

An R1632C variant in the SCN5A gene causing Brugada syndrome

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Abstract. Brugada syndrome (BS) is an electrical disease, inherited in an autosomal dominant manner. BS is caused by mutations in up to 13 different genes. SCN5A is the gene most frequently mutated in BS, although this presents an incomplete penetrance. The present case study investigated the SCN5A gene in a family exhibiting BS. Direct sequencing of the SCN5A gene was performed to identify mutations and a familial investigation was performed. A novel variant was identified in the voltage-sensing domain of the SCN5A protein. This familial investigation revealed one novel asymptomatic carrier in the family. Genetic investigations are useful to classify individuals who require more frequent clinical monitoring and to stratify the risk of developing the disease.

Introduction

Brugada syndrome (BS) is an electrical and hereditary disease, including in a group of cardiac channelopathies characterized by a right bundle branch block (RBBB), ST segment elevation on a 12-lead surface electrocardiogram (ECG) and an increased risk of syncope, seizures, and sudden cardiac arrest in an otherwise healthy individual with a structurally normal heart. In an ECG, the ST segment connects the QRS complex and the T wave and has a duration of 0.080 to 0.120 sec. In BS, the patient may present with 3 types of ECG (I, II or III), only type I (coved-type) is diagnostic of BS and demonstrates an ST segment elevation of ≥ 2 mm and a negative T wave (1). In addition to pathological, diagnostic ECGs, BS patients may present an alternate or normal ECG. A pharmacological unmasking test was performed using inhibitors of the sodium channel.

Mutations in the SCN5A gene have been identified in patients with BS (2-5). Sequencing of the SCN5A gene revealed

that 10-20% of patients exhibited a mutation in SCN5A (6-11). However, certain variations in SCN5A present an incomplete penetrance in familial studies (12). Mutations in other genes, including CACNA1c, CACNB2b, GPD1-L, KCNE3, SCN1B, SCN3B and HCN4, have been associated with the condition to a smaller extent ($\sim 1\%$) (13-17). In ~ 70 -80% of patients with BS, direct sequencing of the associated genes revealed no causative point mutation (18). In the present study, a novel variant and its pathogenicity were investigated by sequencing SCN5A and conducting a cosegregation study with the family, in order to provide a clinical and genetic context for BS.

Materials and methods

Clinical data. The index case was a 26-year-old Latino-American male. The patient was diagnosed following a temporary loss of consciousness for 15 sec and a spontaneous recovery. The patient was admitted to the intensive care unit of the Virgen de la Arrixaca Clinical University Hospital (Murcia, Spain). An ECG was performed and revealed an elevation in ST (V1-V3), with a negative t wave and RBBB. The flecainide drug test was positive and an implantable cardioverter defibrillator was implanted. The ST elevation in V1 was up to 2 mm and 1 mm in V2, so the patient demonstrated a diagnostic ECG (coved-type) in V1 and undifferentiated in V2 (Fig. 1).

To date, no family history of sudden death was observed. The patient's brother (33-year-old) and sister (18-year-old) underwent physical examination, ECG and Doppler ECG investigations, similar to the index patient. The present study was approved by the ethics committee of Virgen de la Arrixaca Clinical University Hospital and signed informed consent was obtained from the proband.

Genetic study. The index case was included in a genetic study of BS. The genomic DNA was extracted from peripheral blood samples using Maxwell[®] 16 Blood DNA purification kit (Promega Corporation, Madison, WI, USA). All known exons in the SCN5A gene were amplified with intronic primers (Bonsai Technologies Group, SA, Alcobendas, Spain) and sequenced in both directions using BigDye v1.1 (Applied Biosystems, Foster City, CA, USA) chemistry in an ABI3130 analyzer (Applied Biosystems). The intronic primers were forward, 5'-CCCTGCTGAGCACTTTCC ATTTG-3' and reverse, 5'-TACAAGTCAGCTGGACGGAGA

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Table I. *In silico* analysis using prediction software.

