**Diagnostic Yield of Hypertrophic Cardiomyopathy in First-degree Relatives of Decedents with Idiopathic Left Ventricular Hypertrophy**

Gherardo Finocchiaroa MD, PhD, Harshil Dhutiaa BSc MRCP, Belinda Graya MD, PhD, Bode Ensama MRCP, Stathis Papatheodoroua MD, Chris Milesa MRCP, Aneil Malhotraa MSc, MRCP, PhD, Zeph Fantona BSc, Paulo Bullerosa BSc, Tessa Homfraya MD, Adam A. Witneya,d PhD, Nicholas Buncea MRCP, MD, Lisa J. Andersona BSc, MD, James S. Warec BA, MBBS, PhD, Rajan Sharmaa BSc, MBBS, MD, Maite Tomea MD, PhD, Elijah R. Behra MA, MD , Mary N. Sheppardb MBBCH, BAO, BSc, MD, Michael Papadakisa \*  MRCP, MD, Sanjay Sharmaa\* BSc, MRCP, MD

Institutions:

a Cardiology Clinical Academic Group, Molecular and Clinical Sciences Research Institute, St George's, University of London, United Kingdom

b Cardiovascular Pathology Department, St George's, University of London, United Kingdom

C National Heart & Lung Institute & NIHR Royal Brompton Cardiovascular Biomedical Research Unit, Imperial College London, United Kingdom

d Bioinformatics Unit, St George’s, University of London, United Kingdom.

\* The last two authors contributed equally to the manuscript

Word count: 3522

Author for correspondence:

Sanjay Sharma, MD

Professor of Clinical Cardiology,

Cardiac clinical academic group

St. George’s, University of London, Cranmer Terrace, London. SW17 0RE. UK,

E-mail: [sasharma@sgul.ac.uk](mailto:sasharma@sgul.ac.uk)

**ABSTRACT (Word count 270)**

**Background and aims**: Idiopathic left ventricular hypertrophy (LVH) is defined as LVH in the absence of myocyte disarray or secondary causes. It is unclear whether idiopathic LVH represents the phenotypic spectrum of hypertrophic cardiomyopathy (HCM) or whether it is a unique disease entity. We aimed to ascertain the prevalence of HCM in first-degree relatives of decedents with idiopathic LVH at autopsy. Decedents also underwent molecular autopsy to identify the presence of pathogenic variants in genes implicated in HCM through.

**Methods:** Families of 46 decedents with idiopathic LVH (125 first-degree relatives) were investigated with ECG, echocardiography, exercise tolerance test, cardiovascular magnetic resonance imaging, 24h-Holter and ajmaline provocation test. Next generation sequencing molecular autopsy was performed in 14 (30%) decedents.

**Results:** Decedents with idiopathic LVH were aged 33±14 years and 40 (87%) were male. Fourteen families (30%) comprising 16 individuals were diagnosed with cardiac disease, including Brugada syndrome (n=8), long QT syndrome (n=3), cardiomyopathy (n=2) and Wolff-Parkinson-White syndrome (n=1). None of the family members were diagnosed with HCM. Molecular autopsy in decedents did not identify any pathogenic or likely pathogenic variants in genes encoding sarcomeric proteins. Two decedents had pathogenic variants associated with long QT syndrome which were confirmed in relatives with the clinical phenotype. One decedent had a pathogenic variant associated with Danon disease in the absence of any histopathological findings of the condition or clinical phenotype in the family.

**Conclusions:** Idiopathic LVH appears to be a distinct disease entity from HCM and is associated with fatal arrhythmias in individuals with primary arrhythmia syndromes. Family screening in relatives of decedents with idiopathic LVH should be comprehensive and encompass the broader spectrum of inherited cardiac conditions, including channelopathies.

**Keywords:** sudden death, left ventricular hypertrophy, channelopathy

**INTRODUCTION**

Sudden cardiac death (SCD) in apparently healthy individuals is commonly due to a diverse spectrum of inherited cardiac diseases including the cardiomyopathies and channelopathies 1,2. Autopsy is an essential preliminary diagnostic step to steer the clinical evaluation of surviving relatives toward inherited structural diseases or primary arrhythmogenic syndromes. The interpretation of the autopsy results, however, is often a complex task and uncertainty may exist about the precise significance of some pathological findings and their causal relationship with SCD3,4.

Idiopathic left ventricular hypertrophy (LVH) is an increasingly recognized finding at autopsy of young decedents from SCD3,5. The entity is used to describe unexplained LVH in the absence of myocardial disarray or secondary causes2,3. Idiopathic LVH may be interpreted as hypertrophic cardiomyopathy (HCM)6 and targeted screening of first-degree relatives is recommended to detect others with quiescent disease. The significance and causal relationship of this entity with SCD is uncertain. Importantly, it is unclear whether idiopathic LVH represents the disease spectrum of HCM. Consequently, there are concerns that families of decedents with idiopathic LVH may be falsely reassured after limited evaluation with a 12-lead ECG and echocardiogram, which would be expected to identify the majority of individuals with HCM.

We aimed to investigate the significance of idiopathic LVH in decedents who experienced SCD through comprehensive clinical evaluation of their first-degree relatives and by conducting molecular autopsy in the decedents. We hypothesised that idiopathic LVH represents the disease spectrum of HCM, which is inherited as an autosomal dominant trait, and therefore expected to detect LVH in a significant proportion of family members.

**METHODS**

**Study setting**

The Cardiac Risk in the Young (CRY) centre for cardiac pathology at St George’s, University of London is led by an expert cardiac pathologist (M.N.S.) and receives over 500 whole hearts each year across the United Kingdom from individuals who have experienced a SCD. General pathologists are likely to refer to the CRY centre when the clinical history is suggestive of inherited cardiac disease, especially when death affects a young or athletic individual or when the cause of death is uncertain after the initial autopsy. The tertiary inherited cardiac conditions unit at St George’s Hospital has the capacity to provide comprehensive clinical investigation of large family units together in one setting.

**Study population**

Cases of idiopathic LVH were reviewed retrospectively between January 2007 and December 2014 and prospectively from 2015 to 2017 from a database of 4,220 cases of SCDs referred to our centre for cardiac pathology (whole heart referred in 65% of the cases). Sudden cardiac death was defined as instantaneous witnessed death within one hour of onset of symptoms, or if unwitnessed, within 12 hours of being seen alive and well. Idiopathic LVH was defined as unexplained LVH (heart weight > 500 g in males and > 400 g in females or increased heart weight as a function of body weight according to established nomograms7 and left ventricular wall thickness > 15 mm, in the absence of myocardial disarray or secondary causes of LVH)2,3. Although there is no consensus about the definition of normal heart weight8 we followed the nomograms derived by Kitzman et al.7. Myocyte disarray was considered significant if >20% in at least 2

tissue blocks of 4 cm2)2. One hundred decedents aged 5 to 65 years old, whose whole heart was examined at our centre, fulfilled our stringent diagnostic criteria for idiopathic LVH at autopsy. We were able to contact 50 families following which, 46 families (constituting 125 individuals) consented to participate in the study.

**Autopsy**

All decedents underwent a full autopsy evaluation performed by a local pathologist. Following the exclusion of extra-cardiac causes, the heart was referred to our centre with written consent of the coroner and the family of the deceased. A toxicology screen was conducted in all cases in accordance with the usual investigation of sudden and unexpected deaths in the UK9,10. Comprehensive macroscopic examination of the whole heart and histological analysis were performed in accordance with the guidelines on “Autopsy practice for sudden death with likely cardiac pathology” of the Royal College of Pathologists and the Association for European Cardiovascular Pathology9,10. All cardiac structures were systematically examined. The heart weight was recorded in grams and ventricular wall thickness and internal cavity dimensions were measured at mid-ventricular level excluding the papillary muscles and fat. A minimum of 10 blocks of tissue were taken for histological analysis as reported previously2. Sections of myocardium were fixed in formalin, embedded in paraffin and stained with haematoxylin and eosin as well as elastic Van Gieson stain to highlight myocardial fibrosis.

