

Is variant pathogenicity in the eye of the beholder? A case of unexplained sudden cardiac arrest highlights the potentially dangerous role of historical rare variant compendia in *SCN5A* rare variant adjudication



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Introduction

Sudden cardiac arrest (SCA) is a leading cause of death globally.^{1,2} Although most SCAs occur in the setting of structural or ischemic heart disease, many seemingly unexplainable cases may stem from primary electrical disorders such as Brugada syndrome (BrS).² However, 5%–10% of SCA survivors have no evidence of a structural or primary electrical disorder and are diagnosed with idiopathic ventricular fibrillation (VF).³

BrS, affecting an estimated 1–5 per 10,000 individuals globally,⁴ is characterized by an abnormal electrocardiogram (ECG) with coved-shape ST elevation involving the right precordial leads V₁–V₃.⁴ The Brugada ECG pattern is often concealed in patients, but can be provoked by sodium channel blockers such as procainamide, flecainide, and ajmaline.⁴ The diagnostic type 1 Brugada ECG pattern is defined as coved-type ST segment, J-point elevation of ≥ 2 mm, and a negative T wave.^{4,5} *SCN5A*-encoded voltage-gated sodium

channel (Nav1.5) loss-of-function mutations have been implicated in 18%–30% of BrS.^{5–7}

Here, we present the case of a survivor of an unexplained SCA diagnosed elsewhere as having BrS following a genetic testing laboratory's overzealous interpretation of an identified *SCN5A* variant as "likely pathogenic." Subsequent electrophysiological functional studies determined the variant to be benign and the diagnosis of BrS was removed.

Case report

An 18-year-old white woman presented initially for the evaluation of sudden onset of palpitations and syncope. Following the appointment, the patient suffered an SCA and was resuscitated by paramedics with 2 shocks. A dual-chamber implantable cardioverter-defibrillator (ICD) was implanted and she was started on nadolol. The patient was challenged with procainamide during the ICD implantation procedure, but no Brugada ECG pattern was observed. Following an unremarkable coronary angiogram and cardiac magnetic resonance imaging, pan-arrhythmia genetic testing was pursued, which identified an ultra-rare p.Glu1240Gln-*SCN5A* missense variant. Based on a 2002 publication,⁸ where the variant was identified in a single patient diagnosed with supposed BrS, the p.Glu1240Gln-*SCN5A* variant was reported to the ordering healthcare providers as "likely pathogenic," despite no functional or phenotype co-segregation data to support this variant interpretation.

Based on the aforementioned variant interpretation, cascade screening of potentially at-risk first- and second-degree relatives was initiated and the patient's asymptomatic father (II2), sister (III3), brother (III4), paternal uncle (II3), and cousins (III5 and III8, Figure 1A) were also p.Glu1240Gln-*SCN5A* positive. Importantly, all p.Glu1240Gln-*SCN5A*-positive family members remain

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KEY TEACHING POINTS

- This study shows another example of the negative effects associated with using the alleged genotype to determine the presumed phenotype. In this case, the patient and her family were led down a treatment path designed for a channelopathy with which they should not have been diagnosed.
- Genetic testing should never be used to make a primary diagnosis of Brugada syndrome (BrS) in isolation, especially when the clinical phenotype of BrS is sorely missing.
- While guidelines have provided a major effort towards the standardization of variant interpretation, inter-laboratory differences in the use and implementation of the American College of Medical Genetics criteria exist.
- Unfortunately, some genetic testing companies still conflate the mere presence of a specific variant in an early manuscript as evidence for pathogenicity, despite these reports lacking (1) adequate numbers of healthy controls, (2) functional characterization of the variant, or (3) illustration of proper co-segregation with the disease phenotype within a multigenerational pedigree; each of which individually, and more so concomitantly, constitutes more definitive evidence in the interrogation of a potentially pathogenic variant.

asymptomatic and have normal ECGs with no manifest Brugada ECG pattern at rest or with high-lead positioning. [Figure 1B](#) shows a representative ECG of the patient's asymptomatic father.

