Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death

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BACKGROUND L-type calcium channel (LTCC) mutations have been associated with Brugada syndrome (BrS), short QT (SQT) syndrome, and Timothy syndrome (LQT8). Little is known about the extent to which LTCC mutations contribute to the J-wave syndromes associated with sudden cardiac death.

OBJECTIVE The purpose of this study was to identify mutations in the CaV1.2 subunits of LTCC (CaV1.2) among 205 probands diagnosed with BrS, idiopathic ventricular fibrillation (IVF), and early repolarization syndrome (ERS).

METHODS/RESULTS Overall, 23 distinct mutations were identified. A total of 12.3%, 5.2%, and 16% of BrS/BrS+SQT, IVF, and ERS probands displayed mutations in α1, β2, and α2δ subunits of LTCC, respectively. When rare polymorphisms were included, the yield increased to 17.9%, 21%, and 29.1% for BrS/BrS+SQT, IVF, and ERS probands, respectively. Functional expression of two CaV1.2 mutations associated with BrS and BrS+SQT led to loss of function in calcium channel current. BrS probands displaying a normal QTc had additional variations known to prolong the QT interval.

CONCLUSION The study results indicate that mutations in the LTCC are detected in a high percentage of probands with J-wave syndromes associated with inherited cardiac arrhythmias, suggesting that genetic screening of CaV genes may be a valuable diagnostic tool in identifying individuals at risk. These results are the first to identify CACNA2D1 as a novel BrS susceptibility gene and CACNA1C, CACNB2, and CACNA2D1 as possible novel ERS susceptibility genes.

KEYWORDS Arrhythmia; Calcium; Electrophysiology; Genetics; Ion channels

ABBREVIATIONS BrS = Brugada syndrome; CHO = Chinese hamster ovary; ERS = early repolarization syndrome; IVF = idiopathic ventricular fibrillation; LQTS = long QT syndrome; LTCC = L-type calcium channel; PCR = polymerase chain reaction; SCD = sudden cardiac death; SNP = single nucleotide polymorphism; SQS = short QT; WT = wild type

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Introduction

Sudden cardiac death (SCD) is often associated with inherited cardiac arrhythmia syndromes.1 Twenty-five percent of all unexplained sudden deaths may be due to inherited cardiac diseases such as Brugada syndrome (BrS), idio-pathic ventricular fibrillation (IVF), and long QT syndrome (LQTS).2 BrS, early repolarization syndrome (ERS), and some forms of IVF represent a continuous spectrum of phenotypic expression that differ with respect to the magnitude and lead location of abnormal J-wave manifestations, which we and others have proposed be termed J-wave syndromes.3

The past decade has witnessed a veritable explosion of information linking inherited cardiac arrhythmia syndromes to cardiac ion channel mutations. BrS has been associated with mutations in seven genes classified as BrS1 through BrS7.4 Mutations in SCN5A, which encodes the Na+1,5 protein forming the α subunit of the sodium channel, have been associated with 11% to 28% of BrS probands by different groups.5 A genotype has not yet been identified in the majority of BrS probands. ERS has thus far been associated with one mutation in KCNJ8, a gene encoding the pore-forming subunit of the I\textsubscript{K\textsubscript{ATP}} channel.6 Expression studies suggesting a functional effect of this mutation has recently been reported.7

Little is known about the contribution of calcium channel gene variations to the etiology of inherited cardiac arrhythmia syndromes. Splawski et al.8,9 first described gain-of-function mutations in CACNA1C, a gene encoding Ca\textsubscript{2+}1.2 protein that forms the α subunit of the L-type calcium channel (LTCC), associated with a multiorgan dysfunction causing long QT intervals, arrhythmias, and autism known as Timothy syndrome (LQT8). Our group first described loss-of-function mutations in the α and β subunits of the cardiac LTCC associated with BrS and shorter than normal QT intervals and SCD.10,11

The LTCC is composed of four subunits: the main pore-forming α1 (Ca\textsubscript{2+}1.2) subunit, which determines the main biophysical and pharmacologic properties of the channel, and three auxiliary subunits, including a cytoplasmic β subunit, encoded by CACNB\textsubscript{1}, α2δ encoded by CACNA2D, and a γ subunit, which is present in skeletal, but not cardiac, muscle.12–14 Although a number of isoforms for the auxiliary subunits have been identified, in this study we focused on β2 (CACNB2), the dominant isoform known to play an essential role in the voltage dependence of LTCC,15,16 and the extracellular α2 and transmembrane δ1 (CACNA2D1), which are linked to each other via disulfide bonds.

