

Sudden unexplained death in the young: epidemiology, aetiology and value of the clinically guided genetic screening

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Aims

To determine the incidence and the causes of sudden death (SD) in persons aged 1–35 years old and the diagnostic yield of clinically guided genetic screening in the sudden arrhythmic death syndrome (SADS) victims' families.

Methods and results

Incidence and causes of SD in the Attica region of Greece in 2002–10 were determined using death certificates and autopsy reports. We evaluated clinically consecutive families of SADS victims and if a clinical diagnosis was established, we proceeded to targeted genetic analysis. Out of 6030 deaths, 56% were due to traumatic or violent causes, 40.5% were natural deaths, and 3.3% were of undetermined cause. There were 349 SD cases. Cardiovascular causes accounted for 65%, non-cardiovascular causes for 17%, and SADS for 18%. Clinical evaluation identified an inherited heart disease in 5/20 SADS families (25%). Targeted genetic analysis identified a causative mutation in all of the five screened families and reconfirmed the diagnosis in three of five proband victims. Clinical and genetic evaluation of 28 family members identified eight affected carriers and eight non-affected carriers. Molecular autopsy failed to identify any of these families.

Conclusion

Sudden death in the young is of cardiovascular origin in the majority of cases. A considerable rate of SD cases remains of unknown cause on post-mortem. Apart from channelopathies, subclinical forms of inherited structural heart diseases would appear to be implicated in SADS. Clinically guided genetic screening has a significant diagnostic yield and identifies affected families that would have been missed by the current suggested molecular autopsy panel.

Keywords

Sudden arrhythmic death syndrome • Clinical genetics • Sudden death in the young • Molecular autopsy • Epidemiology

Introduction

Sudden death (SD) in a young person is an unexpected and devastating event for both the family and the community. Although cardiovascular causes are identified as the major cause in its aetiology; its true

magnitude remains generally unknown with widely variable reported incidence.¹ A relatively significant portion of SD remains unexplained after post-mortem analysis, referring to as sudden arrhythmic death syndrome (SADS). Previous studies managed to detect an inherited cardiac disease in 22–53% of the families of SADS victims.^{2–4}

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What's new?

- We report a clinicopathological assessment of sudden death (SD) in a region-based young population of 12 750 000 people-years in Greece.
- Cardiovascular disease represents the major cause of SD and sudden arrhythmic death syndrome (SADS) in the young.
- The annual incidence rate of sudden cardiac death and SADS in the young was estimated to be 1.8 per 100 000 and 0.5 per 100 000, respectively.
- Beyond channelopathies, subclinical forms of inherited structural heart diseases are implicated in the aetiology of SADS.
- Clinically guided genetic screening must be pursued as a complementary and supportive method in the assessment of SADS families.

Concerning the management of SADS, two main models of assessment emerge in the medical literature. The first model (molecular autopsy) uses the infrastructure and expertise of large pathology centres with a specialty in cardiac pathology as a filter through which structural heart disease is identified. Genetic testing for non-structural disease in the stored DNA of the negative-autopsy victims is then performed (molecular autopsy).^{1,5} In contrast, the second model (clinically guided genetic screening) uses the clinical evaluation of the families as the primary diagnostic tool in order to detect relatives with an inherited cardiac disease.⁶ As soon as an index case is identified, targeted genetic screening is performed in the living member. Genetic screening in the victim's DNA is conducted only if a potential causative mutation (or mutations) is found in another member of the family, and this specific genetic substrate is examined.

The aim of this study was to determine the incidence and the causes of SD in persons aged 1–35 years in a region-based population and to determine the diagnostic yield of clinically guided genetic screening of the SADS victims and their relatives.

Methods

Study design

The study was prospective using the availability of death certificates and autopsy reports in the Attica region of Greece. All deaths in persons aged 1–35 years in this region during 2002–10 (8.5 years) were included. The population was demographically stable and representative of the Greek population. Incidence and causes of SD in this population were determined. Deaths caused by trauma, accidental causes, drowning, drug toxicity or epilepsy and deaths in tourists or non-residents were excluded from this analysis. Fatalities that occurred in individuals who were admitted to a hospital were not considered as SD.

Death certificates

In Greece, when a person dies, a death certificate is always issued and can only be issued by a medical doctor (physician). All death certificates are then registered in the informative civil records of the Ministry of Interior's system. The causes of death are registered according to the International Classification of Diseases-10. The Hellenical Statistical Authority (ELSTAT), responsible for producing periodical census of the population

and health statistics, derives the information required for statistical purposes from the central database of the Ministry on a monthly basis. The deaths in males and females are reported yearly at 5-yearly age intervals. For the purpose of this study, data on mortality were collected from the ELSTAT database.

Autopsies

All forensic autopsies conducted in Attica were reviewed on an individual basis and the cause of death was recorded. According to Greek law regulations, a forensic autopsy is mandatory in every case of SD. Consent to conduct the autopsy is not required and the autopsy is performed on the statutory authority's own motion. All autopsies in Attica are conducted at the Department of Forensic Medicine and Toxicology at the University of Athens and at Forensics Medicines Services of Ministry of Justice (two laboratories).

The autopsy followed a standardized protocol, in which all organs were examined. Toxicology screens and histopathology analysis were performed in every victim. The autopsies were performed by several pathologists practicing at the two departments during this period, but they were all reviewed by the senior authors (C.S. and P.K.).

Definitions

Sudden death was defined in unwitnessed cases as a person last seen alive and functioning normally <24h before being found dead, and in witnessed cases, as an acute change in cardiovascular status with the time to death being <1h. Sudden cardiac death (SCD) was defined as an SD from a cardiac cause. Sudden arrhythmic death syndrome was defined as an SD in which no cause could be established based on autopsy and toxicology. The proband victim was defined as the person who died suddenly in each family. The index case was defined as the first family member in whom a clinical diagnosis of an inherited cardiac disease was established.

