ORIGINAL ARTICLE

Sudden cardiac arrest in patients without overt heart disease: a limited value of next generation sequencing

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KEY WORDS

arrhythmia, cardiac arrest, diagnosis, genetics, next generation sequencing, outcome

ABSTRACT

INTRODUCTION Unexplained sudden cardiac arrest (SCA), occurs in up to 10% of patients and is often attributed to an inherited arrhythmia syndrome. Family screening and genetic testing may help clarify the cause of unexplained SCA.

OBJECTIVES We aimed to assess the usefulness of clinical evaluation and genetic testing in patients after unexplained SCA and in their families.

PATIENTS AND METHODS In the years 2014–2017, we studied 44 unrelated patients after unexplained SCA and 96 of their relatives. All patients and relatives underwent comprehensive cardiac evaluation. In 31 patients with SCA, next generation sequencing (NGS) was performed. The Kaplan–Meier survival curve was constructed to compare the event-free survival depending on clinical diagnosis or genotype. An adverse event was defined as an adequate implantable cardioverter-defibrillator discharge.

RESULTS Based on the clinical evaluation, diagnosis was established in 39% of probands (long QT syndrome, 21%; short QT syndrome, 7%; Brugada syndrome, 7%; catecholaminergic polymorphic ventricular tachycardia, 2%; and early repolarization syndrome, 2%). Ventricular arrhythmia was identified in the relatives of 19% of probands. In 18 of the 31 probands (54.8%), 23 rare gene variants were identified, of which only 2 were classified as pathogenic. The event-free survival over a median of 4.5 years was similar in patients with or without clinical diagnosis and in carriers and noncarriers of a rare genetic variant.

CONCLUSIONS This study shows the significance of an extensive clinical assessment in unexplained SCA survivors and their relatives. Routine genetic testing by NGS has low diagnostic and prognostic value.

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INTRODUCTION Sudden cardiac arrest (SCA) usually occurs in relation to coronary artery disease or structural heart disease. Epidemiological data show that 5% of all cases of SCA occurs in the absence of any form of structural heart disease; this percentage increases to 10% to 12% of cases among patients under the age of 45.[1,2] The major causes of SCA in patients without overt structural cardiac abnormalities include previously undiagnosed inherited arrhythmias, subclinical structural heart disease, or intoxication.[3-5] The CASPER registry (Cardiac Arrest Survivors With Preserved Ejection Fraction Registry)[6] and follow-up data on 200 SCA survivors, free of evidence of coronary artery disease, left ventricular dysfunction, or evident repolarization syndromes demonstrated that advanced testing determined the diagnosis in 34% of patients at baseline, with

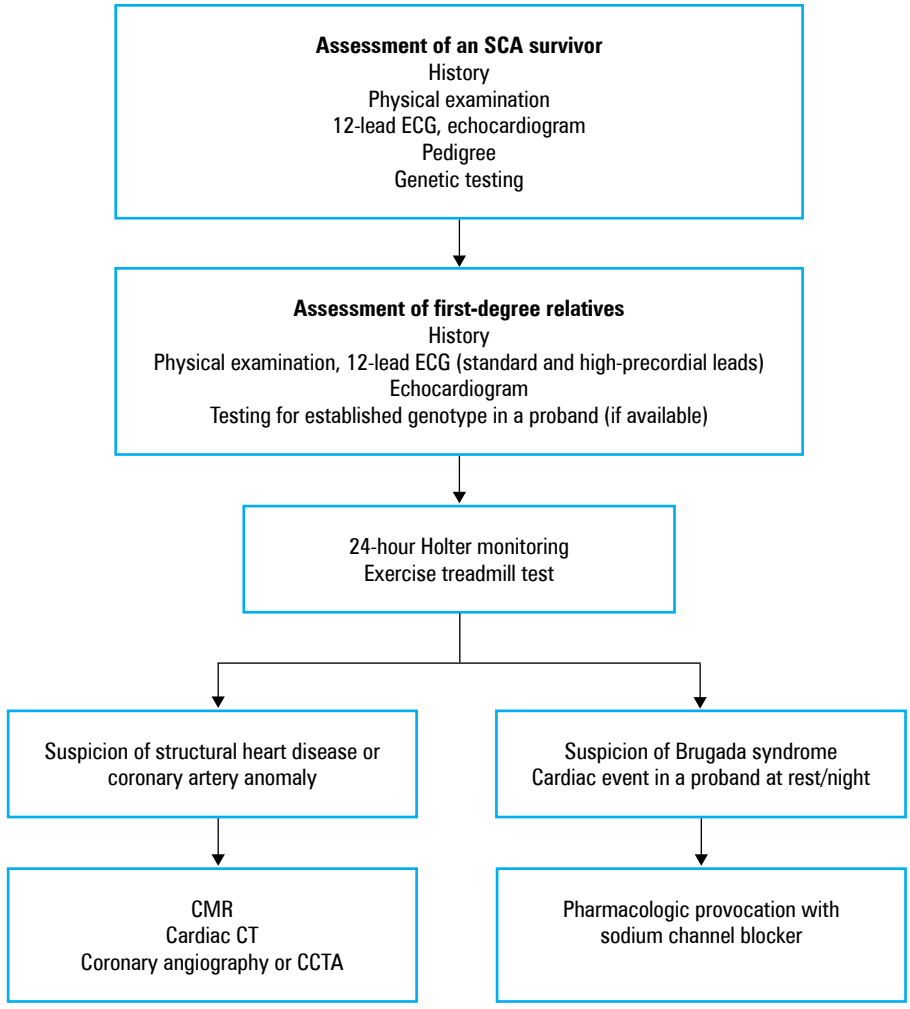

FIGURE 1 Algorithm for screening of patients with sudden cardiac arrest and their families
Abbreviations: BrS, Brugada syndrome; CCTA, cardiac computed tomography angiography; CMR, cardiac magnetic resonance; CT, computer tomography; ECG, electrocardiogram; SCA, sudden cardiac arrest

