

行政院國家科學委員會專題研究計畫 成果報告

台灣兒童 QT 延長症候群之盛行率與分子生物學之診斷

計畫類別：個別型計畫

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計畫主持人：吳美環

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## 一、中文摘要

先天性 QT 間距延長症候群，雖然是罕見之疾病，但其基因學之變化自西元 1991 年首度報告以來，已有相當多在鈉離子通道及鉀離子通道的基因突變被篩檢出，不同基因突變的先天性 QT 間距延長症候群，有可能必須有不同的治療途徑。在台灣，相關之基因突變資料相當有限，由我們之臨床經驗分析，我們有數位罕見之胎兒期發病的先天性 QT 間距延長症候群之病兒，其病程複雜，且不易治療。至於兒童期後發病之病兒其臨床症狀與國外之報告則較為相近。

本研究檢定了先天性 QT 間距延長症候群病兒之基因學變化。我們發現一種從未報告過在 SCN5A 的突變，此種突變雖是 heterozygous 突變，但仍會造成胎兒期發病，病程複雜且不易治療之先天性 QT 間距延長症候群。其功能之研究亦顯示此基因突變之鈉離子電流，有不活化功能之障礙，因此有相當大之 residual current。而於兒童期發病之病兒，目前我們所檢出的為鉀離子通道之突變，例如 KVLQT1 之突變等。具這種突變之病兒，常於游泳時被誘發出心室頻脈及休克。此種臨床表現與過去之報告相似。

**關鍵詞：**先天性 QT 間距延長症候群、心室不整脈、胎兒、兒童

### Abstract

Congenital long QT syndrome (LQTS) with *in utero* onset of the rhythm disturbances is associated with a poor prognosis. The underlying ionic channel abnormalities remained largely unknown. In the present study, we report a novel

LQTS-3 mutation in the early C-terminal domain of SCN5A, VXXXXX, from a newborn with fetal bradycardia and 2:1 atrioventricular block and ventricular tachycardia soon after birth. The 2:1 atrioventricular block improved to 1:1 conduction only after intravenous lidocaine infusion or high dose mexiletine that also controlled the ventricular tachycardia. The mutation occurred in a highly conserved domain within the C-terminus of the cardiac sodium channel, with a X->X substitution at codon xxxx which resulted in an amino acid substitution. The proband was heterozygous but the mutation was absent in both parents and the sister, thus suggesting a *de novo* origin. Expression of the mutant channels in tsA201 mammalian cells revealed a big residual current that was blocked by TTX and lidocaine. These findings suggest that this novel SCN5A channel dysfunction may contribute to a persistent inward current of the cardiac myocytes and clinically congenital LQTS with perinatal onset of the arrhythmias and probably a poor prognosis. As to those with tachycardia (syncope) onset during childhood, a mutation of the KVLQT1 gene (a C→T transversion at the second position of codon 341, with a subsequent substitution of alanine for valine (A341V) in the S6 region of the potassium channel protein) was found. The mutation had been described in the previous studies.

**Keywords:** Congenital long QT syndrome, SCN5 mutation, fetus, atrioventricular block, torsades de pointes

## 二、緣由與目的

The idiopathic or congenital long QT syndrome (LQTS) is an inherited disease characterized by prolonged ventricular repolarization and a high risk for sudden cardiac death due to torsade de pointes.<sup>1-2</sup> Most of the life-threatening arrhythmias in LQTS occur during physical or emotional stress, although in some families, sudden death occurs during sleep. Thus, LQTS has been regarded as an important example of neurally mediated sudden cardiac death. In the infants, the LQTS has been suggested as a major underlying mechanism for sudden infant death syndrome. The prevalence of LQTS is estimated as 1/10,000 to 15,000 live births in the Western countries with a female to male ratio of 2. In Taiwan, we have approximately 250,000 to 300,000 live births each year. However, the reported LQTS patients are few. This may be due to under-diagnosis of the disease or a racial difference. However, we have identified 4 fetuses in recent 3 years with LQTS with unusual early onset and malignant course. The disease pattern is only rarely described. Therefore, the relative low number of the reported cases is more likely related to under-diagnosis of the disease.

The inheritance of LQTS can be autosomal dominant (mostly common), called as Romano-Ward syndrome, or autosomal recessive (less common, around 7% only), called as Jervell-Lange-Nielsen syndrome. The patients with Jervell-Lange-Nielsen syndrome are associated with hearing disturbance. However, about 10% of the patients are due to fresh mutation. To date, more than 200 mutations have been identified in 6 separate ion channels genes that encode the Na or K ( $I_{Kf}$  or  $I_{Ks}$ ) channels.<sup>1-2</sup> According to a recent report based on the mutational analysis from a pool of 262 untreated LQTS individuals, the distribution of the mutations are: KVLQT1, 42%, HERG, 45%, SCN5A, 8%, KCNE1, 3% and KCNE2, 2%. In Taiwan, the related data are very limited. Only an adult patient with possible mutation of the HERG had been described.