Standard definitions for the diagnoses of structural heart disease during autopsy were utilized.⁷ The diagnosis of arrhythmogenic right ventricular (RV) cardiomyopathy, required fibrofatty replacements at histopathology, of hypertrophic cardiomyopathy the presence of myofibrils' disarray and of myocarditis evidence of cardiac inflammation and myocyte necrosis. The toxicological screening included (i) alcohol, (ii) drugs of abuse, and (iii) general screening for organic poisons.

Evaluation of families and clinically guided genetic screening

As many living family members as possible, first- and second-degree relatives of SADS probands underwent cardiac clinical investigation. Victim's parents were approached by the physician who confirmed the SD or the pathologist who conducted the autopsy, usually in a 3 month period after the event. Cardiac assessment included a detailed personal and family history of cardiac events, physical examination, resting, exercise, and ambulatory 24 h electrocardiogram (ECG) and 2D echocardiography. Any further invasive or non-invasive investigations (e.g. provocation testing with sodium channel blocker, signal averaged ECG, cardiac MRI) were performed as indicated by overall clinical findings. The standard protocol (including ECG, 2D echocardiogram, and exercise testing) was used in all 20 SADS families. Provocation testing with procainamide was performed in four relatives from four SADS families after clinical suspicion of Brugada syndrome (BrS) in the resting ECG (Type 2 Brugada pattern). We did not perform any adrenaline provocation testing. Standard criteria were used based on recent guidelines for the diagnosis of typical or sub-clinical forms of inherited heart diseases in families with a history of SADS.^{8,9} All patients who underwent additional cardiogenetic screening signed informed consent prior to study participation.

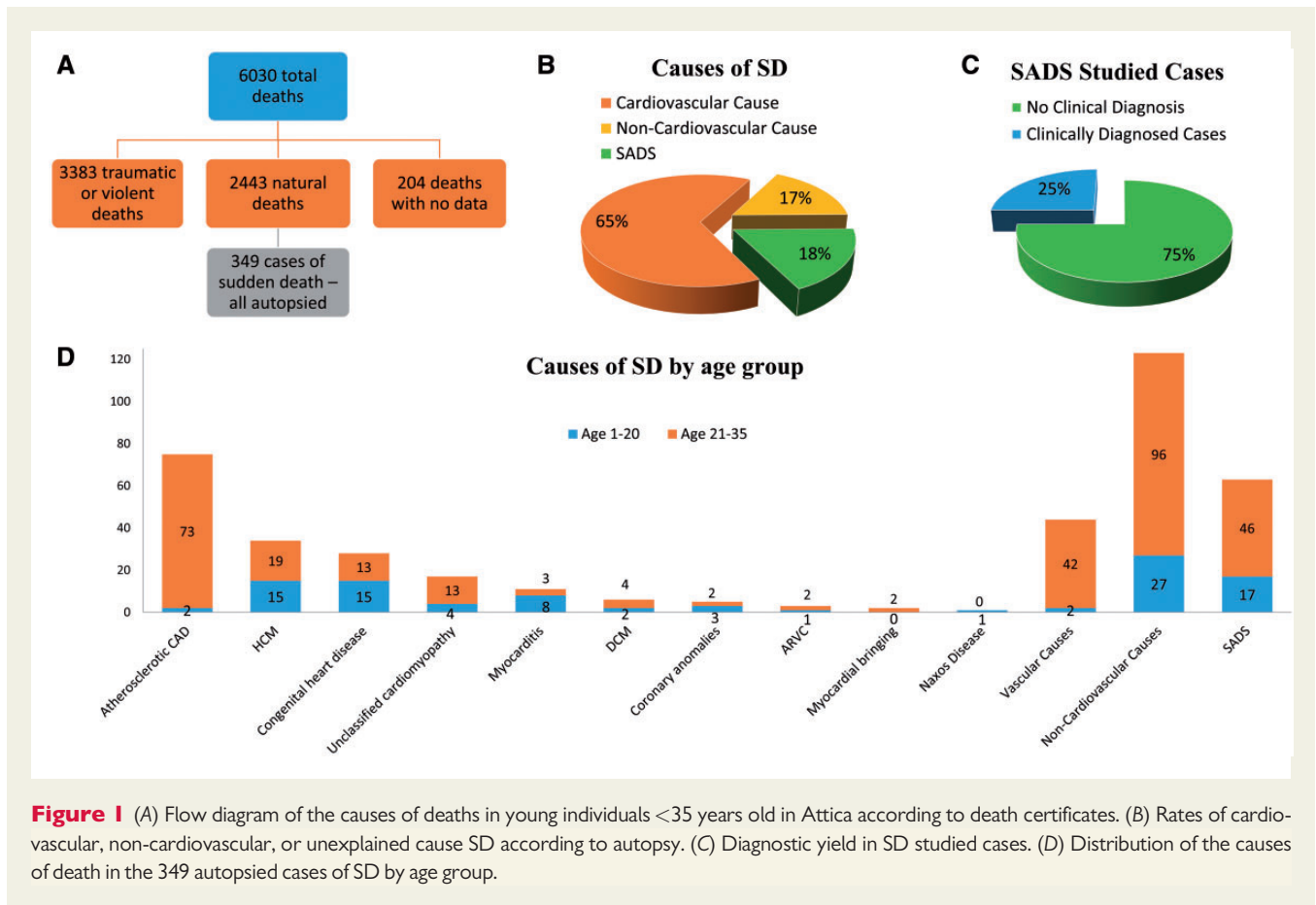


Figure 1 (A) Flow diagram of the causes of deaths in young individuals <35 years old in Attica according to death certificates. (B) Rates of cardiovascular, non-cardiovascular, or unexplained cause SD according to autopsy. (C) Diagnostic yield in SD studied cases. (D) Distribution of the causes of death in the 349 autopsied cases of SD by age group.

