Ventricular tachycardia in a Brugada syndrome patient caused by a novel deletion in SCN5A

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The aim of the present study was to identify the molecular mechanism behind ventricular tachycardia in a patient with Brugada syndrome. Arrhythmias in patients with Brugada syndrome often occur during sleep. However, a 28-year-old man with no previously documented arrhythmia or syncope who experienced shortness of breath and chest pain during agitation is described. An electrocardiogram revealed monomorphic ventricular tachycardia; after he was converted to nodal rhythm, he spontaneously went into sinus rhythm, and showed classic Brugada changes with coved ST elevation in leads V₁ to V₂. Mutation analysis of SCN5A revealed a novel mutation, 3480 deletion T frame shift mutation, resulting in premature truncation of the protein. Heterologous expression of this truncated protein in human embryonic kidney 293 cells showed a markedly reduced protein expression level. By performing whole-cell patch clamp experiments using human embryonic kidney 293 cells transfected with the mutated SCN5A, no current could be recorded. Hence, the results suggest that the patient suffered from haploinsufficiency of Na_v1.5, and that this mutation was the cause of his Brugada syndrome.

Key Words: *Brugada syndrome; Deletion; Electrophysiology; Haploinsufficiency; SCN5A*

Brugada syndrome was first described in 1992 by the Brugada brothers (1). In a series of eight cases, they showed that ST elevation in leads V₁ to V₃ in patients with a structurally normal heart was associated with an inherited form of sudden cardiac death. Mutations in the gene encoding the pore-forming sodium channel SCN5A, giving rise to the Na_v1.5 protein, have been shown to cause 15% to 20% of Brugada syndrome cases (2-4). The pattern of inheritance is autosomal dominant, and the penetrance of the disease is not 100%. Until now, approximately 100 mutations in SCN5A have been reported (<http://pc4.fsm.it:81/card-moc>). Most mutations in SCN5A giving rise to a Brugada phenotype are missense mutations, although splice error, nonsense, deletion and insertion mutations in SCN5A have also been reported. All these mutations are believed to result in a reduced availability of functional Na_v1.5 channels. This can either be due to a reduced number of channels at the cell membrane (5-7) or to alterations in its biophysical properties, such as shift in activation and/or inactivation thresholds (7), or acceleration of intermediate inactivation (8). We describe a Danish Brugada syndrome patient with a frame shift deletion mutation in SCN5A giving rise to a truncated protein unable to conduct current.

Une tachycardie ventriculaire chez un patient atteint du syndrome de Brugada, causée par une nouvelle délétion du gène SCN5A

La présente étude visait à repérer le mécanisme moléculaire de la tachycardie ventriculaire chez un patient atteint du syndrome de Brugada. Chez les patients atteints du syndrome de Brugada, une arythmie se produit souvent pendant le sommeil. Cependant, est présenté le cas d'un homme de 28 ans sans arythmie ou syncope documentée et qui présentait une dyspnée et des douleurs thoraciques lorsqu'il était agité. Un électrocardiogramme a révélé une tachycardie ventriculaire monomorphe. Après conversion au rythme nodal, il est passé spontanément au rythme sinusal et a présenté les changements classiques du syndrome de Brugada avec élévation du segment ST et aspect en dôme aux dérivations V₁ à V₂. L'analyse de mutation du gène SCN5A a révélé une nouvelle mutation, une mutation à trame décalée T de la délétion 3480, entraînant une troncature prématurée de la protéine. L'expression hétérologue de cette protéine tronquée dans les cellules rénales embryonnaires humaines 293 a révélé une réduction marquée du taux d'expression des protéines. Au moyen d'expériences par la méthode patch-clamp à l'aide de cellules rénales embryonnaires humaines 293 transfectées avec le gène SCN5A mutant, on n'a pu enregistrer aucun courant. Ainsi, d'après les résultats, le patient a souffert d'une semi-dominance haploïde du Na_v1.5, et cette mutation était responsable du syndrome de Brugada.

