Usefulness of Genetic Testing in Sudden Cardiac Arrest Survivors With or Without Previous Clinical Evidence of Heart Disease

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Genetic testing in survivors of sudden cardiac arrest (SCA) with a suspicious cardiac phenotype is considered clinically useful, whereas its value in the absence of phenotype is disputed. We aimed to evaluate the clinical utility of genetic testing in survivors of SCA with or without cardiac phenotype. Sixty unrelated SCA survivors (median age: 34 [interquartile range 20 to 43] years, 82% male) without coronary artery disease were included: 24 (40%) with detectable cardiac phenotype (Ph(+)SCA) after the SCA event and 36 (60%) with no clear cardiac phenotype (Ph(−)SCA). The targeted exome sequencing was performed using the TruSight-One Sequencing Panel (Illumina). Variants in 185 clinically relevant cardiac genes with minor allele frequency <1% were analyzed. A total of 32 pathogenic or likely pathogenic variants were found in 27 (45%) patients: 17 (71%) in the Ph(+)SCA group and 10 (28%) in the Ph(−)SCA group. Sixteen (67%) Ph(+)SCA patients hosted mutations congruent with the suspected phenotype, in which 12 (50%) were cardiomyopathies and 4 (17%) channelopathies. In Ph(−)SCA cases, 6 (17%) carried a mutation in cardiac ion channel genes that could explain the event. The additional 4 (11%) mutations in this group, could not explain the phenotype and require additional studies. In conclusion, cardiac genetic testing was positive in nearly 2/3 patients of the Ph(+)SCA group and in 1/6 of the Ph(−)SCA group. The test was useful in both groups to identify or confirm an inherited heart disease, with an important impact on the patient care and first-degree relatives at risk.   © 2019 Elsevier Inc. All rights reserved. (Am J Cardiol 2019;123:2031−2038)

Sudden cardiac arrest (SCA) is a common manifestation of a wide spectrum of inherited and acquired cardiovascular diseases, and extracardiac pathologies.[1] Identifying the cause of SCA is essential for proper management of the arrhythmic substrate to allow a personalized approach. The detection of a specific cardiomyopathy or inherited arrhythmia phenotype in a SCA survivor (Ph(+)SCA) allows genetic testing for confirmation of the clinical diagnosis, risk stratification, and eventually specific therapy.[2,3] Phenotype-guided genetic testing has shown a heterogeneous diagnostic yield of 9% to 60% in SCA survivors, depending on the panel and technology used.[4−6] In the absence of a clear cardiac phenotype, the event is referred to as a phenotype-negative SCA (Ph(−)SCA), or idiopathic ventricular fibrillation.[7] Genetic testing in Ph(−)SCA has been discouraged by the expert consensus (class III) to avoid possible overdiagnosis and unnecessary interventions in families owing to potential misinterpretation of innocent bystander variants.[2,7] Thus, currently the knowledge on genetic basis of Ph(−)SCA is limited. In this study, we sought to evaluate the clinical and genetic characteristics of an unselected cohort of SCA survivors referred for genetic testing from different regions of Switzerland.

Methods

In this partly prospective and partly retrospective study we included SCA patients referred to the Bern University Hospital for genetic testing between January 2014 and May 2018. SCA was defined as an unexpected circulatory arrest

with cardiovascular collapse and documented hemodynamically unstable sustained ventricular tachycardia (VT) or ventricular fibrillation, restored by cardioversion or defibrillation, as appropriate. Internationally accepted criteria were used to diagnose/exclude specific diseases. Initial diagnostic workup included personal and family history, 12-lead resting electrocardiography, echocardiography ,and coronary angiogram/cardiac computed tomography, exercise stress test (whenever possible); additional examination with cardiac magnetic resonance imaging, electrophysiological study, or provocative testing were performed when considered clinically indicated. Patients with coronary artery disease, myocarditis, sarcoidosis, systemic diseases affecting the heart, secondary cardiomyopathies, or reversible causes of SCA were excluded from the study. Survivors of SCA with any suspicious or robust cardiac phenotype (Ph(+) SCA) and those with no identified phenotype (Ph(−)SCA) were included in this study. This study was conducted in full agreement with the principles outlined in the Declaration of Helsinki. The study protocol was approved by the Ethical Commission of the Canton Bern.

Genomic DNA was extracted from ethylene diamine tetraacetic acid-blood using standard procedures. Clinical exome analysis was performed using next-generation sequencing on MiSeq or NextSeq 500 instrument using either the TruSight One or TruSight One expanded Sequencing Panel (Illumina, San Diego, California), which provide comprehensive coverage of exons and flanking intronic sequences of 4,813 and 6,794 clinically relevant genes, respectively. The intronic coverage comprised at least 30 to 50 bp of each intron; the coverage depth of exons was minimum 30x. After sequencing, variants in 185 genes associated with hereditary cardiovascular diseases were analyzed (Supplementary Table 1). Read alignment and local realignment of indels were performed using CLC Genomic Workbench v7.5.1 or CLC Biomedical Genomic