Ultimately, despite the absence of a spontaneous or provoked type I Brugada ECG pattern in the p.Glu1240Gln-SCN5A-positive proband or her variant-positive family members, a diagnosis of BrS was rendered, largely based on her "positive" genetic test. After she received 1 VF-terminating ICD shock 2 months later, the nadolol was discontinued and the patient was placed on metoprolol. Throughout the next year, the patient was trialed on verapamil, quinidine, propranolol, sotalol, atenolol, and amiodarone. Significant ventricular tachycardia (VT) and VF episodes were observed on every drug except amiodarone.

Nine months post SCA, the patient underwent a BrS-specific epicardial ablation. Both procainamide and electrical stimulation were utilized to induce arrhythmias; however, no VT or VF was inducible at baseline. Two abnormal substrates were identified at the inferobasal epicardium and anterior RVOT epicardium, and were ablated subsequently. All drug therapy was discontinued following the ablation. Unfortunately, the patient's symptoms returned shortly after, and

both quinidine and propranolol were reintroduced. Despite continued drug therapy, cardiac events requiring ICD treatment persisted. The patient underwent a flecainide drug challenge test, which also failed to uncover a Brugada ECG pattern.

Lastly, the proband and her siblings were referred to the Mayo Clinic for a second opinion evaluation. Serial ECGs were conducted at rest with QTc values of 454, 462, and 473 ms. [Figure 1C](#) shows a representative ECG tracing. The patient's PR interval was normal, with a value of 150 ms. Additionally, the patient's exercise stress test was normal, with no Brugada ECG pattern or ectopy. Similarly, the patient's sister had normal ECGs before, during, and after exercise with QTc values of 440, 440, and 470 ms. Her PR interval was also normal at 150 ms. The patient's brother had normal QTc values both at rest and during exercise, a normal PR interval, and no visible Brugada ECG pattern.

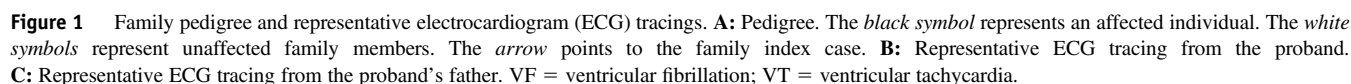
Given the lack of clinical evidence supporting a BrS phenotype in the proband and her variant-positive family members, we questioned whether p.Glu1240Gln-SCN5A was truly pathogenic. Therefore, we performed a molecular and functional characterization of p.Glu1240Gln-SCN5A.

Methods and results

Three *in silico* tools (polymorphism phenotyping v2 [PolyPhen-2], sorting intolerant from tolerant [SIFT], and combined annotation dependent depletion [CADD]) predicted p.Glu1240Gln-SCN5A as damaging. The p.Glu1240Gln variant localizes to the small extracellular loop between transmembrane spanning regions, segments DIII-S1 and DIII-S2. The p.Glu1240Gln-SCN5A has a reported allele frequency of 7.923e-05 in the European population (9/56,800 individuals) and 3.984e-05 in all represented populations (10/125,513 individuals) in the Genome Aggregation Database (gnomAD).⁹

The standard whole-cell patch clamp technique was used to measure SCN5A wild-type (WT) and mutant sodium currents at room temperature (22°C–24°C)¹⁰ with the use of an Axopatch 200B amplifier, Digidata 1440A, and pClamp 10 software (Axon Instruments, Sunnyvale, CA). All data points are shown as the mean values. Bars represent the standard error of the mean. A Student *t* test was performed to determine statistical significance between 2 groups. A *P* < .05 was considered to be significant.

