Novel *SCN5A* frame-shift mutation underlying in patient with idiopathic ventricular fibrillation manifested with J wave in inferior lead and prolonged S-wave in precordial lead

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Abstract. Mutations in the SCN5A gene has been recognized as resulting in a series of life-threatening arrhythmias. However, it also causes idiopathic ventricular fibrillation (IVF) with J wave in inferior leads and prolonged S-wave upstroke in precordial leads, which has not been previously reported. The present study aimed to study the mechanisms of a patient with IVF manifested with J wave in inferior leads and prolonged S-wave upstroke in precordial leads. The electrocardiograms (ECG) of the proband were recorded and genetic testing was conducted. Patch-clamp and immunocytochemical studies were performed in heterologously transfected 293 cells. The VF attacks was documented in a 55-year-old male proband with syncope episodes. 12-lead ECG shown the transient J wave in the inferior leads and prolonged S-wave upstroke in precordial V1-V3 leads in the same timeframe. Genetic analysis revealed a novel 1 base deletion (G) at position 839 in exon 2 in SCN5A gene (C280S*fs61), which causes a severe truncation of the sodium channel. The functional study revealed that in 293 cells transfected with mutant channel, no sodium current could be recorded even though the immunocytochemical experiment confirmed the truncated sodium channel existed in cytosol. The kinetics of the wild-type (WT) channel were not altered when co-transfected with C280S*fs61 mutant which suggested a haploinsufficiency effect of sodium channel in the cells. The present study identified a novel C280Sfs*61 mutation that caused the 'loss of function' of the sodium channel by haploinsufficiency mechanism. The reduced sodium channel function in the heart may cause conduction delay that may underlie the manifestation of J wave and prolonged S-wave upstroke associated with IVF.

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Abbreviations: ECG, electrocardiogram; ER, early repolarization; ERS, early repolarization syndrome; ICD, implantable cardiac defibrillator; SCD, sudden cardiac death; VF, ventricular fibrillation; WT, wild-type

Key words: idiopathic ventricular fibrillation, early repolarization syndrome, SCN5A mutation, ion-channel disease, sudden cardiac death

Introduction

Idiopathic ventricular fibrillation occurs in patients with structural normal heart and causes unexpected cardiac death (1,2). The primary electrical disorders resulting from ion-channel mutations are believed to play a crucial role. In recent years, early repolarization (ER) or J wave have been reported to be associated with idiopathic VF (3-5), 'Gain-of-function' mutations in KCNJ8 (6,7) and 'loss-of-function' mutations in L-type calcium channel genes, including CACNA1C, CACNB2B, and CACNA2D1 (8), have been identified in patients with early repolarization syndrome (ERS). Several missense mutations in SCN5A gene caused ERS have also been reported (9-11).

Although there are clinical studies that reveal the relationship between ER and higher risk of VF (12,13), the mechanism responsible for the J wave (or ER) and its arrhythmogenesis remains controversial. Coronary-perfused wedge preparation shows that the transient outward current (Ito) mediated transmural voltage gradient resulted in the inscription of J wave (14). Conversely, several clinical non-invasive electrophysiological studies support the hypothesis that J waves may be more strongly associated with a 'depolarization-dependent' abnormality (15,16).

The present study presented the electrophysiological characteristics of an novel frame-shift mutation of C280Sfs*61 in the SCN5A gene in a male proband with IVF and transient appearance of J wave in the inferior leads and prolonged S-wave upstroke in precordial leads.

Methods and materials

Study subject. The present study was approved by the Ethics Committee of Fuwai Hospital (approval no. 080133) and all experiments conformed to the principles outlined in the Declaration of Helsinki. Blood samples were obtained after the patient volunteered to participate in this study and provided written informed consent for publication.

