The Role of CAV3 in Long–QT Syndrome
Clinical and Functional Assessment of a Caveolin-3/Kv11.1 Double Heterozygote Versus Caveolin-3 Single Heterozygote

Paula L. Hedley, MSc; Jørgen K. Kanters, MD; Maja Dembic, PhD; Lasse Skibsbye, PhD; Frederik H. Aïdt, MSc; Ole Eschen, MD; Claus Graff, PhD; Elijah R. Behr, MD; Sarah Schlamowitz, MSc; Valerie Corfield, PhD; William J. McKenna, MD; Michael Christiansen; MD

Background—Mutations in CAV3, coding for caveolin-3, the major constituent scaffolding protein of cardiac caveolae, have been associated with skeletal muscle disease, cardiomyopathy, and most recently long–QT syndrome (LQTS) and sudden infant death syndrome. We examined the occurrence of CAV3 mutations in a large cohort of patients with LQTS.

Methods and Results—Probands with LQTS (n=167) were screened for mutations in CAV3 using direct DNA sequencing. A single proband (0.6%) was found to be a heterozygous carrier of a previously described missense mutation, caveolin-3:p.T78M. The proband was also a heterozygous carrier of the trafficking-deficient Kv11.1:p.I400N mutation. The caveolin-3:p.T78M mutation was found isolated in 3 family members, none of whom had a prolonged QTc interval. Coimmunoprecipitations of caveolin-3 and the voltage-gated potassium channel subunit (Kv11.1) were performed, and the electrophysiological classification of the Kv11.1 mutant was carried out by patch-clamp technique in human embryonic kidney 293 cells. Furthermore, the T-wave morphology was assessed in mutation carriers, double mutation carriers, and nonmutation carriers by applying a morphology combination score. The morphology combination score was normal for isolated caveolin-3:p.T78M carriers and of LQT2 type in double heterozygotes.

Conclusions—Mutations in CAV3 are rare in LQTS. Furthermore, caveolin-3:p.T78M did not exhibit a LQTS phenotype. Because no association has ever been found between LQTS and isolated CAV3 mutations, we suggest that LQTS9 is considered a provisional entity. (Circ Cardiovasc Genet. 2013;6:452-461.)

Key Words: arrhythmias, cardiac genetics ion channel long–QT syndrome molecular biology

Caveolae are small (50–100 nm in size) plasma invaginations with a cup-like shape and a composition of cholesterol and other lipids that classify them as lipid rafts. These rafts are enriched in a large number of protein complexes involved in signaling and vesicular trafficking. Caveolae are structurally composed of caveolins, a family of proteins comprising caveolin-1, caveolin-2, and caveolin-3 in humans. They exhibit a ubiquitous tissue distribution; however, caveolin-3 is selectively expressed in the caveolae of heart and skeletal muscle.

Caveolin-3 comprises 151 amino acids and is divided into four domains: the N-terminal domain contains the FEDVIAEP caveolin signature domain (Figure 1). The transmembrane domain loops through the membrane, and both the N and C termini are cytoplasmic. After homo-oligomerization, which occurs in the endoplasmic reticulum, caveolin-3 forms caveolar complexes that subsequently fuse with the plasma membrane to form the caveolae. The scaffolding domain is essential for the homo-oligomerization of caveolin-3.
Figure 1. Caveolin-3 domain organization and mutation map. The primary structure of caveolin-3 in which each amino acid is represented as a stripe, the 4 domains are demarcated by colored blocks and are labeled in the figure (N-terminal [amino acid (aa) 1–53]—blue, scaffolding domain [aa 54–73]—pink, transmembrane domain [aa 74–106]—green, and C-terminal domain [aa 107–151]—orange). The FEDVIAEP caveolin signature domain is indicated within the N terminus. Previously reported mutations have been indicated as green stripes along with the diseases reported to be associated with them. Caveolin-3:p.T78M is represented by a stripe, and the 4 domains are demarcated by colored blocks and are labeled in the figure (N-terminal [aa 1–53]—blue, scaffolding domain (aa 54–73) —pink, transmembrane domain (aa 74–106) —green, and C-terminal domain (aa 107–151) —orange).

