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RESEARCH LETTER

Novel Gain-of-Function Variant in *CACNA1C* Associated With Timothy Syndrome, Multiple Accessory Pathways, and Noncompaction Cardiomyopathy

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Timothy syndrome (TS) is a rare multisystem disorder attributed to rare gain-of-function variants in *CACNA1C*, which codes for the $Ca_V\alpha1$-subunit of the cardiac voltage-dependent L-type calcium channel. The associated phenotype includes extreme QT interval prolongation, hypertrophic cardiomyopathy, congenital heart defects, syndactyly, craniofacial anomalies, and neuropsychiatric disease.[1] The majority of cases are caused by a recurrent de novo variant, p.G406R, located in the proximal portion of the Domain I-Domain II cytoplasmic linker, which results in impaired channel inactivation.[2] We hereby report a case of TS with a novel *CACNA1C* variant resulting in a peculiar biophysical phenotype and associating typical features of TS with left ventricular noncompaction (LVNC) cardiomyopathy and multiple accessory pathways.

At birth, the proband had bilateral hand syndactyly which required surgical correction at age 2, as well as feet syndactyly which remains uncorrected (Figure [A]). He also had persistent ductus arteriosus that required surgical closure at 3 months of age. LVNC was noted since early childhood. Figure [B] shows cardiac magnetic resonance imaging at the age of 17 demonstrating LVNC with diastolic noncompacted/compacted ratio reaching 6.7 (normal<2.3) in the anterior LV with evidence of wall thinning. His ECG was abnormal since birth, with ventricular preexcitation and a prolonged QT interval which at the time was thought to be secondary to preexcitation. At the age of 15, he presented with syncope and preexcited atrial fibrillation, and an electrophysiological study was performed. Two right-sided accessory pathways (anterior and posterolateral) were identified and successfully ablated. During follow-up, the patient presented with intermittent ventricular preexcitation with a different morphology (Figure [C]). Electrocardiographic recordings in the absence of preexcitation still showed important QT interval prolongation (Figure [D]). The patient has an implantable cardiac defibrillator for primary prevention since age 17, with no appropriate therapy as of his last follow-up at age 23. Despite mild neurodevelopmental delay during childhood, he does not have any apparent impairment during adult life. Both parents and the sister had a normal resting ECG and cardiac magnetic resonance imaging. There was no history of cardiomyopathy or other heritable heart disease in the extended family.

Following informed consent in an ongoing study approved by the institutional research ethics committee, we performed quad whole-exome sequencing on genomic DNA of the proband and unaffected parents and sister (Figure [E]). We identified a de

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Nonstandard Abbreviations and Acronyms

LVNC	left ventricular noncompaction
TS	Timothy syndrome

novo heterozygous missense variant in *CACNA1C* (NM_000719): c.1255G>C, p.G419R, as the plausible cause of the phenotype. The variant is absent from the genome aggregation database and ClinVar and has never been published. A different nucleotide change (c.1255G>A) predicted to result in an identical amino acid change was reported in ClinVar as a variant of unknown significance. Sanger sequencing confirmed the presence of the p.G419R variant in the proband (Figure [F]) and absence in family members. Paternity was confirmed using sequencing data.

The functional consequence of *CACNA1C*:p.G419R was studied in HEK293T cells. Compared with the wild type, the recombinant cardiac voltage-dependent L-type calcium channel p.G419R channel displays a 4-fold increase in the peak current density (Figure [G and H]). Unlike the classic TS p.G406R variant located in the same protein region, p.G419R did not exhibit slower inactivation kinetics compared with cardiac voltage-dependent L-type calcium channel wild type, but, in fact, had slightly faster inactivation kinetics (Figure [I]). Importantly, cardiac voltage-dependent L-type calcium channel p.G419R was associated with a ≈—10 mV gain-of-function shift in the activation potential without any change in the inactivation potential. The resulting increase in window currents, within the range where L-type calcium channels operate (from —40 to 0 mV), is proposed to contribute to the gain-of-function phenotype.

