TSC1 and *TSC2* Gene Mutations in Chinese Tuberous Sclerosis Complex Patients Clinically Characterized by Epilepsy

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Objective: Tuberous sclerosis complex (TSC) is a multisystem disease. Variants in the *TSC1* and *TSC2* genes have been reported to be associated with TSC and are considered pathogenic. The purpose of this study was to determine the genetic mutations and expression patterns of *TSC1* and *TSC2* in 21 Chinese patients suffering from TSC who were clinically characterized by epilepsy.

Methods: Peripheral blood samples were taken from 21 patients, their parents, and other family members. Their *TSC1* and *TSC2* genes were sequenced through next-generation sequencing to identify all variants.

Results: We identified variants in 17/21 patients in either their *TSC1* or *TSC2* genes: 6 patients had *TSC1* mutations and 11 had *TSC2* mutations. There were 13 spontaneous mutations, and 3 that had been inherited from a parent. The mutations were classified by types: there were three missense mutations, five frameshift mutations, two splice site mutations, four nonsense mutations, two single codon deletions resulting the loss of an amino acid, and one large fragment deletion. Six of the mutations have not been previously reported.

Conclusion: The genotypic analysis of Chinese TSC patients who are clinically characterized by epilepsy can potentially be useful for genetic counseling and prenatal diagnoses for patients and their families.

Keywords: tuberous sclerosis complex, epilepsy, *TSC1* gene mutation, *TSC2* gene mutation

Introduction

TUBEROUS SCLEROSIS COMPLEX (TSC) is an autosomal dominant hereditary disease caused by mutations in the *TSC1* and *TSC2* genes. It is a multisystem disorder involving the heart, kidneys, lungs, and central nervous system, resulting in intellectual disability, seizures, and autism (Caban *et al.*, 2016). Recent studies estimate a frequency of 1/6000 to 1/10,000 live births (Hong *et al.*, 2016). Both *TSC1* and *TSC2* are tumor suppressor genes. The *TSC1* gene locus is located in the 9q34 region encoding hamartin, and the *TSC2* gene locus is located in the 16p13.3 region encoding tuberin (Yates *et al.*, 2011). Both proteins are widely expressed in tissues, and form a hamartin–tuberin complex *in vivo*. If mutations occur in either of these genes, the function of the hamartin–tuberin dimer will be affected; and the signal transduction pathway of the mammalian target of rapamycin (mTOR) complex 1 will be abnormally activated, resulting in the development of TSC (Van Slegtenhorst *et al.*, 1998; Li *et al.*, 2004; Henske *et al.*, 2016).

Methods

Patient demographic data

We enrolled 21 (11 males and 10 females) TSC patients in this study who were clinically characterized by epilepsy and

hospitalized in our department from 2016 to 2018. All patients had been treated with >3 drugs for a period of >10 months, and all of them were reported to suffer from epilepsy and were refractory to treatment.

Gene detection methods

The DNA isolated from peripheral blood leukocytes was fragmented, and libraries were prepared according to the recommended protocols of Illumina; the library DNA was captured using an Agilent SSELXT Human All Exon V6 kit. Enriched capture libraries were sequenced on a HiSeq X-ten or HiSeq 2500 platform (Illumina) using 150 base paired-end sequencing. The average sequencing depth of the target regions was $\times 100$, and the coverage of the target exons was 99.8%.

Data analysis consisted of filtering out the Illumina sequencing adapters and low-quality reads (<80 bp) using fastq_mcf. Postquality control, the clean reads were mapped to the UCSC GRCh38/hg38 human reference genome using BWA (Burrows Wheeler Aligner). Duplicated reads were removed using Picard Tools, and only unique mapping reads were used for variation detection. Next, single nucleotide polymorphisms (SNPs) and indel variants were detected

FIG. 1. Six unreported gene mutation sites in patients 1, 2, 3, 5, 7, and 15. The *arrows* are gene mutation sites. Color images are available online.

