Chapter 5
Role of Genetic Testing for Sudden Death Predisposing Heart Conditions in Athletes[1]

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Introduction

Sudden death in a young competitive athlete occurs with a prevalence of approximately 1 per 100,000 athletes per year [2, 3]. Despite its rarity, the sudden death of an athlete is devastating to both the family and community. These tragic events often garner widespread attention due to the sudden, unexpected, and seemly paradoxical nature of the death of a trained athlete in good health. This underscores the occult nature of the diseases responsible for such youthful deaths. While the causes of sudden unexpected death (SUD) in the young are diverse, approximately two-thirds are explained following a conventional autopsy while one-third are classified as autopsy-negative SUD [4, 5]. Chief among autopsy-positive SUD are the heritable cardiomyopathies, particularly hypertrophic cardiomyopathy (HCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC). On the other hand, the cardiac channelopathies including long QT syndrome (LQTS) and catecholaminergic polymorphic ventricular tachycardia (CPVT) represent the most common cause(s) of autopsy-negative SUD [6, 7]. For the athlete, however, sudden cardiac death (SCD) stemming from a variety of cardiac conditions predominates and accounts for approximately 90% of "on-the-field" SUD among young athletes with the heritable cardiomyopathies (HCM and ARVC) responsible for nearly half of all such deaths [8].

[1] Dr. Ackerman is a consultant for Boston Scientific, Medtronic, PGxHealth, and St. Jude Medical, Inc. and chairs PGxHealth's Scientific Advisory Board with respect to their FAMILION™ genetic tests for heritable channelopathies and cardiomyopathies. There is a royalty relationship between PGxHealth and Mayo Clinic Health Solutions with respect to genetic testing for long QT syndrome and catecholaminergic polymorphic ventricular tachycardia.

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The last two decades have witnessed tremendous advances in the identification of the genetic perturbations responsible for the pathogenesis of these heritable cardiomyopathies and channelopathies. These mutations offer the unique possibility of identifying the genetic biomarker responsible for disease pathogenesis, even in ostensibly healthy individuals. Thus, clinical genetic testing can play a crucial role in the prevention of SCD in athletes and identification of individuals at risk of SCD with occult underlying disease. Further, in some SCD-associated diseases, genetic testing may directly influence therapeutic options and clinical management.

Hypertrophic Cardiomyopathy Case Vignette

JPA is a highly recruited 6′ 5″ high school basketball player. Although entirely asymptomatic, he has a positive family history of premature SCD secondary to HCM involving his paternal uncle and his older brother. His father has echocardiographic evidence for nonobstructive HCM but is asymptomatic. Since his brother's death 5 years ago, yearly echocardiograms have revealed a left ventricular wall thickness of 10–14 mm with normal diastolic function. Contrast-enhanced cardiac MRI was negative for late enhancement. Nevertheless, his cardiologist disqualified him from all competitive sports except class IA activities (i.e., billiards, bowling, cricket, curling, golf, and riflery) because of the positive family history and his "borderline" wall thickness (Fig. 5.1a). The family sought a second opinion. Evaluation was again unremarkable except for the upper limit of normal wall thickness. Genetic testing of the patient's father, which is positive in 35–65% of cases that meet a clinical diagnosis of HCM, revealed the well-established R92W missense mutation in cardiac troponin T (TNNT2-R92W). Postmortem genetic testing on archived autopsy samples confirmed this mutation in the basketball player's deceased uncle and brother. Mutation-specific confirmatory testing in the high school recruit was negative, and JPA returned to the basketball court.

Summary of Cardiomyopathy Genetic Testing

Hypertrophic Cardiomyopathy

The most common cause of SCD among athletes is HCM, which is characterized as asymmetrical left ventricular hypertrophy in the absence of a clinically identifiable cause such as hypertension. Affecting 1 in 500 people, HCM is one of the most prevalent, heritable cardiovascular diseases and is underscored by both marked genetic heterogeneity and phenotypic heterogeneity in degree of hypertrophy (none to extreme), fibrosis and myocyte disarray (none to extreme), left ventricular outflow tract obstruction (none to severe), morphological subtype (reverse curve-, sigmoidal-, and apical-HCM, for example), symptoms (none to debilitating symptoms refractory to pharmacotherapy), and sudden death susceptibility (asymptomatic longevity to premature sudden death prior to first sports participation evaluation).

