Novel SCN5A Mutation Leading Either to Isolated Cardiac Conduction Defect or Brugada Syndrome in a Large French Family

Florence Kyndt, PharmD*; Vincent Probst, MD*; Franck Potet, BSc*; Sophie Demolombe, PhD; Jean-Christophe Chevallier, MD; Isabelle Baro, PhD; Jean-Paul Moisan, MD, PhD; Pierre Boisseau, PhD; Jean-Jacques Schott, PhD; Denis Escande, MD, PhD; Hervé Le Marec, MD, PhD

Background—The SCN5A gene encoding the human cardiac sodium channel α subunit plays a key role in cardiac electrophysiology. Mutations in SCN5A lead to a large spectrum of phenotypes, including long-QT syndrome, Brugada syndrome, and isolated progressive cardiac conduction defect (Lenègre disease).

Methods and Results—In the present study, we report the identification of a novel single SCN5A missense mutation causing either Brugada syndrome or an isolated cardiac conduction defect in the same family. A G-to-T mutation at position 4372 was identified by direct sequencing and was predicted to change a glycine for an arginine (G1406R) between the DIII-S5 and DIII-S6 domain of the sodium channel protein. Among 45 family members, 13 were carrying the G1406R SCN5A mutation. Four individuals from 2 family collateral branches showed typical Brugada phenotypes, including ST-segment elevation in the right precordial leads and right bundle branch block. One symptomatic patient with the Brugada phenotype required implantation of a cardioverter-defibrillator. Seven individuals from 3 other family collateral branches had isolated cardiac conduction defects but no Brugada phenotype. One patient with an isolated cardiac conduction defect had an episode of syncope and required pacemaker implantation. An expression study of the G1406R-mutated SCN5A showed no detectable Na+ current but normal protein trafficking.

Conclusions—We conclude that the same mutation in the SCN5A gene can lead either to Brugada syndrome or to an isolated cardiac conduction defect. Our findings suggest that modifier gene(s) may influence the phenotypic consequences of a SCN5A mutation. (Circulation. 2001;104:3081-3086.)

Key Words: fibrillation ■ heart block ■ bundle-branch block ■ genetics ■ arrhythmia

The SCN5A gene encoding a voltage-gated Na+ channel is predominantly expressed in the heart, where it plays a key role in the generation and propagation of the cardiac impulse. Autosomal-dominant mutations in the SCN5A gene are responsible for distinct rhythm and conduction disorders, including the long-QT syndrome (LQT3), Brugada syndrome, and isolated cardiac conduction defect (ICCD; Lenègre disease). Distinct ECG phenotypes and risks characterize these syndromes. The LQT3 phenotype is characterized by a prolonged QT interval, potentially leading to torsade de pointes arrhythmias. At the cellular level, the pathophysiology sequence for LQT3 includes slowed inactivation of the Na+ current, resulting in a sustained inward current (gain of function) during the plateau of the cardiac action potential. The Brugada phenotype is characterized by ST-segment elevation in the right precordial leads, often accompanied (albeit not always) by right bundle branch block but a normal QT duration. As originally described, Brugada syndrome is associated with a high mortality resulting from nocturnal ventricular fibrillation. The pathophysiology sequence of the Brugada syndrome remains incompletely understood, although the syndrome possibly results from a loss of function of the mutated sodium channel protein. ICCD is defined by isolated prolongation of the conduction parameter in the His-Purkinje conduction system but no ST-segment elevation or QT prolongation. It is also associated with a risk of complete atioventricular block and Stoke-Adams syncope but no ventricular dysrhythmias. The pathophysiology sequence of ICCD is also unclear but most likely results from a loss-of-function of the cardiac sodium channel.

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An intriguing overlap between LQT3 and Brugada syndromes has been documented by Bezzina et al, who...
identified a A1795indD mutation, which causes both LQT3 and Brugada syndrome in the same family members. Expression studies of the A1795indD mutation showed that the mutation disrupts fast inactivation, causing sustained Na⁺ current during the action potential plateau and prolonging cardiac repolarization. At the same time, the mutation augments slow inactivation, delaying recovery of Na⁺ channel availability between stimuli and reducing the Na⁺ current.

In the present study, we report a novel missense G1406R \( \text{SCN5A} \) mutation in a large French family causing either Brugada syndrome or ICCD, depending on the family collateral branch. Patients with typical ICCD do not show ECG characteristics of Brugada syndrome and also do not respond positively to flecainide challenge used as a provocative test. Expression studies revealed silent mutated channels but normal intracellular trafficking. Our data raise the possibility that the consequence of the same \( \text{SCN5A} \) mutation may be individual-specific or, more precisely, branch-specific. They also raise the possibility that modifier gene(s) or environmental factors may influence not only the severity but also the phenotype induced by a \( \text{SCN5A} \) mutation.

