary cardiomyopathy.

Metabolic disorders, such as glycogen storage diseases, can be caused by mutation in glucosidase α acid-*GAA*[64] and protein kinase AMP_activated non catalytic subunit γ 2-*PRKAG2*[65] genes whereas lysosomal storage disease such as Anderson-Fabry disease and Danon disease are linked to mutation in galactosidase α-*GLA*[66] and lysosomal associated membrane protein 2-*LAMP2*[67] genes respectively.

Mitochondrial disorders caused by mutations in both nuclear and mtDNA cover a broad clinical and genetic spectrum. Friedrich's ataxia for instance is caused by the expansion of intronic trinucleotide GAA repeat in the mitochondrial frataxin-*FXN* gene[68] and inheritance is complex due to unequal proportion of abnormal mitochondria received from the mother, but also due to an unequal segregation of mitochondria during development.

Table 4. Cardiomyopathies and ion channel diseases.

	Gene	Frequency (%)
Hypertrophic cardiomyopathy		
	MYBPC3	~40
	MYH7	~30
	TNNI3	~5
	TNNT2	~5
	TPM1	~3
	ACTC1	~1
	MYL2	~1
	MYL3	~1
Familial dilated cardiomyopathy		
	TTN	12-25
	LMNA	4-8
	MYH7	3-4
	SCN5A	2-3
	RBM20	2
	DES	2
	ACTC1	~1
	TNNT2	~1
	DMD	~1
	DNAJC19	~1
	EMD	~1
	BAG3	~1
	PLN	~1
	TPM1	~1
	TNNI3	~1
	TAZ	~1
Restrictive cardiomyopathy		
	TNNI3	18
	MYH7	14
	MYBPC3	2
Arrhythmogenic cardiomyopathy		
	PKP2	10-45
	DSP	10-15
	DSG2	7-10
	DSC2	2
	JUP	~1
Long QT syndrome		
	KCNQ1	40-55
	KCNH2	30-45
	SCN5A	5-10
Brugada syndrome		
	SCN5A	20-30
	SCN10A	8-16
	CACNA1C	~7
	CACNB2B	~5
	CACNA2D1	~2
Catecholaminergic Polymorphic Ventricular Tachycardia		
	RYR2	~60
	CASQ2	3-5

RASopathies including Noonan syndrome and Costello syndrome, are developmental syndromes caused by mutations in genes involved in the RAS-MAPK pathway (*PTPN11, RAF1, SOS1, KRAS, NRAS, BRAF, HRAS*).[69-71] These conditions share many phenotypic features including dysmorphic facies, cardiac abnormalities, cognitive impairment and a predisposition to malignancy. Neuromuscular disorders represent another important group of diseases associated with HCM. Genes heavily expressed in the heart, such as *DES*, *CRYAB* and *FHL1*, have been associated with coexisting skeletal and cardiac myopathies.[72-74]

Dilated cardiomyopathy (DCM) is a myocardial disorder characterized by increased LV chamber size and systolic dysfunction, in the absence of abnormal loading conditions or coronary artery disease.[75,76] DCM is more common in men than in women, with an overall prevalence of 1:2500 and a conservative annual incidence of 7 per 10,000.[77] The etiologies of DCM span from inherited pathogenic gene mutations to acquired toxic and metabolic insults and chronic myocarditis. A genetic background has been identified in ~40% of familial forms and more rarely in sporadic forms.[78] Acquired forms (*i.e.* toxic, peripartum and tachycardia-induced cardiomyopathy) may also exhibit a genetic predisposition.

Systolic dysfunction is the hallmark pathophysiologic feature of DCM. Reduced sarcomere contractility can increase ventricular volumes to maintain cardiac output through the Frank-Starling mechanism, producing the thin-walled LV appearance that is observed in overt DCM.[79] Diagnostic criteria have been proposed to encompass a broad spectrum of genetic and acquired disorders that manifest with electrical and functional abnormalities that change over time.

