Mutation analysis of KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 genes in Chinese patients with long QT syndrome

DU Rong1, MD, TIAN Li2, MS, YUAN Guohui2, MD, LI Jin2, MD, REN Fxin2, MD, GUI Le2, MD, LI Wei2, MD, ZHANG Shouyan2, MD, KANG Cailian2, MD, YANG Junguo (yangboh)2, BS

1 Department of Rheumatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China
2 Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Abstract Long QT syndrome (LQTS) is the prototype of the cardiac ion channelopathies, which cause syncope and sudden death. Inherited LQTS is represented by the autosomal dominant Romano-ward syndrome (RWS) and the autosomal recessive Jervell and Lange-Nielsen syndrome (JLNS). Multiple heterozygous mutations in several ion channel genes (KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2) have been shown to cause autosomal dominant LQTS [2–4]. Autosomal recessive LQTS, which is associated with deafness, has been shown to occur with homozygous mutations in KCNQ1 or KCNE1 in JLNS families in which QTc prolongation was inherited as a dominant trait.

Methods

2.1 Identification of LQTS patients

Four family constellations were collected from Shandong, Hubei, Jiangsu and Yunnan Provinces in China, including three RWS family constellations and one JLNS. Informed consent was obtained according to the standards established by the United Hospital Committee on Clinical investigations. Family members were evaluated by family history, physical examination and 12-lead electrocardiography. Historical data (the presence of syncope, number of syncopal episodes, age
of onset of symptoms, and occurrence of sudden death) and the duration of QTc interval were obtained. The final diagnostic indices for JLNS positive case are as follows: 1) congenital deafness; 2) a history of apopsychia or torsades de pointes; 3) QTc > 460 ms; 4) QTc > 440 ms accompanied by bradycardia or an abnormal T-wave.

Peripheral vein blood of 2–5 mL from the above members were collected, anticoagulated with EDTA and stored at a temperature of −20°C.

2.2 DNA isolation and mutational analysis

Genomic DNA was isolated from peripheral blood lymphocytes using the DNA Isolation Kits for Mammalian Blood following the manufacturer’s instruction (Roche Biochemical, Inc., USA). The exons of KCNQ1, KCNH2, KCNE1, KCNE2 and SCN5A were PCR-amplified and sequenced for identifying LQTS causing mutations. The PCR primers were designed as described [5,6]. The primers of KCNQ1, KCNH2, KCNE1 and KCNE2 were synthesized in Shanghai and the primers of SCN5A were offered by Dr. Wang Qin of Cleveland University. The PCR reaction conditions were as follows: 50 uL solution initial denaturation at 94°C for 3 min and 30 cycles for amplification (94°C for 1 min, 56°C–66°C for 45 s and 72°C for 20 s), followed by a final extension for 5 min at 72°C. Protease K was purchased from Sigma Co., USA. Taq DNA-polyase and dNTP was purchased from Biostar Co., Canada. The PCR product purification kit and the gelatin extraction kit were from Qiagen Co., USA. After the purification of the PCR products, bidirectional sequencing was applied to detect DNA on a 3700DNA Analyzer. The concentration of sequencing primers was 5 pmol/ul.

Genbank blast was applied to compare sequencing result of KCNQ1 with the whole length of KCNQ1 CDNA to find the mutational sites. Blood DNA of 50 normal people were applied in PCR amplification of correlated fragments of KCNQ1 which was confirmed as mutation or SNP by direct sequencing. The detected mutational site was then checked in the Genbank to assure whether it was a new-found site. In order to confirm the reliability of sequencing, PCR products of the patients with mutations were purified and then transduced into DH5α-competent cells by using the T-vector cloning kit (Promega Co., USA). Bidirectional sequencing was applied to the positive clone plasmids to verify the reliability of sequencing.

3 Results

Four families were characterized with LQTS (including a family with JLNS) from the Chinese population and identified a missense mutation in a RWS family, an SNP and a novel mutation in the KCNQ1 of the JLNS family.

3.1 Mutation of RWS (kindred F1, Fig. 1)

The LQTS-causing mutations were screened in KCNQ1 and KCNH2 in all probands with RWS. A missense mutation was identified in a RWS family (Fig. 2). The proband of a kindred F1 contained a G to A change at nucleotide position 940 (940 G > A). This mutation leads to substitution of a glycine residue by a serine residue at codon 314 (G314S) in the pore region. Mutation G314S was present in affected family members only. This mutation did not be found in 50 normal unrelated individuals. This mutation had earlier been identified in Caucasian and Japanese populations. This subject had repeated episodes of syncopal attacks during exercise and emotional stress. She continued to be asymptomatic after β-receptor blocker therapy. Her father was diagnosed as LQTS after repeated syncopal attacks and died suddenly at the age of 55 during a period of excitement.

3.2 An SNP and a heterozygous novel mutation of JLNS family (kindred F3, Fig. 1)

An SNP G643S in the exon 15 of KCNQ1 was identified in the proband of a subject and her sister which results in substitution of the glycine residue by serine residue (Fig. 3). Their asymptomatic father also had the SNP. The SNP was found in the Japanese population and was demonstrated to be a molecular basis for mild IKs dysfunction. However, this polymorphism has not been found in 50 normal unrelated individuals. A heterozygous novel mutation was found in their KCNQ1 exon 2a: 227 nucleotide C was replaced by T which led to substitution of threonine residue by isoleucine residue and has not been found in 50 normal unrelated individuals (Fig. 3).