METHODS

Clinical data

Informed consent was obtained from study participants according to the guidelines of the medical ethics committee of Copenhagen, Denmark. The present study and all experiments conformed to the Declaration of Helsinki. Each individual or family (in the case of sudden infant death) gave written informed consent for genetic analysis. Clinical methods were used as described in the results.

Molecular genetic analysis

SCN5A was screened for mutations by four-colour multiplex capillary electrophoresis single-strand conformation polymorphism analysis using intronic primers (available on request from mic@ssi.dk) on an ABI 3100 fragment analyzer (Applied Biosystems, USA) (9). DNA sequencing was performed using Big Dye technology (Applied Biosystems). One hundred Danish blood donors were used as population controls. DNA from the child who had suffered from sudden infant death was extracted from the neonatal phenylketonuria card using the QIAamp DNA blood kit (Qiagen GmbH, Germany).

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Functional and biochemical analysis of $\text{Na}_v1.5$ channels

The *SCN5A* mutation (3480AT) was engineered into wild-type complementary DNA (clone hH1a received from Dr R Kass, Columbia University, New York, USA), cloned in pcDNA3.1 (Invitrogen, USA) using the QuikChange kit (Stratagene, USA) and verified by sequencing. For electrophysiological and biochemical studies, human embryonic kidney 293 (HEK293) cells were transiently transfected with 0.6 μg WT or mutant $\text{Na}_v1.5$ construct complementary DNA or as indicated. All transfections included 2.0 μg pIRES-h β 1-CD8 complementary DNA encoding the h β 1 subunit and CD8 antigen as a reporter gene. Cells were transfected using Lipofectamine (Stratagene), and the sodium current (I_{Na}) was measured after 48 h. Anti-CD8 beads (Dyna; Invitrogen) were used to identify transfected cells. Patch clamp recordings were carried out using an internal solution containing cesium chloride 60 mmol/L, cesium aspartate 70 mmol/L, EGTA 11 mmol/L, magnesium chloride 1 mmol/L, calcium chloride 1 mmol/L, HEPES 10 mmol/L and $\text{Na}_2\text{-ATP}$ 5 mmol/L, pH 7.2 with cesium hydroxide; the external solution contained sodium chloride 130 mmol/L, calcium chloride 2 mmol/L, magnesium chloride 1.2 mmol/L, cesium chloride 5 mmol/L, HEPES 10 mmol/L and glucose 5 mmol/L, pH 7.4 with cesium hydroxide. Current recordings were measured 1 min after rupture. Holding potentials were -100 mV, and no leak subtraction was performed. Peak currents were measured during a current-voltage protocol, and I_{Na} densities (picoampere/pico-farad [pA/pF]) were obtained by dividing the peak I_{Na} by the cell capacitance obtained from the pClamp function (Molecular Devices Corp, USA). Measurements were carried out at room temperature.

Western blotting conditions have been previously described (10). The anti- $\text{Na}_v1.5$ affinity-purified antibody recognizes residues 493 to 511 of rat $\text{Na}_v1.5$ (ASC-005; Alomone, Israel); these residues are identical in the human sequence. Anti-actin antibody was purchased from Sigma, USA.

Data analysis

Data are presented as mean $\pm$ SEM. The two-tailed Student's *t* test was used to compare means, and $P < 0.05$ was considered significant.