Mutation screening. The genomic DNA was isolated from leukocytes with TIANamp Blood DNA isolation kit (Tiangen Biotech Co., Ltd.) according to the manufacturer's instructions. The known arrhythmia syndrome suspected genes, such as KCNQ1, KCNH2, KCNE1, KCNE2, KCNE3, KCNJ8, CACNA1C, CACNB2, GPD1L, SCN5A, SCN1B and SCN3B, underwent comprehensive open-reading frame/splice site mutational screening using denaturing high-performance liquid chromatography (DHPLC) and verified by direct DNA sequencing as previously described (9,17,18). From March 2015 to September 2015, a total of 200 unrelated healthy Chinese Han individuals (male 56%, female 44%; aged 18-30 years old) consisted of the control group in Fuwai Hospital (Beijing, China).

Site-directed mutagenesis and heterologous expression. The full-length wild-type (WT) human SCN5A cDNA (GenBank ID: NM198056) was subcloned into pcDNA3.1 vector for mammalian expression in the Pathophysiology Laboratory of Fuwai Hospital (Invitrogen; Thermo Fisher Scientific, Inc.). The mutation (C280Sfs*61) was constructed using a QuikChange site-directed mutagenesis kit (Stratagene; Agilent Sumitomo Dainippon Pharma Co., Ltd.) on the WT-SCN5A background, and verified by direct sequencing. 293 cells were transfected with 0.6 μg cDNA of WT or mutant channels using the Effectene method (Qiagen GmbH) (19,20). 293 cells were cultured in DMEM containing l00 ml/l fetal bovine serum. Cells were spread in 6-well plates 24 h before transfection and the density of cells was 70-80% at the time of transfection. The cells were transfected into pEGFP-SCN5A, pEGFPI.100lQSCN5A and pEGFP-SCN5A/pEGFP-L100lQ SCN5A (1:1) plasmids respectively according to the instructions of I. ipofectaIIIi- neTM2000 and then incubated at 37˚C with 50 ml/l CO_2 for 48 h. Then, subsequent experiments were performed. In the coexpression experiments, WT and mutant were transfected at a 1:1 molar ratio. Enhanced green fluorescent protein gene (0.2 μg) was co-transfected and served as an indicator. All experiments were performed 24-48 h after transfection. Over three independent experiments were conducted to confirm the reproducibility of the results.

Patch-clamp recordings. Sodium current was measured using whole-cell patch clamp techniques with Axonpatch 700B amplifiers (Molecular Devices, LLC.) at a room temperature of 22-24˚C (21,22). Pipette resistance ranged from 1.5-2.5 MΩ when filled with recording solution. The bath solution contained (in mmol/l), NaCl 140, KCl 4, CaCl2 1.8, MgCl2 0.75 and HEPES 5 (pH7.4 set with NaOH). The pipette medium contained (in mmol/l), CsF 120, CsCl 20, EGTA 2.0, MgCl2 1.0 and HEPES 5 (pH 7.4 set with CsOH). The standard voltage clamp protocols are presented with the data and as described in detail previously (10).

Immunocytochemistry. The immunocytochemical experiments were performed in 293 cells transfected with WT or mutant SCN5A plasmid as previously described. Briefly, cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Nonspecific binding was blocked with 5% bovine serum albumin (BSA; Beyotime Institute of Biotechnology) in PBS. Then cells were incubated with anti-Nav1.5 N-terminal monoclonal antibody (1:50; Abcam) and anti-Nav1.5 C-terminal polyclonal antibody (1:200; Alomone Labs) overnight at 4˚C (23). Then the cells were incubated with Cy3-conjugated goat anti-rabbit (1:1,000; Jackson ImmunoResearch Laboratories, Inc.) and FITC-conjugated goat anti-mouse (1:500; Jackson ImmunoResearch Laboratories, Inc.) for 1 h at room temperature in the dark. Confocal images were obtained using a confocal laser scanning microscope (FV1000; Olympus Corporation).