**Clinical information in decedents**

The referring coroner and pathologist were requested to complete a questionnaire inquiring about the demographics of the deceased, past medical history, family history, cardiac symptoms, the nature and level of physical activity and exact circumstances of death. The data were derived from a number of sources including interviews with family members of decedents, potential witnesses of the SCD and reports from the deceased’s family physician. All information relating to the decedents was reviewed during the clinical consultation with the relatives. For the purpose of this study, we took particular care in trying to exclude decedents with hypertension by ascertaining their clinical history.

**Family screening**

First-degree relatives of decedents with idiopathic LVH who were aged between 16 and 75 years were prospectively investigated with tests specifically tailored to diagnose the broad spectrum of the HCM phenotype. These included 12-lead ECG, echocardiogram and cardiovascular magnetic resonance (CMR) imaging, exercise tolerance test (ETT) and 24-hour Holter monitor. Among families where we were unable to diagnose HCM or another cardiac condition associated with SCD, we proceeded to perform an ajmaline provocation test to specifically investigate the possibility of Brugada syndrome3 (Figure 1).

Individuals with phenotypes suggestive of cardiac disease were offered treatment and genetic testing after appropriate counselling as per established protocols11. Families of decedents with idiopathic LVH that encountered extensive diagnostic work-up at a different centre (n=6) were included in the study after gaining their consent to retrieve medical information. When such family members had not been previously investigated as comprehensively as per our study protocol, we offered the appropriate additional cardiac tests in our inherited cardiac disease unit.

*Investigations*

12-lead ECG

A standard resting 12-lead ECG was performed as previously described12. High intercostal leads (third and second intercostal space) were performed in all cases.

Echocardiography

Two-dimensional echocardiography was performed with the subject in the left lateral decubitus position using the following commercially available ultrasound systems: GE Vivid I, GE Vivid 9 (GE Healthcare, Milwaukee, WI, USA) and Philips iE33 (Philips Medical, Bothell, Washington, USA). Standard views were obtained and analysed according to the protocols specified by the European Association of Echocardiography13. Left sided cardiac dimensions were measured from two-dimensional images in the parasternal long-axis and short-axis views 14,15. When measuring septal thickness, care was taken to exclude right ventricular septal bands.

Cardiovascular magnetic resonance

Cardiovascular magnetic resonance imaging was performed with a Philips Achiever 3.0T TX (Amsterdam, The Netherlands) scanner using steady-state, free precession breath-hold cines in long-axis planes and sequential 7mm short-axis slices from the atrioventricular ring to the apex. Ventricular volumes and function and left ventricular mass were measured using standard techniques and analysed using semi-automated software (Extended MR workspace [Philips, Amsterdam, The Netherlands]. All volumes and masses were indexed for age and body surface area (BSA)16. Assessment of left ventricular and right ventricular function was performed by 2 experienced observers. Late gadolinium enhancement images were acquired 10 minutes after intravenous gadolinium-DTPA (0.15 mmol/Kg, Gadovist) in long-axis and short-axis planes using an inversion-recovery gradient echo sequence17. Inversion times were adjusted to null normal myocardium and late gadolinium enhancement images were phase-swapped when necessary to exclude possible artefacts.

Exercise tolerance test

An ETT was performed on a treadmill using a standard Bruce protocol18 in a quiet air-conditioned room with an average temperature of 21° C and full resuscitation facilities. Before the test, all subjects underwent a 3 minutes familiarization test. Participants were encouraged to exercise to the point of exhaustion.

24-hour Holter

All patients were investigated with a 3-lead 24-hour Holter monitor to detect possible arrhythmias.

Ajmaline provocation test

Ajmaline provocation was used to unmask the concealed form of Brugada syndrome19 in family members without phenotypic features of cardiomyopathy or other potentially inherited cardiac diseases, as described elsewhere20. All tests were performed using conventional and high right precordial leads, as per established protocol20. A MAC 5000 or a Cardiosoft recorder (GE Medical, Milwaukee, Wisconsin; 500 samples/s, 4.88 mcV) was used for digital ECG acquisition. All ECGs were subsequently exported to a customized software program where tracings could be magnified to measure basic intervals accurately with on-screen callipers. The test was discontinued if the patient exhibited significant side effects, multiple premature ventricular beats or complex ventricular arrhythmias, a QRS duration increase of more than 50% when compared to the baseline or when the ECG developed the diagnostic type-1 Brugada pattern in the standard or higher right precordial leads20.

**Molecular autopsy in decedents with idiopathic LVH**

Due to historic limitations, post-mortem DNA (from a spleen sample) was available in only 14 (30%) decedents. Twelve decedents underwent research based cardiac gene panel testing using the 174 gene Illumina Trusight Cardiac panel (Supplementary material, Table 1),21 and 2 decedents underwent clinical molecular autopsy using a targeted panel of 19 cardiac genes. Variants with a minor allele frequency (MAF) >0.001% in the Exome Aggregation Consortium (ExAC)22, synonymous variants not located at splice sites and non-truncating variants in titin (TTN) gene were excluded23. Variants were classified according to American College of Medical Genetics (ACMG) criteria24.

**Definitions of clinical diagnoses**

Diagnosis of cardiac disease was made according to international guidelines and recommendations. The diagnosis of HCM was considered in the presence of unexplained LVH with end-diastolic LV wall thickness >15 mm on 2D echocardiography or CMR. A diagnosis of HCM was also considered in individuals with a wall thickness between 13 and 15 mm, in the presence of abnormal repolarisation changes on electrocardiography, or in the context of another family member fulfilling conventional diagnostic criteria for HCM25.

Arrhythmogenic right ventricular cardiomyopathy (ARVC) was diagnosed on the basis of fulfilling the Revised Task Force Criteria published in 201026. Dilated cardiomyopathy (DCM) was considered on the basis of LV dilation (LV end-diastolic diameter >117% of the predicted per age and sex), and reduced systolic function (LV ejection fraction < 55%) in the absence of abnormal loading conditions such as hypertension or valvular disease and in the absence of significant coronary artery disease27. Left ventricular non-compaction (LVNC) was considered if an individual fulfilled the Chin28 or Jenni29 criteria and showed reduced LV systolic function on the echocardiogram and/or fulfilled the Petersen criteria30 on CMR. Brugada syndrome was considered in the presence of a spontaneous Brugada ECG pattern (coved ST-segment elevation ≥2 mm followed by a negative T-wave) in > 1 right precordial leads (V1 to V3) or after intravenous ajmaline 31. Long QT syndrome was diagnosed according to the International criteria31–33. Cases where the QT duration was borderline were subjected to an exercise tolerance test, as per the study protocol. The diagnosis of CPVT was considered following the documentation of polymorphic ventricular tachycardia (but not necessarily typical bidirectional VT with a beat-to-beat 180 degrees rotation of the QRS complex) during exertion and in the absence of ECG features suggestive of an alternative diagnosis31,34. Wolff-Parkinson-White syndrome was diagnosed in presence of a short PR interval and a delta wave (< 120 msec) and symptoms of palpitations or syncope. Pre-excitation was defined by the same ECG changes in the absence of palpitations or syncope35.

**Ethical approval**

Ethical approval for this study was granted by the Fulham Research Committee in London, UK (REC reference is 15/LO//2074). The study protocol was approved by the St George’s NHS trust. All the patients signed a written consent and all data were handled in accordance with the Data Protection Act (1998).

**Statistical analysis**

Statistical analysis was performed using the PASW software (PASW 18.0 Inc, Chicago, IL). Results are expressed as mean ± standard deviation (SD) for continuous variables or as number of cases and percentage for categorical variables. Comparison of groups was performed using Student’s T-test for continuous variables with correction for unequal variance when necessary and Chi-square test or Fisher Exact Test, as appropriate for categorical variables. P values < 0.05 were considered statistically significant.

**RESULTS**

**Demographic and clinical characteristics of decedents with idiopathic LVH**

The mean age at death was 33±14 years. The majority of decedents were male (n=40, 87%) and white (n=43, 93%) (Table 1). Fourteen (30%) decedents were competitive athletes. Two (4%) decedents had a BMI > 30 kg/m2 but none > 35 kg/m2. Although most deaths (n=30, 65%) occurred at rest, a considerable proportion (n=16; 35%) died during exercise. As far as we can ascertain from medical documents and interviews with relatives, none of the decedents reported premorbid cardiac symptoms or had a history of hypertension. The average heart weight was 544 ± 93 grams and LV fibrosis was detected in 14 (30%) cases (Table 1). We were unable to identify a definitive association between athletic activity and prevalence of LV fibrosis in the proband (LV fibrosis in athletes (42%) and non-athletes (25%), p=0.41).

