A missense mutation in the sodium channel β1b subunit reveals *SCN1B* as a susceptibility gene underlying long QT syndrome

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BACKGROUND Long QT syndrome (LQTS) is associated with sudden cardiac death and the prolongation of the QT interval on the electrocardiogram. A comprehensive screening of all genes previously associated with this disease leaves 30% of the patients without a genetic diagnosis. Pathogenic mutations in the sodium channel β subunits have been associated with cardiac channelopathies, including *SCN4B* mutations in LQTS.

OBJECTIVE To evaluate the role of mutations in the sodium channel β subunits in LQTS.

METHODS We screened for mutations in the genes encoding the 5 sodium β subunits (*SCN1B* isoforms *a* and *b*, *SCN2B*, *SCN3B*, and *SCN4B*) from 30 nonrelated patients who were clinically diagnosed with LQTS without mutations in common LQTS-related genes. We used the patch-clamp technique to study the properties of sodium currents and the action potential duration in human embryonic kidney and HL-1 cells, respectively, in the presence of β1b subunits.

RESULTS The genetic screening revealed a novel mutation in the *SCN1Bb* gene (β1bP213T) in an 8-year-old boy. Our electrophysiological analysis revealed that β1bP213T increases late sodium current. In addition, β1bP213T subtly altered $Na_v1.5$ function by shifting the window current, accelerating recovery from inactivation, and decreasing the slow inactivation rate. Moreover, experiments using HL-1 cells revealed that the action potential duration significantly increases when the mutant β1b was overexpressed compared with β1bWT.

CONCLUSION These data revealed *SCN1Bb* as a susceptibility gene responsible for LQTS, highlighting the importance of continuing the search for new genes and mechanisms to decrease the percentage of patients with LQTS remaining without genetic diagnosis.

KEYWORDS *SCN5A*; *SCN1B*; Cardiac sodium channel; β subunits; Sudden cardiac death; long QT syndrome

ABBREVIATIONS **AF** = atrial fibrillation; **APD** = action potential duration; **APD_{90}** = action potential duration at 90% of repolarization; **BrS** = Brugada syndrome; **cDNA** = complementary DNA; **ECG** = electrocardiogram; **GFP** = green fluorescent protein; **HEK** = human embryonic kidney; **I_{Na}** = sodium current; **I_{NaL}** = late sodium current; **LQTS** = long QT syndrome; **QTc** = corrected QT; **SCD** = sudden cardiac death; **SIDS** = sudden infant death syndrome; **SNV** = single nucleotide variant

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Introduction

Long QT syndrome (LQTS) is a rare cardiovascular disorder (1 in 2000 individuals[1]) associated with syncopal episodes, seizures, torsades de pointes, ventricular fibrillation, and sudden cardiac death in a structurally normal heart.[2,3] This syndrome is characterized by QT interval prolongation in the 12-lead surface electrocardiogram (ECG) because of an abnormality in cardiac repolarization.[4,5]

Congenital LQTS follows an autosomal dominant pattern of inheritance. A genetic link can be established in approximately 75% of LQTS cases.[6,7] Ninety percent of the LQTS pathogenic mutations are found in 5 genes (*KCNQ1* and its associated β subunit *KCNE1*, *KCNH2* and its associated β subunit *KCNE2*, and *SCN5A*).[8–12] The remaining pathogenic

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mutations have been reported in other 11 genes: *ANK2*, *KCNJ2*, *CACNA1C*, *CAV3*, *SCN4B*, *AKAP9*, *SNTA1*, *KCNJ5*, *CALM1*, *CALM2*,[13] and *RYR2*.[14]

Several of these genes associated with LQTS determine directly or indirectly the properties of the sodium current (I_{Na}). In cardiomyocytes, the α subunit of the cardiac sodium channel ($Na_v1.5$) is part of a protein complex that includes sodium channel β subunits and other regulatory proteins.[15] Five sodium channel β subunits have been identified: β1, β1b, β2, β3, and β4 subunits, which are encoded by 4 genes: *SCN1B* (for the 2 β1 subtypes), *SCN2B*, *SCN3B*, and *SCN4B*, respectively.[16–21] The sodium channel β1b subunit was described as an alternative splicing of the β1 subunit characterized by a retention of intron 3, which leads to an alternative C-terminal sequence (*SCN1Bb*), resulting in a larger protein (268 residues) than the β1a subunit (218 residues).[18,21] It has been reported that the β1b is anchored to the cell surface when it is coexpressed with $Na_v1.5$.[22,23] Evidence has shown that it is expressed in human ventricles, atria, and Purkinje fibers.[24,25] Also, previous studies revealed an effect of β1b on sodium channel function.[18,21–25]