Statistical analysis. I_{Na} data were analyzed using Clampfit 10.0 (Molecular Devices, LLC.) and non-linear curve fitting was performed with OriginPro 8.5 software (OriginLab Corporation). Data were presented as the means ± standard error of the mean (SEM). Student's t-test analysis was used for the comparison of two means. All statistical analyses were performed with SPSS, version 17.0. (SPSS Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical evaluation. The proband, a 55-year-old otherwise healthy man suffered from agonal respiration during sleep. After being woken by his family, his respiration became smooth. However, the next day he suddenly lost consciousness when talking with his colleagues at work and was admitted to the local emergency room in Weixian People's Hospital. There was no prior syncope episode and no family history of sudden cardiac death (SCD). In the hospital, six episodes of spontaneous VF (Fig. 1) were recorded during daytime and aborted by immediate external defibrillation. However, the diagnosis is not clear, so the patient sent to Fuwai Hospital with information on previous tests. In Fuwai Hospital, laboratory test revealed normal levels of serum electrolytes and cardiac enzymes. The subsequent echocardiogram and coronary angiography showed structurally normal heart (left ventricular ejection fraction was 61%) and normal coronary arteries.

During the hospitalization, the repeated baseline ECG with sinus rhythm exhibited wide and notched P wave (138 msec) and prolongation of QRS wave (140 msec) without signs of bundle branch block. There were also no signs of QT prolongation (QTc interval, 389 msec). The transient J waves, however, were recorded in the inferior leads (II, III and aVF), and prolonged S-wave upstroke appeared in the precordial leads of V1-V3 in the same time-frame (Fig. 2). Typical type I Brugada ECG was not shown in the repeated recordings. In addition, 24-h Holter recording did not reveal bradycardia, atrioventricular block or other arrhythmias. The metoprolol and potassium magnesium aspartata were administered after discharge. Then, three months later, the patient came in for further consultation. Since the sodium channel blockers, such as ajmaline, flecainide and procainamide, were not available in China, drug challenge was not performed. Although strongly

