科技部補助專題研究計畫成果報告
期末報告

KCNQ2癲癇基因在正常兒童與不明原因癲癇兒童的基因型與功能性研究

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 103-2314-B-040-010-
執 行 期 間 ： 103年08月01日至104年07月31日
執 行 單 位 ： 中山醫學大學醫學系兒童學科

計 畫 主 持 人： 李英齊

計畫參與人員： 碩士級-專任助理人員：陳威翰

報 告 附 件 ： 出席國際會議研究心得報告及發表論文

處 理 方 式 ：
1.公開資訊：本計畫涉及專利或其他智慧財產權，2年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：否
3.「本報告」是否建議提供政府單位施政參考：否

中 華 民 國 104 年 11 月 13 日

中 文 摘 要 ： 癲癇佔人口1%，而兒童有60%為不明原因癲癇(idiopathic epilepsy)，其定義為癲癇且無明顯腦部病變與之相關，KCNQ2突變可造成如此。KCNQ2為細胞膜鉀離子通道調控基因。對帶有KCNQ2突變的人，部份會有癲癇與智障，但目前尚未完全瞭解。而最近，KCNQ2突變引起嚴重癲癇腦病變已引起廣泛的關切。因此本研究的目的要建立台灣地區不明原因癲癇KCNQ2基因突變的盛行率資料庫，更進一步的利用細胞模式探討KCNQ2基因突變後所造成的功能影響，來釐清KCNQ2基因在兒童不明原因癲癇所扮演的角色和致病機轉。在過去的計畫，我們已完成50個正常人和54個病人的KCNQ2基因篩檢，經過初步病人組與對照組比較後，發現25(46%)病人帶KCNQ2基因突變，但不知這些突變是否有相關性。再經過SIFT、PolyPhen軟體及功能性分析研究後，會導致的疾病的突變點包括: c.1545 G>C (p.E515D); c. 2264 G>A (p.Y755C); c. 1627G>A (p.V543M); c.1294 C > T (p.R432C)。這些突變分布在8個家族中。而p.Y755C更會造成新生兒的嚴重癲癇腦病變。在功能性分析顯示造成HEK293cell電位的改變，且與疾病的嚴重度有相關性。但不足的是，樣本數尚不夠多與功能性分析尚未全部完成。1.54個兒童不明原因癲癇的次世代基因檢查結果。2.經與正常人比對後， 25(46%)個病人帶有KCNQ2突變。包括c.1545 G>C (p.E515D); c. 2264 G>A (p.Y755C); c. 1627G>A (p.V543M); c.1294 C > T (p.R432C); c.2339 A > C(p.N780T); c. 2235 G > A (p.P745P); 與4個位於intron 16, 11, 10, 1的突變3.再經過SIFT、PolyPhen軟體及功能性分析(functional study)研究後，對於個別的點突變，從54病人中有8(15%)個病人(7個家族，1個為de novo 突變)，帶有病理性突變，對細胞膜電位有影響，得到進一步釐清。4.其中2個病人帶有p.Y755C造成嚴重的新生兒癲癇，導致KCNQ2 腦病變。5.經由功能性分析結果顯示基因型 (genotype)與表現型(phenotype)有關，顯示帶突變的基因造成細胞電流的改變程度與症狀嚴重度有相關性。

中 文 關 鍵 詞 ： KCNQ2；癲癇；功能性研究; 兒童

英 文 摘 要 ： Background and Objectives: Non-lesional epilepsy in children is usually genetically heterogeneous. The common genetic cause includes a KCNQ2 gene mutation The mutant gene is located at 20q13, a voltage-gated potassium-channel gene. KCNQ2 encephalopathy with mental retardation and refractory seizures continues to be reported, which highlights that KCNQ2 is important in children with epilepsy, whereas, the exact mechanism and phenotype of the KCNQ2 mutation and childhood epilepsy are still unknown.
Patients and methods:
We studied the KCNQ2 genotype from 75 non-relative patients (age range: 2 days to 18 years old), whom have non-lesional epilepsy. The study also screens KCNQ2 from 50 healthy controls without epilepsy. We use next generation sequences in 55 patients, and direct sequence in 20 patients to screen KCNQ2 mutation variants. KCNQ2 mutation variants were transfected into HEK293 cell to investigate functional

changes. Positive control is p. R213Q, which is known to cause neonatal seizure with suppression-bursting EEG.
Results and Conclusions:
Eight (8/75, 11%) cases were identified KCNQ2 mutations. All mutation variants were not found in the control group, and are predicated to be deleterious by the computerbased algorithms SIFT and PolyPhen. Mutations, p.V543M and p.R431C, are novel mutations. All patients with p.E515D cause epilepsy, however, their seizures were favorable, and with 3 normal intelligence and one mild intelligent disability. Two patients carrying p.Y755C cause neonatal epilepsy with suppression-bursting EEG in one, and multiple focal spikes in another case. Functional study demonstrated that the current recordings from higher to lower were wild-type, p.V543M, p.E515D, and p.R213Q, in that order.
Conclusion: The KCNQ2 mutation account for about 10% of idiopathic epilepsy in newborns and in children. It should be a candidate gene when diagnosing idiopathic childhood epilepsy. Functional study demostrated the genotype is heavy factor to phenotype.

