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Does sudden unexplained nocturnal death syndrome remain the autopsy negative disorder: a gross, microscopic, and molecular autopsy investigation in Southern China

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Abstract

Objective—To look for previously unrecognized cardiac structural abnormalities and address the genetic cause for sudden unexplained nocturnal death syndrome (SUNDS).

Methods and Results—148 SUNDS victims and 444 controls (matched 1:3 on gender, race, and age of death within 1 year) were collected from Sun Yat-sen University from January 1, 1998 to December 31, 2014 to search morphological changes. Additional 17 Brugada syndrome (BrS) patients collected from January 1, 2006 to December 31, 2014 served as a comparative disease cohort. The Target Captured Next Generation sequencing for 80 genes associated with arrhythmia/cardiomyopathy were performed in 44 SUNDS victims and 17 BrS patients to characterize the molecular spectrum. SUNDS had slight but statistically significantly increased heart weight and valve circumference compared to controls. 12/44 SUNDS victims (*SCN5A, SCN1B, CACNB2, CACNA1C, AKAP9, KCNQ1, KCNH2, KCNJ5, GATA4, NUP155, ABCC9*) and 6/17 BrS patients (*SCN5A, CACNA1C, P*>.05) carried rare variants in primary arrhythmia-susceptibility genes. Only 2/44 SUNDS cases compared to 5/17 BrS patients hosted a rare variant in the most

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None.

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common BrS causing gene, SCN5A (*P*=.01). Using the strict American College of Medical Genetics guideline-based definition, only 2/44 (*KCNQ1*) SUNDS and 3/17 (*SCN5A*) BrS patients hosted a "(likely) pathogenic" variant. The 14/44 SUNDS cases with cardiomyopathy-related variants had a subtle but significantly decreased circumference of cardiac valves, and tended to die on average 5–6 years younger compared to the remaining 30 cases (*P*=.02).

Conclusions—We present the first comprehensive autopsy evidence that SUNDS victims may have concealed cardiac morphological changes. SUNDS and BrS may result from different molecular pathological underpinnings. The distinct association between cardiomyopathy-related rare variants and SUNDS warrants further investigation.

Keywords

sudden unexplained nocturnal death syndrome; arrhythmia; Brugada syndrome; cardiomyopathy; genetic

Since the initial report in 1917, sudden unexplained nocturnal death syndrome (SUNDS) has been considered an autopsy negative disorder with unknown etiology and describes a distinct subgroup of individuals with idiopathic sudden death.[1] SUNDS prevails preponderantly in Southeast Asia and has multiple academic terms in different nations such as in the Philippines (bangungut),[1] Thailand (lai-tai),[2] Japan (pokkuri),[3] and China (sudden manhood death syndrome).[4] The incidence of SUNDS (per 100,000 people years) has been reported to be as high as 43 in the Philippines[5] and 38 in Thailand.[6] Kampuchea, Laos, and Hmong refugees in the United States were also reported to have a high incidence (59, 82, and 92 per 100,000 people years, respectively) of SUNDS.[7,8] The annual incidence of SUNDS is approximately 1–3 per 100,000 people in Southern China.[4,9]

The definition of SUNDS described a perplexing entity with special clinic phenotype:[1–9] (1) predominantly occurs in Southeast Asia or immigrants from Southeast Asia without a significant disease history; (2) prevails preponderantly in apparently healthy males (>90%); (3) >80% of victims are at the ages between 20–40; (4) occurs during nocturnal sleep with typical symptoms such as moaning and tachypnea which last for just a few minutes prior to death; (5) there is no pathological changes to identify the cause of death; (6) most victims were sporadic; (7) death most frequently occurred out of hospital without any clinical record, giving first access to forensic pathologists rather than the clinicians.

Various hypotheses, such as bacterial infection,[10] potassium deficiency,[11] structural or functional abnormalities of the coronary arteries,[3] and nocturnal sleep respiratory disorders,[7] have been postulated by epidemiological studies on SUNDS but need further confirmation.[4,9] While structural diseases such as cardiac conduction system (CCS) abnormalities and acute hemorrhagic pancreatitis account rarely for the death of a SUNDS victim,[1–3,5–9] the vast majority of cases reported were defined as autopsy-negative.[1,3,4,7–9,12–15]

As a special idiopathic sudden cardiac death (SCD), it differs significantly in clinic phenotype from other primary electric disorders such as long or short QT syndrome (LQTS or SQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT), etc. SUNDS and

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Brugada syndrome (BrS) have been considered to be phenotypically, genetically, and functionally the same allelic disorder.[16] We have previously reported postmortem genetic screening for SUNDS,[4,12–15] but these studies were limited by small autopsy numbers or a relative paucity of candidate genes screened. Thus further studies for the morphological and molecular pathological characterizations of SUNDS are justified.

In order to address whether SUNDS is truly morphologically negative, we performed a case-control study on gross and microscopic findings in the largest number of Chinese SUNDS autopsy cases reported to date. In addition, we conducted a next-generation sequencing based 80 genes targeted analysis on consecutive 44 SUNDS victims and 17 BrS patients to characterize the molecular pathological spectrum of Chinese SUNDS compared to BrS.