Materials and Methods

Patients

Unrelated probands, referred from specialist cardiology centers in Denmark and the United Kingdom to Statens Serum Institut for genetic investigation of LQTS (n=167; 61% women), were included in the study. The probands had been screened by capillary-electrophoresis single-strand conformation polymorphism for mutations in KCNQ1, KCNH2, KCNE1, KCNE2, and SCN5A, as described elsewhere.20–22 Fifty-eight (35%) patients had been found to carry a probably damaging mutation in one of the genes. A control panel comprising 50 normal chromosomes was used to verify population frequencies. The information single nucleotide polymorphism [SNP] database and the Exome Variant Server data were used as an in silico control of allele frequencies (Exome Variant Server; NHLBI Exome Sequencing Project, Seattle, WA [http://evs.gs.washington.edu/EVS/, accessed June 2012]).

CAV3 Screening

DNA was extracted from frozen EDTA-blood using a Qiagen kit (Qiagen; QmbH, Hilden, Germany). Screening for mutations in CAV3 was performed by direct sequencing using primers that flanked the coding region (primer sequences and polymerase chain reaction conditions are available on request). Polymerase chain reaction products were sequenced by the Sanger method on an automated sequencer (Applied Biosystems, Foster City, CA) and the 3100 ABI-PRISM automated sequencer (Applied Biosystems). Sequence analysis was performed using the Sequencer 4.8 software (Gene Codes Corporation, Ann Arbor, MI).

Definition of Disease Causation or Association

Genetic variants were considered probably damaging if (1) the nucleotide variation had been deduced to result in a missense mutation, frameshift, and/or abnormal splicing; (2) relevant, the variation affected a conserved amino acid; (3) the variation cosegregated with the disease in affected family members; and (4) the variation was not identified among 189 Danish blood donors. In the absence of available family members for cosegregation studies, disease association was presumed if just criteria 1, 2, and 4 were fulfilled. If the mutation had been associated with disease previously—in accordance with the criteria mentioned here and relevant functional studies—disease causation was presumed when just criteria 1 and 4 were met.

Cell Cultures, Transfections, and DNA Constructs

Human embryonic kidney 293 cells were cultured in Eagle minimal essential medium (MEM) with Glutamax supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/mL; 100 mg/mL) at 37°C and 5% CO2. For Western blot and immunoprecipitation analysis, cells were transfected transiently with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to manufacturer instructions. Cells were plated to be 90% confluent at transfection day and transfected with 4 μg of total DNA in 6-well plates for Western blot.
For electrophysiological recordings, cells were cotransfected in T25 flasks with 2 μg of total amount of DNA together with 0.2 μg of green fluorescent protein for detection of successfully transfected cells. The human KCNH2 and the CA3 cDNA constructs were purchased from OriGene Technologies, Inc. (Rockville, MD). The caveolin-3 cDNA constructs were purchased from Origene Technologies, Inc. (Rockville, MD). The cell culture media was composed of 5.17 mmol/L NaCl, 2 mmol/L EDTA, 10% KCl, 1 mmol/L PMSF, 1 mmol/L Na3VO4, 5 mmol/L NaF supplemented with fresh protease inhibitor cocktail EDTA free (La Roche). Lysates were cleared at 12000 rpm for 15 minutes, and protein concentration was determined by Bradford assay. Equal amounts of protein (20 μg) were heated for 10 minutes at 60°C and subjected to SDS-PAGE on a 7.5% gel followed by transfer on polyvinylidene difluoride membranes. After blocking, membranes were incubated with primary antibodies against αERG (term 1:1000; sc-15968; Santa Cruz Biotechnology Inc, Santa Cruz, CA) or polyclonal IgG isolated from normal goat serum for negative control. Immune complexes were precipitated finally with protein G-agarose for 2 hours at 4°C, collected by centrifugation, washed 5× in washing buffer (Trition 0.1%, NP40 0.1%, 50 mmol/L Tris–HCl pH 7.4, 150 mmol/L NaCl, 2 mmol/L EDTA, and 10% glycerol), and disrupted by incubation in denaturing Laemmli sample buffer. The complexes were separated on 4% to 15% gel and transferred to polyvinylidene difluoride membrane. After blocking, membranes were probed with anti-hERG (1:1000; sc-15968; Santa Cruz) or anti-Cav3 antibody (1:500; sc-5310; Santa Cruz), and the appropriate secondary antibody was conjugated with horseradish peroxidase. A total of 20 μg of each protein extract was also loaded on the gel to check the efficiency of transfection. Signals were detected using an ECL west-pico detection kit (Thermo Scientific, Rockford, IL).