Workbench v5.0 (Qiagen, Redwood City, California). Novel or putative disease-associated variants were analyzed using the following filtering criteria: protein's primary structure, species conservation of the affected amino acid and an allele frequency <1% based on the 1,000 Genome Project database. Exonic variants below 1% frequency in the Exome Aggregation Consortium browser, including +/− 8 bp flanking intronic variants, were further analyzed. For titin (TTN), only radical mutations (i.e., nonsense, frameshift, and splice-site mutations) were considered as putative pathogenic variants. The final interpretation of variants was performed according to the guidelines established by the American College of Medical Genetics and Genomics.[8]

Categorical variables are presented as numbers and percentages; quantitative variables are presented as median (interquartile range) for non-normally distributed variables. Categorical variables were compared using Chi-Square or Fisher's exact test, as appropriate. Continuous variables were compared using Student's t Test or Mann−Whitney U test, as appropriate. To adjust for multiple testing, we used Bonferroni adjustment and set alpha at 0.005. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0. IBM Corp. All tests were 2-tailed, with a p < 0.05 considered significant.

Results

Sixty unrelated SCA patients (median age 34 [20 to 43] years; male−82%) without previously known heart disease, including 24 (40%) with (Ph(+)SCA) and 36 (60%) with Ph(−)SCA, were enrolled (Table 1). The 2 groups were similar in terms of age at SCA and gender distribution. Data regarding the circumstances of SCA were available in 28 (47%) patients. In 13 (22%) cases the event occurred at the time or shortly after exercise activities and in 15 (25%)

Table 1

Demographic characteristics and genetic test results in sudden cardiac arrest survivors referred for cardiovascular genetic testing, grouped based on the displayed cardiac phenotype

Characteristic	All patients with SCA	Patients with Ph(+)SCA	Patients with Ph(−)SCA	p Value*
No. of probands	60 (100%)	24 (40%)	36 (60%)	0.155
Age at SCA, y, median (IQR)	34 (20-43)	36 (20-46)	34 (20-42)	0.490
Male gender	49 (82%)	21 (87%)	28 (78%)	0.274
Patients aged ≤35 yrs	32 (53%)	12 (50%)	20 (56%)	0.673
Male	27 (84%)	10 (83%)	17 (85%)	0.674
Female	5 (16%)	2 (17%)	3 (15%)	
Genetic test results				
Positive	27 (45%)	17 (71%)	10 (28%)	**0.001**
Male	23 (85%)	15 (88%)	8 (80%)	0.561
Female	4 (15%)	2 (12%)	2 (20%)	
VUS	23 (38%)	5 (21%)	18 (50%)	
Male	18 (78%)	4 (80%)	14 (78%)	0.709
Female	5 (22%)	1 (20%)	4 (22%)	
Negative	10 (17%)	2 (8%)	8 (22%)	
Male	8 (80%)	2 (100%)	6 (75%)	0.622
Female	2 (20%)	0 (0%)	2 (25%)	

Ph = phenotype; SCA = sudden cardiac arrest; SD = standard deviation; VUS = variant of uncertain significance.

* p Values are for binomial test, t Test, Fisher's exact test, or Chi-square test, as appropriate. Chi-square test was used to test for gender differences between phenotype groups in the whole population, as well as in subgroups according to age and genetic test results.

occurred unrelated to sports activities. The proportion of exercise-related SCA cases were similar in both groups (19% vs 25%, p = 0.609). Twelve (92%) out of 13 exercise-related SCA cases occurred in males, but males were not more likely to experience a SCA during exercise, than the females (p = 0.263).

Overall, 32 putative pathogenic variants (14 novel and 18 previously reported) were identified in 27 (45%) out of 60 subjects (Table 2). No variants have been identified more than once in our cohort. Patients with exercise-related SCA had a likelihood of having a positive result of genetic testing (54%) similar to those with exercise-unrelated (40%) or unknown circumstances of SCA (44%; p = 0.747).

In the Ph(+)SCA group, dilated cardiomyopathy (DCM, n = 7) was the most common, followed by hypertrophic cardiomyopathy (n = 4) and arrhythmogenic right-ventricular cardiomyopathy (n = 4). Other phenotypes included left-ventricular noncompaction, catecholaminergic polymorphic ventricular tachycardia (CPVT, n = 2), mitral valve prolapse (n = 2), long QT syndrome (n = 1), short QT syndrome (n = 1), and progressive cardiac conduction disease (n = 1). Twenty-one putative pathogenic variants were identified in 17 (71%) out of 24 Ph(+)SCA patients, including 14 (67%)

mutations in cardiomyopathy-associated genes *(LMNA, TNNT2, TNNI3, TTN, FLNC, PKP2, DSP, JPH2, TNXB, LAMP2, BAG3,* and *CAV3)* and 7 (33%) in cardiac ion channel genes *(RYR2, CASQ2, SCN5A, KCNQ1,* and *HCN4).* In 13 out of 17 (76%) genotype-positive patients a cardiomyopathy and in 4 (24%) cardiac channelopathy was suspected (Figure 1). Sixteen (67%) of 24 Ph(+)SCA patients carried mutations in agreement with the observed phenotype, whereas 1 patient, a 31-year-old male, carried a likely pathogenic variant in the *CAV3* (caveolin 3) gene, which so far has not been associated with the identified DCM phenotype; thus it was considered suspicious but not diagnostic.