英文關鍵詞：KCNQ2; epilepsy; associational study)

附件一

科技部補助專題研究計畫成果報告

（□期中進度報告/V 期末報告）

（計畫名稱）

計畫類別：V 個別型計畫　□整合型計畫

計畫編號：MOST　103-2314-B-040-010

執行期間：104　年 8 月 1　日至 105　年 7 月 31　日

執行機構及系所：中山醫學大學醫學系兒童學科

計畫主持人：李英齊

共同主持人：李宣佑

計畫參與人員：楊建洲、陳威翰

本計畫除繳交成果報告外，另含下列出國報告，共 1 份：

□執行國際合作與移地研究心得報告

V 出席國際學術會議心得報告

期末報告處理方式：

1. 公開方式：

　V 涉及專利或其他智慧財產權，V 二年後可公開查詢

2.「本研究」是否已有嚴重損及公共利益之發現：V 否 □是

3.「本報告」是否建議提供政府單位施政參考 V 否 □是，____（請列舉提供之單位；本部不經審議，依勾選逕予轉送）

中　華　民　國 104　年 10　月　30　日

ABSTRACT

Objective: *KCNQ2*-associated seizures are a rare autosomal-dominant neonatal epileptic syndrome. *KCNQ2* mutations can cause different seizure frequencies and neurological consequences; however, *in vitro* functional studies of *KCNQ2* mutations are rare. We studied the *KCNQ2* wild-type and the c.1545G>C (p.E515D), c. 1627G>A (p.V543M), and c.638G>A (p.R213Q) mutations, which cause different phenotypes with mild to severe neurological consequences. The p.V543M mutation caused only mild seizures and a normal neurodevelopmental outcome in one patient and her 3 family members. The p.E515D mutation caused substantially more frequent seizures but normal neurodevelopmental outcome in another patient and his mother. However, the p.R213Q mutation caused frequent seizures, neonatal suppression-bursting electroencephalograms, and poor neurodevelopment in still another patient. Neurodevelopmental outcomes were worst in patients with the p.R213Q mutation, and better in patients with the p.E515D mutation, and best in patients with the p.V543M mutation. The relationship between the phenotype and genotype of *KCNQ2* is still unknown.

Methods: The study used electrophysiological techniques to analyze *KCNQ2* mutation variants that caused different degrees of functional disabilities. *KCNQ2,* wild-type, and mutant *KCNQ2* alleles were transfected into HEK293 cells. Post-transfection cell-conductance voltage was induced in those cells.

Results: The opening threshold shifted to values that were more positive, and the maximal current induced by strong depolarization was higher in cells with the p.R213Q and p.E515D mutations. The half-maximal activation voltage ($V_{½}$) was significantly different ($P < 0.05$) between the cells with wild-type, p.E515D, and p.R213Q.

Significance: Our findings provide evidence that the clinical phenotype of each *KCNQ2* mutation is associated with its genotype. The degree of functional disability in the *KCNQ2* mutations transfected into HEK293 cells was correlated with patient outcomes.

Key words: *KCNQ2*, neonatal epilepsy, functional study, outcome

INTRODUCTION

KCNQ2-associated seizures are a rare, inherited, autosomal-dominant form of neonatal epileptic syndrome; they usually occur in the first week after birth. Benign familial neonatal convulsions (BFNC), a central nervous system channelopathy (ion channel dysfunction), is a monogenic, autosomal dominant, benign familial epilepsy syndrome.[1,2] Patients with BFNC will usually have seizures in the neonatal and infantile periods, but they are predicted to have benign courses.[1,3] The diagnosis depends on family history, clinical features, and genetic studies. The mutant gene is located at 20q13, a voltage-gated potassium channel gene (*KCNQ2* [MIM#602235]). *KCNQ2* genes are expressed predominantly in the brain and are encoding for voltage-gated potassium channel subunits underlying the M-current, a repolarizing current that limits repetitive firing during long-lasting depolarizing inputs.[4]

Each subunit of *KCNQ2* consists of heteromultimeric channels with six transmembrane domains (S1-S6), including a voltage sensor in S4, a loop between S5-S6 that builds the ion channel pore, and a long C-terminal region of mostly unknown function.[5] In *KCNQ2*, about 60% of the mutations are in the C-terminus and predicted to truncate the C-terminus with haploinsufficiency.[5-7] Most *KCNQ2* mutations are in the C-terminal, and, on rare occasion, some are in the S4 and S5 segments and the pore region. Most BFNC seizures will spontaneously disappear during the infant's first 12 months of life.[4] Despite the expectation of typical neurological development, some patients will develop epilepsy, recurrent febrile seizures, or developmental delays. Some affected children will have recurrent febrile seizures, benign childhood epilepsy with centrotemporal spikes (BCECTS), or a rare photosensitive myoclonic epilepsy at follow-up.[8] However, it is not yet possible to accurately predict outcomes in these patients.

