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Case Report

A novel *TSC1* mutation (c.1964delA) in a Chinese patient with tuberous sclerosis complex

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ABSTRACT. Tuberous sclerosis complex is an autosomal-dominant heritable disease caused by mutations in the *TSC1* and *TSC2* genes. We studied a Chinese patient with sporadic tuberous sclerosis complex. The clinical features of this patient included epilepsy, hypomelanotic macules and angiofibromas on his back; a cranial CT scan showed subependymal nodules along the lateral walls of the lateral ventricles. The *TSC1* and *TSC2* genes were studied by PCR and direct sequencing of the entire coding region and exon-intron boundaries of these genes. A novel deletion mutation (c.1964delA) in the *TSC1* gene exon 15 was identified, which was not present in his parents or 100 unrelated normal controls. This is the first report of this c.1964delA mutation of the *TSC1* gene, associated with tuberous sclerosis complex, expanding the spectrum of *TSC1* mutations that cause this disease.

Key words: Tuberous sclerosis complex; Mutation; *TSC1*; Gene

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INTRODUCTION

Tuberous sclerosis complex (TSC; MIM #191100 and MIM #613254), affecting between 1/6000 and 1/10,000 individuals, is an autosomal dominant disorder. The main clinical features of TSC include seizures, mental retardation and the development of hamartomas in a variety of organs and tissues such as the skin, brain, heart, kidney (Sasongko et al., 2008; Mozaffari et al., 2009; Orlova and Crino, 2010). Two causative genes for TSC, *TSC1* gene and *TSC2* gene, have been identified, and over 900 mutations in these genes have been found (Napolioni and Curatolo, 2008; http://www.hgmd.cf.ac.uk/ac/gene.php?gene=TSC1; http://www.hgmd.cf.ac.uk/ac/gene.php?gene=TSC2), which include a broad range of small insertions and deletions, single-base substitutions creating nonsense codons, missense changes, or splicing mutations, and larger genomic deletions (Napolioni and Curatolo, 2008). However, mutational screening of the *TSC1* and *TSC2* genes is still far from the saturation point, and more novel mutations will be identified to enhance our insight into the molecular basis for the pathogenesis of TSC. Here, a novel mutation in the *TSC1* gene was detected in a Chinese patient with TSC.

SUBJECTS AND METHODS

This study was approved without restrictions by the Research Ethics Committee of Qilu Hospital, and authorized by all individuals and the patient.

Patient

A patient with sporadic TSC is a 12-year-old male. He received medical attention for frequent seizures in September 2009, and within the last three months, he experienced seizures four times. Upon physical examination, his height was 148.3 cm, and weight was 39 kg, hypomelanotic macules and angiofibromas were present on his back (Figure 1A). The electroencephalogram revealed a spike wave, spike-slow wave complex in the left parietal region, right prefrontal region and posterior temporal region. He also had subependymal nodules along the lateral walls of the lateral ventricles on a computed-tomography scan of his brain (Figure 1B). This patient had a clinical diagnosis of TSC, as defined by the Tuberous Sclerosis Consensus Conference (Roach et al., 1998). His parents and other family members were not affected by TSC.

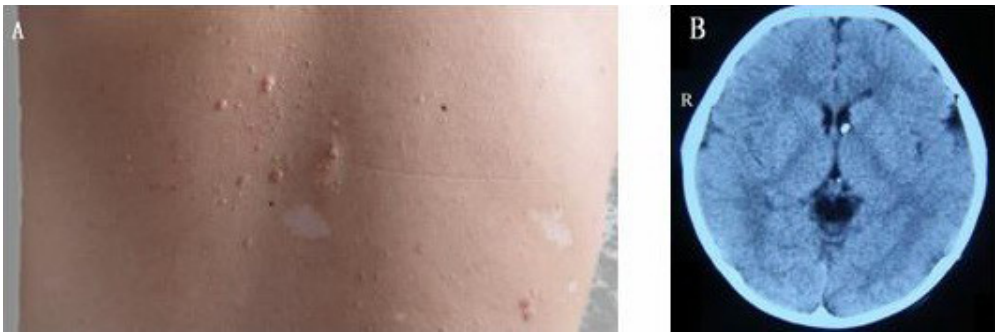

Figure 1. Major features of the patient with tuberous sclerosis complex. **A.** Hypomelanotic macules, angiofibromas on the patient's back. **B**. Cranial CT scan: subependymal nodule along the lateral walls of the lateral ventricles.

