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ORIGINAL ARTICLE

Prevalence of HCM and long QT syndrome mutations in young sudden cardiac death-related cases

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Abstract Cardiomyopathies and channelopathies are major causes of sudden cardiac death. The genetic study of these diseases is difficult because of their heterogenic nature not only in their genetic traits but also in their phenotypic expression. The purpose of the present study is the analysis of a wide spectrum of previously known genetic mutations in key genes related to hypertrophic cardiomyopathy (HCM), long QT syndrome (LQTS), and Brugada syndrome (BrS) development. The samples studied include cases of sudden cardiac death (SCD) in young adults and their relatives in order to identify the real impact of genetic screening of SCD in forensic cases. Genetic screening of described variation in 16 genes implicated in the development of HCM and three more genes implicated in LQTS and BrS was performed by using MassARRAY™ technology. In addition, direct sequencing of the two most prevalent genes implicated in the development of SQTL type 1 and 2 was also carried out. Genetic screening allowed us to unmask four possibly pathogenic mutation carriers in the 49 SCD cases considered; carriers of mutation represent 9% (2/23) of the probands with structural anomalies found after autopsy and 7% (1/14) of the probands with structurally normal hearts after in depth autopsy protocol. One mutation was found among 12 of the recovered SCD cases considered. In people with direct family history of sudden cardiac death, but not themselves, 11 additional mutation carriers were found. Three different mutations were found in six of the 19 LQTS patients, representing three families and two different mutations were found among six patients with previous syncope. Genetic analysis in sudden cardiac death cases could help to elucidate the cause of death, but it also can help in the prevention of future deaths in families at risk. The study presented here shows the importance and relevance of genetic screening in patients with signs of cardiac hypertrophy and in family cases with more than one relative affected.

Keywords Brugada syndrome · Genetic screening · Hypertrophic cardiomyopathy · Long QT syndrome · Mutation detection · Sudden cardiac death

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Introduction

Geneticists, forensic pathologists, and medical doctors, all of them share the necessity of finding the cause of death in sudden death (SD) cases. The most frequent cause of SD is sudden cardiac death (SCD), defined as an unexpected death from a cardiac cause which occurs within a short time, generally within 1 h of symptom

onset, in a person with or without pre-existing heart disease [1].

SCD is one of the most common causes of death in developed countries with an incidence of 30–200/100,000 people each year. Atherosclerosis and acquired forms of cardiomyopathies are the most frequent findings after autopsy in adults with SCD [2], whereas in people under 35 years old, non-ischemic diseases are responsible for a large number of cases [3–5].

In around 5–10% of the SD cases, especially in young people, the cause of death cannot be explained neither after autopsy nor after laboratory tests. The investigation of the causes of these unexpected deaths has become one of the most important objectives of forensic pathologists since, in many cases, the death occurs in previously healthy young people [6]. Inherited heart diseases such as hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and primary electrical diseases such as long QT syndrome (LQTS), Brugada syndrome (BrS), or catecholaminergic polymorphic ventricular tachycardia (CPVT), are the main causes of death in young adults with no previous pathological clinical history. Most often, these inherited cardiac disorders give rise to lethal ventricular arrhythmias and they show an autosomal dominant pattern of inheritance.

Genetic screening of major genes involved may help to determine the cause of death and also to evaluate the potential risk of relatives. Table 1 summarizes some of the most commonly studied genes associated with HCM and familial LQTS.

Our main objective in this study was to try to understand the real impact of genetic causes hidden behind SCD cases. We aim to evaluate if genetic screening in SCD is advisable in negative autopsies, not only to clarify the cause of the death but also to provide clinical and genetic counselling to the relatives carriers of asymptomatic causative mutations, in order to prevent the recurrence of any fatal cardiac event in both individuals diagnosed with one of these pathologies and also in asymptomatic relatives of SCD cases. To achieve this goal, we investigated the prevalence of LQTS and HCM causing mutations in both a cohort with personal history of SCD (group I) and a cohort of relatives of individuals that suffered SCD (group II).

