A novel functional variant residing outside the *SCN5A*-encoded $Na_v1.5$ voltage-sensing domain causes multifocal ectopic Purkinje-related premature contractions

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Introduction

Pathogenic variants in the *SCN5A*-encoded pore-forming α-subunit of the $Na_v1.5$ voltage-gated cardiac sodium channel are associated with primary arrhythmia syndromes including type 3 long QT syndrome (LQT3; $Na_v1.5$ gain-of-function), Brugada syndrome (BrS; $Na_v1.5$ loss-of-function), early-onset atrial fibrillation (AF; $Na_v1.5$ loss- and gain-of-function), primary cardiac conduction disease ($Na_v1.5$ loss-of-function), dilated cardiomyopathy (DCM; $Na_v1.5$ loss- and gain-of-function), and overlap syndromes, whereby the clinical hallmark(s) of more than 1 disorder is observed with the same patient and/or family.[1–5]

Multifocal ectopic Purkinje-related premature contractions (MEPPC; $Na_v1.5$ gain-of-function), a recently described *SCN5A*-mediated cardiac channelopathy, is characterized by frequent premature ventricular complexes (PVCs) originating from the fascicular-Purkinje system, atrial arrhythmias, a predilection for PVC-mediated DCM, and sudden cardiac death (SCD).[2,6] Thus far, only 6 MEPPC-causative *SCN5A* variants (p.Ala204Glu-SCN5A,[3] p.Gly213Asp-SCN5A,[7] p.Arg222-Glu-SCN5A,[6,8,9] p.Arg225Pro-SCN5A,[10] p.Leu828Phe-SCN5A,[11] and p.Met1851Val-SCN5A[2]) have been identified, most within the voltage-sensing domain (VSD) of $Na_v1.5$. In addition, LQT3-causative *SCN5A* variants that localize to $Na_v1.5$'s VSD appear to cause a LQT3-MEPPC overlap phenotype/syndrome that follows a more malignant clinical course.[12]

Here, we describe the clinical phenotype and unique cellular electrophysiological profile associated with a putative MEPPC-causative *SCN5A* variant (c.1256A>C-*SCN5A*; p.Gln419Pro-SCN5A), localizing outside the VSD of $Na_v1.5$, that co-segregates with frequent PVCs and/or early-onset AF in a small kindred.

Case report

A 21-year-old white male subject with no past medical history presented for the abrupt onset of nocturnal palpitations. An initial 12-lead electrocardiogram (ECG) revealed sinus rhythm with frequent PVCs in bigeminy (Figure 1a). A subsequent ambulatory Holter monitor revealed frequent PVCs that accounted for 4% of total beats. No atrial or ventricular arrhythmias were noted. The remainder of the patient's initial work-up was unremarkable.

Despite lifestyle modifications (eg, alcohol, caffeine, and nicotine cessation), the patient's symptoms persisted. Repeat ambulatory Holter revealed sinus rhythm with frequent PVCs (2.4% of total beats) alternating with a low atrial tachycardia. Owing to concern for arrhythmogenic cardiomyopathy, cardiac magnetic resonance imaging was pursued, but no structural abnormalities were observed. After metoprolol exacerbated the patient's symptoms, the atrial tachycardia was ablated successfully.

Over the next several months, the patient's palpitations slowly returned and frequent, multifocal PVCs (14.4% of total beats) were noted on ambulatory Holter and 12-lead ECG (Figure 1b). After unsuccessful trials of sotalol and low-dose

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KEY TEACHING POINTS

- A diagnosis of multifocal ectopic Purkinje-related premature contractions (MEPPC) should be considered in all young, otherwise healthy individuals presenting with a high burden of otherwise unexplained Purkinje-related premature ventricular contractions and early-onset atrial arrhythmias.
- MEPPC-causative gain-of-function variants in the *SCN5A*-encoded Na_v1.5 cardiac sodium channel typically result in an isolated increase in window current and may localize to regions outside the Na_v1.5 voltage sensing domain.
- Treatment of MEPPC with class Ic antiarrhythmic agents such as flecainide appears to be highly efficacious and should be trialed before proceeding to catheter ablation.

flecainide (50 mg twice a day), the patient opted for a watchful waiting approach.