As soon as a clinical definitive or suspected diagnosis of an inherited cardiac disease, targeted genetic analysis was offered to the index case. We analysed the genes that correlated to the clinical phenotype, according to the protocols used in the everyday practice.^{10,11} If a putative mutation or mutations were found, cascade genetic analysis was offered in the other family members. Proband victim's DNA (blood sample or paraffin-embedded tissue) was also screened for the mutation or mutations already found, in order to further verify the cause of death.

Results

Epidemiological data

From 2002–10 (8.5 years), there was a total of 6030 deaths in persons aged 1–35 years in the region of Attica. The mean young population at the study period was 1.5 million inhabitants (Hellenic Statistical Authority). From those, 3383 (56%) were due to traumatic or violent causes and 2443 (40.5%) were natural deaths. In 204 cases (3.3% of the total deaths) there were no data about the cause of death.

There were 349 (5.7%) cases of sudden, non-traumatic death (mean age at death 25.6 years). All of these cases were autopsied. In total, 242 (69%) were males (mean age: 26.9 years) and 107 (31%) were females (mean age: 22.9 years). At age range 1–20 years, SD occurred in 97 cases (28%), whereas in the age range between 21 and 35 years, 252 cases (72%) died suddenly. According to these

data, SD mortality rate in the young was 3.4 per month in this region, and the total incidence rate was 2.7 per 100 000 person-years.

Sudden death was of cardiovascular origin in the majority of the cases (226/349 cases, 65%), whereas 60/349 cases of SD (17%) were attributed to non-cardiovascular causes and in 63/349 cases (18%) no cause of SD was identified at autopsy. The incidence rate of SCD in the young was 1.8 per 100 000 person-years and of SADS in the young was 0.5 per 100 000 person-years. In age group 1–20 years, the most frequent cardiac cause of SD was hypertrophic cardiomyopathy (HCM) (15 cases, 28%) and congenital heart disease (15 cases, 28%). In the age group 21–35 years, atherosclerotic coronary artery disease (CAD), with strong coexisting family history of CAD, was the leading cause of SD (73 cases, 42%) (Figure 1).

SADS cohort

Twenty of the 63 consecutive SADS families accepted to be further investigated (see Supplementary material online, Table S1). Clinical evaluation identified an inherited cardiovascular disease in 5 of the 20 families (25%). Clinical and molecular genetic investigation of 28 family members revealed 8 affected carriers (28%), 8 non-affected carriers (28%), and 12 normal (43%) (Figure 2).

A pathogenic mutation was identified in all five affected families and was reconfirmed in the DNA of three proband victims. Genetic analysis was not conducted in two proband victims due to lack or poor quality of DNA from paraffin blocks.