Few data are available on the extent to which mutations in the various subunits of LTCC contribute to SCD, the extent to which they are associated with ST-segment elevation and QT abbreviation giving rise to the BrS and BrS+SQT phenotypes, and their pathogenicity. The present study sought to identify genetic variations in the α1, β2, and α2δ1 subunits of LTCC among probands diagnosed with BrS, ERS, and IVF and the extent to which they contribute to pathogenesis of these syndromes. We tested the hypothesis that mutations in LTCC genes are relatively common among probands diagnosed with these syndromes. We also examined the hypothesis that LTCC mutation-mediated BrS associated with a normal QTc is attributable to additional genetic variations known to prolong the QT interval.

Material and methods

Diagnosis

The probands and their family members were diagnosed as having BrS, BrS with shorter than normal QT (BrS/SQT), IVF, or ERS based on established criteria.17–20 Diagnosis was made based on 12-lead ECG, personal history of syncope, seizures, or aborted cardiac death, and family history of SCD or arrhythmic events. BrS patients displayed a coved-type ST-segment elevation in at least one right precordial lead under baseline conditions or after sodium channel block challenge with ajmaline or procainamide. Criteria for BrS with shorter than normal QT included QTc ≤ 360 ms for males and QTc ≤ 370 ms for females. An early repolarization pattern was defined as J-point (QRS–ST junction) elevation > 0.1 mV manifested as QRS slurring or notching or a distinct J wave. Patients were categorized as having IVF when no clear established phenotype was discernible in individuals experiencing one or more episodes of ventricular fibrillation. Most, but not all, patients underwent a sodium block challenge to rule out BrS and/or an isoproterenol challenge to rule out catecholaminergic polymorphic ventricular tachycardia.

Mutation analysis

A total of 205 BrS, BrS/SQT, ERS, and IVF probands who tested negative for SCN5A mutations were included in the study. After obtaining informed consent, blood was collected from the probands and family members. Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (Puregene, Gentra Systems, Inc., Minneapolis, MN, USA) and amplified by polymerase chain reaction (PCR) on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). All exons and intron borders of the CACNA1C, CACNB2, and CACNA2D1 genes were amplified and analyzed by direct sequencing. PCR products were purified with a commercial reagent (ExoSAP-IT, USB, Cleveland, OH, USA) and directly sequenced from both directions using an ABI PRISM 3100 Automatic DNA Analyzer (Applied Biosystems). Electropherograms were visually examined for heterozygous peaks and compared with reference sequences for homozygous variations (GenBank accession number NM_000719, NM_201590, NM_000722) using CodonCode Aligner Version 2.0.4 (CodonCode Corporation, Dedham, MA, USA). Fifty-five primer pairs were used to screen 55 exons, including splice alternative variants of CACNA1C, 20 primer pairs were used for CACNB2, and 39 primer pairs were used for CACNA2D1. Probands with calcium channel mutations and rare variants were also screened for KCNH2, KCNQ1, KCNJ8, KCNE1, KCNE2, KCNE3, KCNE4, SCN1B, and
SCN3B for the purpose of identifying additional mutations and/or polymorphisms. Variations were designated as mutations based on the Human Genome Variation Society’s guidelines for nomenclature. To be considered a mutation, a variation must have changed or disrupted the open reading frame (missense, nonsense, insertion/deletion mutation) and been absent in a minimum of 400 reference alleles obtained from more than 200 healthy individuals of similar ethnicity whenever possible. Possible single nucleotide polymorphisms (SNPs) were confirmed in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/projects/SNP). Numbering of residue variations was based on the NCBI database nucleotide reference sequence. The degree to which variations uncovered are conserved among species was determined using VISTA browser (http://pipeline.lbl.gov/cgi-bingateway2).

