Compound Heterozygosity for Mutations (W156X and R225W) in SCN5A Associated With Severe Cardiac Conduction Disturbances and Degenerative Changes in the Conduction System


Abstract—Cardiac conduction defects associate with mutations in SCN5A, the gene encoding the cardiac Na\(^+\) channel. In the present study, we characterized a family in which the proband was born in severe distress with irregular wide complex tachycardia. His older sister died at 1 year of age from severe conduction disease with similarly widened QRS-complexes. Mutational analysis of SCN5A in the proband demonstrated compound heterozygosity for a nonsense mutation (W156X), inherited from the father, and a missense mutation (R225W), inherited from the mother. Genotyping on DNA extracted from tissue from the deceased sibling revealed the same SCN5A genotype. Injection of cRNA encoding the W156X mutation in Xenopus oocytes did not produce any current. The R225W substitution neutralizes the third Arg residue within the voltage-sensing segment of domain I. Expression studies showed that this mutation leads to a severe reduction in \(I_{\text{Na}}\) and is also associated with gating changes. Histological examination of the heart from the deceased sibling revealed changes consistent with a dilated type of cardiomyopathy and severe degenerative abnormalities of the specialized conduction system. The occurrence of compound heterozygosity for these two mutations implies that the proband carries solely severely dysfunctional cardiac Na\(^+\) channels. This explains his severe phenotype and that of his deceased sister who had been a carrier of the same genotype. The morphological changes within the heart of the deceased sibling may have occurred secondary to the Na\(^+\) channel abnormality and contributed to the severity of the disorder in this individual. (Circ Res. 2003;92:159-168.)

Key Words: arrhythmia ■ conduction ■ ion channels ■ electrophysiology ■ cardiomyopathy

Isolated cardiac conduction disease (in absence of gross structural heart disease) can affect various regions within the heart. The disorder has been linked to mutations in SCN5A, which encodes the pore-forming subunit of the cardiac Na\(^+\) channel, and to 2 chromosomal locations (19q13.2-13.3;1,2 16q23-24) at which the involved gene is yet unidentified. One of the SCN5A mutations linked to conduction disease is a splice-site mutation (IVS22+2T>C), described in a family with a progressive form of the disorder.\(^4\) The other mutations, which are associated with a conduction defect from birth, are a deletion (5280delG) leading to premature truncation of the protein\(^4\) and a substitution at codon 514 (G514C).\(^5\) Mutation in the SCN5A gene is also associated with two other distinct clinical presentations, Brugada syndrome and Long QT syndrome (LQTS type 3).\(^6\) Conduction defects are often part of the clinical picture in these disorders.\(^7-9\)

In this study, we characterized a family with a severe conduction disorder presenting from birth, wherein two SCN5A mutations were found to segregate. The effects of these were studied by heterologous expression in Xenopus oocytes. We demonstrate that the occurrence of compound heterozygosity for these mutations very likely accounts for the severity of the disorder in the proband and his deceased sibling. Histological examination of the heart from the deceased sibling revealed degenerative abnormalities, which likely occurred secondary to the Na\(^+\) channel abnormality and are expected to have contributed to the severity of the disorder in this individual. This is the first report of compound heterozygosity in SCN5A, and the first to associate SCN5A mutations to degenerative cardiac abnormalities.

Materials and Methods

Clinical Data

The study was performed according to a protocol approved by the local ethics committee of the AMC, Amsterdam. Informed consent
was obtained from the parents. The index patient came to our attention immediately after birth. Twelve lead ECGs (paper speed 25 mm/s) were taken at rest and in supine position.

Pathology

Permission for a full autopsy excluding the brains was obtained. Description of pathology is limited to the heart. After careful inspection of the heart for gross abnormalities, photography, and documentation of heart weight, fresh tissue samples were taken from both ventricles and frozen in liquid nitrogen. After fixation in 4% buffered formalin, full thickness tissue blocks were taken from both ventricles and a block containing the AV node area and proximal parts of the bundle branches. After paraffin embedding, 6-μm sections were stained with Hematoxylin and Eosin and Elastic van Gieson stains, respectively. The block containing specialized tissue of the conduction system was serial sectioned, and at 50-μm intervals, sections were mounted for staining.

SCN5A Haplotype Analysis

This was done by genotyping of intragenic SCN5A polymorphisms (Figure 3C).