Overall, 11 pathogenic or likely pathogenic variants were detected in 10 (28%) out of 36 Ph(−)SCA survivors (Figure 1), which was significantly lower than the 71% diagnostic yield in Ph(+)SCA patients (p = 0.017). Six (17%) subjects carried mutations in ion channel genes *(SCN5A, RYR2,* and *KCNE2),* that could explain the SCA in the absence of phenotype (concealed channelopathies). Additionally, 5 putative pathogenic variants localized in the *RANGRF, ABCC9, TRPM4, DSP,* and *TTN* did not clarify the SCA event and require further functional studies or segregation analysis.
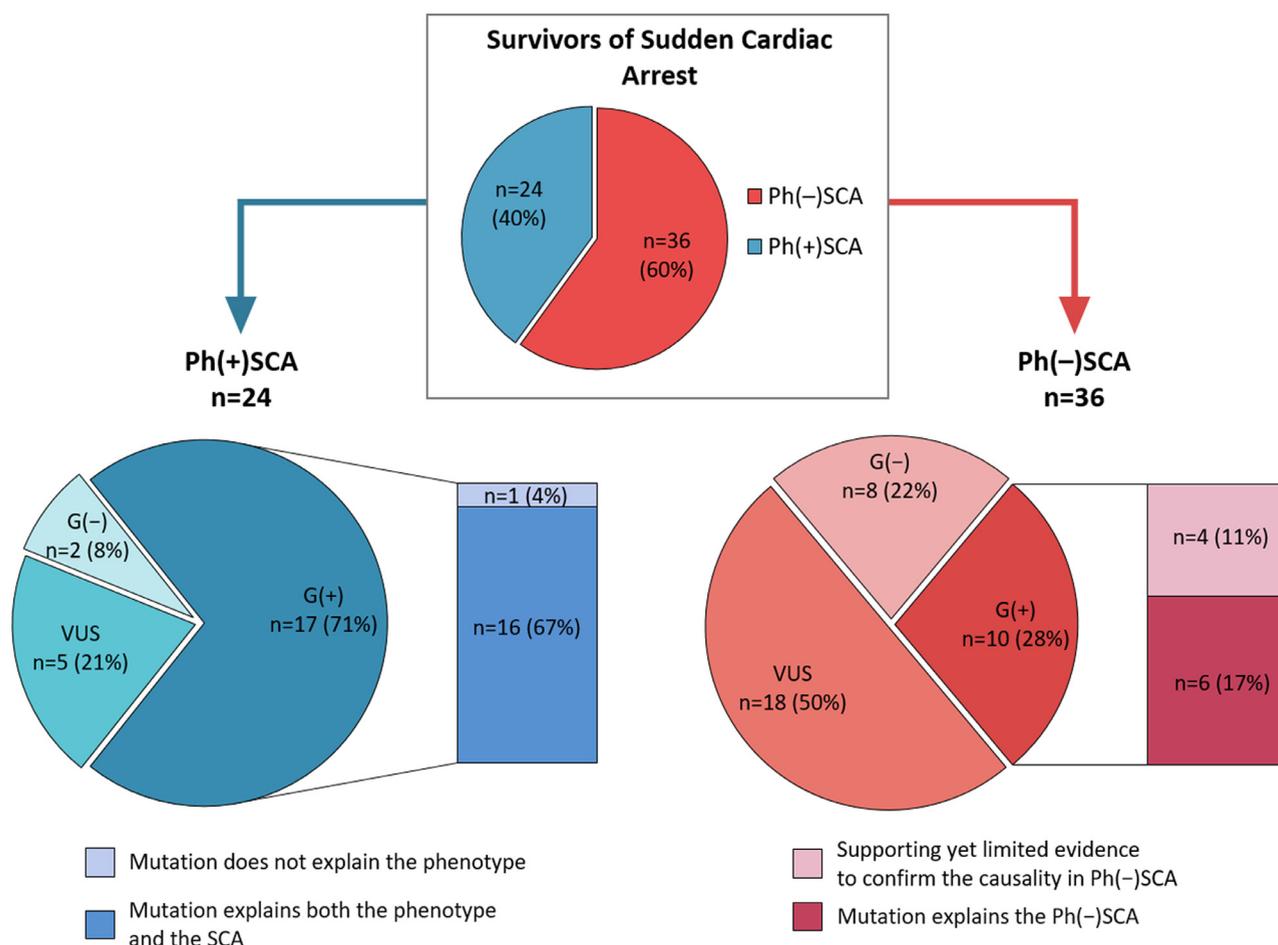

Figure 1. The results and the interpretation of genetic test findings in survivors of sudden cardiac arrest with and without evident clinical cardiac phenotype. G = genotype; Ph = phenotype; SCA = sudden cardiac arrest; VUS = variant of uncertain significance.