DCM appears to be inherited as a monogenic trait with autosomal dominant, autosomal recessive, X-linked and matrilineal modes. More than 50 genes have been related to the disease pathogenesis of familial forms (Table 4).[80] Since the identification of the first mutation in the actin α cardiac muscle 1-*ACTC1* gene associated with familial DCM,[61] various genes encoding for proteins acting at different levels in the cardiomyocyte have been implicated in DCM, *e.g.* sarcomere, cytoskeleton, ion channels, nucleus and intercalated disc complexes. About 13% of patients carry at least 2 mutations in the same gene (compound heterozygosis) or in different genes (digenic heterozygosis), related to a worse prognosis.[80]

Titin-*TTN* truncating mutations have been linked to DCM, accounting for 19-25% of familial forms and 11-18% of sporadic forms.[81] However, *TTN* truncating mutations can be also found in 2-3% of healthy population,[82] making definition of mutations as pathogenic challenging. *TTN* missense mutations, with only few exceptions, are currently considered benign.[83,84] Lamin A/C (*LMNA*) mutations are found in up to 8% of DCM patients characterized by early onset (between 30 and 40 years) and conduction defects, atrial fibrillation, left bundle branch block, major ventricular arrhythmias and SCD, even in the absence of systolic left ventricular (LV) dysfunction.[85-87] Among sarcomeric genes causing DCM, 4-8% of DCM patients carry mutations in myosin protein such as *MYH7* and another 2% in troponin T-*TNNT2.*

Restrictive cardiomyopathies (RCMs) are currently classified according to their etiology as either primary (idiopathic RCM) or secondary (infiltrative such as amyloidosis or storage diseases).[88,89] Indeed, cardiac amyloidosis resulting from extracellular deposition of amyloid fibrils either from misfolded immunoglobulin light chain or from transthyretin-TTR protein, is an increasingly recognized cause of heart failure with preserved ejection fraction, and should be considered in the differential diagnosis of RCM patients. Idiopathic RCM is the least common of all cardiomyopathies, characterized by normal LV chamber size and wall thickness but increased wall stiffness, diffuse fibrosis, myocyte hypertrophy and progressive atrial enlargement.[90] In the largest series of RCM cases reported thus far, the mean age at diagnosis was 64 years (range 10 to 90 years) in the absence of infiltrative disease, long-standing untreated hypertension or other cardiac conditions known to impair diastolic ventricular filling.[91] The risk of death is higher for males with left atrial dimension >60 mm, age >70 years and higher New York Heart Association (NYHA) function class. RCM in adults has a prolonged course of disease as compared to pediatric cases which have often shown poor prognosis with high mortality rate.[91] Familial disease as well as sporadic cases have been described.

Heart failure due to diastolic dysfunction is the most common initial manifestation with a wide range of symptoms such as diminished exercise tolerance, dyspnea, edema, and palpitation. Increased myofilament sensitivity to calcium, marked deposition of collagen type III or of desmin have all been implicated in the pathogenesis of this condition.[92]

Familial RCM is characterized by autosomal dominant inheritance with variable expressivity ranging from skeletal myopathy, particularly affecting distal muscles of the extremities, to atrioventricular block.[88,89] Cardiac troponin I-*TNNI3* was the first gene associated with RCM,[93] and since then missense mutations have been identified in 18% of young patients with marked myofibrillar disarray in the absence of LVH. Up to 14% of RCM patients carry a mutation in β myosin heavy chain-*MYH7* and exhibit a mixed phenotype with HCM, more dyspnea, lower exercise capacity and higher rate of mortality, cardiac

transplantation or implantable cardioverter-defibrillator discharges.[94] Mutations in other sarcomeric (*TNNT2, MYBPC3, MYL2, MYL3* and *ACTC*)[95-97] and non-sarcomeric genes (*MYPN, TTN, FLNC, CRYAB* and *DES*)[98-101] accounting for less than 2% of cases have also been described in RCM (Table 4).