Parameter	MutationTaster	Polyphen-2	PMUT
Results	Prediction disease causing	Probably damaging	Pathological
Score	0.9999	1.0	0.8846

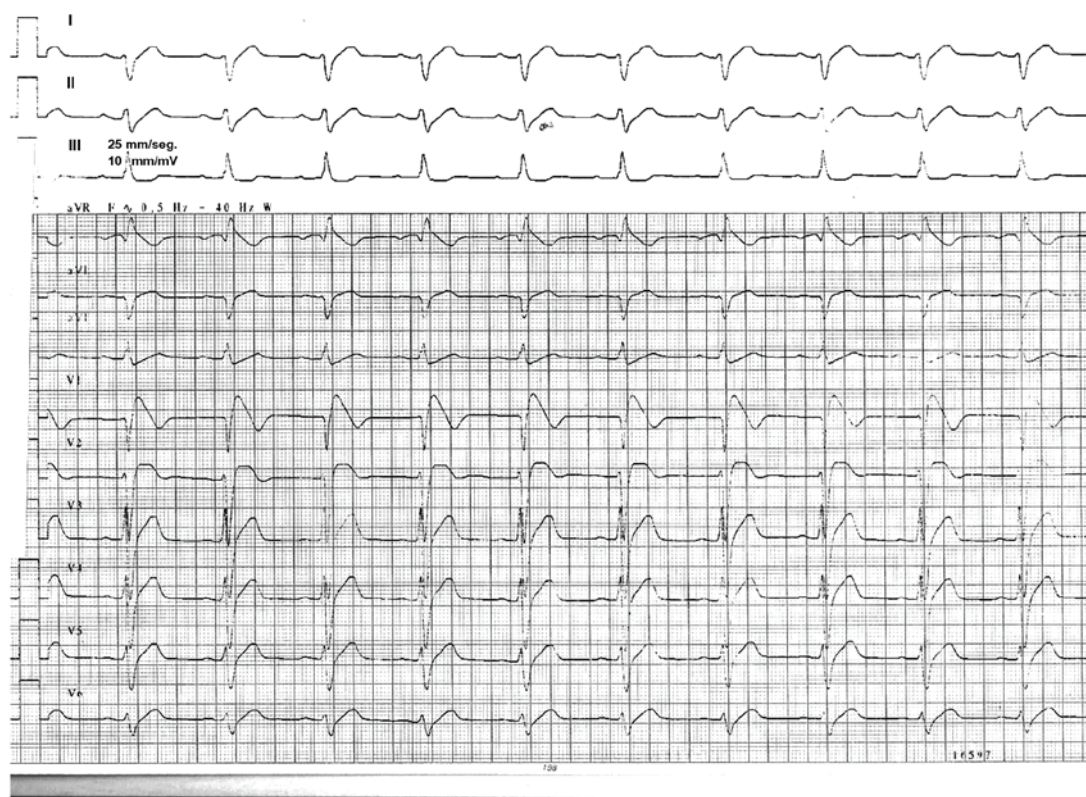


Figure 1. Pathological electrocardiograph of the index case.

AGC-3'. The genomic sequence from the samples obtained from the index patient was compared with the SCN5A sequence in the NCBI sequence database (NM_198056). *In silico* analysis was performed using pathogenicity prediction software: PMUT (<http://mmb.irbbarcelona.org/PMut/>), MutationTaster (<http://www.mutationtaster.org/>) and Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>). The siblings of the index patient also underwent genomic sequencing from obtained blood samples and a cosegregation study was conducted to elucidate the pathogenicity of the variant detected.

Results and Discussion

A total of three variants in the SCN5A gene were identified. Two were known variants, which are expressed in the normal population (p.A29A and p.E1061E). Sequence analysis identified a novel missense variation in heterozygotes (the index case and the patient's sister), c.4894C>T within exon 28, resulting in the replacement of arginine 1632 by cysteine (Fig. 2). The Grantham score for this replacement was 180 (scores between 0-250). The variant, p.R1632C, affected a highly conserved

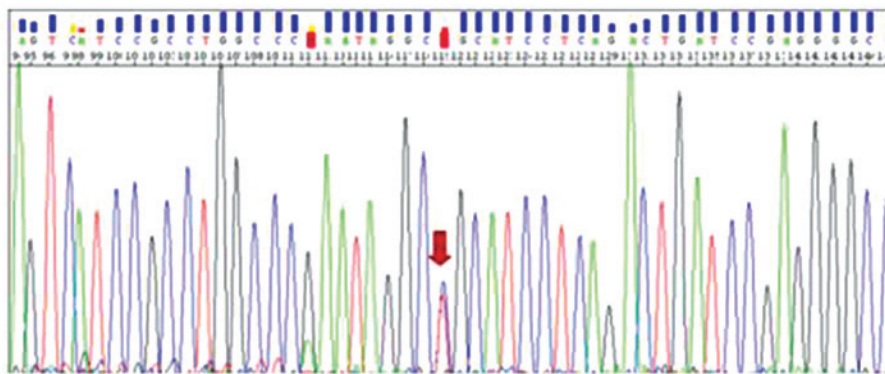
region in the protein, the fourth transmembrane loop (S4) of the IV domain. S4 acts as the 'voltage sensor' and is activated by changes in membrane potential, and is also involved in channel gating.

