A Novel Variant in the SCN5A Gene May Cause Brugada Syndrome

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Abstract

Brugada syndrome is a genetic disease characterized by ST segment elevation in V1-V3 electrocardiographic leads, leading to syncope and sudden cardiac death from ventricular tachyarrhythmias. We report a woman suffering from recurrent palpitation and syncope. The electrocardiogram showed alterations consistent with type I Brugada Syndrome electrocardiographic pattern. Comprehensive genetic analysis using Next Generation Sequencing technology identified two rare variants (p.L143P_DSP and p.C1703S_SCN5A). Clinical and genetic analysis in family members identified both genetic variant in her son. His electrocardiogram also showed electrocardiographic alterations consistent with Brugada Syndrome pattern despite remained asymptomatic. Clinical, genetic and bioinformatic analysis supported a potential pathogenic role of the variant in the SCN5A gene, being the most plausible cause of the disease.

Keywords: Brugada Syndrome; Sudden Cardiac Death; Genetics; SCN5A

Highlights
a. Brugada Syndrome patients showed incomplete penetrance, a hallmark of the disease
b. Next Generation Sequencing allows a comprehensive, cost-effective genetic analysis
c. Several analyses, including family segregation, are crucial to clarify the role of a genetic variant
d. Clinical and genetic assessment is crucial to identify relatives at risk

Introduction

Brugada Syndrome (BrS) is a cardiac arrhythmogenic entity characterized in the electrocardiogram (ECG) by spontaneous or drug-induced ST segment elevation in the right precordial leads (V1-V3), often referred to as a type-I BrS pattern [1]. This electric alteration can cause syncope and sudden cardiac death (SCD) in a structurally normal heart due to rapid polymorphic ventricular tachycardia (VT) or ventricular fibrillation (VF). However, the typical ECG has been also identified in asymptomatic people who are at risk of SCD. Unfortunately, the SCD may be the first manifestation of the disease. The lethal episode usually occurs at rest, 8 to 10 times more common in men and the onset of disease usually occurs around 40 years old. The incidence in global population is estimated to 5 in 10,000 despite the exact prevalence is unknown. It has long been recognized as sudden unexplained death syndrome (SUDS) in Southeast Asia, which has one of the highest reported incidences [2].

BrS is a familial disease that follows an autosomal dominant pattern of inheritance. Currently, more than 300 variants in more than 20 genes have been associated with BrS [3]. Most part of these genes encodes myocyte ion channels (sodium, potassium and calcium) or associated proteins. Therefore, use of Next Generation Sequencing (NGS) technology is the current most cost-effective approach in a genetic analysis. Despite recent advances, a comprehensive genetic analysis only identify the cause of disease in 35% of families and 25% of these usually carry a pathogenic variant in the SCN5A gene [4]. This gene encodes the pore-forming alpha-subunit of the human cardiac voltage-gated sodium channel Na1.5, which mediates the fast inward Na+ current (I Na) that contributes to the rapid depolarization of the cardiac action potential.

In the present manuscript we aim to perform a comprehensive clinical assessment and genetic analysis in a family suffering from BrS.

Material and Methods

This study was approved by the Ethics Committee of our hospital and conforms to the principles outlined in the declaration of Helsinki. All individuals signed a written informed consent to participate, in accordance with international review board guidelines of our institution. All the samples were anonymized. Detailed family history was obtained including age of presentation, initial symptoms, 12-lead ECG and flecainide test.