Arrhythmogenic cardiomyopathy (AC) is a rare disease of the heart muscle pathologically characterized by fibrofatty myocardial replacement and, clinically, by prominent ventricular arrhythmias.[102-105] The estimated prevalence of AC in the general population ranges from 1:2000 to 1:5000.[104-106] AC affects more frequently males than females (up to 3:1). It becomes clinically overt most often in the second to fourth decade of life.[104-106]

The hallmark lesion of AC is the replacement of the ventricular myocardium by fibrofatty tissue.[102,103,107] In AC myocardial atrophy is a genetically determined process that occurs progressively with time, it starts from the epicardium and extends toward the endocardium to become transmural, resulting into progressive wall thinning. It typically displays right ventricular aneurysms located in the so-called *triangle of dysplasia* (*i.e.* inflow, apex and outflow tracts).[107] Biventricular and left-dominant disease variants have been identified extending the spectrum of AC phenotypic expressions that affects both ventricles.[105] The phenotypic expression of AC varies considerably, ranging from the clinical profiles of asymptomatic family members with concealed structural abnormalities and no arrhythmias to symptomatic patients experiencing arrhythmic cardiac arrest or undergoing cardiac transplantation because of refractory heart failure.[104,108-113] 1994 and 2010 task force criteria were developed to diagnose the original right-dominant disease phenotype but did not consider specific criteria for detecting LV involvement and the more recently recognized left-sided phenotypic variants.[114]

AC exhibits an autosomal dominant mode of inheritance even though, recessive forms with and without cutaneous abnormalities have been reported.[115] Heterozygous or compound mutations in genes encoding proteins of desmosomes have been identified in 50% of cases.[115] Other genetic (non-desmosomal) and non-genetic causes of the disease have been also postulated. The first mutation, a deletion in plakoglobin-*JUP*, was identified in a recessive form of AC,[116] followed few years later by the identification of mutation in desmoplakin-DSP in the autosomal recessive and dominant forms.[117] Since then, disease-causing mutations are detected in genes encoding mainly for desmosomal proteins (Table 4). The most common mutant gene is plakophilin 2-*PKP2* (10-45%), followed by *DSP* (10-15%), desmoglein 2-*DSG2* (7-10%), and desmocollin 2-*DSC2* (2%).[118-121] Copy number variations (CNVs) of desmosomal genes have also been linked to AC substantially increasing the diagnostic yield of genetic testing.[122] Screening for non-desmosomal genes marginally increases the rate of detection of gene mutations, despite the fact that some mutations in specific genes such as transmembrane protein 43-*TMEM43* p.S358L and phospholamban-*PLN* p.R14del can be highly prevalent in certain populations due to a founder effect.[123,124] Compound /digenic heterozygosity has been identified in up to 25% of patients accounting for both phenotypic variability and more malignant life-time arrhythmic outcome (*dose-effect*).[125]

Ion channel diseases

Long QT syndrome (LQT) is a cardiac electro-physiologic disorder, characterized by QT prolongation and T-wave abnormalities at the electrocardiogram (ECG), which affects repolarization of the heart. The arrhythmic events occur due to runs of *torsades de pointes* ventricular tachycardia, which, according to its duration, produces syncope and deteriorates into ventricular fibrillation leading to cardiac arrest and SCD. Patients affected by LQTs have been identified all over the world except for black Africans and African-Americans. Among Caucasians, the prevalence of LQTs has been estimated as 1:2000 in apparently healthy live births with a 14 years mean age of presentation.[126]