METHODS

Study Population

148 consecutive SUNDS cases were collected from January 1, 1998 to December 31, 2014 at the National Center for Medicolegal Expertise at Sun Yat-sen University. The inclusion criteria for SUNDS were as previously reported:[4,9,12–15] (1) an apparently healthy individual older than 15 years and without a history of significant disease; (2) who died of a sudden unexpected death during nocturnal sleep; (3) and had a negative autopsy, toxicology, histology, and death-scene investigation that resulted in their death being unexplained. Cases with (1) obvious disease or pathological changes to explain the death; or (2) a non-natural manner of death (such as suicide, homicide, accident) were excluded.

An additional 444 non-SUNDS death cases were collected from the same autopsy case database and served as the control group. These controls represented individuals with an acute non-disease death within 24 hours caused by traffic accident, mechanical asphyxia, electric shock, and carbon monoxide poisoning and were previously healthy, without any significant disease or pathological changes identified by postmortem examinations. The controls were matched 3:1 with the SUNDS cases. The matching criteria were: (1) gender and race identical to the paired case; (2) age of death within 1 year; (3) interval of death date within 3 months.

The consecutive 17 BrS patients during January 1, 2006 to December 31, 2014 from the Department of Cardiology at the First Affiliated Hospital of Sun Yat-Sen University were collected and designed as a comparative disease cohort for the genetic screening with the SUNDS victims. The inclusion criteria for BrS in this study were patients with: (1) a basal ECG showing a BrS type I pattern, (2) and at least one clinical criterion (documented ventricular arrhythmia, family history of SCD or BrS, and/or symptoms secondary to arrhythmia), (3) and no structural heart disease.

Informed consent was obtained from the patients or legal representatives of the victims. The principles outlined in the Declaration of Helsinki were followed. The project was approved for human research by the ethics committee of Sun Yat-sen University.

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Case-Control Study on Autopsy Findings

All death cases had toxicology screening, gross and microscopic autopsy as well as CCS examinations. The collected autopsy findings included gender, age, height, time of death, the investigation record of death scene, and the macroscopic and microscopic examinations of vital organs. The pathological diagnosis for each case was confirmed by at least two forensic pathologists independently.

Molecular Genetic Analysis

Molecular autopsy investigation based on target captured next generation sequencing technology was conducted in the consecutive 44 (collected from January 1, 2010 to December 31, 2014) out of 148 SUNDS cases. This molecular analysis was also performed on 17 BrS patients. The genomic DNA from the blood samples was extracted and quantified, and the target DNA was enriched and sequenced as we previously reported.[17] The alignment was performed using BWA version 0.7.12-r1039[18,19] with hg19 reference, and then applied GATK[20] according to the Best Practices recommendations.[21] Finally, variant calls were annotated for allele frequency and functional effect using publicly available databases.

A total of 80 genes associated with primary arrhythmia- and cardiomyopathy-related disorders were investigated as we described previously.[17] More than 94% of the target base pairs (exon and 10bp of adjacent introns) were covered at least 10×.

Only genetic rare variants leading to non-synonymous amino acid changes (missense, nonsense, frame-shift insertion/deletions, in-frame insertion/deletions, or splice-errors) and with a minor allele frequency (MAF) <.01 observed in any ethnic group among four population databases including the National Heart, Lung and Blood Institute Grand Opportunity (NHLBI GO) Exome Sequencing Project (n=6,503), the 1,000 Genome Project (n=2,504), Exome Aggregation Consortium (ExAC, n=60,706 all ethnicities, n=4,327 East Asian) and a local database (n=2,087, 989 of whom were Chinese, with normal phenotype) were considered for further analysis.

Rare non-synonymous variants were characterized according to the strict variant interpretation guidelines outlined by the American College of Medical Genetics (ACMG).[22] To be considered “pathogenic” or “likely pathogenic”, the variant must first be absent among all aforementioned control population databases. Each variant was then scrutinized for additional supporting evidence for pathogenicity as outlined in the ACMG guidelines[22]. Some of the molecular/functional lines of evidence for pathogenicity were specified in the online-only text in the Supplement. The remaining rare variants were categorized as variants of uncertain significance (VUS). All variants reported were confirmed by Sanger sequencing.

Statistical Analysis

The data were presented as “mean ± SD” for continuous variables or as frequencies and percentages for categorical variables. The continuous variables were examined accordingly with normal distributions and were then compared using the unpaired Student's *t*-test for normal distributions and the Mann-Whitney *U*-test for non-normal distributions. The

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categorical variables were evaluated using the Pearson Chi-square test or Fisher's exact test, with OR (95% CI) given afterwards if appropriate. Statistical analyses were conducted using IBM SPSS version 20.0 (IBM, Chicago IL, USA) and a *P* value <.05 was considered to be significant.

RESULTS

Macroscopic Autopsy Findings

The SUNDS group did not significantly differ from the matched controls in demographic characteristics including gender, age, height, and thickness of the layer of subcutaneous fat of abdominal wall (Table 1). Both SUNDS cases and controls showed no significant macroscopic pathological changes except for non-specific morphological changes related to acute death (such as visceral congestion). Some controls had primary acute violent injuries associated with their cause of death such as electrical injury marks on the skin and muscular contusion.

The average weight of liver, kidneys, brain, and especially the heart in SUNDS cases were significantly greater than those in controls (Table 2). Due to the correction of an individual's body weight and individual organ weights, and the lack of body weight information in our database, we calculated the combined total weight (TW) of all vital organs in each case to normalize individual organ weights. After normalization, only average heart weight in SUNDS showed a significant increase compared to controls (P =.04, Supplemental Table 1 in the Supplement).