Table 2
Summary of clinical and genetic characteristics of sudden cardiac arrest survivors

Case #	Gender	Age at SCA, y	Phenotype category	Cardiac Phenotype	Circumstances of the event	Gene	Reference sequence no.	Nucleotide change	Protein change	Variant type	Homo-/Heterozygous	dbSNP ID	AF in gnomAD (European non-Finnish)	MAF in ExAC	ACMG variant class
1	M	56	Ph(−)SCA	−	exercise unrelated	RANGRF	NM_16492.4	c.198C>A	p.Tyr66*	nonsense	heterozygous	rs1223777741	0.00001758	N/A	LP
						DES	NM_001927.3	c.742C>T	p.Arg248Cys	missense	heterozygous	rs772117708	0.000008920	0.000008	VUS
2	M	17	Ph(−)SCA	−	unknown	SCN5A	NM_001099404.1	c.2441G>A	p.Arg814Gln	missense	heterozygous	rs199473584	0.00003158	0.000018	LP
						SNTA1	NM_003098.2	c.566C>T	p.Ser189Leu	missense	heterozygous	rs144860423	0.0003642	0.0002	VUS
3	F	5	Ph(−)SCA	−	exercise unrelated	RYR2	NM_001035.2	c.6737C>T	p.Ser2246Leu	missense	heterozygous	rs121918597	N/A	N/A	P
4	M	17	Ph(−)SCA	−	exercise-related	ABCC9	NM_020297.3	c.1320+1G>A	p.(?)	splice region	heterozygous	rs139620148	0.00003899	0.000058	LP
5	F	17	Ph(−)SCA	−	exercise-related	RYR2	NM_001035.2	c.365G>A	p.Arg122His	missense	homozygous	rs727503396	N/A	N/A	LP
6	M	47	Ph(−)SCA	−	unknown	RYR2	NM_001035.2	c.1250G>A	p.Arg417Gln	missense	heterozygous	rs751428303	0.00001559	0.000025	LP
7	M	28	Ph(−)SCA	−	exercise unrelated	DSP	NM_004415.2	c.4775A>G	p.Lys1592Arg	missense	heterozygous	rs200421954	0.0003817	0.000185	LP
						TTN	NM_001256850	c.29732-2A>T	p.?	splice region	heterozygous	novel variant	N/A	N/A	P
8	F	34	Ph(−)SCA	−	unknown	TRPM4	NM_017636	c.2387T>C	p.Leu796Pro	missense	heterozygous	rs1191927360	N/A	N/A	VUS
						FKTN	NM_001079802	c.1380dupA	p.Tyr461Ilefs*?	frameshift	heterozygous	rs775366895	0.00004399	N/A	LP*
9	M	38	Ph(−)SCA	−	exercise-related	RYR2	NM_001035.2	c.1346T>G	p.Ile449Arg	missense	heterozygous	rs373331669	0.00001777	0.000008	LP
						DSP	NM_004415.2	c.2462A>G	p.Lys821Arg	missense	heterozygous	novel variant	N/A	N/A	VUS
10†	M	2	Ph(−)SCA	−	unknown	KCNE2	NM_172201.1	c.170T>C	p.Ile57Thr	missense	heterozygous	rs74315448	0.001045	0.0009	P‡
						CACNB2	NM_201593.3	c.410G>A	p.Gly137Asp	missense	heterozygous	rs754596850	0.000008799	0.000008	VUS
11	M	28	Ph(−)SCA	−	unknown	SCN5A	NM_001099404.1	c.3929C>T	p.Pro1310Leu	missense	heterozygous	novel variant	N/A	N/A	VUS
						RYR2	NM_001035.2	c.7570G>A	p.Val2524Ile	missense	heterozygous	rs934248102	0.00003893	N/A	VUS
						TTN	NM_001267550.1	c.1545_1549delAGAAA	p.Lys515AsnfsTer2	frameshift	heterozygous	novel variant	N/A	N/A	LP
12†	M	22	Ph(−)SCA	-	exercise unrelated	KCNQ1	NM_000218.2	c.190_210delCCTGCGT CCCCGGCCGCGCC	p.Pro64_Pro70del	deletion	heterozygous	reported, no dbSNP ID	N/A	N/A	VUS§
						PKP2	NM_004572.3	c.2431C>A	p.Arg811Ser	missense	heterozygous	rs139734328	0.0009214	0.00087	VUS
13	M	19	Ph(−)SCA	−	exercise unrelated	AKAP9	NM_005751	c.10664A>T	p.Asp3555Val	missense	heterozygous	rs139046510	0.001444	0.0009	VUS
						TNXB	NM_019105.6	c.5956A>G	p.Ile1986Val	missense	heterozygous	rs201365475	0.000008873	N/A	VUS
14	F	41	Ph(−)SCA	−	exercise-related	TNNI3	NM_000363	c.349A>G	p.Lys117Glu	missense	heterozygous	novel variant	N/A	N/A	VUS
15	M	28	Ph(−)SCA	−	unknown	DSP	NM_004415.2	c.2723G>A	p.Arg908His	missense	heterozygous	rs142494121	0.0009762	0.0011	VUS
						MYH6	NM_002471.3	c.1138G>A	p.Glu380Lys	missense	heterozygous	rs768924353	N/A	0.000008	VUS