Prolongation of the QT interval is the hallmark of LQTs even though it is not always present. Ventricular repolarization is not only prolonged but often shows bizarre morphologic alterations, some of which tend to be gene-specific. The diagnosis of LQTs is mainly based on the measurement of the corrected QT (QTc). A prolonged QTc >460 ms is sufficient to make a diagnosis of LQTs, in the absence of secondary causes of QTc prolongation that can occur with drugs, acquired cardiac conditions, electrolyte imbalance, and unbalanced diets. A scoring system has been established, which takes into account the age of the patient, medical and family history, symptoms, and QTc and provides a probability of the diagnosis of LQTs.[127] To date 15 subtypes of LQTs have been described which may be grouped into categories based on the mode of inheritance and extracardiac manifestations: Romano-Ward syndrome[128,129] (LQT 1-6, LQT 9-13) is characterized by an isolated prolonged QT interval and comprises the 3 major clinical phenotypes (LQT type 1, 2 and 3) associated with specific triggers of cardiac events: exercise/emotion (LQT1), auditory stimuli (LQT2) and sleep (LQT3).

All syndromes with an extracardiac manifestation are characterized by an extremely prolonged QT interval; Andersen-Tawil syndrome (LQT7)[130] and Timothy syndrome (LQT8)[131] exhibit an autosomal dominant inheritance and facial dysmorphism whereas

the Jervell and Lange-Nielsen syndrome[132] is characterized by congenital deafness and an autosomal recessive inheritance.

Mutations in more than 15 genes have been associated with LQTs, most encoding for subunits of potassium, sodium and calcium voltage-dependent ion channels (Table 4). Genetic screening identifies a disease-causing mutation in 75% of LQTs although 20%-25% of the patients with LQTs confirmed by the presence of an LQTs gene mutation may have a normal range QTc.[133] Three main genes, *KCNQ1*,[134] *KCNH2*,[135] and *SCN5A*,[136] account for 90% of positively genotyped LQT cases.[137] Gene specific therapy does exist underlying a major role for genetic testing in affected patients unlike other inherited CVD.

Short QT syndrome (SQT) is an extremely rare inherited cardiac channelopathy characterized by an accelerated cardiac repolarization, responsible for the development of life-threatening arrhythmias. Global population prevalence is difficult to establish due to the limited number of cases (<200 cases) identified worldwide. Fatal arrhythmias in early phase of SQTs are common, thus SQT frequency and lethality in adults is underestimated.[138]

Patients with SQT show a characteristic reduced adaptation of the QT interval to changes in heart rate. The alterations of the gating properties of the K^+ channels caused by SQT-related variants result in an increased efflux of K^+ during the plateau phase. This globally accelerates the cardiac repolarization and results in a remarkable and homogeneous shortening of the ventricular action potential duration, which represents the mechanism underlying arrhythmic susceptibility and SCD risk.

SQT displays an autosomal dominant pattern of inheritance with high phenotype penetrance.[139] SQT is associated with gain-of-function alterations in genes encoding outward K+ channels (*KCNH2, KCNQ1* and *KCNJ2*)[140-142] and loss-of-function mutations in genes encoding different subunits of cardiac L-type $Ca2^+$ channel (*CACNA1C* and *CACNB2*).[143] Recently 3 different mutations have been identified in SQT patients in genes encoding ion channels and plasma membrane proteins (*CACN2D1*, *SCN5A* and *SLC4A3*),[143-145] however no conclusive data exist concerning the association with SQT. The overall yield of genetic testing is low (range 15-30%), with none of the identified genes affecting more than 5% of the known SQT population.[138]

Brugada syndrome (BrS) is an inherited disease characterized by a coved-type ST-segment elevation in the right precordial ECG leads and increased risk of SCD, in the absence of structural abnormalities.[146,147] The cornerstone of BrS definition is its characteristic ECG pattern that can be present spontaneously or unmasked by drugs. BrS estimated prevalence is about 1:10,000-25,000 worldwide with much higher incidence in Asian and Southeast Asian countries, especially Thailand, the Philippines, and Japan, reaching 0.5-1 per 1000.[148,149] BrS is 8-10 times more prevalent in men than in women.[150]