a diagnosis emerging during a mean follow-up of 3 years in 7% of patients with unexplained SCA. SCA carries a significant cardiovascular risk for first-degree relatives[7] and has a devastating impact on families. Cardiac screening and genetic evaluation in the relatives of families with sudden cardiac death[8] and unexplained SCA may unmask a heritable disorder in a significant proportion of families. The aim of this study was to assess the usefulness of clinical evaluation and genetic testing in patients after unexplained SCA and in their families. We also sought to determine short-term prognosis in the study group.

PATIENTS AND METHODS

Study population

Between January 1, 2014, and December 31, 2016, we enrolled 44 consecutive patients after unexplained SCA and 96 of their relatives (91 first--degree relatives and 5 second-degree relatives).

Definition of unexplained sudden cardiac arrest

Unexplained SCA was defined as occurring in a previously healthy individual with no known history of an inherited cardiac disease, with abrupt and unexpected out-of-hospital loss of consciousness, with loss of vital signs within 1 hour of symptom onset, where resuscitation efforts were successful,[9] and the following conditions had been excluded in follow-up examinations: 1) resting electrocardiogram (ECG) with corrected QT (QTc) >460 ms (men) or >480 ms (women); patients were permitted to have transient QT prolongation immediately after the cardiac arrest if this resolved promptly; 2) presence of a coronary artery stenosis >50% or anomalous coronary arteries; 3) left ventricular ejection fraction <50% that was not transient and related to resuscitation; 4) structural cardiac causes, such as cardiomyopathies, and valvular disease; 5) hemodynamically unstable idiopathic ventricular tachycardia (VT) or hemodynamically stable sustained monomorphic VT with a QRS morphology consistent with recognized forms of idiopathic VT; 6) clinically suspected myocarditis; 7) noncardiac causes, such as pulmonary embolus, cerebral hemorrhage, trauma, or intoxication.

Screening protocol

All probands after unexplained SCA and their families underwent a systematic protocol of screening. The algorithm for cardiac and genetic examination in the studied families is shown in FIGURE 1. A 3-generation family pedigree was drawn; a family history of sudden death and any cardiac disease (eg, cardiomyopathies, pacemaker or defibrillator implantation for conduction system disease), epilepsy, or deafness was noted to identify any potential cause of SCA. Genetic testing was offered to all probands.

TABLE 1 Definition of diagnosis strength

Level of diagnosis strength	Definition
Definite	Diagnosis based on current guidelines[9]
Probable	Any clinical testing positive but not meeting all recognition criteria based on guidelines (for example long QT in 12-lead ECG identified once or in 24-hour Holter monitoring or in exercise treadmill test)

Abbreviations: see **FIGURE 1**

TABLE 2 Clinical characteristics of sudden cardiac arrest survivors (probands) and their relatives at the time of screening (continued on the next page)

Parameter		Probands (n = 44)	Relatives (n = 96)
Age, y, mean (SD)		36.4 (11.7)	40 (16)
Male sex, n (%)		23 (52)	43 (45)
Reported circumstances (total), n (%)		21 (48)	–
Reported circumstances (total), n (%)	Rest/sleep	8 (18)	–
	Exercise	5 (11)	–
	Fever	2 (4.5)	–
	Drugs	3 (7)	–
	Stress	3 (7)	–
First-degree relatives (total), n (%)		–	91 (95)
First-degree relatives, n (%)	Parents	–	32 (35)
	Siblings	–	31 (34)
	Offspring	–	28 (31)
Second-degree relatives, n (%)		–	5 (5)
Premorbid symptoms, total, n (%)		16 (36)	–
Premorbid symptoms, n (%)	Syncope	3 (7)	–
	Presyncope	7 (16)	2 (2)
	Palpitations	10 (23)	19 (20)
SCA, n (%)		–	2 (2)
First documented rhythm, n (%)			
Ventricular fibrillation		34 (77)	–
Short coupled torsade de pointes		3 (7)	–
Other		7 (16)	–
Family history of SUD, n (%)			
≥1 SUD in first-degree relative (age, 1–50 y)		5 (11)	–
Cardiovascular risk factors, n (%)			
Smoking		11 (25)	23 (24)
Dyslipidemia		18 (41)	25 (26)
Systemic hypertension		9 (20)	27 (28)
Obesity		8 (18)	14 (15)
Any other concomitant disease, n (%)			
Endocrinological disease		5 (11)	5 (5)
Psychiatric disease		3 (7)	–
Gastrointestinal disease		–	8 (11)
Diseases of the urinary system		–	7 (7)
Allergy		–	2 (2)
Physical examination, mean (SD)			
Systolic blood pressure, mmHg		120 (15)	127 (19)
Diastolic blood pressure, mmHg		77 (11)	80 (11)
Heart rate, per minute		67 (11)	72 (11)
BMI, kg/m^2		25 (5)	25 (5)