### Data Analysis

Data analysis was performed using Igor Pro Software (WaveMetrics, Lake Oswego, OR). No leak subtraction was used during current recordings, and current densities were normalized to cell capacitance. Deactivation kinetic analysis was performed by calculating the time constants of double exponential functions fitted on the currents measured. Data were fit with the Boltzmann function. The significance of differences between the recordings was calculated using the t test.

### Results

One LQTS proband (0.6%) was identified as a heterozygous carrier of the missense mutation CA3 c.233C>T: p.T78M that has been previously associated with LQTS, as well as sudden infant death syndrome. This threonine residue is highly conserved in mammals.

A further 15 non-disease--causing genetic variants, 3 in the 5-prime untranslated region part of the gene, 5 from the intronic regions around the intron/exon boundaries, and 7 synonymous variants from the coding region, were identified as detailed in Table 1. Ten of the variants described here,
including caveolin-3:p.T78M, had been registered in the SNP database. Some of the variants had a very low prevalence. The frequent genotypes were in Hardy–Weinberg Equilibrium, although the frequency of the rare variants was too low for a reliable Hardy–Weinberg Equilibrium estimation.

Of the variants not previously described in an SNP database, 2 were 5-prime untranslated region SNPs (c.-34C>T and c.-15C>A), 2 were intronic variants (c.114+32A>G and c.115-32delT), and 2 were synonymous variants (c.12A>G:p.E4= and c.240G>A:p.L80=). All these variants were extremely rare and occurred only in the LQTS cohort. There was no appreciable effect on splicing by these variants as assessed by in silico analysis.

The caveolin-3:p.T78M carrying proband was also a heterozygous carrier of the Kv11.1:p.I400N mutation. The pedigree and clinical synopsis are shown in Figure 2. Three family members (II-2, II-4, and III-7) carry the caveolin-3:p.T78M mutation in isolation. All 3 reported syncopal episodes: II-2, an 80-year-old woman (8 years after diagnosis at 72 years), had 2 syncopes and a severely prolonged PQ interval of 0.52 ms, AV block was assumed and a DDD pacemaker was implanted, and the patient has been symptom free since; II-4 had a syncopal episode once during painful delivery; and III-7 fainted once during an accident where her hand was lacerated in a machine. The 3 isolated caveolin-3:p.T78M carriers (II-2, II-4, and III-7) had heart rates of 56, 75, and 75 bpm, respectively. All Kv11.1:p.I400N carriers had a Schwartz score ≥4 except IV-3 with a score of 3. Family members only carrying the caveolin-3:p.T78M mutation had a Schwartz score of 2, indistinguishable from healthy subjects.

Table 1. Genetic Variants in CAV3 and Their Frequency in a White Population and LQTS