No significant differences in the average left and right ventricular thickness were found between the two groups. The average circumferences of all cardiac valves in SUNDS tended to be increased and the mitral valves were significantly increased compared to controls (Figure 1A), which is consistent with the slight increase in average heart weight, and confirming a slightly enlarged heart size in SUNDS.

Microscopic Autopsy Findings

Controls showed no specific disease related microscopic changes except those injuries and morphological changes attributable to the cause of death in some cases. Most non-specific and non-significant histopathologic changes showed no significant difference between SUNDS cases and controls. The thymus is a vestige with prominent atrophy in the adult. Notably, the unwithered thymus (> 25 g) showed higher prevalence (73/148) in SUNDS compared to controls (81/444, P<.001, Supplemental Table 2 in the Supplement). These findings revealed that SUNDS cases were not associated with obvious structural cardiac diseases (such as viral myocarditis and typical cardiomyopathy).

Demographics of BrS Cohort

For the 17 BrS patients, the average age at the time of diagnosis was 45.9 ± 10.7 years, 15/17 were males, 6/17 had a previous family history of BrS, 8/17 had suffered previous syncope, seizures, or nocturnal agonal respiration, and 6/17 had an implantable cardioverter -defibrillator.

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Molecular Autopsy/Genetic Findings

Overall, 22/44 SUNDS victims and 11/17 patients with BrS hosted at least one rare non-synonymous variant (MAF <.01) among the 80 candidate genes analyzed. At least one ultra-rare variant (absent in all publically available control databases) was observed in 7/44 SUNDS cases (1 case with a *CACNA1C* variant, 1 *DSP*, 1 *EYA4*, 1 *GATA4*, 1 *MYBPC3*, 1 *MYH7*, and 1 case with both a *KCNQ1* and *KCNH2* variant) and 6/17 patients with BrS (3 with a *SCN5A* variant, 2 *MYH6*, and 1 *CACNA1C*). However, using the strict ACMG guideline-based definition for pathogenicity, only 2/44 SUNDS cases hosted a "likely pathogenic" variant (p.Q376sp-KCNQ1, p.G626_P631del-KCNQ1) compared to 3/17 patients with BrS that hosted either a "pathogenic" or "likely pathogenic" variant (p.G400R-SCN5A, p.D1275N-SCN5A, T1893Pfs*29-SCN5A ; Table 3).

We examined the distribution of the rare non-synonymous variants in 35 genes associated with primary arrhythmias such as BrS, LQTS, SQTS, CPVT, cardiac conduction disease, familial atrial fibrillation (AF), Wolff-Parkinson-White syndrome, and sick sinus syndrome.[17] At least one rare variant in a primary arrhythmia causing gene was detected in 12/44 SUNDS cases (Table 3) and in 6/17 BrS patients (Table 4). The 12/44 SUNDS victims hosted AF (*GATA4, NUP155, ABCC9*), LQTS (*AKAP9, KCNQ1, KCNH2, KCNJ5*) and BrS (*SCN5A, SCN1B, CACNB2, CACNA1C*) associated rare variants while the 6/17 BrS patients carried only BrS (*SCN5A, CACNA1C, P*>.05) related rare variants. Moreover, only 2/44 (4.5%) SUNDS cases compared to 5/17 (29.4%, *P*=.01) BrS patients hosted at least one rare variant in the most common BrS causing gene *SCN5A*. The difference in distribution of rare variants in primary arrhythmia associated genes indicates that SUNDS and BrS may largely result from different molecular pathological underpinnings.

Considering the latent cardiac structural abnormality identified in this study, we then focused on the rare variants in 48 genes previously associated with cardiomyopathy such as dilated, hypertrophic, restrictive, arrhythmogenic right ventricular cardiomyopathy and left ventricular noncompaction. At least one rare variant in a cardiomyopathy-associated gene was identified in 14/44 SUNDS cases (Table 3) and 7/17 patients with BrS (Table 4). Interestingly, the 14/44 SUNDS cases with variants within cardiomyopathy related genes had a significantly decreased circumference of both pulmonary (*P*=.002) and aortic valves (*P*=.003; this may indicate slight narrowing of both right and left ventricular outflow tract due to slight ventricular hypertrophy), and tended to die on average 5–6 years younger (26.21±8.58 versus 32.10±7.12 years, *P*=.02, Figure 1B, Supplemental Table 3 in the Supplement) than the 30 remaining SUNDS cases. These results prompt speculation that rare variants in cardiomyopathy-related genes may have a slight but actual impact on cardiac morphological changes which may contribute to primary or secondary arrhythmia in SUNDS.

DISSCUSSION

Forensic and clinical pathologists have attempted to uncover plausible pathogenic morphological characteristics underlying SUNDS for nearly a century.[1–3] Several pilot autopsy studies have suggested some cardiac structure changes in SUNDS: Kirschner (1986),[23] Park (1990),[24] and Elfawal (2000) et al[25] observed cardiomegaly or cardiac

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hypertrophy in 14 of 18 SUNDS cases (all with CCS anomalies), 4 of 14 SUNDS decedents (without grossly detectable structural cardiac anomaly), 7 of 22 SUNDS victims (2 cases had coronary stenosis, 7 had cardiac hypertrophy), respectively. Takeichi et al. (2008)[26] found that the circumferences of the coronary arteries and the aorta in proportion to the heart weight were significantly narrower in 20 SUNDS cases compared to 23 controls. However, due to the small sample sizes, the uncertain definition of cardiomegaly (heart weight >350g, but not normalized to body size), the selection of cases (a large portion had structural heart disease), or the lack of controls, these findings and their significance need further confirmation. Structural heart disease were gradually excluded from this disorder and the vast majority of reported SUNDS cases have remained an autopsy-negative enigma.[1,3–5,7–9,12–15]