**Methods**

**Clinical Investigation**

The study was conducted according to the French guidelines for genetic research and was approved by the Nantes University Hospital ethics committee. Informed, written consent was obtained from each family member who agreed to participate to the study. Investigation included a review of medical history, a complete physical examination, and a 12-lead ECG (Mac Vu Marquette Inc). Heart rate, PR interval, QRS, QT, QTc duration, and P and QRS axis were measured at rest. QRS axis was classified as normal when its value was between −30° and 90° and as abnormal when out of this range. The conduction defects were defined using conventional classification. Parietal block was defined as a QRS duration > 115 ms without morphology of left or right bundle branch block. A PR duration > 210 ms was considered prolonged. The diagnosis of ICCD was established only if no morphological cardiac abnormalities (an echocardiograph was performed for all the affected patients) or other diseases associated with the conduction defect were discovered.

In 4 individuals, an invasive electrophysiological test was conducted according to standard methods. A flecainide challenge (2 mg/kg IV administered as a bolus over 10 minutes) was conducted in 4 individuals (patients II-15, III-3, III-9, and III-11).

**Mutation Analysis of \( \text{SCN5A} \)**

Genomic DNA was prepared from peripheral blood lymphocytes by standard methods. Mutation analysis was conducted by direct sequencing of the \( \text{SCN5A} \) gene using an ABI 377 automated sequencer. All 28 exons of the \( \text{SCN5A} \) gene were amplified using intronic primers, as designed by Wang et al. Segregation of the mutation was assessed by restriction fragment length polymorphism (RFLP) because the G1406R mutation creates a new restriction site (Figure 1).

**Functional Studies**

The human wild-type (WT) \( \text{SCN5A} \) was subcloned into the mammalian expression vector pCI (Promega). The WT \( \text{SCN5A} \) cDNA, plus the 5’UTR of the Xenopus \( \beta \)-globin and 1394 bp of the \( \text{SCN5A} \) 3’UTR, were removed from a pSP64T-WT-\( \text{SCN5A} \) plasmid (a kind gift from Dr Al George, Vanderbilt University Medical Center, Nashville, Tenn). The G1406R substitution was introduced into the \( \text{SCN5A} \) cDNA by directed mutagenesis (QuickChange Site-Directed Mutagenesis kit, Stratagene). The human \( \beta 1 \) subunit was subcloned into a pRC vector. The WT and mutant \( \text{SCN5A} \) were also inserted in frame into a pEGFP-N3 plasmid (Clontech) to be expressed as fusion to the N terminus of the enhanced green fluorescent protein (EGFP). All constructs were checked by direct sequencing. COS-7 cells, obtained from the American Type Culture Collection, were transfected using polyethylenimine (22 kDa).

Eight hours after transfection, cells were separated by enzymatic treatment and seeded in plastic Petri dishes bottomed with a coverslip. Using the patch-clamp technique, whole-cell currents were recorded at room temperature, as previously described. Capaciti-
Results

A partial pedigree of this family (overall, this family comprises 71 identified members) is presented in Figure 1. The proband (patient II-11) was referred to our institution because of recurrent episodes of palpitation and dizziness. His familial history noted that 2 of his uncles died suddenly at the ages of 44 and 56 years. Twelve-lead ECG recordings (Figure 2) demonstrated a typical Brugada syndrome phenotype, including ST-segment elevation and rSR', in the precordial leads, associated with a prolonged PR interval (284 ms and 215 ms).

Patient II-11 was given an implantable cardioverter-defibrillator. Although asymptomatic, one of his sons (patient III-18) exhibited ECG signs of Brugada syndrome (Figure 2); however, the ECG of another son (patient III-19) was normal. One brother of the proband (patient II-15), as well as one of his sons (patient III-24), also had a typical Brugada ECG phenotype. In patient II-15, invasive electrophysiological testing showed a prolonged HV interval (80 ms) and inducible ventricular tachycardia. In accordance with this phenotype, patient III-4 was given an implantable pacemaker. After pacemaker implantation, he remained free of symptoms.

