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# Whole exome sequencing in Brugada and long QT syndromes revealed novel rare and potential pathogenic mutations related to the dysfunction of the cardiac sodium channel

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## Abstract

**Background:** Brugada syndrome (Brs) and long QT syndrome (LQTS) are the most observed “inherited primary arrhythmia syndromes” and “channelopathies”, which lead to sudden cardiac death.

**Methods:** Detailed clinical information of Brs and LQTS patients was collected. Genomic DNA samples of peripheral blood were conducted for whole-exome sequencing on the Illumina HiSeq 2000 platform. Then, we performed bioinformatics analysis for 200 genes susceptible to arrhythmias and cardiomyopathies. Protein interaction and transcriptomic co-expression were analyzed using the online website and GTEx database.

**Results:** All sixteen cases of Brs and six cases of LQTS were enrolled in the current study. Four Brs carried known pathogenic or likely pathogenic of single-point mutations, including *SCN5A* p.R661W, *SCN5A* p.R965C, and *KCNH2* p.R692Q. One Brs carried the heterozygous compound mutations of *DSG2* p.F531C and *SCN5A* p.A1374S. Two Brs carried the novel heterozygous truncated mutations (MAF < 0.001) of *NEBL* (p.R882X) and *NPPA* (p.R107X), respectively. Except for the indirect interaction between *NEBL* and *SCN5A*, *NPPA* directly interacts with *SCN5A*. These gene expressions had a specific and significant positive correlation in myocardial tissue, with high degrees of co-expression and synergy. Two Brs carried *MYH7* p.E1902Q and *MYH6* p.R1820Q, which were predicted as “damaging/possibly damaging” and “damaging/damaging” by Polyphen and SIFT algorithm. Two LQTS elicited the pathogenic single splicing mutation of *KCNQ1* (c.922-1G > C). Three LQTS carried a single pathogenic mutation of *SCN5A* p.R1880H, *KCNH2* p.D161N, and *KCNQ1* p.R243S, respectively. One patient of LQTS carried a frameshift mutation of *KCNH2* p.A188Gfs\*143.

**Conclusions:** The truncated mutations of *NEBL* (p.R882X) and *NPPA* (p.R107X) may induce Brugada syndrome by abnormally affecting cardiac sodium channel. *SCN5A* (p.R661W, p.R965C and p.A1374S) and *KCNH2* (p.R692Q) may

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cause Brugada syndrome, while *SCN5A* (p.R1880H), *KCNQ1* (c.922-1G>C and p.R243S) and *KCNH2* (p.D161N and p.A188Gfs\*143) may lead to long QT syndrome.

**Keywords:** Arrhythmia, Brugada syndrome, Long QT syndrome, Natriuretic peptide precursor A, Nebulette

## Background

Inherited primary arrhythmia syndromes (IPAS), a rare disease (prevalence < 1/1,000) also called “channelopathies,” are commonly induced by genetic disorders and result in ventricular tachycardia (VT), torsade de pointe (TdP) and ventricular fibrillation (VF), consequently leading to sudden cardiac death (SCD) and even sudden unexplained death [1, 2]. The Brugada syndrome (Brs) and long QT syndrome (LQTS) are the most frequently observed IPAS in the general population. The prevalence of Brs and type-2/3 Brugada pattern electrocardiogram (ECG) is 0.5/1,000 and 6.1/1,000 and is reported to be the highest in Southeast Asia [3]. According to a study enrolling 44, 596 infants 15 to 25 days old (43, 080 whites) from 18 maternity hospitals, the prevalence of LQT between 451 and 470 ms of QTc might be close to 1:2000 [4]. A literature search reported that the prevalence of LQTS-induced sudden infant death syndrome (SIDS) ranged from approximately 3.9 to 20.6%, with an average of 12% [5]. The poor prognostic factors for mixed populations described in the series of Brs and LQTS, including sex (men for Brs, type-2 LQTS for female), symptoms, ECG characteristics, family history of SCD, genetic mutation, and inducibility of ventricular arrhythmia during the cardiac electrophysiological examination [6, 7].

For drug therapies of Brs, quinidine, blocking  $I_{to}$  and  $I_{Kr}$  channels reduces the arrhythmias incidence, including arrhythmic storms and multiple shocks, or as an alternative to an implantable defibrillator (ICD) in children at risk of arrhythmias. Additionally, isoproterenol, increasing the  $I_{CaL}$  inflow current, has been used successfully in cases of electrical storms [6]. Syncope in patients with LQTS is often triggered by periods of high sympathetic activity, including stress and exercise, for example, swimming. The type-1 LQTS patients should not be allowed to participate in competitive sports, especially swimming, or only cautiously with supervision. Type-2 and -3 LQTS patients are more susceptible to events during sleep. Type-2 LQTS patients are particularly sensitive to startle or sudden noises while sleeping, such as alarm clocks and telephones, and thus should avoid unexpected noises during sleep.  $\beta$  blockers are recommended as the first line of therapy for all patients with LQTS. Patients with type-1 LQTS appear to benefit most from  $\beta$  blockers and should be started on  $\beta$  blockers as the first-line therapy [8]. According to the 2017 AHA/ACC/HRS guidelines, the ICD is the most important treatment for Brs and

LQTS [9, 10]. The left cardiac sympathetic denervation should be considered for LQTS patients with  $\beta$  blockers therapy who continue to have syncope, TdP, recurrent appropriate ICD shocks despite antiarrhythmic drug therapy, or cardiac arrest [8].

Approximately 25 genes associated with Brs have been identified, of which eighteen genes are responsible for encoding ion channel subunits and seven genes for encoding regulatory proteins. Mutations on *SCN5A* are the most dominant for Brs and have more than 300 mutations related to Brs [11]. More than 20 disease-causing genes have been reported in almost 70% LQTS patients, including *KCNQ1* (30.1%, type-1 of LQTS), *KCNH2* (23.2%, type-2 of LQTS) and *SCN5A* (5.7%, type-3 of LQTS). However, the genetic causes for about one-third of LQTS remain unknown [12]. Notably, the genotype of *SCN5A* is a crucial component of the scheme for risk stratification of Brs and LQTS. It encodes  $Na_v1.5$ , a sodium channel protein, wherein type-1 LQTS with mutations affecting the transmembrane domain or C-loop and type-2 and -3 LQTS with missense mutations on the S5-pore-S6 region have a considerably higher risk for cardiac events. Brs with pore-*SCN5A* mutation has a higher event risk than *SCN5A*-negative variants [1]. Based on these researches, the pathogenic genotypes of IPAS, for example, the Brs and LQTS are tightly associated with the risk of malignant cardiac events, especially ventricular arrhythmia and SCD. In this study, we enrolled twenty-two unrelated cases of Brs and LQTS. The potential pathogenic mutations carried by these patients will be identified by Whole Exome Sequencing (WES) to analyze the correlations among pathogenic mutations, clinical phenotypes and their risks. Interestingly, in these cases, we found that some common pathogenic genetic mutations may be related to Brs and LQTS. At the same time, we also first found and speculated that truncated *NEBL* and *NPPA* mutations might lead to Brs by aberrantly affecting the function of the cardiac sodium channels.

## Methods

### Study population and diagnostic criteria

Twenty-two cases of Brs and LQTS were enrolled from June 2015 to June 2017. Detailed clinical information was collected. The clinical information included family history, age of presentation, initial symptoms of VT, physical examination, ECGs, and monitoring of ICD based on

their informed consent. The clinical diagnosis of Brs was based on the presence of typical type I Brugada pattern on the ECGs, characterized by a coved ST-segment and J-point elevation  $\geq 0.2$  mV in the right precordial leads [13]. The ECG's QTc (corrected for heart rate) can be calculated (QTc = QT interval + square root of the RR interval). The QTc interval helps us diagnose LQT. A QTc is prolonged if exceeding 0.47 s in women and 0.45 s in men [14, 15]. According to the Schwartz score, a definite LQTS is defined by an LQTS score  $\geq 3.5$  points [16].

#### Ethics approval

This study was approved by the Guangdong Medical Institutional Review Board and Medical Ethics Committees [No. GDREC2016001H (R1)]. With the consent of the ethics committee, we followed up with the patients under the condition of informed consent and obtained blood samples for genetic analysis.

#### Whole exome sequencing

Peripheral bloods from the patients were extracted for WES. Genomic DNA samples were isolated from peripheral blood using a standard DNA extraction protocol. The isolated genomic DNA was then fragmented into 150–200 bp and subjected to DNA library preparation using established Illumina paired-end protocols. Adaptor-ligated libraries were amplified via PCR. A portion of each library was used to create an equimolar pool. Each pool was amplified to enrich targets sequenced by the Agilent SureSelectXT Target Enrichment System (Agilent Technologies Inc., Santa Clara, CA, USA). According to the manufacturer's protocol, whole-exome capture was performed with the Agilent SureSelectXT Human All Exon 50 Mb Kit (Agilent Technologies Inc.). According to the manufacturer's instructions, the exome-enriched libraries were sequenced with the Illumina HiSeq 2000 platform (Illumina, San Diego, CA, USA), and 100 bp paired-end sequencing reads were generated. Each sample was sequenced per lane to obtain an average theoretical depth of  $100 \times$  [17, 18].

