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Homozygous SCN5A mutation in Brugada syndrome with monomorphic ventricular tachycardia and structural heart abnormalities

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Aims To describe a patient showing monomorphic ventricular tachycardia, ECG aspect of Brugada syndrome, and structural heart abnormalities due to a homozygous missense mutation in SCN5A.

Methods and results Thirteen subjects (six males, seven females, mean age 46 ± 22 years) belonging to the same family underwent physical examination, basal biochemical marker detection, 12-lead ECG, Holter ECG, signal-averaged ECG, echocardiogram and genetic analysis. The proband underwent a stress test together with left and right ventricular angiography and electrophysiological study. Three subjects (the proband, his mother, and one brother) showed on ECG an ST-segment elevation in the right precordial leads with coved type aspect. Moreover, the proband presented a sustained monomorphic ventricular tachycardia (left bundle branch block aspect with superior axis), whereas all other family members were asymptomatic. Imaging techniques documented right ventricular structural abnormalities only in the proband. Mutation screening in SCN5A gene was performed in the proband and in available family members. The proband carries a novel SCN5A mutation, R814Q, in homozygous, whereas the parents and four siblings were heterozygous carriers of the same mutation.

Conclusion This study provides the first evidence of a homozygous missense mutation in SCN5A associated with atypical ventricular arrhythmias and right structural abnormalities.

Introduction

In 1989, Martini *et al.*[1]described a peculiar ECG pattern characterized by right bundle branch block and ST-segment elevation in patients with localized heart structural abnormalities survived of cardiac arrest. Some years later, Brugada and Brugada[2] defined this ECG pattern due to a new distinctive syndrome, called Brugada syndrome (BS) characterized by the high incidence of sudden death in the absence of cardiac pathology.[3] In 1998, BS was found to be linked to mutations in SCN5A gene,[4] encoding for the alpha-subunit of the cardiac sodium channel gene; about 18–30% of subjects with BS were found to carry a SCN5A gene mutation with autosomal dominant inheritance.[3] Thus in the remaining 70–75%, other causes and/or other genes could be involved. In addition, the presence of concealed structural cardiac abnormalities, as part of the phenotype of the BS previously suggested by some authors,[5,6] was later confirmed, at least, in a part of the affected patients.[7–10]

Moreover, mutations of SCN5A gene have been found to be associated also with other arrhythmic disorders (long-QT syndrome and atrioventricular block)[11–15] and even with cardiomyopathies.[16,17]

Patients with BS usually present with polymorphic ventricular tachycardia or ventricular fibrillation. Rarely, a monomorphic ventricular tachycardia has been described.[18–25] Genetic test, when performed,[21,24,25] showed heterozygous mutation in SCN5A gene.[25]

We describe here a patient with BS and monomorphic ventricular tachycardia who, different from previously reported studies, showed a SCN5A mutation in homozygous status associated with heart structural abnormalities.

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Methods

Clinical data

A family composed of 13 subjects (six males, seven females, mean age 46 ± 22 years) underwent physical examination, basal biochemical marker detection, 12-lead ECG, signal-averaged ECG with time-domain analysis (SAECG), 24-h Holter ECG, echocardiogram, and genetic screening for the SCN5A mutation. The family was studied after the investigation of the proband (25-year-old man) admitted to the hospital due to the onset of a sustained monomorphic ventricular tachycardia and in whom baseline ECG showed ST-segment elevation in V1–V2 (coved type) and right bundle branch block, compatible with BS. The proband underwent an exercise stress test and invasive cardiac evaluation with right ventricular angiography and electrophysiological study.[26,27]

The presence of symptoms such as syncope or presyncope, family history of syncopal episodes, and/or sudden death were carefully investigated.

Mutation analysis

Blood samples were obtained after written informed consent. Genomic DNA was isolated from peripheral blood lymphocytes by standard procedures.