Patient III-3, who showed first-degree atrioventricular block and left anterior hemiblock, was challenged with a flecainide test. Flecainide markedly aggravated preexisting conduction anomalies, but no ST-segment elevation occurred. The PR interval increased from 206 to 230 ms, a right bundle branch block appeared, the QRS complexes widened from 100 to 150 ms, and the HV interval increased from 72 to 104 ms. Finally, programmed electrical stimulation did not provoke arrhythmias. A flecainide test was also conducted in patients III-9 (the PR interval increased from 202 to 244 ms and the QRS complexes widened from 120 to 186 ms) and in patient III-11 (Figure 4; the PR interval increased from 208 to 222 ms and the QRS complexes widened from 108 to 136 ms). In no case was ST-segment elevation induced by flecainide.

Overall, in this family, 4 individuals exhibited clinical Brugada syndrome, and 7 members exhibited isolated conduction anomalies. DNA sequencing of exon 23 from the proband (II-11) identified a heterozygote G-to-T mutation at position 4372 (Figure 1) of SCN5A. This missense mutation was predicted to change a glycine for an arginine (G1406R).

Patient III.4

Figure 3. ECG recording from patient III-4 showing a prolonged PR duration (274 ms) and a complete right bundle branch block but no ST-segment elevation.
between the DIII-S5 and DIII-S6 domain of the sodium channel protein (Figure 5A). DNA sequencing of the other exons revealed no further anomalies. In addition, the G1406R mutation was not found in 100 normal alleles from unrelated individuals. RFLP analysis of exon 23 (amplified by polymerase chain reaction; Figure 1) showed a segregation of the mutation in individuals affected by Brugada syndrome or ICCD. In comparison with a group of 22 nongene carriers from the same family, gene carriers had a longer PR interval (256±36 ms for Brugada syndrome and 212±35 ms for ICCD phenotype versus 164±21 ms in controls; P<0.001) and a wider QRS duration (120±10 ms for Brugada syndrome and 133±18 ms for ICCD versus 94±10 ms in controls; P<0.001). Finally, patients with a Brugada phenotype had a longer PR interval (P<0.006) but similar QRS duration (120±10 ms for Brugada syndrome and 133±18 ms for ICCD phenotype). Comparing the heart rate in the 3 different cohorts showed that Brugada patients (64±5 beats/min; n=4) had a significantly slower heart rate compared with ICCD patients (71±3 beats/min; n=7) or nongene carriers (74±2 beats/min; n=19; P<0.05 with 1-way ANOVA).

For functional studies, WT or mutated G1406R SCN5A plasmids were expressed in COS-7 cells. Illustrative whole-cell currents obtained in response to depolarizing steps between −60 and 0 mV are shown in Figure 5. In cells expressing WT SCN5A, the peak current density at −20 mV was −66.7±16.2 pA/pF (n=10). In cells cotransfected with WT SCN5A plus β1-subunit cDNAs, larger currents were recorded (at −20 mV, the peak current amplitude was −120.3±25.2 pA/pF; n=12; P<0.05 in comparison with WT SCN5A alone; Figure 5). Cells transfected with the G1406R mutant exhibited no inward current in the absence (n=14) or presence (n=24) of the β1-subunit (Figure 5C). With the aim of identifying the origin of the G1406R SCN5A dysfunction, WT or G1406R SCN5A-EGFP fusion proteins were expressed in COS-7 cells. Figure 6 illustrates typical epifluorescence and confocal microscopy observations. Both WT and G1406R SCN5A-EGFP fusion proteins localized at the cell membrane. From these experiments, we concluded that although non-functional, the mutated G1406R SCN5A protein was correctly processed to the plasma membrane.

Discussion

We report a large French family in which a single mutation in the SCN5A gene led to either Brugada syndrome (in 2 brothers and their descendants) or to ICCD (in 3 sisters from the same generation and in their descendants). Our report shows that the same SCN5A mutation can lead to 2 different ECG phenotypes and, thereafter, to different syndromes and, most importantly, different risks, ie, tachyarrhythmia or inversely extreme bradycardia, depending on the family collateral branch.