#### Read mapping, variant detection, and functional annotation

After WES, raw reads were collected for quality control, in which low-quality reads were filtered, and 3'/5' adapters were trimmed using the Trim Galore program (version 0.4.4). Clean reads were aligned to the human reference genome (University of California Santa Cruz, UCSC build hg19) using the Burrows-Wheeler Aligner (BWA, version: 0.7.17-r1188) program. The quality scores were recalibrated, and reads were realigned to the reference genome using the Genome Analysis Toolkit (GATK, version: 3.5-0-g36282e4) software package. Following the

exclusion of duplicate reads, insertion-deletions (InDels) and single-nucleotide polymorphisms (SNPs) were called using the GATK or Sequence Alignment/Map tools (SAM tools, Version: 1.3.1). The quality value of variants detected by GATK was 99 (the highest value), and the variant abundance was more than 30% [17, 18].

#### Pathogenic risk classification

The SNPs and Indels were annotated using a pipeline, in which all insertion and deletion variants occurring at coding regions were considered damaging, and nonsynonymous SNPs were predicted by SIFT (<http://sift.jcvi.org/www/>) and PolyPhen-2 (Polymorphism Phenotyping v2, <http://genetics.bwh.harvard.edu/pph2/>) [19]. Subsequently, the common risk genes associated with cardiomyopathies and arrhythmias, as reported in our previous research [18, 20], were detected in the patients. These variants were screened with the following filtering criteria: (1) same variants in the WES data; (2) missense, nonsense, insertion and deletion variants; (3) SNPs with minor allele frequency, not  $\geq 0.01$  according to the SNP database of National Center; excluded variants with allele frequency in 1000genomes (2015 version) higher than 1%, or higher than 5% in house frequency. The potential risk variants were classified as "pathogenic (P)", "likely pathogenic (LP)", "uncertain significance (VUS)", "likely benign (LB)" or "benign (B)" by the Clinvar database [17, 18] and InterVar tool [21] following the 2015 ACMG/ACP guidelines [22]. The detailed ACMG classification was shown in our previous research [18].

#### Protein interaction analysis

Using the online website <https://string-db.org/>, the target gene was input for protein–protein interaction analysis. The combined score between proteins with interaction records was scored by combining other database records, experimental verification, gene fusion, co-localization, co-expression and homology analysis. It is currently the mainstream and high-reliability database of protein–interaction information.

#### Transcriptomic co-expression analysis

In the Genotype-Tissue Expression (GTEx) database [23], the TPM matrix of ventricular tissue, spleen, whole blood, ovary, lung and liver were used for co-expression analysis. The GTEx version was GTEx analysis V8 (dbgap access phs000424.v8.p2). The "Cor" function in the R language was used to calculate the gene correlation matrix. The method parameter used Spearman correlation, in which the correlation threshold was above 0.7, indicating a very close relationship; 0.4–0.7 indicated a close relationship; 0.2–0.4 indicated a general relationship.

## Results

### Genotype–phenotype relationship

In all, sixteen cases of Brs (median onset-age, 46-year-old; IQR 21.5-year-old; 22 to 65-year-old) and six cases of LQTs (median onset-age, 15-year-old; IQR 18-year-old; 6 to 55-year-old) were enrolled in the current study (Table 1). The echocardiograms (ECGs) of these patients showed normal cardiac structure. VT or VF was detected in 19 cases. Two cases of Brs were induced VF by electrophysiological examination (EPS). These patients suffered from clinical symptoms, including dizziness, syncope, palpitation, amaurosis, and chest distress. Nineteen cases were implanted with ICD, while three Brs refused ICD implantation. Two cases of Brs and one case of LQTs had

a familial history of SCD. One case of Brs was the dominant familial inheritance because three siblings had Brugada-like ECGs without clinical symptoms.

The WES detected some known and pathogenic/likely-pathogenic (P/LP) mutations. Four cases of Brs demonstrated single mutations with known or likely pathogenicity, including p.A1374S (Clinic/ACMG=LP/VUS, No.5, VT/VF, ICD therapy), p.R661W (Clinic/ACMG=P/VUS, No.8, VF, ICD therapy), and p.R965C (Clinic/ACMG=LP/VUS, No.10, VF induced by EPS, ICD therapy) on *SCN5A*, and p.R692Q (Clinic/ACMG=LP/VUS, No.18, ICD therapy) on *KCNH2*. One case of Brs carried the compound heterozygous and pathogenic mutations of *DSG2* p.F531C (Clinic/ACMG=LP/

**Table 1** The clinical characteristics of patients with Brugada syndrome and long QT syndrome

No	DS	Sex	Age (years)	Onset of age (years)	Ventricular arrhythmia	Symptoms	ICD therapy	Drugs	Familial history
1	Brs	F	40	38	VT	Dizzy, syncope	refused	Beta blocker	No
2	LQTs	F	61	55	VF	Syncope	ICD	Beta blocker	No
3	Brs	M	72	65	VT	Syncope	refused	Beta blocker	Brother (SCD, 31-year-old)
4	Brs	M	48	47	VF	Amaurosis, syncope	ICD	No	No
5	Brs	M	46	45	VT, VF	Dizzy, amaurosis, syncope	ICD	No	No
6	Brs	M	60	57	VT, VF	Syncope	ICD	Beta blocker, mexiletine	No
7	Brs	M	57	47	No	Syncope	refused	No	Three brothers (Brs)
8	Brs	M	41	13	VF	Syncope	ICD	No	No
9	Brs	M	49	47	VT	Palpitation, chest distress	ICD	No	No
10	Brs	M	63	53	EPS induced VF	Dizzy, amaurosis,	ICD	No	No
11	Brs	M	22	22	VF	Syncope	ICD	No	No
12	Brs	F	51	51	VF	Syncope when wake up	ICD	No	No
13	LQTs	F	13	6	Tdp, VF	Sleeping syncope	ICD	Mexiletine	No
14	LQTs	F	19	16	VF	Palpitation, amaurosis, Syncope	ICD	Beta blocker, potassium magnesium aspartate	No
15	Brs	M	41	34	VF	Syncope	ICD	Beta blocker	No
16	Brs	M	54	53	EPS induced VF	Amaurosis, palpitation	ICD	No	No
17	Brs	M	32	31	VT, VF	Syncope, convulsion	ICD	Beta blocker, potassium	No
18	Brs	M	33	23	No	No	ICD	No	No
19	LQTs	F	35	30	VF	Syncope	ICD	Beta blocker	No
20	Brs	M	41	30	VF, AF	Amaurosis, syncope	ICD	No	No
21	LQTs	F	16	14	VF	Amaurosis, syncope	ICD	Beta blocker, pacing rate of 95 bpm	No
22	LQTs	F	16	12	VF	Syncope, chest distress	ICD	No	Mother (SCD, 33-year-old)

DS diseases, M male, F female, AF atrial fibrillation, VT ventricular tachycardia, VF ventricular fibrillation, Tdp torsades de pointes, EPS electrophysiology study, SCD sudden cardiac death, ICD Implantable Cardioverter-Defibrillator, - loss of follow-up or lack of clinical data due to refuse of hospitalization



LP) and *SCN5A* p.A1374S (Clinic/ACMG=LP/VUS, No.11, VF, ICD therapy). Two cases of LQTS elicited the pathogenic and single splicing mutation of *KCNQ1* c.922-1G>C (Clinic/ACMG=P/P, No.19, VF, ICD therapy). Three cases of LQTS carried a single pathogenic mutation of *SCN5A* p.R1880H (Clinic/ACMG=P/VUS, No.13, TdP and VF, ICD therapy), *KCNH2* p.D161N (Clinic/ACMG=P/LP, No.21, VF, ICD therapy), and *KCNQ1* p.R243S (Clinic/ACMG=P/LP, No.22, familial history of SCD, VF, ICD therapy), respectively (Table 2).

We also found several novel mutations potentially associated with Brs and LQTS. In two Brs patients, we first found the heterozygous p.R882X (Clinic/ACMG=VUS/VUS, No.1, VT, refused ICD therapy) of the *NEBL* gene (at the rs151012132 locus) and p.R107X (Clinic/ACMG=-/LP, No.3, VT, family history of SCD, refused ICD therapy) of the *NPPA* gene (Table 3), respectively, as truncating mutations, which were absent from or found with MAF (minor allele frequency)<0.001 in the 1000genomes population. *NEBL* p.R882X may induce the loss of domains of partial linker and SH3 in NEBL protein (Fig. 1A, B). *NPPA* p.R107X only expressed the pro-peptide (Fig. 1C) but lost the effective structure of atrial natriuretic peptide (ANP).