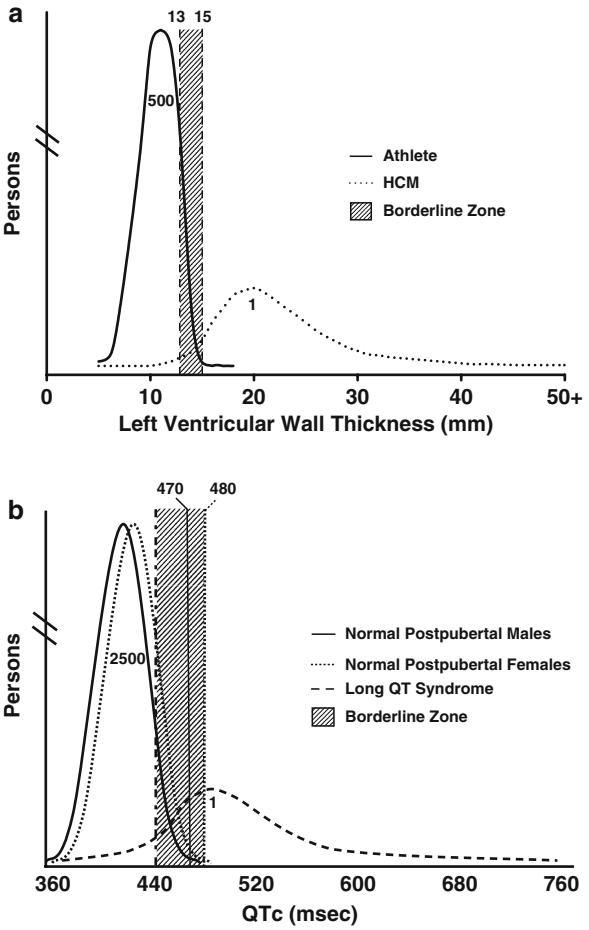

Fig. 5.1 "Borderline" and SCD-predisposing cardiomyopathies/channelopathies. (**a**) Graphical depiction of the overlap zone for left ventricular wall thickness between otherwise healthy athletes and patients with HCM. Note the average left ventricular wall thickness among patients with genetically proven HCM is about 22 mm. An estimated 20–30% of HCM is nonpenetrant, hence the completely normal septal wall thickness measurements. In contrast, a small percentage of normal subjects will have a wall thickness of 13–15 mm, so-called "borderline" hypertrophy. If a diagnosis of HCM has been assigned *solely* because of a wall thickness measurement that resides in this overlap zone, genetic testing may be utilized to provide genetic evidence for HCM or if negative, consider reclassification of the patient as possibly normal. The "athlete" distribution *curve* was adapted from Abernethy et al. [44]. (**b**) Graphical depiction of the normal distribution of QTc values in health and the normal distribution among patients with genetically proven LQTS seen at Mayo Clinic. The *solid line* depicts the distribution of QTc measurements in healthy men, *short dashed line* in healthy women, and the *long dashed line* in LQTS. Note that the so-called "borderline" QTc designation is often applied when the QTc exceeds 440 ms whereas the ~99th percentile values are 470 and 480 ms in males and females, respectively, giving rise to the zone of "borderline QT prolongation" (*oblique lines*). In contrast, the average QTc in our LQTS clinic is about 480 ms and approximately 30–40% of patients with genetically proven LQTS have a QTc < 460 ms. Note also the relative amplitudes of the normal curves and the LQTS curve emphasizing the 1:2,500 estimated incidence of LQTS. Akin to HCM and "borderline" values, if an athlete has been disqualified based solely upon his/her residence in this *gray zone*, a repeat evaluation that may include genetic testing should be considered. The distribution *curves* were adapted from Taggart et al. [43]

Nearly 20 years ago, the first chromosome locus for familial HCM and subsequently mutations involving the *MYH7*-encoded β myosin heavy chain were elucidated as a pathogenic substrate for HCM [9, 10]. Since then, HCM is viewed principally as an autosomal dominant disease with variable expressivity and penetrance. Additional genes encoding components of the cardiac sarcomere have been associated with HCM pathogenesis including the thick myofilament, intermediate myofilament, and thin myofilament. Genes encoding components of the cardiac Z-disc, as well as calcium-handling and regulator proteins, have been associated recently with HCM pathogenesis. Mutations in several genes encoding proteins responsible for cellular metabolic processes have been linked to unexplained left ventricular hypertrophy which can mimic the HCM-phenotype or "phenocopies." In all, hundreds of mutations have been identified in at least 27 putative HCM-susceptibility genes (Table 5.1). Once confined to the research laboratory, genetic testing for HCM-associated mutations has matured into clinically available, diagnostic tests for physicians evaluating and treating patients with this disease.

Currently, Harvard Partners, Correlagen, PGxHealth, and GeneDx offer HCM genetic testing for approximately 9–17 of the known HCM-susceptibility genes including the major HCM-susceptibility genes that encode the critical cardiac myofilaments: *MYH7*, *MYL2*, *MYL3*, *MYBPC3*, *TPM1*, *TNNT2*, *TNNI3*, *TNNC1*, and *ACTC* as well as the genes responsible for three HCM phenocopies: *PRKAG2*, *LAMP2* (Danon's disease), and *GLA* (Fabry's or Anderson Fabry's cardiomyopathy). The HCM-susceptibility genes available for commercial genetic testing are indicated (by superscript alphabet "a") in Table 5.1. These companies utilize a variety of technologies including an oligonucleotide hybridization-based chip methodology (Harvard Partners) or high-throughput direct DNA sequencing with either dideoxy sequencing or next-generation sequencing (Correlagen, PGxHealth, and GeneDx). All HCM genetic tests include mutation-specific family confirmatory testing to determine the presence of a proband-identified mutation in family members. The index test ranges in price from about $3,500–$5,500 (USA), while the family specific test costs between $250 and $900 with insurance reimbursement being quite variable for HCM genetic testing presently.

Regardless of the applied technology, each platform has a diagnostic accuracy exceeding 99%. Although no laboratory has published the results of genotyping the major HCM-susceptibility genes among ostensibly healthy individuals to determine the so-called "background noise rate" of rare genetic variants, the "false-positive" rate is estimated to be quite low («5%). The yield of HCM genetic testing using these commercial tests has declined from its original estimates of 80–90%. Instead, the yield has ranged from 35 to 65% in several different, international research cohorts of unrelated patients who met the clinically accepted definition of HCM [11, 12]. *MYBPC3*-encoded myosin binding protein C and *MYH7*-encoded β myosin heavy chain are, by far, the most common HCM-associated genes with an estimated prevalence of 25–35% for each gene and account for the majority of positive genetic tests. Expansion of commercial testing to include all 27 currently known HCM-susceptibility genes would not enhance the overall yield significantly as the other genes are extremely rare causes of HCM.