**Mutagenesis and functional expression**

The human wild-type (WT) CACNA1C cDNA [(EYFP)Na1c,77] in pcDNA vector was a gift from Dr. Nikolai Soldatov. cDNA of CACNB2b and CACNA2D1 genes, both cloned in pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA), were a gift from Dr. Michael Sanguinetti. Site-directed mutagenesis was performed with QuikChange II XL mutagenesis kit (Stratagene Agilent Technologies Co., La Jolla, CA, USA).

Mutated genes were functionally expressed in either Chinese hamster ovary (CHO) cells or human embryonic kidney (TSA201) cells as previously described. CHO cells were transfected with the cDNA encoding WT or p.V2014I CACNA1C subunits (3 μg) together with the cDNA encoding CACNB2b (12 μg), CACNA2D1 (5.1 μg), and the CD8 antigen (0.5 μg) using FuGENE 6 (Roche Diagnostics, Pittsburgh, PA, USA). The other two calcium variants were expressed in TSA201 cell line as previously described. cDNA of the three LTCC subunits were transfected in a 1:1:1 molar ratio using FuGENE 6 (Roche Diagnostics). In addition, CD8 cDNA was cotransfected as a reporter gene for the experiment involving p.D601E CACNB2b. Because p.E1829_Q1833dup CACNA1C was already tagged with YFP, no extra reporter gene was added. Before experimental use, cells were incubated with polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450, Invitrogen Dynal, Carlsbad, CA, USA) for the experiment of p.V2014I CACNA1C and p.D601E CACNB2b. For protocols involving p.E1829_Q1833dup CACNA1C, cells were directly identified by epifluorescence.

CHO cells were perfused with an external solution containing the following (in mmol/L): NMDG 130, KCl 5, NaCl 15, MgCl2 1, and HEPES 10 (pH 7.35 with HCl). Recording pipettes were filled with internal solution containing the following (in mmol/L): CsCl 120, MgCl2 2, MgATP 2, HEPES 10, CaCl2 5, and EGTA 10 (pH 7.25 with CsOH). Voltage-clamp recordings on TSA201 cells were made with patch pipettes filled with a solution containing the following (in mmol/L): CsCl 110, CaCl2 0.1, HEPES 10, EGTA 10, MgATP 2, and TEA 10 (pH 7.3 with CsOH). Extracellular solution contained the following (in mmol/L): glucose 10, CaCl2 2, MgCl2 1, HEPES 10, and TEA 150 (pH 7.35 with CsOH). Currents were filtered with a four-pole Bessel filter at 5 kHz and digitized at 50 kHz. Series resistance was electronically compensated at 70% to 80%.

**Electrophysiology**

Calcium currents were recorded in CHO or TSA201 cells using whole-cell, patch-clamp techniques at 21°C to 23°C with Axon-200B patch-clamp amplifiers and pCLAMP9 software (Axon Instruments, Chicago, IL, USA). Mean maximum current amplitude and cell capacitance were 0.5 ± 0.1 nA and 12.2 ± 1.5 pF, respectively (n = 16). Capacitance and series resistance were optimized, and ≈80% compensation was usually achieved, leading to uncompensated access resistances of 1.5 ± 0.7 MΩ.

Current–voltage relationships were constructed by applying 500-ms pulses from a holding potential of –70 mV to potentials ranging –50 and +70 mV. Voltage dependence of inactivation was determined using a two-step voltage-clamp protocol with a 500-ms conditioning pulse from –70 mV to potentials between –90 and +50 mV, followed by a test pulse to +20 mV. Inactivation curves were constructed by plotting the current amplitude elicited by the test pulse as a function of the voltage command of the conditioning pulse. Calcium channel conductance (G) was determined from the following relationship:

\[ G = I_{tp}/(V_m - V_R) \]

where \( I_{tp} \) = peak current amplitude at \( V_m \), and \( V_R \) = reversal potential (–67.4 ± 1.1 mV, n = 16). A Boltzmann function was fitted to the conductance–voltage and inactivation curves, yielding the midpoint (\( V_b \)) and slope (k) value of the curves.