Unfortunately, by age 25, the patient's PVC burden had climbed to 52% of total beats. Despite this PVC burden, serial echocardiograms revealed no evidence of PVC-mediated DCM (Figure 1c). Subsequent diagnostic electrophysiology study identified at least 5 distinct PVC morphologies, originating from both the right and left ventricles, that appeared to be triggered by ectopic Purkinje potentials. Three PVCs were ablated successfully. Postablation, flecainide 100 mg 3 times a day was initiated, with a dramatic and sustained reduction in PVC burden (~2%–5%) over the past 5 years (Figure 1d).

Given a clinical suspicion for MEPPC, commercial genetic testing was pursued and an ultra-rare missense variant in *SCN5A* (c.1256A>C-SCN5A; p.Gln419Pro-SCN5A) was identified. This variant was classified originally as a variant of uncertain significance. Unlike most MEPPC-causative variants described to date, which localize to the Na_v1.5 VSD (ie, transmembrane segments 1–4; Figure 2a), p.Gln419Pro-SCN5A localizes to a highly conserved region of the domain I-II linker (Figure 2a and 2b).

To clarify the role of p.Gln419Pro-SCN5A, the patient and his family were referred for further evaluation. Following cascade genetic testing, three p.Gln419Pro-SCN5A-positive relatives were identified (Figure 2d). Of note, the index case's mother and maternal uncle (II-1 and II-2) each had a history of early-onset AF (ie, <60 years of age) and a maternal first cousin (III-4) with a history of palpitations had an MEPPC-like phenotype (Figure 1e–1g). Like the index case (III-2), a dramatic and sustained attenuation of ventricular ectopy (32% to 2% of total beats) was noted following the initiation of flecainide (75 mg twice a day; Figure 1d) and there was no imaging evidence of cardiomyopathy (Figure 1e) or QTc prolongation on 12-lead ECG (Figure 1f). As observed previously in MEPPC patients, the frequent, multifocal PVCs observed at rest were suppressed with increasing workloads (ie, heart rate ~140 beats per minute) during exercise stress testing (Figure 1g).

Although the clinical phenotype observed in p.Gln419Pro-SCN5A-positive individuals appeared to be consistent with Na_v1.5 gain-of-function, given that p.Gln419Pro-SCN5A resides outside of the VSD, we sought to utilize the whole-cell patch-clamp technique to provide additional evidence in support of this variant's pathogenicity.

Methods and results

As described previously, the standard whole-cell patch-clamp technique was used to measure SCN5A wild-type (WT) and p.Gln419Pro-SCN5A (Q419P) sodium currents at room temperature (22–24°C) with the use of an Axopatch 200B amplifier, Digidata 1440A (Molecular Devices, San Jose, CA), and pclamp 10 software.[13,14] 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 deemed to be significant.

Typical inward sodium current (I_{Na}) tracings of voltage-dependent activation from WT and Q419P are shown in Figure 3a with holding potential at -100 mV to various depolarization potentials (Figure 3a). Current-voltage relationship shows that WT and Q419P reached peak at -20 mV. Analysis of the current-voltage relationship revealed that SCN5A current densities were not altered by Q419P (Figure 3b). Furthermore, the late inward sodium current remained unchanged (Figure 3c). However, Q419P significantly shifted $V_{1/2}$ in inactivation by -4.6 mV from -84.5 ± 0.7 mV (WT, n = 10) to -89.1 ± 0.4 mV (Q419P, n = 12, $P < .05$ vs WT) and $V_{1/2}$ in activation by -11.6 mV from -30.9 ± 1.3 mV (WT, n = 10) to -42.5 ± 1.0 mV (Q419P, n = 12, $P < .05$ vs WT) (Figure 3e), resulting in increased window current (Figure 3e).