Table 5.1 Molecular basis of cardiomyopathies

Gene	Locus	Protein	Frequency
Myofilament (sarcomeric) hypertrophic cardiomyopathy (HCM)			
Giant filament			
TTN	2q31	Titin	<1
Thick filament			
MYH6	14q11.2–q12	α-Myosin heavy chain	Rare
MYH7[a]	14q11.2–q12	β-Myosin heavy chain	25–35%
MYL2[a]	12q23–q24.3	Regulatory myosin light chain	Rare
MYL3[a]	3p21.2–p21.3	Essential myosin light chain	Rare
Intermediate filament			
MYBPC3[a]	11p11.2	Cardiac myosin-binding protein C	25–35%
Thin filament			
ACTC[a]	15q14	α-Cardiac actin	Rare
TNNC1[a]	3p21.1	Cardiac troponin C	Rare
TNNI3[a]	19p13.4	Cardiac troponin I	1–5%
TNNT2[a]	1q32	Cardiac troponin T	3–5%
TPM1[a]	15q22.1	α-Tropomyosin	1–5%
Z-disc HCM			
ACTN2	1q42–q43	α-Actinin 2	Rare
CSRP3	11p15.1	Muscle LIM protein	Rare
LBD3	10q22.2–q23.3	LIM binding domain 3	Rare
MYOZ2	4q26–q27	Myozenin 2	Rare
TCAP	17q12–q21.1	Telethonin	Rare
VCL	10q22.1–q23	Vinculin/metavinculin	Rare
Calcium-handling HCM			
JPH2	20q13.12	Junctophilin 2	Rare
PLN	6q22.1	Phospholamban	Rare
CALR3	19p13.11	Calreticulin 3	Rare
CASQ2	1p13.3–p11	Calsequestrin 2	Rare
Metabolic cardiac hypertrophy (HCM mimickers)			
FXN	9q13	Frataxin	Rare
GLA[a]	Xq22	α-Galactosidase A	Rare
LAMP2[a]	Xq24	Lysosome-associated membrane protein 2	Rare
PRKAG2[a]	7q35–q36.36	AMP-activated protein kinase	Rare
RAF1	3p25.2	RAF serine/threonine kinase	Rare
Arrhythmogenic right ventricular cardiomyopathy (ARVC)			
DSC2[a]	18q12	Desmocollin 2	Rare
DSG2[a]	18q12.1–q12.2	Desmoglein 2	10–15%
DSP[a]	6p24	Desmoplakin	10–20%
JUP	17q21	Plakoglobin	Rare
PKP2[a]	12p11	Plakophilin 2	10–40%
RYR2	1q42.1–q43	Ryanodine receptor 2	Rare
TGFB3	14q23–q24	Transforming growth factor-β3	Rare
TMEM43[a]	3p25	Transmembrane protein 43	Rare

[a] Genes available as a commercial genetic test

Instead, echocardiography may help in providing appropriate genetic counseling as to the a priori probability of a positive genetic test (i.e., echo-guided genetic testing). Analysis of the echocardiograms of nearly 400 unrelated patients demonstrated that sigmoidal-septal, in which the septal hypertrophy is greater near the left ventricular outlet (so-called subaortic basal bulge) giving the septum a sigmoidal shape, and reverse curve-septal, in which the hypertrophic septum bulges into the left ventricular cavity "reversing" its usual curvature, morphologies were the two most prevalent anatomical HCM subtypes (47 and 35%, respectively). Apical HCM, in which the hypertrophy is localized to the apex of the heart and left ventricular cavity, constituted 10% of the cohort. In patients demonstrating reverse curve-HCM, the yield of myofilament genetic testing was 80% while sigmoidal-HCM had a 10% yield [13]. In this respect, septal contour was the strongest predictor of a positive HCM genetic test, regardless of age, with an odds ratio of 21 ($p < 0.001$) when reverse curve morphology was present [13].

Unlike the channelopathies, LQTS in particular, where there are clear diagnostic, prognostic, and therapeutic implications with the clinical genetic test, HCM genetic testing contributes primarily as a diagnostic tool. While a negative genetic test is not sufficient to "rule out" HCM, a positive genetic test may help distinguish HCM from physiologic cardiac hypertrophy and the "athlete heart." Further, genetic testing may play a key role in distinguishing HCM from other HCM phenocopies such as Anderson–Fabry's, glycogen (*PRKAG2*) and lysosomal (*LAMP2*) storage, mitochondrial, Noonan and LEOPARD syndromes, for which there are definitive and alternative therapies distinct from HCM treatments.

Most importantly, however, genetic testing of the index case has the potential of providing the diagnostic gold standard for his/her offspring, siblings, parents, as well as more distant relatives. A positive genetic test would then enable systematic scrutiny of the index case's relatives to identify affected individuals who carry the biomarker independent of clinical phenotype. On the other hand, a negative genetic test, as depicted in the case vignette, would effectively "rule out" HCM in this family setting enabling the athlete to once again pursue his/her dreams and forego yearly echocardiographic examinations.

Arrhythmogenic Right Ventricular Cardiomyopathy

In addition to HCM, arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC) is a notable cause of SCD in athletes due to fibro-fatty replacement of ventricular myocardium. Despite its name, ARVC can involve the left ventricle, in addition to the right, and is not necessarily arrhythmogenic [14]. The variable clinical course may include: (1) a subclinical phase with concealed structural abnormalities during which the affected individual may present with SCD as their sentinel manifestation of the disease, (2) an overt electrical disorder with palpitations and syncope due to tachyarrhythmias stemming from the right ventricle often triggered during exertion, and (3) severe right ventricular or biventricular failure requiring cardiac transplantation [15].

Studies have shown that up to 20% of youthful SCD may be attributed to ARVC, and the disease may be even more common in athletes who die suddenly. In one study

comprising 100 ARVC patients from the Johns Hopkins ARVC registry, palpitations and syncope were common presenting symptoms (27 and 26%, respectively) and fatal ventricular arrhythmias were the first manifestation in 22% [16]. Of the 29 patients experiencing SCD, 18 (62%) were involved in a routine activity, 9 (31%) in active exercise, 1 (3.5%) was pregnant, and 1 (3.5%) was in bed at the time of death. Nearly 25% of these SCD victims had other symptoms prior to death and may have benefited from an earlier diagnosis and an implantable defibrillator [16].

About half of all ARVC is recognized as familial with an autosomal dominant inheritance and the now expected disclaimer of incomplete penetrance and variable expressivity. To date, mutations in five ARVC-susceptibility genes that encode essential desmosomal proteins have been identified [14]. Desmosomes, also known as macula adherens, are macromolecular cellular structures composed of plasma membrane proteins involved in linking intracellular keratin filaments into a cell-spanning network allowing for effective transmission of force across cells. Functional deficits in these proteins imparted by genetic mutations result in cardiocyte detachment and cell death while subsequent inflammation results in fibro-fatty remodeling. Three nondesmosomal proteins, and the genes which encode them, have been linked to ARVC pathogenesis through varying mechanisms. Overall, eight genes have been implicated as pathogenic determinants of ARVC (Table 5.1).

