

A novel SCN5A deletion mutation in a child with ventricular tachycardia, recurrent aborted sudden death, and Brugada electrocardiographic pattern

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Summary

A novel *SCN5A* mutation was found in a child with congenital sick sinus disease, a Brugada-like electrocardiogram and recurrent aborted sudden death. The mutation (L1821fs/10) is a 4 base pair deletion (TCTG) at position 5464-5467 in exon 28 of the gene. The novel mutation is predicted to produce a frameshift leading to a premature stop codon after ten missense amino acids upstream that did not allow the generation of the complete protein, and probably producing an incomplete and therefore non functional protein. The resulting alteration in sodium current could explain the clinical phenotype observed in this patient.

Key words: Deletion. Cardiac arrhythmias. Molecular-cardiology. Cardiovascular genetics. Channelopathies. Mutation.

Palabras clave: Delección. Arritmias cardíacas. Cardiología molecular. Genética cardiovascular. Canalopatías. Mutación.

Introduction

Ventricular tachycardia (VT) in children has been associated with congenital heart disease. In the presence

Resumen

DESCRIPCIÓN DE UNA NUEVA MUTACIÓN (DELECCIÓN) EN EL GEN *SCN5A* EN UN NIÑO CON TAQUICARDIA VENTRICULAR, RIESGO DE MUERTE SÚBITA, Y CUADRO ELECTROCARDIOGRÁFICO DE BRUGADA

Identificamos una nueva mutación en el gen *SCN5A* en un niño con disfunción sinusal congénita, un electrocardiograma semejante al encontrado en el síndrome de Brugada y riesgo de muerte súbita. Se trata de una delección de 4 pares de bases (TCTG) en la posición 5464-5467 del exón 28 de este gen (L1821fs/10). La nueva mutación produce un cambio en el marco de lectura que lleva a la generación de un codón de terminación después de 10 aminoácidos aberrantes. Esto impide la síntesis de la proteína completa, y produce probablemente una proteína incompleta y por ende no funcional. La alteración resultante en la corriente de sodio, podría explicar el fenotipo clínico observado en este paciente.

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of structurally normal hearts, it is attributed to abnormalities of the genes encoding cardiac ion channels such as *SCN5A* that create an arrhythmogenic substrate predisposing to the arrhythmia.¹ The *SCN5A* gene consists of 28 exons that

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* The contribution of M. Márquez y D. Cruz-Robles to this work is the same and the authors order is arbitrary.