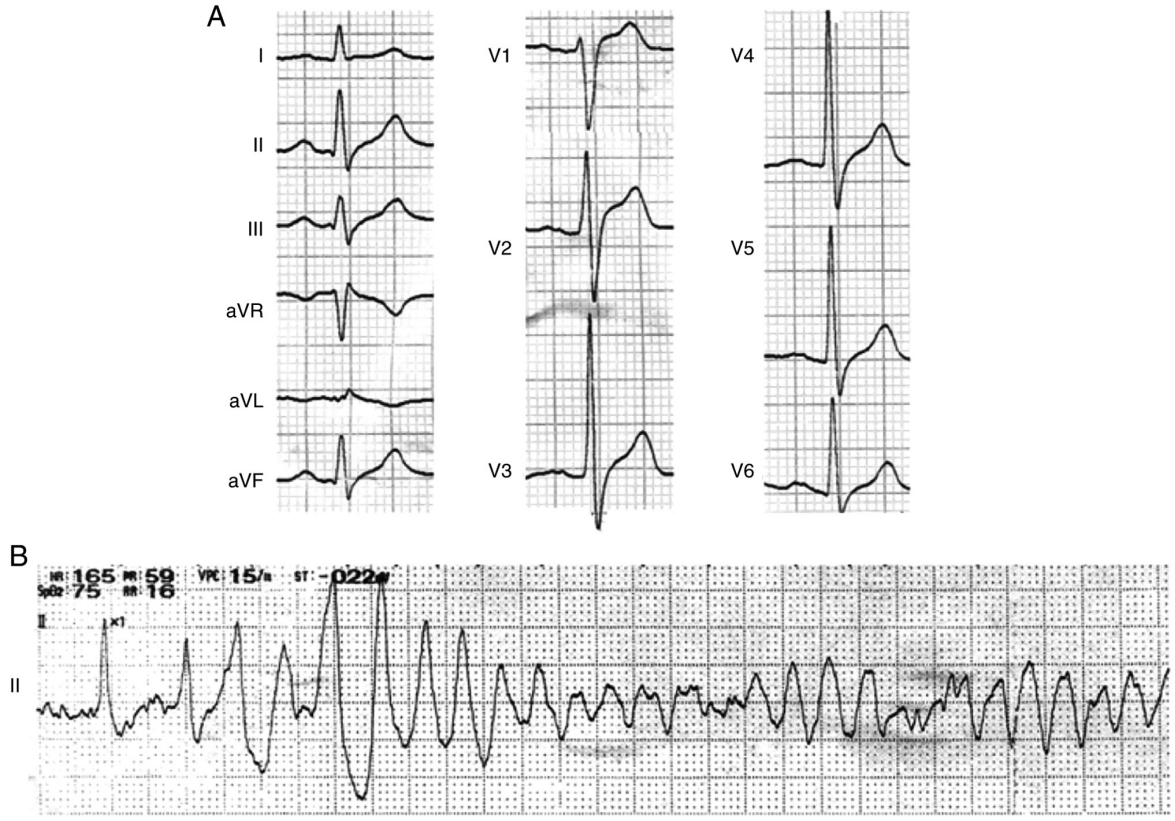

Figure 1. 12-Lead ECG recordings of the proband. (A) The baseline ECG of the proband (10 mm/mV, 25 mm/sec, 62 beats/min) showing a borderline QRS duration of 120 msec, the P-R interval was 184 msec and the QT/QTC was 384/389 msec. (B) ECG monitor strip of a spontaneous onset of ventricular fibrillation recorded in Lead II in a Weixian People's Hospital (Xingtai, China) (10 mm/mV, 25 mm/sec). ECG, electrocardiogram.

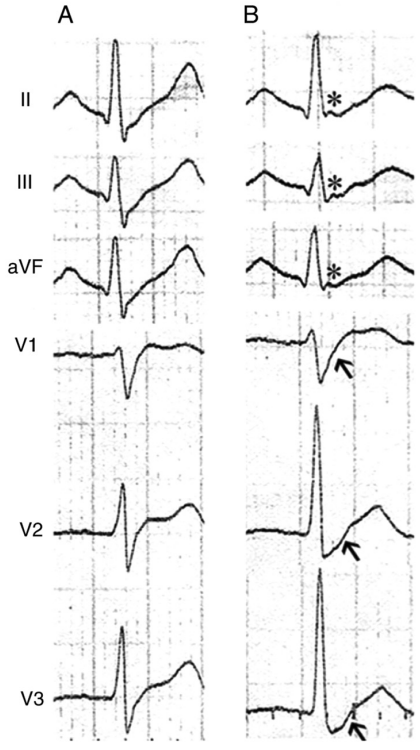

Figure 2. Dynamic changes of the J wave. (A) At baseline routine ECG during the hospitalization (10 mm/mV, 25 mm/sec, 70 beats/min) exhibited a relatively wide P wave (124 msec) and s wave in the inferior leads. (B) In another ECG (10 mm/mV, 25 mm/sec, 74 beats/min), the J wave appeared in leads II, III and AVF (asterisks) accompanied by newly appearing prolonged S-wave upstroke (arrows). ECG, electrocardiogram.

suggested, the patient still refused to receive an implantation of implantable cardiac defibrillator (ICD). Regular one year and a half follow-up showed no subsequent arrhythmic event.

Genetic analysis. A novel heterozygous 1 base (G) deletion at position 839 (c.839delG) locating in exon2 of SCN5A gene was revealed in the proband (Fig. 3). This deletion produced a frame-shift annotated as C280Sfs*61, which indicated the cysteine (C) at position 280 was replaced by serine (S) resulting in 61 amino acids frame-shift before a premature stop codon. Thus, the mutation produced a severe truncated protein which ended at the extracellular domain between segment 5 and 6 in domain I. No other disease-causing mutation was identified in the proband and the mutation was not found in 200 control subjects. Neither his daughter or son inherited this mutation.

Electrophysiological characterization. The transfected 293 cells transiently expressing the Nav1.5-WT or Nav1.5-C280Sfs*61 were voltage clamped after 24-48 h incubation. Given the nature and severity of the truncation mutation, the C280Sfs*61 mutant produced no detectable sodium current as expected, which conformed that the truncated protein was nonfunctional. Fig. 4 showed the representative current traces.