**Statistical analysis**

Results are expressed as mean ± SEM. Data were compared using analysis of variance followed by the Newman-Keuls test. \( P < .05 \) was considered significant.

**Results**

**Clinical characteristics**

A total of 205 unrelated probands enrolled at the Masonic Medical Research Laboratory (MMRL) inherited cardiac arrhythmia registry over the past 5 years diagnosed with BrS, BrS/SQT, IVF, or ERS and their families were included in the study. Figure 1 shows representative 12-lead ECGs from BrS, ERS, and BrS/SQT phenotypes. The cohort consisted of 152 probands diagnosed with BrS, 10 with BrS/SQT, 19 with IVF, and 24 with ERS. Demographic characteristics are given in Table 1. Average age ranged between 30 ± 11 and 43 ± 16 years, and all four categories were male dominated (68%–90%).

**Identification of mutations**

Among all diagnostic groups, 25 probands were identified with one or more mutations in CACNA1C, CACNB2, or
CACNA2D1 genes encoding the three subunits of the L-type Ca channel: 15 BrS, 5 BrS/SQT, 1 IVF, and 4 ERS. Clinical characteristics and demographics of the probands with mutations are summarized in Table 1. Mean age at time of diagnosis and gender among the probands identified with mutations were similar to those of the entire cohort. A majority of probands in all four diagnostic groups were symptomatic, and there was a high incidence of syncope, ventricular tachycardia/ventricular fibrillation, and family history of SCD in all groups. An early repolarization pattern was observed in one or more of the inferior or lateral leads of 26% of BrS probands. Corrected QT intervals were shorter than normal in the BrS/SQT, ERS, and IVF groups but were in the normal range in the BrS group (Table 1).

Of the 23 mutations uncovered, 21 were missense and 2 were deletion/duplication (Table 3). Four of the mutations (p.A39V, p.G490R, p.T11I, p.S481L) were previously reported by our group.\(^{10,11}\) Nine mutations were localized in the \(\alpha_1\) subunit, 10 in the \(\beta_2\) subunit, and 4 in the \(\alpha_2\delta\) subunit.

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Loss-of-function mutations involving LTCC are known to predispose to a phenotype consisting of BrS with an abbreviated QTc. Yet the majority of BrS probands in this study presented with normal QTc intervals. It is noteworthy that a QT-prolonging variation could be identified in 12 (86%) of the 14 BrS cases (Table 3). The most common modulating variation involved the co-presence of a p.D601E polymorphism in \textit{CACNB2b} that augments late ICa (Figures 5C and 5G). Another common variant modulating the manifestation of the QT interval is a common polymorphism in \textit{KCNH2}, p.K897T. Although this SNP has been reported to exert a modifying effect on QTc, whether it confers risk or a protective effect remains controversial. Some studies have shown that it reduces I\textsubscript{Kr} and aggravates LQTS,\textsuperscript{25,26} whereas others have shown it increases I\textsubscript{Kr} and confers a protective effect.\textsuperscript{27,28} Other additional variations typically associated with LQTS include p.T10M-\textit{KCNE2}, p.R1047L-\textit{KCNH2}, p.D76N-\textit{KCNE1}, and p.G643S-\textit{KCNQ1}.\textsuperscript{29–34} In contrast, these QT-prolonging variants are only present in 1 (20%) of the 5 BrS/SQT probands (Table 3).

The total yields of probands with mutations and rare polymorphisms in each of the diagnostic groups is listed in Table 2. A total of 12.3% of BrS and BrS/SQT probands displayed mutations in 1 (5.5%), 2 (4.9%) and 3 (2%) of the 5 BrS/SQT probands (Table 3). The total yields of probands with mutations and rare polymorphisms in each of the diagnostic groups is listed in Table 2. A total of 12.3% of BrS and BrS/SQT probands displayed mutations in 1 (5.5%), 2 (4.9%),
and α2δ (1.8%) subunits of the LTCC; a total of 5.2% of IVF patients had mutations in the β2 subunit; and 16.0% of ERS patients had mutations in the α1 (4.1%), β2 (8.3%), and α2δ (4.1%) subunits. The total yield of probands with mutations and rare polymorphisms together was 17.9% for BrS and BrS/SQT, 21% for IVF, and 29.1% for the ERS group.