An emerging phenotype of neonatal *KCNQ2* epileptic encephalopathy was recently reported.[9,10] Most

cases are de novo mutations, and patients present with severe seizures and grave neurological consequences. Some patients present with burst-suppression or multiple focal spikes in neonatal electroencephalograms (EEGs). Seizures will remit after the infants become a few years older, but they will usually manifest intellectual developmental delays. Despite some case-series reports[10,11], the relationship between the genotype and the phenotype is not well understood. A loss of function because of a haploinsufficiency of the *KCNQ2* gene is presumed to be the major pathomechanism for KCNQ2-associated seizures .[12-14] Because the relationship of genotype and phenotype is still not clear, and because the *in vitro* functional consequences caused by the *KCNQ2* mutation are not fully understood, we studied the correlation of genotype and 3 different phenotypes caused by mutations of *KCNQ2*: c.1545G>C (p.E515D), c.1627G>A (p.V543M), and c.638G>A (p.R213Q).

Patients and materials

From 20 unrelated patients presenting idiopathic neonatal or infant-onset epilepsy, we performed genetic study for *KCNQ2*. We identified 2 cases of *KCNQ2*-associated epilepsy: one patient in family 1 had the c.1627G>A (p.V543M) mutation and one patient in family 2 had the c.1545G>C (p.E515D) mutation. We used a functional study to investigate the phenotype and genotype relationship of the two family members. We also did a functional analysis of the wild-type allele as a negative control and of the p.R213Q mutation as a positive control.

Patient #1, Family #1 with c.1627G>A (p.V543M)

This index case was in a first child of nonconsanguineous parents was born at 39 weeks of gestation at a local hospital by cesarean section because of fetal distress. Her birth weight was 3125 grams, body length was 48 cm, and head circumference was 33 cm. Her Apgar scores were 9 (at 1 min) and 10 (at 5 min). After birth, her condition was unremarkable, and she started feeding with good tolerance. Her first seizure occurred when she was 1 months old, and she was then referred to our hospital. The seizures were unique asymmetrical general tonic-clonic seizures: all four limbs were twitching, her eyes were staring, and the seizures were accompanied by tachycardia. During her first examination, the infant's activity was good, with regular respiration and a normal heart rate. Her appearance, breathing sounds, abdomen, and extremities were unremarkable, except for a mild systolic murmur. She had another seizure when she was 2 months old. No more seizures were reported by the parents after the patient had begun taking oxcarbamezepine. A magnetic resonance imaging (MRI) study was normal. An EEG showed nonspecific, multiple high-voltage sharp waves and spike waves over the bilateral central area. We traced the family history and found that the infant's father and two of her aunts had seizures from birth but were not taking antiepileptic medication. These family members could not remember

how many seizures they had experienced, but they thought the number was about two or three. At present, they are healthy and have no intellectual disabilities. A genetic study for *KCNQ2* showed c.1627G>A (p.V543M) , and no mutation for *KCNQ3*. The c.1627G>A (p.V543M) is a novel mutation and highly conserved . The computer-based algorithms SIFT and PolyPhen predict that p.V543M is deleterious. The patient's family study showed that her father and two aunts had the same c.1627G>A (p.V543M) mutation.

Patient #2, Family #2, c.1545G>C (p.E515D)

This child had his first seizure when he was 2 days old, after which he was referred to our hospital. On the first examination, the infant's activity was good: his respiration was regular and his heart rate was normal. His appearance, breathing sounds, abdomen, and extremities were unremarkable, except for a mild systolic murmur. At 5 days old, he had cluster seizures, with a unique asymmetrical general tonic-clonic seizure, all four limbs were twitching, his eyes were staring, and the seizures were accompanied by tachycardia. Seizure frequency reached more than 20 times per day thereafter. After talking with the previous hospital's medical staff, we determined that the infant had shown the same ictal pattern since his second day of life. A series of examinations (cerebral spinal fluid, electrolyte, and calcium) showed that all values were within normal limits. Cardiac ultrasonography showed a mild ventricular septum defect, and a cardiologist suggested a follow-up. The MRI study was normal. An EEG showed nonspecific, multiple high-voltage sharp waves and spike waves over the bilateral central area. Because of the infant's overall good status, we tried an oral form of phenobarbital (6 mg/kg/day), but the seizures were still frequent on the following day. The patient was given intravenous phenytoin, but the seizures persisted. On his 12th day of life, we added vigabatrin (50 mg/kg/day) and the seizures decreased remarkably. His seizures stopped after two more days. We previously reported on this child.[15] However, he grow older and had 3 recurrent seizures at age of 1, 5, and 6 years old. He is now 7

years old and his intellectual development is normal.

We traced the family history and found that the infant's mother has the same mutation, had seizures from birth, and discontinued taking valproic acid, her only form of drug control, when she was 3 years old. The patient's grandmother has a similar history and currently has no seizures. The genetic study for *KCNQ2* showed c.1545G>C (p.E515D) from the patient and his mother, and no mutation for *KCNQ3*.