Discussion

Following the sentinel description of MEPPC by Laurent and colleagues[6] in 2012, only 5 putative MEPPC-causative *SCN5A* variants (p.Ala204Glu-SCN5A,[3] p.Gly213Asp-SCN5A,[7] p.Arg222Glu-SCN5A,[6,8] p.Arg225Pro-SCN5A,[10] p.Leu828Phe-SCN5A[11]) have been identified to date. In addition, 2 *SCN5A* variants (p.Ile141Val-SCN5A and p.Met1851Val-SCN5A[2]) that confer a similar cellular electrophysiological phenotype (ie, no change in I_{Na} current density with increased window current) to these classic or typical MEPPC-causative variants have been described in patients presenting with an atypical MEPPC phenotype consisting of early-onset atrial arrhythmias, modest PVC burden at rest, and exercise-induced, rather than suppressed, complex ventricular ectopy/ventricular arrhythmias.

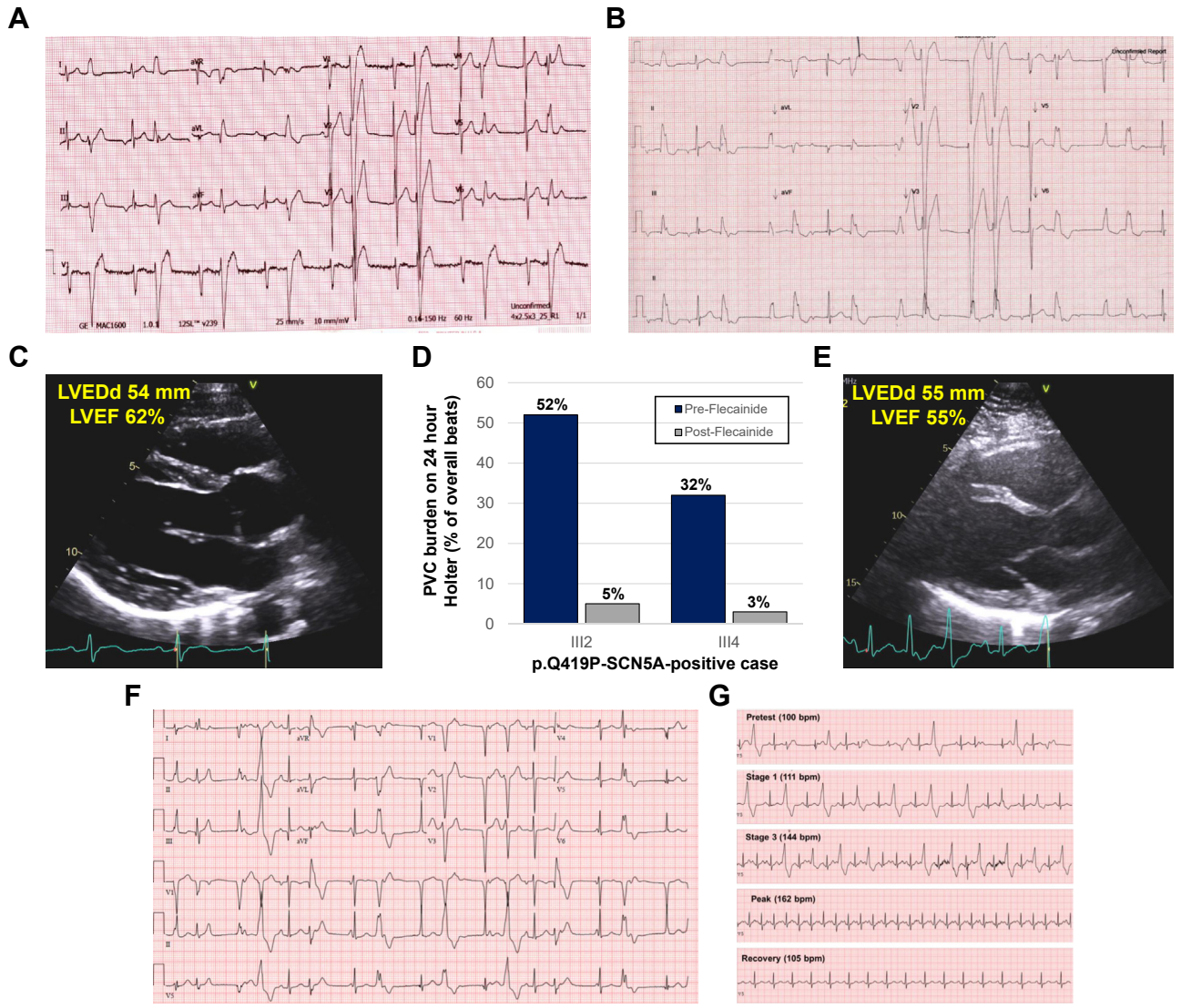

Figure 1 Representative electrocardiographic and echocardiographic data for p.Gln419Pro-SCN5A-positive patients with multifocal ectopic Purkinje-related premature contractions (MEPPC). **a:** Initial 12-lead electrocardiogram (ECG) of the p.Gln419Pro-SCN5A-positive index case (III-2) displaying frequent premature ventricular contractions (PVCs) in a pattern of bigeminy. **b:** Post–atrial tachycardia ablation 12-lead ECG showing an increased burden of frequent, multifocal PVCs in the index case (III-2). **c:** Normal parasternal long-axis view demonstrating normal left ventricular end-diastolic dimension (LVEDd) and ejection fraction (LVEF) in the index case (III-2). **d:** Pre- and postflecainide PVC burden for patients III-2 and III-4. **e:** Normal parasternal long-axis view demonstrating normal LVEDd and LVEF in a p.Gln419Pro-SCN5A-positive maternal cousin with a MEPPC-like phenotype (III-4). **f:** Representative 12-lead ECG of the affected maternal cousin (III-4) displaying frequent, multifocal PVCs. **g:** Pretreatment exercise stress test in the affected maternal cousin (III-4) displaying frequent ectopy at rest that suppresses with increased workload/heart rates.