TABLE 1. GENE MUTATION DATA

Patient	Mutant gene	Mutation type	Exon	Base alteration	Amino acid alteration	Reported or not?	Source	Pathogenicity
1	<i>TSC2</i>	Splice site	Intron40	c.5160+2T>C	No alteration	No	Novel	Pathogenicity
2	<i>TSC2</i>	Nonsense	Exon25	c.2771_2772del	p.Phe924*	No	Unknown	Pathogenicity
3	<i>TSC2</i>	Missense	Exon18	c.1880A>C	p.His627Pro	No	Mother	Pathogenicity
4	<i>TSC2</i>	Missense	Exon24	c.2713C>T	p.Arg905Trp	Yes	Novel	Pathogenicity
5	<i>TSC2</i>	Frameshift	Exon23	c.2634del	p.Ser879Profs*15	No	Novel	Pathogenicity
6	<i>TSC2</i>	Amino acid deletion	Exon38	c.4912_4914del	p.Lys1638del	Yes	Novel	Pathogenicity
7	<i>TSC2</i>	Amino acid deletion	Exon34	c.4452_4454del	p.Thr1485del	No	Father	Pathogenicity
8	<i>TSC2</i>	Missense	Exon27	c.3095G>C	p.Arg1032Pro	Yes	Novel	Pathogenicity
9	<i>TSC2</i>	Frameshift	Exon34	c.4258_4261del	p.Ser1420Glyfs*55	Yes	Novel	Pathogenicity
10	<i>TSC2</i>	Large fragment deletion	Exon10–16	Exon10–16 del	exon10–16 del	Yes	Novel	Pathogenicity
11	<i>TSC2</i>	Nonsense	Exon8	c.684C>A	p.Tyr228*	Yes	Novel	Pathogenicity
12	<i>TSC1</i>	Splice site	Intron9	c.913+1G>A	No alteration	Yes	Novel	Pathogenicity
13	<i>TSC1</i>	Nonsense	Exon21	c.2692C>T	p.Gln898*	Yes	Novel	Pathogenicity
14	<i>TSC1</i>	Frameshift	Exon18	c.2356_2359del	p.Glu787Asnfs*19	Yes	Novel	Pathogenicity
15	<i>TSC1</i>	Frameshift	Exon4	c.127_128dup	p.Asn43Lysfs*20	No	Novel	Pathogenicity
16	<i>TSC1</i>	Frameshift	Exon10	c.989dup	p.Ser331Glyfs*10	Yes	Novel	Pathogenicity
17	<i>TSC1</i>	Nonsense	Exon17	c.2074C>T	p.Arg692*	Yes	Mother	Pathogenicity
18			Not found					
19			Not found					
20			Not found					
21			Not found					

TSC, tuberous sclerosis complex.

with the GATK HaplotypeCaller. Then, the GATK Variant Filtration tool was utilized to filter the variants. The filtering standards were as follows: (1) variants with mapping qualities <30; (2) the total mapping quality zero reads <4; (3) approximate read depth <5; (4) QUAL <50.0; (5) phredscaled *p*-value using Fisher's exact test to detect strand bias >10.0. After these two steps, the data were transformed to a variant call format, and variants were further annotated by ANNOVAR; in addition, they were run against multiple databases, including the 1000 genomes project, ESP6500, dbSNP, EXAC, and HGMD. Variant functions were predicted by SIFT, PolyPhen-2, MutationTaster, and GERP++. Of all the mutations, we focused on mutations that ACMG (American College of Medical Genetics) identified as pathogenic, likely pathogenic, and variant of undetermined significance.

Results

The *TSC* gene was sequenced for all 21 patients enrolled in the study, and mutations in one or the other of the *TSC* genes were found in 17 patients. Six subjects had *TSC1* gene mutations, which accounts for 28.6%, 11 subjects had *TSC2* gene mutations, which accounts for 52.4%, and 4 patients had no *TSC* gene mutations identified accounting for 19.0%. Among all of the variants detected, 13 were spontaneous (76.5%), 3 patients inherited their mutations from 1 of their parents, and 1 patient had an unknown source as no blood samples were taken from his parents. The *TSC2* gene mutations in eight of the patients were spontaneous, two were inherited, and one was undeter-

mined as to origin. The *TSC1* gene mutations in five of the patients were spontaneous, and one was inherited from a parent.