The variants of *MYH7* (p.E1902Q, rs187073962, Clinic/ACMG=-/VUS, No.17, VT/VF, ICD therapy) and *MYH6* (p.R1820Q, rs371222772, Clinic/ACMG=-/VUS, No.15, VF, ICD therapy), predicted as “damaging/possibly damaging” and “damaging/damaging” by Polyphen and SIFT algorithms, were demonstrated in patients of Brs. A patient of LQTS carried a frameshift mutation of *KCNH2* p.A188Gfs\*143 (Clinic/ACMG=-/LP, No.13, TdP and VF, ICD therapy), which did not exist in the 1000genomes population.

#### Literature summary of NEBL and SCN5A interaction

According to previous studies, abnormal desmosome genetic expressions, including desmocollin-2 (*DSC2*), desmoglein-2 (*DSG2*), plakophilin-2 (*PKP2*), desmoplakin (*DSP*), plakoglobin (*JUP*) and desmin (*DES*) participate in the pathogenic mechanism of arrhythmogenic cardiomyopathy (ACM) [17, 18, 20, 24–27]. Interestingly, loss-of-function of *SCN5A* mutations induced complex arrhythmia, including Brs, atrial fibrillation (AF), atrial standstill, VT and sick sinus syndrome [28]. In this study, we first discovered some interesting interactions among desmosome proteins and cardiac sodium channels in cardiomyocytes, including *DSG2* and  $\text{Na}_v1.5$  ( $\alpha$  subunit of the sodium channel, encoded by *SCN5A*), *PKP2* and  $\text{Na}_v1.5$ , *DES* and  $\text{Na}_v1.5$ , *NEBL* and *DES* in the cardiac desmosomes, through literature research using “*NEBL* and *SCN5A* (or  $\text{Na}_v1.5$ , or sodium channel), nebulin and *SCN5A* (or  $\text{Na}_v1.5$ , or sodium channel), each protein

of desmosomes (including *DSG2*, *DSC2*, *PKP2* and *DSP*) and *NEBL* (or nebulin), each protein of desmosomes (including *DSG2*, *DSC2*, *PKP2* and *DSP*) and *SCN5A* (or  $\text{Na}_v1.5$ , or sodium channel), *NEBL* (or nebulin) and Brugada syndrome, each protein of desmosomes (including *DSG2*, *DSC2*, *PKP2* and *DSP*) and Brugada syndrome” in the NCBI PubMed database. We summarized these literatures related to *NEBL*, desmosome proteins and  $\text{Na}_v1.5$  as follows (shown in Fig. 2A, B).

*NEBL* encodes a nebulin-like protein expressed in cardiac muscle. This protein binds to actin, interacting with thin filaments and Z-line-associated proteins in striated muscle and cardiac myofibril assembly. *NEBL* plays a vital role in the dynamics of the DES-*NEBL*-actin complex in cardiac myocytes and maintains the relaxation–contraction cycles of the heart. The *NEBL* exhibits high-affinity interaction and synergic action with DES filaments and is a direct linker between actin and DES. The pathogenic mutations of *NEBL* will induce dilated cardiomyopathy, hypertrophic cardiomyopathy, left ventricular non-compaction cardiomyopathy, and endocardial fibroelastosis [24–27].

Additionally, the pathogenic mutants E245D, T453I, and knockout of *DES* increase binding affinity for *NEBL*, delay filament assembly kinetics, and cause significant attenuation and disruption of cardiac actin-*NEBL*-DES-Z lines filament network as dynamic DES assembly [29]. The pathogenic mutations of *DES* can cause severe impairment of filament formation and induce ACM, consequently complicating rhythm disorder, conduction disease, and heart failure [30]. Therefore, the underlying mechanism of *NEBL*-inducing cardiomyopathies may be comparable to *DES*. According to previous studies, some cases of ACM overlap the phenotype of Brs [31, 32]. Like *DES*, *PKP2* is one of the critical components in desmosomes of the intercalated disk. It is necessary to maintain gap junction integrity and formation through the DES-*DSP*-*PKP2* complex in desmosomes. The lost expression of *PKP2* decreases and disrupts the expression and trafficking of the sodium channel ( $\text{Na}_v1.5$ ) at the intercalated disc, which can degrade cardiac sodium current and subsequently lead to overlapped phenotypes of ACM and Brs [33–36]. Based on these evidences, we proposed that the mutation of *NEBL* might theoretically associate with Brs through the interaction of abnormal *NEBL* protein with the sodium channel, which has not been demonstrated yet.

#### Literature summary of NPPA and SCN5A interaction

We also first discovered obvious interactions between natriuretic peptide precursor A (*NPPA*) and  $\text{Na}_v1.5$  through current summing research from the NCBI PubMed database using “*NPPA* and *SCN5A* (or  $\text{Na}_v1.5$ ,

**Table 2** The known and likely pathogenic mutations of Brugada syndrome and long QT syndrome

ID	DS	Chr	Start	Gene	Amino acid change	Het	1000 g/Esp	SIFT	Polyphen	Clinic	ACMG	Evidence	dbSNP
2	LQTS	chr11	2,604,664	KCNQ1	NM_000218:exon7:c.922-1G>C	±	-	-	-	P	P	PVS1, PM2_Supporting, PP4, PP1	rs387906290
5	Brs	chr3	38,598,739	SCN5A	NM_001160161:exon23:c.G4120T:p.A1374S	±	0.001	0.00(D)	1.00(D)	LP	VUS	PM2_Supporting, PM1, PP3	rs200034939
8	Brs	chr3	38,640,451	SCN5A	NM_000335:exon13:c.C1981T:p.R661W	±	<0.001	0.00(D)	1.00(D)	P	VUS	PM2_Supporting, PP3	rs199473139
10	Brs	chr3	38,622,757	SCN5A	NM_000335:exon17:c.C2893T:p.R965C	±	0.001	0.00(D)	1.00(D)	LP	VUS	PM2_Supporting, PS4_M, PS3_Supporting, PP3	rs199473180
11	Brs	chr18	29,116,333	DSG2	NM_001943:exon11:c.T1592G:p.F531C	±	-	0.00(D)	1.00(D)	LP	LP	PM2_Supporting, PM3_Strong, PS3_Supporting	rs2000484060
13	LQTS	chr3	38,598,739	SCN5A	NM_001160161:exon23:c.G4120T:p.A1374S	±	0.001	0.00(D)	1.00(D)	LP	VUS	PM2_Supporting, PS4_Supporting, PP3	rs200034939
13	LQTS	chr3	38,592,170	SCN5A	NM_001099405:exon27:c.G5639A:p.R1880H	±	<0.001	0.06(T)	0.99(D)	P	VUS	PM2_Supporting, PS4_Supporting, PP3	rs370694515
18	Brs	chr7	150,644,473	KCNH2	NM_172057:exon9:c.G2075A:p.R692Q	±	0.001	0.58(T)	1.00(D)	LP	VUS	PM2_Supporting, PP2	rs199473020
19	LQTS	chr11	2,604,664	KCNQ1	NM_000218:exon7:c.922-1G>C	±	-	-	-	P	P	PVS1, PM2_Supporting, PP4, PP1	rs387906290
21	LQTS	chr7	150,649,569	KCNH2	NM_001204798:exon2:c.G481A:p.D161N	±	-	0.00(D)	1.00(D)	P	LP	PM2_Supporting, PS4_M, PS3_Supporting, PP2, PP3	rs199472912
22	LQTS	chr11	2,593,286	KCNQ1	NM_000218:exon5:c.C727A:p.R243S	±	-	0.00(D)	1.00(D)	P	LP	PM2_Supporting, PM5, PM1, PP3	rs199472713

DS diseases, LQTS long QT syndrome, Brs Brugada syndrome, Chr chromosome, 1000G/Esp 1000genomes (2015 version) or Esp6500 database, SNP single nucleotide polymorphism, PP polyphen-2, D damaging, B benign, T tolerated, ± heterozygous carrier, P pathogenic, LP likely pathogenic, - no report