As such, gain-of-function *SCN5A* variants that result in an isolated increase in window current appear to cause a spectrum of diseases related to Purkinje cell hyperexcitability at rest and with exercise. Interestingly, 6 of the 7 variants associated with typical (resting) or atypical (exercise-induced) MEPPC reside within or near the S4 transmembrane segment of $Na_v1.5$'s VSD. Thus far, only the atypical MEPPC-causative p.Met1851Val-SCN5A variant resides outside the MEPPC "hotspot" in the S4 transmembrane segments.

Therefore, the identification of p.Gln419Pro-SCN5A, which localizes to the highly conserved domain I-II linker of $Na_v1.5$, in a small kindred with typical MEPPC (ie, frequent, complex Purkinje-related PVCs at rest that suppress with exercise, early-onset atrial arrhythmias, etc) represents the first MEPPC-causative *SCN5A* variant to be identified outside the canonical $Na_v1.5$ VSD. Of note, the cellular electrophysiological and clinical phenotypes associated with p.Gln419Pro-SCN5A resemble that of the sentinel p.Arg222Gln-SCN5A variant.[6]

As such, this study provides the first evidence that *SCN5A* variants outside the VSD of $Na_v1.5$ can cause typical MEPPC. As awareness of MEPPC increases and more young patients with otherwise unexplained Purkinje-related PVCs undergo genetic testing, this study highlights the importance of not discounting ultra-rare *SCN5A* variants that reside outside the canonical VSD of $Na_v1.5$. With time, it is anticipated that (1) additional MEPPC-causative variants that localize outside the VSD of $Na_v1.5$ will be identified, and