Patient #3, c.638G>A (p.R213Q)

This patient[9] had the c.638G>A (p.R213Q) mutation. Her seizures began when she was two days old. She had multiple seizures daily[9]; to control them, phenobarbital and phenytoin were prescribed. Her EEG showed a suppression-bursting pattern and severe asymmetric spastic quadriplegia as a neurodevelopmental outcome.

***In vitro* functional study**

Expression in HEK293 cells and whole-cell patch-clamp analysis.

HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Biowhittaker, Walkersville, MD) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), streptomycin (100 U/ml), and 2 mM L-glutamine (Lonza, Walkersville, MD). Mutations in *KCNQ2* were made using a kit (QuickChange; Stratagene, La Jolla, CA) and verified using sequencing.[16]

Whole-cell patch-clamp analysis.

For electrophysiological analysis, HEK293 cells were bathed in modified Tyrode's solution containing 125 mM NaCl, 5.4 mM KCl, 1.8 mM CaC_{l2}, 1 mM $MgCl_2$, 6 mM glucose, and 6 mM HEPES (pH 7.4). Patch-pipettes had a resistance of 3-4 Ω when filled with a pipette solution containing 125 mM potassium gluconate, 10 mM KCl, 5 mM HEPES, 5 mM EGTA, 2 mM MgC_{l2}, 0.6 mM $CaCl_2$, and 4 mM adenosine

5′-triphosphate disodium salt hydrate (Na2ATP) (pH 7.2). To measure the voltage dependence of activation, cells were clamped by applying 3-s conditioning voltage pulses to potentials between −80 mV and +40 mV in 10-mV increments from a holding potential of 0 mV. Data acquisition and analysis were done using Clampex 10.0 (Molecular Devices, Sunnyvale, CA).

Results

A comparison of three different phenotypes with those mutations showed that the seizure frequencies and neurodevelopmental outcomes were worst in patients carrying the p.R213Q, the p.E515D, and the p.V543M, in that order (Table 1).

To investigate the functional consequences of the p.V543M, p.E515D, and p.R213Q mutations, macroscopic currents were recorded with the whole-cell configuration of the patch-clamp technique in HEK293 cells transfected with cDNAs encoded for the wild-type or one of the mutants: c.1545G>C (p.E515D), c.1627G>A (p.V543M), and c.638G>A (p.R213Q). We analyzed the electrophysiological properties of the human wild-type mutant *KCNQ2*'s heteromeric channels transiently expressed in HEK293 cells. Representative current recordings of cells transfected with the wild-type and mutant ion channels showed that the current recordings from higher to lower were wild-type, p.V543M, p.E515D, and p.R213Q, in that order (Fig. 1). The current recording of cells was significantly lower in more HEK293 cells transfected with the p.R213Q mutant than in the other HEK293 cells (Fig. 1; right lower).

An analysis of the half-maximal activation voltage ($V_{½}$) showed that in the wild-type variant, the M current was significantly ($P < 0.05$) lower than in the p.E515D and p.R213Q variants.

The conductance-voltage curves show that the wild-type variant is electrophysiologically superior to the p.V543M, p.E515D, and p.R213Q variants, in that order (Fig. 2). $V_{½}$ is significantly different ($P < 005$) between the wild-type, p.E515D, and p.R213Q variants. The conductance-voltage curves of p.R213Q and p.E515D shifted the opening threshold to values that were more positive and slightly increased the maximal current induced by strong depolarization (Fig. 2).

DISCUSSION

We confirmed in the present study that the p.E515D, p.V543M, and p.R213Q mutations of *KCNQ2* caused functional changes correlated with clinical seizure activity and neurodevelopmental outcomes. We previously reported[15] on the p.E515D mutation, and we found the novel p.V543M mutation in the present study. The p.R213Q and p.E515D mutations changed the cell voltage conductance more than did the p.V543M mutation. This caused the patient with the p.E515D variation to have more seizures than the patient with the p.V543M variation had. The patient with the p.R213Q variation had severe functional and neurological damage. Because of all these findings, we hypothesize that *KCNQ2*-associated neonatal epilepsy is closely correlated in phenotype and genotype.

Another important finding from our functional study is compatible with the finding that the M current is more unstable in the p.E515D and p.R213Q variants than in the wild-type. We found that all six cases of BFNC from the 2 families carrying p.E515D and p.V543M were currently benign; that seizure remission usually occurred after the family members turned 3 years old; and that none of the family members had any intellectual disabilities because of their BFNC. The delineation of the course of BFNC, in addition to a family history of the *KCNQ2* mutation, is a neonatal onset, particularly during the first week of life, with symmetrical or asymmetrical general seizures, unlike focal seizures, which are relatively common in newborns.