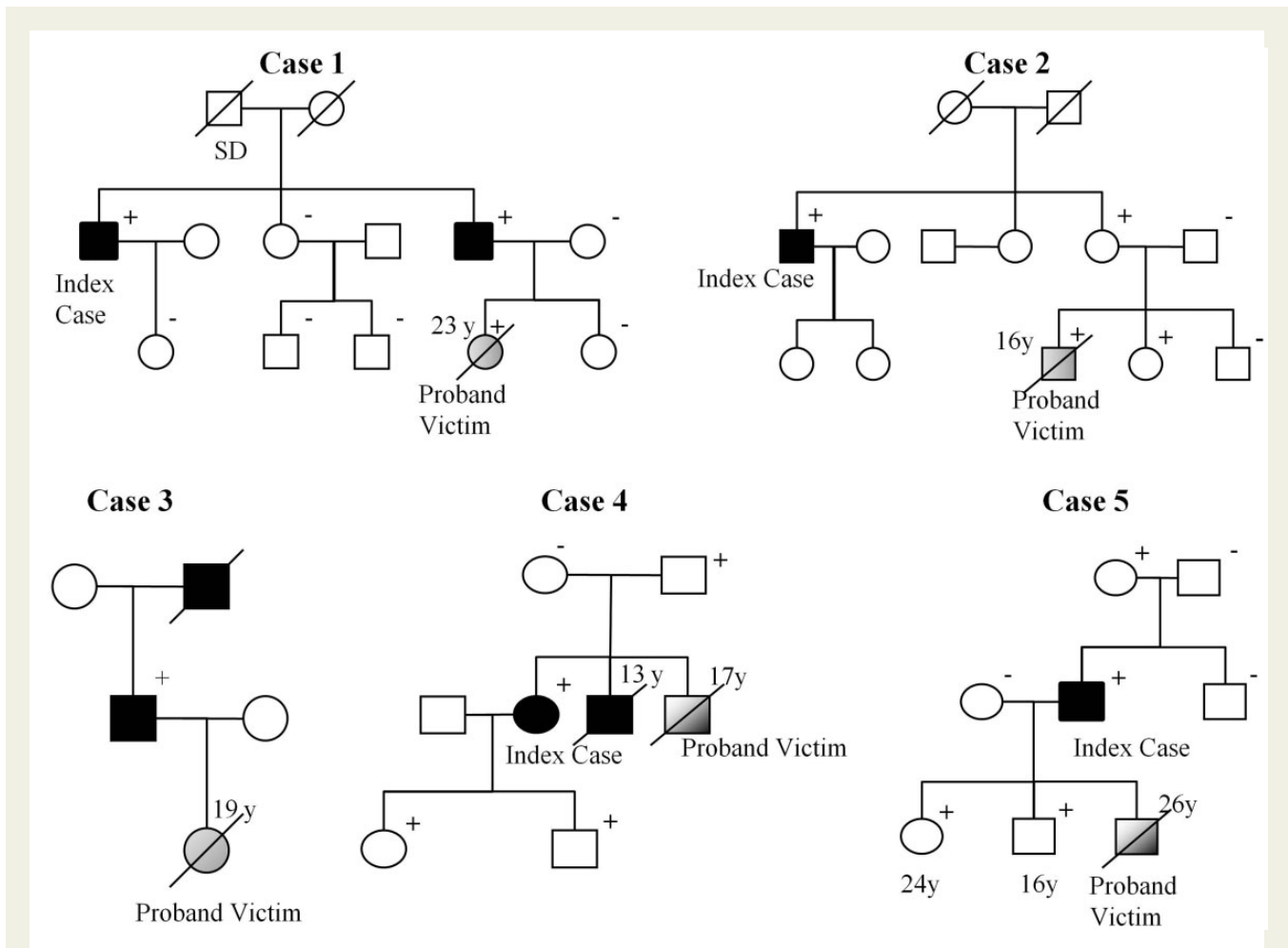


Figure 2 Family trees of the five affected SADS cases. Circles indicate female; squares indicate male; symbols with a slash indicate deceased subjects; and coloured black symbols indicate clinically diseased persons. Symbols with a positive sign indicate mutation carriers whereas a minus sign indicates mutation-free subjects. The age reported in subjects with SD indicates the age at the event.

SADS cases

Case 1 was a 23-year-old female, with a history of pre-syncope episodes, who died suddenly following physical activity. Post-mortem examination was negative. The 12-lead ECG performed 6 months prior to her death showed inverted T-waves in leads V1 and V3, inverted/flat T-waves in inferior leads, and low voltage in leads V4–V6. The proband victim's father (T-wave inversion in leads V1–V5, positive signal average ECG, NSVT in Holter, mild RV dilatation, and dyskinetic area in the RV free wall) and uncle (T-wave inversion in leads V1–V4, positive signal average ECG, >1000 ectopic beats in 24 h Holter, RV dilatation, and aneurysmatic region in the RV apex) fulfilled clinical criteria for arrhythmogenic RV cardiomyopathy/dysplasia (ARVC/D) diagnosis (Figure 3). The genetic screening revealed a single-base deletion (c.2509delA) in exon 13 of the *PKP2* gene in both. Following the results of the genetic testing in the family, the proband victim's DNA, extracted from a paraffin-embedded myocardial sample, was positive for c.2509delA.

Case 2 was an 18-year-old male who died suddenly as he was playing football. Cardiac autopsy showed mild left ventricular

hypertrophy without histopathological criteria of HCM. His maternal uncle (index case) fulfilled major criteria for ARVC/D (Figure 4) (dilated RV, RV free wall aneurysm, inverted T-wave V1–V4) and the consequent genetic testing detected a *PKP2* splice site mutation in exon 11 (c.2146-1G>C). Cascade genetic analysis identified the mutation in the victim's mother and sister. Genetic analysis of DNA extracted from a paraffin block revealed the same *PKP2* mutation in the proband victim.

Case 3 was a 19-year-old female athlete (swimmer) that died while sleeping, during a period of intense training. An intermittent prolongation of QTc interval has been recorded in a previous ECG on retrospective review of her medical records (Figure 5). The proband victim's father was diagnosed with a definite Long QT syndrome (LQTS), gathering 6.5 points according to the Schwartz criteria. Genetic analysis confirmed the diagnosis, detecting a missense mutation (c.202T>C) in exon 2 of *KCNH2* gene. Long QT syndrome was also diagnosed in the proband victim's grandfather. As a result, the proband victim score increased to 4 points (QTc = 500 ms-3p, family member with a definite LQTS-1p) leading

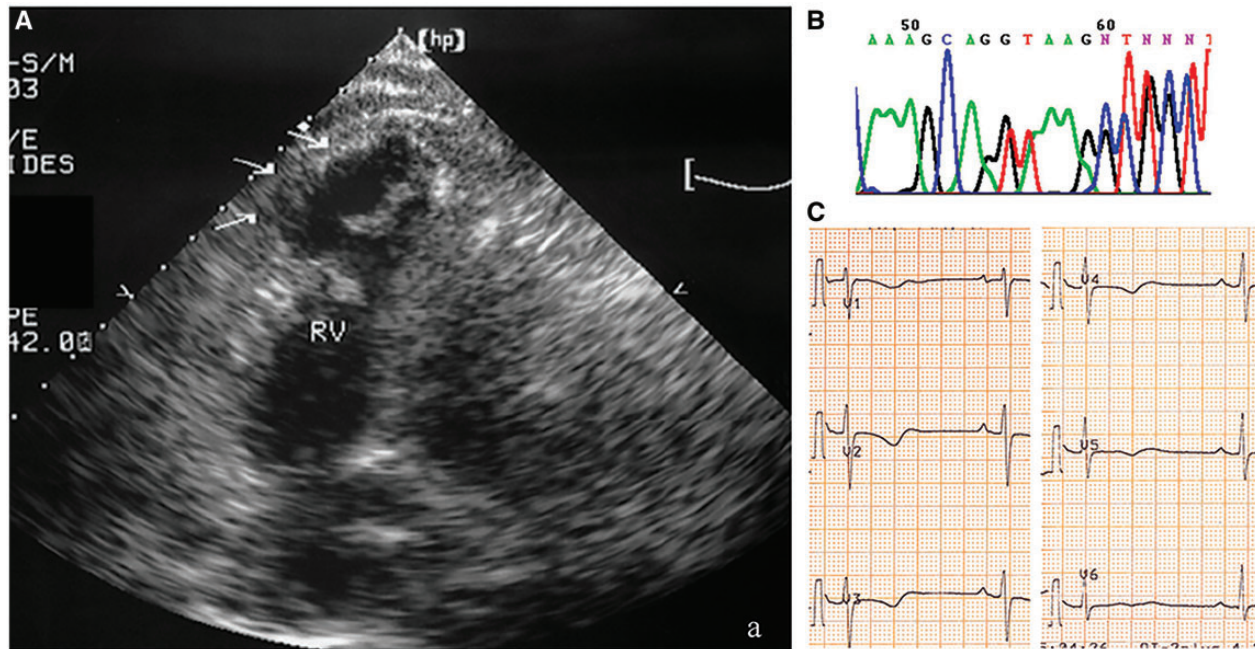


Figure 3 Case 1: (A) Two-dimensional echocardiogram, modified apical four-chamber view showing bulging of the right ventricle (white arrows); (B) 2509delA mutation in *PKP2b* gene; (C) ECG of the proband's father index case showing inverted T-waves in V1–V5.