16	M	24	Ph(−)SCA	−	unknown	CTNNA3	NM_001127384	c.2573T>C	p.Leu858Ser	missense	heterozygous	rs41313840	0.0003953	0.0003	VUS
						KCNE1	NM_001270402.1	c.253G>A	p.Asp85Asn	missense	heterozygous	rs1805128	0.01223	0.0092	VUS
17	M	35	Ph(−)SCA	−	exercise-related	KCNH2	NM_000238	c.1653C>G	p.Phe551Leu	missense	heterozygous	novel variant	N/A	N/A	VUS
18	M	56	Ph(−)SCA	−	exercise-related	FLNC	NM_001458.4	c.1616C>T	p.Pro539Leu	missense	heterozygous	rs375570393	0.000	0.000017	VUS
19	M	41	Ph(−)SCA	−	unknown	ANK2	NM_001148.4	c.4799T>C	p.Ile1600Thr	missense	heterozygous	rs764150279	0.00002342	0.000033	VUS
						SCN2B	NM_004588.4	c.356C>T	p.Pro119Leu	missense	heterozygous	rs767589740	N/A	0.000200	VUS
20	M	38	Ph(−)SCA	−	unknown	KCNJ2	NM_000891.2	c.973C>T	p.Arg325Cys	missense	heterozygous	rs202067116	0.00006197	0.000041	VUS
21	M	31	Ph(−)SCA	−	unknown	KCNJ5	NM_000890	c.155G>A	P.Arg52His	missense	Heterozygous	rs144062083	0.00004396	0.000025	VUS
						FBN1	NM_000138	c.1307A>G	p.Tyr436Cys	missense	heterozygous	novel variant	N/A	N/A	VUS
22	M	34	Ph(−)SCA	−	exercise unrelated	SCN10A	NM_006514.3	c.3675T>G	p.Ile1225Met	missense	heterozygous	rs371834340	0.00008519	0.000017	VUS
						DSG2	NM_001943.3	c.1642C>T	p.Arg548Cys	missense	heterozygous	rs550400909	0.00003125	0.000009	VUS
						KCNE1	NM_000219.4	c.314C>T	p.Ser105Leu	missense	heterozygous	rs780041404	0.00002639	0.000091	VUS
23	M	46	Ph(−)SCA	−	exercise-related	RYR2	NM_001035.2	c.11018G>A	p.Arg3673Gln	missense	heterozygous	rs778111237	0.00003552	0.000025	VUS
						SCN1B	NM_199037	c.716C>A	p.Thr239Asn	missense	heterozygous	novel variant	N/A	N/A	VUS
						IDH2	NM_002168.3	c.293T>C	p.Ile98Thr	missense	heterozygous	rs139512088	0.00007035	0.000033	VUS
24	F	57	Ph(−)SCA	−	exercise unrelated	MYH7	NM_000257.3	c.3078G>T	p.Lys1026Asn	missense	heterozygous	novel variant	N/A	N/A	VUS
25	M	48	Ph(−)SCA	−	unknown	MYH6	NM_002471.3	c.4429C>T	p.Arg1477Cys	missense	heterozygous	rs201989347	0.00006166	0.000017	VUS
26	M	33	Ph(−)SCA	−	unknown	KCNH2	NM_000238.3	c.3304A>C	p.Thr1102Pro	missense	heterozygous	novel variant	N/A	N/A	VUS
						KCNH2	NM_000238.3	c.3265A>C	p.Thr1089Pro	missense	heterozygous	novel variant	N/A	N/A	VUS
27	M	24	Ph(−)SCA	−	exercise unrelated	TMEM43	NM_024334.2	c.323T>C	p.Val108Ala	missense	heterozygous	rs182351748	0.000	0.0002	VUS
						MYH6	NM_002471.3	c.982G>A	p.Glu328Lys	missense	heterozygous	rs144422878	0.00007752	0.000058	VUS
28	F	39	Ph(−)SCA	−	unknown	SCN10A	NM_006514	c.1355C>T	p.Ser452Phe	missense	heterozygous	novel variant	N/A	N/A	VUS
						SNTA1	NM_003098	c.692C>T	p.Pro231Leu	missense	heterozygous	rs559396761	0.000	0.000008	VUS
						TNXB	NM_019105	c.12373G>A	p.Glu4125Lys	missense	heterozygous	novel variant	N/A	N/A	VUS
29	M	60	Ph(−)SCA	−	unknown	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
30	M	13	Ph(−)SCA	−	unknown	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
31	F	39	Ph(−)SCA	−	unknown	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
32	M	19	Ph(−)SCA	−	exercise-related	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
33	M	18	Ph(−)SCA	−	unknown	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
34	M	36	Ph(−)SCA	−	exercise unrelated	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35	M	42	Ph(−)SCA	−	unknown	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 2 (***Continued***)