Typical I_{Na} SCN5A tracings of voltage-dependent activation from WT and Glu1240Gln mutants are shown in [Figure 2A](#) with holding potential at -100 mV to various depolarization potentials. Current–voltage relationship shows that SCN5A-WT and SCN5A-Glu1240Gln reached peak at -20 mV. At peak current density, SCN5A-WT was -356.19 ± 59.95 (n = 15) and SCN5A-Glu1240Gln was -420.07 ± 52.01 (n = 16, *P* = .426). No significant differences in current density across the voltage were observed from -60 to +60 mV between SCN5A-WT and SCN5A-Glu1240Gln ([Figure 2B](#)). Analysis of inactivation curve ([Figure 3A](#)) and activation curve ([Figure 3B](#)) showed no significant shifts in $V_{1/2}$ between WT and mutant. $V_{1/2}$ of inactivation in WT cells



with clinically diagnosed BrS,⁸ the variant was never shown to co-segregate with disease, functionally characterized, or reassessed in the context of large public exome/genome databases such as gnomAD. Instead, based on this 2002 report by a highly reputable source⁸ and the predictions derived from in silico variant assessment tools, the genetic testing company interpreted/graded this variant as “likely pathogenic” for BrS. Despite the discordance between the variant interpretation and the patient’s clinical phenotype, the local physicians diagnosed this patient with BrS. This declared diagnosis changed the patient’s treatment plan and led to the misdiagnosis of her 6 family members also hosting the variant.

Though the patient has a clear history of ventricular arrhythmias, no Brugada ECG pattern was observed at rest, with high lead positioning, or with provocative drug challenges using 2 different sodium channel blockers. Additionally, none of the 6 family members that host the p.Glu1240Gln-SCN5A variant shows a Brugada ECG pattern, and all remain asymptomatic.

Unfortunately, 2% of otherwise healthy whites and 4%–6% of nonwhites host rare missense variants in *SCN5A*.¹² Accordingly, the best signal-to-noise ratio associated with *SCN5A* in the setting of BrS genetic testing is 9–12:1.^{13,14} As such, any given *SCN5A* rare variant identified even in a patient with a bona fide diagnosis of BrS still has a 8%-to-11% chance of being a rare, and likely innocuous, background genetic variant. Therefore, 2–3 of

Genetic testing for channelopathies has become widely used.¹¹ There are many benefits to using genetic testing in clinical settings, including individualized treatment options, conclusive diagnoses, and identification of at-risk family members.¹¹ However, along with these benefits comes the potential for misdiagnoses and inaccurate treatment.

Here, we describe a family misdiagnosed with BrS after the identification of a rare p.Glu1240Gln-SCN5A variant in the patient who presented with an unexplained SCA and suffered recurrent episodes of idiopathic VT/VF. Although p.Glu1240Gln-SCN5A was reported previously in a patient