Mutation Analysis

Coping regions of NKX2.5, GJA5, GJA1, GJA7, KCNE1, and SCN1B were analyzed by DNA sequencing. Mutation analysis of SCN5A was done by single strand conformation polymorphism analysis followed by sequencing of aberrant conformers as described previously.10 LMNA (lamin A/C) was analyzed by denaturing gradient gel electrophoresis except for exon 1, which was analyzed by sequencing.

Isolation of Genomic DNA From Fixed Tissue

DNA was isolated from paraffin-embedded heart tissue using the QIAamp DNA Mini Kit (Qiagen).

Functional Expression

Mutant Na⁺ channel cDNAs were prepared by mutagenesis on the pSP64T-hH1(sp) plasmid11,12 using the QuikChange kit (Stratagene). Extensive sequence analysis was performed to ensure that the selected clones were free of polymerase errors. Wild-type (WT) and mutant constructs were linearized, and cRNAs were synthesized from the selected clones free of polymerase errors. Wild-type (WT) and mutant constructs were linearized, and cRNAs were synthesized from the selected clones free of polymerase errors. Wild-type (WT) and mutant constructs were linearized, and cRNAs were synthesized from the selected clones free of polymerase errors. Wild-type (WT) and mutant constructs were linearized, and cRNAs were synthesized from the selected clones free of polymerase errors. Wild-type (WT) and mutant constructs were linearized, and cRNAs were synthesized from the selected clones free of polymerase errors.

Electrophysiology

Stage V-VI Xenopus laevis oocytes were isolated and injected with 5 to 45 ng of WT or mutant hH1 cRNA using a Nanoject Oocyte Injector (Drummond Scientific) according to standard methods.13 For coexpression of the Na⁺ channel with the connexin (Cx) 40 (GJA1), Cx43 (GJA3), and Cx45 (GJA7) were screened for mutations in the proband.

Results

Clinical Features

The index patient (Figure 1, III-3; at birth: length, 49.5 cm; weight, 3050 g) presented immediately after birth in severe distress secondary to broad complex tachycardia with low output (Figure 2A). The tachycardia was slightly irregular (CL 280 to 320 ms), frequently terminated spontaneously, but always resumed after 1 or 2 sinus node beats. In the presence of severe underlying conduction disease (see following section) the origin of the tachycardia, ie, whether origin was ventricular or supraventricular, could not be determined with certainty. On infusion of lidocaine (3 mg bolus), all ventricular activity ceased and broad P-waves remained (not shown). The patient was successfully resuscitated. Adenosine (200 μg bolus) terminated the tachycardia instantly (Figure 2B) and broad P-waves followed by broad QRS complexes appeared for 30 seconds after which the tachycardia resumed. This response was taken as suggestive of c-AMP-dependent arrhythmia,18 and β-blockade was successfully installed and maintained till today. The boy is now 6.5 years old and has not experienced any further episodes of tachyarrhythmias. Serial ECG analysis shows that conduction delay is progressive at both the atrial and ventricular levels (Figure 2C).

Echocardiographic analysis of the boy showed that he was born with a small muscular ventricular septal defect (VSD). The index’s sister (Figure 1, III-2) was born 2 years earlier with the same conduction disorder but initially without significant arrhythmias. At the end of her first year of life, conduction worsened, 2:1 AV block developed, and isoproterenol infusion was given. Subsequently, she developed severe arrhythmias alike the one shown in Figure 2A (respectively, this is in agreement with the working hypothesis concerning the arrhythmogenic mechanism described above). The arrhythmia proved to be intractable and she died shortly after. The oldest sister (III-1) is now 9 years old. She is in good health. Her ECG is normal (Figure 1).

Both parents are without any symptoms and their family histories are unremarkable with regard to pacemaker therapy and/or sudden cardiac death. Their ECGs are virtually normal with exception of a left axis deviation in the father (Figure 1). Based on 24-hour recordings, range of heart rate for the mother and father were 49 to 165 and 36 to 111 bpm, respectively (no arrhythmias were recorded in both cases). Both had no limitations with exercise (maximum heart rate reached: mother, 178 bpm; father, 158 bpm). Echocardiographically, the hearts of both parents were normal, except for mitral valve prolapse of the posterior leaflet and mild mitral regurgitation in the mother.