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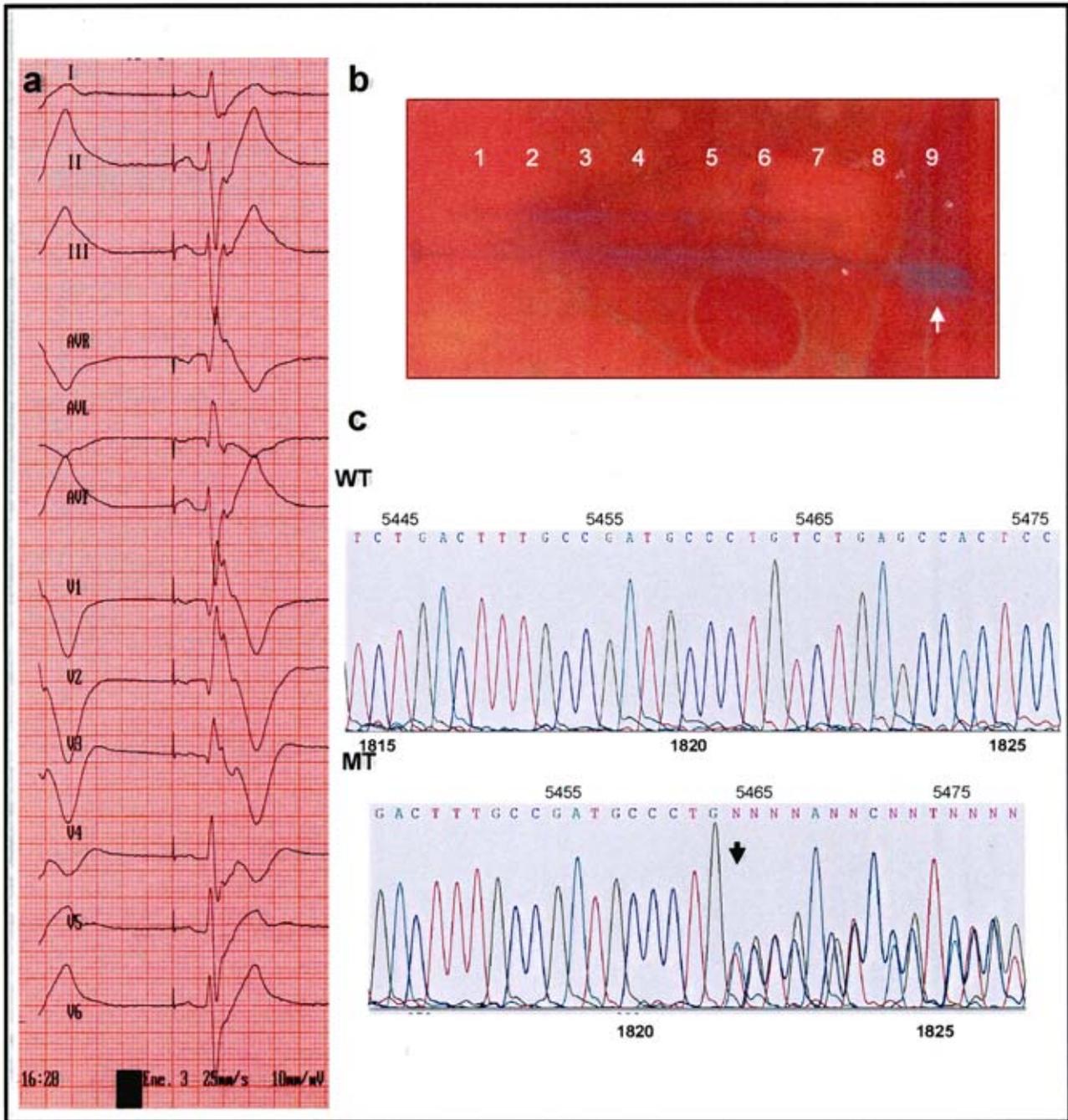


Fig. 1. a) 12-lead ECG with the pacemaker on AAI mode at 40 bpm. PR interval 260 ms (first-degree AV block); right bundle branch block pattern with a QRS duration of 240 ms and ST segment elevation in leads aVR, V1, and V2; prolonged QT interval (760 ms); **b)** SSCP analysis of the *SCN5A* gene (exon 28c) in the patient (arrow) and healthy controls; **c)** Wild-type (WT) and mutated (MT) sequences. In the MT sequence, we can observe a sequence abnormality (double peaks) at base 5464 (codon 1821), which is produced by a deletion of 4 base pairs (TCTG) (arrow).

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span 80 kb and encodes a protein of 2016 amino acids whose structure consists of four homologous domains (DI-DIV), each of which contains six membrane-spanning segments (S1-S6), similar to the structure of the potassium channel α -subunits.² Expression in *Xenopus* oocytes demonstrated that *SCN5A* mutations act through a gain-of-function mechanism.³ The so called "channelopathies", include diseases of several cardiac ion channels, affected by multiple genetic defects with different functional consequences. Phenotypic characteristics give rise to diseases such as the long QT syndrome (LQTS), short QT syndrome, Brugada syndrome (BrS OMIM#601144), catecholaminergic polymorphic VT, and Lenègre disease. Families with overlapping phenotypes of LQTS, BrS, sinus node disease, and conduction defects have been described.⁴⁻⁷ The BrS is characterized by ventricular fibrillation and sudden cardiac death associated with the electrocardiographic pattern of ST-segment elevation in leads V1-V3. Right bundle branch block (RBBB) morphology is also often observed.⁸ This syndrome is a monogenic disorder with an autosomal dominant inheritance and is associated with mutations in the *SCN5A* gene. However, *SCN5A* has been excluded as the gene causing the Brugada syndrome in at least one family, leading to the speculation that genetic heterogeneity exists in this syndrome. The aim of this study was to screen for *SCN5A* gene mutations in an 8-year-old male child with recurrent ventricular tachycardia (VT) and recurrent aborted sudden death.