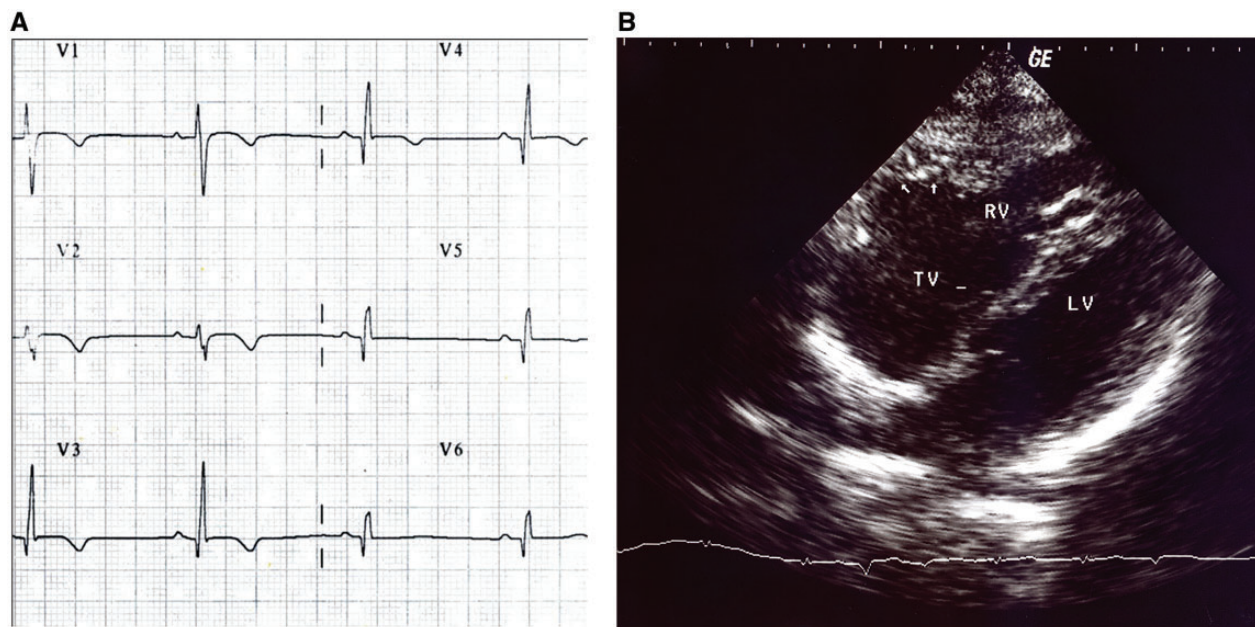


Figure 4 Case 2: (A) Proband victim's ECG showing inverted T-waves in precordial leads V1–V4; (B) Two-dimensional echocardiogram, sub-costal view showing dilation and hypokinetic bulging of right ventricle free wall (white arrows).

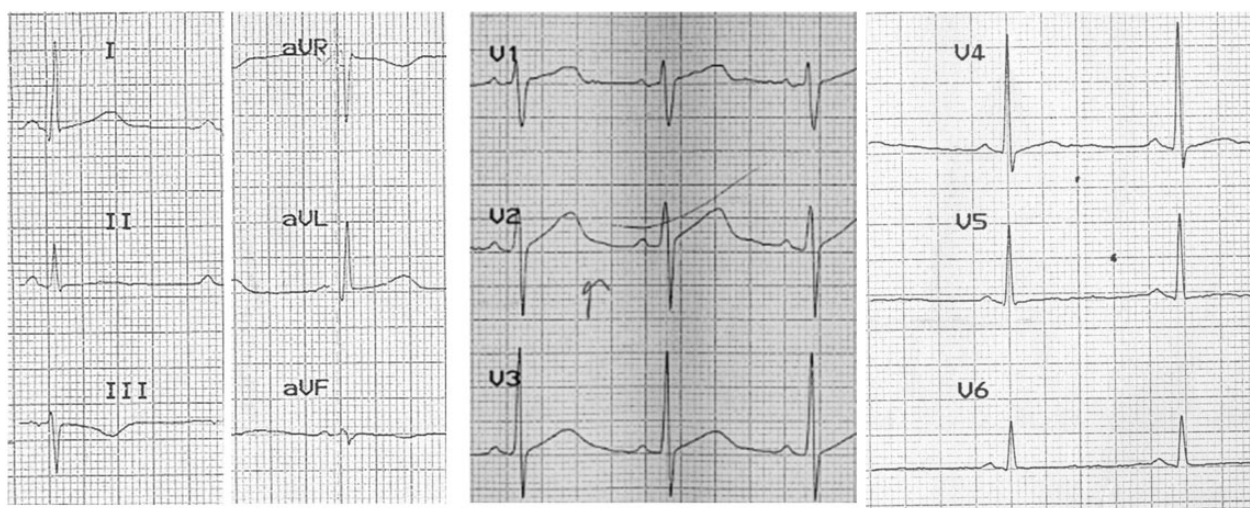


Figure 5 Case 3: (A) Resting ECG of the proband victim showing a QT-corrected interval prolongation of 500 ms.

to a diagnosis of a definite LQTS. Paraffin block was not available, so no testing was conducted in the proband victim's DNA.