**Families diagnosed with an inherited cardiac condition**

We evaluated 125 first-degree relatives. Their mean age was 41 ± 17 years and 57 (46%) were male. A potentially inherited cardiac condition was identified in 14 (30%) families based on the presence of the respective phenotype in 16 relatives (Figure 2). Of these, none demonstrated the HCM phenotype although 6 individuals, from 5 separate families, revealed mild LVH in the context of hypertension but had no other phenotypic features of HCM following extensive investigation. Channelopathies accounted for most (80%) diagnoses. Brugada syndrome was the most common diagnosis (8 families; 9 relatives), followed by long QT syndrome (3 families; 4 relatives) and dilated cardiomyopathy, arrhythmogenic cardiomyopathy and Wolff-Parkinson-White syndrome (1 family; 1 relative each) (Figure 2, Table 2). Brugada syndrome was diagnosed in 4 out of the 6 families (66%) where the decedent died during sleep. Decedents where comprehensive family screening led to a diagnosis of an inherited heart disease were younger (27 ± 12 vs 36 ± 14 years, p=0.04) and less frequently exhibited LV fibrosis at autopsy (7% vs 41%, p=0.04) compared with decedents where family screening revealed normal results.

**Molecular autopsy**

None of the 14 decedents who underwent molecular autopsy showed pathogenic or likely pathogenic variants in genes encoding sarcomeric proteins implicated in HCM. Three (21%) decedents revealed a pathogenic variant and 11 (78%) revealed at least one variant of uncertain significance (VUS) (Supplementary Table 2). Two decedents had pathogenic variants in genes implicated in long QT syndrome, which were also identified in relatives with the long QT syndrome phenotype (Figure 3). The third decedent with a pathogenic variant had a large ~136kb deletion of band q24 of chromosome X which contained part of LAMP2 gene known to be causative of X-linked dominant Danon disease, a known HCM mimic. She was 26 years old and died at rest whilst 28 weeks pregnant. She had a history of intellectual disability and atrial arrhythmias prior to her death. Typical changes of Danon disease such as accumulation of autophagic material and glycogen in myocytes were not observed at autopsy. Subsequently three of her first-degree relatives were screened (mother, father and brother) and none showed evidence of disease phenotype. All 3 family members underwent cascade genetic testing and all were negative for the LAMP2 deletion indicating that this pathogenic variant occurred de novo*.* (Table 2).

**Combined Yield**

The combination of family screening and molecular autopsy led to a familial diagnosis of an inherited cardiac condition in 15 families (33%)

**Other diagnoses**

Among 4 family members from 4 respective families, cardiac investigations led to uncertain findings which were not related to a specific disease process (Table 3). Additionally we identified hypertensive heart disease, coronary artery disease, atrial fibrillation or alcohol related cardiomyopathy in 12 individuals from separate families. Specifically, 6 family members showed features of hypertensive heart disease with a wall thickness of up to 13 mm at CMR, 4 had a past medical history of coronary artery disease or an ETT with inducible myocardial ischemia, 1 individual had atrial fibrillation and 1 showed compatible with alcohol induced cardiomyopathy.

**DISCUSSION**

Sudden cardiac death from a potentially inherited cardiac disorder in young and previously healthy individuals instigates cardiac evaluation of first-degree relatives. In a significant proportion of such deaths, the pathologist may observe findings that partially fulfil diagnostic criteria for structural cardiac disease, leaving uncertainty about causality and subsequent investigation of surviving relatives. Left ventricular hypertrophy in the absence of myocyte disarray is reported in up to 10% of deaths in young or athletic individuals and is described as idiopathic LVH2. Although myocyte disarray is a recognized histological hallmark of HCM due to pathogenic variants within genes encoding sarcomeric proteins, the heterogenic nature of the disease means that myocyte disarray may be minimal in some cases and go undetected, therefore many such cases could actually represent HCM. Given that HCM is inherited as an autosomal dominant trait, we sought to assess the diagnostic yield of LVH in first-degree relatives of decedents with idiopathic LVH. We also had the opportunity to perform molecular autopsy in almost one third of the decedents using large gene panels which included numerous genes implicated in HCM.

Clinical evaluation of 125 first-degree relatives of 46 decedents with idiopathic LVH did not reveal a single case of HCM despite comprehensive clinical evaluation, including CMR imaging, exercise testing and prolonged ECG monitoring, which would be expected to identify even milder phenotypes of HCM.

Molecular autopsy is increasingly used both in the clinical and research setting36. Studies on molecular autopsy in SADS have emphasised the dominant role of channelopathy genes 37,38. Since cardiomyopathies are expected to be diagnosed at autopsy, systematic testing of genes associated with structural cardiac disease was rarely performed. However, among one third of the decedents with idiopathic LVH where DNA was available, molecular autopsy failed to identify pathogenic variants in any of the genes encoding sarcomeric proteins implicated in HCM. This is in clear contrast with existing literature in HCM where a pathogenic variant in genes encoding sarcomeric proteins is identified in 40 to 72% of clinically diagnosed cases25,39. Even among sporadic cases of HCM, we would have expected a genetic yield of at least 20%. We identified a pathogenic variant in LAMP2 in a young woman who died suddenly in her sleep. LAMP2 is known to cause X-linked dominant Danon disease, a known HCM mimic40. Typical changes of Danon disease such as accumulation of autophagic material and glycogen in myocytes were not found at autopsy. None of her first-degree relatives showed evidence of disease or the same pathogenic mutation. It is plausible that the young girl tragically developed a malignant arrhythmogenic phenotype of disease prior to developing overt cardiomyopathy. We did not find any other mimics of HCM.

**Significance of idiopathic LVH**

Our observations suggest that idiopathic LVH is a separate entity from conventional HCM, which raises questions about the pathogenesis of idiopathic LVH. In contrast with none of the first-degree relatives being diagnosed with HCM, we found a 23% clinical diagnostic yield of inherited arrhythmia syndromes in family members of decedents with idiopathic LVH and a 14% yield of pathogenic variants in genes encoding cardiac myocyte ion channels at molecular autopsy. Specifically, Brugada syndrome was the most common diagnosis, followed by long QT syndrome. It is possible that idiopathic LVH may be a modifier of the risk for fatal arrhythmias in the context of a channelopathy by altering ion channel expression or promoting QT dispersion and premature ventricular complexes41,42. A recent study showed that individuals harbouring a pathogenic variant in the SCN5A gene were more likely to experience sudden death in the context of hypertension and/or LVH, suggesting that myocardial hypertrophy may exert a modulatory effect on the risk of fatal arrhythmias 41.

We were unable to identify monogenic diseases in 70% of families suggesting that idiopathic LVH may also be acquired, or, result from an interplay between a polygenic genetic background and environmental factors. Almost one third of our decedents were competitive athletes. Long-standing vigorous exercise may result in pathological remodelling of the heart. Repeated bouts of life-long endurance exercise have been associated myocardial fibrosis within the left ventricle and may predispose to fatal arrhythmias43,44. It is also possible that some athletes may develop pathological LVH in response to vigorous exercise which may be driven by environmental influences and/or confounding genetic factors.

**The role of the expert cardiac pathologist**

Idiopathic LVH may be easily confused with HCM in the absence of a thorough macroscopic and microscopic inspection of the heart. Indeed, some SCD series in athletes have categorised such deaths as probable HCM deaths45,46. Our cases were diagnosed by an expert cardiac pathologist using stringent criteria which included a thorough histopathological assessment with quantification of myocardial disarray in all cases.