Genetic analysis

Blood (3 mL) was drawn from the patient, his parents and one hundred unrelated normal controls. Genomic DNA was extracted from whole blood using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, Co. Ltd., China). Genomic DNA was amplified by polymerase chain reaction (PCR) with 25 pairs of PCR primers for exons 1 to 23 of the *TSC1* gene and 36 pairs of PCR primers for exons 0 to 41 of the *TSC2* gene; the primers were designed based on published sequence data (GenBank accession Nos. NP_000359.1 and NP_000539.2) and spanned the entire coding region and exon-intron boundaries of these genes. The sequences of the primers used in this study are shown in Tables 1 and 2.

Table 1. Primers used for polymerase chain reaction, sequencing reactions and amplification conditions for the *TSC1* gene.

Exons	Sense (5'-3')	Antisense (5'-3')	Fragment size (bp)	Annealing (°C)
1	GTCCAACCCACATCGTCAG	CGGAGATAGCGTGTAATAAGAG	424	56
2	AATGTAGACGGGGAGAGGC	GCTGTGAAGAAGAAAGTAACGC	463	56
3	GAAACCCTCTTCATAAACTCG	GTGGCTCTAAAGTCAATCTCT	455	52
4	TGGATTTTGTGACAGGAAGC	TGGGAACAATGTCATCAGTG	320	52
5	GAGATTGGAGCACATCATTG	CATTCAAATCCTTACAAACATC	265	52
6	GGTGTGTTGTAAATGGTCCT	TATTCCCTCATCTGTCTGGT	521	53
7	CTCCTCAATCTGTCTCCAAC	CCAGTCACAAAAGACCACAT	439	54
8	GCCACTAATCTGTTTTCTGTC	CAAGTGGACTGATTCTGTAAG	441	52
9	GCTATCAGAGTTCCGTGGCT	CAATCAGGTAGCAGACCAAGG	484	57
10	TTGTCCCCAAGGATGTCAGT	AGTGGCAAAGGAATGCTAAG	600	54
11	CTGTTGCCTCTTAGATTCAT	CAAGTTTACAACAGCAAGTG	407	50
12	CTGGGGCTTTCAGAATGTGT	GCAGAGGGATAGCAGACGAG	334	56
13	AACTACTTTACAATATGCTC	GGGGATACTACAAAAGACTG	473	56
14	TATCCCCAGGACAGAGCCAT	AGCAGAGCGAGGGTCAGGTT	301	58
15-1	ATGCCACCCAAACTGCCTAG	AGGCTTGCTTTGGTGTGTCAG	292	57
15-2	TACACTCCTCCCTGGACAAG	CTGACAAACAGCAGAGAACC	523	55
16	AGGCTTCTCCTCTAACTCTC	AATGCTGACTTGGCAACACT	378	54
17	AGGGGGCTTGATTGAACCAT	AACTCTGACCTCCTCGGCTG	328	57
18	TGAAATGTTCGCAGTGTGTG	AAAGTTGGGGAACCTCTGTC	449	54
19	AAGAAAGTAGAGCCGTTGAG	TCTGAAGGAAGAATGTTAGC	294	52
20	CAGGTGTTCTGTGGGTGGTC	TGTCTGGGTCTGAAACGCTT	450	56
21	GCCTTCTCAGTCCTTCTTAC	AGATACAGACCAGCCAGAAT	358	53
22	GGCAAGGTAACTTTCATCAG	CGTGACACAGTCCTTATGCT	314	54
23-1	GACTAGACAGGCTGCAACAC	CTGGCTCTCGCTCTTATTAC	671	57
23-2	CAGAGAAACCCCCACACCAG	AATGCCAGATCCAAAAACCGT	528	56

PCR cycling was performed on a DNA thermal cycler (Gene Amp 9700, Perkin-Elmer, USA) with 2X Hotstart Taq PCR Mastermix kit (Tiangen Biotech). In a 50-µL reaction mix, 300 ng genomic DNA was used with 2.0 µL of each primer (10 µM), and 25 µL 2X PCR Mastermix. Genomic DNA was first denatured at 94°C for 3 min, followed by 31 cycles of 94°C for 35 s, 50° to 62°C for 35 s, and 72°C for 50 s. The PCR products were extended at 72°C for 5 min. The products were gel-purified with an agarose gel DNA purification kit (Tiangen Biotech), and the purified PCR products were sequenced using the forward and reverse primers. Automated sequencing was performed according to our previous approach (Wang et al., 2010). Every mutation was confirmed by sequencing of the products from several independent PCRs. After a mutation within an exon was detected in the patient, this exon was amplified by PCR specifically in his parents and one hundred unrelated normal controls.