<table>
<thead>
<tr>
<th>NCBI SNP identifier</th>
<th>NM_001234.3</th>
<th>NP_001225.1</th>
<th>NCBI SNP: MAF</th>
<th>EVS EA: MAF</th>
<th>DK control MAF</th>
<th>LQTS (n=167)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs116840771</td>
<td>c.-37G&gt;A</td>
<td>...</td>
<td>A=0.005</td>
<td>A=0.003</td>
<td>...</td>
<td>0.006</td>
</tr>
<tr>
<td>...</td>
<td>c.-34C&gt;T</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.003</td>
</tr>
<tr>
<td>...</td>
<td>c.-15C&gt;A</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.003</td>
</tr>
<tr>
<td>...</td>
<td>c.12A&gt;G</td>
<td>p.Glu4=</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.003</td>
</tr>
<tr>
<td>rs1974763</td>
<td>c.27C&gt;T</td>
<td>p.Leu9=</td>
<td>T=0.059</td>
<td>T=0.125</td>
<td>T=0.124</td>
<td>0.159</td>
</tr>
<tr>
<td>rs1008642</td>
<td>c.99C&gt;T</td>
<td>p.Asn33=</td>
<td>T=0.371</td>
<td>T=0.239</td>
<td>T=0.265</td>
<td>0.254</td>
</tr>
<tr>
<td>rs11922879</td>
<td>c.114+26G&gt;A</td>
<td>...</td>
<td>A=0.048</td>
<td>A=0.049</td>
<td>A=0.063</td>
<td>0.073</td>
</tr>
<tr>
<td>...</td>
<td>c.114+32A&gt;G</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.003</td>
</tr>
<tr>
<td>rs139242554</td>
<td>c.115-47_115-31del</td>
<td>...</td>
<td>del=0.091</td>
<td>0.114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>c.115-32delT</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>rs57159780</td>
<td>c.115-23G&gt;C</td>
<td>...</td>
<td>C=0.060</td>
<td>C=0.089</td>
<td>C=0.130</td>
<td>0.079</td>
</tr>
<tr>
<td>rs13087941</td>
<td>c.123T&gt;C</td>
<td>p.Phe41=</td>
<td>C=0.167</td>
<td>C=0.241</td>
<td>C=0.250</td>
<td>0.239</td>
</tr>
<tr>
<td>rs61147808</td>
<td>c.171G&gt;A</td>
<td>p.Val57=</td>
<td>A=0.014</td>
<td>A=0.0001</td>
<td>...</td>
<td>0.003</td>
</tr>
<tr>
<td>rs72546668</td>
<td>c.233C&gt;T</td>
<td>p.Thr78Met*</td>
<td>T=0.001</td>
<td>T=0.004</td>
<td>T=0.006</td>
<td>0.003</td>
</tr>
<tr>
<td>...</td>
<td>c.240G&gt;A</td>
<td>p.Leu80=</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.003</td>
</tr>
<tr>
<td>rs139985460</td>
<td>c.336C&gt;T</td>
<td>p.Ile112=</td>
<td>T=0.002</td>
<td>T=0.013</td>
<td>T=0.006</td>
<td>0.006</td>
</tr>
</tbody>
</table>

NCBI SNP: MAF are generated from 1000 genomes data. EVS EA data are generated from the exome sequencing of 8600 individuals. DK indicates Denmark; EA, European American; EVS, Exome Variant Server; LQTS, long–QT syndrome; MAF, minor allele frequency; NCBI, National Center for Biotechnology Information; and SNP, single-nucleotide polymorphism.

*Only variant previously reported in the literature.3,9–11
were lower in Kv11.1:p.I400N channels when compared with WT (Figure 4D). No difference was observed between WT and Kv11.1:p.I400N in the deactivation rates.

Interaction between Kv11.1 and caveolin-3 was confirmed by coimmunoprecipitation analysis (Figure 5A). Furthermore, the interaction between caveolin-3 and Kv11.1 is not compromised by either caveolin-3:p.T78M or Kv11.1:p.I400N mutations (Figure 5B).

**Discussion**

**Clinical Genetics**

We have identified 1 CAV3 variant (c.233C>T; p.T78M) in 167 LQTS cases. This variant falls within the central hydrophobic transmembrane domain (amino acids 75–106) of caveolin-3 and has previously been reported to be disease causing. Of the 16 CAV3 variants identified in the LQTS population, 10 of them occurred at a frequency of <1% in this population. Three of these rare variants were identified in the Danish control group, and 2 occurred with a frequency of <1% in this group. Only 1 of these, caveolin-3:p.T78M, was also identified in the 1000 genomes cohort at a frequency of 0.1%. The significance of these rare variants is unclear. In silico analyses of these variants suggest that they all have the possibility of disrupting exon splicing silencer or enhancer sites and thereby affecting splicing. However, without further experimental evidence, it is difficult to ascribe pathophysiological significance to these variants.