In this study, we found six novel mutation sites (Fig. 1) accounting for 35.3% of all *TSC* mutations characterized in this study, among which five were in the *TSC2* gene: c.5160+

FIG. 2. MRI manifestation. MRI showing manifestations in patient 3 with high signal in left central tectum on T2 flair. MRI, magnetic resonance imaging.

2T>C, no amino acid alteration; c.2771_2772del, p.Phe924*; c.1880A>C, p.His627Pro; c.2634del, p.Ser879Profs*15; c.4452_4454del, p.Thr1485del; and one in the *TSC1* gene: c.127_128dup, p.Asn43Lysfs*20.

The likely pathogenic variants included five frameshift mutations (29.4%), three missense mutations (17.6%), four nonsense mutations (23.5%), two splice site mutations (11.8%), two codon (single amino acid) deletions (11.8%), and one large fragment deletion of multiple exons (5.9%) (Table 1).

DNA sequencing of the causative mutations for the probands resulted in improved diagnoses and genetic counseling for the patients' families in some cases. Patient 3 had a family history of TSC. The patient's brother had a history of epilepsy, for which he received antiepileptic treatment, but had not been diagnosed with TSC despite the fact that he had white spots and a coffee spot on his skin (Fig. 2). Blood samples from other family members showed that the proband's grandfather, mother, and niece had the same mutation in their *TSC2* gene; interestingly, none of them suffered from epilepsy.

Discussion

Epilepsy is a significant cause of morbidity and mortality (Jeong and Wong, 2016), and one of the most common neurological symptoms in patients with TSC, with a reported prevalence from 62% to 93% (Krueger, 2013). The gain of function associated with mutations in the *TSC1*/*TSC2* proteins, which are directly involved in mTOR regulation, affects many functions during development and maturation, including cell growth and proliferation, energy metabolism, inflammation, and synaptic plasticity (Yasin *et al.*, 2013). Such a hyperactivation of the mTOR pathway generates dysmorphic neurons that are as "epileptic generators" in TSC (Kannan *et al.*, 2016). Whether the mutations we reported are more likely to cause refractory epilepsy is uncertain.

Spontaneous mutations in the *TSC* genes were responsible for 76.5% of the TSC cases in this study; this is similar to previous reports (Sancak *et al.*, 2005). Therefore, we conclude that spontaneous mutations are the principal driver of new cases. Among the TSC patients who had no reported familial history, a genetic analysis can aid in making definite diagnoses both pre- and antenatally (Yates, 2006; Schwartz *et al.*, 2007).

Previous studies have reported that the average rate of TSC patients with no *TSC* gene mutations was 14.4% (Nabbout *et al.*, 2018); our findings of 19% are consistent with this. Among all gene mutations, frameshift mutations were the most common type in our study, accounting for 29.4%.

It was reported that *TSC2* gene mutations are more common than *TSC1* gene mutations (Peron *et al.*, 2018a, 2018b) and that they are associated with an increased risk of seizures (Dabora *et al.*, 2001). The fact that our patients had intractable epilepsy may partially account for our finding more *TSC2* gene mutations than *TSC1* gene mutations in our cohort.

This study is not without its limitations. First, the protein changes predicted to be encoded by the gene and the expression of individual mutant genes were not analyzed. In future studies we plan to further study protein function. In

addition, this study had a relatively small sample size and, therefore, cannot fully represent the characteristics of TSC among the Chinese people.

Conclusion

In this study, the majority of the mutations identified in the *TSC* genes were spontaneous. For suspected TSC patients, gene detection can be performed before epileptic seizures occur to achieve an early and definite diagnosis. This study provides important data for family-based diagnoses and genetic counseling for TSC.

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Author Disclosure Statement

No competing financial interests exist.

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