Previously published primer pairs were used to amplify all exons of SCN5A gene from genomic DNA.[28] PCR amplicons were screened by direct sequencing, using the Big Dye Terminator chemistry and analysed on an ABI Prism 3730XL DNA sequencer (Applied Biosystems, Foster city, CA). CHROMAS software (release 1.5; Technelsium) and the LASERGENE software package (DNASTAR) were used to edit and assemble sequences. A control group of 200 healthy and unrelated subjects (400 alleles) from the Italian population was used to exclude that the detected mutation could be a common DNA polymorphism. Mutation screening of genes known to be more frequently involved in arrhythmogenic right ventricular cardiomyopathy [desmoplakin (DSP), plakophilin-2 (PKP2), and desmoglein-2 (DSG2)] was performed as previously described.[29–31]

Haplotype analysis

The Genethon microsatellite markers D3S1260 and D3S3521 and the additional markers EF077480, EF077481, EF077482, and EF077483 were PCR-amplified using forward primers labelled with different florochromes, and migrated on an ABI3730XL automated sequencer. Results were analysed by the Genotyper software (Applied Biosystems).

Results

Proband

The proband (III,3 *Figure 1A*) was a 25-year-old man admitted to the hospital due to a sustained monomorphic ventricular tachycardia with left bundle branch block morphology and superior axis (*Figure 2*) terminated with DC shock. Electrocardiogram showed sinus rhythm, prolonged PQ and QRS intervals (right bundle branch block), and ST-segment elevation in V1–V2 close to 2 mm, coved type (*Figure 3*). The patient refused the Flecainide test. Biochemical markers and chest X-ray were under normal limits.

SAECG demonstrated the presence of late potentials at 40 Hz filter-setting (*Figure 4*) and bicycle stress test performed at submaximal exercise (85% of age-predicted maximum heart rate) did not show significant ventricular arrhythmias, whereas it induced an increasing of ST-segment elevation (*Figure 5*).

Two-dimensional echocardiogram demontrated normal left ventricular dimensions and kinetics with the absence of a valve disease. The right ventricle was mildly enlarged (end-diastolic volume: 67 mL/m^2) with localized kinetic alterations on the posterior subtricuspid wall (akinetic bulging) and at the apex (mild dilatation and akinesia). These findings were confirmed by angiographic study, mainly with right anterior oblique and left lateral views (*Figure 6A* and *B*).

Electrophysiological investigation proved an involvement of the main conduction system with a prolonged HV interval (65 ms).[32] The triple extrastimuli technique performed in the infundibular and apical regions of the right ventricle did not induce sustained ventricular arrhythmias.

The patient refused the implantation of a cardioverter defibrillator; therefore he started a therapy with a beta-blocker (Atenolol 100 mg daily). Continuous ECG monitoring performed during antiarrhythmic therapy documented rare and isolated premature ventricular beats, while 24-h ambulatory ECG showed sinus rhythm with sporadic polymorphic ventricular beats. The patient was discharged with beta-blocker therapy and a 6-month clinical and instrumental follow-up was planned. During the follow-up (3 years),

