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RESEARCH ARTICLE

Identification of a Novel *SCN5A* gene variant in a young female with atrioventricular canal defect in the absence of classical Brugada syndrome phenotype

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ABSTRACT

Background: Brugada syndrome is generally considered a cardiac channelopathy disorder characterized by syncope or sudden cardiac death. The sodium voltage-gated channel alpha subunit 5 (*SCN5A*) gene is the most commonly mutated gene associated with Brugada syndrome. Recent discoveries of new variants of this gene, along with current guidance of family screening, have identified several asymptomatic carriers with potentially causative mutations.

Case presentation: We present the case of a 25-year-old female patient without any family history of Brugada syndrome nor related congenital cardiovascular disorders, with an extensive atrioventricular canal defect, who tested positive for a novel heterozygous variant NM_198056.3: c.3169G>C (p. Asp1057 His) in the *SCN5A* gene. She had no history of syncope or aborted sudden cardiac death except for recurrent chest infections since her early childhood. Intriguingly, she did not show a type I Brugada electrocardiogram pattern.

Conclusions: This report provides a novel heterozygous variant NM_198056.3: c.3169G>C (p. Asp1057 His) in the *SCN5A* gene, which may have a potential detrimental effect.

Keywords: Brugada syndrome, atrioventricular canal, atrial fibrillation, novel variant, whole genome sequencing, Sanger sequencing.

BACKGROUND

Brugada syndrome is a heritable cardiac channelopathy characterized by a distinctive electrocardiographic (ECG) pattern and an increased risk of sudden cardiac death due to ventricular arrhythmias.[1,2]

First described in 1992, Brugada syndrome is associated with mutations in the sodium voltage-gated channel alpha subunit 5 (*SCN5*A),[3-7] which encodes the alpha subunit of the primary cardiac sodium channel Nav1.5 This channel has four domains (DI-DIV), each with six segments (S1-S6).[8] The syndrome exhibits autosomal dominant inheritance with incomplete penetrance and a prevalence that varies geographically, being more common in Southeast Asian populations.[3-7] Despite extensive research, the pathophysiological mechanisms underlying Brugada syndrome remain complex, involving a combination of genetic, molecular, and electrophysiological factors.[1-7]

We report the case of a young female patient without any family history of Brugada syndrome or any related congenital cardiovascular disorders who tested positive for a novel heterozygous variant NM_198056.3: c.3169G>C (p. Asp1057 His) in the *SCN5A* gene.

CASE PRESENTATION

A 25-year-old nondiabetic normotensive female was admitted to the emergency department with fever, chest discomfort, and shortness of breath for the last 15 days, which was exaggerated for the previous few hours. On examination, her heart rate was 112/min, blood pressure 90/60 mmHg, respiratory rate 34/min, SpO2 84% in room air, capillary blood glucose 138 mg/dl, and temperature 39.5°C. A general survey revealed grade three nail clubbing with moderate cyanosis and abdominal tenderness. Cardiopulmonary examination revealed variable splitting of first heart sound (S1); bilateral vesicular breath sound was found to be decreased with prominent basal crepitations predominantly over the right hemithorax. The chest X-ray revealed pulmonary congestion with white infiltrates, predominantly over the right middle lobar area. She was immediately initiated on oxygen support with a face mask, intravenous fluid, and broad-spectrum antibiotics. 12 lead electrocardiogram revealed low amplitude of P wave on the rhythm strip along and right ventricular hypertrophy **(Figure-1a)**. Laboratory tests showed a white blood cell count of 12.3 10^9 /µL with 81% neutrophils, serum C reactive protein of 38 mg/dl (normal range, 0.3 - 1 mg/dl), serum procalcitonin level of 0.7 ng/ml (normal range, less than 0.05 ng/ml), and normal high sensitivity cardiac troponin levels. 2D echocardiography with Doppler was conducted, which revealed an atrioventricular canal defect, single atrium with single atrioventricular valve, large 37 mm ventricular septal defect, mild regurgitation through an atrioventricular valve, right ventricular hypertrophy with partial outflow tract obstruction defect and hypoplastic left ventricular cavity (**Figure-1b and 1c**). Detailed history-taking revealed recurrent episodes of chest infection since her early childhood, for which she was treated accordingly. Besides, an evidential history of preterm delivery with significant developmental retardation became clear. There was no significant history of smoking or any substance abuse.