To mimic the heterozygous state of the proband, the I_{Na} was also recorded in cells co-transfected with NaV1.5-WT and NaV1.5-C280Sfs*61 at a 1:1 ratio. Comparing with WT channels, the peak current density of heterozygous state showed a significant reduction by ~55%, but the kinetics of steady-state activation and inactivation were not altered (Fig. 4). Similarly,

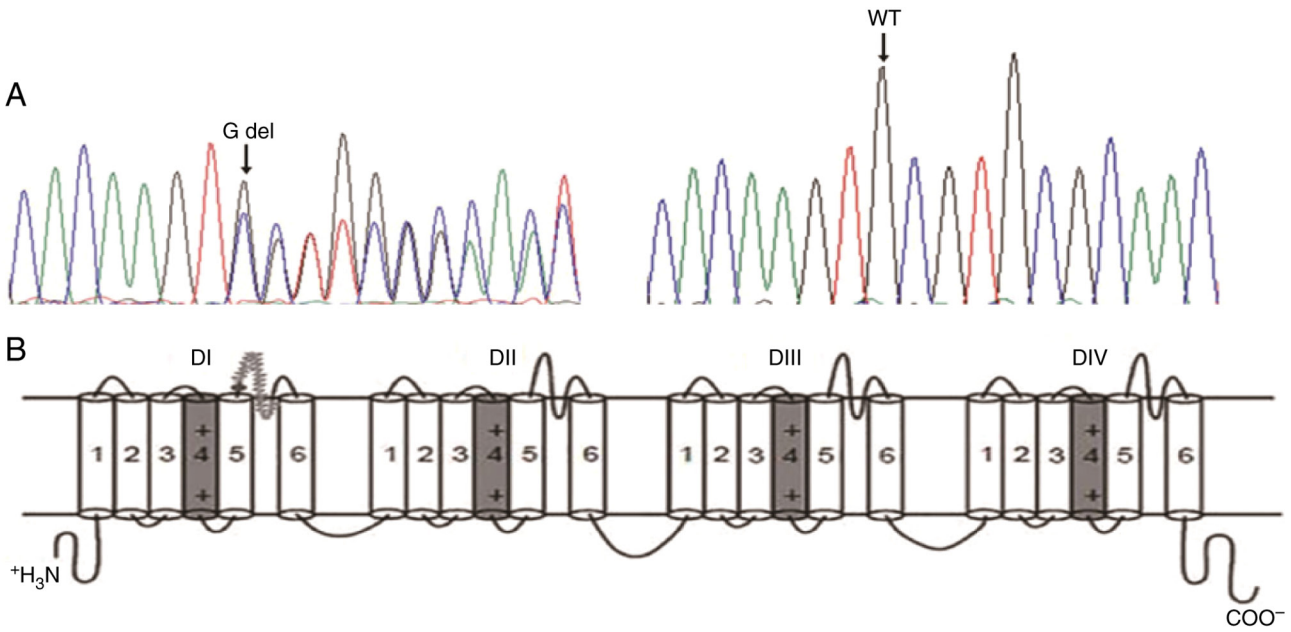

Figure 3. DNA sequence analysis of the proband and schematic representation of the α subunit of the sodium channel. (A) DNA sequence analysis of the proband showing a G base deletion at position 839 of the gene SCN5A, which resulted in the replacement of amino acid cysteine at position 280 by serine followed by a 61 frame-shift amino acids before a premature stop codon. (B) Location of C280Sfs*61 in the predictive topologic structure of the SCN5A channel. Grey circles and zigzag line denote the locations of the mutations.

