A Chinese Tuberous Sclerosis Complex Family and a Novel Tuberous Sclerosis Complex-2 Mutation

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Tuberous sclerosis complex (TSC) is a relatively common autosomal dominant genetic disorder affecting 1/14,000–1/6000 Western populations. The incidence of TSC in Chinese population is still unknown although case reports of Chinese TSC patients were documented.[1] The main clinical features of TSC include seizures, mental retardation, and the development of hamartomas in multiple organs such as the skin, brain, lung, heart, and kidney. Indeed, the disease virtually manifests in every organ.[2] Two causative genes for TSC, TSC1 gene on chromosome 9q34 and TSC2 gene on chromosome16p13, have been identified in 1997 and 1993 respectively. Approximately, 70% of cases of TSC are *de novo* mutations.[3] Chinese TSC patients are more likely to have TSC2 missense and frame shift mutations. Here, we record one Chinese TSC family and it is novel frame shift mutation of TSC2.

One TSC family was identified through a proband from Sichuan province of China. The proband, a 16-year-old male, first presented with frequent focal motor seizures at age of 6 years and was clinically diagnosed as TSC at age of 9 years. Physical examination found forehead fibrous plaque, facial angiofibromas [Figure 1a], hypopigmented macules, and shagreen patches on his back. [Figure 1b]. He has shown moderate mental retardation and failed to pass any academic examinations. Electroencephalogram (EEG) revealed multifocal spike wave and spike-slow wave complex discharges. Cardiac ultrasound was normal, but renal ultrasound detected multiple small nodular hamartomas. His seizure was successfully controlled by sodium valproate and oxcarbazepine. The father of the proband is 48-years-old without epilepsy or mental retardation. However, physical examination revealed facial angiofibromas, shagreen patches on his back, and confetti-like hypopigmented patches on his legs. Renal ultrasound revealed multiple hamartomas obviously larger than the proband. The third patient of the family was the younger brother of the proband. He was born when the proband was 11-year-old without any prenatal molecular diagnosis. At the age of 1 month, he began to experience frequent focal motor seizures, which could not be controlled by the combined treatment of sodium valproate, oxcarbazepine and topiramate, and demonstrated severe mental retardation. Physical examination was almost the same as the proband. EEG revealed focal and generalized epileptiform discharges. Multiple cardiac rhabdomyoma [Figure 1c] and nodular renal hamartomas were identified by ultrasound. In all the three patients, brain computed tomography showed multiple subependymal calcifications [Figure 1d] and brain magnetic resonance imaging detected multiple cortical tubers as well as subependymal nodules along the lateral walls of the lateral ventricles [Figure 1e and f].

According to the Diagnostic Criteria of the National Institutes of Health Consensus Conference, the presence of two major features or one major and two minor features is sufficient for definitive diagnosis of TSC. In this family, each patient presented more than two major features. Therefore, all the three patients had a definite clinical diagnosis of TSC.

This study was approved without restrictions by Medical Ethics Committee of Sichuan University, and informed consents were obtained from the patients or their parents. Blood (3 ml) was drawn for Sanger sequencing from the patients, their healthy family members, and 100 unrelated normal controls. By sequencing the polymerase chain reaction fragments of the entire coding region and the exon-intron boundaries of TSC1 and TSC2, we detected a

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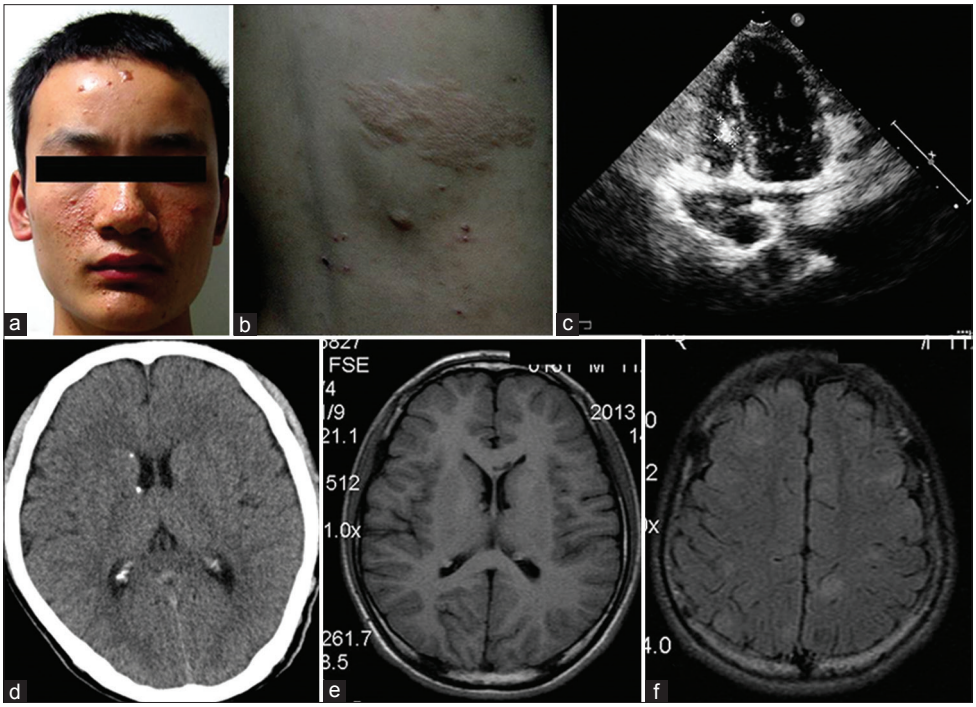