Meanwhile, her condition improved after three days of closed intensive observation. Based on the findings of the significant cardiac anomaly on echo and disproportionate metacarpophalangeal ratio with grade-3 nail clubbing, we considered among the initial diagnoses both the Holt-Oram syndrome, also referred to as the heart-hand syndrome and the nonsyndromic atrioventricular canal defect. However, the hands' digital X-rays showed no brachy-mesophalangia, triphalangeal thumb, or radial deficiency. Henceforth, whole exome genetic sequencing was planned.

Figure-1

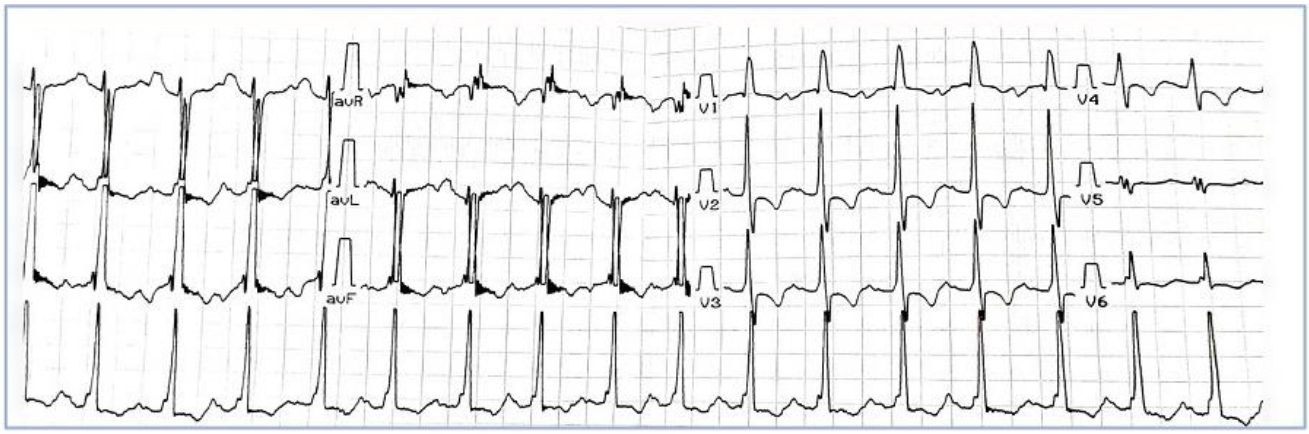

Figure- 1a

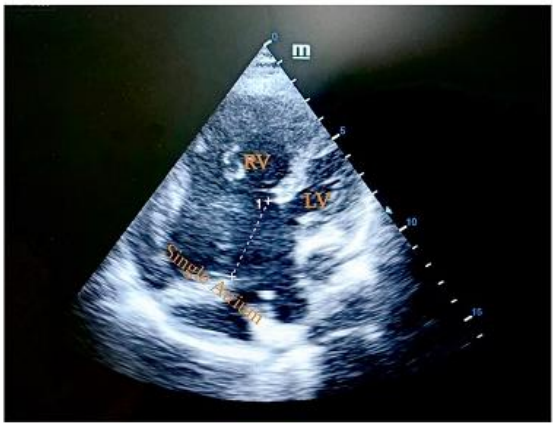

Figure- 1b

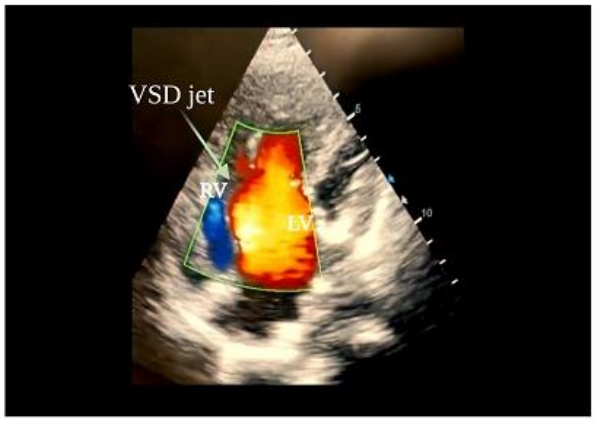

Figure- 1c

Figure 1a reveals a low amplitude of P waves on the rhythm strip and right ventricular hypertrophy. Figure-1b and Figure-1c reveal a single atrium with a significant ventricular septal defect (in dotted line and color doppler-flow), right ventricular (RV) hypertrophy with partial outflow tract obstruction defect, and hypoplastic left ventricular (LV) cavity (four apical chamber view on 2D-echocardiography).

Genetic Assessment:

Whole exome analysis revealed the presence of a novel transversion type single nucleotide variation in the *SCN5A* gene at exon 17 (c.3169G>C), resulting in a missense type mutation from aspartic amino acid to histidine amino acid at 1057 position of the primary protein structure. Sanger sequencing data confirmed revealed a nucleotide change at chromosome 3 (c.3169G>C). We have also performed multiple sequence alignments to determine whether aspartic acid is conserved across the species, and the result of alignment revealed that this particular amino acid is highly conserved (Table 1) **(Figures 2a and 2b).** The detailed sequencing procedure is mentioned in Supplementary 1.