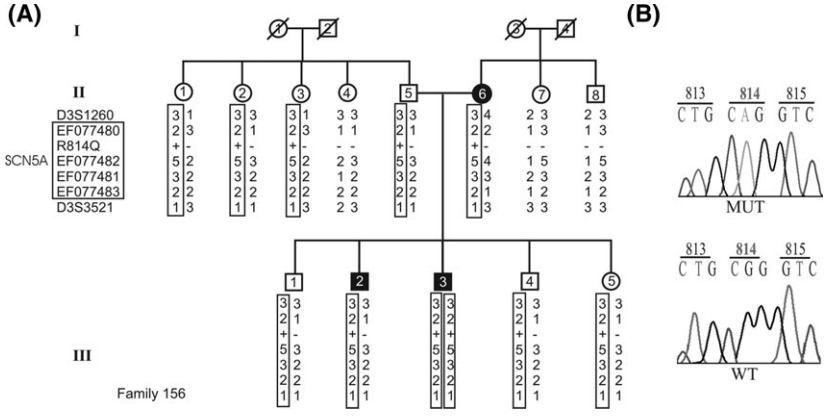

Figure 1 (*A*) Pedigree of family 156. Black and white symbols represent clinically affected and unaffected individuals, respectively. Haplotypes are indicated where DNA was available. The presence (+) or the absence (−) of the SCN5A mutation (R814Q) and six microsatellite markers (D3S1260, EF077480, EF077481, EF077482, EF077483, and D3S3521) are shown below each individual. The common aplotype is boxed. (*B*) Sequence electropherograms of exon 16 show the homozygous missense mutation (MUT) compared with the wild-type (WT).

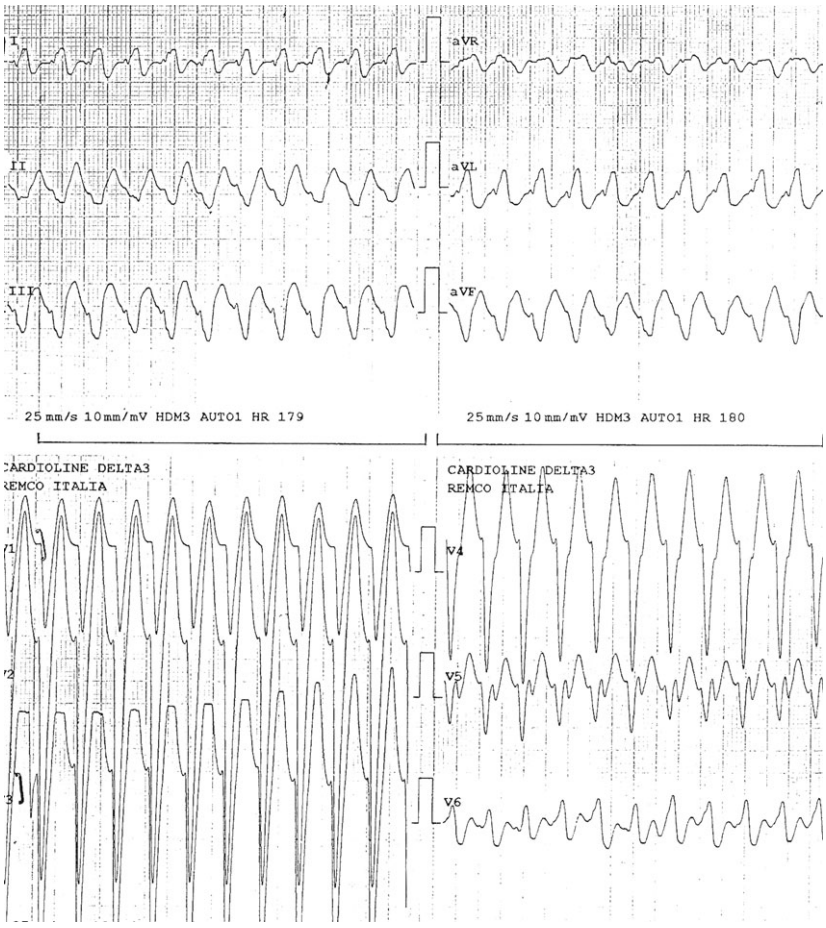

Figure 2 Recordings of the sustained monomorphic ventricular tachycardia documented at the admission in the proband. The arrhythmias shows left bundle branch block morphology and superior axis.