To date, more than 60 different *KCNQ2* genotypes have been described.. Most are over the C-terminal and are missense mutations. The next most common are truncated and splice-site mutations. Most *KCNQ2* mutations are in the C-terminal, but occasionally they are found in the S4 and S5 segments and the pore region. There are more case reports from Asia, which suggest that the *KCNQ2* mutation is pan-ethnic despite

the first reports of *KCNQ2* mutations being from Europe and the USA. Recently, however, our conductance-voltage *in vitro* experiments indicated that the genotype is related to the phenotype. Although most patients with *KCNQ2* have seizures during the neonatal period, a silent *KCNQ2* SNP (rs1801545) was found overrepresented both in rolandic epilepsy and in idiopathic generalized epilepsy samples in one study.[3] The exact mechanism is still unknown. The mutations did not show any protein alterations, but they might have an influence on gene expression or channel gating. Additional *in vitro* functional studies and animal studies are required to confirm the mutation-induced seizures. The phenotype and genotype correlation is complex, including the interplay of genetic and perinatal risk and environmental factors. However, based on our findings, the genotype should be a substantial factor in the phenotype. Borgatti et al.[17] (2004) reported a mutation of *KCNQ2,* c.G1620A (p.K526N), that rendered a different phenotype, including BFNC and early-onset epileptic encephalopathy (EOEE). The mechanism is unknown, however, considered an interplay of pathogenic mutations, modifier genes, and other environmental factors.

The different phenotypes of *KCNQ2* mutations, including BFNC, EOEE, and benign infantile familial convulsion (BFIC), are probably determined by the degree of functional disability. One functional study[16] of the p.V589X, p.T359K, and p.P410fs12X variants showed that p.P410fs12X caused the most functional damage because of cell electrophysiology. The p.P410fs12X mutation caused a more severe phenotype that included hemiplegic migraine and neonatal convulsions. The p.T359K variant caused a moderate developmental delay in a 4-year-old patient. The mutation variants p.R213Q and p.R213W were located in the same codon, but each contained a different amino acid substitution, one in a Japanese family[18] and one in a western European family[9]. Each yielded distinct outcomes: p.R213W caused BFNC and p.R213Q caused a burst-suppression EEG. Parental germline mosaicism, genetic modifiers, or environmental factors are possible

explanations. However, the functional change was significantly less in the p.R213Q carrier than in the p.R213W carrier.[19] In the study, the functional change in p.R213Q was a positive control, and the results of our conductance-voltage study were similar. Taking all these findings together, we conclude that the p.R213Q mutation has a dominant negative effect on the current amplitude of homomeric wild-type and mutant *KCNQ2* constructs, which correlated with clinical seizure frequencies and neurodevelopmental outcomes.

CONCLUSION

We suggest, based on our functional test of cell conductance-voltage, that the clinical phenotype, including the seizure frequency and outcome, is associated with the genotype.

Acknowledgments

Ethical approval of the study was provided by the hospital's IRB (CS13036).

Disclosure of conflict of interest

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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FIGURE LEGENDS

Figure 1. Sample traces of the cell conductance voltage current induced in HEK293 cells transiently transfected with *KCNQ2* constructs: wild-type, the mutants p.E515D, p.V543M, and p.R213Q. Cells were clamped between −80 mV and +40 mV in 10-mV increments from a holding potential of 0 mV (wild-type [left upper], p.E515D [right upper], p.V543M [left lower], p.R213Q [right lower]). Cell conductance voltage current was lower in the p.R213Q variant than in the wild-type, p.V543M, and p.E515D variants. S indicates second.

