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## CALM3 Mutation Associated with Long QT Syndrome

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Each year, more than 400,000 cases (>1,000 per day) of sudden cardiac death (SCD) are reported in the United States.<sup>1</sup> The majority of these SCD cases and more than 10% of all natural deaths are due to cardiac arrhythmias.<sup>2</sup> One condition that affects approximately 1 in 2,000 individuals and is known to cause these potentially lethal arrhythmias is long QT syndrome (LQTS).<sup>3</sup>

LQTS, a disorder of ventricular myocardial repolarization, is characterized by the prolongation of the heart rate-corrected QT interval (QTc) on an ECG, which can precipitate torsades de pointes (a subtype of polymorphic ventricular tachycardia) and as a result syncope, seizures, or SCD, in the setting of a structurally normal heart.<sup>2</sup> LQTS is an inherited disorder, and approximately 80% of LQTS has been elucidated genetically. Accordingly, its genetic basis remains elusive in as many as 20% of patients.<sup>4</sup>

Here, we describe a case of severe LQTS in a young subject exhibiting profound QT prolongation (QTc > 650 ms) in which commercial genetic testing was negative. Recently, whole exome sequencing (WES) analysis has been used to successfully pinpoint the novel genetic cause of a variety of diseases when the genetic cause is still unknown.<sup>4, 5</sup> Due to a

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**Conflicts of Interest:**

Michael J. Ackerman is a consultant for Boston Scientific, Gilead Sciences, Medtronic, and St. Jude Medical and receives sales based royalties from Transgenomic for their FAMILION-LQTS and FAMILION-CPVT genetic tests. The other authors have no conflicts of interest relevant to this article to disclose.

negative family history, seemingly healthy parents, and normal parental ECGs, WES was performed on the trio (index case and his parents), and the results were filtered to include either novel sporadic or rare recessive variants. A sporadic p.D130G mutation in *CALM3* was identified as the most likely cause of the patient's LQTS.

## CASE REPORT

The proband was the first child born to a Caucasian 34-year-old female and a Hispanic 35-year-old male. The mother had Factor V Leiden deficiency which was treated with aspirin throughout pregnancy. She has had no known miscarriages or spontaneous abortions and has never had syncope. There is no family history of sudden death. His father is adopted and there is little known about this family history; however he has never had syncope.

A fetal ultrasound at 27 weeks was reported to be normal with no mention of fetal bradycardia. He was born at 38.2 weeks gestational age via spontaneous vaginal delivery with reported decelerations and meconium stained fluid at the time of delivery. Apgar scores were 7 at one minute and 8 at five minutes. He was noted to have heart rates into the 60s and had hypotonia during the first eight hours of life. At ~8 hours of age, he was noticed to be slightly dusky, and the preductal saturations were 96–97 and the postductal saturations were 70–80. Chest x-ray demonstrated slight cardiomegaly.

At this point, the proband was airlifted to a Children's Hospital where his first ECG demonstrated bradycardia with sinus rhythm, functional 2:1 second-degree AV block, T-wave alternans, and profound QT prolongation, with a corrected QT interval of 690 ms (Figure 1A). An echocardiogram demonstrated a moderate sized perimembranous ventricular septal defect (VSD), a moderate patent ductus arteriosus (PDA), right ventricular dilatation with normal function, and normal left ventricular size and systolic function.

He continued to have ECGs with evidence of functional second-degree AV block due to extremely prolonged QT intervals. He was monitored at the hospital for about one month, during which time propranolol treatment was initiated and a single chamber pacemaker was placed. He had no arrhythmias, however he was treated for pulmonary hypertension. An ECG at five weeks revealed that the QTc had stabilized at 579 ms, with a normal heart rate and the 2:1 AV block had resolved (Figure 1B).

At seven weeks of age his weight was 5 kg (14th percentile) and height was 54.0 cm (1st percentile). At his most recent follow-up at six months, his marked QT prolongation persisted (Figure 1C); however no arrhythmias have been detected by ECG or device interrogation. In addition, the pulmonary hypertension resolved, his PDA has closed, and his VSD is small.