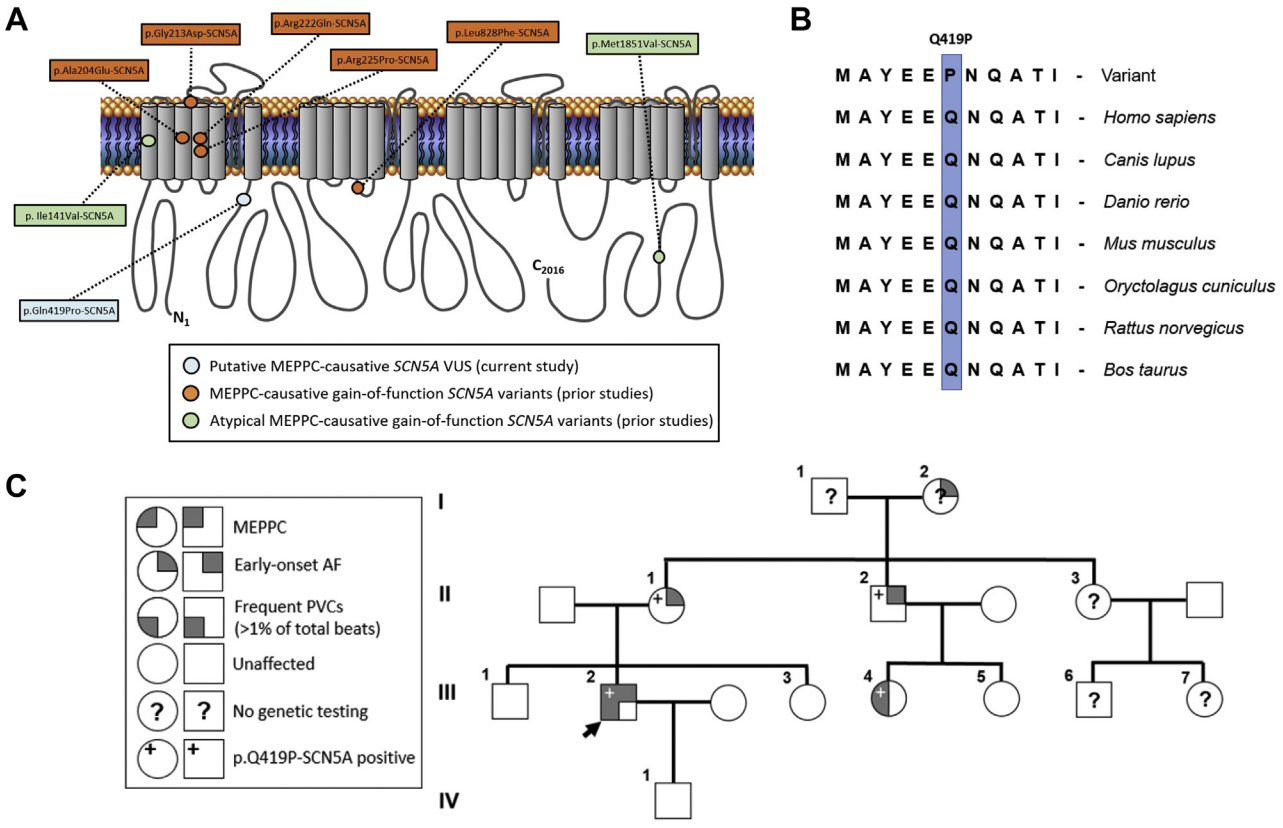

Figure 2 Localization, conservation, and co-segregation of p.Gln419Pro-SCN5A. **a:** Localization of p.Gln419Pro-SCN5A (*blue*) as well as classical multifocal ectopic Purkinje-related premature contraction (MEPPC)-causative (p.Ala204Glu-SCN5A, p.Gly213Asp-SCN5A, p.Arg222Gln-SCN5A, and p.Leu828Phe-SCN5A; *orange*) and nonclassical MEPPC-causative (p.Ile141Val-SCN5A and p.Met1851Val-SCN5A; *green*) variants. **b:** Multiple alignment analysis shows conservation of the p.Gln419 (Q419) amino acid among species. **c:** Co-segregation of p.Gln419Pro-SCN5A in small, multigenerational pedigree with early-onset atrial arrhythmias and/or frequent, multifocal premature ventricular contractions (PVCs). Members affected with MEPPC are indicated with solid upper left quarter. Solid upper right quarter indicates members with early-onset atrial arrhythmia. Solid bottom right quarter indicates members with QTc prolongation. Solid bottom left quarter indicates members with frequent PVCs (>1% of total beats). Open circle/square indicates unaffected persons. Question mark indicates persons with no genetic testing. p.Gln419Pro-SCN5A-positive individuals are indicated with a plus sign.