First-degree relatives who provided written informed consent to participate in the study underwent the following protocol: medical history, physical examination, resting 12-lead ECG with standard and high precordial leads, transthoracic echocardiogram, ambulatory 24-hour Holter monitoring, and symptom-limited exercise testing (Bruce protocol). A positive exercise test for long QT syndrome (LQTS) was defined as end-recovery (4 minutes into recovery) QTc >480 ms. The QT assessment was performed according to the maximal slope technique using lead V_5 or lead II.[10]

Further testing was undertaken as indicated by the findings of this initial screening. This included cardiac magnetic resonance imaging to exclude myocarditis,[11] hypertrophic cardiomyopathy (HCM), or arrhythmogenic right ventricular cardiomyopathy (ARVC), if findings suggestive of HCM or ARVC were noted, and the sodium channel blocker provocation testing for Brugada syndrome (BrS) (if clinically suspected). Standard diagnostic criteria were used according to current European Society of Cardiology (ESC) guidelines for the conditions sought (Supplementary material, *Table S1*). The strength of diagnosis was divided into definite diagnosis or probable diagnosis. The definite diagnosis was based on the available ESC guidelines, and the probable diagnosis was made when not all standard criteria were present but any of the clinical tests was positive (**TABLE 1**).

Based on the above criteria, a new clinical diagnosis of a previously unknown primary inherited arrhythmia syndrome in the probands and their relatives was made, whenever possible. When the new diagnosis of a primary electric disorder in first-degree relatives was made, the relatives were offered comprehensive care and cascade family screening.

All patients and relatives signed written informed consent in accordance with the Declaration of Helsinki. The study was approved by the local Bioethics Committee of the Institute of Cardiology (approval no., 1407).

Molecular genetic assessment In 31 unrelated patients with unexplained SCA, we performed next generation sequencing (NGS) (26 had TruSight One panel, involving 4813 genes, and 5 had whole exome sequencing, WES) on an Illumina HiSeq 1500 sequencer. WES sequencing libraries were prepared using TruSeq Exome Enrichment Kit (Illumina, San Diego, California, United States) or Nextera Rapid Capture Exome (Illumina), as described previously.[12] A mutation was considered damaging when located in the coding or splicing region of one of the 53 analyzed genes associated with arrhythmia (Supplementary material, *Table S2*) and 28 genes associated with HCM (Supplementary material, *Table S3*), of a frequency no greater than 0.001 in any of the 3 databases (1000Genomes, ESP, and EXaC), and classified

TABLE 2 Clinical characteristics of sudden cardiac arrest survivor (probands) and their relatives at the time of screening (continued from the previous page)

Parameter	Probands (n = 44)	Relatives (n = 96)
12-lead standard ECG		
Sinus rhythm, n (%)	37 (84)	96 (100)
Heart rate, per minute, mean (SD)	62 (11)	66 (10)
PR duration, ms, mean (SD)	157 (31)	150 (24)
QRS duration, ms, mean (SD)	99 (12)	97 (10)
QTc duration, ms, mean (SD)	417 (37)	405 (31,5)
2-dimensional echocardiography, mean (SD)		
LVDD, mm	51 (5)	49 (6)
IVSD, mm	10 (1.3)	10 (1.6)
PWDD, mm	9 (1.6)	9 (1.5)
RVDD, mm	25 (6)	26 (5)
LVEF, %	62 (6)	64 (6)
Laboratory tests, mean (SD)		
Na, mmol/l	141 (2)	141 (2)
K, mmol/l	4.5 (0.3)	4.4 (0.3)
Mg, mmol/l	0.9 (0.05)	0.9 (0.24)
TnT, ng/l	5.5 (3)	5.2 (2.3)
Treatment, n (%)		
ICD	41 (93)	2 (2)
β-blocker	37 (84)	25 (26)

Abbreviations: BMI, body mass index; ICD, implantable cardioverter-defibrillator, IVSD, intraventricular septum diastolic diameter; LVDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; PWDD, posterior wall diastolic diameter; QTc, corrected QT; RVDD, right ventricular diastolic diameter; SUD, sudden unexplained death; others, see FIGURE 1

as damaging by at least 3 of the algorithms supplied by the ANNOVAR software.[13] Missense variants in the *TTN* gene were excluded due to uncertainty of their clinical importance. A mutation was considered novel when absent from the HGMD database (release 2016.2). Thus, identified variants were followed with Sanger sequencing using a 3130xL Genetic Analyzer (Applied Biosystems, Foster City, California, United States) and BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. The results were analyzed with the Mutation Surveyor 3.30 Software (SoftGenetics, State College, Pennsylvania, United States). Selected rare variants were assessed for pathogenicity according to American College of Medical Genetics and Genomics (ACMG) standards.[14] Once a mutation was identified, screening was offered to consenting relatives. Whenever possible, we looked for segregation in the studied families. We studied the "trios" of parents and the index case to better assess the yield of a sporadic and recessive disease. The Kaplan–Meier survival curve was constructed to compare the event-free survival depending on genotype.

Follow-up was offered to patients and their first-degree relatives with clinical and noninvasive cardiac assessment. Data on adequate implantable cardioverter-defibrillator (ICD) interventions were noted, and the end of follow-up was considered as an adequate ICD discharge or the date December 31, 2017.

Statistical analysis Descriptive statistics for qualitative variables were presented as the absolute numbers and percentages, and for quantitative variables, as means and standard deviation (age) or median and interquartile range (IQR; follow--up time).

The comparison of patient characteristics with and without clinical diagnosis was assessed by the χ^2 test or the Fisher exact test (in case of a minimum expected count lower than 5) for categorical data and unpaired *t* test or nonparametrical Mann–Whitney test for numerical, normally, or irregularly distributed data, as appropriate.

