An R1632C variant in the SCN5A gene causing Brugada syndrome

ESPERANZA GARCÍA-MOLINA¹, MARÍA SABATER-MOLINA¹, CARMEN MUÑOZ²,
FRANCISCO RUIZ-ESPEJO¹ and JUAN R. GIMENO²

Departments of ¹Clinical Analysis and ²Cardiology, Virgen de la Arrixaca University Hospital,
El Palmar, 30120 Murcia, Spain

Received October 20, 2014; Accepted July 10, 2015

DOI: 10.3892/mmr.2016.5100

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

Correspondence to: Dr María Sabater-Molina, Department of Clinical Analysis, Virgen de la Arrixaca University Hospital, Ctra Murcia-Cartagena s/n., El Palmar, 30120 Murcia, Spain
E-mail: mariasm79es@hotmail.com

Key words: Brugada syndrome, genetics, genotype-phenotype correlation, SCN5A

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.

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

The authors would like to thank Mr. David Lopez (Hereditary Cardiopathies Unit, Virgen de la Arrixaca University Hospital) and Dr Roberto Barriales (Hospital Marítimo de Oza, Coruña, Spain) for their assistance with the clinical work during the present study. The current study was supported, in part, by a national grant from the Sociedad Española de Cardiología and by the Cardiovascular Research Network (RECAVA)

from the Carlos III Health Institute (grant nos. C03/01, RD06/0014/0017 and RD06/0014/0018).

References

- Brugada R, Campuzano O, Sarquella-Brugada G, Brugada J, Brugada P: Brugada syndrome. *Methodist Debakey Cardiovasc J* 10: 25-28, 2014.
- Potet F, Mabo P, Le Coq G, Probst V, Schott JJ, Aïraud F, Guïhard G, Daubert JC, Escande D and Le Marec H: Novel brugada SCN5A mutation leading to ST segment elevation in the inferior or the right precordial leads. *J Cardiovasc Electrophysiol* 14: 200-203, 2003.
- Chen Q, Kirsch GE, Zhang D, Brugada R, Brugada J, Brugada P, Potenza D, Moya A, Borggrefe M, Breithardt G, *et al*: Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature* 392: 293-296, 1998.
- Antzelevitch C: Ion channels and ventricular arrhythmias: Cellular and ionic mechanisms underlying the Brugada syndrome. *Curr Opin Cardiol* 14: 274-279, 1999.
- Antzelevitch C: Genetic basis of brugada syndrome. *Heart Rhythm* 4: 756-757, 2007.
- Moric E, Herbert E, Trusz-Gluza M, Filipceki A, Mazurek U and Wilczok T: The implications of genetic mutations in the sodium channel gene (SCN5A). *Europace* 5: 325-334, 2003.
- Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, *et al*: Natural history of Brugada syndrome: Insights for risk stratification and management. *Circulation* 105:1342-1347, 2002.
- Schulze-Bahr E, Eckardt L, Breithardt G, Seidl K, Wichter T, Wolpert C, Borggrefe M and Haverkamp W: Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: Different incidences in familial and sporadic disease. *Hum Mutat* 21: 651-652, 2003.
- Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Brignole M, Giordano U, Giovannini T, Menozzi C, Bloise R, *et al*: Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. *Circulation* 102: 2509-2515, 2000.
- Bai R, Napolitano C, Bloise R, Monteforte N and Priori SG: Yield of genetic screening in inherited cardiac channelopathies: How to prioritize access to genetic testing. *Circ Arrhythm Electrophysiol* 2: 6-15, 2009.
- Kapplinger JD, Tester DJ, Alders M, Benito B, Berthet M, Brugada J, Brugada P, Fressart V, Guerchicoff A, Harris-Kerr C, *et al*: An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm* 7: 33-46, 2010.
- Campuzano O, Allegue C, Iglesias A and Brugada R: Genetic basis of Brugada syndrome. *J Genet Syndr Gene Ther* 4: 7, 2013.
- Delpón E, Cordeiro JM, Núñez L, Thomsen PE, Guerchicoff A, Pollevick GD, Wu Y, Kanters JK, Larsen CT, Hofman-Bang J, *et al*: Functional effects of KCNE3 mutation and its role in the development of Brugada syndrome. *Circ Arrhythm Electrophysiol* 1: 209-218, 2008.
- London B, Michalec M, Mehdi H, Zhu X, Kerchner L, Sanyal S, Viswanathan PC, Pfahnl AE, Shang LL, Madhusudanan M, *et al*: Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na⁺ current and causes inherited arrhythmias. *Circulation* 116: 2260-2268, 2007.
- Watanabe, H, Koopmann, TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, Demolombe S, Probst V, Anselme F, Escande D, *et al*: Sodium channel β_1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin. Invest* 118: 2260-2268, 2008.
- Antzelevitch, C, Pollevick, GD, Cordeiro, JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, *et al*: Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation* 115: 442-449, 2007.
- Weiss R, Barmada MM, Nguyen T, Seibel JS, Cavlovich D, Kornblit CA, Angelilli A, Villanueva F, McNamara DM and London B: Clinical and molecular heterogeneity in the Brugada syndrome: A novel gene locus on chromosome 3. *Circulation* 105: 707-713, 2002.
- Hedley PL, Jørgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA and Christiansen M: The genetic basis of Brugada syndrome: A mutation update. *Hum Mutat* 30: 1256-1266, 2009.
- Gui J, Wang T, Jones RPO, Trump D, Zimmer T and Lei M: Multiple loss-of-function mechanisms contribute to SCN5A-related familial Sick Sinus Syndrome. *PLoS ONE* 5: e10985, 2010.