Genetic Analysis

Because gap junction channels are important for conduction, the genes encoding connexin (Cx) 40 (GJA5), Cx43 (GJA1), and Cx45 (GJA7) were screened for mutations in the proband. No mutations were detected. Sequencing of the minK gene (associated with the conduction system),19 and the gene for the Na⁺ channel β₁ subunit, also revealed no mutations. Mutations in the NKX2.5 (OMIM 108900) and LMNA...
Figure 1. Pedigree of the family with ECGs. ECG of the index (III-3, at 2 days old) shows sinus rhythm (rate 130 bpm), indeterminate electrical axis, and prolonged conduction intervals (P-wave duration, 160 ms; PR interval, 240 ms; QRS, 140 ms). T-wave morphology is accordingly abnormal. ECG of his (deceased) sister (III-2, at 1 year of age) is similarly abnormal (ventricular rate 75 bpm, atrial rate 150 bpm, ie, 2:1 AV block, P-wave duration, 150 ms, PR interval; 200 ms; QRS, 160 ms). ECG of his eldest sister (III-1, at 6 years of age) shows an indeterminate electrical axis and normal conduction intervals. ECGs of both parents (father II-2, at 35 years old; mother II-3, at 34 years old) are within normal limits (with a leftward shift of the electrical axis in II-2).
Figure 2. A. 12-lead ECG of the index patient (III-3) recorded shortly after birth. A wide complex tachycardia (rate 190 to 200 bpm, LBBB, intermediate axis) terminates spontaneously and resumes after 2 intervening sinus beats. B. Tachycardia terminates on infusion of 200 μg adenosine. Sinus rhythm resumed but shortly after the tachycardia started again (not shown). C. Graphical representation of the progressive aspect of the conduction disease in the index.
(OMIM 150330) genes, associated with conduction abnormalities and structural heart disease were also excluded.

Screening of SCN5A in the proband firstly revealed a nonsense mutation: G>A substitution in codon 156 leading to a stop codon at that position (W156X; Figure 3A). Segregation analysis (Figure 3C) showed that it arose de novo in the electrocardiographically unaffected father and was also inherited by the unaffected sibling (III-1). Haplotype analysis of intragenic SCN5A polymorphisms (Figure 3C) demonstrated that the proband and the unaffected sibling inherited different alleles from their mother, raising the possibility of inheritance of a second SCN5A mutation (from the maternal side) by the proband. Indeed, on further analysis, a C>T substitution in codon 225, leading to the substitution of arginine by tryptophan (R225W; Figure 3B) was found in the SCN5A gene of the proband. Restriction digestion with BstNI (for W156X) and HpaII (for R225W) was used as an independent genotyping method to validate sequence analysis results. In both cases, the mutation abolished a recognition site for the respective enzyme. Neither mutation was detected in 200 normal chromosomes analyzed by restriction enzyme digestion.

Mutation Analysis in Postmortem Heart Tissue From III-2

Genotyping for the W156X and R225W mutations on DNA extracted from paraffin-embedded tissue from the deceased sibling (III-2) revealed that she had been heterozygous for these two mutations.

Autopsy Findings in the Heart of the Deceased Sibling (III-2)

On gross examination, the heart showed ventricular hypertrophy (heart weight 69 g; normal for age 50±6 g) and massive 4-chamber dilation. There were no congenital malformations of cardiac chambers, valves, or great vessels. There was marked endocardial fibroelastosis of the left atrium and ventricle and to a lesser extent the right ventricle. Histology of the myocardium revealed bundles of hypertrophic myocardium and fiber bundle stretching consistent with severe dilatation. Subendocardially, patchy areas of replacement fibrosis with sparse microcalcifications were found in the left ventricular myocardium. Mural thrombus, partially organized, was present around trabeculae of the left ventricular wall. In addition, there was myocardial edema and widespread subendocardial coagulative necrosis with minimal granulocytic infiltration of the left and right ventricular wall (Figure 4A). These areas showed decreased immunostaining with anti-actin and increased immunostaining with anti-fibrinogen antibodies. The conduction system revealed marked degenerative changes of the atroventricular nodal tissue and of both bundle branches, characterized by interstitial fibrosis, mainly collagenous with focal depositions of fine fibrillar elastin (Figure 4B).