Cumulative event rates (probabilities of an adequate ICD intervention) were estimated by the Kaplan–Meier method. The log-rank test was conducted to verify the homogeneity of the curve and to find any differences in event-free survival between the subgroups.

All hypotheses testing was 2-tailed with $P < 0.05$ type I error. All analyses were performed using the SAS 9.2 software (SAS Institute Inc, Cary, North Carolina, United States).

RESULTS Clinical characteristics of sudden cardiac arrest probands and relatives A total of 44 probands were enrolled into the study (mean [SD] age, 36.4 [11.7] years; men, 52%). The most often documented rhythm during unexplained SCA was ventricular fibrillation (77%); in 18% of probands, SCA was present during rest. The basic characteristics of probands and their relatives are shown in TABLE 2. A definite or probable diagnosis was made in 17 SCA probands (39%; definite in 25% and probable in 14%). The majority was diagnosed with LQTS (n = 9; 21%), followed by short QT syndrome (SQTS) (n = 3; 7%), and BrS (n = 3; 7%). Catecholaminergic polymorphic VT and early repolarization syndrome were present in only 1 proband (2%) each (FIGURE 2). Probands with unexplained SCA with and without diagnosis are compared in TABLE 3. There was no significant difference in clinical characteristics between these groups. The probands with or without a rare variant are compared in Supplementary material, *Table S4*. There was no significant difference in clinical characteristics between these groups either.

Family history was positive in 8 probands (18.2%). Sudden unexplained death was present in 5 probands' families (11.4%) and SCA in another 3 (6.8%). In 7 of the 91 relatives (8%), a probable diagnosis was made. The most frequent diagnoses were the early repolarization pattern (n = 4, 4%), SQTS (n = 2, 3%), BrS (n = 1, 1%), and LQTS (n = 1, 1%). In 1 person, SQTS and the early repolarization pattern were present. In 3 of the 7 relatives (coming from different families) with the probable diagnosis, the clinical diagnosis in the proband was present, while in 4 relatives from 3 families, there was no diagnosis in

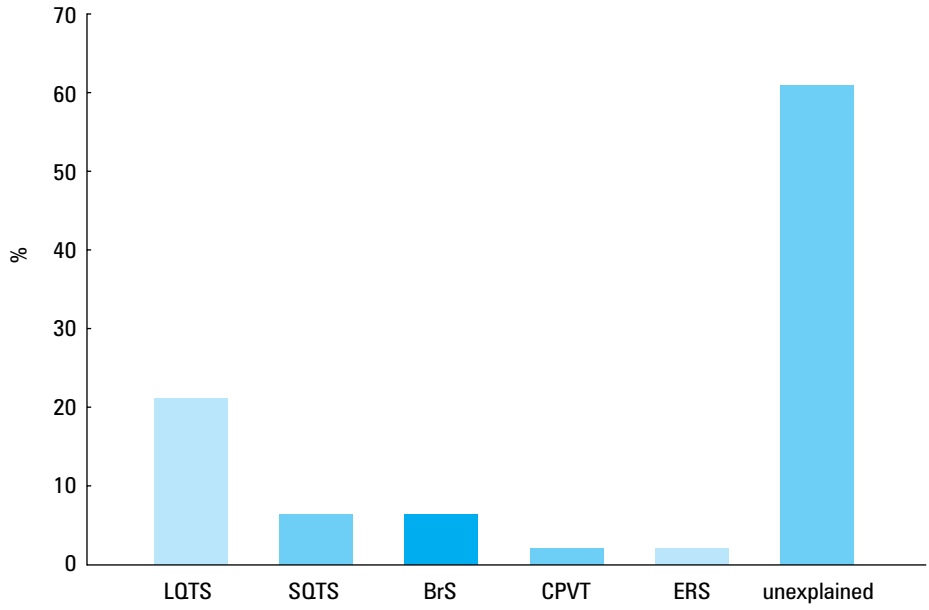

FIGURE 2 Percentage of definite or probable clinical diagnosis in sudden cardiac arrest survivors
Abbreviations: CPVT, catecholaminergic polymorphic ventricular tachycardia; ERS, early repolarization syndrome; LQTS, long QT syndrome; SQTS, short QT syndrome; others, see FIGURE 1

TABLE 3 Comparison of clinical and family data in sudden cardiac arrest survivors with and without clinical diagnosis

Parameter	SCA survivors with diagnosis (n = 17)	SCA survivors without diagnosis (n = 27)	*P* value
Age, y, mean (SD)	34.4 (10.4)	37.6 (12.5)	0.40
Male sex, n (%)	7 (41.2)	16 (59.3)	0.24
Premorbid symptoms			
Palpitations, n (%)	5 (29.4)	5 (18.5)	0.47
Presyncope, n (%)	4 (23.5)	3 (11.1)	0.40
Syncope, n (%)	0	3 (11.1)	0.16
Circumstances			
Rest/Sleep, n (%)	1 (5.9)	7 (25.9)	0.13
Exercise, n (%)	1 (5.9)	4 (14.8)	0.63
Fever, n (%)	1 (5.9)	1 (3.7)	1.00
Drugs, n (%)	1 (6)	2 (7.4)	1.00
Stress, n (%)	1(5.9)	2 (7.4)	1.00
Family history			
SUD in first-degree relative (aged 1–50 y), n (%)	1 (5.9)	4 (14.8)	0.37
SCA in first-degree relative, n (%)	2 (11.82)	1 (3.7)	0.31
Genetic test, n (%)	11 (64.7)	20 (74.1)	0.51
ICD intervention, n (%)	6 (35.3)	8 (39.6)	0.78

Abbreviations: see FIGURE 1 and TABLE 2

the proband. Of note, unexplained ventricular arrhythmia was identified in 9 first-degree relatives in 7 of 32 families (19.4%), 3 of which only had the probable diagnosis.