Case 4 consisted of a young male that died suddenly at the age of 17. The victim had reported some syncopal episodes following fear or emotional stress. His younger brother had also died suddenly age 13. Their sister, who is still alive, aged 36, has a history of syncopal episodes during emotional stress in her work environment. She had a normal resting ECG, ambulatory ECG monitoring, 2D echocardiography, and her signal averaged ECG was negative for late potentials. During exercise testing, bidirectional ventricular ectopy in couplets–triplets (Figure 6) has been recorded consisting with catecholaminergic polymorphic ventricular tachycardia (CPVT). Targeted genetic analysis for CPVT showed a missense mutation (c.7226T > A) in exon 48 of cardiac ryanodine-2 receptor gene. Genetic analysis in the family has revealed other three carriers of the mutation so far, following a dominant trait.

Case 5 was a 26-year-old male who died suddenly reporting no prior clinical symptoms. His resting ECG was abnormal, showing a marked rightward axis and right bundle branch block (RBBB), although not indicating a specific cardiac disease. Through familial screening, his father was diagnosed with a mild apical HCM. Repolarization abnormalities were recorded in leads V3–V6 in the father's resting ECG (Figure 7) and 2D echocardiography showed left ventricular apical hypertrophy. His coronary angiography was normal. Genetic analysis identified a heterozygous missense mutation (c.240 + 4481C > T) in exon 1 of *TPM1* gene. Cascade genetic screening revealed another three asymptomatic carriers of the mutation so far. The mutation was confirmed also in the proband victim's DNA.

Discussion

The aim of this study was to determine the incidence and the causes of SD in the young and the diagnostic yield of clinically guided genetic

screening in the assessment of SADS. Genetic screening, driven by clinical findings, had a significant diagnostic yield and identified families that would have been missed by arrhythmia syndrome-focused molecular autopsy.

Incidence and causes of SD in the young

A regional-based clinicopathological study of SD in a young population of 12 750 000 people-years is presented. The clinical evaluation of the families gave us a useful diagnostic tool in order to determine whether there is inherited cardiovascular disease (diagnosed or suspected) and guided the genetic investigation in the family. The identification of the specific mutation or mutations in the DNA of the victim helped confirm the suspected cause of SCD. Conversely, the identification of other affected family members and mutation-carriers gave us the opportunity to organize the task of secondary prevention programmes. Relatives with a definite inherited disease were given the appropriate treatment, whereas the identification of asymptomatic mutation carriers justified the need for their constant follow-up.^{12,13,14} Relatives with a negative clinical/genetic testing were reassured that they have a low risk of SD. In this way, SADS families held an essential, dominant and dual role in the evaluation of a sudden unknown-cause death, being both the subject and the object of the study.

In agreement with previous studies in unselected populations,^{15,16} the majority of SD in individuals <35 years in Attica was of cardiovascular origin (65%). Atherosclerotic CAD with coexisting family history of CAD was the leading cause of SD in the 20–35 years' subgroup, whereas HCM and congenital heart disease dominated the ages below 20 years old. The incidence rate of SCD was found to be at least 1.8 per 100 000 person-years.

Considerable disparities in the findings of the major preceding studies of SCD reflect different approaches to inclusion and exclusion criteria, age limit used, genetic or local disparities and selection of specific groups of victims as competitive athletes or military recruits. Most importantly, no specific methodology for pathological

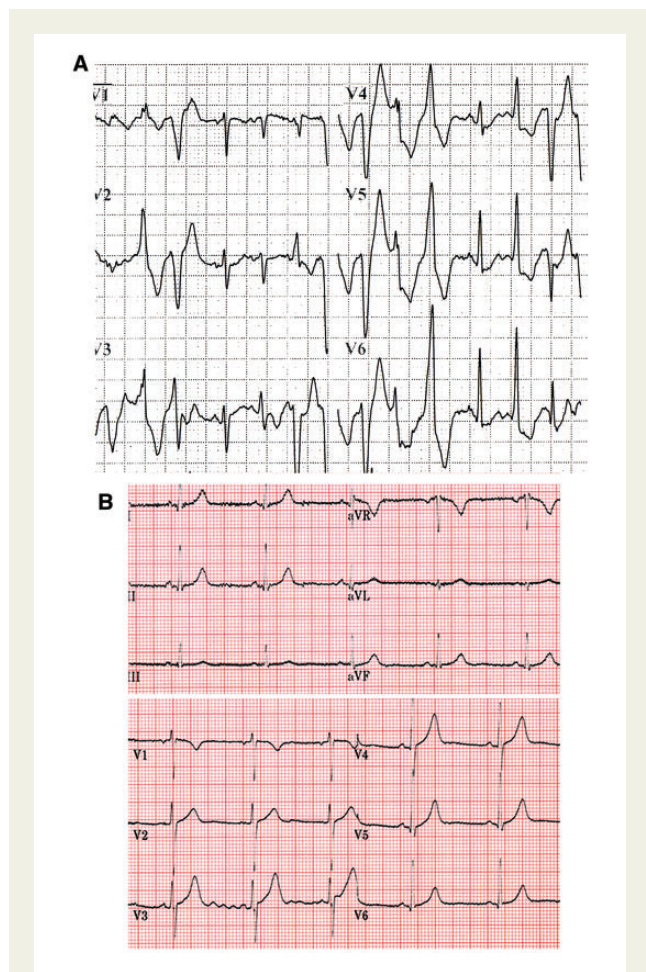


Figure 6 Case 4: (A) Exercise testing ECG of the index case recording bidirectional ectopic couplets–triplets; (B) Normal resting ECG of the same person.

examination was used, ranging from expert examination to no examination.¹⁷ In our study, all the proband victims underwent a routine pathological examination.