## METHODS and RESULTS

When commercially available LQTS genetic testing (*AKAP9*, *ANK2*, *CACNA1C*, *CAV3*, *KCNE1*, *KCNE2*, *KCNH2*, *KCNJ2*, *KCNQ1*, *SCN4B*, *SCN5A*, and *SNTA*) was unable to identify any variants to determine the cause of the patient's LQTS, this family was directed to the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory for further

research-based genetic testing. After written consent for this Institutional Review Board-approved study, WES was initiated in attempt to determine the root cause of the proband's LQTS.

Due to the normal ECG status of each parent (Figure 2A), dominant sporadic or recessive inheritance patterns were suspected. Therefore, a case-parent trio based WES approach was utilized in an attempt to determine the patient's underlying LQTS phenotype, and two distinct filtering strategies were employed based on those two hypothesized inheritance patterns (Figure 2B). Ingenuity Variant Analysis (Qiagen, Valencia, CA) was utilized to complete the following filtering strategy. In total, 135,932 single nucleotide variants and insertions/deletions (In/Dels) were called within the trio. In both strategies, the variants were filtered to remove poor quality variants by eliminating variants with a call quantity of less than 20 and variants found in the top 1% of genes with high variability. Second, variants were filtered for rarity using the NHLBI GO Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>) and 1,000 Genomes Project (<http://browser.1000genomes.org/index.html>) databases. Third, all synonymous variants (encoding for the same amino acid residue) were excluded to focus on non-synonymous (protein altering) variants or In/Dels which commonly cause LQTS.<sup>6</sup>

Next, familial filtering strategies utilizing the case-parent trio were employed. The first familial sequencing strategy was dominant sporadic, in which only rare variants unique to the child were kept as viable candidates. The second familial sequencing strategy was recessive, focusing on either homozygous variants or compound heterozygous variants. Homozygous variants occur when the same mutant allele is inherited from each parent, and compound heterozygous variants exist when two distinct variants are inherited from each parent, or when a sporadic variant is acquired in combination with a distinct inherited variant in the same gene. Dominant sporadic variants had to have a frequency of less than 0.01%, to identify only those variants that were extremely rare. Compound heterozygous and homozygous recessive variants were required to have a frequency of less than 1%. Compound heterozygous and homozygous recessive variants had a higher background frequency because these variants are likely not deleterious by themselves and therefore may be present at a higher frequency in the general population.

The final filtering step utilized the 1629 gene LQTS nodal network, which includes all genes which are significantly related to 13 known LQTS-susceptibility genes in terms of protein-protein interactions and signaling pathways, and are ranked based on association.<sup>7</sup> Focusing specifically on genes within this network, we identified nine variants in seven genes (*CACNA1A*, *CALM3*, *CAMKK2*, *CYP4F2*, *KRT18*, *PTPN21*, and *WAS*). Overall, *CALM3* had the highest ranking in the 1629 gene LQTS nodal network of the seven genes identified through our filtering process (Table 1). Within *CALM3*, we identified a variant, c.389A>G, which is predicted to encode for an aspartic acid to glycine change at protein position 130 (p.D130G). DNA Sanger sequencing was used to confirm that the mutation was present in the proband and absent in both of the unaffected parents, matching the sporadic inheritance pattern (Figure 2C).

## DISCUSSION

WES can be a very useful tool to uncover the genetic cause of a diagnosed disease. In this report, we described a severe case of LQTS in which clinical genetic testing was negative, and familial WES elucidated nine variants in seven potential LQTS-associated genes within our patient. Three independent algorithms ranked the *CALM3* missense mutation as the most likely causative mutation. Although an in-frame deletion was identified in *CACNA1A*, the encoded pore-forming P/Q calcium channel subunit, this channel has negligible expression in the heart in contrast to the cardiac specific *CACNA1C*-encoded  $\text{Ca}_v1.2$  L-type calcium channel. Thus, based on the previously published literature and its overall importance in the heart,<sup>7-8, 12-14</sup> the sporadic p.D130G variant in *CALM3*-encoded calmodulin (CaM) is the most likely root cause for the patient's phenotype.