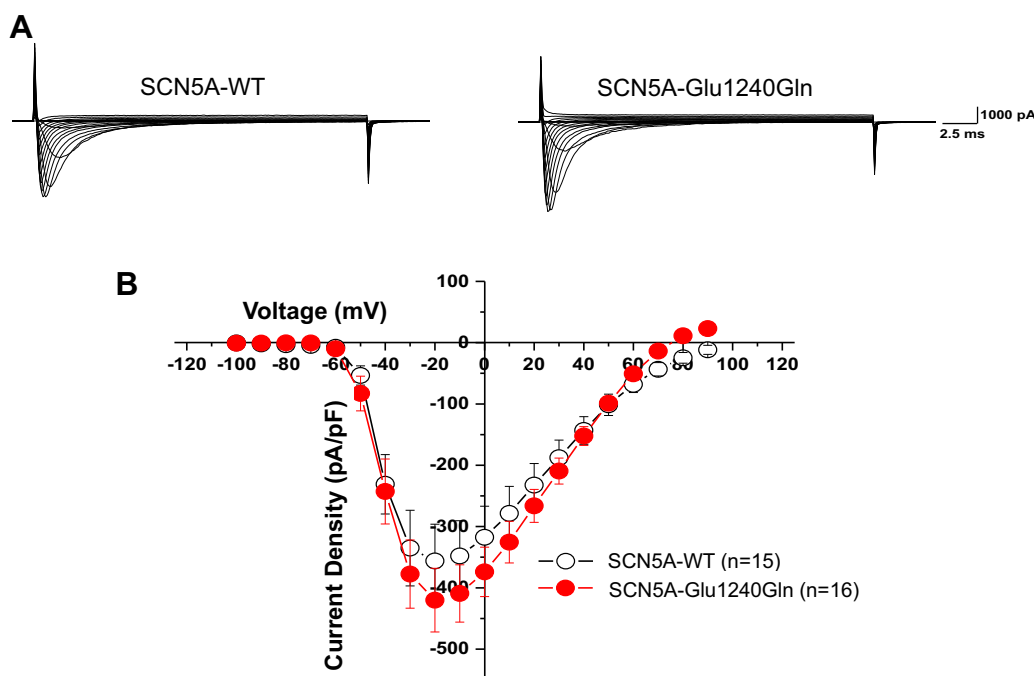


Figure 2 The p.Glu1240Gln-SCN5A variant did not significantly change current density in TSA201 cells. **A:** The whole-cell SCN5A current representative tracings showing wild-type (WT) and Glu1240Gln from a holding potential of -100 mV to testing potential of +90 mV in 10-mV increments with 24 ms duration. **B:** Current–voltage relationship for SCN5A-WT (n = 15) and Glu1240Gln variant (n = 16).

the 28 *SCN5A* variants reported in the 2002 BrS study,⁸ which was used to elevate p.Glu1240Gln-SCN5A to “likely pathogenic” status, potentially represent background genetic noise rather than true “pathogenic” mutations. In fact, including p.Glu1240Gln, 11 of the 28 (39%) “mutations” reported in the 2002 manuscript have now been seen in gnomAD, with 3 variants (p.Arg27His, p.Pro1293Ser, p.Val1951Leu) having a minor allele frequency far greater than the entire prevalence of BrS1 in the general population (~1:25,000). Furthermore, in patients without a discernible BrS phenotype (ie, unexplained SCA/idiopathic VF cases), the signal-to-noise ratio associated with *SCN5A* genetic testing is anticipated to be substantially lower than 9–12:1.¹³ Therefore, even though the recent HRS/EHRA/APHRS guidelines equate the presence of an unequivocal disease-causative mutation in *SCN5A* with the diagnosis of BrS,¹⁵ genetic testing should never be used to make a primary diagnosis of BrS in isolation, especially when the clinical phenotype of BrS is sorely missing.

Predictably, the p.Glu1240Gln-SCN5A variant identified and characterized in this study exhibited a WT in vitro electrophysiological phenotype. First, this variant was identified in an unexplained SCA survivor without evidence of a discernible spontaneous or procainamide/flecainide-induced type 1 Brugada ECG pattern, QT prolongation, or evidence of nonischemic dilated cardiomyopathy. Second, the variant was present in multiple asymptomatic family members. Lastly, the variant was observed in public exomes/genomes at a frequency (10/125,513 individuals overall, or 1:12,551) that far exceeds the estimated prevalence of *SCN5A*-mediated BrS (~1:10,000). Furthermore, no single

SCN5A variant has been demonstrated to account for more than 1% of patients with BrS. In fact, based on Whiffin and colleagues¹⁶ statistical framework for frequency-based filtering of candidate disease-causing variants, accounting for disease prevalence (1/2000 for BrS), genetic/allelic heterogeneity (0.01; ie, no variant accounts for more than 1% of cases), inheritance mode (dominant), and penetrance (assuming 0.5 for BrS), the predicted maximum allele account of an *SCN5A* BrS-causing variant in gnomAD (n = 141,456 individuals) would be only 3. The p.Glu1240Gln variant was seen 10 times, thus exceeding this predicted maximum allele account threshold.