Cardiac examinations in patients with unexplained sudden cardiac arrest and their relatives The cardiac examinations performed in survivors of unexplained SCA and their relatives are shown in Supplementary material, *Table S5*. In 7 of the 9 probands with LQTS, the diagnosis was made by using 12-lead ECG (78%), and in 2 probands, during 24-hour Holter monitoring (22%). In 2 of the 3 probands with BrS, the diagnosis was made by 12-lead ECG (67%), and in the other proband, by flecainide challenge. The diagnosis of SQTS was made by 12-lead ECG in 2 of 3 cases (67%) and by 24-hour Holter monitoring in 1 case (33%). Therefore, the most valuable clinical test in the group was ECG.

Results of genetic testing Genetic testing identified 23 (19 novel) rare variants in genes associated with arrhythmia (*KCNE1*, *KCNH2*, *SCN5A*, *AKAP9*, *SCN10A*, and *SNTA1*) or cardiomyopathies (*DSP*, *MYH7*, *MYH6*, *MYL2*, *FLNC*, *DES*, *SYNE1*, and *ACTN2*) in 18 of the 31 probands (54.8%), of whom 5 probands had 2 rare variants. Of these, 2 variants in the *FLNC* gene were considered pathogenic (these 2 probands did not have any diagnosis), and the remaining 21 variants were classified as variants of unknown significance. In 2 probands, the genetic diagnosis corresponded with the clinical diagnosis, and in 1 proband without the clinical diagnosis, the genetic test revealed a known rare variant for BrS, yet classified as a variant of unknown significance. The identified variants and their frequency in the ExAC database, together with the assessment of pathogenicity using bioinformatics tools, are presented in Supplementary material, *Table S6*. The pedigrees of the 3 families in which we could trace the significance of 2 rare variants in at least 2 generations are shown in FIGURE 3.

In family SCA013 (FIGURE 3A), the proband, a woman aged 32 years at the time of unexplained SCA, had a probable clinical diagnosis of LQTS identified during 24-hour Holter monitoring. She had 2 rare variants in genes associated with arrhythmia (*KCNH2*) and HCM (*MYL2*). The first came from her mother and the latter from her father, who did not have any cardiomyopathy or electrocardiographic abnormalities suggestive