**Table 3** The risk mutations of Brugada syndrome and long QT syndrome

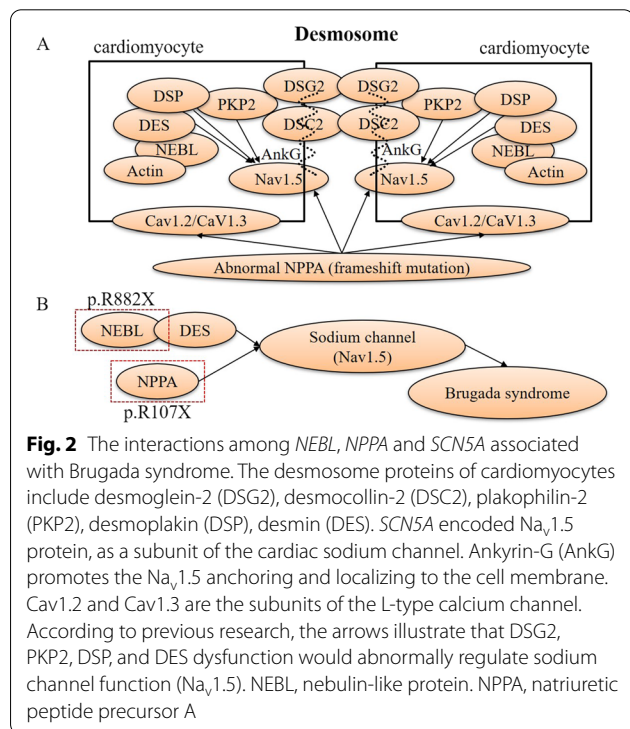
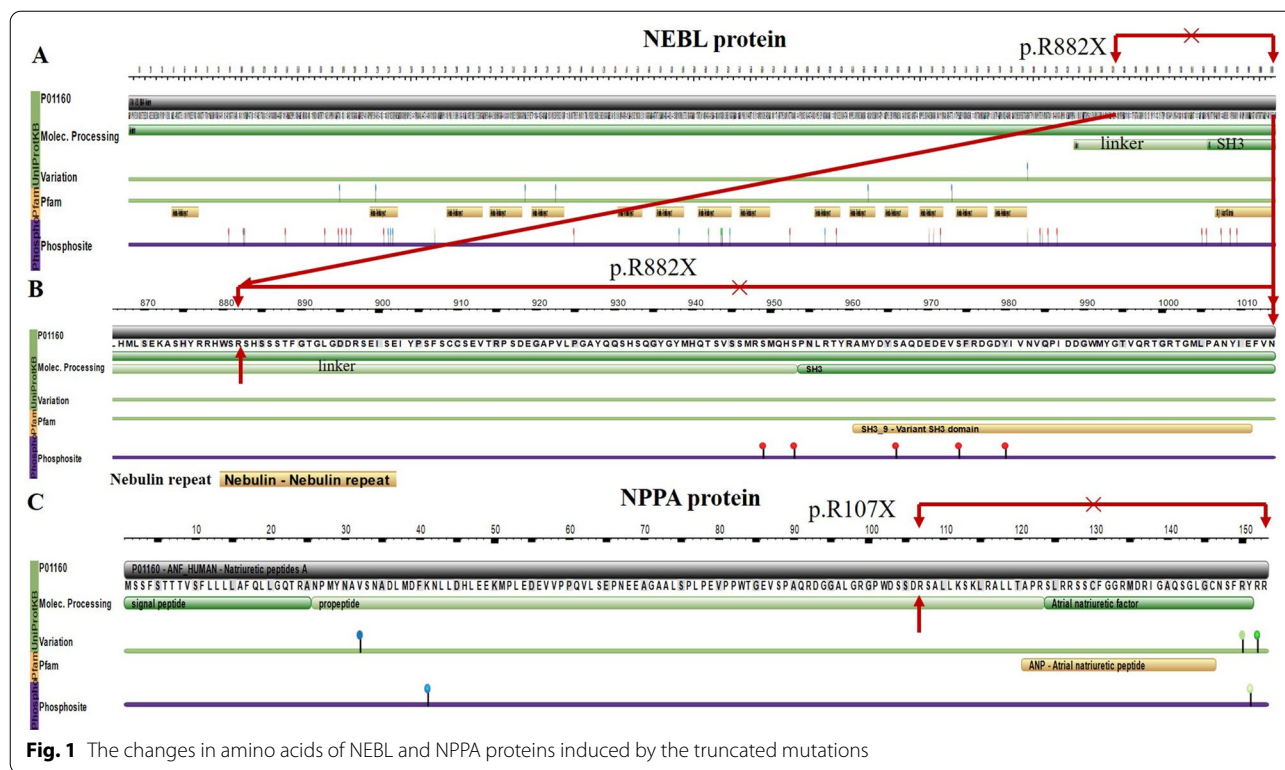
ID	DS	Chr	Start	Gene	Amino acid change	Het	1000 g/Esp	SIFT	Polyphen	Clinic	ACMG	Evidence	dbSNP
1	Bis	chr1	3,342,629	PRDM16	PNM_199454:exon14:c.G3124A:p.G1042R	±	-	0.00(D)	1.00(D)	-	VUS	PM2_Supporting	-
		chr10	21,097,556	NEBL	NM_006393:exon26:c.C2644T:p.R882X	±	<0.001	-	-	VUS	VUS	PM2_Supporting	rs151012132
		chr2	179,447,747	TTN	NM_003319:exon141:c.G38588A:p.R12863Q	±	-	0.04(D)	1.00(D)	VUS	VUS	PM2_Supporting	-
3	Bis	chr2	179,460,249	TTN	NM_003319:exon123:c.A30637G:p.I10213V	±	-	0.36(T)	0.95(D)	-	VUS	PM2_Supporting	rs56025724
		chr21	18,919,405	CXADR	NM_001207063:exon2:c.A104G:p.E35G	±	-	0.05(T)	1.00(D)	-	VUS	PM2_Supporting	-
		chr1	11,907,301	NPPA	NM_006172:exon2:c.C319T:p.R107X	±	-	-	-	-	LP	PM2_Supporting, PVS1	-
4	Bis	chr1	228,467,100	OBSCV	NM_001098623:exon27:c.T7351G:p.F2451V	±	-	0.27(T)	0.93(D)	-	VUS	PM2_Supporting	-
		chr1	228,547,344	OBSCV	NM_052843:exon81:c.C18751T:p.R6251W	±	-	0.02(D)	0.74(P)	-	VUS	PM2_Supporting	-
		chr1	228,559,174	OBSCV	NM_001098623:exon94:c.C20695T:p.R6899W	±	-	0.00(D)	0.64(P)	-	VUS	PM2_Supporting	-
6	Bis	chr2	179,640,347	TTN	NM_003319:exon27:c.G6106A:p.E2036K	±	-	0.27(T)	1.00(D)	-	VUS	PM2_Supporting	-
		chr12	114,793,662	TBX5	NM_080717:exon8:c.C1082T:p.T361I	±	<0.001	0.13(T)	0.46(P)	-	VUS	PM2_Supporting	rs267603320
		chr2	179,432,053	TTN	NM_003319:exon154:c.C5161T:p.S17204F	±	-	0.00(D)	0.84(P)	-	VUS	PM2_Supporting	-
7	Bis	chr15	39,885,760	THBS1	NM_003246:exon19:c.C3158T:p.T1053M	±	<0.001	0.02(D)	0.71(P)	-	VUS	PM2_Supporting	rs267604168
		chr17	39,915,014	JUP	NM_002230:exon9:c.C1606G:p.Q536E	±	-	0.03(D)	0.30(B)	-	VUS	PM2_Supporting	-
		chr3	71,015,109	FOXP1	NM_001244813:exon14:c.C1521G:p.N507K	±	-	0.04(D)	0.83(P)	-	VUS	PM2_Supporting	-
9	Bis	chr19	16,593,346	CALR3	NM_145046:exon7:c.G833A:p.R278H	±	-	0.03(D)	0.00(B)	-	VUS	PM2_Supporting	-
		chr21	18,937,961	CXADR	NM_001338:exon7:c.C1049T:p.A350V	±	-	0.102(T)	0.949(D)	-	VUS	PM2_Supporting	-
		chr4	111,539,442	PITX2	NM_000325:exon3:c.G814A:p.A272T	±	-	0.41(T)	0.95(D)	-	VUS	PM2_Supporting	-
12	Bis	chr5	251,519	SDHA	NM_001294332:exon12:c.A1586C:p.Q529P	±	-	0.02(D)	0.99(D)	-	VUS	PM2_Supporting	-
		chr5	37,333,576	NUP155	NM_001278312:exon13:c.C1507T:p.L503F	±	-	0.01(D)	1.00(D)	-	VUS	PM2_Supporting	-
		chr7	128,481,334	FLNC	NM_001127487:exon12:c.G1924A:p.V642I	±	<0.001	0.82(T)	0.67(P)	-	VUS	PM2_Supporting	rs369387744
14	LQTS	chr10	112,581,622	RBM20	NM_001134363:exon11:c.T3245G:p.L1082R	±	-	0.00(D)	0.08(B)	-	VUS	PM2_Supporting	-
		chr20	33,345,504	NCOA6	NM_001242539:exon7:c.G1047C:p.L349F	±	-	0.02(D)	0.89(P)	-	VUS	PM2_Supporting	-
		chr1	228,467,732	OBSCV	NM_001098623:exon28:c.G7607C:p.G2536A	±	-	0.01(D)	1.00(D)	-	VUS	PM2_Supporting	-
14	LQTS	chr3	38,739,348	SCN10A	NM_001293307:exon26:c.T5069C:p.M1690T	±	-	0.00(D)	0.99(D)	-	VUS	PM2_Supporting	-
		chr3	38,770,058	SCN10A	NM_001293307:exon14:c.C2321T:p.T774M	±	-	0.93(T)	0.02(B)	-	VUS	PM2_Supporting	-
		chr7	150,655,499	KCNH2	NM_000238:exon4:c.563_564del:p.A188Gfs*143	±	-	-	-	-	LP	PM2_Supporting, PVS1	-