In this study we provide from a large SUNDS cohort, the first morphological evidence of a slight but significantly larger heart size. Although these subtle structural cardiac changes did not meet the criteria for cardiomyopathy, this finding poses additional questions: 1) Is the slightly enlarged cardiac size a primary cause or a secondary change for SUNDS? 2) Does this slight structural abnormality underlie arrhythmia or SCD in SUNDS? Although epidemiological studies on the impact of body mass index to SCD risk are conflicting,[27] over 60% of chronic ischemia heart disease associated SCD were reported to have overweight hearts.[28] Cardiomegaly was identified to be the sole arrhythmogenic substrate in approximately 40% of structural heart disease related SCD without specific cardiomyopathy.[29] Indexed left ventricular mass by body surface area was reported as an independent predictor of SCD and may help improve the risk prediction of SCD beyond routine cardiovascular risk factors.[30] For SUNDS with apparently normal heart, whether or not there are more specific and detailed characteristics to establish pathological markers based on slightly increased heart mass for diagnosing SUNDS or predicting SCD risk deserves further investigation. Most recently, Nademanee et al. linked the significant cardiac morphological changes (fibrosis, loss of gap junctions) with the BrS phenotype and life-threatening arrhythmia.[31] Our findings initially highlight the possible important role of subtly increased heart size in the pathogenesis of SUNDS, and provide the direct morphological evidence for the hypothesis that ion channel diseases without obvious cardiac structural abnormality (such as BrS, LQTS, CPVT, and SUNDS) may be a subtype of caridomyopathies.[31–33]

The genetic etiology of SUNDS has been focused on BrS which reportedly shares the type 1-BrS ECG pattern with about 60% of “survived” SUNDS patients.[2,34] The *SCN5A* gene is the most common BrS-susceptibility gene accounting for 20–30% of the disorder. Mutations in *SCN5A* were originally linked to SUNDS (2002) in 3 of 10 (30%) probands with clinical evidence of SUNDS, and thus SUNDS and BrS were considered to be the same allelic disorder.[16] However, we reported only a 6.5% prevalence of SCN5A putative pathogenic variants in a much larger cohort of 123 SUNDS victims (2014).[13] In current analysis, only 4.5 % of SUNDS cases hosted SCN5A variants (the incidence of SCN5A mutation was comparable to our previous study[13]) compared to 29.4% of BrS patients (this incidence was also consistent with our previous studies).[35,36] Moreover, according to the strict ACMG guideline definition, none of SUNDS cases had “pathogenic” or likely “pathogenic” SCN5A variant compared to 18% of our BrS cohort. Notably, the present study has strongly

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suggested that the molecular pathological spectrum for SUNDS may be much broader than BrS. While phenotypically similar, SUNDS and BrS may not be genetically and functionally the same allelic disorder.

Increasing evidence has shown that cardiomyopathy shares some common susceptibility genes (*SCN5A, PKP2, RYR2, ABCC9*) with primary arrhythmia disorders (BrS, AF).[37,38] Recently, the rare variants in cardiomyopathy causing genes *CASQ2, DSG2, JUP*, and *DSP* were identified to be potentially associated to BrS.[17,39] The yield of rare variants in the 48 cardiomyopathy-associated genes in both BrS and SUNDS in this study highlights again the increasingly recognized potential intrinsic linkage between cardiomyopathy and primary arrhythmia with apparently normal heart. Notably, we observed that these rare variants were associated with altered heart structure and a tendency for earlier average age at death by approximately 5 years. These findings suggest the possibility that cardiomyopathy-susceptibility gene variants may contribute to lethal arrhythmia of SUNDS, and suggest this gene pool may be expected to yield additional novel genetic causes for SUNDS and BrS.

LIMITATIONS

Although the present study was the largest Chinese SUNDS autopsy case series to characterize the morphological changes and the primary genetic spectrum, the relatively small sample size for genetic testing as well as the lack of clinic records including ECG and genetic screening of family members limited a deeper analysis of association between clinical phenotype, morphological changes and genetic findings. Although there was no significant difference between SUNDS and the matched controls in gender, age, height, and thickness of the layer of subcutaneous fat of abdominal wall, the body mass index for each individual was unknown due to the lack of body weight. This may limit a more precise assessment of normalized organ weight. The absence of clinic information including ECG data is always a study limitation that is inherent to the vast majority of investigations on postmortem cases, especially with SUNDS victims where by their very definition are apparently healthy individuals who die suddenly and unexpectedly. We are presently enlarging the sample size of SUNDS cases, collecting available clinic data, following up with the families of SUNDS victims, and performing functional studies to establish a SUNDS database that includes morphological, molecular pathologic, electrophysiological, and clinic data. This future database will benefit us for accurately understanding the pathogenesis of SUNDS.