The bioinformatics analysis of the novel variant was performed using three online tools, MutationTaster, PolyPhen-2 and PMUT, to predict the effect of an amino acid substitution on the structure and function of the protein (Table 1). These three software programmes predicted that R1632C is pathogenic. Furthermore, the variant is localized in a key domain for channel formation, and amino acid substitution results in severe physiochemical changes. The scores in the table are the probability of the prediction, a score close to 1 indicates a high likelihood that the prediction is correct.

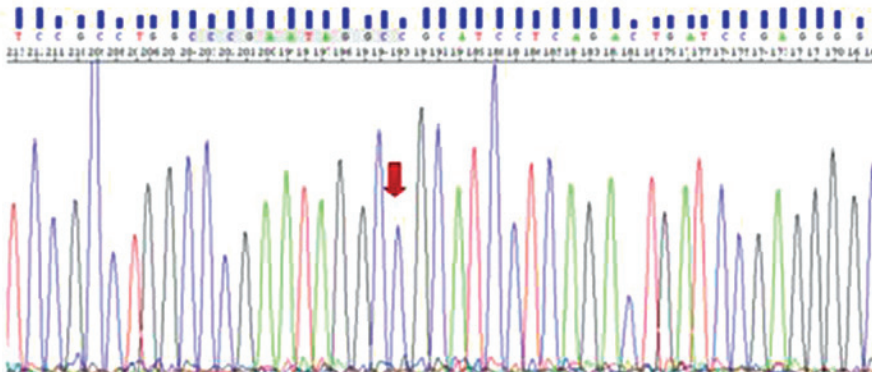
The genetic analysis was performed on his siblings. A healthy carrier, the patient's sister, exhibited a normal baseline and drug challenged ECG.

This variant was not present in public databases, including HGMD, ClinVar and Exome Variant Server. However, in the identical position, there was a R1632H (c.4895G>A) disease causing variation (rs199473286). This variant was investigated by Gui *et al* (19) using whole-cell patch clamp experiments.

Index case. Exon 28 R1632C



Brother. Exon 28 WT



Sister. Exon 28 R1632C

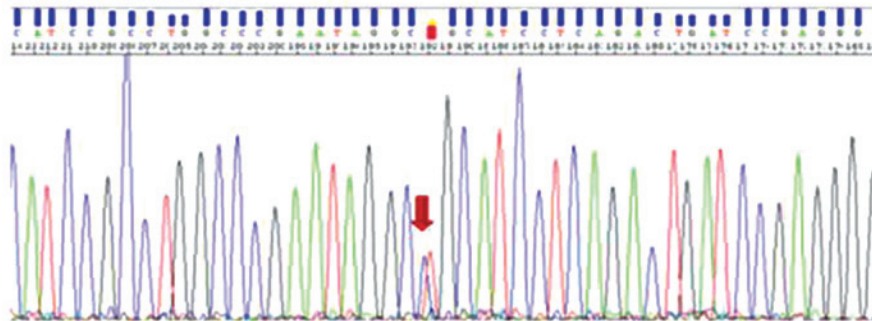


Figure 2. Sequence chromatograms of the SCN5A coding regions from the index patient and siblings. The arrows indicate the nucleotide substitution, c.4894C>T, resulting in the replacement of arginine 1632 by cysteine. The brother does not exhibit this mutation, whereas the index patient and the unaffected sister express the variant.

The expression of this variant causes inactivated channels at physiological membrane potentials and normal heart rates in patients with Sick Sinus Syndrome.

The present case report, identified a novel amino acid change located in a known replacement position. Although the replacement is not identical and in the majority of cases, distinct changes in the identical position causes similar pathological result, this variation may cause a different phenotypic expression. The present study revealed no evidence that this variant co-segregated with the disease, therefore, more extensive familial analysis is required. Also, it is necessary to perform a more frequent clinical follow up with the novel carriers to assess their risk of developing the disease.

When a novel variation is detected, *in silico* analysis can assist with determining the pathogenicity of the novel

alteration, however, a genetic and clinical familial study is crucial to elucidate the causality of the variation. Genotypic investigation of family members is required when a potential pathogenic variant in a proband is identified, to assess the risk of developing the characteristic phenotype.

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