Case #	Gender	Age at SCA, y	Phenotype category	Cardiac Phenotype	Circumstances of the event	Gene	Reference sequence no.	Nucleotide change	Protein change	Variant type	Homo-/ Heterozygous	dbSNP ID	AF in gnomAD (European non-Finnish)	MAF in ExAC	ACMG variant class
36†	F	43	Ph(−)SCA	–	unknown	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
37	F	37	Ph(+)SCA	borderline ARVC	unknown	RYR2	NM_001035.2	c.12665_12667delAGA	p.Lys4223del	deletion	heterozygous	novel variant	N/A	0.000091	LP
38	F	24	Ph(+)SCA	LVNC/DCM	exercise unrelated	LAMP2	NM_002294.2	c.928+1G>T	p.(?)	splice region	heterozygous	novel variant	N/A	N/A	P
39	M	62	Ph(+)SCA	LQTS	exercise unrelated	KCNQ1	NM_000218.2	c.691C>T	p.Arg231Cys	missense	heterozygous	rs199473457	0.00006497	N/A	LP
40	M	15	Ph(+)SCA	suspected CPVT	unknown	RYR2	NM_001035.2	c.8741C>T	p.Thr2914Met	missense	heterozygous	rs766484260	0.00001773	0.000035	VUS
								c.12257G>A	p.Arg4086His	missense	heterozygous	rs752977469	0.000008947	0.000009	LP
41	M	19	Ph(+)SCA	DCM/Laminopathy	unknown	BAG3	NM_004281	c.803C>A	p.Ser268*	nonsense	heterozygous	novel variant	N/A	N/A	P
42	M	69	Ph(+)SCA	possible HCM	unknown	TNNT2	NM_001001430.2	c.860G>A	p.Trp287*	nonsense	heterozygous	rs727504247	0.000	0.000011	P
43	M	41	Ph(+)SCA	PCCD	unknown	SCN5A	NM_198056.2	c.3840+1G>A	p.(?)	splice region	heterozygous	rs1366120635	0.000008801	N/A	P
						CTNNA3	NM_013266.2	c.2097C>G	p.Asn699Lys	missense	heterozygous	rs142942346	N/A	N/A	VUS
						ANK2	NM_001148.5	c.10825C>A	p.Leu3609Ile	missense	heterozygous	novel variant	N/A	N/A	VUS
44	M	45	Ph(+)SCA	LVNC	unknown	HCN4	NM_005477.2	c.1435A>G	p.Ile479Val	missense	heterozygous	novel variant	N/A	N/A	P
								c.1454C>A	p.Ala485Glu	missense	heterozygous	novel variant	N/A	N/A	P
45	M	22	Ph(+)SCA	CPVT	unknown	CASQ2	NM_001232.3	c.872_880delTTGCCCGGG	p.Val291_Arg294del	deletion	homozygous	novel variant	N/A	N/A	P
46	M	23	Ph(+)SCA	HCM	exercise-related	TNNT2	NM_001001430.2	c.275G>T	p.Arg92Gln	missense	heterozygous	novel variant	N/A	N/A	P
47	M	35	Ph(+)SCA	ARVC	unknown	PKP2	NM_001005242	c.148_151delACAG	p.Thr50Serfs*61	frameshift	heterozygous	rs397516997	N/A	N/A	P
48	M	14	Ph(+)SCA	DCM	unknown	LMNA	NM_170707.3	c.1621C>T	p.Arg541Cys	missense	heterozygous	rs56984562	N/A	N/A	P
49	M	14	Ph(+)SCA	HCM	exercise-related	TNNI3	NM_000363	c.485G>C	p.Arg162Pro	missense	heterozygous	rs397516354	0.00005302	N/A	P
50	M	31	Ph(+)SCA	DCM	exercise-related	CAV3	NM_001234.4	c.*6+2T>G	p.(?)	splice region	heterozygous	novel variant	N/A	N/A	LP
						MYH6	NM_002471.3	c.4193G>A	p.Arg1398Gln	missense	heterozygous	rs150815925	0.0005653	0.0004	VUS
51	M	63	Ph(+)SCA	DCM	exercise-related	DSP	NM_004415.2	c.5326G>T	p.Glu1776*	stop-gain	heterozygous	novel variant	N/A	N/A	P
						EYA4	NM_172103	c.1340G>T	p.Gly447Val	missense	heterozygous	novel variant	N/A	N/A	VUS
52	M	31	Ph(+)SCA	DCM	exercise-related	DSP	NM_001008844	c.3562T>C	p.Tyr1188His	missense	heterozygous	rs141508330	0.0001401	0.000067	LP
						FLNC	NM_001127487	c.5199+1G>T	p.?	splice region	heterozygous	rs1465588989	0.00006484	N/A	P
						JPH2	NM_020433	c.1210G>C	p.Ala404Pro	missense	heterozygous	novel variant	N/A	N/A	LP
						TNXB	NM_019105.6	c.10990G>A	p.Val3664Met	missense	heterozygous	novel variant	N/A	N/A	LP
53	M	43	Ph(+)SCA	DCM	unknown	TTN	NM_001256850	c.44440dupA	p.Thr14814Ansfs*7	frameshift	heterozygous	novel variant	N/A	N/A	LP
54	M	9	Ph(+)SCA	possible ARVC	unknown	DSG2	NM_001943.2	c.877A>G	p.Ile293Val	missense	heterozygous	rs2230234	0.08733	0.0687	VUS
						DSP	NM_004415.2	c.6208G>A	p.Asp2070Asn	missense	heterozygous	rs41302885	0.005636	0.0039	VUS
55	M	42	Ph(+)SCA	MVP	exercise unrelated	TNXB	NM_019105.6	c.8734G>A	p.Gly2912Ser	missense	heterozygous	novel variant	N/A	0.0007	VUS
						MYLK	NM_053025.3	c.4169A>C	p.Asn1390Thr	missense	heterozygous	novel variant	N/A	N/A	VUS
						HCN4	NM_005477.2	c.2800C>T	p.Arg934Cys	missense	heterozygous	rs199638465	0.00008081	0.0006	VUS
56	M	40	Ph(+)SCA	HCM	unknown	TMPO	NM_003276	c.689G>A	p.Arg230His	missense	heterozygous	rs758666714	0.00002324	0.000025	VUS
						AKAP9	NM_005751	c.9830T>C	p.Ile3277Thr	missense	heterozygous	rs144021475	0.0001937	0.000008	VUS
						GJD4	NM_153368	c.259C>T	p.Leu87Phe	missense	heterozygous	rs76906304	0.01812	0.013	VUS
57	F	17	Ph(+)SCA	suspected SQTS	unknown	CACNA2D1	NM_000722	c.2264G>C	p.Ser755Thr	missense	heterozygous	rs151327713	0.0006633	0.0008	VUS
58	M	47	Ph(+)SCA	MVP	exercise unrelated	MYH6	NM_002471	c.2383C>T	p.Arg795Trp	missense	heterozygous	rs202120238	0.0002014	0.000074	VUS
59	M	50	Ph(+)SCA	possible ARVC	exercise-related	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
60	M	59	Ph(+)SCA	DCM	exercise unrelated	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