PKP2, *DSP*, and *DSG* harbor the majority of identified mutations accounting for approximately 40% of ARVC cases in studies from the Netherlands [17], Italy [18], and the USA [16]. *PKP2* represents the most common gene mutated in ARVC, with a prevalence ranging from 40% (identified as the sole cause of ARVC in the cohort) [16, 17] to approximately 10% [18]. Harvard Partners, PGxHealth, and GeneDX currently offer ARVC clinical genetic testing with an estimated yield of approximately 50%.

Long QT Syndrome Case Vignette

LLH is a Division I, female collegiate swimmer with a history of dizziness, presyncope, and two fainting episodes associated with the sight of blood but not during exercise (including swimming). A pre-sports participation electrocardiogram demonstrated a QTc of 480 ms (99.5th percentile for postpubertal women, Fig. 5.1b). Her family history was negative but surveillance ECGs on her first-degree relatives revealed that her asymptomatic mother's QTc was 470 ms. Her cardiologist concluded that she has long QT syndrome, disqualified her from swimming, and implanted an ICD. One year later, her ICD lead fractured resulting in inappropriate ICD shocks and significant psychological sequelae. She and her family sought a second opinion that was unremarkable except for a resting QTc of 480 ms. Her treadmill stress test demonstrated normal shortening of her QTc during exercise and recovery. Her epinephrine QT stress test failed to demonstrate paradoxical prolongation of her absolute QT interval characteristic for patients with type 1 LQTS. Genetic testing elucidated an LQT3-causing mutation. Following explantation of her defective ICD lead and given her asymptomatic state and LQT3 status, LLH returns to the sport she loves consistent with recommendation no. 2 of the Bethesda Conference guidelines (task force 7) for LQTS [19].

Summary of Channelopathy Genetic Testing

Long QT Syndrome

Congenital long QT syndrome (LQTS), with a prevalence as high as 1 in 2,500 persons, comprises a distinct group of cardiac channelopathies characterized by delayed cardiac repolarization and increased risk for syncope, seizures, and SCD. Individuals with LQTS may or may not manifest QT prolongation on a resting 12-lead surface electrocardiogram (ECG, Fig. 5.1b). For virtually every heart beat in a person's life, this repolarization abnormality is without direct consequence; however, should the affected individual be exposed to triggers such as physical exertion (swimming), emotion, auditory stimuli (alarm clocks, door bells, etc.), or during the postpartum period, the depolarization delay can electrically destabilize the heart leading to the potentially lethal dysrhythmia of *torsades de pointes*. While in most incidences, the heart's normal sinus rhythm spontaneously returns, resulting in syncope, 5% of untreated and unsuspecting LQTS individuals succumb to a fatal arrhythmia as their sentinel event. Upon additional evaluation, it is estimated that nearly half of the victims of a LQT-triggered sudden death had exhibited prior warning signs, such as exertional syncope or a family history of premature sudden death, that went unrecognized [20].

LQTS, previously known as Romano-Ward syndrome, is a genetically heterogeneous disorder most often inherited in an autosomal dominant manner. Rarely, LQTS presents as the recessive trait first described by Drs. Jervell and Lange-Nielsen and is characterized by a severe cardiac phenotype and sensorineural hearing loss. In addition, 5–10% of LQTS results from a spontaneous germline mutation. To date, hundreds of mutations have been identified in 12 LQTS-susceptibility genes with approximately 75% of clinically robust LQTS due to mutations in three genes: *KCNQ1*-encoded I_{Ks} potassium channel (Kv7.1, LQT1, loss-of-function), *KCNH2*-encoded I_{Kr} potassium channel (Kv11.1, LQT2, loss-of-function), and *SCN5A*-encoded I_{Na} sodium channel (NaV1.5, LQT3, gain-of-function) that are responsible for the orchestration of the cardiac action potential [21]. The remainder of genotype-positive LQTS stems from mutations in genes that encode either other cardiac channels and channel interacting proteins or structural membrane scaffolding proteins that modulate channel function (Table 5.2). These minor genes contribute <5% of LQTS. Thus, 20–25% of LQTS remains genetically elusive.

Since 2004, LQTS genetic testing of the three major LQTS-susceptibility genes: *KCNQ1*, *KCNH2*, and *SCN5A*, and two of the minor genes: *KCNE1* and *KCNE2*, has been a clinically available diagnostic test offered by PGxHealth (FAMILION™). The yield is approximately 75% [22]. Thus, akin to HCM genetic testing, a negative genetic test cannot categorically exclude LQTS as a stand-alone test. Like with HCM genetic testing, the diagnostic accuracy is >99.5%. Approximately 5% of healthy volunteers possess a rare missense mutation of uncertain functional/clinical significance when genotyped through these five LQTS-susceptibility genes and this potential for a "false positive" must be kept in sharp focus especially as the veracity of the evidence for a clinical diagnosis of LQTS softens [23, 24]. Similar to HCM

genetic testing, the index case genetic testing for LQTS costs ~$5,400. In contrast to HCM genetic testing, LQTS genetic testing has been acknowledged already as a bonafide clinical test, rather than an "investigational test," having diagnostic, prognostic, and therapeutic implications. Consequently, the LQTS genetic test is reimbursed by many private health insurance companies, is a Medicare-covered test, and is a Medicaid-approved test in 37 states as of December 2008.

Much like septal morphology can be used to guide genetic testing in HCM, the emergence of phenotype–genotype associations has facilitated rapid mutational analysis of the LQTS-causing genes. Swimming and exertion-induced cardiac events strongly suggest mutations in *KCNQ1*, whereas auditory triggers and events occurring during the postpartum period should prompt suspicion for LQT2, and an event during sleep is associated with LQT3 [25–29]. Importantly, the underlying genetic basis heavily influences the response to standard LQTS pharmacotherapy (β blockers), where β blockers are extremely protective in LQT1 patients, moderately protective in LQT2, and may not provide sufficient protection for those with LQT3 [30].