CONCLUSION

We present the first comprehensive autopsy evidence that SUNDS victims had concealed cardiac morphological changes, and that cardiomyopathy-related rare genetic variants may contribute conceivably to the cardiac abnormal structure and lethal arrhythmia underlying SUNDS. The broader molecular spectrum in arrhythmia associated genes indicates that SUNDS is only partially an allelic disorder to BrS. Our findings provide a new insight into approaches to morphologically and genetically diagnose patients with potential high risk for SUNDS. Deeper on-going investigations on morphological, molecular pathological, and electrophysiological characteristics in larger SUNDS cohorts to address the etiology and

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mechanism of sudden death may contribute to the increasingly important precision medicine for SCD.[40,41]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACMG	American College of Medical Genetics
AF	familial atrial fibrillation
BrS	Brugada syndrome
CCS	cardiac conduction system
CPVT	catecholaminergic polymorphic ventricular tachycardia
LQTS	long QT syndrome
MAF	minor allele frequency
SCD	sudden cardiac death
SUNDS	sudden unexplained nocturnal death syndrome
VUS	variants of uncertain significance

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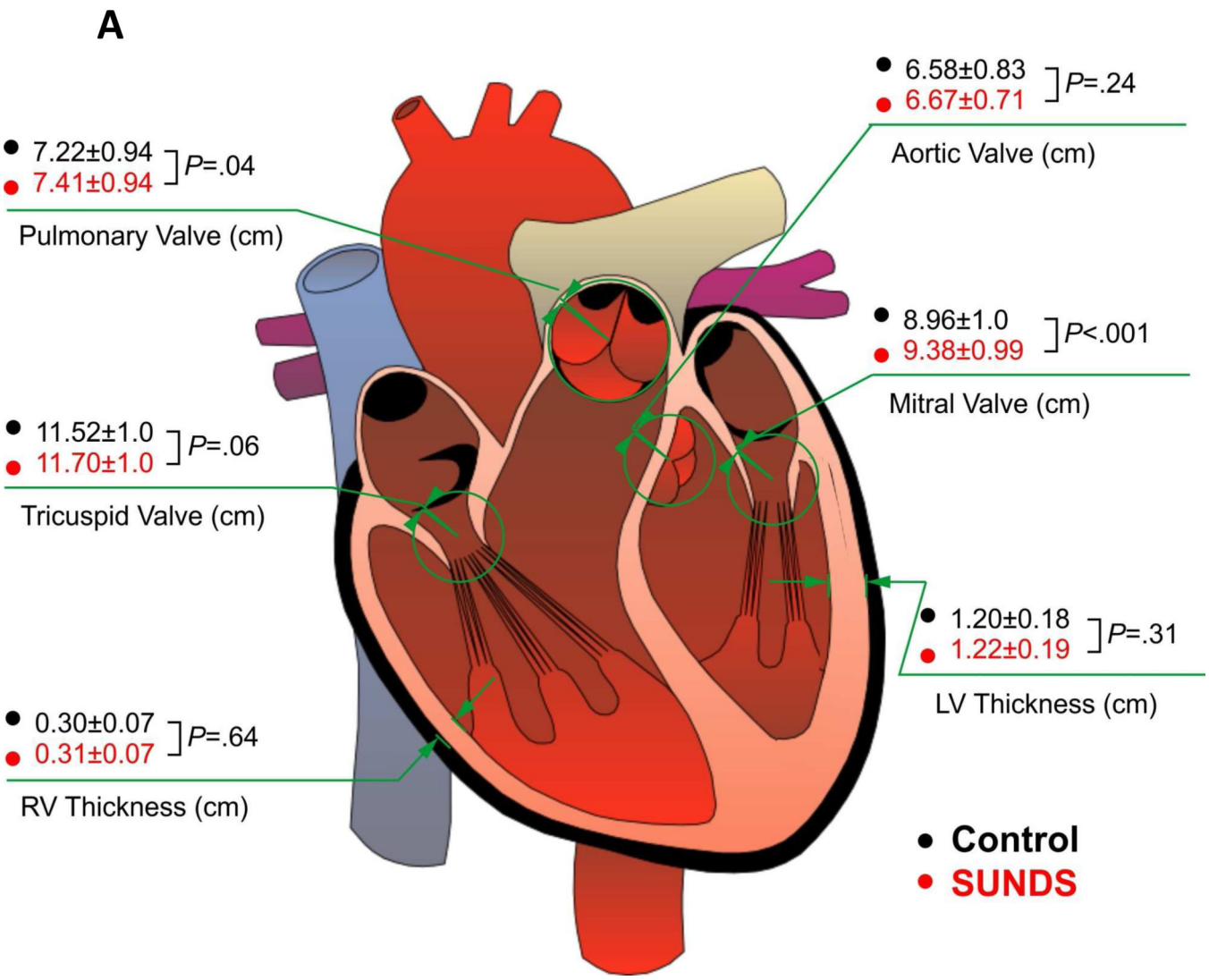