ACMG = American College of Medical Genetics and Genomics; ARVC = arrhythmogenic right ventricular cardiomyopathy; DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy; LP = likely pathogenic; LVNC = left ventricular noncompaction; MVP = mitral valve prolapse; P = pathogenic; Ph = phenotype; SCA = sudden cardiac arrest; SD = standard deviation; SQTS = short QT syndrome; VUS = variant of uncertain significance. N/A denotes 'not applicable'.

* *FKTN*-mediated diseases (DCM or muscular dystrophy-dystroglycanopathy) are transmitted as an autosomal-recessive trait; therefore, the identified heterozygous pathogenic variant is not considered to have a direct link to the SCA.

† Indicates patients who were successfully resuscitated, but died during hospitalization due to neurological complications caused by prolonged cerebral ischemia.

‡ This variant has been initially interpreted as pathogenic; however, the recent evidence suggests contradictory results, and the variant may also be considered as VUS.

§ This variant has been initially interpreted as pathogenic based on the available literature, but has been later reclassified as a VUS after additional in vitro patch clump experiments and segregation studies revealed no evidence of pathogenicity.[25]

Discussion

In a cohort of 60 SCA survivors without previously known heart disease, the cardiac panel genetic testing detected 32 putative pathogenic variants in 27 (45%) cases. In patients with an identifiable cardiac phenotype, the genetic test could explain the phenotype and the SCA in 16 (67%) cases, whereas in those with no phenotype, the genetic test could clarify the cause of the SCA in 6 (17%) cases. Approximately 20% of all patients with SCA were found to have an association with sports activities or physical work.

Our Ph(+)SCA cohort included cases with a wide variety of cardiac phenotypes, which once again highlights the broad spectrum of heritable heart diseases underlying SCA. As expected, the diagnostic yield was 2-fold higher in the Ph(+)SCA group than in the Ph(−)SCA. Interestingly, the diagnostic yield of 71% in the Ph(+)SCA group surpassed the reported general yield for each of the diseases suspected in these patients probably because stronger phenotypes are usually associated with higher diagnostic yield of the genetic test. All but one of the genotype-positive cases of the Ph(+)SCA group, carried pathogenic variants consistent with the suspected heart disease, underscoring that phenotyping remains central to the diagnosis of the cause of SCA. Clarifying the clinical role of the potentially disease-causing variants in genes unrelated to the phenotypes, such as the *CAV3*-variant in a patient with DCM, requires further research and highlights the utility of broader genetic panels for research purposes.

The possible genetic nature of SCA in the absence of measurable heart disease has been first recognized in 2000, when the p.Ser1710Leu mutant sodium channel has been shown to be the likely molecular substrate for Ph(−)SCA.[9] Subsequently, detailed experimental and familial studies have established the causality for the Dutch founder haplotype in *DPP6*,[10] mutations in *CALM1*,[11] *IRX3*,[12] and *RYR2*.[13] Moreover, recent studies on Ph(−)SCA cohorts found mutations in cardiac ion channel genes *(RYR2, KCNH2, KCNQ1, KCNE1, SCN5A,* and *CACNA1C)* and cardiomyopathy-associated genes *(LMNA, TTN, DSP, PLN, DSC2, MYBPC3, MYH7, TNNT2)*, with a diagnostic yield of up to 15%, thus further expanding the spectrum of genes potentially associated with Ph(−)SCA.[3,14] We identified a genetic substrate that explains nearly 1/6 of our Ph(−)SCA cases. More than half of these mutations were located in principal cardiac ion channel genes. This yield is consistent with previous reports,[3,14,15] and highlights that concealed cardiac channelopathies may manifest with malignant arrhythmias even in the absence of a detectable cardiac phenotype.

Four of 6 mutations detected in our Ph(−)SCA cohort were located in *RYR2*, making CPVT the most prevalent diagnosis in this group (11%). Two CPVT patients (#3 and #9) had a history of syncopal episodes preceding SCA with no direct link to exercise. Another patient (#5) had frequent premature ventricular contractions and a nonsustained VT at maximum exercise load, which did not raise a suspicion of CPVT. The fourth patient (#6) had a negative exercise test but experienced a VT at follow-up terminated with an implantable cardioverter defibrillator shock. These data, in line with recent reports,[15] indicate the need for a low threshold to suspect CPVT and to perform a confirmatory genetic test in Ph(−)SCA survivors with equivocal stress test findings.