Altogether, the present report extends the genetic, biophysical, and clinical spectra of TS, a rare highly lethal multisystem genetic disease caused by recurrent rare gain-of-function variants in *CACNA1C* that are often de novo. Quad whole-exome sequencing identified a previously unreported de novo *CACNA1C* variant (c.1255G>C, p.G419R), now classified as pathogenic according to American College of Medical Genetics and Genomics guidelines. Functional studies of the variant demonstrate a peculiar biophysical phenotype with larger peak current densities, increased window currents, and slightly faster inactivation kinetics. Such a biophysical phenotype is unique for a TS-causing variant. The p.I1166T variant located in the Domain III-Domain IV cytoplasmic linker has also been shown to result in increased window current,[3] although changes in peak current density have been inconsistent. Clinically, the patient manifests typical features of TS, such as syndactyly, persistent ductus arteriosus, and important QT prolongation, in addition to novel features, namely LVNC and ventricular preexcitation with multiple accessory pathways. Only a single case report describes LVNC with hypertrophic cardiomyopathy in a patient with TS due to the p.G406R variant.[4] Ventricular preexcitation has never been described in TS. These novel features may be underpinned by a cytosolic calcium overload that could alter transcriptional regulation and development[5] as is the case in our patient. Further studies will be required to demonstrate and delineate the mechanism underlying increased L-type calcium currents and the development of both LVNC and accessory pathways.

Data supporting the findings of this study are available upon request from the corresponding authors.

ARTICLE INFORMATION

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Disclosures

None.

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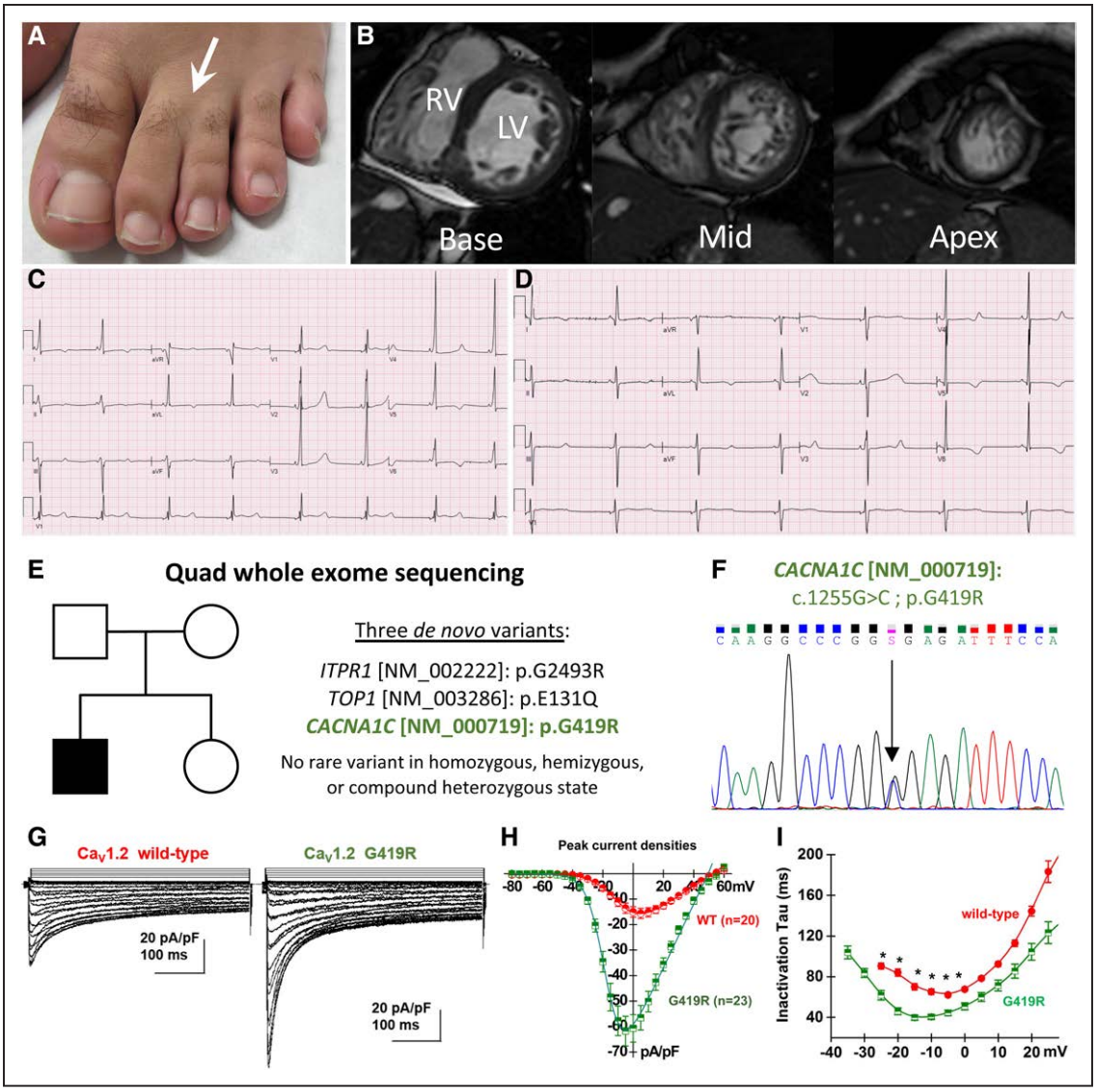