Methods

Inclusion criteria

Samples recruited from deceased persons less than 35 years old were collected from several institutes of legal medicine distributed along Spain. In these samples, the cause of the death could not be determined after the scene investigation and after a rigorous autopsy, although complementary

Table 1 Genes evolved in HCM and LQTS included in the study

Disease	Gene name	Encoded protein	% of disease	Mutations tested
HCM	MYH7	β-Myosin heavy chain	~25%	279
HCM	MYBPC3	Myosin-binding protein C	~25%	249
HCM	TNNI3	Troponin I type 3	~3–5%	35
HCM	TNNT2	Troponin T type 2	~3–5%	43
HCM	TPM1	α-Tropomyosin	~1%	14
HCM	TNNC1	Troponin C type 1	Rare	4
HCM	ACTC	α-Actin	Rare	10
HCM	MYH6	α-Myosin heavy chain	Rare	6
HCM	MYL2	Myosin light chain 2	Rare	14
HCM	MYL3	Myosin light chain 1	Rare	6
HCM	TCAP	Titin-cap (telethonin)	Rare	3
HCM	GLA	α-Galactosidase	–	1
HCM	PRKAG2	Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	–	4
HCM	TTN	Titin	Rare	10
HCM	MYLK2	Myosin light chain kinase 2	–	1
HCM	MYO6	Myosin VI	–	1
LQTS	KCNQ1	Potassium voltage-gated channel, KQT-like subfamily, member 1	30–35%	151
LQTS	KCNH2	Potassium voltage-gated channel, subfamily H, member 2	25–30%	149
LQTS/BrS	SCN5A	Sodium channel, voltage-gated, type V, alpha subunit	5–10%	133

HCM hypertrophic cardiomyopathy, *LQTS* long QT syndrome, BrS Brugada syndrome

laboratory tests such as histological and toxicological studies were performed [7]. We have also included into the study some clinical cases recovered after successful resuscitation after a life-threatening event similar to SCD. These samples were referred to our laboratory from cardiologists who contacted with us from several Spanish hospitals. In all these cases, after an exhaustive examination, it was not possible to infer the cause of the arrest.

Specifically, samples included in the study were grouped as follows:

Group I This group includes individuals with personal history of sudden cardiac death, including deceased individuals (37 individuals) or individuals who recovered after SCD event (12 individuals).

Group II This group includes 57 relatives of an unexplained SCD case with a negative autopsy and where the after autopsy samples were not available (except in two cases). From these 57 individuals, 34 belonged to 12 families with more than one relative. In the remaining 23 cases, only one of the relatives was available.

Figure 1 summarizes the distribution of the samples into the two groups. Autopsy reports, family history review, and clinical history were collected. Circumstances in which the cardiac arrest took place were assessed not only in sudden cardiac death cases but also in aborted cardiac death events.

Evaluation and diagnosis were conducted by cardiologists and forensic scientist following the standard procedures in all cases [8]. This study was developed according to the recommendations of the Declaration of Helsinki and approved by the local ethics committees of the participating institutions.

Genetic screening

After an appropriate counselling, DNA genetic analysis was offered to family relatives or to the index patient in resuscitated cases. DNA was extracted from blood, frozen tissues, or paraffin-embedded material when the fresh sample was not available.

A mutation screening strategy was developed by means of mass spectrometry in Sequenom MassARRAY™ genotyping platform (Sequenom, San Diego, CA), which is based on allelic discrimination by a single base extension reaction, using Iplex Gold technology that