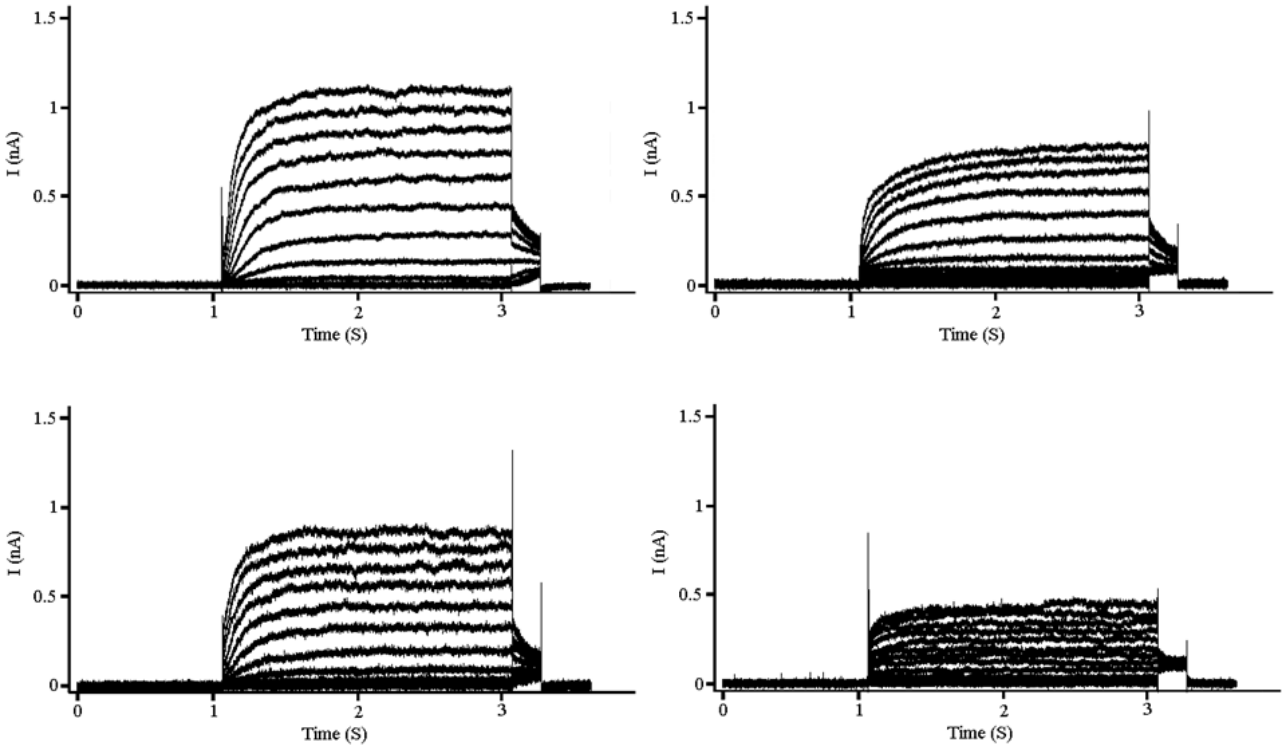

Figure 2. Current-voltage relation of the *KCNQ2*-mediated currents in the transfected cell with wild type (n=15), p.V543M (n=13), p.E515D (n=13), and p.R213Q (n=3). The transfected cells with the p.R213Q and

p.E515D mutations shifted the opening threshold to values that were more positive and increased the maximal current induced by strong depolarization. $V_{½}$ is significantly different ($P < 0.05$) between the wild-type, E515D, and R213Q.

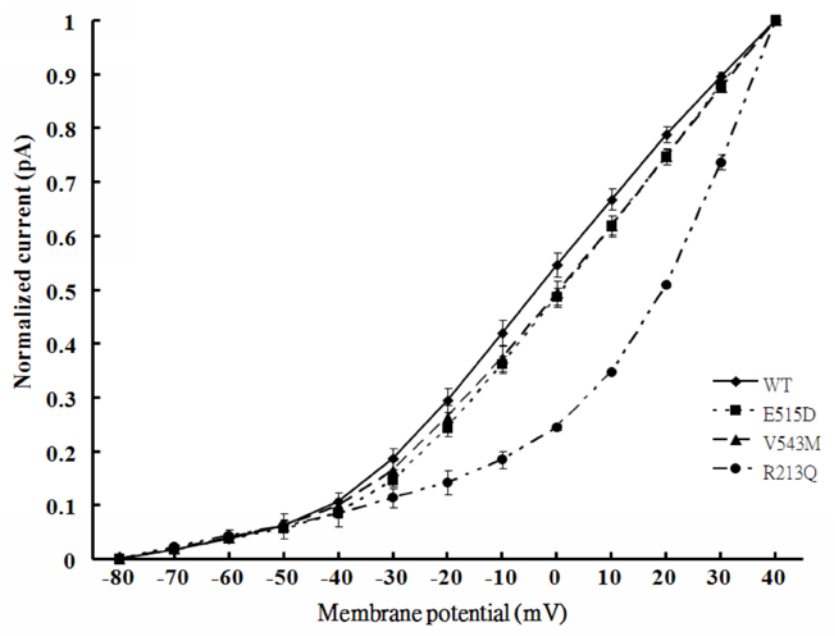

Table 1. The differences in the phenotypes of the three mutations.

Genotype	Case 1 c.1627G>A (p.V543M)	Case 2 c.1545G>C (p.E515D)	Case 3[9] c.638G>A (p.R213Q)
Functional domain	C-terminal	C-terminal	S4
First seizure day	Day 30	Day 2	Day 2
Other genetic study	*KCNQ3*	*KCNQ3*	Karyotype, *PCDH19*, *CDKL5*, array CGH
Seizure frequency before drug control	+	+++	+++
Drug control	OXC	PB, SAB	PB, PHT
Seizure frequency after drug control	-	+ (3 seizures at age of 1, 5, 6 years old)	+++
Family history	+	+	+ (father: mosaic)
Age when seizure-free	2 months	never	14 months
Seizure type	General tonic	General tonic	Apnea, generalized tonic
EEG at age of first seizure	Temporal sharp waves	Multiple high-voltage sharp/spikes in the bilateral central area	In sleep: Burst-suppression pattern
MRI	Normal	Normal	Basal ganglion hyperintensities, relatively small frontal lobe
Neurodevelopmental outcomes	Normal	Normal	Poor (macrocephaly, asymmetric spastic quadriplegia)

PHT, phenytoin; OXC, oxcarbazepine; PB, phenobarbital; SAB, vigabatrin; MRI, magnetic resonance imaging; EEG, electroencephalography