Overall, there are three calmodulin genes, *CALM1* (chr14q31), *CALM2* (chr2p21), and *CALM3* (chr19q13), that all encode for an identical calmodulin protein (CaM), which have been demonstrated to have differential expression levels in the human heart.<sup>8</sup> CaM is a  $\text{Ca}^{2+}$  sensing, signal-transducing protein that modulates several ion channels. CaM is most well-known for its direct interaction with the  $\text{Ca}_v1.2$  L-type calcium channel; deactivating  $\text{Ca}_v1.2$  when CaM binds to free  $\text{Ca}^{2+}$ .<sup>13</sup> Mutations in two of the three calmodulin genes (*CALM1* and *CALM2*) have been discovered recently in arrhythmia syndromes. The first discovery, in 2012, identified a CaM mutation in a large pedigree of catecholaminergic polymorphic ventricular tachycardia (CPVT).<sup>9</sup> Subsequent cohort analysis identified an additional individual with sporadic CaM mutation. Since then, mutations have also been identified in cases of LQTS,<sup>8, 10</sup> LQTS/CPVT overlap phenotypes,<sup>10</sup> as well as a pedigree of idiopathic ventricular fibrillation (IVF).<sup>11</sup> However, to date, no *CALM3* mutations have been identified in any sudden death predisposing disorder.

This severe case of LQTS presented with several distinctive identifiers: presentation at birth, an extremely prolonged QTc of 690 ms, fetal bradycardia, T-wave alternans, and a 2:1 AV block. Much like the case described here, this precise missense mutation, p.D130G, was described originally in the *CALM1*-encoded CaM protein, and was identified in a 6-month-old female who had suffered from cardiac arrest, and was subsequently found to have a QTc of 630 ms, frequent episodes of T-wave alternans, and intermittent 2:1 AV block. Other LQTS cases with identified CaM mutations generally harbor a similar set of characteristics including extremely prolonged QTc intervals (often > 550 ms) and young age at presentation (typically < 10 years of age).<sup>6, 11</sup>

Many functional assays have been completed on a subset of the identified CaM mutations, including calcium binding assays, calcium transient assays, as well as interactions with ion channels such as  $\text{Ca}_v1.2$  and RyR2.<sup>12-14</sup> Because p.D130G has been identified previously in *CALM1*, functional assays using both mouse fetal ventricular myocytes and adult guinea pig cardiomyocyte models have been used to characterize this mutation. D130G-CaM reduces CaM's  $\text{Ca}^{2+}$  binding affinity, causes a calcium cycling disturbance in the form of increased calcium transients, results in electrical alternans, and prolongs the action potential.<sup>8, 12, 13</sup> Because *CALM1* and *CALM3* encode a completely identical CaM, we confidently conclude that the pathophysiology of D130G-CALM3 equals that of D130G-CALM1.

One of the most interesting paradoxes surrounding the concept of CaM-mediated arrhythmogenesis centers around the peculiar finding that there exist 3 distinct *CALM* genes on 3 different chromosomes that nevertheless encode an identical calmodulin protein, and that disruption of only one of six possible *CALM* alleles could have such substantial phenotypic implications in human disease. Novel studies have begun to elucidate the important intricate balance of mutated and wildtype CaM proteins, and how 1/6<sup>th</sup> mutant product is sufficient to disrupt the L-type calcium channel's deactivation.<sup>12</sup> However, future research will be necessary to continue to understand the role of CaM, their differential expression *in vivo*, and how these mutated proteins contribute to sudden death predisposing diseases.

## CONCLUSION

Overall, the identification of the genetic substrate responsible for sudden death predisposing disorders can help provide proper genotype specific therapies for affected patients. In addition, these genotypes can prevent sudden cardiac death by both identifying at risk individuals who could benefit from prophylactic treatment strategies and help generate a molecular substrate for novel therapies. Based on the findings of this report, in addition to screening both *CALM1* and *CALM2* for genetic mutations in cases of LQTS, CPVT and IVF, *CALM3* should also be genetically interrogated for LQTS and possibly other sudden death related disorders.