Figure. Clinical, genetic, and electrophysiological findings.
A, Incomplete simple syndactyly with partial fusion of second and third digits of the left foot. The patient also has syndactyly in the right foot and had surgical correction for syndactyly of third and fourth digits in both hands at the age of 2 y old (not shown). **B**, Cardiac magnetic resonance imaging performed at 17 y old demonstrating left ventricular noncompaction cardiomyopathy (LVNC). Balanced steady-state free precession images in contiguous short-axis slices of the right ventricle (RV) and left ventricle (LV) from base to apex showing prominent circumferential trabeculations in the LV wall, only sparing the basal septum and the mid anteroseptal segment. Biventricular volumes and systolic function were within normal limits, and there was no evidence of fibrosis (not shown). **C**, ECG recording at 16 y old, 9 mo following ablation of an anterior and posterolateral right-sided accessory pathways, showing sinus bradycardia with ventricular preexcitation with a different QRS morphology compared to the preablation ECG. **D**, ECG recording at 17 y old showing sinus bradycardia with no manifest preexcitation despite a slightly shortened PR interval, and a severely prolonged QT interval (732 ms) at a heart rate of 40/min for a heart rate corrected QT (QTc) of 598 ms and 639 ms, using the Bazett and Fridericia correction methods, respectively. **E**, Quad whole-exome sequencing was performed, including the proband, his parents, and his sister followed by filtering for protein altering variants with a maximal population allele frequency $<1\times10^{-4}$ in genome aggregation database (gnomAD), in an autosomal dominant de novo as well as recessive inheritance models. Three protein altering de novo variants were identified, among which *CACNA1C* [NM_000719], c.1255G>C, p.G419R suspected to be causal of the phenotype. Note that paternity was confirmed using sequencing data. **F**, Sanger-sequencing chromatographs confirming presence of *CACNA1C* (NM_000719: c.1255G>C; p.G419R; arrow) in the proband in heterozygous state. The variant was absent in both parents and in the sister (not shown). **G**–**I**, Functional studies where HEK293T cells were transiently transfected with $Ca_V\beta2a$ + $Ca_V\alpha2\delta1$ and either cardiac voltage-dependent L-type calcium channel ($Ca_V1.2$) wild-type (WT) or $Ca_V1.2$ p.G419R. **G**, Whole-cell Ca^{2+} current traces were recorded at room temperature in the presence of 2 mmol/L Ca^{2+} from a holding potential of −100 mV. Both data sets were measured under the same experimental conditions. **H**, Averaged current-voltage relationships. Peak current densities vs voltage relationships were measured for $Ca_V1.2$ WT (red circles) and $Ca_V1.2$ p.G419R (green squares). Peak current densities were on average 4-fold larger with peak current densities of −16 ± 2 pA/pF ($Ca_V1.2$ WT) and −64 ± 6 pA/pF ($Ca_V1.2$ p.G419R; $P<0.01$). **I**, Inactivation time constants (inactivation Tau) as a function of applied voltage. Inactivation time constants were estimated from fitting whole-cell current traces to exponential functions. Inactivation kinetics of $Ca_V1.2$ are typically U shaped being faster at the peak voltage. When measured at the peak voltage, inactivation time constants are significantly different (*$P<0.01$) with inactivation Tau =62±2 ms for $Ca_V1.2$ WT at −5 mV and inactivation Tau =40±2 ms for $Ca_V1.2$ p.G419R at −15 mV ($P<0.01$).