Catecholaminergic Polymorphic Ventricular Tachycardia

Among the other channelopathies whose genetic substrates are summarized in Table 5.2, the channelopathy most relevant to the SCD of an athlete is catecholaminergic polymorphic ventricular tachycardia (CPVT), a heritable arrhythmia syndrome that classically manifests with exertion-induced syncope or sudden death [31, 32]. CPVT is associated with a normal resting ECG, with possible bradycardia and U waves, and is electrocardiographically suspected following either exercise or catecholamine-induced ventricular ectopy. Like LQTS, CPVT is generally associated with a structurally normal heart. However, CPVT is more lethal with a positive family history of juvenile (<40 years) SCD for more than 30% of CPVT individuals and up to 60% in families hosting mutations in *RYR2* [31].

CPVT stems from genetic mutations in genes encoding components of the macromolecular intracellular Ca^{2+} release channel complex within the sarcoplasmic reticulum (SR) of the cardiocyte. Mutations in the *RYR2*-encoded cardiac ryanodine receptor 2/calcium release channel (RyR2 or CRC, CPVT1) represent the most common genetic subtype of CPVT accounting for approximately 50–60% of cases [31]. Gain-of-function mutations in RyR2 lead to SR Ca^{2+} leakage resulting in excessive store Ca^{2+} release, particularly during sympathetic stimulation. Ultimately, this may precipitate Ca^{2+} overload, delayed depolarization, and ventricular arrhythmias [31]. A rare subtype of CPVT is mediated in an autosomal recessive manner with mutations in *CASQ2*-encoded calsequestrin 2 (CPVT2) [33].

RYR2 is one of the largest genes in the human genome containing 105 coding exons. In this regard, current CPVT genetic testing has focused on a targeted mutation analysis of *RYR2*. PGxHealth offers genetic testing of 38 *RYR2* exons (3, 8–15, 37, 41, 44–50, 83, and 87–105) with a purported yield of 50–55% of CPVT cases. Prevention Genetics analyzes 20 *RYR2* exons (8, 14, 15, 44, 45, 46, 47, 49, 50, 83,

Table 5.2 Molecular basis of cardiac channelopathies

Gene	Locus	Protein	Frequency
Long QT syndrome (LQTS)			
KCNQ1 (LQT1)[a]	11p15.5	Kv7.1	30–35%
KCNH2 (LQT2)[a]	7q35–36	Kv11.1	25–30%
SCN5A (LQT3)[a]	3p21–p24	NaV1.5	5–10%
ANKB	4q25–q27	Ankyrin B	Rare
AKAP9	7q21–q22	Yotiao	Rare
CACNA1C	12p13.3	L-type calcium channel	Rare
CAV3	3p25	Caveolin 3	Rare
KCNE1[a]	21q22.1	MinK	Rare
KCNE2[a]	21q22.1	MiRP1	Rare
KCNJ2	17q23	Kir2.1	Rare
KCNJ5	11q24	Kir3.4	Rare
SCN4B	11q23.3	Sodium channel β4 subunit	Rare
SNTA1	20q11.2	Syntrophin α1	Rare
Catecholaminergic polymorphic ventricular tachycardia (CPVT)			
RYR2 (CPVT1)[a]	1q42.1–q43	Ryanodine receptor 2	50–60%
CASQ2	1p13.3–p11	Calsequestrin 2	Rare
Brugada syndrome (BrS)			
SCN5A (BrS1)[a]	3p21–p24	NaV1.5	20–30%
CACNA1C	12p13.3	L-type calcium channel	~5–10%
CACNB2	10p12	L-type calcium channel β2 subunit	~5–10%
GPD1L	3p22.3	Glycerol-3-phosphate dehydrogenase 1-like	Rare
KCNE3	11q13.4	MiRP2	Unknown
SCN1B	19q13.1	Sodium channel β1 subunit	Unknown
Short QT syndrome (SQTS)			
KCNH2[a]	7q35–36	Kv11.1	Unknown
KCNJ2	17q23	Kir2.1	Unknown
KCNQ1[a]	11p15.5	Kv7.1	Unknown

[a]Genes available as a commercial genetic test

88, 90, 93, 95, 97, 100, 101, 102, 103, and 105). The false-positive potential is estimated to be low but results from genotyping a large cohort of healthy subjects have not been published so far.

Brugada Syndrome

Brugada syndrome (BrS) is another heritable arrhythmia syndrome associated with SCD characterized by an ECG pattern of coved type ST-segment elevation in the right V_1 through V_3 precordial leads and an increased risk for sudden death resulting from episodes of polymorphic ventricular tachyarrhythmias [34]. The penetrance and expressivity are highly variable, ranging from entirely asymptomatic individuals to SCD during the first year of life. Fever represents the most common precipitating factor for arrhythmic cardiac events, including syncope and SCD [35].

Approximately 20–25% of BrS stems from loss-of-function mutations in the *SCN5A*-encoded cardiac sodium channel (NaV1.5, BrS1). In addition to primary mutations of the sodium channel, mutations in genes (*GPD1L* [36] and *SCN1B* [37]) that modulate the sodium channel function have been associated with BrS. Recently, mutations involving the L-type calcium channel α (*CACNA1C*) and β (*CACNB2B*) subunits have been implicated in nearly 10% of BrS cases [38] while mutations in the putative β-subunit of the transient outward potassium channel (Ito, *KCNE3*) have been reported as a rare cause [39]. These are summarized in Table 5.2. To date, only PGxHealth offers genetic testing for only type 1 BrS analyzing all 27 translated exons of *SCN5A*.