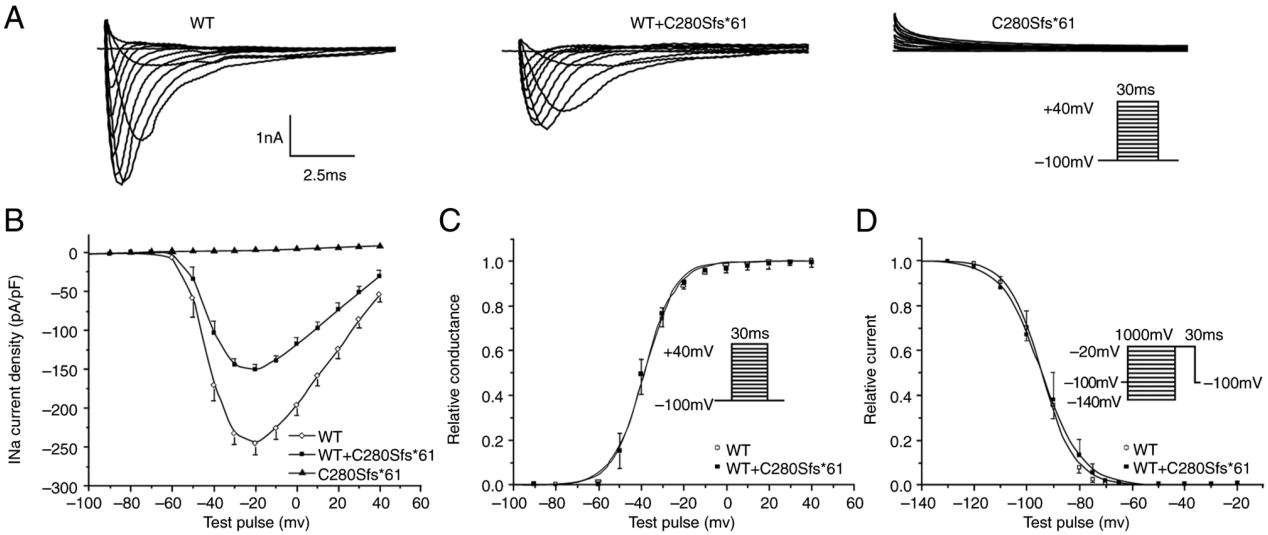

Figure 4. Electrophysiological characterization of Nav1.5 channels. (A) Representative sodium current (I_{Na}) recordings of WT, C280Sfs*61 and WT + C280Sfs*61. (B) Current-voltage relationships for WT, C280Sfs*61 and WT + C280Sfs*61 channels. (C) Voltage-dependence of activation for WT (n=8) and WT + C280Sfs*61 (n=10). The line represents a fit to the Boltzmann function $G_{Na}=[1+\exp(V1/2-V)/\kappa]-1$, where V1/2 and κ are the midpoint and the slope factor, respectively, and $G_{Na}=I_{Na}(norm)/(V-Vrev)$, where Vrev is the reversal potential and V is the membrane potential. The results showed the mutant channel had no effect on the steady state activation. (D) Steady-stage inactivation relationships for WT (n=10) and WT + C280Sfs*61 (n=10) fitted with Boltzmann function: Ina=INa-max[1+exp(Vc-V1/2)/κ]-1, where the V1/2 and κ are the midpoint and the slope factor, respectively, and Vc is the membrane potential. and there was no significant difference between WT and WT + C280Sfs*61 channels. All data points are shown as the mean value, and the bars represent the standard error of the mean. WT, wild-type.

no difference in time-dependent recovery from inactivation was found (data not shown). These results suggested that the haploinsufficiency rather than dominant negative effect of the C280Sfs*61 mutation of the sodium channel was the probable cause of clinical phenotype.

Confocal imaging. To investigate the cellular localization of the WT and mutant channels, immunocytochemical experiments were performed. 293 cells transfected with the WT or C280Sfs*61 channels were double-stained by anti-Nav1.5 N-terminal and anti-Nav1.5 C-terminal antibodies. As expected, cells expressing the mutant channels showed no fluorescence staining identified using the anti-C-terminal antibody, indicating that the truncated peptide of mutant channel was located in the plasma and not functioning (Fig. 5).