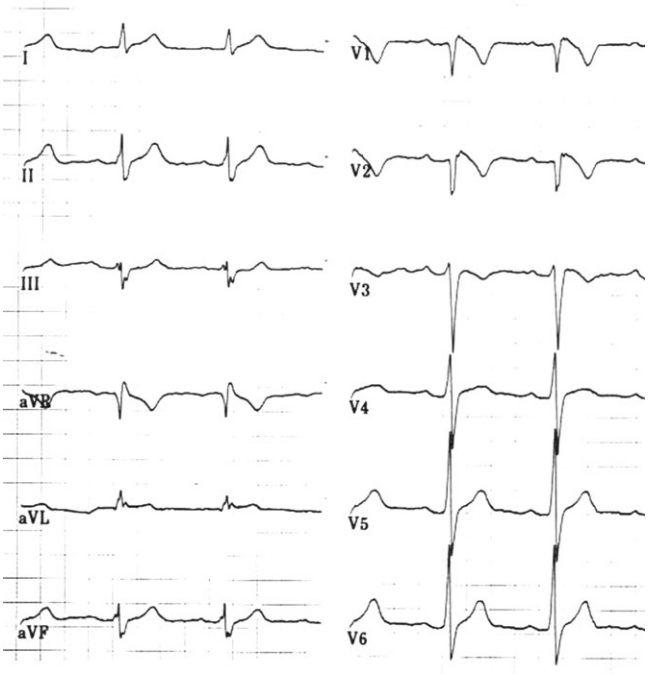

Figure 3 Basal 12-lead ECG of the proband, characterized by sinus rhythm, right bundle branch block, ST-segment elevation (coved-type) and negative T wave in right precordial leads. The ECG showed also P–R interval at the upper limits and left-axis deviation.

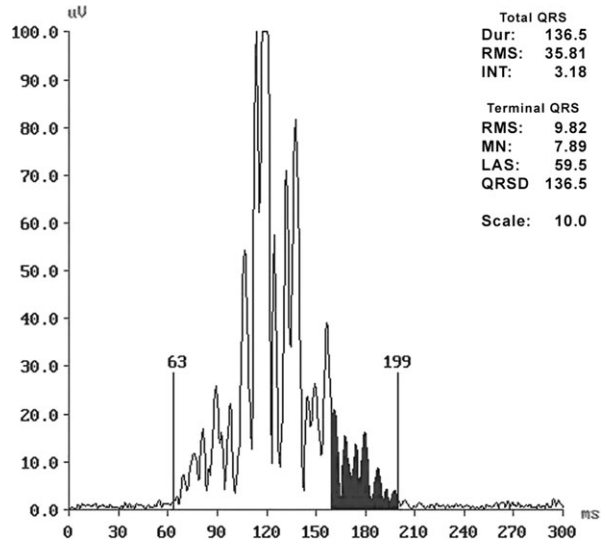

Figure 4 The signal-averaged ECG of the proband, with the presence of late potentials at 40 Hz filter setting.

the patient remained asymptomatic with sporadic premature ventricular beats.

Mutation screening was performed on genomic DNA in the coding sequences and splicing sites of SCN5A, DSP, PKP2, and DSG2 genes by direct sequencing and DHPLC analysis. No pathogenic mutations have been detected in DSP, PKP2, and DSG2 genes. Sequence analysis of exon 16 of SCN5A gene revealed a G-to-A base substitution in homozygous at nucleotide 2441, leading to an amino acid substitution of an arginine by a glutamine at codon 814 (R814Q) (*Figure 1B*). The R814Q mutation lies on the fourth transmembrane segment of domain II (DIIS4), the electrically charged segment that probably is the voltage-sensing region of the domain, characterized by a series of positively charged amino acids at every third position.[33] The detected nucleotide change was absent in 200 control individuals (400 chromosomes).

Family members

Family history reported an unexplained juvenile death (a female cousin of subject II,6 who died suddenly at the age of 20). No clinical or autoptic data were available.

All family members were asymptomatic with normal physical examination and biochemical markers.