Table 1: Multiple sequence alignment showing conserved aspartic amino acid residue across different species.

Sl No	Genbank Id	Species	Start Position	Amino acid Sequence	End Position
1	Case	*Homo sapiens sapiens*	1050	AVAESDTHDQEEDEENSLGTEEESSKQ	1076
2	KAI2528893	*Homo sapiens sapiens*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
3	PNI74006	*Pan troglodytes*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
4	ACX32327	*Synthetic construct*	-	AVAESDTDDQEEDEENSLGTEEESSKQ	-
5	XP_057157671	*Pan paniscus*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
6	XP_055237269	*Gorilla gorilla gorilla*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
7	XP_030667968	*Nomascus leucogenys*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
8	XP_058298838	*Hylobates moloch*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
9	XP_011854877	*Mandrillus leucophaeus*	1049	AVAESDTDDQEEDEENSLDTEEESSKQ	1075
10	XP_025231014	*Theropithecus gelada*	1050	AVAESDTDDQEEDEENSLDTEEESSKQ	1076
11	XP_003894712	*Papio anubis*	1050	AVAESDTDDQEEDEENSLDTEEESSKQ	1076
12	XP_037844370	*Chlorocebus sabaeus*	1050	AVAESDTDDQEEDEENSLDTEEESSKQ	1076
13	XP_055138561	*Symphalangus syndactylus*	1050	AVAESDTDDQEEDEENSLGTEEESSKQ	1076
14	XP_030790807	*Rhinopithecus roxellana*	822	AVAESDTDDQEEDEENSLDMEEESSKQ	848
15	EHH16500	*Macaca mulatta*	916	AVAESDTDDQEEDEENSLDTEEESSKQ	942
16	XP_028699800	*Macaca mulatta*	889	AVAESDTDDQEEDEENSLDTEEESSKQ	915
17	XP_011889640	*Cercocebus atys*	1050	AVAESDTDDQEEDEENSLDTEEESSKQ	1076

Figure-2

Gene Name	Variant Reported in Index Patient	Variant Status	Inheritance
SCN5A	chr3:c.3169G>C, (p.Asp1057His)/Heterozygous	Present (Heterozygous)	Autosomal Dominant