(2) further study of these variants may provide important, and potentially targetable, insights regarding the role of the DI-DII linker and other structures in the regulation of Na_v1.5 activation/inactivation gating, particularly within Purkinje cells.

In addition to increasing awareness of MEPPC as a clinical entity and expanding the MEPPC variant spectrum, the current study also highlights several pitfalls and pearls regarding the diagnosis and clinical management of patients with MEPPC. First, although yet to be directly assessed, the risk of SCD in patients with MEPPC appears to be lower than in those with other *SCN5A*-mediated cardiac channelopathies such as BrS1 and LQT3. Nevertheless, the high PVC burden observed in MEPPC can cause distressing symptoms and, despite not being observed in the current study, can result in a PVC-induced DCM.[10,15] As the PVC burden in MEPPC is attenuated dramatically by class I antiarrhythmic drugs, most notably flecainide and quinidine,[3,6,7,11] and not surprisingly appears—at least anecdotally—to be refractory to catheter-based ablation, it is critical that a diagnosis of MEPPC be considered in any young patient presenting with otherwise unexplained frequent, multifocal PVCs. Exclusion of alternative causes (ie, structural heart disease, drugs, etc), determination of the origin of observed PVCs, acquisition of detailed family history, and, if appropriate, pursuit of genetic testing that includes *SCN5A* can help establish or exclude a diagnosis of MEPPC.

Importantly, as highlighted by the small kindred detailed in the current study, the incomplete penetrance and variable expressivity observed in other *SCN5A*-mediated cardiac channelopathies also extend to MEPPC. As such, more subtle phenotypes such as the early-onset atrial arrhythmias observed in the mother and maternal uncle of the index patient in the current study may function as the only clinical manifestation(s) of an MEPPC-causative *SCN5A* variant in some individuals.

Ultimately, given the rare nature of MEPPC, the formation of a multicenter International MEPPC Registry is needed to (1) better understand the prevalence of isolated MEPPC and MEPPC overlap syndromes, (2) delineate the spectrum of clinical and electrophysiological phenotypes associated with putative MEPPC-causative variants, (3) provide more robust data regarding the clinical management of MEPPC patients, and (4) explore the genetic and environmental