Brugada syndrome is characterized by ST-segment elevation associated with the rSR’ aspect in the right precordial leads (V1 to V3). In most patients, however, the typical widened S wave in the left lateral lead is absent, suggesting that the rSR’ aspect is not a true right bundle branch block. Since the original description of the Brugada syndrome, mild intraventricular conduction defects with prolonged PR and HV intervals have been reported. Because the Brugada syndrome is thought to be caused by a loss of function of the Na+ channel, it is not surprising that conduction defects coexist with the canonical ST-segment elevation in precordial leads. Four male patients from our family exhibited the typical Brugada ECG pattern. In the 2 Brugada patients in
who electromyographical studies were conducted, ventricular tachycardia was inducible. Inversely, in the patients with ICCD but no precordial signs of Brugada, we were unable to provoke ventricular fibrillation or ventricular tachycardia. These observations further support the concept that ICCD differs from Brugada syndrome, not only because of the absence of ST-segment elevation, but also because the risk for life-threatening ventricular arrhythmias remains a major characteristic of Brugada syndrome. Three patients with ICCD had a negative flecainide challenge, a test that has been reported to identify Brugada syndrome patients with a normal ECG but no precordial signs of Brugada. Most recently, however, pharmacological challenge with Na$^+$ channel blockers have been accused of having poor value to reveal Brugada syndrome in most gene carriers with a normal ECG. 

We previously found that heterologous expression of SCN5A mutants responsible for ICCD produces no detectable Na$^+$ current and that, in ICCD, the affected allele supposedly produces no functional proteins, resulting in haploinsufficiency. This profile is consistent with the ICCD phenotype because the sodium channel is a major factor for propagation of the cardiac impulse. Other investigators have also shown that SCN5A mutations producing nonfunctional channels such as R1432G can lead to Brugada syndrome. Therefore, it is not surprising that the G1406R mutant, which also produces silent channels, can lead to both Brugada syndrome and ICCD.

The Brugada syndrome has been reported to have a low penetrance, with as many as 84% of the gene carriers having a normal ECG. This means that the same SCN5A mutation can have a very different impact on cardiac electrophysiology. A logical explanation would be that as-yet unidentified “modifier” gene(s) and/or environmental factors might influence the occurrence of the ECG phenotype.

Furthermore, some patients with typical Brugada ECG pattern at rest or during flecainide challenge experience ventricular tachycardia or ventricular fibrillation, whereas other patients with the same mutation and the same ECG profile remain free of symptoms. 

This also suggests that some factors may influence not only the occurrence of the phenotype, but also the incidence of sudden death in gene carriers and, thereafter, the severity of the syndrome. In the family reported here, the syndrome itself (Brugada or ICCD) varied depending on the collateral branches. Therefore, a logical deduction would be that within the same kindred, unknown factors can impact the consequences of a SCN5A mutation, inasmuch as the same mutation can lead to (1) a normal ECG and phenotype (silent gene carriers), (2) symptomatic or inversely asymptomatic patients sharing the same ECG phenotype, (3) and finally, different syndromes. In particular, it is possible that variable and regional expression of the mutant allele may contribute to different patient disease phenotypes. Control of expression of the disease allele could be considered a function of modifier genes.

In contrast to the woman-preponderance of symptomatic long-QT syndrome, Brugada men outnumber women by far (in a series of 163 patients with Brugada syndrome reported by Alings and Wilde, 1750 were male and only 13 were female; in a prospective study by Priori et al, 75% of patients with Brugada syndrome were male). In the reported family, 4 of 4 patients with Brugada syndrome were male, whereas 6 of 7 ICCD patients were female. In one ICCD patient, the severity of the conduction defects was such that it required pacemaker implantation; this patient was male (patient III-4). Sex differences may be related to genetic factors influencing phenotype. It is also well established that sex hormones can impact cardiac electrophysiology and, more particularly, cardiac repolarization. In vivo experiments support the idea that the heterogeneity in repolarization across the wall of the right ventricular outflow tract contributes to ST-segment elevation in the precordial leads and to the genesis of arrhythmias. Therefore, it is conceivable that sex can influence the phenotype via the effects of sex hormones on repolarizing potassium currents. This hypothesis will require further evaluation.

In summary, our study shows that the same SCN5A mutation can lead to Brugada syndrome or to ICCD. A previous study by Bezzina et al showed that a single SCN5A mutant can lead to LQT3 and Brugada syndrome. Thus, the 3 syndromes related to SCN5A mutation (Brugada, ICCD, and LQT3) clearly overlap. One consequence of the recent progress made in genotyping is that the frontiers between clinical syndromes become less sharp.

Acknowledgments

This work was supported by special grants from the Institut National de la Santé et de la Recherche Médicale (INSERM) to Drs Escande and Le Marec (PROGRES 4P099D) and from the Association Franchise contre les Myopathies. We thank Christine Fruchet for assistance and the family for participation. We also thank Béatrice...
Leray, Marie-Joseph Louerat, and Fabrice Aireau for expert technical assistance with cell cultures, plasmid amplifications, and DNA sequencing.

References

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_Circulation_. 2001;104:3081-3086
doi: 10.1161/hc5001.100834

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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