In order to assist in the interpretation of identified variants, the 2015 American College of Medical Genetics (ACMG) guidelines provide a framework for variant classification by incorporating a variety of weighted factors that lead to a final delineation of pathogenic, likely pathogenic, variant of uncertain significance, likely benign, or benign variant.¹⁷ Using strict criteria, variants are assessed for “very strong,” “strong,” “moderate,” or “supporting” evidence for pathogenicity. Points earned in each category are combined in a variety of ways to reach a final variant classification. While these guidelines have provided a major effort towards the standardization of variant interpretation, inter-laboratory differences in the use and implementation of the ACMG criteria exist.

In fact, p.Glu1240Gln-SCN5A has been submitted to ClinVar (variant ID: 67818) by 3 independent testing labs, with varying interpretations of pathogenicity (Invitae, “uncertain significance”; Clinical Molecular Genetics Laboratory, Johns Hopkins All Children’s Hospital, “likely

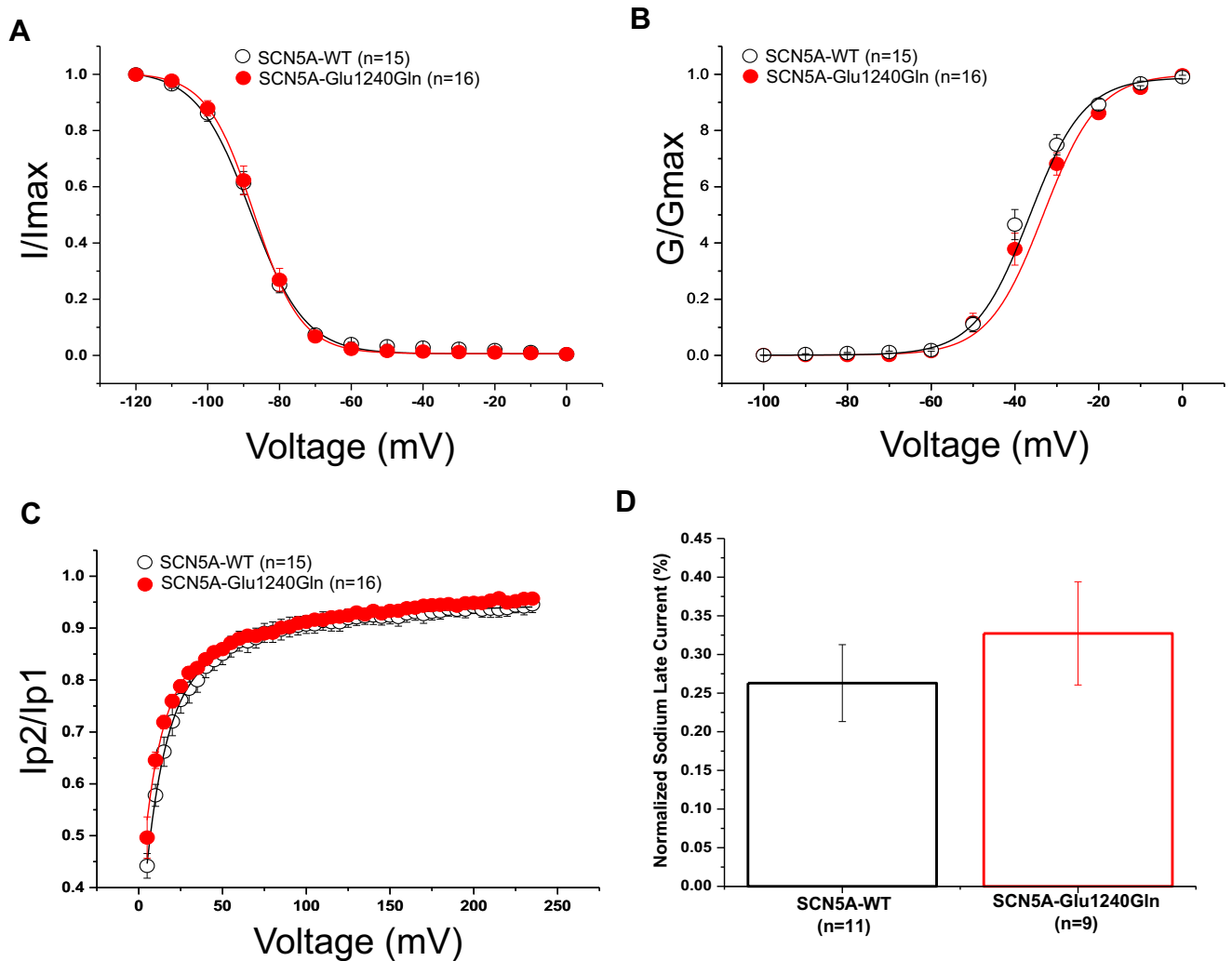


Figure 3 The p.Glu1240Gln-SCN5A variant did not significantly shift $V_{1/2}$ of inactivation or activation, fast component of recovery from inactivation, or the sodium late current. **A:** Inactivation curves of SCN5A-WT ($n = 15$) and Glu1240Gln variant ($n = 16$). I/I_{max} represents normalized sodium current. **B:** Activation curves of SCN5A-WT ($n = 15$) and Glu1240Gln variant ($n = 16$). G/G_{max} represents normalized conductance. **C:** Recovery from inactivation for SCN5A-WT ($n = 7$) and Glu1240Gln variant ($n = 7$) from a holding potential of -120 mV to prepulse of -20 mV with 700 ms duration, with increased recovery interval, followed by a test pulse of -20 mV with 20 ms duration. **D:** Summary data of SCN5A-WT ($n = 11$) and Glu1240Gln variant ($n = 9$) with respect to the late/persistent sodium current.

pathogenic”; and Cardiovascular Biomedical Research Unit, Royal Brompton & Harefield NHS Foundation Trust, interpretation “not provided”).

Additionally, while it is possible to use genetics to define a phenotype, the level of evidence needed to classify such variants is far higher than when assessing patients with a definitive diagnosis. ACMG-type guidelines are generally more effective for patients with definitive phenotypes, rather than for secondary findings or inconclusive phenotypes such as described herein.