Previous studies have documented the

benefits of chronic  $\beta$ -blocker therapy, left stellate ganglionectomy, pacemaker implantation and implantable cardioverter-defibrillator therapy.<sup>2</sup> However, the prognosis of those with prenatal or neonatal onset remains poor.<sup>3-4</sup> The optimal therapy may be achieved by tailoring to the specific ion channel defects.<sup>5-6</sup> The SCN5 gene encodes for the  $\alpha$  subunit of the human cardiac voltage dependent sodium channel. Mutations in SCN5A may cause a persistent cardiac Na current and subsequent delayed repolarization that is responsible for a particular type of congenital LQTS, designated LQT 3.<sup>7</sup> This form has been associated with a lower rate of cardiac events but a higher rate of lethal events.<sup>1</sup> We described here the first case of LQTS with perinatal onset of fetal bradycardia as well as the neonatal AV block and ventricular tachycardia due to a de novo heterozygous mutation in C-terminal domain of SCN5A. Our previous studies have shown the presence of racial differences in the types of congenital heart disease. We have more cardiac children with subpulmonary ventricular septal defect, double inlet ventricle with right ventricular morphology and right isomerism, as compared to those found in Western countries. Therefore, it is highly possible that the mutations of the ion channels in Taiwanese LQTS may also vary from those reported from the Western countries. Although the efficacy of conventional therapy, including chronic  $\beta$ -blocker therapy, left stellate ganglionectomy, pacemaker implantation and implantable cardioverter-defibrillator therapy, has been validated. The annual mortality remains high, especially for those with prenatal or neonatal onset. The optimal therapy should be tailored to the specific ion channel defects, especially for those with high risks. For example, patients with mutation of the SCN5A and a subsequent continued depolarizing Na current can be improved by Na channel blocker instead of  $\beta$ -blockers. Potassium channel opener may be helpful in patients with mutation over the HERG. Therefore, the genetic analysis will be extremely helpful for the treatment

strategy.

### 三、結果與討論

#### Definition of Phenotype

Patients and the family members received clinical evaluation and 12-lead electrocardiogram. QT interval was measured on the surface electrocardiogram in lead II and corrected for the heart rate using Bazett's formula. Genetic studies were performed in concordance with the recommendations of the ethics committee of the institution. The investigation conforms to the principles outlined in the Declaration of Helsinki.

#### Mutational Analysis

Genomic DNA was isolated from venous EDTA blood of the infant and the family members by means of standard procedures. Previously published primer pairs were used to amplify all exons of KVLQT1, HERG, and SCN5A from genomic DNA. Selected exons were screened for the presence of nucleotide sequence polymorphisms by single-strand conformation polymorphism. Amplification reactions were carried out using 40ng of template DNA, 8 pmol of primers, 2µl dNTPs (2.5 mM), 0.8µl Mg<sup>2+</sup> (25 mM) and *Taq* polymerase. Single-strand conformation polymorphism analysis was performed according to the recommendations of the manufacturer (Pharmacia Biotech). Then PCR products were analyzed by single-strand conformation polymorphism on 12.5% nondenaturing polyacrylamide gels run at 5°C and 15°C, as described in GeneExcel protocols (Pharmacia Biotech). Mutations were detected by differences in migration patterns compared with the wild type. When abnormal patterns were observed, PCR products were reamplified and sequenced by the dideoxynucleotide chain termination method (DNA Sequencing Kit – BigDye Terminator Cycle Sequencing v 2.0, PE Biosystems) with fluorescent dideoxynucleotides on an ABI-Prism 373 DNA sequencer (Applied Biosystems), and the result was analyzed with the Genotyper program (PE Biosystems).

### RESULTS

#### Phenotype characterization

##### *Patients with perinatal onset:*

Five newborns from three families were evaluated for prenatal bradycardia and postnatal 2:1 atrioventricular (AV) block. Ventricular tachycardia (torsade de pointes ventricular tachycardia) was noted in 4. There were 2 pairs of siblings among these patients. All revealed prolonged QT intervals and functional AV block or alternating bundle branch block. Positive family history, including aborted sudden death, sudden death or prolonged QT interval was noted in 2 families. Lidocaine had been given as a therapeutic approach in 2 patients. One restored the functional AV block and the other developed ventricular tachycardia. Four patients received beta-blockers and two also received pacing therapy. In the patient who responded to lidocaine, mexiletine was also given. One additional patient received propranolol and mexiletine. Audio and visual evoked potential test, screening for anti-Ro and anti-La antibodies and anti-nuclear antibodies were all negative. Two patients died suddenly during the infancy and one died at the age of 14 months.

##### *Patients with onset during childhood*

Five children from three families were found to have syncope and prolonged QT interval. Among a family, the 3 children (one elder sister and a pair of twins) were found to have syncope during swimming. The father was also found to have syncope that had been less after some medications for hypertension. In another two patients from two families, syncope occurred mainly during exercise and emotional stress.

#### Genotype analysis

##### *Patients with perinatal onset*

In one patient with equivocal family history, the SSCP analysis of KVLQT1, HERG, did not reveal any abnormal conformer. Aberrant band was found in an exon of SCN5A only in the proband. This abnormality was absent in the other 10 family members including his parents. Bidirectional sequencing of the subsequent aberrant DNA fragments revealed a single base transition, which was expected to cause a nonconservative change at the

codon xxxx within the C-terminal domain of the sodium channel. The patient was heterozygous for this substitution.

The electrophysiological properties of the mutation by expression in the mammalian cell line showed a big residual current that could be blocked by lidocaine.

In one patient with positive family history and who developed ventricular tachycardia after lidocaine infusion, the SSCP analysis of KVLQT1, HERG, and SCN5 did not reveal any abnormal conformer.

#### *Patients with onset during childhood*

In the family with syncope that was closely associated with swimming, the DNA sequencing of KVLQT1 gene for the twin probands revealed a C→T transversion at the second position of codon 341, which predicted a substitution of alanine for valine (A341V) in the S6 region of the potassium channel protein. The same heterozygous mutation was also found in the father and the 9-year-old elder sister.

## **DISCUSSION**

We have identified a novel mutation of the SCN5A that may result in a type of congenital long QT syndrome. This mutation although only being heterozygous was associated a malignant clinical course. The patient may have ventricular tachycardia and functional AV block during the fetal stage. In those with tachycardia onset during the childhood, none experienced functional AV block. The mutation may be quite similar to those identified in the previous reports.

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