**Limitations**

Our study has some limitations. Although a history of hypertension or valvular heart disease were exclusion criteria for idiopathic LVH, we cannot exclude that some decedents had undiagnosed hypertension or other subclinical non-cardiac causes of pressure and volume overload. However, considering the young age of SCD victims and relatively low prevalence of hypertension in the first 4 decades of life, we believe this is an unlikely explanation for most of our cases. We were unable to screen all the first-degree family members of all the families recruited, as some individuals were not available or refused to be investigated. This may have led to an underestimation of the prevalence of cardiac disease in families of decedents with idiopathic LVH. Finally, we could only perform molecular autopsy in less than one third of the decedents, however we feel that this number is sufficient in relation to investigation for HCM. Moreover, although several studies have investigated the role of molecular autopsy in the setting of SADS, there is limited knowledge concerning the role of molecular autopsy in decedents exhibiting features of idiopathic LVH according to our stringent criteria (macroscopic and microscopic examination in all cases, exclusion of secondary causes of LVH).

**Clinical impact**

The significance of idiopathic LVH in decedents of sudden death is unknown. Unexplained LVH in this context may be attributed to HCM. We investigated first degree relatives of decedents with idiopathic LVH and could not identify phenotypic features of HCM in any of them. Molecular autopsy was also performed in 30% of decedents using a comprehensive gene panel which failed to identify pathogenic variants in sarcomeric genes. Our study suggests that idiopathic LVH and HCM are separate disease entities. Indeed, we identified ion channel disease in almost one third of cases, suggesting that the investigation of first-degree relatives of decedents with idiopathic LVH should encompass a wide spectrum of inherited heart disease, including channelopathies.

**CONCLUSIONS**

In this study, none of the family members of decedents with idiopathic LVH showed phenotypic features of HCM following comprehensive cardiovascular evaluation. Similarly, molecular autopsy in 14 decedents failed to reveal pathogenic variants in genes encoding sarcomeric proteins which are commonly implicated in HCM. Therefore, idiopathic LVH and HCM are likely to represent two distinct pathological entities. The familial diagnosis of other cardiomyopathies and channelopathies in some families suggests that the presence of idiopathic LVH should prompt comprehensive assessment of families to encompass the broader spectrum of inherited structural heart disease and channelopathies.

**Funding:** GF is funded by the Charles Wolfson Charitable Trust and Cardiac Risk in the Young (CRY). CM is funded by the British Heart Foundation (BHF Clinical Research Training Fellowship FS/18/28/33549).

**Conflict of Interest:** none declared.

**REFERENCES**

1. Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, *et al.* A Prospective Study of Sudden Cardiac Death among Children and Young Adults. *N Engl J Med* 2016;**374**:2441–52.

2. Finocchiaro G, Papadakis M, Robertus J-L, Dhutia H, Steriotis AK, Tome M, *et al.* Etiology of Sudden Death in Sports Insights from a United Kingdom Regional Registry. *J Am Coll Cardiol* 2016;**67**.

3. Papadakis M, Raju H, Behr ER, Noronha S V. De, Spath N, Kouloubinis A, *et al.* Sudden cardiac death with autopsy findings of uncertain significance: Potential for erroneous interpretation. *Circ Arrhythmia Electrophysiol* 2013;**6**:588–96.

4. Raju H, Parsons S, Thompson TN, Morgan N, Zentner D, Trainer AH, *et al.* Insights into sudden cardiac death: exploring the potential relevance of non-diagnostic autopsy findings. *Eur Heart J* 2018;

5. Harmon KG, Asif IM, Maleszewski JJ, Owens DS, Prutkin JM, Salerno JC, *et al.* Incidence, Cause, and Comparative Frequency of Sudden Cardiac Death in National Collegiate Athletic Association Athletes: A Decade in Review. *Circulation* 2015;**132**:10–9.

6. Noronha S V de, Behr ER, Papadakis M, Ohta-Ogo K, Banya W, Wells J, *et al.* The importance of specialist cardiac histopathological examination in the investigation of young sudden cardiac deaths. *Europace* 2014;**16**:899–907.

7. Kitzman DW, Scholz DG, Hagen PT, Ilstrup DM, Edwards WD. Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity): A quantitative anatomic study of 765 specimens from subjects 20 to 99 years old. *Mayo Clin Proc* 1988;**63**:137–46.

8. Wingren CJ, Ottosson A. Postmortem heart weight modelled using piecewise linear regression in 27,645 medicolegal autopsy cases. *Forensic Sci Int* 2015;**252**:157–62.

9. Osborn M, Lowe J, Sheppard MN SS. Guidelines on autopsy practice: sudden death with likely cardiac pathology. *R Coll Pathol* 2015;**G145**.

10. Basso C, Aguilera B, Banner J, Cohle S, D’Amati G, Gouveia RH de, *et al.* Guidelines for autopsy investigation of sudden cardiac death: 2017 update from the Association for European Cardiovascular Pathology. *Virchows Arch* 2017;**471**:691–705.

11. Priori SG, Blomström-Lundqvist C, Mazzanti A, Blom N, Borggrefe M, Camm J, *et al.* 2015 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: The Task Force for the Management of Patients with Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death of the Europe. *Eur Heart J* 2015;**36**:2793–867.

12. EINTHOVEN W, FAHR G, WAART A DE. On the direction and manifest size of the variations of potential in the human heart and on the influence of the position of the heart on the form of the electrocardiogram. *Am Heart J* 1950;**40**:163–211.

13. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, *et al.* Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2015;**16**:233–70.

14. Finocchiaro G, Haddad F, Pavlovic A, Magavern E, Sinagra G, Knowles JW, *et al.* How does morphology impact on diastolic function in hypertrophic cardiomyopathy? A single centre experience. *BMJ Open* 2014;**4**.

15. Maron BJ, Maron MS. The Remarkable 50 Years of Imaging in HCM and How it Has Changed Diagnosis and Management: From M-Mode Echocardiography to CMR. *JACC Cardiovasc Imaging* 2016;**9**:858–72.

16. George KP, Birch KM, Pennell DJ, Myerson SG. Magnetic-resonance-imaging-derived indices for the normalization of left ventricular morphology by body size. *Magn Reson Imaging* Elsevier Inc.; 2009;**27**:207–13.

17. Mewton N, Liu CY, Croisille P, Bluemke D, Lima J a C. Assessment of myocardial fibrosis with cardiovascular magnetic resonance. *J Am Coll Cardiol* Elsevier Inc.; 2011;**57**:891–903.

18. BRUCE RA, LOVEJOY FW. Normal respiratory and circulatory pathways of adaptation in exercise. *J Clin Invest* 1949;**28**:1423–30.

19. Govindan M, Batchvarov VN, Raju H, Shanmugam N, Bizrah M, Bastiaenen R, *et al.* Utility of high and standard right precordial leads during ajmaline testing for the diagnosis of Brugada syndrome. *Heart* 2010;**96**:1904–8.

20. Papadakis M, Papatheodorou E, Mellor G, Raju H, Bastiaenen R, Wijeyeratne Y, *et al.* The Diagnostic Yield of Brugada Syndrome After Sudden Death With Normal Autopsy. *J Am Coll Cardiol* 2018;**71**.

21. Pua CJ, Bhalshankar J, Miao K, Walsh R, John S, Lim SQ, *et al.* Development of a Comprehensive Sequencing Assay for Inherited Cardiac Condition Genes. *J Cardiovasc Transl Res* 2016;**9**:3–11.

22. About Exome Aggregation Consortium (ExAC). Available at: http://exac.broadinstitute.org/about.

23. Herman DS, Lam L, Taylor MRG, Wang L, Teekakirikul P, Christodoulou D, *et al.* Truncations of Titin Causing Dilated Cardiomyopathy. *N Engl J Med* 2012;**366**:619–28.

24. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;**17**:405–24.

25. Elliott PM, Anastasakis A, Borger MA, Borggrefe M, Cecchi F, Charron P, *et al.* 2014 ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy. *Eur Heart J* 2014;**35**:2733–79.

26. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke D a., *et al.* Diagnosis of Arrhythmogenic Right Ventricular Cardiomyopathy/Dysplasia: Proposed Modification of the Task Force Criteria. *Circulation* 2010;**121**:1533–41.

27. McNally EM, Mestroni L. Dilated Cardiomyopathy: Genetic Determinants and Mechanisms. *Circ Res* 2017;**121**:731–48.

28. Chin TK, Perloff JK, Williams RG, Jue K, Mohrmann R. Isolated noncompaction of left ventricular myocardium. A study of eight cases. *Circulation* 1990;**82**:507–13.