**Table 2. Clinical Characteristics of Caveolin-3:p.T78M Carriers, An Aggregation of Data From Vatta et al** and the Present Study

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age at Diagnosis, y</th>
<th>Nucleotide Change</th>
<th>Caveolin-3 Variant</th>
<th>Other LQTS Mutations</th>
<th>Presenting Symptom</th>
<th>QTc, ms</th>
<th>MCS</th>
<th>Other ECG Abnormalities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>M</td>
<td>8</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>...</td>
<td>Nonexertional syncope</td>
<td>433</td>
<td>n.a.</td>
<td>Marked sinus bradycardia</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>40</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>...</td>
<td>None</td>
<td>456</td>
<td>n.a.</td>
<td>...</td>
<td>9</td>
</tr>
<tr>
<td>II-2</td>
<td>F</td>
<td>72</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>...</td>
<td>AV block</td>
<td>410</td>
<td>n.a.</td>
<td>1° AV block</td>
<td>This study</td>
</tr>
<tr>
<td>II-4</td>
<td>F</td>
<td>64</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>...</td>
<td>Syncope during birth delivery</td>
<td>420</td>
<td>0.53</td>
<td>...</td>
<td>This study</td>
</tr>
<tr>
<td>III-7</td>
<td>F</td>
<td>38</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>...</td>
<td>Syncope during emotional stress</td>
<td>410</td>
<td>0.60</td>
<td>...</td>
<td>This study</td>
</tr>
<tr>
<td>III-4</td>
<td>F</td>
<td>48</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>p.I400N-KCNH2</td>
<td>Nonexertional syncope</td>
<td>500</td>
<td>1.78</td>
<td>Atrial pacing, notches</td>
<td>This study</td>
</tr>
<tr>
<td>III-6</td>
<td>F</td>
<td>44</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>p.I400N-KCNH2</td>
<td>None</td>
<td>470</td>
<td>1.80</td>
<td>Notches, abnormal U-waves</td>
<td>This study</td>
</tr>
<tr>
<td>IV-2</td>
<td>F</td>
<td>28</td>
<td>c.233 C&gt;T</td>
<td>p.T78M</td>
<td>p.I400N-KCNH2</td>
<td>Syncope</td>
<td>550</td>
<td>n.a.</td>
<td>...</td>
<td>This study</td>
</tr>
</tbody>
</table>

AV, atrioventricular; F, female; LQTS, long–QT syndrome; M, male; and MCS, morphology combination score.
Furthermore, caveolin-3:p.T78M carriers do not manifest an SCN5A ST-T wave pattern. Although all the 3 single caveolin-3:p.T78M carriers had experienced syncpe, these could be attributed to AV block or a neurocardiogenic mechanism.