Material and methods

Subjects: The index case was a 8-year-old male child with recurrent aborted sudden death. The patient and two previous generations had been born in Mexico City. His personal history included fetal bradycardia and atrial flutter during his first year of life. After ablation of the atrial flutter, a sinus node disease was detected. A double-chamber pacemaker was implanted at age 5 because of a sustained monomorphic VT, considered to be bradycardia-dependent. However, because syncopal events due to VT continued, the patient was referred to our institution in 2001. Structural heart disease was ruled out by physical examination, echocardiographic evaluation and CT scan. Baseline ECG is shown in *figure 1A*. As a comparison group, 100 individuals without any previous diagnosis or any car-

diovascular disease symptom were included. The present study was approved by the Bioethics and Research Committee from the Instituto Nacional de Cardiología and all study subjects voluntarily signed an informed consent letter.

DNA extraction: Genomic DNA was extracted from peripheral blood lymphocytes by means of the high salt extraction method.⁹

Single Strand Conformational Polymorphism (SSCP): Exons 11, 17, 21, 23, 25, 27, and 28 of *SCN5A* gene were amplified by polymerase chain reaction (PCR) with previously reported specific primers.² The sizes of the obtained fragments were visualized in an UV light transilluminator using a known molecular weight marker. The amplifying process was done on a Perkin Elmer 9700 thermocycler. Each amplified exon was run on polyacrylamide gels to visualize the formation of single strain polymorphisms as previously described.¹⁰ We decide analyze those *SCN5A* exons gene because responsible mutations of BrS and related disorders have been reported in those regions.^{2,6}

Aberrant SSCP conformers sequencing: After the PCR-SSCP was done, we detected the fragments that migrated differently on the acrylamide gel. These were then amplified again with the correspondent primers and sequenced. Once we obtained the PCR amplified products, they were purified using a kit (Wizard, Promega, Madison, WI). The purified products were used to do a sequencing PCR with the Dye terminator kit (Applied Biosystems, Foster City, USA). Finally, the products were sequenced by direct sequencing in a Perkin Elmer 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Results

The PCR-SSCP analysis revealed, only in the patient's DNA, an abnormal conformer in exon 28 (*Fig. 1b*). DNA sequence analysis demonstrated a 4 base pair deletion (TCTG) at position 5464-5467 at codon 1821 (L1821fs/10) (Genbank Accession No. EF063680). *Figure 1c* shows the sequence of the patient with the deletion and another individual with the wild-type sequence. The sequence shows that the deletion is present in a heterozygous state. The mutated sequence analysis allowed us to establish that the deletion caused a frameshift leading to a premature stop codon after ten missense amino acids upstream. The deletion was not present in any of the 100 analyzed healthy controls.

Discussion

Channelopathies have been associated with mutations in the *SCN5A* gene encoding a cardiac voltage-dependent sodium channel α -subunit. The case that we reported is an atypical BrS as only the electrocardiographic pattern of RBBB with ST segment elevation is found. Some clinical manifestations of the disease, including the presence of AV block and sinus node dysfunction, are not usually associated with BrS. Also, the clinical presentation of the ventricular arrhythmias are not commonly observed in BrS. Two types of mutations have been described, those that possibly lead to a decrease of functional Na⁺ channels at the sarcolemma, and the missense mutations that give rise to Na⁺ channels with altered biophysical properties. In the present work we detected a novel mutation (c.5464-5467delTCTG) in exon 28 of the *SCN5A* gene. This deletion mutation produces a frameshift leading to a premature stop codon after ten missense amino acids upstream that did not allow the generation of the complete protein, producing an incomplete and perhaps a non functional protein. However, the frameshift *SCN5A* mutation detected in the patients would yield a truncated protein

product if the mutant allele were transcribed and translated. Alternatively, the mutant allele might be a null allele due to degradation of the mutant mRNA via nonsense-mediated decay. To our knowledge this mutation has not been previously reported. The mutation was detected in exon 28 of the gene that codes for the cytoplasmic C-terminus region of the α -subunit of the cardiac Na⁺ channel. The biophysical analysis of this novel mutation is in progress, but the analysis of another mutation located in the distal part of the cytoplasmic C-terminal domain (near the mutation detected in the present work) in *Xenopus* oocytes demonstrated a negative voltage shift of the steady-state activation curves.¹¹ The mutation detected in our study could have the same effect on the Na⁺ channels, however, the mechanisms whereby these functional abnormalities give rise to clinical features are still unclear.

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