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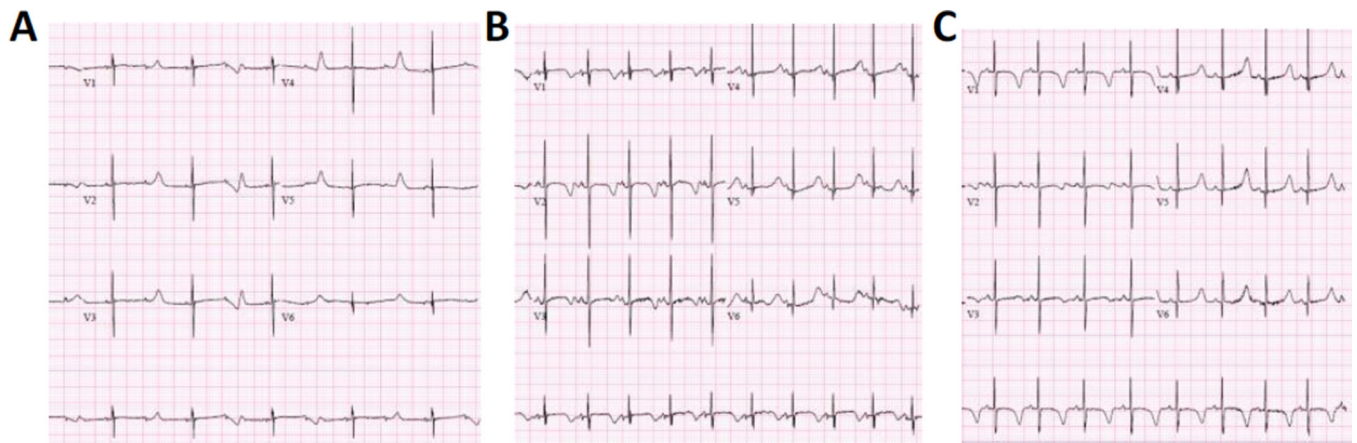
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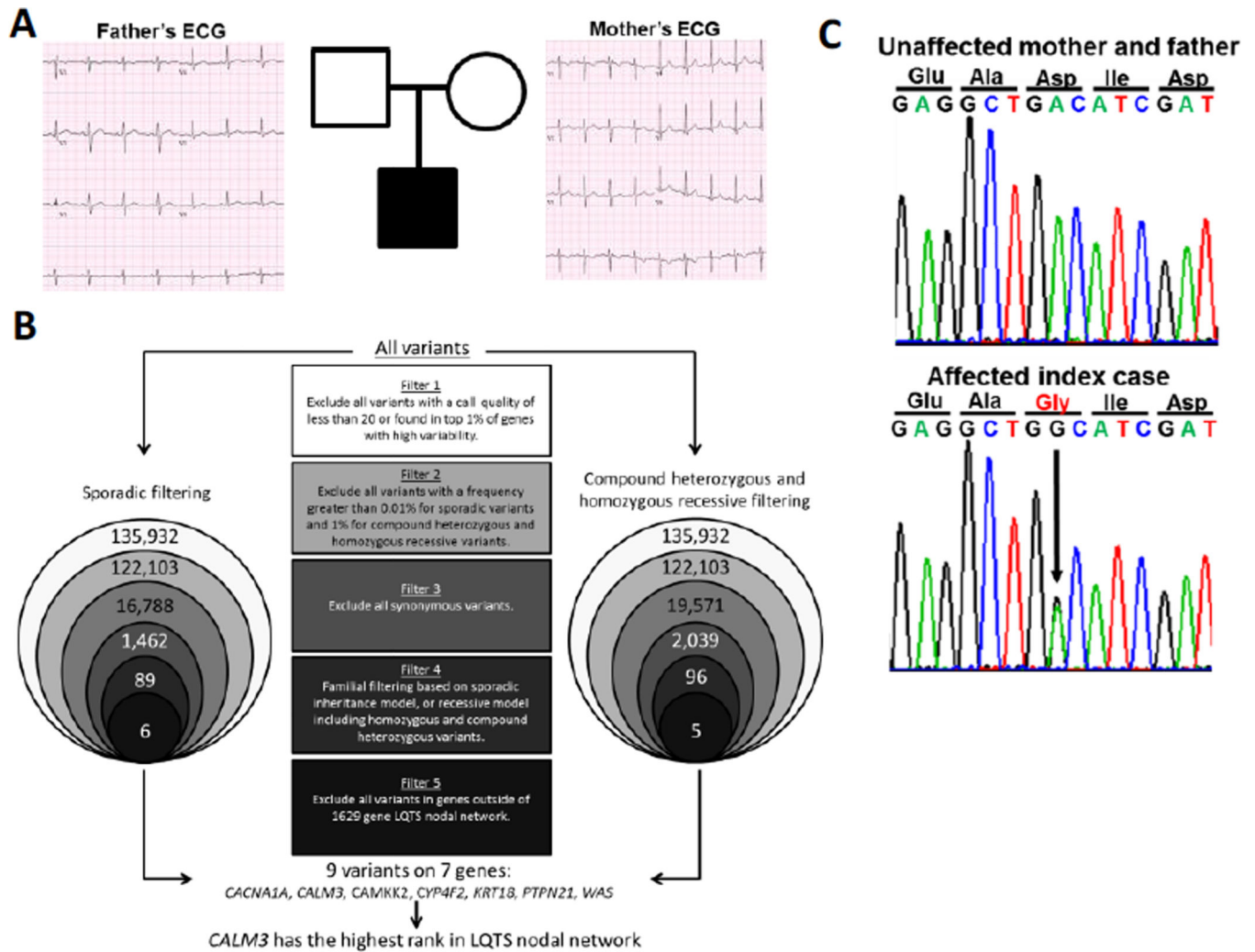
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**Figure 1. Electrocardiograms of the Proband**