附件二

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否有嚴重損及公共利益之發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估 V 達成目標 □ 未達成目標（請說明，以 100 字為限） □ 實驗失敗 □ 因故實驗中斷 □ 其他原因 說明：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：V 已發表 V 未發表之文稿 □撰寫中 □無 專利：□已獲得 □申請中 □無 技轉：□已技轉 □洽談中 □無 其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性），如已有嚴重損及公共利益之發現，請簡述可能損及之相關程度（以 500 字為限）

在過去我們的初步研究結果，發現在不明原因癲癇兒童中，約有46%帶有*KCNQ2* 基因突變，突變的方式包括missense mutations 與silent mutation，但是尚未了解這些突變是否與癲癇疾病有關。在我們初步的報告中，異常突變點有c. 1545 G>G/C (E515D); c. 912 C>C/T (E304E); c. 2235 G>G/A(P745P); c. 2264 G>G/A (Y755C); c. 2339 A>C (N780T); c.1914C>T (P638L); 1627G>A (V543M);2338A>C (T780P)。而其中c. 912 C>C/T(E304E)在控制組正常人中約40%,顯示它是一個常見的良性基因突變(polymorphism); 另一方面在病人組突變點也包括三個新突變點c. 1545 G>G/C(E515D)、1627G>A (V543M)，與c.1294 C > T (p.R432C)，其中c. 1545 G>G/C (E515D)已由本院發表在SCI 期刊(Lee, et al., 2009)，而其他突變點在控制組正常人並未出現，因此需要做功能性分析來確定這些突變點是否會造成細胞膜電位改變。我們已初步完成正常細胞的*KCNQ2* 與未帶*KCNQ2* 基因的電位分析，顯示我們已達成實驗室操作的技術，未來將做基因突變植入細胞的功能性分析。

在 2014 年，我們初步的病人組與對照組比較，再經過SIFT、PolyPhen 軟體及功能性分析研究後，會導致的疾病的突變點包括: c. 1545 G>G/C (E515D); c. 2264 G>G/A (Y755C);c. 1627G>A (V543M);c.1294 C > T (p.R432C)。這些突變分布在8 個家族中。而c. 2264 G>G/A (Y755C)更會造成新生兒的嚴重癲癇腦病變。在功能性分析(functional study)研究報告，顯示會造成HEK293cell 電位的改變。且與疾病的嚴重度有相關性。這些初步的結果已於2014 年美國癲癇年會獲得late-breaking abstract 報告。並獲得2015年日本嬰幼兒癲癇年會最佳海報金質獎。

附件三

無

附件四

科技部補助專題研究計畫執行國際合作與移地研究心得報告

無

附件五

科技部補助專題研究計畫出席國際學術會議心得報告

日期：104 年 11 月 10 日

計畫編號	MOST 103-2314-B-040-010		
計畫名稱	KCNQ2 癲癇基因在正常兒童與不明原因癲癇兒童的基因型與功能性研究 Genotype and functional study of mutation variants of KCNQ2 in normal and idiopathic epileptic children		
出國人員姓名	李英齊	服務機構及職稱	中山醫學大學醫學系兒童學科 助理教授,兒童神經科主任
會議時間	2014 年 12 月 5 日 至 2014 年 12 月 9 日	會議地點	西雅圖, 美國
會議名稱	(中文) 2014 年美國癲癇年會 (英文) 2014, Annual Meeting of American Epilepsy Society		
發表題目	(中文) KCNQ2 突變在兒童不明因癲癇的相關性 (英文) **Mutation variants of *KCNQ2* in children epilepsy without identified causes in Taiwan**		

二、與會心得

2014 年美國癲癇年會(AES)年會於 12 月 5 日到 12 月 9 日在美國西雅圖舉行。這次參加美國癲癇年會主要目標為論文報告，並吸收癲癇一些新的發展。AES 2014 給我們帶來了多項研究和重要報告，描繪出未來癲癇治療的輪廓。包括新的藥物治療，手術方法等等。會議共舉行五天。目前發展的先一代無副作用的藥包括 bethametadine 及一些 NMDA、AMPA 的 antagonist。而對於癲癇基因檢查，新一代的次世代定序（next generation sequence）設計成 panel 加上傳統的基 因診斷，是可以考慮的方法。

會中與各國研究人員交換彼此的研究心得，也與美國 KCNQ2 協會主席 Dr. Copper 交換 KCNQ2 基因突變造成癲癇的研究心得。對於未來的研究方向有所助益。

三、發表論文全文或摘要

ABSTRACT

Background and Objectives: Non-lesional epilepsy in children is usually genetically heterogeneous. The common genetic cause includes a *KCNQ2* gene mutation The mutant gene is located at 20q13, a voltage-gated potassium-channel gene. *KCNQ2* encephalopathy with mental retardation and refractory seizures continues to be reported, which highlights that *KCNQ2* is important in children with epilepsy, whereas, the exact mechanism and phenotype of the *KCNQ2* mutation and childhood epilepsy are still unknown.