29. Jenni R, Oechslin E, Schneider J, Attenhofer Jost C, Kaufmann PA. Echocardiographic and pathoanatomical characteristics of isolated left ventricular non-compaction: a step towards classification as a distinct cardiomyopathy. *Heart* 2001;**86**:666–71.

30. Petersen SE, Selvanayagam JB, Wiesmann F, Robson MD, Francis JM, Anderson RH, *et al.* Left ventricular non-compaction: insights from cardiovascular magnetic resonance imaging. *J Am Coll Cardiol* 2005;**46**:101–5.

31. Priori SG, Wilde AA, Horie M, Cho Y, Behr ER, Berul C, *et al.* Executive summary: HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes. *Europace* 2013;**15**:1389–406.

32. Schwartz PJ, Crotti L, Insolia R. Long-QT Syndrome: From Genetics to Management. *Circ Arrhythmia Electrophysiol* 2012;**5**:868–77.

33. Schwartz PJ, Ackerman MJ. The long QT syndrome: a transatlantic clinical approach to diagnosis and therapy. *Eur Heart J* 2013;**34**:3109–16.

34. Lahat H, Eldar M, Levy-Nissenbaum E, Bahan T, Friedman E, Khoury A, *et al.* Autosomal recessive catecholamine- or exercise-induced polymorphic ventricular tachycardia: clinical features and assignment of the disease gene to chromosome 1p13-21. *Circulation* 2001;**103**:2822–7.

35. BOYER NH. The Wolff-Parkinson-White syndrome. *N Engl J Med* 1946;**234**:111–4.

36. Tester DJ, Ackerman MJ. Genetic Testing for Potentially Lethal, Highly Treatable Inherited Cardiomyopathies/Channelopathies in Clinical Practice. *Circulation* 2011;**123**:1021–37.

37. Ackerman MJ, Siu BL, Sturner WQ, Tester DJ, Valdivia CR, Makielski JC, *et al.* Postmortem molecular analysis of SCN5A defects in sudden infant death syndrome. *JAMA* 2001;**286**:2264–9.

38. Tester DJ, Medeiros-Domingo A, Will ML, Haglund CM, Ackerman MJ. Cardiac channel molecular autopsy: insights from 173 consecutive cases of autopsy-negative sudden unexplained death referred for postmortem genetic testing. *Mayo Clin Proc* 2012;**87**:524–39.

39. Velzen HG van, Schinkel AFL, Baart SJ, Oldenburg RA, Frohn-Mulder IME, Slegtenhorst MA van, *et al.* Outcomes of Contemporary Family Screening in Hypertrophic Cardiomyopathy. *Circ Genomic Precis Med* 2018;**11**:e001896.

40. Arad M, Maron BJ, Gorham JM, Johnson WH, Saul JP, Perez-Atayde AR, *et al.* Glycogen storage diseases presenting as hypertrophic cardiomyopathy. *N Engl J Med* 2005;**352**:362–72.

41. Rivaud MR, Jansen JA, Postema PG, Nannenberg EA, Mizusawa Y, Nagel R van der, *et al.* A common co-morbidity modulates disease expression and treatment efficacy in inherited cardiac sodium channelopathy. *Eur Heart J* 2018;**39**:2898–907.

42. Oikarinen L, Nieminen MS, Viitasalo M, Toivonen L, Wachtell K, Papademetriou V, *et al.* Relation of QT interval and QT dispersion to echocardiographic left ventricular hypertrophy and geometric pattern in hypertensive patients. The LIFE study. The Losartan Intervention For Endpoint Reduction. *J Hypertens* 2001;**19**:1883–91.

43. Tahir E, Starekova J, Muellerleile K, Stritzky A von, Münch J, Avanesov M, *et al.* Myocardial Fibrosis in Competitive Triathletes Detected by Contrast-Enhanced CMR Correlates With Exercise-Induced Hypertension and Competition History. *JACC Cardiovasc Imaging* 2017;

44. Merghani A, Maestrini V, Rosmini S, Cox AT, Dhutia H, Bastiaenan R, *et al.* Prevalence of Subclinical Coronary Artery Disease in Masters Endurance Athletes With a Low Atherosclerotic Risk Profile. *Circulation* 2017;**136**:126–37.

45. Maron BJ, Doerer JJ, Haas TS, Tierney DM, Mueller FO. Sudden Deaths in Young Competitive Athletes: Analysis of 1866 Deaths in the United States, 1980-2006. *Circulation* 2009;**119**:1085–92.

46. Peterson DF, Siebert DM, Kucera KL, Thomas LC, Maleszewski JJ, Lopez-Anderson M, *et al.* Etiology of Sudden Cardiac Arrest and Death in US Competitive Athletes: A 2-Year Prospective Surveillance Study. *Clin J Sport Med* 2018;

**FIGURE LEGENDS**

**Figure 1.** Summary of the study.

**Figure 2**. Family screening in SCD victims with an autopsy suggestive of idiopathic LVH (percentages of families affected). The dots represent the family members, family members belonging to the same family are contained in the same circle. **Abbreviations:** ACM: arrhythmogenic cardiomyopathy; BrS: Brugada syndrome; DCM: dilated cardiomyopathy; ICC: inherited cardiac condition; HCM: hypertrophic cardiomyopathy; LQTS: long QT syndrome; WPW: Wolff-Parkinson-White syndrome.

**Figure 3**. Results of the genetic test in sudden death victims with an autopsy consistent with idiopathic LVH. **Abbreviations**: VUS: variants of uncertain significance.

**Table 1.** Characteristics of the decedents.

|  |  |
| --- | --- |
|  | **Decedents (n=46)** |
| **Mean age (years) (range)** | 33±14 (8-62) |
| **Age ≤ 35 years n (%)** | 29 (63) |
| **Male gender n (%)** | 40 (87) |
| **BMI**  **BMI ≥ 30 n (%)** | 27±5  2 (4) |
| **Height (cm)** | 177±15 |
| ***Ethnicity:***  **Caucasian n (%)**  **Afro-Caribbean n (%)**  **Asian n (%)** | 43 (93)  2 (4)  1 (2) |
| ***Mode of death:***  **Asleep n (%)**  **Rest n (%)**  **Exercise n (%)** | 6 (13)  24 (52)  16 (35) |
| **Competitive athlete n (%)** | 14 (30) |
| **Heart weight (g)** | 544±93 |
| **Left ventricular fibrosis n (%)** | 14 (30) |