BrS is diagnosed in patients with ST-segment elevation with type 1 morphology ≥2 mm in ≥1 lead in the right precordial leads V1, V2, positioned in the 2nd, 3rd, or 4th intercostal space. This occurs either spontaneously or after a provocative drug test with intravenous administration of class I antiarrhythmic drugs (sodium channel blocking agents: ajmaline, flecainide, pilsicainide, or procainamide). Patients with a spontaneous type I ECG at baseline (without conditions known to unmask the signature sign, *i.e.*, drugs and fever) have high risk of cardiac arrhythmic events at follow-up.[150]

Inheritance of BrS occurs via an autosomal dominant mode of transmission. Since the identification of the first loss-of-function mutation in *SCN5A*[151] 17 more genes (Table 4) have been linked to the disease with anecdotal frequencies. In all 18 genotypes, either a decrease in the inward sodium or calcium current or an increase in one of the outward potassium currents has been shown to be associated with the BrS phenotype. To date, more than 500 loss-of-function mutations in the *SCN5A* gene are known to cause BrS, accounting for 20% to 30% of BrS patients. The vast majority are single nucleotide substitutions (missense) or small insertion/deletions. These mutations alter the structure of ion channels made with the SCN5A protein and disrupt the flow of sodium ions into cardiac muscle cells. Other mutations prevent the *SCN5A* gene from producing any functional ion channels, which also reduces the inward flow of sodium ions.[152,153]

Progressive cardiac conduction defect (PCCD), also known as Lenègre disease,[154] is characterized by the progressive slowing of conduction velocity through the His-Purkinje system usually in older individuals.

PCCD is clinically characterized by a prolonged P-wave duration, PR interval, and QRS widening with axis deviation on the surface ECG. The diagnosis is based on clinical data together with family history and 12-lead ECG, nevertheless congenital heart disease or cardiomyopathies should be investigated. Indeed, the majority of cases present normal cardiac structure and contractile function, but complete atrio-ventricular block may lead to LV dilatation and heart failure.

PCCD exhibits an autosomal dominant trait with incomplete penetrance and variable expressivity. Mutations in *SCN5A, TRPM4, SCN1B, GJA5*[155,156] genes have been identified in patients with familial PCCD, presenting a structural normal heart, with only subtle fibrosis. Instead, in the presence of concomitant congenital heart defects, mutations have been localized in

early cardiac transcription factor such as *NKX2.5 GATA4* or *TBX5*.[157,158] Mutations in *LMNA* have also been reported in patients affected by severe PCCD without skeletal muscle involvement and dilated LV.[159]

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare inheritable arrhythmogenic disorder characterized by adrenergic-induced bidirectional and polymorphic ventricular tachycardia. The estimated prevalence of the disease is about 1:10,000 but a systematic study population is lacking.[125]

Clinical manifestations often occur in the first or second decade of life and usually triggered by physical activity or emotional stress.[160] CPVT patients present a normal basal ECG and Echo making diagnosis challenging. Indeed fainting episode may be attributed to neurologic disorder. Family history of exercise-related syncope, and SCD are reported in 30% of cases and may help directing the diagnosis. The first-line therapeutic preference for patients is beta-blockers without intrinsic sympathomimetic activity, and exercise restriction. Flecainide might be considered in case of β-blockers inefficacy,[161,162] as well as left cardiac sympathetic denervation in beta-blockers intolerant patients.[163,164]

CPVT is manly inherited as an autosomal dominant trait caused by mutations in the ryanodine receptor-*RYR2,*[165-167] however also a recessive form has been identified linked to mutation in the cardiac calsequestrin-*CASQ2*.[168] A causative mutation is identified in almost 60% of patients suggesting the presence of other factors involved in the disease pathogenesis. Recently, mutations in other genes *KCNJ2, ANK2, TRDN* and *CALM1* have been reported in patients with clinical features resembling CPVT but their role is still under investigation.[169-173]

Congenital heart disease

Congenital heart diseases are characterized by structural abnormality of the heart and great vessels that is present at birth.[174,175] CHD are considered multifactorial in origin, including genetic and non-genetic acquired risk factors. Genetic testing for CHD is increasingly becoming part of standard care. Phenotyping and family history should strongly guide the type of testing suggested.