Patients and methods:

We studied the *KCNQ2* genotype from 75 non-relative patients (age range: 2 days to 18 years old), whom have non-lesional epilepsy. The study also screens *KCNQ2* from 50 healthy controls without epilepsy. We use next generation sequences in 55 patients, and direct sequence in 20 patients to screen *KCNQ2* mutation variants. *KCNQ2* mutation variants were transfected into HEK293 cell to investigate functional changes. Positive control is p. R213Q, which is known to cause neonatal seizure with suppression-bursting EEG.

Results and Conclusions:

Eight (8/75, 11%) cases were identified *KCNQ2* mutations. All mutation variants were not found in the control group, and are predicated to be deleterious by the computerbased algorithms SIFT and PolyPhen. Mutations, p.V543M and p.R431C, are novel mutations. All patients with p.E515D cause epilepsy, however, their seizures were favorable, and with 3 normal intelligence and one mild intelligent disability. Two patients carrying p.Y755C cause neonatal epilepsy with suppression-bursting EEG in one, and multiple focal spikes in another case. Functional study demonstrated that the current recordings from higher to lower were wild-type, p.V543M, p.E515D, and p.R213Q, in that order.

Conclusion: The *KCNQ2* mutation account for about 10% of idiopathic epilepsy in newborns and in children. It should be a candidate gene when diagnosing idiopathic childhood epilepsy. Functional study demostrated the genotype is heavy factor to phenotype.
(Keyword: *KCNQ2*; epilepsy; associational study)

四、建議

無

五、攜回資料名稱及內容

KCNQ2 association in USA

年會摘要與論文集

六、其他

科技部補助專題研究計畫執行國際合作與移地研究心得報告

無

附件五

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日期：104 年 11 月 10 日

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(Keyword: *KCNQ2*; epilepsy; associational study)

四、建議

無

五、攜回資料名稱及內容

KCNQ2 association in USA

年會摘要與論文集

六、其他

科技部補助計畫衍生研發成果推廣資料表

日期:2015/11/13

科技部補助計畫	計畫名稱: KCNQ2癲癇基因在正常兒童與不明原因癲癇兒童的基因型與功能性研究
	計畫主持人: 李英齊
	計畫編號: 103-2314-B-040-010- 學門領域: 小兒科

無研發成果推廣資料

103年度專題研究計畫研究成果彙整表

計畫主持人：李英齊			計畫編號：103-2314-B-040-010-				
計畫名稱：KCNQ2癲癇基因在正常兒童與不明原因癲癇兒童的基因型與功能性研究							
成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	碩士助理1位
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	2	2	100%		2014, American seizure society, USA 2015, infant seizure society, Japan, 日本嬰幼兒癲癇年會最佳海報金質獎。
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果		2015, infant seizure society, Japan, 日本嬰幼兒癲癇年會最佳海報金質獎					

（無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）	

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形：
   論文：□已發表 ■未發表之文稿 □撰寫中 □無
   專利：□已獲得 □申請中 ■無
   技轉：□已技轉 □洽談中 ■無
   其他：（以100字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500字為限）
   在過去我們的初步研究結果，發現在不明原因癲癇兒童中，約有46%帶有KCNQ2基因突變，突變的方式包括missense mutations 與silent mutation，但是尚未了解這些突變是否與癲癇疾病有關。在我們初步的報告中，異常突變點有c. 1545 G>G/C (E515D); c. 912 C>C/T (E304E); c. 2235 G>G/A(P745P); c. 2264 G>G/A (Y755C); c. 2339 A>C (N780T); c.1914C>T (P638L); 1627G>A (V543M);2338A>C (T780P)。而其中c. 912 C>C/T(E304E)在控制組正常人中約40%，顯示它是一個常見的良性基因突變(polymorphism); 另一方面在病人組突變點也包括三個新突變點c. 1545 G>G/C(E515D)、1627G>A (V543M)，與c.1294 C > T (p.R432C)，其中c. 1545 G>G/C (E515D)已由本院發表在SCI期刊(Lee, et al., 2009)，而其他突變點在控制組正常人並未出現，因此需要做功能性分析來確定這些突變點是否會造成細胞膜電位改變。我們已初步完成正常細胞的KCNQ2 與未帶KCNQ2 基因的電位分析，顯示我們已達成實驗室操作的技術，未來將做基因突變植入細胞的功能性分析。
   在 2014 年，我們初步的病人組與對照組比較，再經過SIFT、PolyPhen 軟體及功能性分析研究後，會導致的疾病的突變點包括: c. 1545 G>G/C (E515D); c. 2264 G>G/A (Y755C);c. 1627G>A (V543M);c.1294 C > T (p.R432C)。這些突變分布在8 個家族中。而c. 2264 G>G/A (Y755C)更會造成新生兒的嚴重癲癇腦病變。在功能性分析(functional study)研究報告，顯示會造成HEK293cell 電位的改變。且與疾病的嚴重度有相關性。這些初步的結果已於

2014 年美國癲癇年會獲得late-breaking abstract 報告。並獲得2015年日本嬰幼兒癲癇年會最佳海報金質獎。