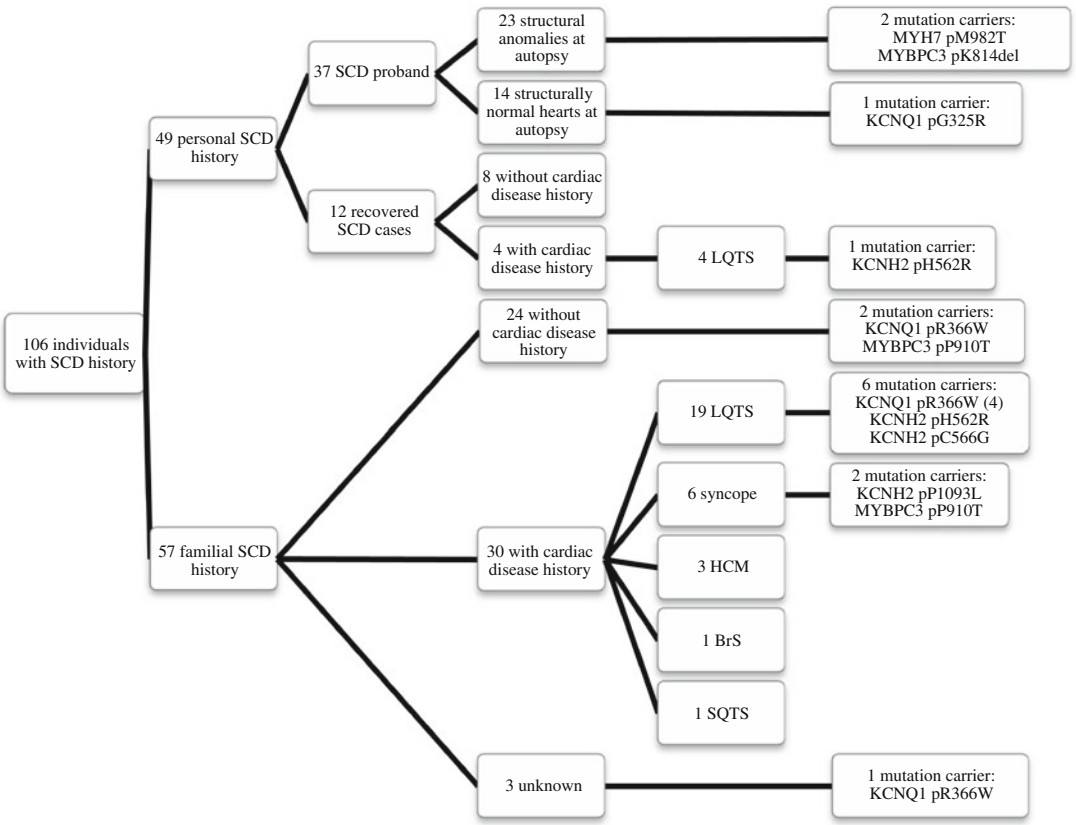

Fig. 1 Flow diagram which describes the probands and relatives included in the study, their autopsy findings or clinical diagnosis and the successful genetic testing results

allows you to multiplex up to 40 mutations and subsequently, it makes possible to detect the extended products by means of mass spectrometry. Two different genetic approaches were performed for HCM and LQTS. The genes considered in the study are shown in Table 1.

Described mutations on HCM-implicated genes were analysed by using the MassARRAY genotyping System of Sequenom. The genotyping strategy used was an upgrade of the one described by Brion et al. [9], which currently includes 680 genetic variant in 16 genes associated with HCM. All the mutations detected with the genotyping platform were subsequently confirmed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and a 3730xl DNA analyzer (Applied Biosystems).

Long QT syndrome approach, described by Allegue et al. (2010) [10] was used for the genetic mutation screening on KCNQ1, KCNH2, and SCN5A genes. Additionally, exons and flanking intronic regions of KCNQ1 and KCNH2 genes (GeneBank: AF000571 and U04270, respectively) were analyzed by direct sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and a 3730xl DNA analyzer in those samples in which no positive genetic variation was detected in the Sequenom MassARRAY® System genotyping platform.

After an exhaustive revision of the clinical history, autopsy results, and/or family history, each sample was added to the most suitable genetic screening strategy.

Results

Samples considered in group I include 37 autopsy cases with structural anomalies detected in the autopsy (23 cases) or with structural normal hearts (14 cases). Structural anomalies included hypertrophy and disarray; none of the samples showed signs consistent with ARVC. From these 37 cases, ten cases showed previous abnormal cardiac symptoms, 13 of them did not show any symptom, and there is no information available of the remaining cases. Group I also includes 12 cases recovered after a sudden death event, four of them had previous cardiac symptoms and eight of them had no warning signs.

We found out that most of group I cases were males (71%), in most of them there is no information available about the circumstances of death, nine of the cases occurred while sleeping or resting, and 15 of which occurred while performing some activity, either working, driving, or during or after playing sports. Table 2 summarises the SCD index cases characteristics. From the 57 individuals of group II, 30 had cardiac disease history, 24 did not show any cardiac pathological background, and the clinical history was not available in three cases.

We present the results obtained from both genetic screening strategies, HCM and LQTS. Among the group I—deceased or recovered individuals—four mutation carriers were detected. Two genetic variants were found in the most frequently mutated genes in arrhythmogenic diseases: KCNQ1, responsible of LQT type 1 and KCNH2, responsible of LQT type 2; and two more genetic variants were present in the most frequently mutated genes in HCM: MYBPC3 and MYH7.