The proband's mother (II,6 *Figure 1A*) and one brother (III,2 *Figure 1A*) showed ECG features characterized by ST-segment elevation in right precordial leads and right bundle branch block suggestive for BS (*Figure 7A* and *B*, respectively). The mother's ECG features were enhanced with Flecainide test. SAECG demonstrated the presence of late potentials at 40 Hz filter setting only in the brother (III, 2) (*Figure 7C*).

Subjects II, 3 and II, 4 (65 and 72 years old, respectively) were found to be affected by a coronary heart disease with localized left ventricular kinetic alterations in the site of previous myocardial infarction.

The remaining family members had normal baseline ECG, SAECG, ambulatory ECG, and 2D-echocardiogram.

Family members (II,1; II,2; II,3), the parents and the siblings (III,1; III,2; III,4; III,5) were heterozygous for the same SCN5A mutation (R814Q) previously found in the proband. Then, we looked for a founder effect by performing haplotype analysis on chromosome 3 containing the SCN5A locus with six microsatellite markers. The same haplotype was transmitted by the parents to the proband, which was homozygous for all the microsatellite markers (*Figure 1A*). As the intragenic haplotype is the same, this result suggests a founder effect.

The conduction values at baseline ECG of heterozygous carriers (PQ: 0.20 ± 0.02, QRS: 0.12 ± 0.01 ms) were compared with those of non-carriers (PQ: 0.16 ± 0.01, QRS: 0.10 ± 0.01), and the *P*-values were 0.08 and 0.05, respectively.

Discussion

Heterozygous mutations of SCN5A gene are involved in several cardiac diseases, such as BS, LQT3, isolated cardiac conduction defects, and dilated cardiomyopathy.[34] In the present study, we describe a novel SCN5A missense mutation, R814Q, in the S4 transmembrane segment, identified at the homozygous state in a symptomatic male with sustained monomorphic ventricular tachycardia.

The mutation reported in the present study involves a residue located within a highly conserved domain and consists of a glutamine substitution for the third basic residue in S4 of the second domain (D2), counting from the extracellular (amino) end of S4. The highly charged S4 segments in each domain are postulated voltage sensors for gating. Chen *et al.*[35] made a series of charge-neutralizing or reversing substitutions for basic residues in the S4 segments of each domain of human SCN5A and examined their consequences on the gating of the channel in *Xenopus* oocytes. One of the examined substitutions was R814Q and its effect was a reduction of the net positive charge of the D2 domain and a significant decrease in the voltage dependence of the activation. Moreover, R814Q mutant gave alteration in the slopes and mid-points of steady-state inactivation. These results indicate effects on both activation and inactivation of Na+ channel potentially leading to reduced sodium channel current. The differences in phenotype among family members seem to be in favour of a more important effect on the gating of the channel in the homozygote status compared with the heterozygote. The proband carrying the mutation in homozygosis showed the ECG alteration typical of the BS, a sustained monomorphic ventricular tachycardia with left bundle branch block morphology and a cardiac pathology of the right ventricle. However, no mutations in known ARVC genes where identified. The other family members carrying the mutation in heterozygosis were asymptomatic and did not show any structural cardiac alterations. The analysis of conduction parameters at baseline ECG showed minor abnormalities (prolonged PQ and QRS intervals) in heterozygote carriers compared with non-carriers, due to reduction of sodium current for the heterozygous status. Nevertheless, within the carriers, only two individuals showed ECG abnormalities characterized by ST-segment elevation with coved type aspect in right precordial leads.