Denham and colleagues¹⁸ have recently performed a systematic reevaluation of 425 *SCN5A* variants associated with BrS using ACMG guidelines specifically adapted to BrS and have concluded that p.Glu1240Gln would at best be considered a variant of uncertain significance. However, with the addition of our functional data demonstrating no damaging effect on the sodium channel and the absence of

segregation of the variant with a definitive phenotype in the pedigree, we can demote p.Glu1240Gln further to a benign variant in accordance with the ACMG guidelines.

In the early 2000s, hundreds of case-derived *SCN5A* missense variants were published with the inclusion of missense variants contingent upon their absence in 50–400 purportedly healthy controls and some without the consideration of functional effect.^{8,19} During this era, it was believed that disease-causing genes such as *SCN5A* would be functionally intolerant to mutation.¹³ Unfortunately, some genetic testing companies still conflate the mere presence of a specific variant in an early manuscript as evidence for pathogenicity, despite these reports lacking (1) adequate numbers of healthy controls, (2) functional characterization of the variant, or (3) illustration of proper co-segregation with the disease phenotype within a multigenerational pedigree; each of

which individually, and more so concomitantly, constitutes more definitive evidence in the interrogation of a potentially pathogenic variant.

Though the potential benefits for the genetic testing of channelopathies are extensive, the responsibility for accurate interpretation of these results is great. This study shows another example of the negative effects associated with using the alleged genotype to determine the presumed phenotype. In this case, the patient and her family were led down a treatment path designed for a channelopathy (BrS) that they should not have been diagnosed with. The importance of maintaining strict guidelines for variant interpretation is increasingly evident and poses a large problem for medical professionals.

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