**Table 3** (continued)

ID	DS	Chr	Start	Gene	Amino acid change	Het	1000 g/Esp	SIFT	Polyphen	Clinic	ACMG	Evidence	dbSNP
15	Brs	chr14	23,853,757	MYH6	NM_002471:exon36:c.G5459A;p.R1820Q	±	< 0.001	0.01(D)	1.00(D)	–	VUS	PM2_Supporting	rs371222772
		chr19	39,406,284	SARS2	NM_017827:exon16:c.C1519T;p.R507W	±	< 0.001	0.01(D)	0.54(P)	–	VUS	PM2_Supporting	rs143316017
		chr6	152,472,791	SYNE1	NM_033071:exon134:c.C24134T;p.A8045V	±	–	0.12(T)	0.98(D)	–	VUS	PM2_Supporting	–
16	Brs	chr14	74,970,636	LTBP2	NM_000428:exon31:c.4573_4575del;p.1525del	±	–	–	–	–	VUS	PM2_Supporting, PM4_Supporting	–
		chr2	179,453,729	TTN	NM_003319:exon132:c.G35528A;p.R11843Q	±	0.001	0.10(T)	1.00(D)	–	VUS	PM2_Supporting	rs377203669
		chr2	179,455,524	TTN	NM_003319:exon132:c.C33733T;p.R11245C	±	0.001	0.00(D)	1.00(D)	–	VUS	PM2_Supporting	rs200898955
		chr7	140,624,425	BRAF	NM_004333:exon1:c.G79A;p.A27T	±	–	0.57(T)	0.48(P)	–	VUS	PM2_Supporting	–
		chr8	106,573,686	ZFPM2	NM_012082:exon4:c.A397G;p.M133V	±	–	0.60(T)	0.01(B)	–	VUS	PM2_Supporting	rs77117583
17	Brs	chr1	228,547,680	OBSO1	NM_052843:exon81:c.G19087A;p.G6363S	±	< 0.001	1.00(T)	0.02(B)	–	VUS	PM2_Supporting	–
		chr14	23,883,054	MYH7	NM_000257:exon39:c.G5704C;p.E1902Q	±	< 0.001	0.08(D)	0.61(P)	–	VUS	PM2_Supporting	rs187073962
20	Brs	chr6	112,506,509	LAMA4	NM_001105206:exon9:c.A1007G;p.K336R	±	–	0.57(T)	0.52(P)	–	VUS	PM2_Supporting	–

DS diseases, LQTs long QT syndrome, Brs Brugada syndrome, Chr chromosome, 1000G/Esp 1000genomes (2015 version) or Esp6500 database, SIFT single nucleotide polymorphism, PP polyphen-2, D damaging, B benign, T tolerated, ± heterozygous carrier, P pathogenic, LP likely pathogenic, – no report





or sodium channel), natriuretic peptide precursor A and *SCN5A* (or  $Na_v1.5$ , or sodium channel), ANP and *SCN5A* (or  $Na_v1.5$ , or sodium channel), Brugada syndrome and *NPPA* (or ANP, or natriuretic peptide precursor A)". The literature summary related to *NPPA* and  $Na_v1.5$  was as follows (shown in Fig. 2A, B).

*NPPA* encodes ANP, expressed in the embryo's atrial and ventricular myocardium. *NPPA* is also expressed in the adult heart but is downregulated in the ventricles around birth to become restricted to the atria and the ventricular conduction system. In a previous study, for atrial myocyte of transgenic mice carrying a frameshift mutation of *NPPA*, the expression, currents ( $I_{Na}$  and  $I_{CaL}$ ) and action potential duration of cardiac sodium ( $Na_v1.5$ ) and L type calcium ( $Ca_v1.2/Ca_v1.3$ ) channels were significantly reduced. In contrast, the rectifier potassium channel current ( $I_{Ks}$ ) markedly increased compared to the wild type of *NPPA*. The malignant changes induced by the frameshift *NPPA* mutation create an atrial substrate of recurrent AF. It is worth noting that ANP is expressed in the atrium and the ventricle. Especially, ANP expression is more significantly re-induced in the ventricles in response to

pathological cardiac stress, such as cardiac hypertrophy or myocardial infarction [37]. There were also obvious interactions among *NPPA*,  $\text{Na}_v1.5$  and  $\text{CaV1.2/CaV1.3}$  ( $\text{I}_{\text{CaL}}$ ). The truncated *NPPA* may induce Brs through the impact on the function of the sodium channel.

#### **NEBL and NPPA interact and co-expressed with SCN5A**

The genes of cardiac desmosome components include *DSG2*, *DSC2*, *PKP2*, *DSP*, *JUP* and *DES*. We analyzed the protein interactions corresponding to these genes to test our hypothesis. In the existing interaction database, PPI interaction network analysis shows that these genes have significant interaction (Fig. 3A). The genes including *DSG2*, *PKP2*, *DSP* and *JUP* directly interact with *SCN5A*. There is indirect interaction between *NEBL/DES* and *SCN5A*, while *DES* has indirect interaction with *SCN5A* through *DSG2*. *VCL* connects the indirect interaction between *NEBL* and *SCN5A*. Vinculin protein encoded by *VCL* is a cytoskeleton protein related to extracellular matrix adhesion and connection, and its mutation may lead to dilated and hypertrophic cardiomyopathy.

In addition, we downloaded the expression data from six different tissue sources (including ventricular tissue, whole blood, spleen, ovary, lung and liver) from the public database of GTEx, and calculated the correlation of the expression of these genes (including *SCN5A*, *NEBL*, *NPPA*, *DSP*, *DES*, *DSG2* and *PKP2*) in each tissue (Fig. 3B–G). It was found that the expression of these genes had the highest correlation in cardiac tissue. The correlation between *SCN5A* and *NEBL* reached 0.83, and the correlation between *SCN5A* and *NPPA* also reached 0.53. The correlations between *SCN5A* and other genes (including *DSP*, *DES*, *DSG2* and *PKP2*) are significantly positive between 0.41 and 0.94, with high degrees of co-expression and synergy. In other tissues, these genes' co-expression has low or no correlation. Therefore, we verified significant co-expression and protein interaction between *NPPA*, *NEBL*, *SCN5A*, *DSP*, *DES*, *DSG2* and *PKP2* genes.

#### **Discussion**

Our study enrolled twenty-two cases of Brs and LQTS and conducted WES for these cases to explore the potential pathogenic mutations. Interestingly, according to genotype-phenotype, protein interaction and transcriptomic co-expression analysis, we first found that the truncated mutations of *NEBL* and *NPPA* might induce Brs through the abnormal impact on the function of the cardiac sodium channel. Additionally, *SCN5A* (p.R661W, p.R965C and p.A1374S) and *KCNH2* (p.R692Q) may

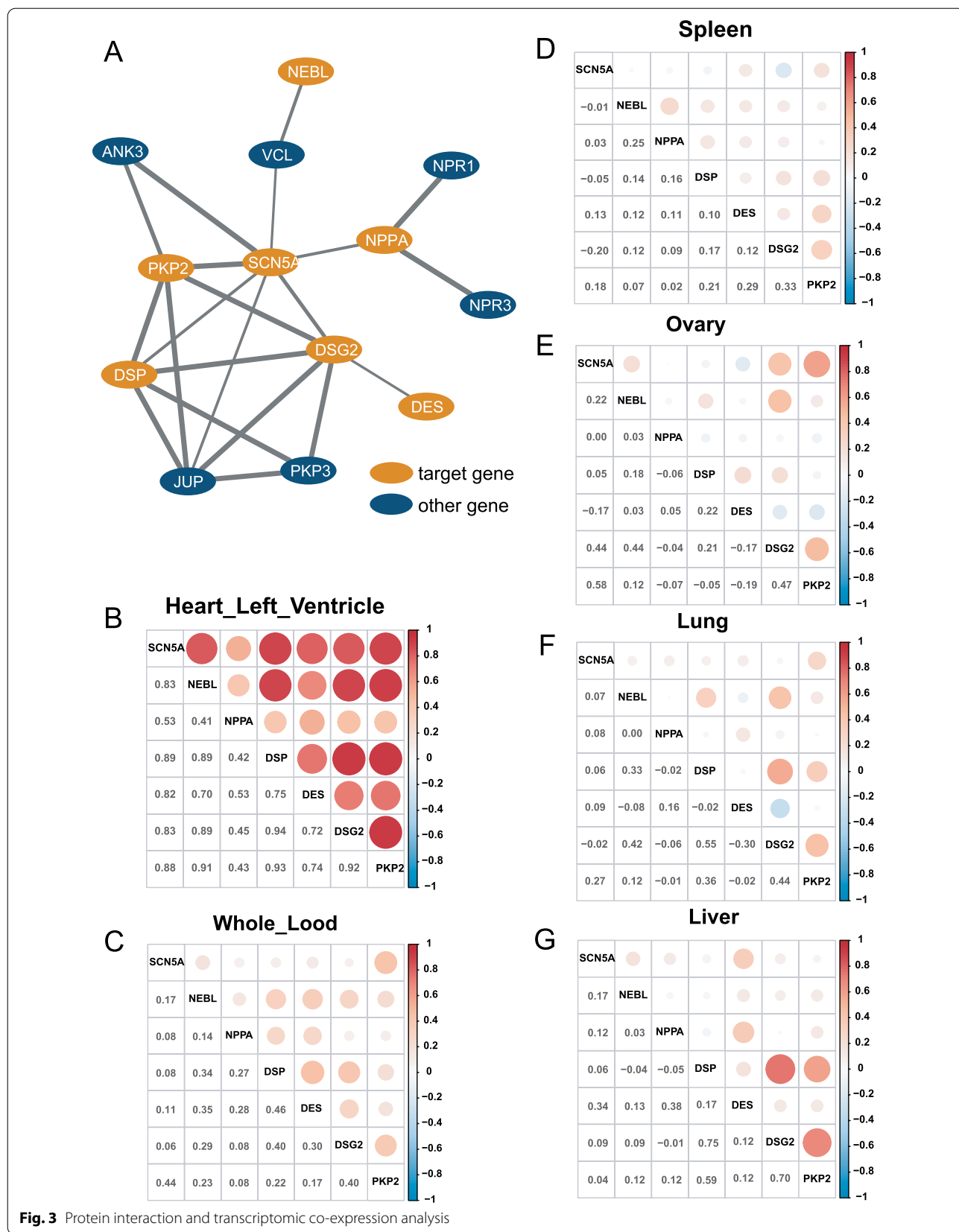
cause Brs, while *SCN5A* (p.R1880H), *KCNQ1* (c.922-1G>C and p.R243S) and *KCNH2* (p.D161N and p.A188Gfs\*143) may lead to LQTS.