**Functional expression studies**

Expression studies probing the functional consequences of mutations in LTCC are limited. Previous studies have shown a loss of function of $I_{\text{Ca}}$ as the basis for BrS associated with mutations in $\text{CACNA1C}$ and $\text{CACNB2b}$. As a further test of the hypothesis that loss-of-function mutations in LTCC underlie BrS as well as ERS and some forms of IVF, we are in the process of performing functional studies of the variants uncovered. We present two cases here.

The first case is a 41-year-old woman of Panamanian descent who presented with palpitations, incomplete right bundle branch block, and a history of presyncope (MMRL219). A diagnosis of BrS was confirmed following a positive procainamide challenge (Figure 4A). Family history was negative for SCD but positive for stroke and coronary disease. Genetic testing identified a heterozygous substitution of a valine for isoleucine at position 2014 of $\text{CACNA1C}$ and a polymorphism, p.D601E, in $\text{CACNB2}$ (Figure 4B and Table 3). The husband and two sons were negative for the p.V2014I mutation (Figure 4A). The same mutation was present in another BrS patient (MMRL793; Table 3) together with a common polymorphism, p.H558R, in $\text{SCN5A}$. This proband presented with a BrS type I ECG following sodium block challenge and has a family history of sudden death of undetermined cause at a young age. A valine at position 2014 is highly conserved among species (Figure 4C).

To determine the consequences of this mutation, we evaluated $I_{\text{Ca}}$ characteristics in CHO cells transfected with WT or p.V2014I $\text{CACNA1C}$. Figure 5A shows that the p.V2014I mutation significantly reduced peak current density at potentials between 0 and $-10\text{ mV}$, with a 61% reduction at $-10\text{ mV}$ ($-72.3 \pm 19.0\text{ pA/pF}$ vs $-28.2 \pm 10.6\text{ pA/pF}$, $n = 8$ in each group, $P < .05$; Figure 5D). The voltage at which the maximum peak current was achieved remained unchanged. As illustrated in Figure 5E, the mutation significantly reduced conductance of the calcium channel at potentials between 0 and $-30\text{ mV}$ ($P < .05$) without modifying $V_h$ or $k$ values of the activation curve ($-0.5 \pm 3.3\text{ mV}$ and $5.9 \pm 0.8\text{ mV}$, $n = 8$) compared to WT ($-1.5 \pm 1.4\text{ mV}$ and $6.0 \pm 0.9\text{ mV}$, $n = 8$, $P > .05$).

Figure 5B shows current traces recorded using a protocol designed to examine the voltage dependence of inactivation. $I_{\text{Ca}}$ density recorded during the test pulse to $-20\text{ mV}$ was significantly smaller with conditioning pulses to potentials between $-90$ and $-20\text{ mV}$ in cells expressing p.V2014I. The mutation shifted half-inactivation voltage to more negative potentials ($-23.0 \pm 1.2\text{ mV}$ vs $-30.5 \pm 4.2\text{ mV}$, $n = 8$ in each group, $P < .01$) without modifying $k$ values ($7.2 \pm 0.4\text{ mV}$ vs $7.9 \pm 0.6\text{ mV}$, $P > .05$; Figure 5F).

Interestingly, this proband also had a p.D601E polymorphism in $\text{CACNB2}$. To examine the functional effect of this variant, we expressed it in human embryonic kidney (TSA201) cells. Figures 5C and 5G show the effect of p.D601E in $\text{CACNB2}$ in significantly increasing late $I_{\text{Ca}}$, which is known to prolong QT. The modulatory effect of
Yield of probands with mutations in \( \alpha_1, \beta_2, \) and \( \alpha_2 \delta \) subunits of L-type calcium channel

