Genetics and cardiovascular disease

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In recent years, comprehensive developments in genetics improved our knowledge of inherited diseases, including hereditary cardiac disorders. During the past, only one to several candidate genes could be studied to search for disease-causing mutations; currently Next Generation Sequencing (NGS) techniques enable the analysis of all variants within an individual genome, including those predisposed to disease.

These new genotyping techniques provide the possibility to identify variants which influence disease development, either by leading to more severe symptoms or protecting carriers of pathogenic variants from getting seriously ill.

Cardiogenetics is a branch of medical genetics in which molecular and cellular biology is applied to search for an alteration of cellular functions during development and for variants inside the genes that can explain this dysfunction. Based on these findings, it is important to establish a comparison between patient's phenotype and genotype and try to find a connection between them.

Over the past two decades, researchers in the field of cardiogenetics have acquired a complex understanding of the pathophysiological basis of inherited cardiac disease. Since the discovery of the first cardiomyopathy-associated gene in 1990 and the first cardiac channelopathy-associated genes in 1995, genetic testing for these heritable diseases has advanced from basic scientific level to clinical application (1).

Inherited cardiac diseases, distinguished in Cardiomyopathies and Channelopathies, are genetic disorders that often cause sudden cardiac death

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(SCD) at young age. SCD must be well investigated because it may occur from different causes like also Nemaline myopathy (2). Clinical genetic testing may improve risk stratification among patients affected by cardiomyopathy or channelopathy, especially for arrhythmia and SCD (3).

Cardiomyopathies

Inherited cardiomyopathies are characterized by structural and functional abnormalities of the ventricular myocardium that are unexplained by coronary artery disease or abnormal loading conditions (4). This group of cardiovascular disorders is classified based on ventricular morphology and function and include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) (1). Common complications of cardiomyopathies can include heart failure and SCD (5).

Hypertrophic Cardiomyopathy

Hypertrophic Cardiomyopathy (HCM) has a population prevalence estimated of 1:500 (6). HCM is typically characterized by the presence of unexplained left ventricular hypertrophy (LVH) with a maximum wall thickness ≥15 mm in adults or a z-score >3 in children. If there is a family history of HCM, or if genetic testing confirms that a relative has inherited the family's pathogenic variant, a maximum LV wall thickness ≥13 mm supports diagnosis.

The diagnosis of HCM is established with cardiac imaging, including echocardiography and/or cardiac magnetic resonance imaging (cardiac MRI) and rests on the detection of increased LV wall thickness, but the disease phenotype also includes myocardial fibrosis, morphologic abnormalities of the mitral valve apparatus, abnormal coronary microcirculatory function and electrocardiographic abnormalities.

While asymmetric septal hypertrophy is the most common pattern of hypertrophy, the degree and location of hypertrophy vary. It can be concentric or confined to other walls or the LV apex.

Clinical manifestations of HCM are highly variable, from asymptomatic LVH to arrhythmias to refractory heart failure. Moreover, manifestations can vary even within the same family. An important but relatively rare consequence of HCM is SCD, most likely related to ventricular tachycardia or ventricular fibrillation.

Pathogenic variants in one of the genes encoding a component of the sarcomere, are found in approximately 50-60% of probands with a family history of HCM, and 20-30% of probands without a family history of HCM. Approximately 3-5% of affected individuals have more than one sarcomere gene variant, although fewer than 1% will have more than one pathogenic or likely pathogenic variant (7-9). Double mutations (compound or double heterozygotes) usually produce a more-malignant phenotype than single mutations (1).

Most variants (80%) identified in patients with HCM occur in two genes encoding β-myosin heavy chain 7 (MYH7) or cardiac myosin-binding protein C (MYBPC3). Other genes encoding sarcomeric proteins are also implicated in HCM, including cardiac troponin T2 (TNNT2), cardiac troponin I (TNNI3), α-tropomyosin (TPM1), myosin regulatory light chain (MYL2), myosin essential light chain (MYL3), and cardiac a-actin (ACTC1) (7, 10). Genetic testing is recommended to confirm the diagnosis in individuals with clinical evidence that is suggestive of HCM and to allow cascade screening of at-risk relatives (11, 12).

Dilated cardiomyopathy

Dilated Cardiomyopathy (DCM) has a population prevalence estimated of 1:250 (13). DCM is typically characterized by ventricular dilatation and depressed myocardial contractility, clinically evident by a reduced ejection fraction, without a clear cause such as coronary artery disease.

The diagnosis of DCM is established by the presence of left ventricular enlargement and systolic dysfunction with a reduction in the myocardial force of contraction (8). Frequently, adults manifest in the fourth to sixth decade, although it may present at any age. DCM is genetically heterogeneous with many affected cellular proteins and diverse pathologic mechanism leading to specific phenotype. (9) Pathogenic variants have been reported in more than 30 genes in 40-50% of probands. The detection rate of variants is about 27% (14-16).