Table 2. Primers used for polymerase chain reaction, sequencing reactions and amplification conditions for the *TSC2* gene.

Exons	Sense (5'-3')	Antisense (5'-3')	Fragment size (bp)	Annealing (°C)
0	GCGGCACAGAACTACAACTC	TAAAACTAAGGGATGCTCGG	301	56
1	AATGTAGACGGGGAGAGGC	CTGAACCAGGTCACCAAGAT	508	56
2	GCGGCTCGTCAAGTGAATCT	GTGAGCCAAGATTGTGCCAG	421	56
3	GAGACACAGGAGATACGAGC	CCCAGAACATCCCATAAGAG	509	56
4	GGTCCCGACACACAGCAGT	CCACCTCCCAGTGACAGAAC	676	59
5	GACTGATGATGGGGTTTCTG	CAACTTTATTCACTGCGGAG	305	54
6	GTTGTTTGTTTAGAATGTTGC	CGGCTATCCTGACTTACAT	441	51
7	CAGCATCAATGACCCACAGT	CACCCCAAGAATCAGACAAC	441	55
8	GTCATTTTCCCAGGCAGTTG	TAAGTAGTTGGGGAGCACCG	546	56
9	CGCAGGAGTGAACAAGAGTG	CCAAGCAGAAAGAGCAGAAC	528	56
10	GGGGGATGTTGTCTTTGTGC	GGTGGAAGGTGGATGAGCG	646	58
11	CGGTGGGTGTGTAGCGAG	AGGTGGGAGGAAACTGATGG	432	58
12	CTTTGGGCTTTGGCTGGTG	TCATCGGGCATCCTGAGAC	661	58
13	CTCTTTTCGGGGGTCGTCT	GTGCCTTCCTTGCTACTTGG	481	57
14	ATTGGAAGTGTCACGAGATG	GATGCCCTTTATACCCGAGT	603	54
15	TCAAGACCATCCTGGCTAAC	AACAGACTCCAACACAACGC	513	55
16	GCACGAGGTTGGGTTTTACT	CAGGCAGAGGGAAGGACAC	492	57
17	TTTCTGAGTGCCTGTGGTGC	CAGCAGGAACGGAACAGACT	342	57
18	GTCTGTTCCGTTCCTGCTGC	CAGCGTCAAGGGCTATGGAG	424	59
19	CTGTCCTGACGCCTCCTCTC	TTTGGGGAAAAACCCTACTG	205	58
20	ACTCCCACCACTCCGAAAG	CAGAGCCAACTCACTCATCC	429	57
21	CAGAACCAGGGGATGAGAGC	AGAGAGGGCACCAGAGAAGC	526	59
22-23	GGCTCCCCTGACCACCCTCT	CCCCAAACACCCTCCCACTG	523	61
24	AGGCTGTGTCTCTCGGTAGG	CACAGGACCCATTTCCACTC	504	58
25	CTGTCTCTTCCCCGCTAACT	AACCAGCCCGTGCTCATAC	501	57
26-27	GGGTCTTTCCGAGCGAGGTC	GTCCCCAGGCTGGTACGAGG	607	62
28	ACGTGGCACCCTCGTACCAG	GAAGGCTCACCCCAGAGTCAG	322	61
29	GCATCAGGTAAGTGGTGGTC	GGGTGACTGGCAGAAAGATG	304	57
30	CAGAGATGGGTAAGGGGAGGT	AGGAGCCACATTGCCGTCAC	338	59
31	CACTCGGCACCGTGCTTCT	GTGGGCTTCCCCCTAAACAG	684	59
32	GTGGATGGCAGCAGTAAGCAG	CTAAGGAGCCCCCCGAGGT	317	62
33	GGGATGGAGGACAGATAGG	AGCCCACAGGGAGGAACAC	768	58
34-35	GAACCTGGTGCCTCACTTGC	CCACGCTAACCTGTCACTCG	760	61
36	GCTGCTGGAATGGATGGTCT	CCAGTGGTCCTCGGCTCTC	548	58
37	CTCCCATCCAGTCCTGCTAC	GCCAGTTACTCCTGACAGACAC	608	60
38-40	CAACCAGGCAGTAGCCGAGA	GCTGAGGGAGCCCCATATTC	562	60
41	ACGCCTCCCAGACTTAG/CTGC	GCATCAGGTCTGGGCAAGTAG	602	60