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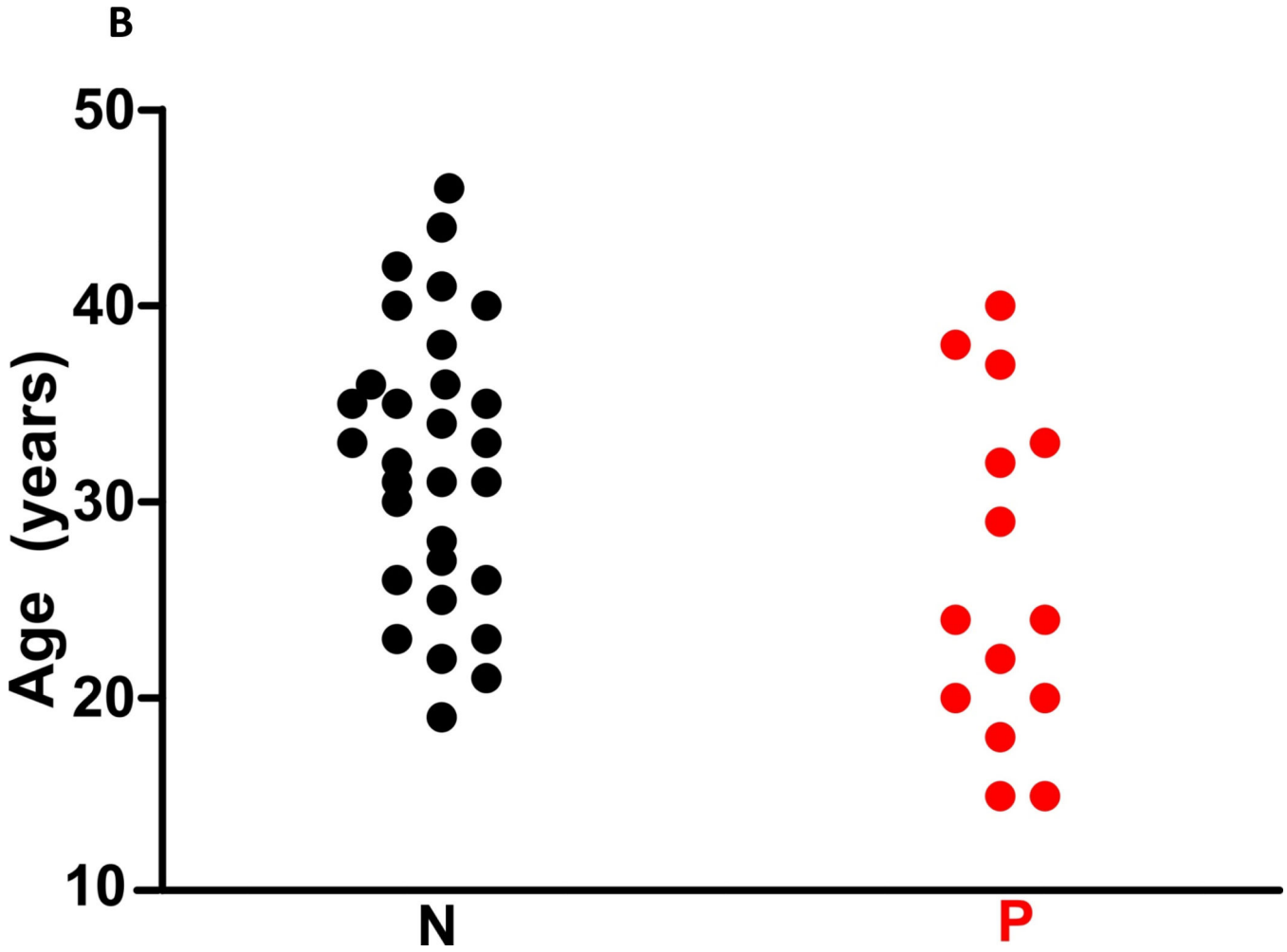

Figure 1. The heart structure and death age in SUNDS victims

(A) 148 SUNDS cases showed statistically significant increased circumferences of cardiac valves versus 444 controls. (B) Compared with 30 SUNDS cases without rare variants in cardiomyopathy associated genes (N, 26.21±8.58 years), the 14 SUNDS cases with rare variants (P, 32.10±7.12 years) tended to die on average 5–6 years younger.

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TABLE 1

Demographics of SUNDS victims and controls[a]

Variable	SUNDS (N=148)	Control (N=444)	*P* value
Gender ratio (M:F)	143:5	429:15	1.0
Average age (years)	30.7±7.4	30.9±7.3	.85
Average height (cm)	168.1±5.9	167.5±6.5	.37
Average TFA (cm)	1.7±0.8	1.6±1.0	.68

[a]F = female; M = male; TFA = thickness of the layer of subcutaneous fat of abdominal wall.

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TABLE 2

The average weight of vital organs

Organ weight (g)	SUNDS (N=148)	Control (N=444)	*P* value
Brain	1457.8±133.9	1426.0±143.2	.02
Left lung	608.6±125.8	597.7±217.9	.57
Right lung	696.6±150.6	678.2±240.1	.39
Heart	352.0±54.4	328.6±60.5	<.001
Liver	1539.0±278.2	1453.7±353.7	.01
Spleen	176.0±72.1	170.5±92.0	.52
Left kidney	153.0±38.0	143.1±36.2	.006
Right kidney	146.9±35.2	139.2±36.5	.03
Pancreas	118.9±26.6	117.2±34.6	.59

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TABLE 3

The rare variants identified in primary arrhythmia or cardiomyopathy susceptible genes in SUNDS victims[a,b,c]