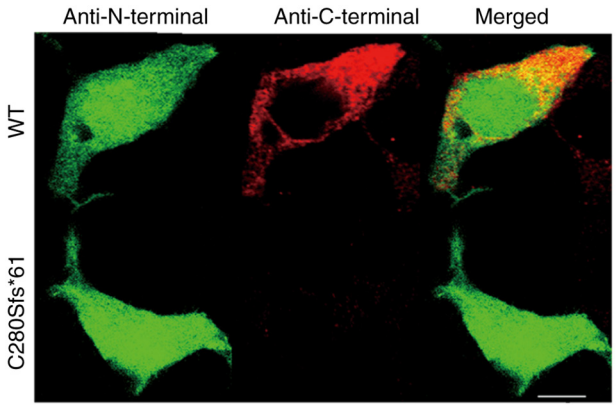

Figure 5. Representative confocal imaging of WT and mutant channels. Each cell was double-stained by anti-Nav1.5 N-terminal antibody (green) and anti-Nav1.5 C-terminal antibody (red). Cells expressing WT channel showed both of the fluorescence, whereas cells transfected with C280Sfs*61 only exhibited the green fluorescence. Scale bar, 10 μm. WT, wild-type.

Discussion

Main findings. In the present study, a novel SCN5A frame-shift mutation of C280Sfs*61 was identified in a patient suffered from spontaneous VF episodes. The patch clamp studies revealed that C280S*fs61 mutant channel failed to produce any sodium current. The ECGs of the proband revealed the distinguishing electrocardiographic anomalies of transient J wave in inferior leads along with prolonged S-wave upstroke in precordial leads. In the absence of type I Brugada ECG and other well-defined electrophysiological disorders, this patient was diagnosed as ERS according to the expert consensus statement (24).

'Loss-of-function' SCN5A mutation. SCN5A encodes the α-subunit of cardiac sodium channel, which drives the impulse conduction in the heart. 'Loss-of-function' in SCN5A is associated with a wide range of inherited arrhythmia syndromes, such as Brugada syndrome, progressive cardiac conduction disease and sick sinus syndrome (25-27). In Brugada syndrome, SCN5A mutations are responsible for 15-20% of the cases (28-30). Among these mutations, frame-shift mutations are less commonly seen as other mutations. In accordance with prior reported early truncated mutations (30-32), the C280S*fs61 mutant channel identified in the present study was not functional. In addition, in the heterozygous state, the current density was reduced by half without affecting the biophysical gating characteristics of WT channel. Accordingly, the truncated C280S*fs61 channel may not be able to reach to cell membrane, but be degraded by nonsense-mediated mRNA decay (NMD) mechanism (9,18).

Depolarization disorder underlying J wave. As a risk factor of SCD, J wave or early repolarization is attracting more attention. The association between J-waves and idiopathic VF has been described in case-control studies (33,34). In the patient of the present study, the transient appearance of J wave in the inferior leads were recorded, meanwhile, a simultaneous remarkable prolongation of the S-wave upstroke in leads V1-V3 was noticed.