Figure- 2a

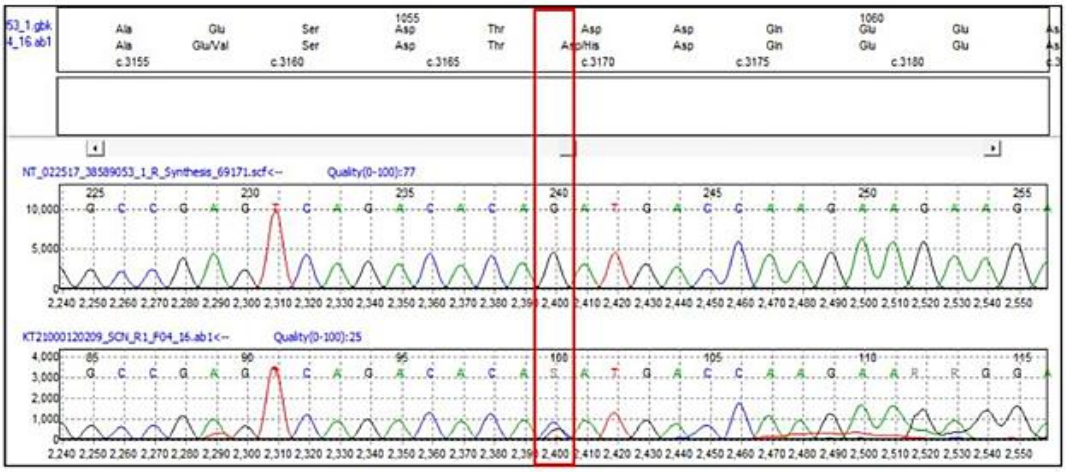

Figure- 2b

Figure- 2c

Figure 2a: Sanger Sequencing for the confirmation of variant in the provided sample detected in the SCN5A gene in the index patient. The analysis was performed only for the variant at c.3169G>C (p.Asp1057His) in the SCN5A gene.

Figure-2b: Sanger sequencing data (electropherogram) for the provided sample showing nucleotide change at chromosome-3: c.3169G>C, (p.Asp1057His) in *SCN5A* gene.

Figure 2c: CFSSP prediction of the local secondary structure of the sodium ion transport associated domain (InterPro ID – IPR10526, Pfam ID – PF06512) comprising 953rd to 1200th amino acid. The D1057H change in the protein corresponding to D105H in this domain (underlined with blue in the figure) shifts the second turn to the following amino acid, causing local structural instability.

In-silico Predictions:

The secondary structure tolerance prediction analyzer tool, Sorting Intolerant From Tolerant (SIFT),[9] was used to predict the functional tolerance of the variation in comparison to the wild type. Predictive changes in the secondary structure of proteins due to nucleotide variations were analyzed using the Chou and Fasman Secondary Structure Prediction Server (CFSSP)[10] and Disorder Including Mutation Prediction (DIM_PRED).[11] The analysis found that substituting aspartate at the 1057 amino acid position of the *SCN5A* protein can only be tolerated functionally when replaced with amino acids like phenylalanine, glutamine, and glutamic acid. With a SIFT tolerance score of a moderately higher value of 0.68, the rest of the amino acids, including histidine, are predicted to be non-tolerable at the 1057 aa position of *SCN5A* polypeptide. This SCN5A encoded polypeptide is the alpha subunit of the voltage-gated sodium channel 5 expressed in cardiac cells. The protein has seven functional domains, including four ion transport domains, one cytoplasmic domain of sodium ion channel, one inactivation gate, and one sodium ion transport associated domain (InterPro ID – IPR10526, Pfam ID – PF06512) comprising 953rd to 1200th amino acid where the change was found in the 1057th position of the protein. This 248 amino acids long domain harbours the change of D to H (Asp to His) at its 105th position. CFSSP server analysis of this domain revealed that the wild-type protein has a secondary turn position comprising 106th amino acid D (1058aa of the protein).

In contrast, D1057H variation altered the turn position to comprise 107th amino acid Q (1059 aa of the protein). The DIM_Pred tool (Prediction of protein disorder on amino acid substitutions) of IIT, Madras, India, was used to identify any functional change of the D1057H substitution. DIM_Pred predicted an order to disorder change due to this substitution. Therefore, the presently reported novel variation with a non-tolerable substitution at the 1057th position is expected to be functionally damaging. **(Figure 2c)**

DISCUSSION

The case presented here highlights a 25-year-old female with a novel *SCN5A* variant (c.3169G>C, p. Asp1057His) who presented with an atrioventricular canal defect without the classical Brugada syndrome phenotype. This case adds to the growing body of literature on the genetic variability and phenotypic heterogeneity of Brugada syndrome.