RESULTS

Extraction of genomic DNA and PCR of exons 1 to 23 of the *TSC1* gene and exons 0 to 41 of the *TSC2* gene

OD_{260}/OD_{280} of genomic DNA from blood specimens was 1.7~1.9, which meant that the genomic DNA was suitable for use as a template for PCR. PCR fragments of exons 1 to 23 of the *TSC1* gene and exons 0 to 41 of the *TSC2* gene were checked by electrophoresis on 20 g/L agarose gels, and the gels were photographed, as shown in Figures 2 and 3.

Sequence analysis

By sequencing the PCR fragments of exons 1 to 23 of the *TSC1* gene and exons 0 to 41 of the *TSC2* gene, we detected a deletion mutation (c.1964delA) in the *TSC1* gene exon 15 in the patient with TSC (Figure 4). This mutation in the cDNA at position 1964 truncated the predicted TSC1 protein by 510 amino acids. There is no such sequence variant at that posi-

tion in his unaffected parents and one hundred unrelated normal controls, suggesting that the sequence aberration is pathogenic.

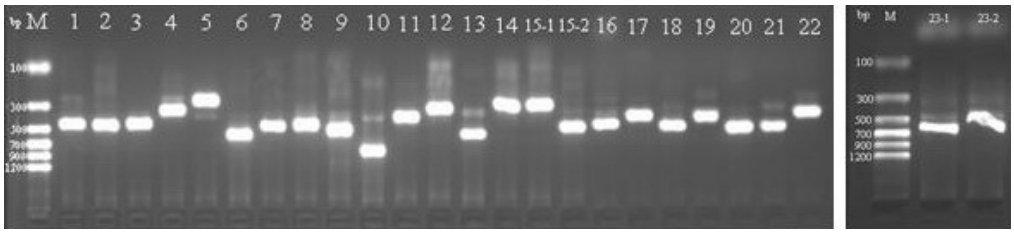

Figure 2. Electrophoresis of PCR fragments of exons 1 to 23 of the *TSC1* gene. *Lane M* = DNA marker. *Lanes 1* to *23-2* = PCR fragments of exons 1 to 23, respectively.

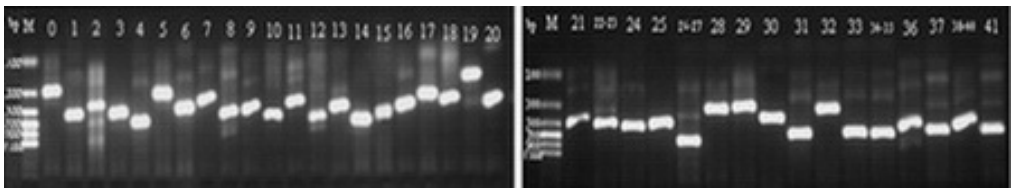

Figure 3. Electrophoresis of PCR fragments of exons 0 to 41 of the *TSC2* gene. *Lane M* = DNA marker. *Lanes 0* to *41* = PCR fragments of exons 0 to 41, respectively.

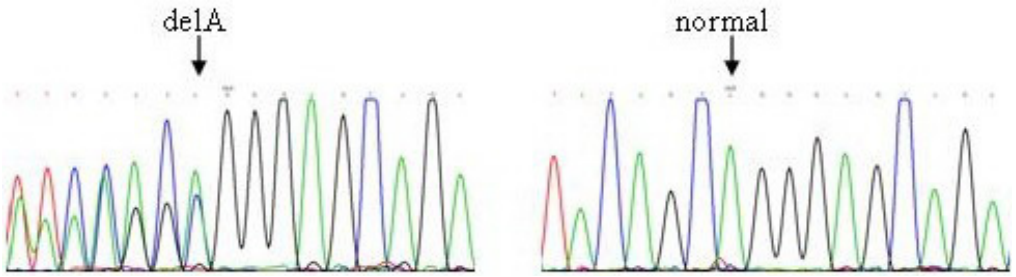

Figure 4. Sequencing of exon 15 of the *TSC1* gene showed a deletion mutation in c.1964.