Short QT Syndrome

Short QT syndrome (SQTS), a relatively new clinical syndrome first described in 2000, is associated with a short QT interval (<320 ms) on a 12-lead ECG, paroxysmal atrial fibrillation, syncope, and an increased risk for SCD [40]. Cardiac arrest has been the most common initial presentation among 29 patients with SQTS while nearly one-third presented with atrial fibrillation [41]. Symptoms such as syncope or cardiac arrest occurred most often during periods of rest/sleep. To date, gain-of-function mutations in the potassium channel encoding genes *KCNH2*, *KCNQ1*, and *KCNJ2* have been identified as pathogenic substrates for SQTS [42] (Table 5.2).

Indications for Genetic Testing in the Athlete

The "Borderline" Patient

Genetic testing plays a universal, yet variable, role in the diagnosis of SCD-associated cardiomyopathies and channelopathies. While wise clinical judgment remains paramount, genetic testing can offer evidence to support (move towards) or refute (move away from) a contemplated cardiomyopathic/channelopathic diagnosis which can be particularly important in questionable cases. For example, while a negative genetic test is not sufficient to "rule out" either HCM or LQTS all by its own, genetic testing might help distinguish borderline pathologic HCM from extreme physiologic hypertrophy/athlete heart (both between 13 and 15 mm septal thickness, Fig. 5.1a). Indeed, a positive *MYH7* or *MYBPC* genetic test in an athletic adolescent with borderline left ventricular hypertrophy would confirm the diagnosis of HCM while a negative test might provide an independent, objective data point to move away from this consideration especially if the sole determinant for considering HCM in the first place was a borderline wall thickness measurement. Further, genetic testing may play a key role in distinguishing HCM from treatable, metabolic diseases such as Fabry's disease. Correct diagnosis of these diseases would guide the use of definitive and alternative therapies, such as recombinant enzyme-replacement therapy in Fabry's disease.

Similar to "borderline hypertrophy," genetic testing may assist in the evaluation of the athlete who has been disqualified solely because of "borderline QT prolongation" (Fig. 5.1b). Recently, we demonstrated that 40% of unrelated patients coming to Mayo Clinic with the diagnosis of LQTS have left without the diagnosis, the vast majority being reclassified as otherwise normal with only neurocardiogenic presyncope/syncope [43]. Among this subset of diagnostic reversals, no LQTS-causative mutations have been identified so far providing an independent, objective piece of evidence falling more in line with our clinical judgment than the patient's originally rendered diagnosis.

"Gold Standard" Diagnostic Marker for the Asymptomatic Athlete Who Is Part of a Family with a Sudden Death Predisposing Heart Condition

One of the most important roles for genetic testing is potential identification of an at-risk, disease-conferring genetic biomarker in the index case which can be used as the diagnostic gold standard in the clinical evaluation of his/her offspring, siblings, parents, as well as more distant relatives. This is especially crucial in SCD-associated cardiomyopathies/channelopathies in which SCD can be the sentinel event. A positive genetic test would enable systematic scrutiny of the index case's athletic and nonathletic relatives to identify genetically affected individuals. In this way, the genetic testing of an HCM index case, for example, permits separation of family members into two management groups: (1) close surveillance of the genotype-positive, preclinical individual and (2) casual observation or dismissal of the genotype-negative/phenotype-negative relative (like case vignette no. 1) and his/her future progeny.

Finally, the results of familial genetic testing should be communicated to the affected individuals in the context of appropriate genetic counseling by either genetic counselors, cardiovascular geneticists, or genetic/molecular cardiologists. It is critical that the effect(s) of the genetic test on lifestyle, physical activities, insurance, and family planning be considered in the context of each individual. Thus, while genetic testing may play an important role in the evaluation of the athlete being investigated for a possible SCD-predisposing cardiomyopathy/channelopathy, the wise use and even wiser interpretation of such genetic testing is of paramount importance.

Glossary

Dideoxy DNA sequencing	To directly determine a DNA sequence, a primer binds to the region of interest on the sample DNA and initiates polymerization of DNA which is terminated in small amounts after each nucleotide addition. Each resulting oligonucleotide is labeled with a fluorescent tag identifying the sequence of the final nucleotide. The collection of fluorescent products is separated based on size,

	where the smallest fragment represents the first sequenced nucleotide, and the sequence is determined by the order of the nucleotide-specific fluorescent signals. Generally, this can yield a DNA sequence up to 800 nucleotides per reaction.
Exon	DNA sequence of a gene that is present in the mature mRNA after splicing removal of introns. Generally, the exonic DNA sequence ultimately codes for the corresponding amino acid sequence of the protein. There is exonic sequence at the termini of the mature mRNA of variable lengths which do not code for protein in this manner and remain untranslated (5′ and 3′ untranslated regions).
Gene	The basic hereditary unit of life which, in humans, is composed of a DNA sequence traditionally containing "coding" exonic sequence that is ultimately *transcribed* into mRNA and *translated* into protein as well as intervening "noncoding" intronic sequences which are removed during transcription and do not code for protein.
Genome	The hereditary information of an organism encoded by nucleic acid including all chromosomes. In humans, there are two complete homologous sets of DNA – one paternal and one maternal – and are thus considered diploid genomes.
Germline	The cells within an organism that pass genetic information to progeny. In humans, this refers to sperm and egg cells specifically.
Intron	A gene's DNA sequence that is removed during splicing of the mature mRNA and does not ultimately code for protein during translation.
Mutation	Changes in the DNA sequence of an organism. In humans, mutations can generally be described as synonymous (does not ultimately alter the coding protein sequence) or nonsynonymous (does ultimately alter the original coding sequence). Nonsynonymous mutations include missense (altering a single amino acid), nonsense (changes an amino acid to a stop codon which truncates the protein), and insertion/deletion (addition or subtraction of DNA which often shifts the open reading frame of the gene resulting in an altered, "frame-shifted," protein sequence after that point and usually an early truncation of the protein). For the hypertrophic cardiomyopathy-associated missense mutation, TNNT2-R92W, for example, the DNA mutation has changed an arginine amino acid (R) to a tryptophan (W) at position 92 of the protein troponin T which is encoded by *TNNT2*.