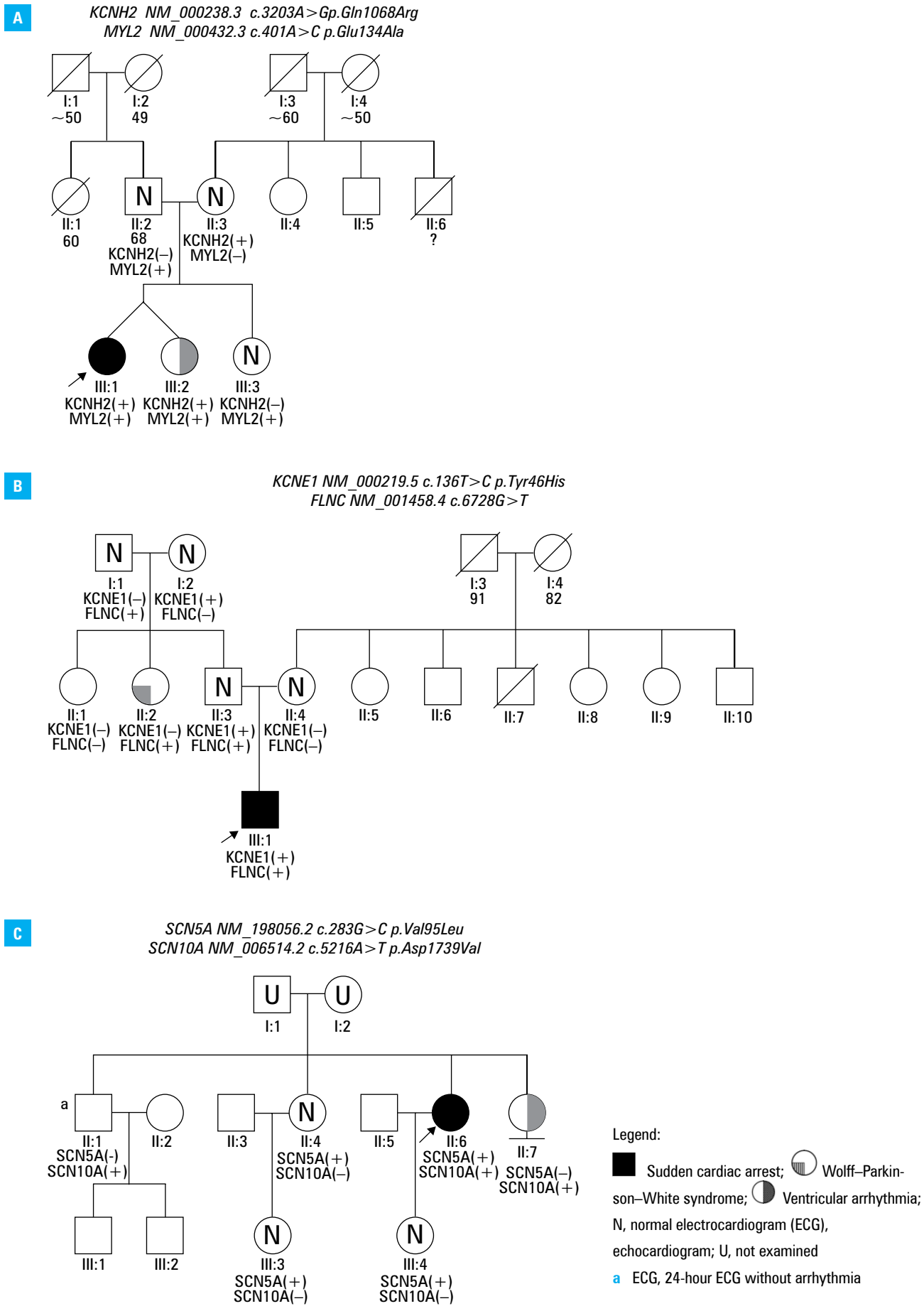

FIGURE 3 Pedigrees of 3 families of sudden cardiac arrest survivors who had 2 rare variants: **A** – family SCA013 with *KCNH2* and *MYL2* variants; **B** – family SCA004 with *KCNE1* and *FLNC* variants; **C** – family SCA031 with *SCN5A* and *SCN10A* variants

of an inherited arrhythmia syndrome at the time of genetic testing (the mother at the age of 56 years and the father at the age of 62 years). The proband's twin sister also had the 2 variants in *KCNH2* and *MYL2* genes. She had complex ventricular arrhythmia (couplets) and received a β-blocker. She did not show long QT either in standard ECG or after provocation with the exercise test or during 24-hour Holter monitoring.

Similarly, in family SCA004 (FIGURE 3B), the proband, a man aged 25 years at the time of unexplained SCA, without clinical diagnosis, also had 2 rare variants, a missense one in the gene associated with arrhythmia (*KCNE1*) and a splicing variant in the gene associated with arrhythmia and cardiomyopathy (*FLNC*). We followed the 2 variants in the antecedent generations. The proband's father (II-3), aged 59 years at the time of the genetic testing, was also a carrier of the 2 variants but did not suffer from SCA and showed no abnormalities on noninvasive cardiac examinations. In the same generation, the proband's aunt (II--2) was treated for WPW syndrome and ventricular arrhythmia. We further managed to trace the variants from generation I by genetic examination of buccal swabs of the paternal grandfather and paternal grandmother and found that these variants alone allowed to reach the age of 90 years (grandfather) and 85 years (grandmother) without a history of heart disease. Thus, in these 2 families, even in the presence of 2 rare variants, the effect of strong environmental factors should be considered.

In turn, in family SCA031 (FIGURE 3C), the proband, a woman aged 47 years at the time of unexplained SCA, had 2 rare arrhythmic variants in the genes *SCN5A* and *SCN10A*. Based on the positive drug test, she was diagnosed with BrS. In her family, 5 relatives were carriers of one variant only, either in *SCN5A* (3) or in *SCN10A* (2). The proband's daughter, aged 16 years, had the rare variant in *SCN5A*. She had a negative drug test for BrS. Both the proband's elder sister (II:4) and her daughter (III:3), with the variant in *SCN5A* alone, did not have features typical for BrS on standard ECG and did not have any arrhythmic events. The provocative drug test is planned. The proband's younger sister (II:7), with the rare variant in *SCN10A*, aged 45 years at the time of the genetic testing, had unexplained complex ventricular arrhythmia, and her brother, with the rare variant in *SCN10A*, was an asymptomatic carrier at the age of 53 years with normal ECG and 24-Holter monitoring. In this family, the most probable coexistence of these 2 rare variants contributed to the occurrence of SCA in the proband. The role of the *SCN10A* variant alone is unclear.

Follow-up In the whole group, the median follow--up was 1772 days (IQR, 1084–2329 days). During this period, 14 patients (31.8%) had adequate ICD discharge. The Kaplan–Meier survival analysis did not show any difference in event-free survival between patients with and without clinical diagnosis (FIGURE 4A). Interestingly, we found a trend towards a worse event-free survival in patients who had an absolute value of left ventricular end-diastolic diameter of 55 mm or higher ($P = 0.06$, FIGURE 4B). In the group who underwent genetic testing (n = 31), the median follow-up was 1802 days (IQR, 138–2357 days), and there was no difference in the event-free survival between the groups with or without the identified rare variant (FIGURE 4C). There were also no differences in the event-free survival among patients with clinical diagnosis, those with an identified rare variant, and those with both clinical diagnosis and identified rare variant (FIGURE 4D).

DISCUSSION

In this study, we showed that the diagnostic yield of the comprehensive cardiac examination in the families of SCA survivors (mean age, 36 years; range, 18–50 years) was 39% (17 of the 44 probands). In a study by Van der Werf et al[15] on a young population (age, 1–50 years), the diagnostic yield for the identification of the cause of unexplained SCA event was higher (61%); however, unlike in our study, the authors did not exclude structural or coronary heart disease. Similarly, Kumar et al,[16] based on family screening and genetic evaluation, established a diagnosis in 32 of 52 families with unexplained SCA (yield, 62%), the majority of which had LQTS and BrS. However, the authors also included patients with HCM (n = 6) and ARVC (n = 4), and if the diagnoses had been excluded, the yield would be 42% (22 of 52 families), which is comparable with our results. In the CASPER registry involving both unexplained SCA survivors and sudden unexplained death victims, the cardiac screening revealed abnormalities in 30% of first-degree relatives with a clear working diagnosis in 17%.[17] Similarly, in our study group, the screening of relatives showed abnormalities (unexplained ventricular arrhythmia) in 17.8% of the families; however, a probable diagnosis was made in 8%. The screening of the relatives did not contribute to the definite diagnosis in any of the families.