In recent years, several pathogenic mutations in sodium channel β subunits have been related to arrhythmogenic diseases: mutations in *SCN1B*, *SCN1Bb*, and *SCN2B* have been associated with Brugada syndrome (BrS), atrial fibrillation, and sudden infant death syndrome (SIDS),[23,24,26,27] but not LQTS. It has also been shown that a pathogenic mutation in *SCN3B* causes a trafficking defect of $Na_v1.5$, resulting in BrS and SIDS.[28,29] Finally, mutations in *SCN4B* have been associated with LQTS and SIDS.[29]

In this work, we performed a study of 30 nonrelated patients with LQTS with no pathogenic mutations in *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* to screen for mutations in the genes that encode the 5 sodium channel β subunits (*SCN1B*, isoforms *a* and *b*, *SCN2B*, *SCN3B*, and *SCN4B*). We found a novel single nucleotide variant (SNV) in *SCN1Bb* (*SCN1Bb* p.(Pro213Thr)) in a patient clinically diagnosed with LQTS. The biophysical analysis of *SCN1Bb* p.(Pro213Thr) revealed *SCN1Bb* as a susceptibility gene responsible for LQTS.

Methods

This study was approved by the Ethics Committee of Hospital Josep Trueta (Girona, Spain) and conforms to the principles outlined in the Declaration of Helsinki.

Sample population

Our population of 30 nonrelated patients with LQTS who are negative for pathogenic mutations in *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, and *KCNE2* genes belongs to a large population that was previously described[30]. Clinical data including 12-lead ECG, personal history of syncope, and family history were collected. The corrected QT (QTc) value was obtained using Bazett's formula. The diagnosis of LQTS was assessed by using the reevaluated Schwartz diagnostic criteria.[2,13]

Written informed consent to participate in the study was obtained from all patients in accordance with review board guidelines of Hospital Josep Trueta and Universitat de Girona (Girona, Spain).

Genetic analyses

Total genomic DNA was isolated from blood samples and directly sequenced (3130XL Genetic Analyzer, Applied Biosystems, Austin, TX,). The DNA sequence obtained was compared with the reference sequence of *SCN1B* (NM_001037.4 for isoform *a* and NM_199037.3 for isoform *b*), *SCN2B* (NM_004588.4), *SCN3B* (NM_018400.3), and *SCN4B* (NM_174934.3) by using SeqScape v2.6 software (Applied Biosystems) (see Online Supplemental Material).

Plasmid constructs and site-directed mutagenesis

The pcDNA3 vector harboring the complementary DNA (cDNA) of human *SCN5A* was a generous gift from Dr Matteo Vatta (Baylor College of Medicine, Houston, TX). The pcDNA3 vector harboring the cDNA of enhanced green fluorescent protein (GFP) was a generous gift from Dr Kirstin Callø (University of Copenhagen, Copenhagen, Denmark). A bicistronic vector encoding *SCN1Bb*-Red (pIRES-Red-SCN1Bb) was constructed using human *SCN1Bb* cDNA obtained from total cDNA from human right ventricle. The resulting pIRES-Red-*SCN1Bb* was used as a template to engineer the mutated vector *SCN1Bb* p.(Pro213Thr) (see Online Supplemental Material).

Cell culture and transfection

Human embryonic kidney (HEK)-293 tsA201 cells were used for the heterologous expression of sodium channel subunits in whole-cell recordings. HEK cells were transfected with 2 μg of DNA as follows: *SCN5A*:*GFP* ($Na_v1.5$ alone), *SCN5A*:*SCN1Bb*_WT ($Na_v1.5$+β1bWT), or *SCN5A*:*SCN1Bb*_P213T ($Na_v1.5$+β1bP213T) at 1:1 molar ratios. HL-1 cells (a generous gift from Dr Javier Díez-Juan, INCLIVA, València, Spain) were used for measuring the action potential duration (APD) from cells overexpressing GFP (as a control), β1bWT, or β1bP213T (see Online Supplemental Material).

Electrophysiological studies

Electrophysiological studies were performed 48 hours after transfection. Whole-cell I_{Na} values in HEK cells were measured at room temperature using whole-cell patch-clamp technique as in our previous studies.[27] Action potential recordings from transfected single HL-1 cells were registered using the perforated patch technique at room temperature. APs were recorded in response to brief (2 ms) depolarizing current injections (1 nA). All our recordings were done at a pacing frequency of 2 Hz. APD was determined at 90% of repolarization (APD_{90}). Solutions, protocols, and acquisition systems are specified in Online Supplemental Material.