A 19-year-old man who died suddenly and had previously both personal and family history of LQTS, showed a p.G325R (c.973G>A) heterozygous mutation in KCNQ1 [11–14]. The p.H562R (c.1685A>G) genetic variant in KCNH2 [15] was found in heterozygosis in a male with a history of syncope at rest and a remarkably long QT interval leading to the diagnosis when he was 20 years old. His grandfather, who had recurrent syncope and was recovered from a sudden death at age 71, was also included in the study and he was found to carry the same heterozygous p.H562R gene mutation.

Table 2 SCD index cases characteristics

		HCM mutation carriers (%)	LQTS mutation carriers (%)
Total SCD cases	49	2 (4.1%)	2 (4.1%)
Autopsy cases	37 (75.5%)	2 (5.4%)	1 (2.7%)
Aborted cases	12 (24.5%)		1 (8.3%)
Males	35 (71.4%)	2 (5.7%)	1 (2.9%)
Mode of death			
At rest	9 (18.4%)		
On exertion	15 (30.6%)	1 (6.7%)	
Unknown/others	25 (51.0%)	1 (4.0%)	2 (8.0%)
Antecedent symptoms			
Present	15 (30.6%)		2 (13.3%)
Absent	21 (42.9%)	2 (9.5%)	
Unknown	13 (26.5%)		

HCM hypertrophic cardiomyopathy, *LQTS* long QT syndrome, *SCD* sudden cardiac death

The p.M982T (c.2945T>C) mutation in the MYH7 gene has been associated in the literature with increased left ventricular wall thickness and family history of SCD [16]. In the present study, the mutation was detected in heterozygosis in a 22-year-old man who died suddenly, with no personal or family history of cardiac disease. After an autopsy report, only a slight dilatation of the cavities could be found. The widely described p.K814del (c.2440_2442delAAG) in the MYBPC3 gene [17, 18] was also found in heterozygosis in a 23-year-old man who died suddenly while playing football. His medical history showed none associated medical record, but the autopsy report was coincident with HCM.

Among the individuals with direct family history of sudden cardiac death—referenced as the group II in the "Method" section—11 mutation carriers were identified. Most of them (8/11) were individuals with some previous cardiac disease history, specially LQT syndrome and syncope but two of them never showed any cardiac disease sign.

The most frequent mutation was the p.R366W (c.1096C>T) in KCNQ1 [19–22], widely described in the literature. It was found in heterozygosis in a family where the index SCD case was not available (a young woman who died suddenly), but the genetic screening was performed in nine first degree relatives, four of them were LQTS affected. Among nine family members, six of them resulted to be heterozygous mutation carriers; four of them were previously diagnosed with LQTS, one 55-year-old brother of the deceased woman who was asymptomatic with no pathological QT interval, and a niece with unknown clinical history.

Among the three genetic variants found in the LQT2 gene, two of them were previously described: p.H562R also detected in SCD case included in the group I [15], and p.P1093L (c.3278C>T) [15, 23] found in a young male with a personal and family history of deafness and recurrent syncope. In addition, the p.C566G (c.1696T>G) variant in the KCNH2 gene was found in heterozygosis in an asymptomatic long QT syndrome affected woman, whose sister was also LQTS affected and who had suffered recurrent syncope. Her father died at age 30 in the bed, victim of an unknown cause. It has not been found that any information about this mutation were published in the literature or in any of the databases available online, but it should be mentioned that it has been described in another different mutation in the same amino-acidic position [24], resulting in a substitution of cysteine by serine, instead of cysteine by glycine as in this described case. This genetic variant affects to a highly conserved residue and the PolyPhen-Polymorphism Phenotyping (http://genetics.bwh.harvard.edu/pph/), which is an automatic tool for predicting the possible impact of an amino acid substitution on the structure and function of a human protein, classify the variant as "possibly damaging".