Genomic DNA was extracted from whole blood (Perkin Elmer Inc). DNA was fragmented and library preparation was performed according to the manufacturer's instructions (Sure Select XT Target Enrichment System for Illumina Paired-End Sequencing Library protocol, Custom 1-499 Kb library, Agilent Technologies Inc). Sequencing protocol was developed on MiSeq System (Illumina). We analyzed the most prevalent genes involved in SCD-associated pathologies (ABCC9, ACTC1, ACTN2, AKAP9, ANK2, BAG3, CACNA1C, CACNA2D1, CACNB2, CASQ2, CAV3, CRYAB, CSRP3, DES, DMD, DSC2, DSG2, DSP, EMD, FKTN, GLA, GPD1L, HCN4, JPH2, JUP, KCNBD1, KCNE1, KCNE2, KCNE3, KCN5, KCN7, KCNQ1, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEBL, NEXN, NOS1AP, PDLIM3, PKP2, PLN, PRKAG2, RANGRF, RBBM20, RyR2, SCN1B, SCN2B, SCN4B, SCN5A, SCDG, SLMAP, SNTA1, TAZ, TCAF, TGB3, TMEM43, TMAP3, TNCL1, TTTS12, TP63, TPM1, TRDN, TRPM4, TTN, and VCL) [5]. Variants were annotated with dbSNP human Build 142 IDs (http://www.ncbi.nlm.nih.gov/SNP/); NHLBI Exome Sequencing Project (ESP) ESP6500SI-V2 data release (http://evs.gs.washington.edu/ESV/); 1000 Genomes browser Phase 3 data release (http://www.1000genomes.org/); Exome Aggregation Consortium (ExAC) v0.3 data release (http://exac.broadinstitute.org/); Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php); ClinVar archive (http://www.ncbi.nlm.nih.gov/clinvar/); Ensembl information (http://www.ensembl.org/index.html), and in-home database. Variants were annotated and allelic frequency was consulted using the

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same databases. In-silico pathogenicity of novel genetic variations was assessed using CONDEL, PROVEAN, and Mutation Taster. Alignment among species was also performed using the Universal Protein Resource database. Non-common (Minor Allele Frequency or MAF < 1%) genetic variants were confirmed by Sanger method. CNV analysis was performed using an algorithm capable to identify this type of genetic alterations from custom enrichment gene panel designs. Familial cosegregation of rare genetic variants was also performed using Sanger technology. All coding exons and flanking intronic sequences of SCN5A and DSP were also sequenced using Sanger technology. Regions were amplified by PCR, purified and directly sequenced in both directions (3130XL Applied Biosystems). SeqScape Software v2.5 (Life Technologies) was used to compare results with the reference sequence from Hg19.

Results

Phenotypical Characteristic of Patients

A 46 year-old woman presented with recurrent palpitations at rest. Family history showed a brother died suddenly during sleep at 42 years of age without previous symptoms. Autopsy did not identify any alteration and cardiac arrhythmia was concluded as the most plausible cause of death. However, no molecular autopsy was performed. Taking all data into account, comprehensive clinical assessment was performed in the patient and her initial ECG revealed electric alterations in ST segment but not diagnostic. Flecainide test unmasked a typical ECG showing BrS type-I pattern. Family assessment (husband and two sons) identified a son, 25 years of age with a basal ECG type-I pattern, diagnostic of BrS (figure 1 and 2). However, the son remained asymptomatic, without any previous symptom. Neither the husband nor other the son showed any ECG alteration. Father’s family history was negative for seizures, pregnancy loss, neonatal death, or SCD.

Genotype Results

Next Generation Sequencing analysis revealed a call rate of 99.85% at 30X (12 exons failed but they were sequenced by Sanger technology). Mean coverage was 473, and the percentile 25 and 75 was 350 and 590, respectively. We identified two rare missense variants in two different genes (SCN5A and DSP) (figure 3). The first rare variant (p.C1703S_SCN5A) was never reported so far and is novel. The amino acid Cysteine (Cys, C) change to Serine (Ser, S) at position 1703 (nucleotide 5107, where T changes to A, c.5107T > A). The position is highly conserved between species (figure 4), and predicted in-silico as deleterious/pathogenic by all in-silico tools used. The second rare variant (p.L143P_DSP) was reported previously in global population databases (Allele frequency: 1/121158). The amino acid Leucine (Leu, L) change to Proline (Pro, P) at position 143 (nucleotide 428, where T changes to C, c.428T > C). The position is not highly conserved between species, and predicted in-silico as Neutral by all in-silico tools used. No CNVs were identified in any of genes analyzed.

Figure 1: Family pedigree. The generations are indicated in the left column and all individuals are identified with a pedigree number. In all other family members, clinical and genetic analysis was performed. Black round/square means clinically diagnosed patients. White round/square means negative on clinically assessment. Plus signs indicate genetic carriers. Minus signs indicate non-genetic carriers. Arrow indicates the index case.