RESULTS

Case history of the proband

On October 13, 2004, a 28-year-old Caucasian man was admitted to the emergency room at Copenhagen University Hospital (Copenhagen, Denmark) with ventricular tachycardia (VT). During a dinner party 3 h before admission, the patient had been drinking beer and smoking cigarettes, after which he attended a soccer match. By the end of the game, he experienced shortness of breath and chest pain associated with excitement, but no dizziness or palpitations. Paramedics immediately transported him to the hospital. On admission, he was awake and agitated. His heart rate was 250 beats/min, and his blood pressure and temperature were normal. Infusion of amiodarone and subsequently, magnesium, converted the sustained monomorphic VT to an irregular accelerated nodal rhythm at 120 beats/min (Figure 1A). A right bundle branch block and ST elevation in leads V_1 to V_2 of the coved type developed, the nodal rhythm spontaneously converted into sinus rhythm, and ST elevation remained. The patient was suspected of having Brugada syndrome, thus he was admitted to the cardiology department for further investigation. Plasma electrolytes and heart enzymes were normal, and plasma glucose was 9 mmol/L. A signal-averaged electrocardiogram (ECG) investigation for late potentials was positive. Coronary angiography, echocardiography and a stress test showed normal results. One week later, the patient underwent electrophysiological investigations, whereby stimulation at the apex of the right ventricle induced ventricular fibrillation. Intravenous administration of flecainide, a type 1c antiarrhythmic drug, had no effect on the ST elevation in leads V_1 to V_3 . An automatic cardioverter defibrillator was implanted the following day, and blood was drawn for mutation analysis of the sodium channel gene *SCN5A*. The patient had no documented arrhythmia. Four years before admission, he had experienced symptoms

Figure 1 A Precordial electrocardiograms showing the arrhythmia of the proband. On admission, the patient had a ventricular tachycardia, which was converted to a nodal and consecutively irregular sinus rhythm with ST elevation in leads V_1 to V_2 by amiodarone and magnesium infusion. The next day, the electrocardiogram was normal (arrow indicates ST elevation suspicious for Brugada syndrome). B Holter monitoring showing the proband's paroxysmal atrial fibrillation

like those leading to the current admission, but an ECG was found to be normal and no further action was taken. His medical history included hypertension and bipolar affective disorder. His blood pressure was well controlled with calcium and beta-blocking agents for two years before treatment was discontinued. Five years before admission, the patient was diagnosed with bipolar affective disorder, and treatment was started with oral venlafaxine 150 mg daily. The patient consumed alcohol and smoked cigarettes only on social occasions, and he denied any use of drugs. When seen for follow-up in 2006, atrial fibrillation was noted (Figure 1B).

Family history (Figure 2): The patient's father (II:4) had endocarditis at the age of 12 years. At 18 years of age, he experienced palpitations and was treated by a psychiatrist for heart neurosis. He later developed hypertension. The patient's 40-year-old brother (III:3), and his 63-year-old mother (II:3) and two of her brothers (II:1 and II:2) had unremarkable medical histories except for hypertension (II:1), with normal ECGs. A third maternal uncle (II:5) had an unexplained syncope two years before dying of a subarachnoid hemorrhage at 35 years of age, and his only child (III:4) died from sudden infant death syndrome at nine months of age. On the patient's mother's side, the grandfather (I:1) suffered sudden cardiac death at 35 years of age, and the grandmother and her new husband (II:2 and II:3) died of causes other than sudden cardiac death.

Figure 2) The pedigree is shown with the symbol for each family member (squares represent male members and circles represent female members; arrow represents proband)

Figure 3) Genomic analysis of the SCN5A gene. **A** Capillary electrophoresis single-strand conformation polymorphism chromatogram showing exon 19 (upper and lower panels show the wild type and patient, respectively). **B** Sequence analysis of exon 19; the white/grey boundary denotes the 3480 deletion T frame shift mutation (numbering according to the start codon)

Genetic testing of family members

Genetic screening of family members revealed that the asymptomatic mother carried the mutation (Figure 2). The maternal uncle with a history of hypertension also tested positive, whereas the mutation was not found in DNA of the deceased infant (III:4). The maternal uncle (II:1) subsequently did not respond to a flecainide challenge. Another maternal uncle with no cardiac symptoms was not examined. The

Figure 4) **A** The SCN5A heart sodium channel embedded in the cell membrane (black arrow indicates the mutation). **B** Schematic drawing of the truncated SCN5A with changes in nucleotide level (n) and amino acid level (p)

proband had a seven-month-old daughter without any cardiac symptoms. The family was counselled by experts in clinical genetics and pediatric cardiology, as well as by the cardiologists treating the proband. After reviewing the family history, the nature of the genetic defect and the therapeutic options, the family chose to not proceed with genetic testing of their daughter.