The HCM genetic screening among the group II samples only reported one genetic variant, the p.P910T (c.2728C>A) variant in the MYBPC3 gene, found in heterozygosis in a 33-year-old man subjected to beta blocker therapy because of having suffered syncopal episodes since childhood, and with a family history of two brothers who died suddenly at 8 and 14 years of age. His parents were included in the genetic screening although they had no medical case record. Their mother was found to be a heterozygous carrier of the genetic variant. This amino-acidic change, p.P910T has been reported twice in HCM patients with additional mutations, so its implication as a disease causal mutation remains unknown [25, 26].

In order to summarize the results, Table 3 includes genetic variants with possible pathogenic effect detected in the samples recruited in this study. Five genetic variants were detected in the genes responsible of arrhythmogenic diseases, three in KCNH2 gene responsible of LQT type 2 and two in KCNQ1 gene responsible of LQT type 1. Three genetic variants were present in the most frequently mutated genes in HCM, MYBPC3 gene and MYH7 gene, with two and one mutations, respectively.

Discussion

It has been described that in nearly half of young SCD victims from 1 to 35 years of age, no obvious cause of death can be found after legal autopsy, and sudden death in these young people often occurs as a first symptom without notice. Recent advances in molecular biology demonstrate that a significant proportion of these deaths are due to mutations in genes leading to inherited cardiac diseases that may provoke a sudden cardiac death.

Genetic screening allowed us to unmask the underlying genetic variation implicated in the inherited disease possibly responsible of the sudden death event in four of the 49 SCD cases considered. Unfortunately, family members were not available to perform genetic screening in three of the cases, and only one of them—one relative also LQTS affected—was included in the examination resulting also to be a mutation carrier. It should be borne in mind that the real number of mutations in these cases would be probably higher if a complete sequencing of all the genes involved in SCD was performed. However, the strategy used allows an easy and fast analysis of genetic mutations [10].

Many studies have highly demonstrated that genetic and clinical screening of SCD first and second degree case relatives, even in asymptomatic ones, are useful in preventive treatment and diagnosis or prognosis [27–30].

Table 3 Genetic variants detected

Genetic variant	Gene	Sex	SCD (age[a])	Autopsy findings[b]	Personal and family medical history (age[a])	Relatives (mutation carriers)
p.G325R	KCNQ1	M	Yes (19)	Structurally normal heart	Familial LQTS	No
p.R366W	KCNQ1	F	No	na	Familial LQTS, SCD in a daughter (<35)	8 (5)
p.H562R	KCNH2	M	No	na	LQT with syncopes at rest	1 (1)
p.C566G[c]	KCNH2	F	No	na	Familial LQTS, SCD in the father (30)	No
p.P1093L	KCNH2	M	No	na	Familial deafness, syncopes	No
p.M982T	MYH7	M	Yes (22)	Hypertrophic heart	None	No
p.K814del	MYBPC3	M	Yes (23)	Hypertrophic heart	None	No
p.P910T	MYBPC3	M	No	na	Syncopal episodes since childhood, beta-blocker treatment. SCD in two brothers (8 and 14)	2 (1)

SCD sudden cardiac death, *F* female, *M* male, *na* not applicable, *HCM* hypertrophic cardiomyopathy, *LQTS* long QT syndrome

[a] Age at the SCD event

[b] Relatives of a SCD case)

[c] Novel mutation described in the present study

In our study, five additional mutations were found in people with direct family history of sudden cardiac death, but not themselves. Mutations were detected in nine individuals that belong to four different families, so 11.4% of the families considered showed a possible pathogenic mutation. It is worth mentioning that among group II, approximately 53% of the cases had some personal cardiac medical history and, among these, about 27% were carriers of a mutation, all of them on the cardiac ion channel-codifying genes (Fig. 1).

Previous studies such as the one developed by Tan et al. in 2005 [30] described the importance of cardiological and genetic examination in surviving first- and second-degree relatives to increase the likelihood of establishing family diagnosis, especially in families in which at least in two cases the SCD took place at age under 40 years old. In our study, only two families fulfilled these conditions: first, a single individual with two siblings who died at 25 and 26 years of age, in which we did not find any of the mutations analyzed and second, three members of the same family, father and mother, both asymptomatic, and one son with syncope history whose two brothers died suddenly at ages of 8 and 14. The son and the mother were carriers of the p.P910T mutation in heterozygosis in MYBPC3 gene, although the pathogenicity of this genetic variation is still uncertain [25, 26].