Figure 2: Electrocardiogram showing basal BrS type I pattern.
Association of Genotype with Brugada Syndrome Phenotype

DNA samples from three family members were analyzed for both rare variants identified in index case. Both variants were identified only in one of the son who also showed a BrS ECG pattern. The husband and the other son did not carry either of the rare variants. As mentioned above, both relatives also showed normal ECG.

Discussion

In the present report we identified a family showing BrS pattern in the ECG. Our index case presented with palpitations, an usual symptom of BrS but ECG showed a doubtful type 1 Brugada ECG; following current clinical guidelines, subsequent Flecainide test (sodium channel blocker) unmasked the type-I pattern, diagnostic of BrS [6]. A comprehensive genetic test identified two rare variants, one in the DSP gene and other in the SCN5A gene. The DSP gene codifies desmoplakin, a desmosomal protein of myocytes associated with cardiomyopathies (mainly arrhythmogenic cardiomyopathy). No report about pathogenic DSP variants associated with BrS was reported so far. The potential non-pathogenic role was supported by the evidence that there is low conservation of the amino acid across species, resulting in in-silico tools predicting this change to be neutral. However, we do not exclude a possible contribution of the DSP variant as BrS phenotype modifier. The second variant was a novel, not previously identified in any population database and supported in-silico analysis as pathogenic. Concerning the novel variant in the SCN5A gene (amino acid 1703), the change occurs in the domain IV of the protein (from amino acid 1524 to 1772) concretely in segments S5-S6 (from amino acid 1660 to 1772) which encode the pore region and selectivity filter of the ion channel. To date, more than 40 pathogenic variants have been reported inside these segments, most of them associated with BrS (22 variants), Long QT syndrome (16 variants) or SCD (5 variants). In near amino acids around novel variant identified (from 1690 to 1722), all cases are associated with BrS, suggesting a hot-spot for BrS [7]. This potential pathogenic role agrees with current ACMG guidelines of pathogenic classification [8].

Family assessment in her husband and two sons identified
that all they remained asymptomatic. This fact is also reported in BrS patients in whom variable expressivity and incomplete penetrance are hallmarks of disease and even SCD may be the first manifestation of the disease [9]. Family history showed a sudden death during sleep in index case's brother at young age, accordingly with epidemiological characteristics of BrS [2]. Unfortunately, neither additional clinical data nor samples from other relatives were available. One of sons of the index case also carried the both variants, despite being asymptomatic. Neither husband nor the other son carried any of variant supporting the potential deleterious role of both variants. Hence, family segregation supports a potential pathogenic role of both variants but additional analysis suggests that the novel variant in the \textit{SCN5A} gene is the most plausible cause of BrS, at least in our family. Preventive and therapeutic strategies in this family are based on close follow-up and avoidance of drugs (www.brugadadrugs.org) and situations (fever) reported as triggers of arrhythmias in BrS patients. Current guidelines recommend early genetic testing for clinical management and therapeutic decisions involving family members at any age because BrS may be presented as SCD as the first manifestation of the disease (even in infants) [10]. Hence, molecular autopsy in cases with a no conclusive cause of death after complete autopsy should be performed, as recommended by current guidelines [11]. Both situations are crucial in order to preventive identification of relatives at risk and thus, avoid new potential cases of syncope. In addition, it is therefore of vital significance that a multidisciplinary team discusses the clinical interpretation of each novel variant, being very cautious before translation into clinical practice.

Finally, some limitations should be mentioned concerning this study. The first one is the lack of clinical and genetic data about index case’s brother. In addition, no functional studies have been performed to clarify the pathophysiological mechanisms of both variants such as in-vitro studies, induced pluripotent stem cells analysis or even in-vivo analysis. Other limitation is that although we have analyzed the known genes associated with SCD, it is possible that additional genetic alterations that predispose to BrS could be present in unknown genes which have not been analyzed in our study.

Conclusions

In conclusion, BrS is an inherited cardiac arrhythmic disorder associated with syncope and SCD, despite some patients may remain asymptomatic. We identified a family clinically diagnosed of BrS who carry two genetic variants potentially pathogenic but additional data suggest p.C1703S in the \textit{SCN5A} as the most plausible cause of the disease. Early clinical assessment and genetic identification of relatives at risk may help to avoid episodes of syncope adopting preventive therapeutic measures.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


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