Genetics Visser et al[18] used NGS with a large panel of more than 200 genes to identify the cause of primary ventricular fibrillation in 33 patients. They found a single likely pathogenic mutation and detected one or multiple variants of uncertain clinical significance in 15% of patients.

Mellor et al[19] performed genetic testing (single or multiple gene panels) in 174 patients from the CASPER registry. The patients were classified as phenotype-positive (n = 72) or phenotype--negative (n = 102). The pathogenicity of the variants was assessed using ACMG criteria.[14] Pathogenic variants were identified in 29 patients (17%; channelopathy-associated genes, 60%, and cardiomyopathy-associated genes, 40%), and 70 variants of unknown significance were identified in 32 patients (18%). Previous syncope and a family history of sudden death were

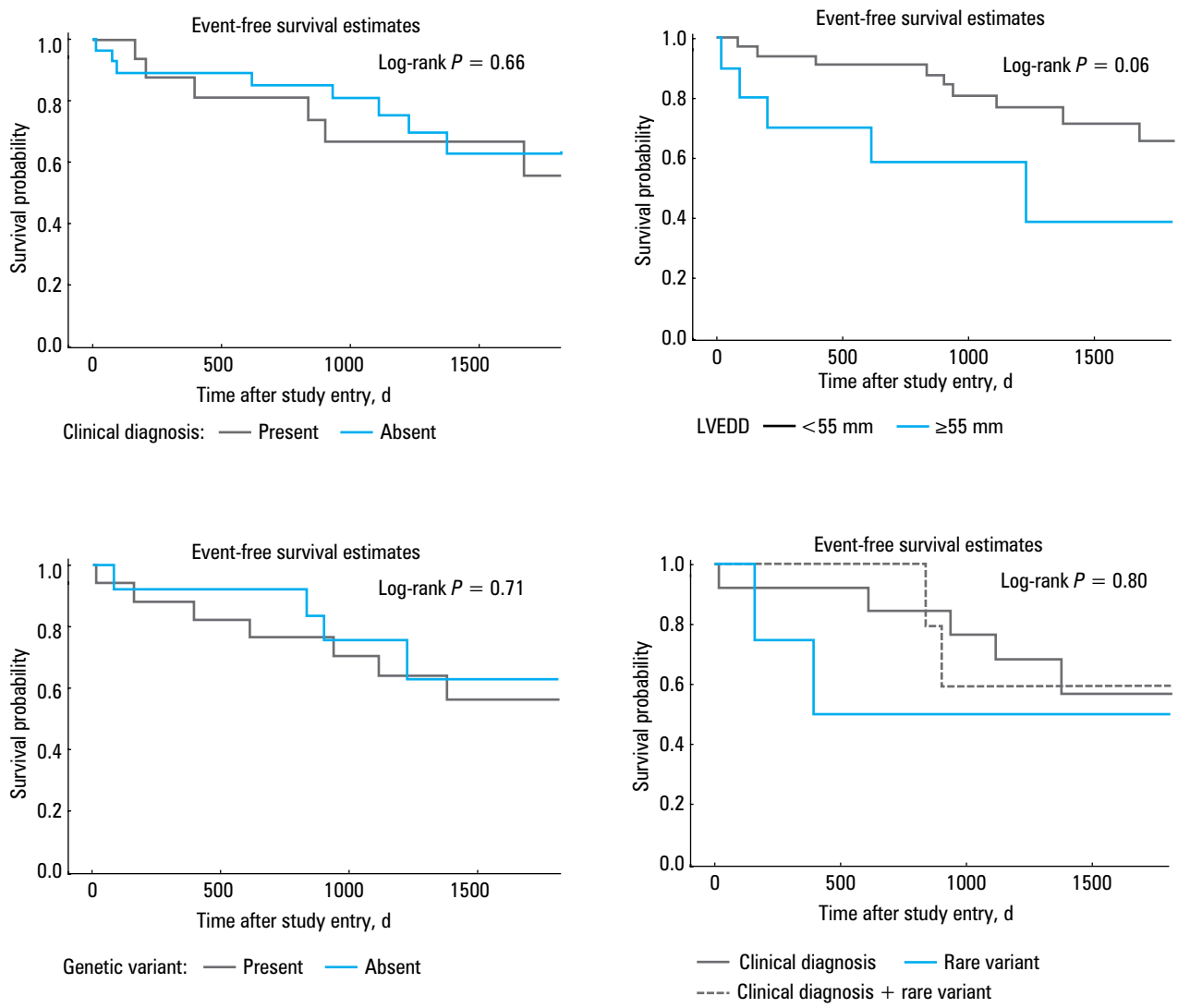

FIGURE 4 Kaplan–Meier survival analysis showing freedom from endpoint (adequate implantable cardioverter-defibrillator discharges) in different group of patients; comparison of survival by log-rank test: **A** – patients with and without clinical diagnosis. Median follow-up in the whole group, 1772 days (interquartile range [IQR], 1084–2329 days); **B** – patients with and without absolute left end-diastolic diameter (LVEDD) ≥55 mm in the whole group; **C** – patients with and without the identified genetic variant. Median follow-up in the group genetically examined, 1802 days (IQR, 1138–2357 days); **D** – patients with clinical diagnosis, patients with the identified rare variant, patients with both clinical diagnosis and the identified rare variant