**CAV3 Mutations and Disease Association**

The caveolin-3:p.T78M mutation was reported originally in 3 unrelated patients with LQTS, 1 of whom also carried Kv11.1:p.A913V. On the basis of clinical presentation of 2 of the patients, the authors concluded that caveolin-3:p.T78M may modulate the I_{Na} and may reduce the sinus rate producing clinical features characteristic of LQT3. However, of the 3 patients carrying only the caveolin-3:p.T78M mutation, 2—a 14-year-old girl and an 8-year-old boy—had normal QTc intervals of 405 and 433 ms, respectively. The last, a 40-year-old man, had a QTc interval of 456 ms that could be considered borderline prolonged; however, as reported by Drew et al, QTc intervals of 470 ms in postpubertal men and 480 ms in postpubertal women are considered the upper limits of normal. Data aggregated from Vatta et al and the present study (Table 2) indicate a paucity of evidence supporting CAV3 as an LQTS-causing gene. A subsequent study by Cronk et al31 identified caveolin-3:p.T78M in a sudden infant death syndrome case. The authors concluded that, as caveolin-3 colocalizes with β_{1a}-adrenoreceptors in ventricular and sinoatrial myocytes, disruption of caveolae by mutations in caveolin-3 might affect the β-adrenergic responsiveness and the excitation–contraction coupling of cardiac myocytes. The caveolin-3 p.T78M mutation has also been found in a subject with idiopathic hyperCKemia, although in this case immunohistochemical analysis of caveolin-3 in a muscle biopsy was normal.35 Traverso et al described a case where a homozygous caveolin-3:p.T78M carrier developed severe limb-girdle muscular dystrophy with dilated cardiomyopathy, whereas a heterozygous offspring was asymptomatic, suggesting an autosomal recessive mode of inheritance for this trait. In vitro studies showed that caveolin-3:p.T78M impairs the ability of the caveolin-3 hydrophobic domain to assemble caveolin-3 homo-oligomers units for the formation of caveolae in muscle cells.36,37 Furthermore, the first case of a heterozygous caveolin-3:p.T78M mutation associated with rippling muscle disease and proximal weakness was reported by Ricci et al in which immunohistochemical analysis of skeletal muscle confirmed reduced caveolin-3 immunolabeling. The patient was also affected by mild facioscapulohumeral muscular dystrophy that was explained by a D4Z4 partial deletion. However, weakness of the pelvic girdle muscles, a complaint not usually present in facioscapulohumeral muscular dystrophy, and the rippling phenomenon, a specific sign of caveolinopathy, has never been associated with D4Z4-reduced allele expression. Subsequently, Spadafora et al questioned the association of caveolin-3:p.T78M with disease. They presented a case in which caveolin-3:p.T78M was found in a woman presumed to suffer from distal myopathy. A final diagnosis of mild frontotemporal dementia was determined, and as the proband’s father was suspected to have died with parkinsonism, it was surprising that the proband had inherited the caveolin-3:p.T78M variant from her clinically nonaffected mother. The authors also point out that caveolin-3:p.T78M has a frequency of 1.5% in Italy and hypothesize that either the association of this variant with disease is incidental or specific population factors are required to modify the effect of this variant. Collectively, these data do not support the hypothesis that the caveolin-3:p.T78M mutation is pathogenic in the heterozygous state. It may be that caveolin-3:p.T78M exerts an effect in association with other genetic factors. Genotype–phenotype correlation studies in caveolinopathies suggest that genetic modifiers may contribute to the phenotype.

As seen in Table 2, the caveolin-3:p.T78M is not independently associated with a prolonged QT or with previous syncope, despite its previously described electrophysiology effect (a 4-fold increase in the late I_{Na}). Several cardiac ion channels have been reported to be localized within caveolae: HCN4, Nav1.5, Kv1.5, Cav1.2, and Kv11.1. The complex regulation of these ion channels within macromolecular complexes in caveolae suggests that the effect CAV3 mutations might exert on individual channels would produce a cumulative effect on the currents that make up the action potential. The ion channels listed above play critical roles in different phases of the cardiac action potential. Therefore, it is meaningless to assess the effects that CAV3 mutations have on a single ion channel without being able to assess the effect on the action potential as a whole. The findings that 2 of the caveolin-3:p.T78M carriers had reported a previous syncope and 1 had a slight 1° AV block could, however, indicate that CAV3 mutations may increase the propensity for numerous types of arrhythmia depending on the involved interacting ion channel.

An interesting possibility is that CAV3 mutations affect the I_{Kr} current predominantly under hypokalemic conditions.
conditions, where CAV3 is known to play a role in the availability of Kv11.1 at the cell membrane. This would be in accordance with the known sensitivity of the I_{Ks} current to external hypo- and hyperkalemia. This interdependence between hypokalemia and CAV3 function may explain the highly variable phenotypic expression of CAV3 mutations.

However, in the absence of an LQTS phenotype in any of the isolated caveolin-3:p/I78M carriers, despite that this mutation has the signature in vitro electrophysiological effect of LQTS9, it would seem reasonable to consider LQTS9 a provisional entity until an association between the clinical LQTS phenotype and CAV3 mutations has been established.
Cellular Electrophysiology

Cellular protein synthesis is a tightly controlled process where incorrectly processed proteins may be retained until they are correctly processed, retrogradely transported to an earlier compartment, and targeted for degradation. The processing of Kv11.1 channel proteins includes 2 glycosylation steps. Immature Kv11.1 proteins undergo N-linked core glycosylation in the endoplasmic reticulum (135 kDa), whereas complex glycosylation occurs in the Golgi apparatus (155 kDa); this mature form of Kv11.1 is inserted into the cell membrane.47,48 Using this defective glycosylation as a representation of defective trafficking, Anderson et al49 reported that most Kv11.1 mutations were trafficking deficient. Our results suggest that Kv11.1:p.I400N results in a trafficking deficiency of the Kv11.1 channel subunit.