**Table 2.** Results of family screening in decedents with idiopathic LVH.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **N** | **Decedent** | **Family member** | **Diagnosis/findings** | **Genetics (family members)** |
|  | **Sex, circumstances, age** | **Family member, age** |  |  |
| 1 | male, died during exercise, 47, MF | sibling, 45  sibling, 51 | Negative  Negative |  |
| 2 | male, died at rest, 39 | father, 67  mother, 65  sibling, 35 | Mild LVH, hypertension  Mild LVH, hypertension  Negative | Negative |
| 3 | male, died during sleep, 37 | father, 66  sibling, 42 | Possible IHD  Concealed Brugada | Negative |
| 4 | male, died during exercise, 18, MF | father, 40  mother, 53  sibling, 21 | Negative  Negative  Negative |  |
| 5 | male, died at rest, 42 | sibling, 48 | DCM |  |
| 6 | male, died at rest, 29 | mother, 59  sibling, 22 | Negative  Negative |  |
| 7 | male, died during exercise, 25 | father, 59  mother, 55  sibling, 25 | Negative  Negative  Negative |  |
| 8 | male, died at rest, 28 | father, 69  mother, 67  sibling, 47 | Possible IHD  Negative  Negative |  |
| 9 | male, died at rest, 34, MF | father, 73  mother, 73 | Negative  Negative |  |
| 10 | male, died during exercise, 28 | father, 63  mother, 59 | Atrial fibrillation  Negative |  |
| 11 | male, died at rest, 48 | sibling, 49 | Negative |  |
| 12 | male, died during exercise, 36, MF | sibling, 48  sibling, 70  sibling, 46 | Negative  Negative  Negative |  |
| 13 | male, died at rest, 21, MF | father, 59  mother, 58  sibling, 19 | Negative  IHD  Negative |  |
| 14 | male, died at rest, 57, MF | son, 30  son, 34 | Negative  Negative |  |
| 15 | male, died during exercise, 38 | mother, 63  father, 64  sibling, 36  sibling, 35 | Negative  Possible IHD  Negative  Cardiac mass |  |
| 16 | male, died at rest, 50 | sibling, 65 | Negative |  |
| 17 | male, died during exercise, 28 | father, 63  mother, 50  sibling, 27 | Negative  WPW  Negative |  |
| 18 | male, died during sleep, 25 | father, 50  mother, 48  sibling, 25  sibling, 21 | Mild LVH, hypertension  Concealed Brugada  Negative  Negative | Negative |
| 19 | male, died during exercise, 24 | father, 50  mother, 50 | Mild LVH, hypertension, steroid use  Negative | Negative |
| 20 | male, died at rest, 27, KCNQ1 | mother, 57  father, 61 | LQTS  Mild LVH, hypertension | KCNQ1 |
| 21 | female, died during exercise, 8 | father, 58  mother, 57  sibling, 33  sibling, 33 | Negative  Negative  Negative  AC | Lamin A/C |
| 22 | male, died during exercise, 55, MF | son, 30  daughter, 28 | Negative  Negative |  |
| 23 | female, died at rest, 25 | mother, 62  father, 55 | Negative  Tri-fascicular block |  |
| 24 | male, died during exercise, 21 | father, 55  sibling, 27  sibling, 23  sibling, 22 | Mild LVH, hypertension  VE burden 9%  Concealed Brugada  Negative | Negative  Negative |
| 25 | male, died at rest, 57 | daughter, 32  daughter, 21  son, 25  daughter, 27 | Negative  Negative  Negative  Negative |  |
| 26 | male, died during sleep, 20 | sibling, 20 | Negative |  |
| 27 | male, died at rest, 15 | father, 64  brother, 35 | Negative  Negative |  |
| 28 | male, died during exercise, 59, MF | daughter, 16  daughter, 17  daughter, 16  daughter, 36  daughter, 22  son, 19  son, 23 | Negative  Negative  Negative  Negative  Negative  Negative  Negative |  |
| 29 | male, died during exercise, 22, MF | father, 56  sibling,28  sibling, 24  sibling, 21  sibling, 19  sibling, 16 | Negative  Negative  Negative  Negative  Negative  Negative |  |
| 30 | male, died during exercise, 19 | sibling, 47 | LQTS |  |
| 31 | male, died during sleep, 24 | mother, 54  father, 59  sibling, 28 | Negative  Concealed Brugada  Negative |  |
| 32 | male, died at rest, 23 | mother, 45  sibling, 23 | Negative  Negative |  |
| 33 | male, died at rest, 30 | mother, 56  father, 56  sibling, 28 | Negative  Concealed Brugada  Negative |  |
| 34 | female, died at rest, 23 | mother, 45  father, 48  sibling, 22 | Concealed Brugada  Negative  Negative | Negative |
| 35 | male, died during exercise, 18, MF | mother, 48  sibling, 24  sibling, 24 | Negative  Negative  Negative |  |
| 36 | female, died at rest, 26, LAMP2 | mother, 56  father, 58  brother, 30 | Negative  Negative  Negative |  |
| 37 | male, died at rest, 18 | mother, 47  father, 56  sibling, 22 | VE burden 9%  Alcoholic CMP  Negative | Negative |
| 38 | male, died at rest, 26 | mother, 58  father, 65  sibling, 36  sibling, 32 | Negative  Concealed Brugada  Negative  Negative | Negative |
| 39 | female, died at rest, 52, MF | son, 29 | Negative |  |
| 40 | female, died at rest, 21, KCNH2 | mother, 41  father, 55  sibling, 15  sibling, 10 | Negative  LQTS  LQTS  Negative | KCNH2  KCNH2 |
| 41 | male, died during exercise, 52, MF | sibling, 50  sibling, 52 | Negative  Negative |  |
| 42 | male, died at rest, 28 | mother, 62  father, 60  sibling, 21  sibling, 25 | Negative  Negative  Negative  Negative |  |
| 43 | female, 62, died at rest, MF | daughter, 30  daughter, 28 | Brugada syndrome  Brugada syndrome |  |
| 44 | male, died at rest, 34 | son, 17 | Negative |  |
| 45 | male, died during sleep, 35 | sibling, 33  sibling, 31 | Negative  Negative |  |
| 46 | female, died at rest, 56, MF | sibling, 58  sibling, 60  son, 29 | Negative  Negative  Negative |  |

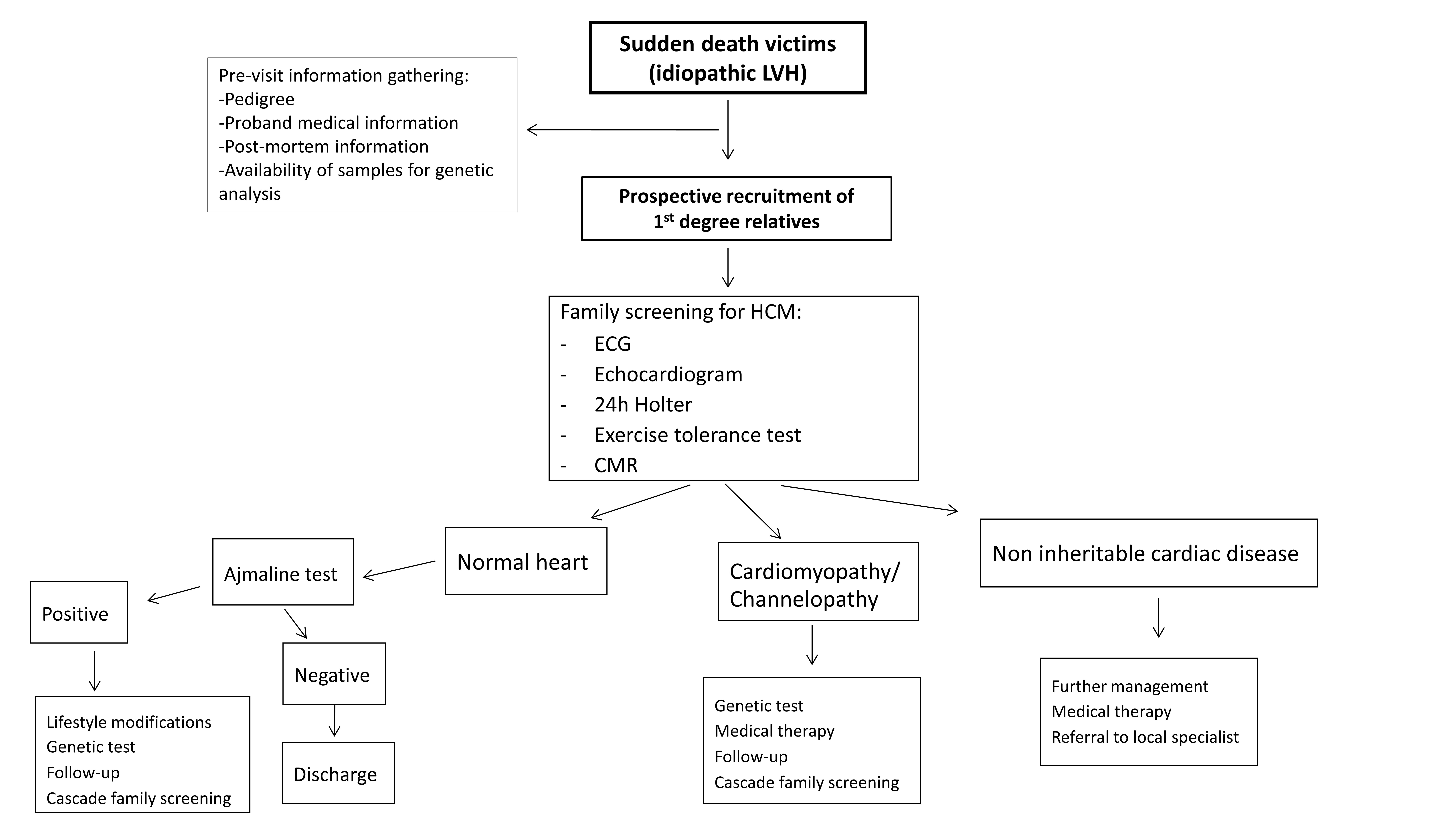
**Abbreviations:** AC: arrhythmogenic cardiomyopathy; CMP: cardiomyopathy; IHD: ischemic heart disease; LVH: left ventricular hypertrophy; LV: left ventricular; LQTS: long QT syndrome; MF: myocardial fibrosis; VE: ventricular ectopics; WPW: Wolff-Parkinson-White syndrome.