Abbreviations: see **TABLE 2**

independently associated with the presence of a pathogenic variant. In phenotype-negative patients, broad multi-phenotype genetic testing showed more variants of unknown significance (55% vs 5% in phenotype-positive patients; P <0.01).[19]

The only 2 pathogenic variants classified according to ACMG criteria in our series of patients were related to *FLNC* and were found in patients without any specific diagnosis. Our carriers had complex ventricular arrhythmia, left ventricular end-diastolic diameter at the upper limit of normal, and normal left ventricular ejection fraction at the age of 25 years, indicating that progression to dilated cardiomyopathy (DCM) is possible; however, it did not occur in the antecedent generation of the proband with the splice *FLNC* variant. Of note, the proband's father with the coexistence of splice *FLNC* and *KCNE1* variants had normal left ventricular function.

Ortiz-Genga et al[20] studied *FLNC* variants in 2877 patients with inherited cardiovascular diseases by NGS. Twenty-three truncating mutations in *FLNC* were identified in 28 probands previously diagnosed with dilated, arrhythmogenic, or restrictive cardiomyopathies. Of interest, the authors found ventricular arrhythmias in the majority of *FLNC* mutation carriers (82%) and frequent sudden cardiac death (40 cases in 21 of the 28 families). Similarly, Janin et al[21] analyzed a panel of 48 cardiomyopathies-causing genes in a cohort of 222 patients. Truncating variants in *FLNC* were found in 10 DCM patients (4.5%), showing that these variants are important in the pathogenesis of DCM.[21]

In this study, we also investigated the clinical significance of 2 rare variants in 2 or more generations in 2 other families. First, we found a novel variant, p. Val95Leu *SCN5A*, in the BrS family with the concomitant *SCN10A* variant p.Asp1739Val.

Since 1998, when the first mutation in BrS was discovered, *SCN5A* mutations have been found in up to 30% of families with BrS. They are loss-of-function mutations and result in a variety of abnormalities in the sodium channel activity, including failure of expression, alteration in the voltage and time dependence of activation, and accelerated or prolonged recovery of inactivation.[22-24] In turn, Hu et al[25] identified mutations in *SCN10A*, the gene that encodes the Nav1.8 subunit of the neuronal sodium channel. The mutations were reported in 25 of 150 BrS probands (16.7%), and 23 of the 25 probands had overlapping phenotypes. The coexpression of the mutant *SCN10A* gene with wild-type *SCN5A* in HEK cells led to a significant reduction in the sodium channel current and a slower recovery from inactivation, causing a major loss of function of the sodium channel. *SCN5A* and *SCN10A* mutations have been associated with an overlap syndrome with affected patients exhibiting the sick sinus syndrome or complete heart block as well as BrS.[22,25]

In another family, we found the coexistence of 2 rare variants, one related to arrhythmia (in *KCNH2*) and the other to HCM (in *MYL2*), which underlay unexplained SCA in the proband and ventricular arrhythmia in her twin sister. *KCNH2* p.Gln1068Arg has been reported as a polymorphism by Anson et al,[26] who also found that p.Gln1068Arg in *KCNH2* channels inactivated and recovered from inactivation faster than wild channels. In turn, *MYL2* p.Glu134Ala was published first in a series of patients with HCM,[27] and Burghardt and Sikkink[28] showed, in an experimental model of papillary muscle fibers, that the regulatory light chain harboring the mutation p.Glu134Ala is not able to maintain normal isometric force or normal stiffness suggesting that actin binding in contraction is compromised by the p.Glu134Ala mutation. We did not find any evidence of HCM in the sisters. Of note, p.Ile451Met in the desmin gene has been related first[29] to idiopathic DCM, and an elegant study using transgenic mice expressing the mutant desmin (p.Ile451Met) in the cardiac tissue revealed that mutant desmin loses its Z-disc localization but it can still be associated with the intercalated discs, which have an altered architecture, resembling DCM.[30] In our family, we did not find DCM in the 2 generations.

In our series, the follow-up did not show any difference in major adverse cardiac events between probands with and without clinical diagnosis, or between carriers and noncarriers of rare variants. However, this may be related to a small sample size or a relatively short follow-up. Of interest, we found that patients with a greater size of the left ventricle tended to have shorter event-free survival ($P = 0.06$).

In conclusion, our study showed that extensive clinical workup allows a definite or probable diagnosis in nearly 40% of patients without overt heart disease after unexplained SCA. Family screening is important since cardiac abnormalities have been identified in the relatives of nearly 20% of families. With regard to the genetic background, pathogenic variants were found only in 2 of the 31 examined patients (6.5%), thus showing limited diagnostic value of NGS in the study group. Interestingly, we identified different variants of unknown significance in the genes related to arrhythmia or cardiomyopathies; however, the presence of rare variants had no prognostic significance in mid-term follow-up. In addition, our study suggested the role of double heterozygosity underlying the SCA event in a few families.

SUPPLEMENTARY MATERIAL Supplementary material is available with the article at www.pamw.pl.

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CONTRIBUTION STATEMENT MS-W, JS, and ZTB conceived the concept for the study. MS-W, JS, AL, and ZTB contributed to the design of the research. All authors were involved in data collection. ZTB, MS-W, MB, RB, and EKB analyzed clinical data. MR, JP, GT, and RP performed genetic examinations. MR, JP, GT, MS-W, ZTB, and RP analyzed genetic data. IK performed statistical analysis. MS-W coordinated funding for the project. All authors edited and approved the final version of the manuscript.

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