Electrophysiological assessment of the Kv11.1:p.I400N mutation indicated that there is no effect on the gating of the mutant and confirmed the Western blot analysis that the mutation results in a reduced number of total functional channels present on the membrane.

Limitations

This study may be limited with respect to the method used for mutation screening. Direct sequencing of the coding regions of CAV3 cannot identify large deletions or duplications that would be more suitably identified by MLPA; it is also limited in identifying intronic variants as only the intronic regions close to the intron/exon boundaries are interrogated by this method. Consequently, we cannot comment on the role of CAV3 structural rearrangements or the genetic variation CAV3 regulatory regions may play in LQTS.

Conclusions

CAV3 mutations are present in LQTS, but they are rare and may not have any clinical consequence, despite an electrophysiology in vitro phenotype. There is no evidence indicating that the caveolin-3:p.T78M mutation is disease causing. As caveolin-3:p.T78M has been shown to generate the signature electrophysiological effect of LQT5, and we have demonstrated that this effect is not associated with LQTS, it is most reasonable to consider LQTS5 a provisional entity until an association with clinical LQTS can be established. In this study, all symptomatic patients are heterozygote carriers of
the Kv11.1:p.I400N mutation, which has shown to be associated with trafficking deficiency and reduced peak current. This functional effect is the clinical pathogenic mechanism in LQTS2.5,51 CAV3 analysis need not be performed routinely in the genetic workup of patients with LQTS, and the findings of rare variants in CAV3 should be handled with caution, particularly with respect to their clinical significance.

Sources of Funding
We acknowledge the financial support of The Danish Strategic Research Council, Novo Nordisk, and The Lundbeck Foundation.

Disclosures
Dr McKenna is partly supported by the National Institute for Health Research University College London Hospitals Biomedical Research Center. The other authors report no conflicts.

References
32. Balijepalli RC, Foell JD, Hall DD, Hell JW, Kamp TJ. Localization of cardiac L-type Ca(2+) channels to a cardiac macromolecular signaling complex is required for beta(2)-adrenergic regulation. Proc Natl Acad Sci USA. 2006;103:7500–7505.


**CLINICAL PERSPECTIVE**

We examined the occurrence of *CAV3* mutations in 167 long-QT syndrome (LQTS) probands by sequencing the coding regions of the gene. We describe a family with double heterozygosity, harboring mutations in *CAV3* and *KCNH2*; however, caveolin-3:p.T78M did not exhibit a LQTS phenotype, and functional characterization indicates that the *KCNH2* mutation is disease causing. Our findings in aggregate with published reports on *CAV3* mutations suggest that, despite an in vitro electrophysiology phenotype, *CAV3* mutations may not have any clinical consequence. There is no evidence indicating that the caveolin-3:p.T78M mutation is disease causing, and as caveolin-3:p.T78M has been shown to generate the signature LQTS9 electrophysiological effect, it is reasonable to consider LQTS9 a provisional entity until an association with clinical LQTS can be established, and the findings of rare variants in *CAV3* should be handled with caution, particularly with respect to their clinical significance.

Paula L. Hedley, Jørgen K. Kanters, Maja Dembic, Thomas Jespersen, Lasse Skibsbye, Frederik H. Aidt, Ole Eschen, Claus Graff, Elijah R. Behr, Sarah Schlamowitz, Valerie Corfield, William J. McKenna and Michael Christiansen

_Circ Cardiovasc Genet._ 2013;6:452-461; originally published online September 10, 2013; doi: 10.1161/CIRCGENETICS.113.000137

_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/6/5/452

Data Supplement (unedited) at:
http://circgenetics.ahajournals.org/content/suppl/2013/09/10/CIRCGENETICS.113.000137.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation: Cardiovascular Genetics_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation: Cardiovascular Genetics_ is online at:
http://circgenetics.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Legend to Figure 1 in Supplemental Material

**Figure 1.** ECG traces of family members solely carrying the caveolin 3:p.T78M mutations. A II2, B II4 and C III7.