The proband of the family presented a sustained monomorphic ventricular tachycardia with left bundle branch block morphology, that could be in keeping with

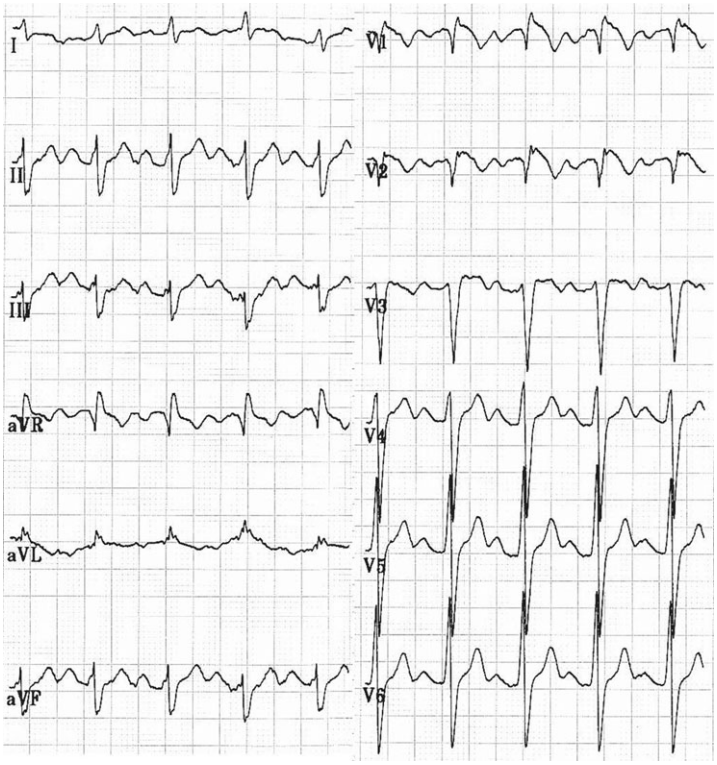

Figure 5 ECG recorded during bicycle stress test (proband). The trace shows an enhancement of ECG abnormalities documented at baseline, with particular regard to ST-segment elevation and right bundle branch block.

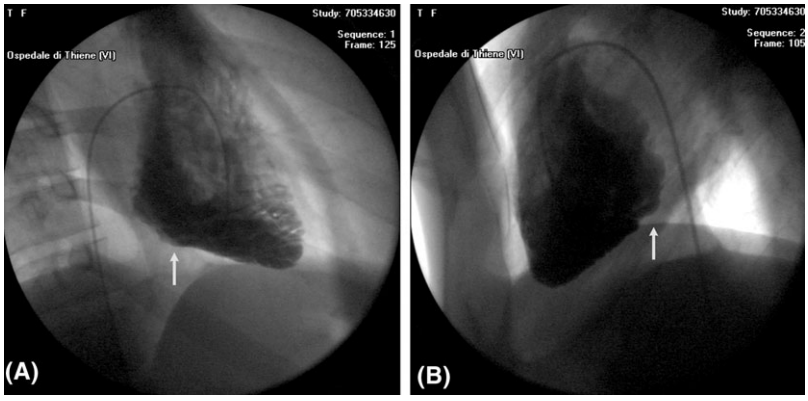

Figure 6 (*A*) Angiographic RAO view of the right ventricle (proband) that shows an akinetic bulge localized on the posterior subtricuspid wall. (*B*) Angiographic left lateral view of the right ventricle (proband). The presence of an akinetic bulge in the posterior subtricuspid wall is confirmed.

the re-entrant circuit due to the presence of an organic substrate characterized by the contiguity of areas of myocardium with normal conduction and areas with low conduction. It is noteworthy that this arrhythmia was different from the typical polymorphic ventricular arrhythmias usually detected in BS patients. Moreover, exercise stress test provoked an increase of the ST-segment. The response to exercise of the ST elevation in the BS is variable, even if usually the exercise decreases the amplitude of ST-segment.[36] The increase of the ST-segment during exercise has been already described.[37] Bezzina *et al.*[12] reported an inherited C-terminal SCN5A mutation (1795insD) causing QT-interval prolongation at slow heart rates and distinctive ST-segment elevation with exercise in affected individuals. Veldkamp *et al.*[38] showed that the mutation altered both the fast and slow components of inactivation. In particular, it induces depolarized Na+ channels to undergo excessive slow inactivation during the sustained depolarization period intrinsic to the cardiac action potential. This abnormal behaviour reduces Na+ channel availability primarly at rapid heart rates and underlies the pronounced ST-segment elevation seen in carriers during exercise.