Genetic analysis

Electrophoresis single-strand conformation polymorphism analysis showed an aberrant peak pattern in SCN5A exon 19 in one allele of the proband (Figure 3A). Sequencing of exon 19 revealed a thymidine nucleotide deletion at position 3480 (3480ΔT) located in the intracellular domain between domains 2 and 3 (DII to DIII loop). The 3480 deletion of thymidine led to a frame shift, causing a stop codon 163 base pairs further downstream (Figures 3B, 4A and 4B). No other mutations in SCN5A were found in this patient. The mutation was not found in 100 healthy blood donors used as controls.

Electrophysiological characterization of the novel 3480ΔT SCN5A mutation

The functional consequences of the SCN5A mutation were studied using HEK293 cells transiently transfected with mutant complementary DNA. Because the 3480ΔT mutation leads to a frame shift followed by a stop codon 163 base pairs further downstream, the encoded protein is expected to have a size of approximately 134 kDa. Figure 5 shows the results of a Western blot experiment in which the mutant protein migrated according to its predicted size and the wild-type channel to approximately 220 kDa. The protein expression level of the 3480ΔT mutant protein was drastically reduced (repeated in three independent experiments). The reduced amount of the 3480ΔT Na_v1.5 protein indicates an increased degradation rate of the messenger RNA or truncated protein, potentially induced by a reduced stability or folding efficiency, which could be caused by the lack of the C-terminal half part of the Na_v1.5 protein (Figure 5). Electrophysiological characterization of mutant and wild-type Na_v1.5

Figure 5) Biochemical expression of wild type (WT) and 3480 Δ T $\text{Na}_v1.5$ channels expressed in human embryonic kidney 293 cells. Western blot of human embryonic kidney 293 cell lysates expressing WT and 3480 Δ T channels. Controls are nontransfected cells

channels was performed by whole-cell patch clamp measurements (Figure 6). HEK293 cells transfected with 3480 Δ T *SCN5A* did not reveal sodium currents significantly higher than nontransfected cells (voltage-clamping with increasing depolarizing steps, see inserted protocol in Figure 6A). As expected, $\text{Na}_v1.5$ wild-type channels gave rise to very fast activating and inactivating inward sodium currents, with a maximal peak at approximately -20 mV. In cells co-transfected with wild-type and 3480 Δ T *SCN5A*, there was no change compared with wild-type alone, indicating that there was no dominant negative effect of the 3480 Δ T *SCN5A*. These data strongly suggest that the deletion of the thymidine nucleotide in position 3480 of *SCN5A* induces haploinsufficiency. Hence, the cause of the patient's Brugada syndrome was probably low or no expression of the mutated *SCN5A* allele.

DISCUSSION

Brugada syndrome was originally associated with high mortality caused by nocturnal ventricular fibrillation (1), and ST elevation in Brugada syndrome has been reported to be enhanced by vagal stimulation (ie, mainly at night) (11). VT during daytime, as found in the proband, is unusual. To our knowledge, this is the first case of VT possibly induced by agitation in a Brugada syndrome patient with haploinsufficiency of *SCN5A*.

Neither the proband nor his genotype-positive uncle responded with ST elevation while being challenged with flecainide during electrophysiological studies. It has been previously reported that a class Ic blocker test is not always positive in patients with Brugada syndrome (12). Thus, the value of the class Ic blocker test in Brugada syndrome is still being debated, and the specificity of sodium channel blockers in identifying patients at risk is currently not clear (13-16). The fact that the ST elevations are dynamic is a well-established phenomenon in Brugada syndrome patients (17), but difficulties in their recognition make the Brugada syndrome difficult to diagnose.