Over 300 *SCN5A* variants have been found, including missense, nonsense, insertion/deletion, and splice site variations associated with Brugada syndrome.[3-7,12] In this present study, we report for the first time the NM_198056.3: c.3169G>C (p. Asp1057 His) heterozygous variant in the *SCN5A* gene and its segregation with Brugada syndrome. The findings support a likely harmful effect of this variant and contribute an essential milestone to advancing our diagnostic capabilities in patients with similar variants. In silico analysis predicted that the identified *SCN5A* variant is likely deleterious, potentially affecting the protein's function. This aligns with previous findings that many *SCN5A* mutations result in a loss of function of the sodium channel, contributing to the arrhythmogenic substrate in Brugada syndrome.[4] However, the clinical significance of this variant requires further validation through functional studies and family screening.

The absence of a type 1 Brugada ECG pattern in our patient, despite the presence of an *SCN5A* mutation, underscores the incomplete penetrance and variable expressivity of the syndrome. Although an electrophysiological study was planned, the patient denied it. Besides, we decided not to perform any drug provocation test with a sodium channel blocking agent provided that the patient's condition was acute and that a possible positive test would not alter the treatment in the absence of any arrhythmic syncope or aborted sudden cardiac death. The current definition of only type-I morphology is diagnostic of Brugada syndrome, while the other types, including type-II and type-III electrocardiogram patterns, do not support its diagnosis.[12] We took a more conservative

approach as the patient did not consent to an electrophysiological study with regular follow-ups and lifestyle recommendations. Considering the patient's poor financial status and normal phenotypical characteristics on detailed clinical examination, including 12-lead electrocardiogram and 2D-echocardiography among first-degree relatives of the patient, we also decided not to go for whole-exome sequencing of the first-degree family members.

The coexistence of significant structural heart anomalies, such as the atrioventricular canal defect in our patient, raises intriguing questions about the interplay between genetic mutations and cardiac development. While structural heart defects are not typically associated with Brugada syndrome,[1,2] this case suggests that certain *SCN5A* mutations might have broader implications for cardiac morphology and function. Atrioventricular canal defects can be classified into complete and partial, based on the extent of anatomical abnormalities, representing approximately 7% of all congenital heart diseases.[13] Most of such cases are usually associated with underlying genetic abnormalities like trisomy-21/ heterotaxy, yet 40% of such cases exist without these usual genetic associations; of these, 30% of patients have identifiable chromosomal or gene disorders, 10% have extracardiac anomalies, and the remaining do not have extracardiac abnormalities (nonsyndromic atrioventricular canal defect).[14] According to a recent study,[15] five out of 36 patients with Brugada syndrome revealed ventricular septal defects for which they were taken forward for genetic research, showing *SCN5A* with missense, non-functional variant, and loss-of-function variants.

In summary, this case interestingly reveals an uncanny combination of Brugada syndrome-related genetic variance with extensive atrioventricular canal malformation. Intriguingly, such extensive cardiac malformation in our patient could not be apprehended, and whether this was related to this novel *SCN5A* variant. Thus, it highlights that clinical decision algorithms may sometimes be more complex and stepwise protocol-based, necessitating more comprehensive individualized investigations and management. Additionally, complete atrioventricular canal defect in association with the novel heterozygous variant NM_198056.3: c.3169G>C (p. Asp1057 His) in the *SCN5A* gene segregates with Brugada syndrome; this finding may add crucial human data to understand the pathogenesis of Brugada syndrome for patients with this variant. Such novel variants may also be significant for understanding the population not associated with typical genotype or nonsyndromic atrioventricular canal defect.

Finally, this case emphasizes the need for comprehensive genetic screening and personalized management strategies in patients with suspected Brugada syndrome, particularly those with atypical presentations. Future research should focus on elucidating the functional impacts of novel SCN5A variants and exploring their potential roles in congenital heart defects.

Conflict of Interest Statement:

None

Study funding:

Nil.

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Author contributions:

All authors contributed significantly to the creation of this manuscript; each fulfilled the criterion as established by the ICMJE.

Ethics statement:

Written informed consent was obtained from the patients to publish this article and any accompanying images.

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