Familial CHD mutations may occur as autosomal dominant, recessive, or X-linked traits and are characterized by high penetrance associated with variable clinical manifestations. Aneuploidies were the earliest identified genetic causes of CHD, observed in 35% to 50% of live born children with trisomy 21, 60% to 80% of live born children with trisomy 13 and trisomy 18, and 33% with monosomy X.[176]

Syndromic CHD have been demonstrated to be caused by several well-characterized large CNVs such as the 3Mb deletion del22q11 characterized by a variable phenotype encompassing palate abnormalities, hypocalcemia, immunodeficiency, characteristic facial features, and neurodevelopmental abnormalities including learning disabilities and psychiatric disorders, also known as DiGeorge syndrome and velo-cardio-facial syndrome.[177-179] Other CHD-associated CNVs are the deletion del8p23, which includes the cardiac transcription factor GATA4 characterized by developmental delay; the deletion del7q11 causing haploinsufficiency for elastin and William syndrome,[180,181] and the deletion del11q24-25 resulting in Jacobsen syndrome.[182,183] Besides syndromes associated with CNVs, their global contribution to CHD has been investigated in several large cohorts of patients with CHD such as, tetralogy of Fallot,[184] heterotaxy,[185] and hypoplastic left heart,[186-188] all of which show an over-representation of rare CNVs, and *de novo* CNVs compared with controls.[189]

Approximately 2% of CHD is due to inherited point mutations and many of the genes first implicated in inherited CHD are members of a core group of cardiac transcription factors that includes NKX2.5, the GATA family of zinc-finger proteins, T-box factors including TBX5 and TBX1 and MEF2 factors.[190-192]

The explosion of NGS enlarges the understanding of CHD complex genetics, allowing the identification of mutations that were undefinable through traditional genomic methods, such as *de novo*. *De novo* mutations account for approximately 10% of CHD and, in general, are more deleterious and cause more significant comorbidities than the mutations seen in Mendelian CHD.[193] Mosaic *de novo* variants have been shown to contribute up to 20% of sporadic cases in several developmental disorders, including Sturge-Weber syndrome,[194] facioscapulohumeral muscular dystrophy,[195] and segmental neurofibromatosis.[196] There have also been clinical reports suggesting pathogenic mosaic CNVs in patients with CHD.[197] Finally, detection limitations of CNVs and single-nucleotide variants may lead to underestimation of their contribution in some CHDs which are likely to be the result of multi-locus inheritance, or caused by mutations in noncoding DNA.

Genetic testing in cardiovascular diseases and the need of multidisciplinary cardio-genetic referral centers

NGS-based platforms for identifying causative variants increased significantly the success rate of genetic testing. Indeed, the yield of genetic testing is variable across CVDs, ranging from a very low value of 2-5% in valvular heart disease up to 75% in LQT syndrome. On the other hand, NGS increased also the difficulty in genetic variants interpretation, since the analysis of large numbers of genes may lead to the