\* variants mentioned in this table are pathogenic.; \*\* mild LVH is defined as maximal wall thickness ≤ 13 mm.

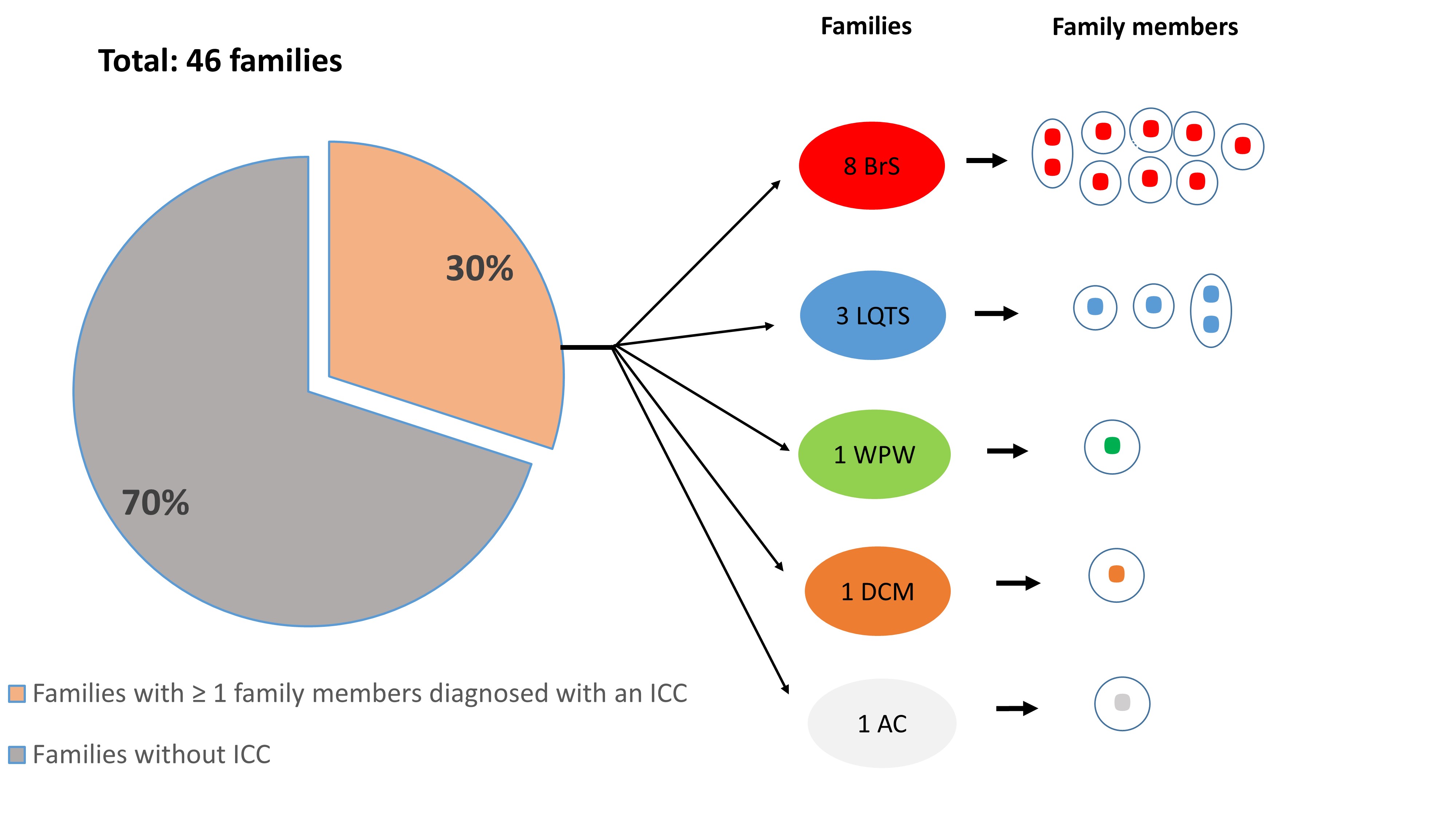
**Table 3.** Family members with findings of uncertain significance.

|  |  |  |
| --- | --- | --- |
| **Proband (age and sex)** | **Family member (age and relationship with proband)** | **Finding** |
| 39, male | 65, father | Hypertension, normal ECG, septal bulge (maximal wall thickness 14 mm), no pathogenic variants |
| 24, male | 50, father | Hypertension, reported long-term use of anabolic steroids, normal ECG, interventricular septum wall thickness of 15 mm, no pathogenic variants |
| 21, male | 28, brother | Athlete, significant ventricular ectopic burden (9%), mild dilatation of the right ventricular outflow tract, no pathogenic variants |
| 18, male | 47, mother | Palpitations, significant ventricular ectopic burden (10%), structurally normal heart. |