The main gene involved in DCM is TTN, encoding titin, the largest protein expressed in the heart. Rare variant in TTN are estimated to account for 10-20% of DCM. Other important genetic causes include variants in LMNA gene, encoding nuclear envelope protein lamins A and C. Rare variant in LMNA are estimated to account for 6% of DCM and is mostly associated with arrhythmic risk. Missense mutations of the LMNA gene can affect nuclear function and may also result in apoptosis and premature cell death of adipocytes, thereby causing lipodystrophy (17).

Other genes encoding sarcomeric, nuclear envelope and cytoskeleton proteins are also involved in DCM (10). Molecular genetic testing should be offered to individual of any age with non-ischemic DCM, including those with peripartum or pregnancy-associated cardiomyopathy (PPCM/PACM).

Molecular diagnosis of asymptomatic first-degree family members of an affected individual can allow early detection of DCM, initiation of treatment and improvement in long-term outcome. Genetic risk assessment and cardiac surveillance of at-risk relatives are important for detection of early treatable manifestations of DCM (16).

Arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) has a population prevalence estimated of 1:1000-1250 in the general population. ARVC is characterized by progressive fibrofatty replacement of the myocardium that predisposes to ventricular tachycardia and SCD. It primarily affects the right ventricle and it may also involve the left ventricle. The presentation of disease is highly variable even within family members.

The common genetic causes known to be associated with ARVC are genes encoding components of desmosome: plakophilin2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), desmoscollin-2 (DSC2), and junctional-plakophilin (JUP). Rare variant in PKP2 are estimated to account for 30-70% of ARVC. Less common genetic causes include variants in TMEM43, CTNNA3, DES, LMNA, PLN, RYR2, TGFB3 and TTN (18).

Molecular genetic testing should be considered in individuals who are suspected of having ARVC. If the pathogenic variant have been identified in an affected family member, it is appropriate to offer molecular genetic testing to relatives at risk for ARVC (even those age <18 years) because morbidity and mortality can be reduced by early diagnosis and treatment.

Cardiac channelopathies

Cardiac Channelopathies are a group of primary electrical disorders, resulting from the dysfunction of ion channels, associated with a risk of arrhythmia and SCD. These include Brugada Syndrome (BS), Long QT Syndrome (LQTS) and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). A few rare channelopathies characterized by clinical overlap have been associated also with pathogenic variant in ion-channel genes. These conditions include short QT syndrome, early repolarization syndrome, premature cardiac conduction disease, idiopathic ventricular fibrillation, familial atrial fibrillation, sinus node disease and multifocal ectopic Purkinje-related premature contractions (1).

Brugada syndrome

Brugada syndrome (BS) is caused by genetic changes in transmembrane ion channels which create action potentials, leading to an increased risk of cardiac arrhythmia. BS is characterized by cardiac conduction abnormalities (ST-segment abnormalities in leads V_1-V_3 on ECG and a high risk for ventricular arrhythmias) that can result in sudden cardiac death. It presents during adulthood, although age at diagnosis may range from infancy to late adulthood. The mean age of sudden death is approximately 40 years. Clinical presentations may also include sudden infant death syndrome (SIDS; death of a child during the first year of life without an identifiable cause) and the sudden unexpected nocturnal death syndrome (SUNDS).

Other conduction defects can include first-degree AV block, intraventricular conduction delay, right bundle branch block, and sick sinus syndrome (19). The prevalence of the disease manifesting with clinical symptoms is estimated to be 1:5000-10000, but the prevalence of clinically silent of type 1 Brugada ECG pattern is likely much higher (20).

The diagnosis of BS is established in a proband with Type 1 Brugada ECG pattern (elevation of the J wave ≥2 mm with a negative T wave and ST segment that is coved type and gradually descending) in more than one right precordial lead (V_1-V_3) with or without administration of a sodium channel blocker (i.e. flecainide or ajmaline) and at least one of the following manifestations: documented ventricular fibrillation, self-terminating polymorphic ventricular tachycardia, family history of SCD, coved-type ECGs in family members, electrophysiologic inducibility, syncope or nocturnal agonal respiration, and may include identification of a pathogenic variant in one of the genes involved (19).

In approximately 75% of probands, the diagnosis is established based on clinical history and ECG results. The most common gene involved is *SCN5A*, which encodes the α subunit of $Na_v1.5$ sodium channel. Rare variant in *SCN5A* are estimated to account for 15-35% of BS. Less common genetic causes include pathogenic variants in ABCC9, CACNA1C, CACNA2D1, CACNB2, FGF12, GPD1L, HCN4, KCND2, KCND3, KCNE5, KCNE3, KCNH2, KCNJ8, PKP2, RANGRF, SCN1B, SCN2B, SCN3B, SCN10A, SEMA3A, SLMAP, and TRPM4 genes (19).