#### **NEBL and NPPA mutations may induce Brugada syndrome by aberrantly affecting the cardiac sodium channel**

The cardiac actin-NEBL-DES-Z lines filament network participates in the maintenance of the desmosome junction and the stability of the myocardial structure. As reported before, *NEBL* p.G202R can augment desmosome separation. The *NEBL* p.A592E presents abnormal ultrastructure changes and DES downregulation [38]. A GWAS analysis has revealed that *NEBL* p.A219D (rs2296610) is significantly correlated with AF [39], suggesting that the *NEBL* mutation may probably associate with an increased risk of arrhythmia. *NPPA* mutation has been disclosed to link with familial AF, increasing the risk of AF [40] and stroke (*NPPA* p.V32M) [41]. The heterozygous mutation of *NPPA* p.S64R caused refractory AF due to the augmented potassium current and shortened atrial action potential [42, 43]. The homozygous mutation of *NPPA* p.R150Q is associated with dilated cardiomyopathy with atrial standstill [44]. *NPPA* p.I138T causes AF by activating TNF- $\alpha$ , NF- $\kappa$ B, and IL-1 $\beta$  signaling, inflammation, and fibrosis [45]. The mice with frameshift *NPPA* mutation elicited the most dramatic prolongation of QRS wave, slightly attenuated atrioventricular conduction and ventricular repolarization through the downregulation of the sodium channel in the atrium, ventricle, and atrioventricular junction [46]. In addition, ANP can reduce mRNA expression of  $\text{Na}_v1.5$  in the epithelium [47] and modulate *KCNQ1* expression [48]. Loss-of-function of  $\text{Na}_v1.5$  induced by its abnormalities of expression, trafficking, and location to the membrane, will lead to decreased sodium current, delayed activation, or earlier/faster inactivation, which can thus cause Brugada-like ECG or Brugada syndrome [49]. *NPPA* (p.R107X) and *NEBL* (p.R882X) mutations were identified in Brs patients. Our further analysis showed the indirect interaction between *NEBL* and *SCN5A* and the direct interaction between *NPPA* and *SCN5A*. Interestingly, there are high degrees of co-expressions among *NEBL*, *NPPA* and *SCN5A* in myocardial tissue. Therefore, we proposed that truncated mutations of *NPPA* (p.R107X) and *NEBL* (p.R882X) may induce Brugada syndrome by aberrantly affecting the cardiac sodium channel, similar to loss-of-function of the sodium channel.

#### **The common ionic-channel genetic mutations caused Brugada syndrome and Long QT syndrome**

Our study also identified several pathogenic or likely pathogenic mutations of *SCN5A*, *KCNH2*, and *KCNQ1*



in Brs and LQTs. The mutations of *SCN5A* (p.A1374S, p.R661W, and p.R965C) and *KCNH2* p.R692Q may be associated with Brs, which is consistent with previous studies [50–54]. *SCN5A* p.R965C can cause hyperpolarized inactivation and slower recovery from the inactivation of the sodium channel [55]. However, the mechanisms of how the mutations of *SCN5A* (p.A1374S and p.R661W) and *KCNH2* p.R692Q induce Brs are still unknown. Up to date, there is no functional research on the splicing mutation (c.922-1G>C) [56] and p.R243S [57–62] of *KCNQ1* demonstrated in LQTs. *KCNQ1* p.R243C can induce slower activation and the voltage dependence of activation and inactivation, which may shift to more positive potentials in the  $I_{Ks}$  channel. It can also impair the regulation by PKA and  $I_{Ks}$  channel-PIP<sub>2</sub> (phosphatidylinositol 4, 5-bisphosphate) interactions. Therefore, it increases the risk of life-threatening events while having pronounced benefits from  $\beta$ -blocker treatment [57, 59, 60]. *SCN5A* p.R1880H (or p.R1898H), predicted to be a pathogenic mutation, has been previously reported in LQTs and Brs. It can dramatically reduce the sodium channel current [63, 64] and the abundance of Na<sub>v</sub>1.5 and N-Cadherin clusters at the intercalated disc, which is associated with ACM [65]. *KCNH2* p.D161N (similar to D501N) has been reported in cases of LQTs, even in a five-year-old boy of the ventricular non-compaction with LQTs [63, 66–69]. The *KCNH2* encodes 1159 amino acids of the  $\alpha$ -subunit of voltage-dependent potassium channel mediator for the rapid component of delayed rectifying  $I_{Kr}$  current. For one LQTs case in our study, we also detected a novel and pathogenic frameshift mutation of *KCNH2* (p.A188Gfs\*143). However, more than sixty patterns of frameshift mutations in *KCNH2* have been reported in LQTs [69]. For example, *KCNH2* p.G1006fs\*49 can cause a significant delay in the voltage-sensitive transition to the channel open state, faster-inactivating kinetics, and quicker recovery from the inactivation for the delayed rectifying  $I_{Kr}$  current [70].

#### ***MYH7* and *MYH6* variants were identified in Brugada syndrome**

According to a previous report, *DSG2* and *MYH7* have been identified as new potential Brs candidates [71]. The mutations of *MYH7* have been demonstrated in approximately 25% of patients with the overlap of hypertrophic cardiomyopathy and LQTs. Meanwhile, rare mutations of *MYH6* have also been identified in these patients [72]. In our study, *MYH7* (p.E1902Q) and *MYH6* (p.R1820Q) were predicted as "damaging/possibly damaging" and "damaging/damaging" by Polyphen and SIFT algorithms

and were also identified in cases of Brs. However, whether these two variants cause Brs remains unclear, which needs further confirmation by more research center data and functional research.

#### **Limitations**

The WES of blood DNA from these patients was completed before June 2017. This study was a retrospective study. We did not carry out the verification by Sanger sequencing for these mutations and variants. Our study needs further family genotype–phenotype co-segregation analysis and cell/animal research to investigate how the Brs and LQTs are associated with potential pathogenic mutations of *NEBL*, *NPPA*, *SCN5A*, *KCNH2* and *KCNQ1*.

#### **Conclusions**

In our study, we first reported the indirect interaction between *NEBL* and *SCN5A* and the direct interaction between *NPPA* and *SCN5A*. There are high degrees of co-expressions among *NEBL*, *NPPA* and *SCN5A* in myocardial tissue. The truncated mutations of *NEBL* (p.R882X) and *NPPA* (p.R107X) may induce Brs by abnormally affecting the cardiac sodium channel. *SCN5A* (p.R661W, p.R965C and p.A1374S) and *KCNH2* (p.R692Q) may cause Brs, while *SCN5A* (p.R1880H), *KCNQ1* (c.922-1G>C and p.R243S) and *KCNH2* (p.D161N and p.A188Gfs\*143) may lead to LQTs. Additionally, *MYH7* (p.E1902Q) and *MYH6* (p.R1820Q) were identified in Brs. However, further pedigree and functional research related to these mutations and variants are needed.