Case	Age (years)	Gene	Transcript	Nucleotide change	Amino acid change	dbSNP	MAF					Condel prediction	Associated diseases	ACMG Definition	Ref
							NHLBI ESP	1000 Genomes	Local Database	ExAC Overall	ExAC East Asian				
1	38	DMPK	NM_004409.3	c.1868C>T	p.Pro623Leu	rs200199039	-	0.0005	0.0013	-	-	deleterious	LVNC	VUS	
3	31	AKAP9	NM_005751.4	c.11135G>A	p.Arg3712Gln	rs186148498	-	0.0009	-	0.0006264	0.0083	deleterious	LQTS	VUS	
4	37	SCN5A	NM_198056.2	c.4018G>A	p.Val1340Ile	rs199473605	-	-	-	4.951E-05	0.00035	deleterious	BrS	VUS	1
		AKAP9	NM_005751.4	c.1642A>G	p.Arg548Gly	rs147247719	-	0.0014	0.0013	0.0001408	0.0019	deleterious	LQTS	VUS	
		LDB3	NM_001171610.1	c.1457C>G	p.Pro486Arg	rs12761754	-	-	0.0004	0.0000827	0.0012	deleterious	DCM, VNC	VUS	
		MYBPC3	NM_000256.3	c.787G>A	p.Gly263Arg	-	0.000084	-	-	0.0002344	0.0018	neutral	HCM	VUS	2
		MYH7	NM_000257.2	c.3341G>A	p.Arg1114His	-	-	-	-	-	-	deleterious	DCM,HCM,RCM	VUS	
		SGCD	NM_000337.5	c.848A>G	p.Gln283Arg	-	-	-	-	0.0004696	0.0064	deleterious	DCM	VUS	
		DSP	NM_004415.2	c.7735G>C	p.Asp2579His	-	-	-	0.0004	-	-	deleterious	ARVC	VUS	
7	24	DSP	NM_004415.2	c.373A>T	p.Ile125Phe	-	-	-	-	-	-	deleterious	ARVC	VUS	
11	24	GATA4	NM_002052.3	c.544G>C	p.Gly182Arg	-	-	-	-	-	-	deleterious	AF	VUS	
		DSP	NM_004415.2	c.269A>G	p.Gln90Arg	rs188516326	-	0.0023	0.0049	0.0006768	0.0096	deleterious	ARVC	VUS	3
15	20	LMNA	NM_170707.3	c.1718C>T	p.Ser573Leu	rs60890628	-	-	0.0004	0.0001055	-	neutral	DCM,FPLD2	VUS	4~6
		MYH6	NM_002471.3	c.4207G>A	p.Glu1403Lys	-	-	-	-	3.296E-05	0.00012	deleterious	DCM, HCM	VUS	
16	33	ACTN2	NM_001103.2	c.1162T>A	p.Trp388Arg	-	-	-	0.0009	4.119E-05	0.00046	deleterious	DCM	VUS	7
		DSP	NM_004415.2	c.4071G>C	p.Glu1357Asp	-	-	-	0.0004	4.975E-05	0.0007	deleterious	ARVC	VUS	
17	31	GATA4	NM_002052.3	c.544G>C	p.Gly182Arg	-	-	-	-	-	-	deleterious	AF	VUS	
18	32	ANKRD	NM_014391.2	c.545G>A	p.Arg182His	-	-	-	0.003	0.0001074	0.0013	deleterious	DCM	VUS	
21	40	KCNQ1	NM_000218.2	c.1876_1893del	p.Gly626_Pro631del	-	-	-	-	-	-	-	LQTS	LP	8
		KCNH2	NM_000238.3	c.3110A>T	p.Asp1037Val	-	-	-	-	-	-	deleterious	LQTS	VUS	
24	20	MYBPC3	NM_000256.3	c.3579C>G	p.Ile1193Met	-	-	-	-	-	-	deleterious	DCM,HCM,LVNC	VUS	
		MYH7	NM_000257.2	c.3235C>T	p.Arg1079Trp	rs192722540	-	0.0005	0.0005	4.944E-05	0.00069	deleterious	DCM,HCM,RCM	VUS	
26	26	NUP155	NM_153485.1	c.1507C>T	p.Leu503Phe	-	-	-	0.0004	8.238E-06	0.00012	deleterious	AF	VUS	
27	18	ACTN2	NM_001103.2	c.1162T>A	p.Trp388Arg	-	-	-	0.0009	4.119E-05	0.00046	deleterious	DCM	VUS	7
29	35	SCN1B	NM_001037.4	c.566C>T	p.Thr189Met	rs2305748	-	-	0.0022	0.000173	0.0016	deleterious	BrS, AF	VUS	9,10
30	29	MYBPC3	NM_000256.3	c.104G>A	p.Arg35Gln	-	-	-	-	7.909E-05	0.00093	deleterious	HCM	VUS	11