Functional Properties of R225W and W156X Channels

The R225W mutation caused a drastic reduction in \(I_{\text{Na}}\). Injection of 5 ng WT-cRNA, resulted in peak Na\(^{+}\) currents of 1.4±0.25 µA within 1 to 2 days and 9.1±2.1 µA in 4 to 6 days (mean±SE, Figure 5A). In contrast, injection of similar amounts of R225W-cRNA did not result in measurable Na\(^{+}\) currents during 2 to 6 days. Increasing the amount of injected R225W-cRNA to 10 to 25 ng resulted in peak currents of 1.1±0.12 µA after 3 to 6 days, comparable to currents found 1 to 2 days after injection of 5 ng WT-cRNA. To enable a better comparison of peak currents, oocytes were also injected with 10 to 20 ng WT-cRNA, and Na\(^{+}\) currents were measured 4 to 6 days after injection. In these experiments, mean WT-current was 10.7±2.9 µA, ie, 10 times larger than in oocytes injected with the same amounts of R225W-cRNA. Furthermore, after injecting as much as 45 ng R225W-cRNA, only 4 out of 10 oocytes had currents larger than 2 µA within 3 to 6 days. Mean current in these 4 oocytes was 5.8±0.97 µA (Figure 5A). These data show that the R225W mutation leads to ~90% reduction in \(I_{\text{Na}}\). Whereas coinjection of 150 ng of h\(\beta_3\), with 10 ng WT-cRNA resulted in a 2.5-fold increase in peak current, no significant increase was observed on coinjection of 150 ng h\(\beta_3\), with 15 ng of R225W-cRNA (Figure 5B). As expected, oocytes injected with up to 30 ng W156X-cRNA (n=20) did not exhibit any inward Na\(^{+}\) currents at all.

Voltage dependence of steady-state activation and inactivation was investigated next. Half-maximal voltages (\(V_{1/2}\)) for activation and inactivation were not significantly different between WT and R225W channels expressed in the absence of the \(\beta_3\) subunit (Table). The slope factor (expressed as gating charge, \(e_0\)) of steady-state activation was however significantly reduced with one elementary charge for R225W channels (Table). In the presence of the \(\beta_3\) subunit, compared with WT, \(V_{1/2}\) of activation and inactivation for R225W channels were significantly shifted by +14 mV and +11 mV, respectively, and the \(e_0\) of activation and inactivation was reduced by approximately 1 (Figure 6A).

R225W channels displayed no difference in slow inactivation (Figure 6B). Also, the time constant for recovery from inactivation was not different between WT (14.5±1.1 ms, n=4) and R225W channels (13.1±1.7 ms, n=6).

Preliminary experiments (not shown) in human embryonic kidney (HEK) 293 cells provided evidence that \(I_{\text{Na}}\) is also drastically reduced in the mammalian expression system, and as in the oocytes system, \(V_{1/2}\) of activation and inactivation for R225W channels (n=11) were shifted to positive potentials compared with WT (n=10).

Discussion

Nature and Effect of Mutations

Mutations in SCN5A have been associated with three forms of primary electrical disorders: LQTS\(_3\), Brugada syndrome, and cardiac conduction defects.\(^6\) All mutations reported thus far for these disorders occurred heterozygously in affected individuals, except in one family wherein the proband, who was homozygous for V1777M, presented with major QT-prolongation and 2:1 atrioventricular block.\(^20\) In the present study, we report compound heterozygosity in SCN5A associated with a severe conduction defect in the proband and his (deceased) sister who inherited the W156X mutation from the father and the R225W from the mother.
Figure 3. Sequence analysis of exon 4 (A) and exon 6 (B) from a normal control and from the proband. C, Pedigree of the family displaying intragenic SCN5A haplotypes. Dashes (·) indicate that the genotype at that particular locus was not determined.
The stop codon (W156X) occurred at the S1-S2 linker of domain I. It is likely that mRNA encoded by this allele gets eliminated by nonsense-mediated mRNA decay, whereby the decay rate of mRNAs transcribed from genes containing nonsense mutations (especially when such mutations are present early in the gene, as in this case), is accelerated. Nevertheless, if mRNA encoded by this allele would escape such surveillance mechanisms, it would result in a truncated protein, consisting only of the amino-terminal end and the first transmembrane segment and would not conduct any current. In line with this, injection of cRNA encoding this mutant in oocytes failed to produce any current. In theory, and as evidenced in mice heterozygous for targeted disruption of \( \text{Scn5a} \), this should behave as a "loss-of-function" allele, leading to a 50\% reduction in \( I_{\text{Na}} \).