It is noteworthy that the mutation in the same amino acid (R814) was reported by Olson *et al.*,[39] leading to dilated cardiomyopathy and atrial fibrillation. This was a *de novo* mutation (R814W) identified in a young woman with

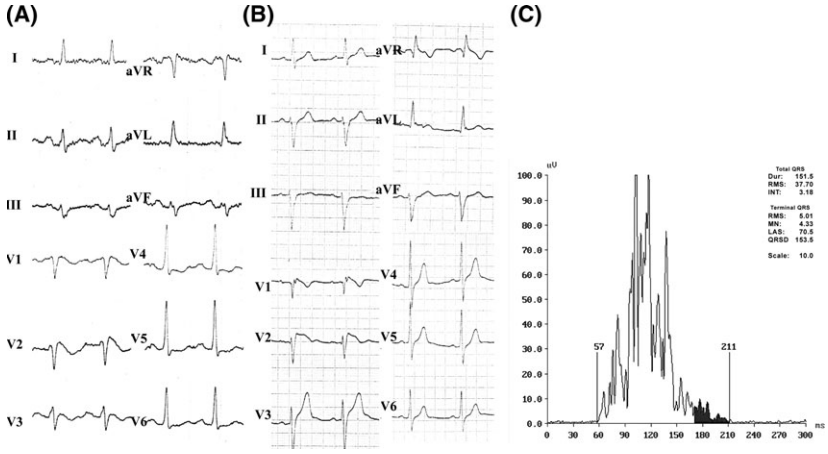

Figure 7 (*A*) Subject II,6 (mother of the proband). The basal 12-lead ECG showed sinus rhythm, ST-segment elevation, right bundle branch block, and negative T-wave in right precordial leads. A left-axis deviation has also been present. (*B*) Subject III,2 (brother of the proband). The ECG showed similar characteristic of his mother. In particular, there is a more emphasized left-axis deviation. (*C*) Subject III,2 (brother of the proband). The signal-averaged ECG showed late potential at 40 Hz filter setting.

dilated cardiomyopathy, atrial flutter, and short runs of sustained ventricular tachycardia. Cardiac biopsy revealed mild-to-moderate myocellular hypertrophy and mild interstitial fibrosis.

Up to now, only one homozygous SCN5A gene mutation (V1777M) has been described, linked with long-QT syndrome and functional two-to-one atrioventricular block (2:1 A-V block).[40] The proband presented an homozygote status, whereas the parents and two siblings were heterozygote carriers. Functional studies demonstrated that residual sodium current was more important in the homozygote model compared with the heterozygote model. Long-QT with 2:1 A-V block conduction has been reported often in infants or young children with a major QTc prolongation, but without any positive family history.

Bezzina *et al.*[41] described a case with two sodium channel abnormalities (compound heterozygosity), also leading to a severe cardiac phenotype. In particular, conduction was affected and structural abnormalities have been demonstrated.

The mechanisms by which similar or identical SCN5A mutations lead to variable expression of heart disease remain unknown. Indeed, it has been shown that the same mutation can cause either isolated cardiac conduction defect or BS within the same family.[15]

As far as the therapeutic strategy is concerned, in our study, asymptomatic family members with diagnostic ECG did not use any drug. On the contrary, in the proband, we suggested an implantable cardioverter defibrillator that was refused by the patient. Thus, he started a beta-blocker therapy that seems to be effective so far, even if the follow-up is still too short to evaluate its efficacy.

In conclusion, this study suggests that homozygous mutations in SCN5A gene may be associated with an atypical BS phenotype showing monomorphic ventricular tachycardia and structural heart abnormalities.

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