Supraventricular arrhythmia, exemplified by the later development of atrial fibrillation in the proband, occurs in approximately 20% of patients with Brugada syndrome (17). For the patient, this may create a fear of reoccurrence of VT or ventricular fibrillation and therefore represents a nontrivial clinical challenge for the clinician.

We have identified a novel mutation in a patient with Brugada syndrome. Two family members were found to have the same genotype, but not the Brugada syndrome phenotype. Intrafamilial phenotypic variability has previously been reported in Brugada syndrome (13,18). Overall, male patients have a 5.5-fold higher risk of sudden death (17). However, a recent report by Hong et al (13) delineated a large European family with higher prevalence in the female members of the family. Hence, unidentified modifiers, environmental cofactors

Figure 6) Functional characterization of wild type (wt) and 3480 Δ T $\text{Na}_v1.5$ channels expressed in human embryonic kidney 293 cells. **A** Current traces recorded from cells expressing wt or 3480 Δ T channels, as well as nontransfected cells (control), in response to a series of 10 ms test pulses (inset). **B** Averaged peak currents of cells expressing wt and 3480 Δ T channels ($n=8$ cells from two independent experiments). I_{Na} Sodium current; pA/pF Picoampere/picofarad

and genes may influence whether a carrier of an *SCN5A* mutation will present with Brugada syndrome. In our case, the family members found to carry the mutant gene were advised to undergo further testing, but were not tested with invasive electrophysiology because they were asymptomatic and because the value of the invasive electrophysiology is still debated (19,20). In other cardiac channelopathies, such as long QT syndrome, the discovery of a mutation in the potassium channel in relatives of an affected deceased patient may lead to prophylactic treatment, even if these family members are clinically asymptomatic (21). If the father of the proband in our case had been found to be positive for the mutation, the clinical consequence would have been an invasive electrophysiological study due to his symptoms. Cardiac symptoms in relatives of a patient with Brugada syndrome therefore influence the level of clinical testing.

Approximately 20% of Brugada syndrome cases are caused by a mutation in *SCN5A*, coding for the α -subunit of the voltage-activated sodium channel in the heart (17). Mutations, mainly missense, have been found to cause either loss of function or altered biophysical characteristics of the channel. In a patient with Brugada syndrome, we found a 3480 deletion T frame shift mutation in *SCN5A*. To date, no hot spots in the *SCN5A* gene have been identified in the population of Brugada syndrome with *SCN5A* mutations, and only two *SCN5A* mutations approximately 100 nucleotides upstream of 3480 have been reported in patients with Brugada syndrome (4,16). Investigations of the electrophysiological properties of the $\text{Na}_v1.5$ channel carrying the 3480 Δ T mutation in a heterologous expression system (HEK293 cells) revealed a lack of sodium current, suggesting a nonfunctional channel. Accordingly, we found a highly reduced protein level of 3480 Δ T compared with wild-type $\text{Na}_v1.5$. It has recently been shown that an insertion mutation of the four nucleotides TGGG in the noncoding region leads to a splice site

change of exon 27, resulting in an in-frame deletion of 32 amino acids (22). Due to this frame shift, the mutant protein sequence was also changed in the C-terminal end of the protein.

CONCLUSION

We have identified a novel mutation in the *SCN5A* gene. The 3480 Δ T mutation in *SCN5A* was shown to cause haploinsufficiency in a heterologous expression system, which most likely caused the Brugada syndrome patient's VT.