#### **Abbreviations**

IPAS: Inherited primary arrhythmia syndromes; VT: Ventricular tachycardia; VF: Ventricular fibrillation; SCD: Sudden cardiac death; Brs: Brugada syndrome; LQTs: Long QT syndrome; WES: Whole exome Sequencing; ECGs: Electrocardiograms; ICD: Implantable cardioverter-defibrillators; SNPs: Single-nucleotide polymorphisms; ACM: Arrhythmogenic cardiomyopathy; PKP2: Plakophilin 2; DES: Desmin; DSP: Desmoplakin; NPPA: Natriuretic peptide precursor A; ANP: Atrial natriuretic peptide; AF: Atrial fibrillation; NEBL: Nebulette; KCNQ1: Potassium voltage-gated channel subfamily Q member 1; SCN5A: Sodium voltage-gated channel alpha subunit 5; MYH6: Myosin heavy chain 6; MYH7: Myosin heavy chain 7; KCNH2: Potassium voltage-gated channel subfamily H member 2.

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#### **Author contributions**

JC, HL, ZY and YBL: whole-exome sequencing, bioinformatics analysis and writing; SCG, YTM and YCC: protein interaction and transcriptomic co-expression analysis; JJZ, HJG, YPL and FW: case collection and follow up; SPS, KH, HY and YBL: quality control of clinical data and clinical design. All authors read and approved the final manuscript.



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### Availability of data and materials

The data used in this study is not publicly available, but it might be available from the corresponding author upon reasonable request and permission from relevant Chinese Authorities.

### Declarations

#### Ethics approval and consent to participate

This study was approved by the Guangdong Medical Institutional Review Board and Medical Ethics Committees [No. GDREC2016001H (R1)]. With the consent of the ethics committee, we followed up with the patients and their family members under the condition of informed consent and obtained blood samples for genetic analysis.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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### References

- Aiba T. Recent understanding of clinical sequencing and gene-based risk stratification in inherited primary arrhythmia syndrome. *J Cardiol*. 2019;73(5):335–42.
- Gando I, Yang HQ, Coetzee WA. Functional significance of channelopathy gene variants in unexplained death. *Forensic Sci Med Pathol*. 2019;15(3):437–44.
- Vutthikraivit W, Rattanawong P, Putthapiban P, Sukhumthammarat W, Vathesatogkit P, Ngarmukos T, et al. Worldwide prevalence of brugada syndrome: a systematic review and meta-analysis. *Acta Cardiol Sin*. 2018;34(3):267–77.
- Schwartz PJ, Stramba-Badiale M, Crotti L, Pedrazzini M, Besana A, Bosi G, et al. Prevalence of the congenital long-QT syndrome. *Circulation*. 2009;120(18):1761–7.
- Ioakeimidis NS, Papamitsou T, Meditskou S, Iakovidou-Kritsi Z. Sudden infant death syndrome due to long QT syndrome: a brief review of the genetic substrate and prevalence. *J Biol Res*. 2017;24:6.
- Arbelo E, Brugada J. Risk stratification and treatment of brugada syndrome. *Curr Cardiol Rep*. 2014;16(7):508.
- Zareba W. Sex and genotype in long QT syndrome risk stratification. *JAMA Cardiol*. 2019;4(3):254–5.
- Aleong RG, Milan DJ, Ellinor PT. The diagnosis and treatment of cardiac ion channelopathies: congenital long QT syndrome and Brugada syndrome. *Curr Treat Options Cardiovasc Med*. 2007;9(5):364–71.
- Al-Khatib SM, Stevenson WG, Ackerman MJ, Bryant WJ, Callans DJ, Curtis AB, et al. 2017 AHA/ACC/HRS guideline for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American heart association task force on clinical practice guidelines and the heart rhythm society. *J Am Coll Cardiol*. 2018;72(14):e91–91e220.
- Mascia G, Bona RD, Ameri P, Canepa M, Porto I, Parati G, et al. Brugada syndrome and syncope: a practical approach for diagnosis and treatment. *Europace*. 2021;23(7):996–1002.
- Garcia-Elias A, Benito B. Ion channel disorders and sudden cardiac death. *Int J Mol Sci*. 2018;19(3):692. <https://doi.org/10.3390/ijms19030692>.
- El-Sherif N, Turitto G, Boutjdir M. Congenital long QT syndrome and torsade de pointes. *Ann Noninvasive Electrocardiol*. 2017;22(6):e12481.
- Wilde AA, Behr ER. Genetic testing for inherited cardiac disease. *Nat Rev Cardiol*. 2013;10(10):571–83.
- Steinberg C. Diagnosis and clinical management of long-QT syndrome. *Curr Opin Cardiol*. 2018;33(1):31–41.
- Shah SR, Park K, Alweis R. Long QT syndrome: a comprehensive review of the literature and current evidence. *Curr Probl Cardiol*. 2019;44(3):92–106.
- Wilde A, Amin AS, Postema PG. Diagnosis, management and therapeutic strategies for congenital long QT syndrome. *Heart*. 2022;108(5):332–8.
- Chen J, Ma Y, Li H, Lin Z, Yang Z, Zhang Q, et al. Rare and potential pathogenic mutations of LMNA and LAMA4 associated with familial arrhythmogenic right ventricular cardiomyopathy/dysplasia with right ventricular heart failure, cerebral thromboembolism and hereditary electrocardiogram abnormality. *Orphanet J Rare Dis*. 2022;17(1):183.
- Lin Y, Huang J, Zhu Z, Zhang Z, Xian J, Yang Z, et al. Overlap phenotypes of the left ventricular noncompaction and hypertrophic cardiomyopathy with complex arrhythmias and heart failure induced by the novel truncated DSC2 mutation. *Orphanet J Rare Dis*. 2021;16(1):496.
- Dong C, Wei P, Jian X, Gibbs R, Boerwinkle E, Wang K, Liu X. Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. *Hum Mol Genet*. 2015;24:2125–37.
- Lin Y, Zhang Q, Zhong ZA, et al. Whole genome sequence identified a rare homozygous pathogenic mutation of the DSG2 gene in a familial arrhythmogenic cardiomyopathy involving both ventricles. *Cardiology*. 2017;138(1):41–54.
- Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet*. 2017;100:267–80.
- Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehml HL. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–24.
- Human genomics. The genotype-tissue expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science*. 2015;348(6235):648–60. <https://doi.org/10.1126/science.1262110>.
- Lin Y, He S, Liao Z, Feng R, Liu R, Peng Y, et al. Whole exome sequencing identified a pathogenic mutation in RYR2 in a Chinese family with unexplained sudden death. *J Electrocardiol*. 2018;51(2):309–15.
- Yang Z, Li T, Xian J, Chen J, Huang Y, Zhang Q, et al. SGLT2 inhibitor dapagliflozin attenuates cardiac fibrosis and inflammation by reverting the HIF-2 $\alpha$  signaling pathway in arrhythmogenic cardiomyopathy. *FASEB J*. 2022;36(7):e22410.
- Lin Y, Huang J, Zhao T, He S, Huang Z, Chen X, et al. Compound and heterozygous mutations of DSG2 identified by whole exome sequencing in arrhythmogenic right ventricular cardiomyopathy/dysplasia with ventricular tachycardia. *J Electrocardiol*. 2018;51(5):837–43.
- Lin Y, Huang J, He S, Feng R, Zhong Z, Liu Y, et al. Case report of familial sudden cardiac death caused by a DSG2 p.F531C mutation as genetic background when carrying with heterozygous KCNE5 p.D92E/E93X mutation. *BMC Med Genet*. 2018;19(1):148.
- Lin Y, Qin J, Shen Y, Huang J, Zhang Z, Zhu Z, et al. Identification of rare heterozygous linkage R965C–R1309H mutations in the pore-forming region of SCN5A gene associated with complex arrhythmia. *Mol Genet Genomic Med*. 2021;9(5):e1613.