Molecular genetic testing can confirm the diagnosis of BS. The genetic testing to search a pathogenic variant in family members of affected patient is useful to exclude clinical phenotypes in genotype-negative individuals to allow discharge from follow-up. Identifying pathogenic mutation carriers can have an important role in predicting the development of conduction disease and risk stratification. Genetic testing is not indicated in the case of an isolated type 2 or type 3 Brugada ECG pattern (1).

Long QT syndrome

Long QT syndrome (LQTS) is a cardiac electrophysiologic disorder, characterized by QT prolongation and T-wave abnormalities on the ECG associated with tachyarrhythmias, typically the ventricular tachycardia *torsade de pointes* (TdP). TdP is usually self-terminating, thus causing a syncopal event, the most common symptom in individuals with LQTS. Such cardiac events typically occur during exercise and emotional stress, less frequently during sleep, and usually without warning. TdP can degenerates to ventricular fibrillation and causes aborted cardiac arrest (if the individual is defibrillated) or SCD. (21)

Cardiac events are most common from the preteen years through the 20s but may also occur from infancy through middle age. Diagnosis of LQTS is established by prolongation of the QTc interval in the absence of specific conditions known to lengthen it and/or by molecular genetic testing that identifies a diagnostic change in one or more genes known to be associated with LQTS. Pathogenic variants in KCNH2 (LQTS types 2), KCNQ1 (LQTS types 1), and SCN5A (LQTS types 3), are the most common. Genotype-phenotype correlations have established circumstances associated with risk and ECG characteristics. Less common genetic causes include AKAP9, ANK2, CACNA1C, CALM1, CALM2, CAV3, KCNE1, KCNE2, KCNJ2, KCNJ5, SCN4B, SNTA1. Approximately 20% of families meeting clinical diagnostic criteria for LQTS do not have detectable pathogenic variants in a known gene (21).

Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease characterized by cardiac electrical instability exacerbated by acute activation of the adrenergic nervous system. CPVT is characterized by episodic syncope occurring during exercise or acute emotion in individuals without structural cardiac disease. The underlying cause of syncope is the onset of ventricular tachycardia (bidirectional or polymorphic). Spontaneous recovery may occur when these arrhythmias self-terminate.

In other instances, ventricular tachycardia may degenerate into ventricular fibrillation and cause SCD if cardiopulmonary resuscitation is not readily available. The mean age of onset of symptoms (usually a syncopal episode) is between seven and twelve years; onset as late as the fourth decade of life has been reported. If untreated, CPVT is highly lethal, as approximately 30% of affected individuals

experience at least one cardiac arrest before the age of 30 years, and up to 80% one or more syncopal spells. SCD may be the first manifestation of the disease. (22). The prevalence of CPVT in the population is not known. An estimate prevalence is 1:10,000. Approximately 50-55% of probands carry a mutation in RYR2 gene, which encodes the cardiac ryanodine receptor. Pathogenic variants in RYR2 gene are associated with early onset of symptoms. CASQ2 gene encoding calsequestrin account for a small number of rare autosomal recessive cases (2-5%). Cardiac ryanodine receptor and calsequestrin are involved in intracellular and sacroplasmic reticulum, which leads to intracellular calcium overload. This dysregulation can result in triggered activity and subsequently bidirectional ventricular tachycardia (TV). Other genetic causes include CALM1 and TRDN. RYR2 and CALM1-related CPVT are inherited in an autosomal dominant manner. CASQ2 and TRDN-related CPVT is typically inherited in an autosomal recessive manner. Identification of heterozygous pathogenic variants in RYR2 or CALM1, or of biallelic pathogenic variants in CASQ2 or TRDN can establish the diagnosis (22).

Hereditary cardiac disorders share characteristics that have a direct relevance to the clinical utility of genetic analysis and family screening. Because of variable expression and reduced penetrance, clinical phenotype can vary within family members sharing the same mutation, from a patient having no clinical manifestation to severe disease.

Genetic testing for inherited cardiac diseases need to be considered as one component of a multidisciplinary cardiogenetic evaluation in which the certainty of diagnosis, the probabilistic nature of genetic testing and the need for pre-test genetic counselling to inform the patient of the intrinsic uncertainties of genetic testing, and the need to obtain a family history to evaluate disease penetrance and expressivity, are addressed with care (1). Genetic analysis result has the potential to influence clinical decisions, so it is important to communicate it carefully during genetic counselling, the communication process that deals with the risk of occurrence of a genetic disorder in the family and should be regarded as an integral part of the genetic testing process, even in the field of cardiogenetics (23-26).

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