Next-generation DNA sequencing	A conglomeration of recently developed DNA sequencing methodologies capable of sequencing over 20×10^9 nucleotides per reaction depending on the platform [1].
Oligonucleotide hybridization mutation detection	Sample (patient) DNA is fluorescently labeled and exposed to a microarray chip containing thousands of short sequences of DNA (oligonucleotides) corresponding to mutated sequences of the genes of interest. If a mutation is present in the patient, it will bind (hybridize) to the known oligonucleotide on the chip and deliver an identifiable fluorescent signal.

References

1. Morozova O, Marra MA. 2008. Applications of next-generation sequencing technologies in functional genomics. *Genomics*. 92(5):255–264.
2. Maron BJ, Gohman TE, Aeppli D. 1998. Prevalence of sudden cardiac death during competitive sports activities in Minnesota High School athletes. *J Am Coll Cardiol*. 32(7):1881–1884.
3. Corrado D, Basso C, Schiavon M, Thiene G. 2006. Does sports activity enhance the risk of sudden cardiac death? *J Cardiovasc Med*. 7(4):228–233.
4. Puranik R, Chow CK, Duflou JA, Kilborn MJ, McGuire MA. 2005. Sudden death in the young. *Heart Rhythm*. 2(12):1277–1282.
5. Ellsworth EG, Ackerman MJ. 2005. The changing face of sudden cardiac death in the young. *Heart Rhythm*. 2(12):1283–1285.
6. Tester DJ, Ackerman MJ. 2007. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. *J Am Coll Cardiol*. 49(2):240–246.
7. Tester DJ, Ackerman MJ. 2006. The role of molecular autopsy in unexplained sudden cardiac death. *Curr Opin Cardiol*. 21(3):166–172.
8. Maron BJ. 2003. Sudden death in young athletes. *N Engl J Med*. 349(11):1064–1075.
9. Jarcho JA, McKenna W, Pare JA, Solomon SD, Holcombe RF, Dickie S, Levi T, Donis-Keller H, Seidman JG, Seidman CE. 1989. Mapping a gene for familial hypertrophic cardiomyopathy to chromosome 14q1. *N Engl J Med*. 321(20):1372–1378.
10. Geisterfer-Lowrance AA, Kass S, Tanigawa G, Vosberg H, McKenna W, Seidman CE, Seidman JG. 1990. A molecular basis for familial hypertrophic cardiomyopathy: A beta cardiac myosin heavy chain gene missense mutation. *Cell*. 62:999–1006.
11. Marian AJ, Roberts R. 2001. The molecular genetic basis for hypertrophic cardiomyopathy. *J Mol Cell Cardiol*. 33:655–670.
12. Van Driest SL, Ommen SR, Tajik AJ, Gersh BJ, Ackerman MJ. 2005. Yield of genetic testing in hypertrophic cardiomyopathy. *Mayo Clin Proc*. 80(6):739–744.
13. Binder J, Ommen SR, Gersh BJ, Van Driest SL, Tajik AJ, Nishimura RA, Ackerman MJ. 2006. Echocardiography-guided genetic testing in hypertrophic cardiomyopathy: septal morphological features predict the presence of myofilament mutations. *Mayo Clin Proc*. 81(4):459–467.

14. Sen-Chowdhry S, Syrris P, McKenna WJ. 2007. Role of genetic analysis in the management of patients with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J Am Coll Cardiol*. 50(19):1813–1821.
15. Thiene G, Corrado D, Basso C. 2007. Arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Orphanet J Rare Dis*. 2:45.
16. Dalal D, Molin LH, Piccini J, Tichnell C, James C, Bomma C, Prakasa K, Towbin JA, Marcus FI, Spevak PJ, Bluemke DA, Abraham T, Russell SD, Calkins H, Judge DP. 2006. Clinical features of arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in plakophilin-2. *Circulation*. 113(13):1641–1649.
17. van Tintelen JP, Entius MM, Bhuiyan ZA, Jongbloed R, Wiesfeld ACP, Wilde AAM, van der Smagt J, Boven LG, Mannens MMAM, van Langen IM, Hofstra RMW, Otterspoor LC, Doevendans PAFM, Rodriguez L-M, van Gelder IC, Hauer RNW. 2006. Plakophilin-2 mutations are the major determinant of familial arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Circulation*. 113(13):1650–1658.
18. Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, Lorenzon A, Frigo G, Vettori A, Valente M, Towbin J, Thiene G, Danieli GA, Rampazzo A. 2006. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation*. 113(9):1171–1179.
19. Zipes DP, Ackerman MJ, Estes Iii NAM, Grant AO, Myerburg RJ, Van Hare G. 2005. Task Force 7: Arrhythmias. *J Am Coll Cardiol*. 45(8):1354–1363.
20. Tester DJ, Ackerman MJ. 2007. Postmortem long QT syndrome genetic testing for sudden unexplained death in the young. *J Am Coll Cardiol*. 49(2):240–246.
21. Tester DJ, Will ML, Haglund CM, Ackerman MJ. 2005. Compendium of cardiac channel mutations in 541 consecutive unrelated patients referred for long QT syndrome genetic testing. *Heart Rhythm*. 2:507–517.
22. Tester DJ, Will ML, Haglund CM, Ackerman MJ. 2006. Effect of clinical phenotype on yield of long QT syndrome genetic testing. *J Am Coll Cardiol*. 47(4):764–768.
23. Ackerman M, Tester D, Jones G, Will M, Burrow C, Curran M. 2003. Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. *Mayo Clin Proc*. 78(12):1479–1487.
24. Ackerman MJ, Splawski I, Makielski JC, Tester DJ, Will ML, Timothy KW, Keating MT, Jones G, Chadha M, Burrow CR, Stephens JC, Xu C, Judson R, Curran ME. 2004. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: Implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm*. 1(5):600–607.
25. Ackerman MJ, Tester DJ, Porter CJ. 1999. Swimming, a gene-specific arrhythmogenic trigger for inherited long QT syndrome. *Mayo Clin Proc*. 74(11):1088–1094.
26. Wilde AA, Jongbloed RJ, Doevendans PA, Duren DR, Hauer RN, van Langen IM, van Tintelen JP, Smeets HJ, Meyer H, Geelen JL. 1999. Auditory stimuli as a trigger for arrhythmic events differentiate HERG- related (LQTS2) patients from KVLQT1-related patients (LQTS1). *J Am Coll Cardiol*. 33(2):327–332.
27. Moss AJ, Robinson JL, Gessman L, Gillespie R, Zareba W, Schwartz PJ, Vincent GM, Benhorin J, Heilbron EL, Towbin JA, Priori SG, Napolitano C, Zhang L, Medina A, Andrews ML, Timothy K. 1999. Comparison of clinical and genetic variables of cardiac events associated with loud noise versus swimming among subjects with the long QT syndrome. *Am J Cardiol*. 84(8):876–879.
28. Schwartz PJ, Priori SG, Spazzolini C, Moss AJ, Vincent GM, Napolitano C, et al. 2001. Genotype-phenotype correlation in the long-QT syndrome: gene-specific triggers for life-threatening arrhythmias. *Circulation*. 103:89–95.
29. Khositseth A, Tester DJ, Will ML, Bell CM, Ackerman MJ. 2004. Identification of a common genetic substrate underlying postpartum cardiac events in congenital long QT syndrome. *Heart Rhythm*. 1:60–64.
30. Khositseth A, Ackerman MJ. Clinical evaluation, risk stratification, and management of congenital long QT syndrome. In: Gussak I, Antzelevitch C, eds. *Contemporary Cardiology: Cardiac Repolarization: Bridging Basic and Clinical Science*. Totowa: Humana; 2003.