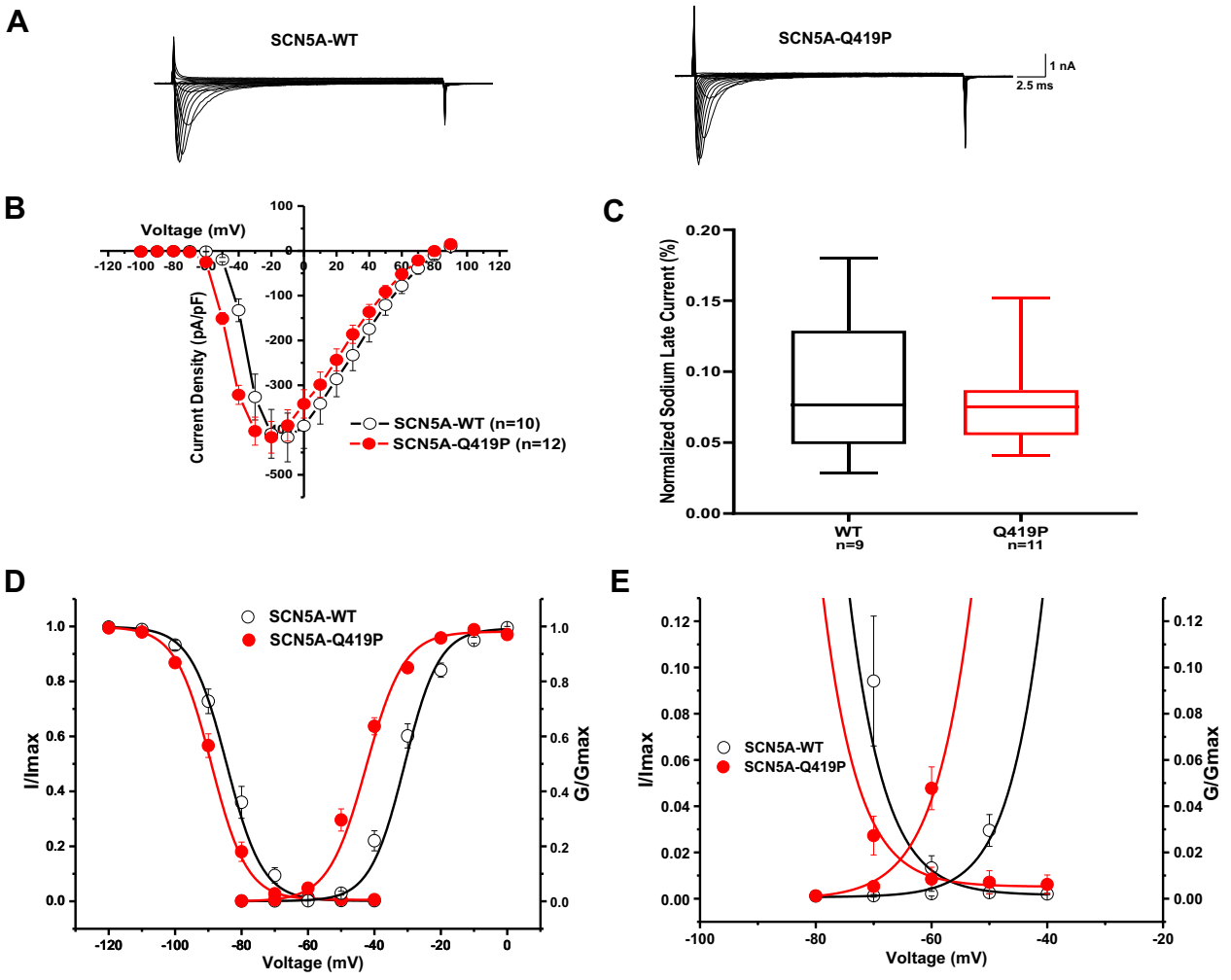

Figure 3 The putative multifocal ectopic Purkinje-related premature contraction (MEPPC)-causative p.Gln419Pro-SCN5A variant increases sodium window current. **a:** Whole-cell SCN5A current representative tracings from TSA201 cells expressing wild-type (WT)-SCN5A p.Gln419Pro-SCN5A. **b:** Current-voltage relationship for WT-SCN5A (n = 10) and SCN5A-Q419P (n = 12). **c:** Sodium late current normalized to peak current representing WT-SCN5A (n = 9) and p.Gln419Pro-SCN5A (n = 11). **d:** Steady-state inactivation curves and voltage dependence of activation curves from WT-SCN5A (n = 10) and p.Gln419Pro-SCN5A (n = 12). **e:** Window current from WT-SCN5A (n = 10) and p.Gln419Pro-SCN5A (n = 12).

underpinnings that influence the phenotypic expression and risk of SCD/PVC-induced cardiomyopathy in patients with putative MEPPC-causative *SCN5A* variants.

Conclusion

p.Gln419Pro-SCN5A represents only the seventh MEPPC-causative variant described to date and the first associated with a typical MEPPC phenotype localizing outside the $Na_v1.5$ VSD. As class I antiarrhythmic drugs are highly efficacious in MEPPC, this clinical entity merits consideration in all young patients with a high burden of Purkinje-related PVCs.

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