Since Miyazaki *et al* described the association between ER and idiopathic VF in the year of 2008 (3), studies have demonstrated that inferior/inferior-lateral location of the J wave is associated with higher risk of ventricular arrhythmias (33-36). However, the mechanisms of J wave formation remains to be elucidated. There are two hypotheses regarding the formation of the J wave: The repolarization hypothesis and the depolarization hypothesis. J-wave can be the phenotypic expression of either delayed depolarization or early repolarization. The mechanism of repolarization suggests that J-waves can result from different distributions and functions of transient outward currents as a consequence of transmembrane repolarization gradients. A late-depolarization J-wave is more likely associated with gene variants in the Na channel, connexins and structural proteins, while mutations in the ion channels carrying Ito, IK-ATP or ICa are more associated with early repolarization (37). With the wedge preparation of canine ventricle, Yan and Antzelevitch (14,38) proposed that, on the basis of uneven level of transient outward current (Ito) in epi- and endocardia wall, a larger inward notch in action potential phase 1 in epicardium was responsible for J-point elevation in surface ECG. However, there are still questions regarding the 'repolarization-disorder theory' (39). Wellens (40) states that 'abnormality at the end of QRS could be interpreted as early repolarization or as delayed activation of depolarization'. Clinicians report that J wave could be depolarization-dependent' (41,42). For example, Abe *et al* (16) shows no association between repolarization parameters (TWA and QTD) and J-wave. However, the late potentials reflecting the abnormal depolarization were commonly observed in ERS patients. In addition, Kamakura *et al* (43) divided the inferior lateral ERS into two groups: ER group A, the inferiolateral ER plus non-type 1 anterior ER (anterior ER consisting of notching or saddleback ST-segment elevation in any of the right precordial leads) and ER group B, the pure inferolateral ER group. They found in ER group A patients that the J waves were augmented by sodium channel blockers and showed saddleback ST-segment elevation in the anterior lead, clinical profiles similar to Brugada syndrome. The positive J-wave responses to sodium channel blockers accompanied with frequent VF episodes in the parasympathomimetic status is considered to indicate a repolarization abnormality. By contrast, in ER group B patients, most J waves were attenuated or disappeared along with QRS prolongation under sodium channel blockers and the VF episodes occurred in an awaken state, which does not seem to indicate the presence of significant transmural dispersion of repolarization in the ventricle. The J waves in this group seem to be an expression of a depolarization abnormality in some ventricular areas, although subsequent ST-segment elevation is explainable by transmural dispersion of repolarization (44).

The prolongation of S-wave upstroke in leads V1-V3 in our patient, which could be a minor diagnostic criteria of arrthymogenic right ventricular cardiomyopathy (ARVC) (45), represented the activation delay or abnormal depolarization of the right ventricle. The co-existence of J wave and prolonged S-wave, to some extent, may support the hypothesis that J wave is associated with abnormal depolarization rather than repolarization.

In the present study, the C280Sfs*61 mutation causing haploinsufficiency of sodium channel has been identified.

In a study of animal model of SCN5A+/- haploinsufficiency mice, the increased fibrosis was more severe in the right ventricle, which may cause conduction delay and prolonged S-wave in right precordal leads (46). Similarly, concealed disarrangement of cardiomyocytes and interstitial fibrosis was also reported in a young man with SCN5A mutation-related ERS (11). Considering the age of our patient, it may also be hypothesized that there concealed abnormalities existed in the right ventricle that may have led to conduction delay and provide the substrate for ventricular fibrillation (46). How loss-of-function of sodium channels explains the manifestation of Brugada or ER syndromes is still not fully understand at the molecular level. Other genetic or environmental factors may be involved in modifying the clinical phenotype (9).

Moreover, Papadotos *et al* (47) reported that slow conduction and significant impairment of impulse propagation were identified in SCN5a+/- mice within the atrium, which is in accordance with the present study, in that the ECG of the proband showed wide and notched P wave with normal atrium size.

The present study identified a novel frame-shift mutation of C280Sfs*61 in SCN5A gene in a patient manifested transient J wave in the inferior leads and prolonged S-wave upstroke in precordial leads follow by ventricular fibrillation. Functional study revealed that the mutation caused haploinsufficiency effect of the sodium channel. The results suggested that the depolarization delay may underly the mechanism of the J wave in the inferior leads and prolonged S-wave upstroke in the precordial leads.

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Availability of data and materials

All data generated and/or analyzed during the present study are included in this published article.

Authors' contributions

JP conceived and designed present study. YC was responsible for administrative and financial support. LR and JP were responsible for the provision of study materials and the patient. LR, JH and YZ were responsible for collection and assembly of data. LR, XZ, YC and JP were responsible for data analysis and/or interpretation. XZ was responsible for writing the manuscript. LR and XZ confirm the authenticity of all the raw data. All authors read and approved the final manuscript. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Fuwai Hospital (approval no. 080133) and all experiments conformed to the principles outlined in the Declaration of Helsinki. The patient volunteered to participate in this study and provided written informed consent for publication

Patient consent for publication

The patient volunteered to participate in this study and provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

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