<table>
<thead>
<tr>
<th>Subunit</th>
<th>BrS, BrS/SQT</th>
<th>IVF</th>
<th>ERS</th>
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<tbody>
<tr>
<td>( \alpha_1 )</td>
<td>( 162 )</td>
<td>( 19 )</td>
<td>( 24 )</td>
</tr>
<tr>
<td>( \beta_2 )</td>
<td>9</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>( \alpha_2 \delta )</td>
<td>5.5%</td>
<td>4.9%</td>
<td>4.1%</td>
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<tr>
<td>( \alpha_1 )</td>
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<td>2</td>
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<tr>
<td>( \beta_2 )</td>
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<tr>
<td>Total yield of probands with mutations</td>
<td>20/162</td>
<td>1/19</td>
<td>4/24</td>
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<tr>
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<tr>
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<tr>
<td>Yield</td>
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<td>2</td>
<td>4.3%</td>
</tr>
<tr>
<td>No. of probands with rare polymorphism for ( \beta_2 )</td>
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<td>2</td>
</tr>
<tr>
<td>Total yield of probands with mutations and rare polymorphisms</td>
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<td>4/19</td>
<td>7/24</td>
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<tr>
<td>( \alpha_1 )</td>
<td>17.9%</td>
<td>21%</td>
<td>29.1%</td>
</tr>
</tbody>
</table>

Total number (n) and yield (%) of mutations and rare polymorphisms identified for each subunit of calcium channel and diagnostic group.

BrS = Brugada syndrome; BrS/SQT = Brugada syndrome with shorter than normal QT; ERS = early repolarization syndrome; IVF = idiopathic ventricular fibrillation.

Discussion

This study is the first comprehensive attempt to associate inherited cardiac arrhythmia syndromes with genetic variations in the cardiac LTCC. We identified 23 mutations in three genes encoding the three subunits of the LTCC in 25 unrelated probands and six rare polymorphisms in 17 additional probands diagnosed with BrS, BrS/SQT, IVF, or ERS. A total of 12.3%, 5.2%, and 16% of BrS/BrS+SQT, IVF, and ERS probands displayed mutations in \( \alpha_1 \), \( \beta_2 \), and \( \alpha_2 \delta \) subunits of LTCC, respectively. The total yield of probands with mutations and rare polymorphisms is 17.9% for BrS and BrS/SQT, 21% for IVF, and 29.1% for ERS diagnostic groups (Table 2). The yield of probands with LTCC mutations associated with BrS (12.3%) is second only to \( SCN5A \) mutations, which have been reported to range between 11% and 28% at different international centers.\(^{5}\) In the case of ERS, \( CACNA1C, CACNB2, \) and \( CACNA2D1 \) represent the second, third, and fourth genes proposed to underlie this phenotype, the first one being \( KCNJ8.6 \)

Topologically, it is interesting that no mutations were detected in any of the transmembrane regions of \( \text{Ca}_1.2 \) (Figure 3). Six of the nine mutations were located in the N- or C-terminus of the \( \alpha_1 \) subunit. Relevant to this finding is the demonstration by Soldatov’s group of voltage-gated mobility of the C-and N-cytoplasmic tails of \( \text{Ca}_1.2 \) and their important regulatory role in voltage- and \( \text{Ca}^{2+} \)-dependent inactivation.\(^{35,36}\) In addition, cleavage of the C-terminus of native \( \text{Ca}_1.2 \) channels has been shown to result in a proteolytic fragment that is able to act as a repressor of \( \text{Ca}_1.2 \) promoter activity.\(^{37,38}\) Thus, mutations in the C-terminus could have significant effects on the regulation of expression level and on function of the \( \text{Ca}_1.2 \) channel. Another mutation of great interest is p.E1115K because it is proposed to underlie this phenotype, the first one being \( KCNJ8.6 \)

The probability of a nonsynonymous mutation causing a genetic disease increases with a higher degree of evolutionary conservation of the mutated site.\(^{39}\) The majority of our mutated sites were located in highly conserved regions (Table 3), suggesting that many of the variations...
uncovered likely are disease-causing. Twelve of the 14 probands with rare polymorphisms had variations in residues that were either highly conserved or conserved among large mammals (Table 3). Moreover, excellent genotype–phenotype correlation was seen among available families, with pathogenic phenotypes co-segregating with a positive genotype (Figure 2). Failure to do so in all but one case could be attributed to female gender and/or young age, both of which are known to diminish expression of the disease phenotype.