The remaining putative pathogenic variants affected genes encoding the ion channel TRPM4 *(TRPM4)*, ion channel interacting proteins *(RANGRF, ABCC9)* or myocardial structural proteins *(DSP, TTN)*. Mutations in the TRPM4 channel gene have been implicated in progressive familial heart block, atrioventricular block, right bundle-branch block, and Brugada syndrome (BrS).[16] Although the TRPM4 channel is known to be involved in cellular calcium regulation, its exact role in the cardiac physiology and pathophysiology remains unclear. Loss-of-function *RANGRF* mutations have been reported to cause BrS through impairing the trafficking of Nav1.5 channel.[17] Likewise, gain-of-function mutations in *ABCC9*, a critical gene for the activation and regulation of the K_{ATP} channels, have been shown to result in increased I_{KATP} current and manifest with BrS.[18] Mutations in *DSP* (desmoplakin) have been implicated in arrhythmogenic right-ventricular cardiomyopathy[19] and DCM.[20] Desmoplakin disease is known to cause connexin mislocalization and result in significant alterations in conduction-repolarization abnormalities prior to morphological changes.[21] Radical mutations in *TTN* have been linked mainly to DCM,[22] with overlaps with other cardiomyopathies and are known to confer high arrhythmic potential. Despite the presumed pathogenic role of these variants, their potential causality in Ph(−)SCA is subject to further evaluation in functional and follow-up studies. These patients would therefore need close surveillance for detection of any related phenotype at follow-up.

Our findings are particularly interesting in the context of the current Heart Rhythm Society/European Heart Rhythm Association guidelines, which discourage the use of genetic testing in Ph(−)SCA.[2,7] On the contrary, consideration of genetic testing in sudden arrhythmic death syndrome (SADS) is recommended (IIa).[2,7] The main difference between these 2 groups is that survivors reach the hospital alive and SADS victims to the morgue. Previous studies have found a genetic cause in 25% of SADS cases.[23,24] In such cases, cascade screening reveals a cardiac disease in nearly half of relatives,[6] who can benefit from regular surveillance, lifestyle recommendations, and preventive therapy. Our results indicate that the yield of the genetic test in survivors of Ph(−)SCA (17%) is similar to those in SADS victims. Additionally, our analysis uncovered cardiomyopathy gene mutations in Ph(−)SCA patients, which is in accordance with studies on SADS and requires further studies.[24] Predictive genetic testing in Ph(−) SCA patients' family members can therefore be as useful as in relatives of SADS victims.[2,7]

Similar to previous studies, the use of large gene panels in our study led to high yield of variant of uncertain significance, particularly in the Ph(−)SCA group (47%). The clinical implication of these variants remains elusive and requires further studies. Mutations in minor cardiomyopathy or channelopathy genes, defined as implicated in <5% of disease cases, were identified in 8 SCA patients (13%). These included mutations in *RANGRF, ABCC9, FKTN,*

KCNE2 in patients with Ph(−)SCA and *LAMP2, BAG3, HCN4, CAV3, FLNC,* and *JPH2* in patients with Ph(+)SCA.

Our results suggest that genetic testing is useful in all SCA survivors with and without evidence of cardiac disease. Nevertheless, the test should be performed only by experienced and accredited laboratories to avoid potential erroneous assignment of pathogenicity.[3] Longitudinal prognostic studies of a large cohort of genetically tested SCA survivors and their family members are necessary to determine the potential role of mutation status for risk stratification. The progress in the understanding of the genotype-phenotype associations and the evolving variant classification schemes will optimistically solve the puzzle of uncertain variants and provide more reliable criteria for assessment of pathogenicity.

Our study has several limitations. The number of enrolled patients in the present study was relatively small, an inherent problem in studies conducted on rare diseases. Our cohort included only SCA survivors referred for genetic testing. Although the classification of the variants stringently followed the American College of Medical Genetics and Genomics guidelines, we cannot definitely conclude on their pathogenicity in the absence of segregation or functional studies, although this may be hampered by an incomplete penetrance of the variants. We did not investigate whether a carrier status of a putative pathogenic variant confers a risk for recurrent cardiac events, or whether mutation-carrier Ph(−)SCA patients develop a phenotype at follow-up. Finally, we lack clinical and genetic data of the families, which could help better interpret the impact of many variants.

In conclusion, comprehensive cardiac panel genetic testing identified mutations linked to channelopathies and/ or cardiomyopathies in nearly half of SCA survivors without previous diagnosis of cardiac disease. Genetic testing revealed a putative pathogenic variant compatible with the phenotype in 67% of Ph(+)SCA cases. In the Ph(−)SCA group, genetic test revealed the cause of arrest in 17% of cases, similar to the yield reported in SADS. Majority of mutations identified in Ph(−)SCA patients were associated with channelopathies, most commonly CPVT, whereas in the Ph(+)SCA group, the mutations were mostly located in cardiomyopathy-associated genes. Since genetic testing facilitated the understanding of 1/6 of Ph(−)SCA cases, the test is recommended in all SCA survivors, with or without a cardiac phenotype, as it allows to guide treatment decisions. This study also highlights the need for long-term follow-up studies to examine the prognostic value of genetic findings in SCA survivors.

Disclosures

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2019.02.061.

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