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REFERENCES

- Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: A distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol* 1992;20:1391-6.
- Schulze-Bahr E, Eckardt L, Breithardt G, et al. Sodium channel gene (*SCN5A*) mutations in 44 index patients with Brugada syndrome: Different incidences in familial and sporadic disease. *Hum Mutat* 2003;21:651-2.
- Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* 1998;392:293-6.
- Priori SG, Napolitano C, Gasparini M, et al. Natural history of Brugada syndrome: Insights for risk stratification and management. *Circulation* 2002;105:1342-7.
- Baroudi G, Acharfi S, Larouche C, Chahine M. Expression and intracellular localization of an *SCN5A* double mutant R1232W/T1620M implicated in Brugada syndrome. *Circ Res* 2002;90:E11-6.
- Baroudi G, Pouliot V, Denjoy I, Guicheney P, Shrier A, Chahine M. Novel mechanism for Brugada syndrome: Defective surface localization of an *SCN5A* mutant (R1432G). *Circ Res* 2001;88:E78-83.
- Itoh H, Shimizu M, Mabuchi H, Imoto K. Clinical and electrophysiological characteristics of Brugada syndrome caused by a missense mutation in the S5-pore site of *SCN5A*. *J Cardiovasc Electrophysiol* 2005;16:378-83.
- Wang DW, Makita N, Kitabatake A, Balser JR, George AL Jr. Enhanced Na^+ channel intermediate inactivation in Brugada syndrome. *Circ Res* 2000;87:E37-43.
- Hofman-Bang J, Behr ER, Hedley P, et al. High-efficiency multiplex capillary electrophoresis single strand conformation polymorphism (multi-CE-SSCP) mutation screening of *SCN5A*: A rapid genetic approach to cardiac arrhythmia. *Clin Genet* 2006;69:504-11.
- van Bemmelen MX, Rougier JS, Gavillet B, et al. Cardiac voltage-gated sodium channel $\text{Na}_v1.5$ is regulated by Nedd4-2 mediated ubiquitination. *Circ Res* 2004;95:284-91.
- Mizumaki K, Fujiki A, Tsuneda T, et al. Vagal activity modulates spontaneous augmentation of ST elevation in the daily life of patients with Brugada syndrome. *J Cardiovasc Electrophysiol* 2004;15:667-73.
- Kyndt F, Probst V, Potet F, et al. Novel *SCN5A* mutation leading either to isolated cardiac conduction defect or Brugada syndrome in a large French family. *Circulation* 2001;104:3081-6.
- Hong K, Berruezo-Sanchez A, Pongvarin N, et al. Phenotypic characterization of a large European family with Brugada syndrome displaying a sudden unexpected death syndrome mutation in *SCN5A*. *J Cardiovasc Electrophysiol* 2004;15:64-9.
- Brugada R, Brugada J, Antzelevitch C, et al. Sodium channel blockers identify risk for sudden death in patients with ST-segment elevation and right bundle branch block but structurally normal hearts. *Circulation* 2000;101:510-5.
- Shimizu W, Antzelevitch C, Suyama K, et al. Effect of sodium channel blockers on ST segment, QRS duration, and corrected QT interval in patients with Brugada syndrome. *J Cardiovasc Electrophysiol* 2000;11:1320-9.
- Priori SG, Napolitano C, Gasparini M, et al. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. *Circulation* 2000;102:2509-15.
- Antzelevitch C, Brugada P, Borggreffe M, et al. Brugada syndrome: Report of the second consensus conference: Endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation* 2005;111:659-70.
- Weiss R, Barmada MM, Nguyen T, et al. Clinical and molecular heterogeneity in the Brugada syndrome: A novel gene locus on chromosome 3. *Circulation* 2002;105:707-13.
- Brugada P, Brugada R, Brugada J. Should patients with an asymptomatic Brugada electrocardiogram undergo pharmacological and electrophysiological testing? *Circulation* 2005;112:279-92.
- Priori SG, Napolitano C. Should patients with an asymptomatic Brugada electrocardiogram undergo pharmacological and electrophysiological testing? *Circulation* 2005;112:279-92.
- Ackerman MJ, Tester DJ, Porter CJ, Edwards WD. Molecular diagnosis of the inherited long-QT syndrome in a woman who died after near-drowning. *N Engl J Med* 1999;341:1121-5.
- Hong K, Guerchicoff A, Pollevick GD, et al. Cryptic 5' splice site activation in *SCN5A* associated with Brugada syndrome. *J Mol Cell Cardiol* 2005;38:555-60.