In previous studies we demonstrated a loss of function of ICa for four of these mutations (marked with an asterisk in Figures 2 and Figure 3 and Table 3).10,11 In the present study, we demonstrated a loss of function of ICa in BrS and BrS/SQT probands carrying a p.V2014I or p.E1829_Q1833dup mutation in CACNA1C. The BrS proband, unlike the BrS/SQT proband, was also found to carry a rare polymorphism, p.D601E, in CACNB2b, which when expressed was found to augment late ICa, thereby explaining the absence of an abbreviated QTc in this proband. QT-prolonging variations (p.D601E-CACNB2b, p.K897T-KCNH2, p.T10M-KCN2, p.R1047L-KCNH2, p.D76N-KCNE1, p.G643S-KCNQ1) were found in 12 of the 14 BrS probands presenting with a normal QTc (Table 3).25,26,33

Our study results suggest that mutations in all three subunits of the LTCCs are detected in a relatively high percentage of probands with inherited cardiac arrhythmia syndromes, including BrS, ERS, and some forms of IVF. These findings suggest that genetic screening of Ca, genes may prove to be a valuable diagnostic tool for identifying individuals who might be at risk. CACNA1C, CACNB2, and CACNA2D1 should be included in the genotyping of patients who have diseases with a high occurrence of sudden death, particularly in cases where J-wave syndromes are suspected.3

Study limitations
The LTCC subunit genes, especially CACNB, have multiple isoforms. Our focus on CACNB2 in this study may have resulted in an underestimation of linkage of LTCC mutations to inherited cardiac arrhythmia disease. Thus far, a total of seven genes have been identified as associated with BrS.40 Our findings of three BrS probands associated with mutations in highly conserved residues of CACNA2D1 suggest that it may be a new gene for BrS. In support of this hypothesis, our preliminary functional expression studies indicate that the double mutation in CACNA2D1 [p.D550Y and p.Q917H (MMRL194)] reduces ICa to 25% of normal (Barajas et al, unpublished observation).

Mutations in only one gene, KCNJ8, have thus far been associated with ERS.6,7 The present study identifies four probands in whom mutations in highly conserved residues...
**Table 3** Summary of L-type calcium channel mutations and rare polymorphisms in CACNA1C, CACNB2, and CACNA2D1

<table>
<thead>
<tr>
<th>No.</th>
<th>Amino acid change</th>
<th>Nucleotide change</th>
<th>Mutation type</th>
<th>Conserv.</th>
<th>Exon</th>
<th>Location</th>
<th>Probands (n) Diagnosis Additional variations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutations in CACNA1C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>p.E850 del</td>
<td>c.2548-550del GAG</td>
<td>Deletion</td>
<td>HC</td>
<td>19</td>
<td>DII/DIII</td>
<td>422</td>
</tr>
<tr>
<td><strong>Mutations in CACNB2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>p.L399F</td>
<td>c.1195 C&gt;T</td>
<td>Missense</td>
<td>HC</td>
<td>7b</td>
<td>Hook region</td>
<td>289</td>
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<tr>
<td><strong>Mutations in CACNA2D1</strong></td>
<td></td>
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<tr>
<td><strong>Rare SNP</strong></td>
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</tr>
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<td>1</td>
<td>CACNA1C</td>
<td>p.G37R</td>
<td>c.109 G&gt;A</td>
<td>Missense</td>
<td>HC</td>
<td>2</td>
<td>N-terminus</td>
</tr>
<tr>
<td>2</td>
<td>CACNA1C</td>
<td>p.P817S</td>
<td>c.2449 C&gt;T</td>
<td>Missense</td>
<td>NC</td>
<td>17</td>
<td>DII/DIII</td>
</tr>
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<td>3</td>
<td>CACNA1C</td>
<td>p.A1717G</td>
<td>c.5150 C&gt;G</td>
<td>Missense</td>
<td>CM</td>
<td>42</td>
<td>C-terminus</td>
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<tr>
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<td>CACNA1C</td>
<td>p.T1178M</td>
<td>c.5360 C&gt;T</td>
<td>Missense</td>
<td>NC</td>
<td>42</td>
<td>C-terminus</td>
</tr>
<tr>
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<td>CACNA1C</td>
<td>p.R1973Q</td>
<td>c.5918 G&gt;A</td>
<td>Missense</td>
<td>HC</td>
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<td>C-terminus</td>
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<td>6</td>
<td>CACNB2</td>
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<td>c.1654 C&gt;G</td>
<td>Missense</td>
<td>CM</td>
<td>14</td>
<td>C-terminus</td>
</tr>
</tbody>
</table>