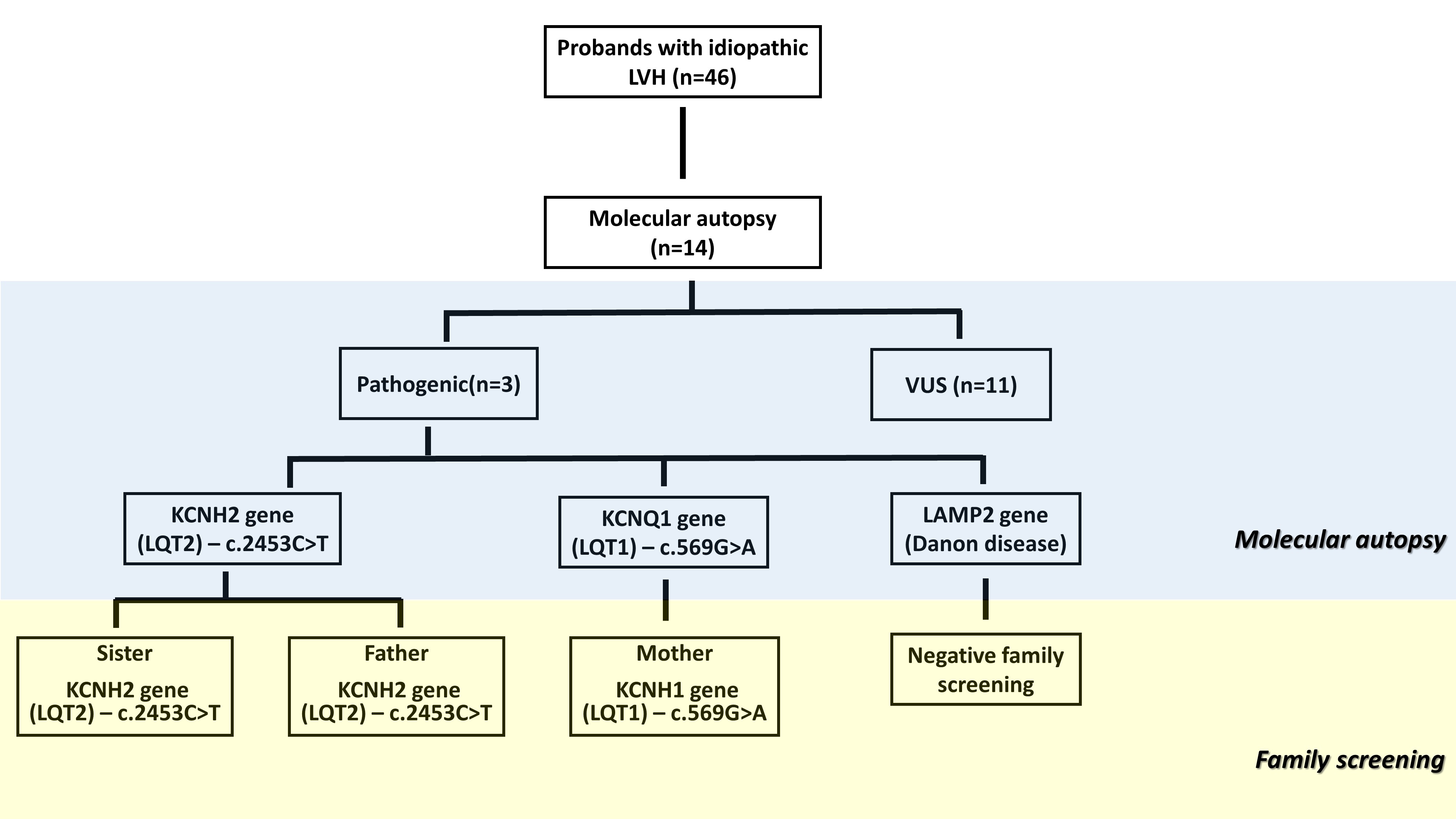
**Figure 1.**

****

**Figure 2.**



**Figure 3.**



**SUPPLEMENTARY MATERIAL**

**Table 1.** Gene List: Illumina TruSight Cardio (174 genes).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| ABCC9 | COL5A1 | GATAD1 | LAMP2 | PCSK9 | SMAD4 |
| ABCG5 | COL5A2 | GCKR | LDB3 | PDLIM3 | SNTA1 |
| ABCG8 | COX15 | GJA5 | LDLR | PKP2 | SOS1 |
| ACTA1 | CREB3L3 | GLA | LDLRAP1 | PLN | SREBF2 |
| ACTA2 | CRELD1 | GPD1L | LMF1 | PRDM16 | TAZ |
| ACTC1 | CRYAB | GPIHBP1 | LMNA | PRKAG2 | TBX20 |
| ACTN2 | CSRP3 | HADHA | LPL | PRKAR1A | TBX3 |
| AKAP9 | CTF1 | HCN4 | LTBP2 | PTPN11 | TBX5 |
| ALMS1 | DES | HFE | MAP2K1 | RAF1 | TCAP |
| ANK2 | DMD | HRAS | MAP2K2 | RANGRF | TGFB2 |
| ANKRD1 | DNAJC19 | HSPB8 | MIB1 | RBM20 | TGFB3 |
| APOA4 | DOLK | ILK | MURC | RYR1 | TGFBR1 |
| APOA5 | DPP6 | JAG1 | MYBPC3 | RYR2 | TGFBR2 |
| APOB | DSC2 | JPH2 | MYH11 | SALL4 | TMEM43 |
| APOC2 | DSG2 | JUP | MYH6 | SCN1B | TMPO |
| APOE | DSP | KCNA5 | MYH7 | SCN2B | TNNC1 |
| BAG3 | DTNA | KCND3 | MYL2 | SCN3B | TNNI3 |
| BRAF | EFEMP2 | KCNE1 | MYL3 | SCN4B | TNNT2 |
| CACNA1C | ELN | KCNE2 | MYLK | SCN5A | TPM1 |
| CACNA2D1 | EMD | KCNE3 | MYLK2 | SCO2 | TRDN |
| CACNB2 | EYA4 | KCNH2 | MYO6 | SDHA | TRIM63 |
| CALM1 | COL5A3 | KCNJ2 | MYOZ2 | SEPN1 | TRPM4 |
| CALR3 | COL5A4 | KCNJ5 | MYPN | SGCB | TTN |
| CASQ2 | COX16 | KCNJ8 | NEXN | SGCD | TTR |
| CAV3 | COL5A3 | KCNQ1 | NKX2-5 | SGCG | TXNRD2 |
| CBL | COL5A4 | KLF10 | NODAL | SHOC2 | VCL |
| CBS | COX16 | KRAS | NOTCH1 | SLC25A4 | ZBTB17 |
| CETP | CREB3L4 | LAMA2 | NPPA | SLC2A10 | ZHX3 |
| COL3A1 | COL5A3 | LAMA4 | NRAS | SMAD3 | ZIC3 |

**Table 2.** Rare variants in sudden death victims.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **N** | **Gene** | **Protein Name** | **c.DNA change** | **p. amino acid change** | **ExAC MAF** | **CADD** | **ACMG** |
| 20 | KCNQ1 | Potassium voltage gated channel 1 | c.569G>A | p.Arg190Gln | 0 | 35 | Pathogenic |
| 40 | KCNH2 | Potassium voltage gated channel 2 | c.2453C>T | p.Ser818Leu | 0 | / | Pathogenic |
| 36 | LAMP2 | Deletion of ~136kb of chromosome X, band q24 | | | | | Pathogenic |
| 41 | RYR1 | Ryanodine Receptor, skeletal muscle | c.1901T>C | p.Ile634Thr | 0.00001 | 12 | VUS |
| 41 | CBL | Casitas B-lineage lymphoma | c.2461G>A | p.Glu821Lys | 0.00002 | 20 | VUS |
| 25 | FBN1 | Fibrillin 1 | c.442+15G>T |  | 0.00006 | 2 | Likely benign |
| 3 | LAMA2 | Laminin alpha 2 | c.2611C>T | p.Leu871Phe | 0.000008 | 22 | VUS |
| 14 | MYOZ2 | Myozenin 2 | c.302C>A | p.Ser101\* | 0.00002 | 36 | VUS |
| 14 | COL5A1 | Collagen Type V Alpha-1 | c.2493C>T | p.Ile831Ile | 0.0001 | 8 | Likely benign |
| 16 | APOB | Apolipoprotein B | c.9140C>T | p.Thr3047Met | 0.00004 | 8 | VUS |
| 18 | ZBTB17 | Zinc finger and BTB domain protein 17 | c.646G>C | p.Glu216Gln | 0.000008 | 10 | VUS |
| 18 | VCL | Vinculin | c.1048A>C | p.Met350Leu | 0 | 18 | VUS |
| 38 | LTB2 | Latent transforming growth factor beta | c.2995G>A | p.glu999lys | 0.00002 | 24 | VUS |
| 19 | ZHX3 | Zinc fingers and homeobox protein 3 | c.886C>T | p.Leu296Phe | 0.00003 | 16 | VUS |
| 16 | SALL4 | Sal-like protein 4 | c.203G>A | p.Arg68Gln | 0.0003 | 12 | VUS |
| 25 | CBS | Cystathionine beta-synthase | c.935C>A | p.Pro312His | 0.00000008 | 17 | VUS |
| 41 | TXNRD2 | Thioredoxin reductase2 | c.940G>T | p.Asp314Tyr | 0.00008 | 13 | VUS |
| 24 | SCN5A | Voltage gated sodium channel | c.392+3A>G |  | 0 | 16 | VUS |
| 16 | MYL3 | Myosin light chain | c.502G>A | p.Glu168Lys | 0 | 24 | VUS |
| 41 | HFE | Human hereditary haemochromatosis | c.764G>A | p.Arg255His | 0.00008 | 8 | VUS |
| 41 | DPP6 | Dipeptidyl aminopeptidase-like protein 6 | c.203G>A | p.Arg68Gln | 0 | 10 | VUS |
| 16 | DSP | Desmoplakin | c.4303G>C | p.Ala1435Pro | 0.000008 | 15 | VUS |
| 38, 20 | SDHA | Succinate dehydrogenase | c.1337T>C | p.Val446Ala | 0.00009 | 18 | VUS |
| 38, 20 | SDHA | Succinate dehydrogenase | c.1202C>T | p.Ala401Val | 0.00009 | 22 | VUS |
| 16 | CACNB2 | Voltage dependent calcium channel | c.372\_39delAGTCA | p.Ser13\_Tyr14del | 0 | Null | VUS |
| 22 | CRYAB | Alpha B Crystallin | c.343delT | p.Ser115Profs | 0 | Null | VUS |
| 22 | LAMA2 | Laminin, alpha 2 | c.5750\_5753del | p.1918del | 0 | Null | VUS |
| 22 | SCN10A | Voltage gated sodium channel type X | c.71T>C | p.Ile24Thr | 0 | 15 | VUS |

**Abbreviations:** ACMG: American College of Medical Genetics; CADD: Combined Annotation-Dependent Depletion**.**