Figure 1: (a) Forehead fibrous plaque and facial angiofibromas of the proband. (b) Shagreen patches on the back of the proband. (c) Heart ultrasound of the younger brother detects multiple cardiac rhabdomyoma. (d) Brain computed tomography of the father shows multiple subependymal calcifications. (e) Brain magnetic resonance imaging (MRI) of the proband reveals subependymal nodules along the lateral walls of the lateral ventricles. (f) Brain MRI of the proband demonstrates multiple cortical tubers.

novel frame-shift mutation (c. 3576_3577insA) in the TSC2 gene exon 29 in each of the three patients [Figure 2a]. The mutation was not observed in other healthy family members and the unrelated normal controls [Figure 2b].

Tuberous sclerosis complex 1 and TSC2 genes, the pathogenic genes of TSC, are indicated as tumor suppressor genes due to their function in regulation of cell growth and differentiation by inhibiting the mammalian target of rapamycin (mTOR) in the Akt-mTOR-S6kinase cell growth pathway. Mutations including nonsense mutations, small deletions, splice site changes, missense mutations, and sometimes large deletions or rearrangements can occur over the entire regions of TSC1 and TSC2. However, no hotspots have been found.[3] In this report, the family had a frame-shift mutation (c. 3576_3577insA) in exon 29 of TSC2 gene detected by Sanger sequencing, the gold standard to detect gene mutations. The fact that this mutation was observed only in the patients of the family but not in other healthy family members and the 100 unrelated normal controls suggests that this mutation is pathogenic. This mutation cannot be found in the leiden open variation database, suggesting the sequence aberration is novel.

Gene mutations often cause essential alterations of its coded protein. The product of TSC2 gene, tuberin, which consists of 1807 amino acids, is known to have seven domains including a leucine zipper region, two small coiled-coil domains (CCD1, CCD2), a small region of similarity with GTPase-activating protein (GAPD), two transcriptional activation domains (TAD1, TAD2), and a calmodulin-binding site (CaMD) in the carboxyl terminus of tuberin. The

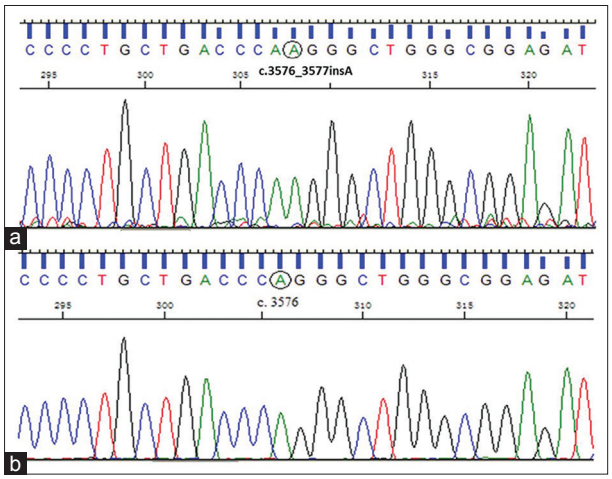

Figure 2: (a) DNA sequencing of exon 29 of the tuberous sclerosis complex (TSC2) gene finds a c. 3576_3577insA mutation in three patients. (b) Sequence of exon 29 of the TSC2 gene in normal subjects.

GAP activity of tuberinis thought to be essential for its physiological function. GAPD of tuberin activates GTPase, reducing stimulation of ras homologue enriched in brain and its downstream protein mTOR.[4] The frame-shift mutation (c. 3576_3577insA) of TSC2 in the family is predicted to truncate the TSC2 protein by 614 amino acids and add 39 amino acids, resulting in an alternative protein that lacks four of the seven domains: TAD1, GAPD, TAD2, and CaMD. The alternated tuberin could not effectively act the function of tumor suppressor gene when combined with hamartin, thus causing uncontrolled cell growth and tumorigenesis.

Tuberous sclerosis complex patients with the same genotype can have different clinical phenotypes.[5] In this family, patients are characterized by similar physical examination with different severity of symptoms. On one hand, the three patients had almost the same major features of TSC and the shagreen patches of the proband and his brother was nearly at the same position on the back. While some other features of TSC, such as non-traumatic ungula or periungual fibroma, were absent in the family. Consequently, it is probable that the same inheritable mutation of TSC has caused similar manifestations among family members. On the other hand, in each patient, the severity of the disease is completely different. The symptoms of the father are almost absent, whereas the third son, the proband's little brother with TSC, are most severe, and the proband are between them. The phenomenon of mosaicism can explain the difference in severity between the father and his children, but cannot explain that between the two children. Therefore, whether TSC caused by the same mutation would aggravate itself when transmitted to the next generation and result in severity differences between siblings needs to be further explored.

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