Conserv. = degree of conservation for the mutated site among multiple species: CM = conserved among large mammals; HC = highly conserved; NC = not conserved. BrS = Brugada syndrome; BrS/SQT = Brugada syndrome with shorter than normal QT; ERS = early repolarization syndrome; IVF = idiopathic ventricular fibrillation; MMRL ID no. = three-digit Masonic Medical Research Laboratory identification number; SNP = single nucleotide polymorphism.

*Previously published mutations.10,11
Figure 5  The p.V2014I-CACNA1C mutation causing a loss of function of $I_{Ca}$ together with a p.D601E-CACNB2b single nucleotide polymorphism causing a gain of function of late $I_{Ca}$ result in Brugada syndrome (BrS) with normal QTc (MMRL219).  A: Representative calcium current traces recorded in Chinese hamster ovary (CHO) cells transfected with wild-type (WT; left) or p.V2014I (right) CACNA1C subunits in response to the voltage clamp protocol shown at the top. B: $I_{Ca}$ recorded in response to the inactivation protocol shown. C: Overlapping calcium traces recorded from human embryonic kidney (TSA201) cells expressing WT and p.D601E-CACNB2b rare polymorphism. D: Current–voltage relationship. E: Activation curve showing conductance–voltage. F: Normalized inactivation curves in WT or p.V2014I CACNA1C. G: Bar graph showing $I_{Ca}$ current density recorded with WT versus p.D601E CACNB2b at different times (100, 200, and 300 ms) into the depolarized testing pulse at 0 mV (protocol inset). *$P < .05$, **$P < .01$ vs WT data. Each datapoint/bar represents mean ± SEM of 6–8 experiments.

Figure 6  A: Duplication of five amino acids in Cav1.2 leads to loss of function of $I_{Ca}$ resulting in Brugada syndrome (BrS) with shorter than normal QT interval (QTc = 346 ms). ECG recorded in leads V1–V3 of patient #300 at baseline. B: Electropherogram of wild-type (WT) and mutant CACNA1C showing duplication of five amino acids EETSQ. C: Representative calcium current traces recorded in human embryonic kidney (TSA201) cells transfected with WT (left) and p.E1829_Q1833-dup mutant (right) CACNA1C subunits by applying the protocol shown at the top. D: Current–voltage relationship (I–V curve) p.E1829_Q1833-dup mutant effect in Cav1.2 channels. Data are given as mean ± SEM of at least eight cells. *$P < .05$. 

[Figure 5: Graphs showing calcium current traces, conductance-voltage, inactivation curves, and current density with statistical analysis.]

[Figure 6: Graphs showing ECG waveform, electropherogram, calcium current traces, and current-voltage relationship with statistical analysis.]
of CACNA1C, CACNB2, and CACNA2D1 are associated with ERS, suggesting linkage of these genes with ERS. Although many of the mutations in these genes occur in highly conserved residues and genoty-pe-phenotype correlation among male members of available families is excellent, confirmation of these hypotheses must await the availability of functional expression studies. The requirement for such studies is underscored by the study of Kapa et al. suggesting that, in the case of LQTS, mutations of highly conserved residues may not always be disease-causing. It is possible that the same may be true in the case of calcium channel mutations associated with BrS and ERS. 41

We present functional expression data for two of the phenotypes evaluated. Although functional studies for the other mutations are in process, the data likely will not be available for many months, and it would be unreasonable to delay reporting of these results until that time.

Although in most cases of IVF we made a diligent effort to exclude the diagnosis of known channelopathies, we recognize that these tests are not always definitive and that patients whom we categorize as IVF may properly belong to another category.

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References