The most striking feature of the R225W channel was the severe (\( \approx 10\)-fold) reduction in maximum \( I_{\text{Na}} \) amplitude. Although any gating effects of the R225W substitution are likely overwhelmed by this severe reduction in current, from a structure-function point of view, the location of R225 makes analysis of gating parameters interesting and worthwhile. The R225W substitution neutralizes the third of four positive charges within the S4 segment of domain I. Such positively charged residues (located in each domain) confer voltage sensitivity to the channel, bringing about outward movement of S4 segments in response to depolarization, leading to activation gating. The R225W mutation is thus expected to impact on voltage gating. Indeed, the R225W channel showed a decreased voltage dependence of activation by one elementary gating charge. This most probably reduces recruitment of Na\(^{+}\) channel availability during the fast depolarization phase of the action potential. In coexpression studies with the \( \beta_1 \) subunit, gating charge for steady-state inactivation was also reduced, which is expected in view of coupling between the activation and inactivation processes of the channel.
Steady-State Activation and Inactivation Parameters of Na⁺ Currents Through Wild-Type (WT) and R225W Channels

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<tr>
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<th>Activation</th>
<th>Inactivation</th>
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<tbody>
<tr>
<td></td>
<td>V₁/₂, mV</td>
<td>Slope, e₀</td>
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<tr>
<td>WT (n=8)</td>
<td>-35.2±5.2</td>
<td>4.7±0.6</td>
</tr>
<tr>
<td>R225W (n=11)</td>
<td>-36.1±5.6</td>
<td>3.6±0.6*</td>
</tr>
<tr>
<td>+β-subunit</td>
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<tr>
<td>WT+β (n=4)</td>
<td>-39.9±4.3</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>R225W+β (n=6)</td>
<td>-25.9±2.5†</td>
<td>2.7±0.2†</td>
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* e₀ for R225W was significantly different from that of WT (Mann-Whitney Rank Sum test, P=0.0022).
† Values are significantly different from those of WT+β-subunit (unpaired Student t test, unequal variances, P<0.05). All data are expressed as mean±SEM.

As part of an elaborate structure-function study to explore the relevance of the basic residues in S4 domains of the cardiac Na⁺ channel, Chen et al. substituted R225 by two other residues: the negatively charged glutamate (R225E) and the uncharged yet polar glutamine (R225Q). As expected, the charge-reversing substitution (R225E) was associated with the most deleterious effect, resulting in nonfunctional channels. The charge-neutralizing substitution (R225Q) resulted in a decrease of voltage dependence of activation by 1.7 elementary gating charges. It appears that in spite of its bulky side chain, substitution to the uncharged residue tryptophan (R225W) exerts a less adverse effect. Although both residues have uncharged side chains, the fact that that of tryptophan is nonpolar, whereas that of glutamine is polar, could underlie at least some of the difference in severity.

The positive shift of the steady-state inactivation curve (availability curve) in the presence of the β subunit (Figure 6A) results in an availability of R225W channels at normal resting potentials that is about twice that of the WT. The concomitant positive shift of the activation curve of the R225W channel likely results in an increased threshold for action potential generation. Nevertheless, both for mutant as well as WT, all available channels will be activated during the action potential upstroke because the whole trajectory of activation is followed. Summarizing, the observed changes in kinetics suggest a “gain-of-function” for the R225W channel, which however (as pointed above) is likely outweighed by the severe reduction in current amplitude.

Genotype-Phenotype Correlation

Considering that the W156X allele theoretically causes a 50% reduction in functional channels, and the R225W allele reduces current amplitude to 10% of WT, one could envision that Iₙa in the proband is reduced to 5% to 10% of normal. Such drastic reduction in Iₙa explains the severe phenotype in this individual and his deceased sibling. In a modeling study on the role of Iₙa, IₖCaL, and gap junctional coupling on conduction efficacy in a theoretical multicellular cardiac fiber, Shaw and Rudy calculated that a 10-fold reduction of Iₙa, reduced action potential upstroke velocity by 90% and conduction velocity by 70%. In this model, any further reduction of Iₙa below these values leads to complete conduction block. This is in line with the finding of severe conduction disturbance in the index patient and his deceased sister.