We identified an 18% rate of SADS in the region. Mean population of the region remained stable during the study allowing for a relatively accurate estimation of the rates reported. Variable SADS rates were found in the major preceding studies of SCD (6–35%).^{1,17–19} van der Werf *et al.*¹⁷ while reviewing the main clinicopathological series of SD in the young reported an SADS rate of about 18%, a rate that agrees to our finding. Unlike the findings of larger studies from the Netherlands and the UK,^{1,3,4} the dominant cause found in our study was structural heart disease. The limited number of families included, the characteristics of the cohort (only 20% of the investigated SADS deaths occurred during sleep), and comparatively low routine use of provocation testing with a sodium channel blocking agent could explain this disparity. However, it also reflects true findings behind consecutive SADS clinical evaluation in a non-expert cardiac pathology centre cohort where subtle pathological abnormalities can be more easily missed during autopsy. Non-specific changes of uncertain

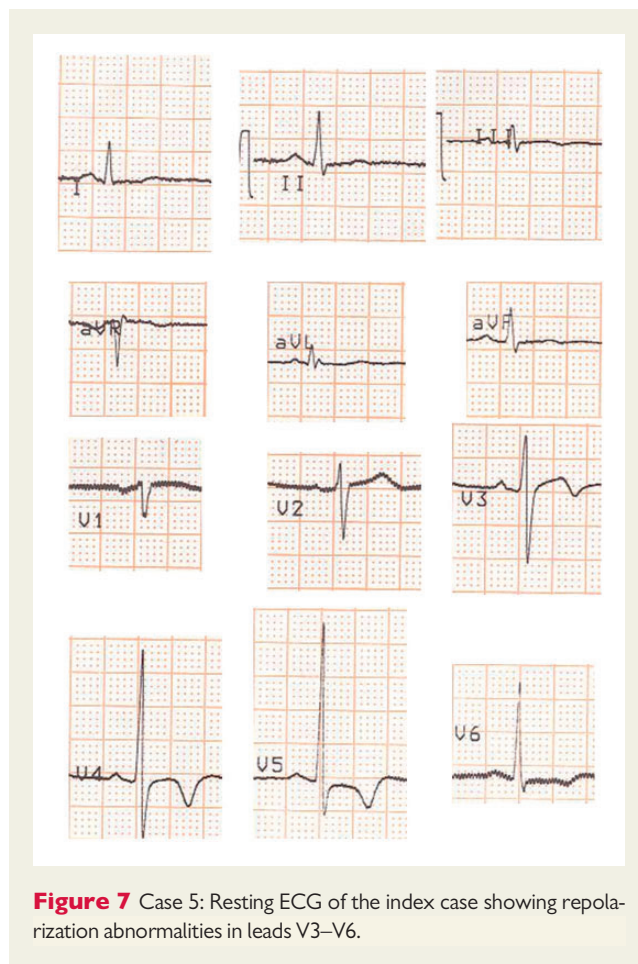


Figure 7 Case 5: Resting ECG of the index case showing repolarization abnormalities in leads V3–V6.

clinical significance or minor pathological findings after a cardiac autopsy are common even in expert pathology centres,²⁰ allowing for erroneous interpretation. In our study, real-world consecutive cases of unexplained death from routine pathological examination were included. The centres that performed the post-mortem analysis are scientifically high level, but they are not special centres for cardiovascular pathology analysis.²¹ All the families included were collected from a region-based population, eliminating a probable referral bias.

Clinically guided genetic screening and molecular autopsy

Genetic testing has been used to an increasing extent as a diagnostic tool in the study of SADS.^{10,11} Most recent expert consensus statement noted that comprehensive or targeted ion channel genetic testing (*RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A* genes) may be considered in SADS cases and is recommended if circumstantial evidence points toward a clinical diagnosis of LQTS or CPVT specifically.²² Population-based studies suggest a detection rate for four-gene molecular autopsy up to 15–20%.^{9–11,18,22,23} Molecular autopsy for cardiomyopathies is not recommended because of the expected detection of the disease through the pathology.⁹ However, mutations in genes encoding structural heart disease have been identified in victims of SADS through exome sequencing.²⁴ A recent large population-based study, conducted in Australia and New Zealand,

Table 1 Efficacy of the different methods approaching the SADS families found with an inherited cardiac disease

Cases N:5	Clinical screening 5/5	Clinically guided genetic screening 5/5	Verification in proband victim's DNA 3/5	Molecular autopsy model (if conducted) 0/5
1	ARVC/D	c.2509delA - PKP2 ^a	+	–
2	ARVC/D	2146-1G>C - PKP2 ^b	+	–
3	LQTS	c.202T>C - KCNH2 ^c	–	No paraffin block
4	CPVT	c.7226T>A - RyR2 ^d	–	Bad quality of paraffin block's DNA
5	HCM	c.240 + 4481C>T TPM1 ^d	+	–

^awww.arvcdatabase.info/mutationdetails.aspx?VariantID=7505

^bwww.arvcdatabase.info/mutationdetails.aspx?VariantID=7491, ExAc database entry: (0.00004944 splice acceptor 12:32955491 C/G 2146).

^cwww.fsm.it/cardmoc/hergmut

^dThe variant has not been previously described in any paper, communication, or database, but the available data indicate a mutation highly probably associated with the disease.

identified clinically relevant cardiac gene mutations in 31 of 113 SADS cases (27%) in which genetic testing was performed. Notably, only 10 variants (9%) were found in the four molecular autopsy genes (*RYR2*, *KCNQ1*, *KCNH2*, and *SCN5A*) whereas 20 genetic variants were found when the major, minor, and rare cardiomyopathy genes were analysed.¹⁸ The authors concluded that a thorough clinical evaluation of surviving at-risk family members is strongly recommended and may be supplemented by a molecular autopsy.