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Case	Age (years)	Gene	Transcript	Nucleotide change	Amino acid change	dbSNP	MAF					Condel prediction	Associated diseases	ACMG Definition	Ref
							NHLBI ESP	1000 Genomes	Local Database	ExAC Overall	ExAC East Asian				
		VCL	NM_014000.2	c.282G>A	p.Met94Ile	-	-	-	-	1.647E-05	0.00023	deleterious	DCM, HCM	VUS	
32	22	KCNQ1	NM_000218.2	c.1128+5G>A	-	rs76735093	-	-	0.0004	8.308E-06	-	-	LQTS	LP	12
		ABCC9	NM_020297.2	c.3589C>T	p.Arg1197Cys	-	-	-	0.0018	9.061E-05	0.00012	deleterious	AF	VUS	
33	40	KCNJ5	NM_000890.3	c.536A>G	p.Asn179Ser	rs147070381	0.0002	-	0.0004	4.118E-05	0.00023	deleterious	LQTS	VUS	
		EYA4	NM_004100.4	c.1622A>T	p.Asn541Ile	-	-	-	-	-	-	deleterious	DCM	VUS	
35	35	CACNA1C	NM_000719.6	c.4393T>C	p.Phe1465Leu	-	-	-	-	-	-	deleterious	BrS	VUS	
36	22	LDB3	NM_001171610.1	c.1333T>C	p.Ser445Pro	-	-	-	0.0004	-	-	deleterious	DCM, LVNC	VUS	
		JUP	NM_002230.2	c.958C>T	p.Arg320Cys	rs200740462	-	0.0005	0.0013	0.0001895	0.0022	deleterious	ARVC	VUS	
38	15	CACNB2	NM_201590.2	c.1354C>T	p.Arg452Cys	-	0.00015	-	-	0.000033	-	deleterious	BrS	VUS	
		MYH6	NM_002471.3	c.4504C>T	p.Arg1502Trp	-	-	-	-	8.238E-06	-	deleterious	DCM,HCM	VUS	
		TNNT2	NM_000364.2	c.877C>T	p.Arg293Cys	-	-	-	-	1.119E-05	0.00015	-	DCM	VUS	13~15
39	15	MYH6	NM_002471.3	c.1154C>T	p.Ser385Leu	-	-	-	-	0.0001652	0.0022	neutral	DCM,HCM,ASD	VUS	16
40	46	SCN5A	NM_198056.2	c.3292G>T	p.Val1098Leu	rs199473191	-	-	-	0.0001476	0.002	neutral	LQTS,SUNDS	VUS	9,17
		CACNA1C	NM_000719.6	c.5329C>T	p.Arg1777Cys	-	-	-	-	6.183E-05	0.00061	neutral	BrS	VUS	

[a]ASD = atrial septal defects; FPLD2 = familial partial lipodystrophy subtype 2; LP = Likely pathogenic; MAF = Minor allele frequency; Ref = references associated with the corresponding variant, which were listed in the online-only references in the Supplements; VUS = Rare variant of uncertain significance.

[b]sign "–" indicates that the variants of interest is absent from the corresponding database.

[c]All cases list above were males; age refers to death age. all variants were heterozygous.

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TABLE 4

The rare variants identified in primary arrhythmia or cardiomyopathy susceptible genes in Brugada syndrome patients[a,b]

Case	Age (years)	Gene	Transcript	Nucleotide change	Amino acid change	dbSNP	MAF					Condel prediction	Associated diseases	ACMG Definition	Ref
							NHLBI ESP	1000 Genomes	Local Database	ExAC Overall	ExAC East Asian				
1	25	MYH6	NM_002471.3	c.5539C>T	p.Arg1847Trp	-	-	-	-	-	-	deleterious	DCM,HCM	VUS	
2	30	SCN5A	NM_198056.2	c.1198G>A	p.Gly400Arg	-	-	-	-	-	-	deleterious	BrS	LP	18
3	48	SCN5A	NM_198056.2	c.3823G>A	p.Asp1275Asn	rs137854618	-	-	-	-	-	deleterious	CCD	P	19,20
		DSP	NM_00100884	c.943C>T	p.Arg315Cys	rs200476515	0.00008	0.0005	0.0005	0.00007456	0.00047	deleterious	ARVC	VUS	
		DTNA	NM_001198938.1	c.1891C>T	p.Arg631Cys	-	-	-	-	0.00002482	0.00012	deleterious	LVNC	VUS	
4	39	SCN5A	NM_198056.2	c.4282G>T	p.Ala1428Ser	rs200034939	-	0.0005	-	0.00002486	0.00035	deleterious	BrS,LQTS	VUS	21,22
9	38	CACNA1C	NM_001129843.1	c.4393T>C	p.Phe1465Leu	-	-	-	-	-	-	deleterious	BrS	VUS	
11	40	SCN5A	NM_198056.2	c.4282G>T	p.Ala1428Ser	rs200034939	-	0.0005	-	0.00002486	0.00035	deleterious	LQTS, BrS	VUS	21,22
12	57	JUP	NM_002230.2	c.1807G>T	p.Val603Leu	rs200327969	-	0.0005	0.0023	0.0006604	0.0055	deleterious	ARVC	VUS	23
13	60	MYH6	NM_002471.3	c.1111G>A	p.Glu371Lys	-	-	-	-	-	-	deleterious	DCM,HCM	VUS	
14	53	SCN5A	NM_198056.2	c.5676delC	p.Thr1893Profs*29	-	-	-	-	-	-	-	BrS	P	
		SCN5A	NM_198056.2	c.5692C>T	p.Arg1898Cys	-	0.00008	-	-	0.00002484	0.00012	neutral	BrS	VUS	
		MYO6	NM_004999.3	c.3824A>G	p.Tyr1275Cys	rs146461956	0.00023	0.0037	0.0058	0.005352	0.0029	deleterious	HCM	VUS	
15	55	ACTN2	NM_001103.2	c.1192C>T	p.Arg398Cys	rs148189507	0.00023	-	-	0.00003295	0.00012	deleterious	DCM	VUS	
17	50	MYH7	NM_000257.2	c.77C>T	p.Ala26Val	rs186964570	0.000077	0.0032	0.0085	0.0005687	0.0069	neutral	HCM	VUS	24,25

[a] LP = Likely pathogenic; MAF = Minor allele frequency; P = Pathogenic; Ref = references associated with the corresponding variant, which were listed in the online-only references in the Supplements; VUS = Rare variants of uncertain significance.

[b] All cases list above except No.12 and 17 were males; age refers to the age at the time of diagnosis; all variants were heterozygous.

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