DISCUSSION

TSC is characterized by hamartomas in multiple organ systems, including the brain, skin, heart, kidneys, and lung. Central nervous system manifestations include epilepsy, autism, learning difficulties, and behavioral problems. Renal lesions, usually angiomyolipomas, can cause renal failure. Skin lesions include melanotic macules, facial angiofibromas, and patches of connective tissue nevi. The diagnosis of TSC is made based on the above clinical manifestations that are categorized into major and minor features. The presence of 2 major features, or one major and 2 minor features, is sufficient for a definitive diagnosis (Roach et al., 1998). In this research, the patient suffered from 3 major features: hypomelanotic macules, angiofibromas and subependymal nodule, and thus the diagnosis for TSC is definite.

The genetic basis of TSC has been determined to be heterogeneous with two unlinked causative genes, *TSC1* gene and *TSC2* gene (Povey et al., 1994; van Slegtenhorst et al., 1997). The human *TSC1* gene on chromosome 9q34 consists of 23 exons and encodes a 130-kDa protein, TSC1 (or hamartin), and this protein spans 1164 amino acids (van Slegtenhorst et al., 1997). TSC1 consists of the following regions: a putative transmembrane domain at amino acids 127-144; a coiled coil domain spanning amino acids 719-998, which is necessary for protein-protein interactions between the TSC1 and TSC2 proteins; a Rho-activating domain at amino acid residues 145-510 for activation of Rho GTPase, and amino acid residues 881-1084 that interact with the N-terminal of the ezrin-radixin-moezin (ERM) family of actin-binding proteins (Napolioni and Curatolo, 2008). The *TSC2* gene, which is located on chromosome 16p13.3, contains 41 exons and encodes a 200-kDa protein with 1807 amino acid (Povey et al., 1994); this protein, which is called TSC2 or tuberin, contains a calmodulin-binding domain and an estrogen receptor-a-binding domain (Napolioni and Curatolo, 2008). TSC1 and TSC2 form a complex that activates the GTPase activity of rheb, preventing the rheb-GTP-dependent stimulation of cell proliferation, adhesion, growth, differentiation, or migration through mTOR (Garami et al., 2003; Hung et al., 2006). The molecular basis of TSC is a functional impairment of the TSC1-TSC2 complex; TSC1 in the complex is required for maintaining the stability, activity and correct intracellular localization of the complex (Huang and Manning, 2008; Mozaffari et al., 2009).

The mutation spectra of the TSC genes are very heterogeneous. Up to now, more than unique 200 *TSC1* gene variants and 700 unique *TSC2* gene variants have been reported (Napolioni and Curatolo, 2008; http://www.hgmd.cf.ac.uk/ac/gene.php?gene=TSC1; http://www.hgmd.cf.ac.uk/ac/gene.php?gene=TSC2). Approximately 10 to 30% of cases of TSC are due to mutations in the *TSC1* gene (van Slegtenhorst et al., 1997). In this study, we screened all coding regions and exon-intron boundaries of the *TSC1* and *TSC2* genes. A 1-bp deletion (c.1964delA) was identified in the *TSC1* gene exon 15, and the resulting frameshift would truncate the predicted TSC1 protein by 510 amino acids, and added a novel sequence of 9 amino acids, ending in a new stop codon. After searching the SNP database and the human gene mutation database, we found that c.1964delA is absent from the two databases. Unaffected parents of the patient and one hundred unrelated normal controls did not carry the same mutation, suggesting that the deletion mutation is pathogenic. Therefore, we demonstrated that the present patient carries a novel deletion mutation.

Because the deletion mutation (c.1964delA) in the *TSC1* gene exon 15 is predicted to produce a truncated protein product with the coiled-coil domain lost, the TSC1/TSC2 complex fails to form, leading to uncontrolled cell growth and cell proliferation and consequent malformations consisting of disorganized arrangement of tissue types, such as hypomelanotic macules, angiofibromas and subependymal nodules.

In conclusion, we identified a novel mutation, c.1964delA, in the *TSC1* gene in a Chinese patient with TSC. This mutation is predicted to produce a truncated TSC1 protein that is unable to interact with the TSC2 protein, which impairs the inhibitory function of the TSC1/TSC2 complex, leading to phosphorylation of the downstream effectors of mTOR, and thereby resulting in uncontrolled cell growth and tumorigenesis. The identification of the c.1964delA mutation in this Chinese patient further expands the clinical phenotypes and mutation spectrum of TSC, and will contribute to prenatal molecular diagnosis and preimplantation genetic diagnosis.

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