Our data, supported by previous similar findings,^{1,17,18} show that inherited structural heart disease is implicated in SADS and can be detected through familial evaluation. In fact, the five presented families would all have been missed if a molecular autopsy model approach had been followed. Genetic analysis was not possible to be conducted in the two proband victims with channelopathies due to lack (Case 3) or poor quality (Case 4) of DNA. In the other three proband victims, a presumed arrhythmia syndrome mutation screening (CPVT, LQTS, and BrS) would have been negative, missing the identification of the inherited subclinical structural disease found via the clinical evaluation of families. Although a direct comparison is not feasible, the molecular autopsy model would have shown a relatively week efficacy in our cohort (Table 1).

Furthermore, the sensitivity of genetic testing of inherited cardiac disease is limited (range 25–75%) even when the clinical diagnosis is definite.¹⁰ It is well established that the presence of a genetic mutation alone cannot provide clinical evidence and especially missense mutations should be interpreted with great caution. Genetic tests must be viewed in most cases by clinicians as probabilistic tests, not binary (positive/negative) tests.¹³ Therefore, this model presents some serious disadvantages for implementation in routine health care that increases further if the high cost of non-targeted genetic testing is also considered. Recently Bai et al.²⁵ showed that the blind/not clinically guided screening of family members of SCD victim on LQTS and BrS genes is largely ineffective and costly.

In our opinion, clinically guided genetic testing through the initial clinical familial evaluation allows the conduction of a decentralized job, using the readily accessible and affordable diagnostic tools of clinical cardiology. The vast majority of the autopsies in the victims of SD are conducted at peripheral forensic centres with a wide spectrum of experience in cardiac pathology. So, in everyday practice, we experience the case of an SADS autopsied in these centres, families with

varied economic status and varied opportunity to access victim's DNA (that is not always in a good quality). Unlike non-targeted genetic analysis, cascade genetic testing in a family where the genetic basis of the disease has already been identified can be considered essentially 100% sensitive.¹⁰ The verification of the presence of the mutation in the victim provides more solid evidence of the cause of SADS and its genetic substrate compared with a mere mutation without any clinical data, completing the puzzle of this devastating event. Efforts for standardization of the autopsy protocol along with the development of more expert pathology centres, closely cooperating with inherited heart disease centres, and clinical genetics must be made.

Advances in next-generation sequencing technologies allow large panels of genes, including the cardiomyopathy genes, to be screened fast and at reduced cost.^{11,18} Thus, molecular autopsy could provide critical insights in the pathogenesis of SADS by identifying novel loci and genes involved in arrhythmogenesis, unveiling the complex, heterogeneous, and currently largely unknown genetic architecture of SADS. Whole-exome sequencing however comes with the caveat of the identification of a large number of genetic variants of unknown significance that need to be interpreted with caution, given the dramatic consequences of potential 'false-positive results'. In addition, co-segregation studies are not always easy to be performed, given the by-definition absence of any phenotype in the proband victims, small family sizes, and the expected variable expressivity and incomplete penetrance of inherited cardiac disease. Further large-scale, prospective population studies and international collaborations involving comprehensive genotype–phenotype correlation and analysing the cost-effectiveness of different and extended genetic approach are needed in order to shape future strategies in the genetic assessment of SADS victims. Until then, genetic testing must be pursued as a complementary and supportive method, driven by initial clinical findings.

Limitations of the study

A main limitation of the study was the small number of families with SADS examined. Insufficient guidance from general practitioners and/or forensic scientists, cost of movement to our centre along with cost of medical examinations, and unwillingness of families from specific regions due to fear of stigmatization constituted the main reasons for poor SADS families' recruitment. Moreover, our protocol

used provocation testing with a sodium channel blocking agent with standard lead positioning only upon clinical suspicion of BrS, which potentially led to the absence of BrS in this cohort. A more aggressive approach of provocation testing may have increased our diagnostic yield.

In addition, the use of real-world pathology examination possibly overestimated the incidence of sudden unexplained death leading to a reduced accuracy in the initial diagnosis. The support of expert cardiac pathologists could have improved the detection of subtle disease in the autopsy and therefore the accuracy of the diagnosis. However, special cardiac pathology centres may not be readily accessible to all. Conversely, the exclusion of drowning victims and victims with epilepsy could have concealed possible cases of unexplained death due to channelopathies.

The authors did only assess death certificates on an individual basis in the 349 SD cases, potentially resulting in inaccuracies arising from errors related to the medical diagnosis, the selection of the main cause of death, and the coding of the cause of death in the mortality data. The authors also acknowledge that few SD cases possibly did not undergo an autopsy, despite established medico-legal regulations. It is however plausible to assume that the majority of SDs were eventually autopsied given their youth and the promoting for autopsy legal framework in Greece.

Conclusion

In conclusion, SD in the young is of cardiovascular origin in the majority of cases (65%). A considerable rate of SD cases in the young remains of unknown cause on post-mortem (18%). Apart from channelopathies, subclinical forms of inherited structural cardiovascular diseases may cause SADS. Clinically guided genetic screening has a significant diagnostic yield (at least 25%) and seems to identify cases that through an arrhythmia syndrome-focused molecular autopsy, *per se*, would have been missed. The development of inherited cardiovascular diseases' centres, studying the SD victims' families, could give us the opportunity to identify the cause of SADS and enable secondary prevention in the affected relatives in a wider area with more reliable results, combining the existent clinical and genetic tools.^{4,6}

Supplementary material

Supplementary material is available at *Europace* online.

Conflict of interest: none declared.

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