29. Hernandez DA, Bennett CM, Dunina-Barkovskaya L, et al. Nebulette is a powerful cytolinker organizing desmin and actin in mouse hearts. *Mol Biol Cell*. 2016;27(24):3869–82.
30. Klauke B, Kossmann S, Gaertner A, et al. De novo desmin-mutation N116S is associated with arrhythmic right ventricular cardiomyopathy. *Hum Mol Genet*. 2010;19(23):4595–607.
31. Kataoka S, Serizawa N, Kitamura K, et al. An overlap of Brugada syndrome and arrhythmic right ventricular cardiomyopathy/dysplasia. *J Arrhythm*. 2016;32(1):70–3.
32. Hoogendijk MG. Diagnostic dilemmas: overlapping features of brugada syndrome and arrhythmic right ventricular cardiomyopathy. *Front Physiol*. 2012;3:144.
33. Novelli V, Malkani K, Cerrone M. Pleiotropic phenotypes associated With PKP2 variants. *Front Cardiovasc Med*. 2018;5:184.
34. Cerrone M, Lin X, Zhang M, et al. Missense mutations in plakophilin-2 cause sodium current deficit and associate with a Brugada syndrome phenotype. *Circulation*. 2014;129(10):1092–103.
35. Sato PY, Musa H, Coombs W, et al. Loss of plakophilin-2 expression leads to decreased sodium current and slower conduction velocity in cultured cardiac myocytes. *Circ Res*. 2009;105(6):523–6.
36. Moncayo-Arlandi J, Guasch E, Sanz-de M, et al. Molecular disturbance underlies to arrhythmic cardiomyopathy induced by transgene content, age and exercise in a truncated PKP2 mouse model. *Hum Mol Genet*. 2016;25(17):3676–88. <https://doi.org/10.1093/hmg/ddw213>.
37. Zhang Z, Zhang Q, Lal H, Nam YJ. Generation of Nppa-tagBFP reporter knock-in mouse line for studying cardiac chamber specification. *Genesis*. 2019;57(6):e23294.
38. Maiellaro-Rafferty K, Wansapura JP, Mendsaikhan U, et al. Altered regional cardiac wall mechanics are associated with differential cardiomyocyte calcium handling due to nebulin mutations in preclinical inherited dilated cardiomyopathy. *J Mol Cell Cardiol*. 2013;60:151–60.
39. Low SK, Takahashi A, Ebana Y, et al. Identification of six new genetic loci associated with atrial fibrillation in the Japanese population. *Nat Genet*. 2017;49(6):953–8.
40. Gudbjartsson DF, Arnar DO, Helgadóttir A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. *Nature*. 2007;448(7151):353–7.
41. Pereira NL, Tosakulwong N, Scott CG, et al. Circulating atrial natriuretic peptide genetic association study identifies a novel gene cluster associated with stroke in whites. *Circ Cardiovasc Genet*. 2015;8(1):141–9.
42. Disertori M, Masè M, Narula N, et al. Atrial fibrillation and NPPA gene p.S64R mutation: are cardiologists helpless spectators of healthy mutation carriers? *J Cardiovasc Med*. 2016;17(3):177–80. <https://doi.org/10.2459/JCM.0000000000000302>.
43. Abraham RL, Yang T, Blair M, Roden DM, Darbar D. Augmented potassium current is a shared phenotype for two genetic defects associated with familial atrial fibrillation. *J Mol Cell Cardiol*. 2010;48(1):181–90.
44. Disertori M, Quintarelli S, Grasso M, et al. Autosomal recessive atrial dilated cardiomyopathy with standstill evolution associated with mutation of natriuretic peptide precursor A. *Circ Cardiovasc Genet*. 2013;6(1):27–36.
45. Cheng C, Liu H, Tan C, Tong D, Zhao Y, Liu X, Si W, Wang L, Liang L, Li J, Wang C, Chen Q, Yimei D, Wang QK, Ren X. Mutation in NPPA causes atrial fibrillation by activating inflammation and cardiac fibrosis in a knock-in rat model. *FASEB J*. 2019;33(8):8878–91. <https://doi.org/10.1096/fj.201802455R>.
46. Menon A, Hong L, Savio-Galimberti E, et al. Electrophysiologic and molecular mechanisms of a frameshift NPPA mutation linked with familial atrial fibrillation. *J Mol Cell Cardiol*. 2019;132:24–35.
47. Luo Y, Xia Q, Xia Z, Tang Y. Atrial natriuretic peptide reduces the  $\alpha$ -subunit of the epithelial sodium channel mRNA expression in the mouse stria vascularis. *Biomed Rep*. 2015;3(2):159–62.
48. Zhang J, Zhao Z, Zu C, et al. Atrial natriuretic peptide modulates the proliferation of human gastric cancer cells via KCNQ1 expression. *Oncol Lett*. 2013;6(2):407–14.
49. Aam W, Amin AS. Clinical spectrum of SCN5A mutations: long QT syndrome, Brugada syndrome, and cardiomyopathy. *JACC Clin Electrophysiol*. 2018;4(5):569–79.
50. Kapplinger JD, Tester DJ, Alders M, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm*. 2010;7(1):33–46.
51. Sommariva E, Pappone C, Martinelli BF, et al. Genetics can contribute to the prognosis of Brugada syndrome: a pilot model for risk stratification. *Eur J Hum Genet*. 2013;21(9):911–7.
52. Yoshikane Y, Yoshinaga M, Hamamoto K, Hirose S. A case of long QT syndrome with triple gene abnormalities: digenic mutations in KCNH2 and SCN5A and gene variant in KCNE1. *Heart Rhythm*. 2013;10(4):600–3.
53. Silva D, Martins FM, Cavaco D, et al. Natural history of Brugada syndrome in a patient with congenital heart disease. *Rev Port Cardiol*. 2015;34(7–8):493.e1–4.
54. Priori SG, Napolitano C, Gasparini M, et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: a prospective evaluation of 52 families. *Circulation*. 2000;102(20):2509–15.
55. Hsueh C-H, Chen W-P, Lin J-L, Tsai C-T, Liu Y-B, Juang J-M, Tsao H-M, Ming-Jai S, Lai L-P. Distinct functional defect of three novel Brugada syndrome related cardiac sodium channel mutations. *J Biomed Sci*. 2009. <https://doi.org/10.1186/1423-0127-16-23>.
56. Murray A, Donger C, Fenske C, et al. Splicing mutations in KCNQ1: a mutation hot spot at codon 344 that produces in frame transcripts. *Circulation*. 1999;100(10):1077–84.
57. Matavel A, Medei E, Lopes CM. PKA and PKC partially rescue long QT type 1 phenotype by restoring channel-PIP2 interactions. *Channels*. 2010;4(1):3–11.
58. Kapa S, Tester DJ, Salisbury BA, et al. Genetic testing for long-QT syndrome: distinguishing pathogenic mutations from benign variants. *Circulation*. 2009;120(18):1752–60.
59. Franqueza L, Lin M, Shen J, Splawski I, Keating MT, Sanguinetti MC. Long QT syndrome-associated mutations in the S4–S5 linker of KvLQT1 potassium channels modify gating and interaction with minK subunits. *J Biol Chem*. 1999;274(30):21063–70.
60. Barsheshet A, Goldenberg I, O-Uchi J, et al. Mutations in cytoplasmic loops of the KCNQ1 channel and the risk of life-threatening events: implications for mutation-specific response to  $\beta$ -blocker therapy in type 1 long-QT syndrome. *Circulation*. 2012;125(16):1988–96.
61. Amin AS, Giudicessi JR, Tijssen AJ, et al. Variants in the 3' untranslated region of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. *Eur Heart J*. 2012;33(6):714–23. <https://doi.org/10.1093/eurheartj/ehr473>.
62. Liu JF, Goldenberg I, Moss AJ, et al. Phenotypic variability in Caucasian and Japanese patients with matched LQT1 mutations. *Ann Noninvasive Electrocardiol*. 2008;13(3):234–41.
63. Napolitano C, Priori SG, Schwartz PJ, et al. Genetic testing in the long QT syndrome: development and validation of an efficient approach to genotyping in clinical practice. *JAMA*. 2005;294(23):2975–80.
64. Hermida JS, Dassonville E, Six I, et al. Prospective evaluation of the familial prevalence of the brugada syndrome. *Am J Cardiol*. 2010;106(12):1758–62.
65. Te RAS, Agullo-Pascual E, James CA, et al. Multilevel analyses of SCN5A mutations in arrhythmic right ventricular dysplasia/cardiomyopathy suggest non-canonical mechanisms for disease pathogenesis. *Cardiovasc Res*. 2017;113(1):102–11.
66. Jongbloed R, Marcellis C, Velter C, Doevendans P, Geraedts J, Smeets H. DHPLC analysis of potassium ion channel genes in congenital long QT syndrome. *Hum Mutat*. 2002;20(5):382–91.
67. Ogawa K, Nakamura Y, Terano K, Ando T, Hishitani T, Hoshino K. Isolated non-compaction of the ventricular myocardium associated with long QT syndrome: a report of 2 cases. *Circ J*. 2009;73(11):2169–72.
68. Nagaoka I, Shimizu W, Itoh H, et al. Mutation site dependent variability of cardiac events in Japanese LQT2 form of congenital long-QT syndrome. *Circ J*. 2008;72(5):694–9.
69. Kapplinger JD, Tester DJ, Salisbury BA, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION long QT syndrome genetic test. *Heart Rhythm*. 2009;6(9):1297–303.
70. De Zio R, Gerbino A, Forleo C, et al. Functional study of a KCNH2 mutant: novel insights on the pathogenesis of the LQT2 syndrome. *J Cell Mol Med*. 2019;23(9):6331–42. <https://doi.org/10.1111/jcmm.14521>.
71. Di Resta C, Pietrelli A, Sala S, et al. High-throughput genetic characterization of a cohort of Brugada syndrome patients. *Hum Mol Genet*. 2015;24(20):5828–35.
72. Allegue C, Gil R, Blanco-Verea A, et al. Prevalence of HCM and long QT syndrome mutations in young sudden cardiac death-related cases. *Int J Legal Med*. 2011;125(4):565–72.

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