Behr et al. [27] described that syncope was the only significant predictor they found useful for diagnosis. Our study also confirms syncope as good predictor since 33% of the individuals with SCD family history and syncope history were also mutation carriers.

Gimeno et al. [31] described statistical association between male sex and sudden death and a higher prevalence of exercise-related sudden deaths at young age. They also described greater association between sudden deaths during exercise–stress–normal daily activities in cardiomyopathy than channelopathy. In our study, the proportion of men who suffered SCD events was higher than in women.

Christiaans et al. [32] carried out an in-depth literature review of the clinical risk markers in hypertrophic cardiomyopathy. Literature, as recommended international guidelines, supports the use of six major risk factors—previous cardiac arrest or sustained ventricular tachycardia, non-sustained ventricular tachycardia, extreme left ventricular hypertrophy, unexplained syncope, abnormal blood pressure response, and family history of sudden death—to establish risk stratification of SCD. They propose the addition of left ventricular outflow tract obstruction as it seemed to be associated to a higher risk of SCD. We suggest to include the HCM genetic screening as a possible predictor of SCD risk, since two of the 23 deceased individuals at the ages of 22 and 23, showed structural anomalies in the heart at autopsy although they did not show any previous symptoms while alive, nevertheless they were carrier of HCM-described mutations.

Despite all the above recommendations for the implementation of genetic screening in the current forensic autopsy practices, a recent survey conducted mainly in European countries has shown a very limited application of genetic testing in cases of sudden cardiac death in routine forensic investigation, only 40% of the responders have the possibility to perform the genetic testing [33]. This survey also shows that many of the problems involved in the adequate investigation of SCD cases are financial in origin,

Taking into account the efficiency of both MassArray strategies, it was clearly demonstrated that LQTS strategy [10], should be upgraded, since none of the mutations presented in our cases were included in the panel considered for the MassArray mutation screening; all of them were detected by direct sequencing of KCNQ1 and KCNH2 genes. In addition, it should be mentioned that probably the number of novel mutations detected by conventional sequencing in those genes was low because of the reduced number of clear LQTS affected cases. Only 23 individuals showed clinical history of LQTS, they belong to 16 different families and three different mutations were found (18.7%) being one of them a novel mutation.

However, among the samples with suspected HCM, the MassArray strategy allowed to detect two mutation carriers among 13 suddenly deceased individuals with some sign of HCM. This represents the 15% of individuals probably deceased who were HCM affected, whose analysis with conventional strategies, such as sequencing will require much more time and expenses.

Although at present, the new next generation sequencing technologies are replacing the classic strategies, reducing costs and time of analysis, one of the main advantages of the mutation detection using the MassArray system is its speed, since all mutations are analyzed within 48 h, allowing for a rapid sample screening, avoiding sequencing in those cases in which a clear pathogenic mutation is found. In addition, the MassARRAY System allows analyses of low quality DNA samples, as it is the case of paraffin-embedded tissues, the only available material for many SCD cases.

Conclusion

As we get more precise diagnosis, each of the hereditary diseases in the clinical protocol and the sort of cases of sudden cardiac death is more accurate—ruling out possible causes, such as myocarditis or congenital anomalies among others—genetic screening in young adults who died suddenly and also in their relatives is becoming an increasingly feasible study. Specifically, the study presented here shows the importance and relevance of genetic screening in patients with signs of cardiac hypertrophy and in family cases in which more than one relative is affected.

The genetic screening proposed enables to provide a fast screening of mutations in the major genes involved in sudden cardiac death in young adults. Although it is widely known that most of the mutations described in pathologies such as long QT syndrome are only described in one family, the sequencing of the genes KCNQ1 and KCNH2 allows detecting novel mutations, which thanks to the platform flexibility may be incorporated in subsequent updates of the genotyping strategy.

Therefore, our genetic study contributes to clarify the importance of the genetic screening in the establishment of SCD risk. Genetic screening in suddenly dead individuals without premonitory symptoms and with signs of hypertrophic cardiomyopathy at autopsy would help to determine the cause of death, and also to establish a risk factor in asymptomatic relative carriers of the mutation. In addition, genetic screening is also advisable in SCD cases with structurally normal hearts at autopsy, but with family history of SCD and arrhythmogenic disease. The progressive technological development we are experiencing at the present time seems to indicate that the available genotyping strategies will help in the near future more extensive studies and lower costs [34].

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