The fact that the father and the eldest sibling (III-1) have a virtually normal ECG, in spite of carrying the W156X allele, is intriguing. This nonfunctional allele is expected to lead to a 50% reduction in Iₙa; however, contrary to what is expected, this does not affect conduction as is apparent from normal clinical conduction parameters. Several possibilities can be envisioned to explain the apparently normal phenotype in these individuals: (1) the cardiomyocyte has an abundance of Na⁺ channels such that even a severe reduction in Na⁺ channels can be tolerated; (2) expression from the other allele is upregulated; and (3) there is compensation by upregulation of expression of another Na⁺ channel.

Another intriguing observation is that although heterozygosity for the W156X allele is not associated with a clinical
phenotype, a distinct clinical phenotype has been reported for other SCN5A mutations generating a premature termination codon. These mutations include 4196delA:V1397X associated with Brugada syndrome and 5280delG:1786X associated with conduction disease, which are expected to generate premature termination codons at positions 1397 and 1786, respectively. Differences in disease penetrance and expressivity could relate to effect of genetic background. However, the reason why the W156X mutation is phenotypically silent in the heterozygous state, whereas heterozygosity for 4196delA:V1397X or 5280delG:1786X is associated with a clear phenotype, could be related to the different positions of the stop codons within the respective mRNAs. The W156X codon occurs earlier in the transcript (exon 4) compared with the V1397X codon (exon 23) and the 1786X codon (exon 28). Studies in β-thalassemia and Marfan syndrome have demonstrated the expression level of mutant transcripts (β-globin and fibrillin 1, respectively) and, consequently, the severity of the disease is related to the position of the premature termination codon, with those occurring early within the transcript being associated with decreased levels of transcript and a milder phenotype.

**Ion Channel Defect and Structural Heart Disease**

An extreme type of dilated cardiomyopathy with replacement fibrosis in combination with areas of coagulative necrosis was found in the heart of the deceased sibling (II-2; carrier of the same SCN5A genotype as the index). Necrosis indicates episodes of ischemia presumably due to intractable arrhythmia and to lack of oxygen supply in the severely dilated cardiac chambers. It likely reflects end-stage pathology, which resulted in death. A tachycardiohypertrophy seems unlikely because persistent arrhythmias were only present for a short period. In the proband, however, it might have contributed to ventricular dilatation at birth because of possible recurrent periods of tachyarrhythmia in utero.

To some extent, similarities in cardiac pathology can be noticed with earlier observations on the heart of a stillborn child of 36 weeks gestation with homozygous truncation of the hERG protein leading to a severe form of LQTS. This patient, of which we could only review the histological slides of the myocardium, also showed dilation of both ventricles, with repetitive signs of myocardial infarction in utero, which we interpreted as due to ischemia secondary to sustained arrhythmia.

The dilated cardiomyopathy and degenerative changes of the specialized conduction system possibly relate to the severe nature of the ion channel defect. However, this case is the first description of cardiac pathology associated with SCN5A mutation in humans, and therefore, any cause-effect relation remains speculative. The only documentation on SCN5A-related structural abnormalities of the heart thus far concerns mice with targeted disruption of this gene. In these mice, homozygous disruption of Scn5a caused intrauterine lethality with severe defects in ventricular morphogenesis, which consisted of reduced chamber size, reduced trabeculation of the ventricular wall, and a reduced number of thin spindle-like cardiomyocytes compared with hearts from control mice. In the heart of our deceased patient, ventricular trabeculation appeared normal, despite dilatation and endocardial fibrosis.

The severe degenerative (fibrotic) changes of the specialized tissues of the conduction system of the present patient are expected to have severely affected conduction, thereby further exacerbating the conduction defect already severely compromised by the ion channel abnormality in this child’s heart. Moreover, they could contribute to the progressive nature of the conduction disorder. The severity of structural abnormalities likely relates to the severe nature of the ion channel defect.

These observations, taken together with the fact that autopsies from patients with LQTS have revealed structural changes, in particular in the conduction system, and the fact that an ion channel (hRyR2) defect has been found to underlie one form of arrhythmogenic right ventricular dysplasia (ARVD2), begs the consideration that ion channel defects may result in morphological changes and some form of cardiomyopathy.

The observation of a small muscular VSD in the boy is noteworthy. Although the co-occurrence of the electrical disorder and the VSD could represent two independent events, the fact that there have been several reports in the literature describing occurrences of VSDs in combination with an electrical disorder (LQTS) hints to a possible link. Moreover, through findings in transgenic mice harboring homozygous disruption of specific ion channels, it is becoming increasingly recognized that electrical integrity of the heart could represent a prerequisite for proper cardiac development.

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**References**


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