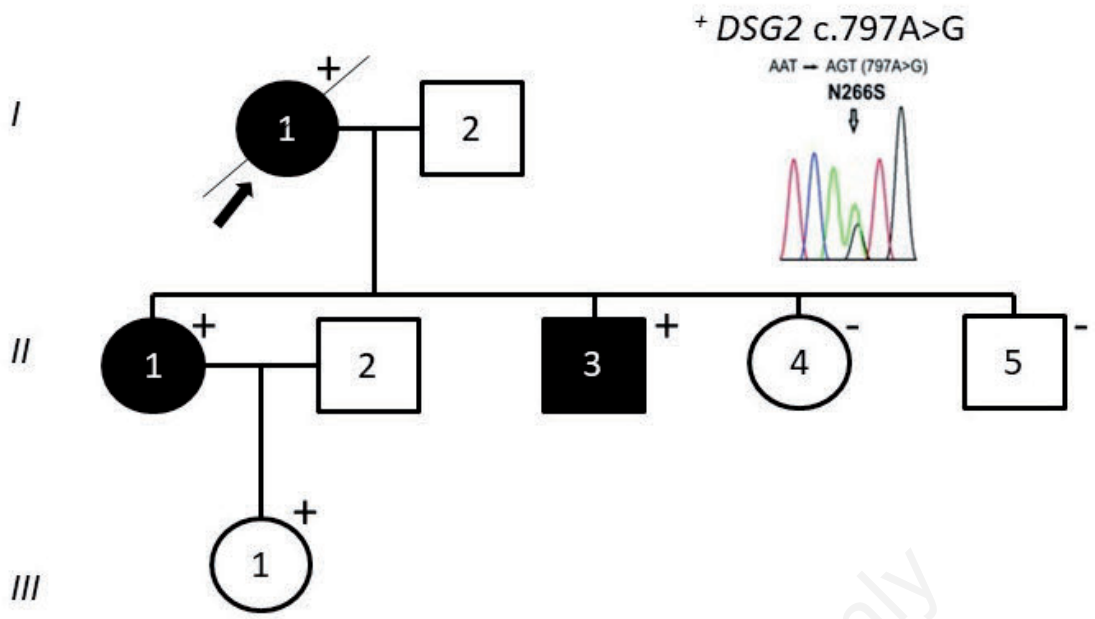

Figure 1. Family pedigree with a missense mutation in *Desmoglein-2* gene and autosomal dominant inheritance of the arrhythmogenic cardiomyopathy. Black and white symbols represent affected and unaffected individuals, respectively, Presense (+) or absence (-) of the DSG2 mutation is indicated and arrow highlights the proband. *Modified from Pilichou* et al.*, 2006.*[120]

identification of a large number of sequence variants with uncertain clinical significance (VUS). Thus, a VUS in a gene known to cause disease can create significant clinical equipoise regarding its use in predictive testing and diagnosis. As such, genetic testing and its interpretation should be performed by genetic counselors in dedicated cardio-genetic centers, with pre- and post-counseling facilities. Characterizing the underlying genetic cause of these cases reassures the patient, directs family screening and fertility planning, and in selected cases can guide therapy.[1] For example, identifying the causal gene in LQT allows target therapy in subsets of disease caused by potassium versus sodium channel dysfunction. Further, clarifying that is due to variants in *TTR* or Fabry's disease can allow targeted therapies including RNA silencing, isoform stabilizers and enzyme replacement.

Conclusions

Genetic testing can be used in a clinically affected patient to confirm diagnosis, or to formulate a differential diagnosis among overlapping phenotypes or between hereditary and acquired (nongenetic) forms of CVD. Moreover, after the identification of a pathogenic mutation in the proband, cascade genetic screening is recommended in clinically unaffected relatives, to identify asymptomatic carriers for early diagnosis and preventive strategies (Figure 1). Finally, a precise molecular diagnosis can help risk stratification in specific diseases and may guide management such as in LQT syndrome. Many knowledge gaps still exist in our understanding of CVDs. Establishing clear genotype-phenotype correlations remains a challenge, as the presently known heritable factors only partially explain the multiple cardiovascular phenotypes.

However, precision medicine is becoming increasingly possible with integration of the genome into the medical record and high throughput sequencing platforms for gene expression. The potential role of modifier genes, environmental and epigenetic factors in disease onset, progression and variable phenotype may soon unravel novel pathogenetic mechanisms.

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