(A) ECG taken at 1 day of age showing extreme QT prolongation ( $QTc = 690$  ms), 2:1 atrioventricular block, bradycardia (59 bpm), and T wave alternans. (B) ECG at 5 weeks of age showing QT prolongation ( $QTc = 586$  ms) at a normal heart rate (111 bpm) and resolution of the 2:1 AV block. (C) ECG at 6 months of age showing marked QT prolongation ( $QTc = 556$  ms).



**Figure 2. Familial Electrocardiograms, WES Filtering Strategy, and Mutation Confirmation** (A) Pedigree of family utilized for WES, including ECGs for the mother and father of the proband, showing normal corrected QT intervals. (B) Filtering strategy utilized for WES, separated by inheritance pattern, to include filtering for sporadic inheritance (left) and recessive inheritance (right). Some variants were identified in both sequencing strategies; please see Table 1 for more information regarding the identified variants. (C) DNA Sanger sequencing confirmation of the p.D130G mutation in the proband (lower) and the p.D130G mutation's absence in the parents (upper), supporting a sporadic inheritance pattern.



**Table 1**

Variants Identified through WES after Filtration and Ranked Using the LQTS Nodal Network.

<b>Gene</b>	<b>Variant</b>	<b>Rank in LQTS Nodal Network</b>	<b>Inheritance Pattern</b>
<i>CALM3</i>	p.D130G	88	Sporadic
<i>CACNA1A</i>	p.H2219del	150	Sporadic
<i>WAS</i>	p.H180N	349	X-linked recessive
<i>KRT18</i>	p.I67M	383	Sporadic
<i>CAMKK2</i>	p.G539fs*5	822	Sporadic
<i>PTPN21</i>	p.Y569H	1367	Sporadic/compound heterozygous
<i>PTPN21</i>	p.A725T	1367	Compound heterozygous
<i>CYP4F2</i>	p.T472A	1507	Compound heterozygous
<i>CYP4F2</i>	p.A483G	1507	Sporadic/compound heterozygous

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