31. Priori SG, Napolitano C, Memmi M, Colombi B, Drago F, Gasparini M, DeSimone L, Coltorti F, Bloise R, Keegan R, Cruz Filho FE, Vignati G, Benatar A, DeLogu A. 2002. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia [see comment]. *Circulation*. 106(1):69–74.
32. Tester DJ, Kopplin LJ, Will ML, Ackerman MJ. 2005. Spectrum and prevalence of cardiac ryanodine receptor (RyR2) mutations in a cohort of unrelated patients referred explicitly for long QT syndrome genetic testing. *Heart Rhythm*. 2(10):1099–1105.
33. Eldar M, Pras E, Lahat H. 2003. A Missense mutation in the CASQ2 gene is associated with autosomal-recessive catecholamine-induced polymorphic ventricular tachycardia. *Trends Cardiovasc Med*. 13:148–151.
34. Chen PS, Priori SG. 2008. The Brugada syndrome. *J Am Coll Cardiol*. 51(12):1176–1180.
35. Probst V, Denjoy I, Meregalli PG, Amirault JC, Sacher F, Mansourati J, Babuty D, Villain E, Victor J, Schott JJ, Lupoglazoff JM, Mabo P, Veltmann C, Jesel L, Chevalier P, Clur SA, Haissaguerre M, Wolpert C, Le Marec H, Wilde AA. 2007. Clinical aspects and prognosis of Brugada syndrome in children. *Circulation*. 115(15):2042–2048.
36. London B, Michalec M, Mehdi H, Zhu X, Kerchner L, Sanyal S, Viswanathan PC, Pfahnl AE, Shang LL, Madhusudanan M, Baty CJ, Lagana S, Aleong R, Gutmann R, Ackerman MJ, McNamara DM, Weiss R, Dudley SC, Jr. 2007. Mutation in glycerol-3-phosphate dehydrogenase 1-like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. *Circulation*. 116(20):2260–2268.
37. Watanabe H, Koopmann T, Le Scouarnec S, Yang T, Ingram C, Schott J, Demolombe S, Probst V, Anselme F, Escande D, Wiesfeld A, Pfeufer A, Kääb S, Wichmann H, Hasdemir C, Aizawa Y, Wilde A, Roden D, Bezzina C. 2008. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest*. 118(6):2260–2268.
38. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros EP, Jr., Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu LF, Haissaguerre M, Schimpf R, Borggrefe M, Wolpert C. 2007. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation*. 115(4):442–449.
39. Delpon E, Cordeiro JM, Nunez L, Bloch Thomsen PE, Guerchicoff A, Pollevick GD, Wu Y, Kanters JK, Larsen CT, Burashnikov E, Christiansen M, Antzelevitch C. 2008. Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. *Circ Arrhythm Electrophysiol*. 1(3):209–218.
40. Gussak I, Brugada P, Brugada J, Wright RS, Kopecky SL, Chaitman BR, Bjerregaard P. 2000. Idiopathic short QT interval: a new clinical syndrome? *Cardiology*. 94(2):99–102.
41. Giustetto C, Di Monte F, Wolpert C, Borggrefe M, Schimpf R, Sbragia P, Leone G, Maury P, Anttonen O, Haissaguerre M, Gaita F. 2006. Short QT syndrome: clinical findings and diagnostic-therapeutic implications. *Eur Heart J*. 27(20):2440–2447.
42. Brugada R, Hong K, Dumaine R, Cordeiro J, Gaita F, Borggrefe M, Menendez TM, Brugada J, Pollevick GD, Wolpert C, Burashnikov E, Matsuo K, Wu YS, Guerchicoff A, Bianchi F, Giustetto C, Schimpf R, Brugada P, Antzelevitch C. 2004. Sudden death associated with short-QT syndrome linked mutations in HERG. *Circulation*. 109:30–35.
43. Taggart NW, Haglund MC, Tester DJ, Ackerman MJ. 2007. Diagnostic miscues in congenital long-QT syndrome. *Circulation*. 115(20):2613–2620.
44. Abernethy WB, III, Choo JK, Hutter AM, Jr. 2003. Echocardiographic characteristics of professional football players. *J Am Coll Cardiol*. 41(2):280–284.