Two Novel Gross Deletions of TSC2 in Malaysian Patients with Tuberous Sclerosis Complex and TSC2/PKD1 Contiguous Deletion Syndrome

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Tuberous sclerosis complex is an autosomal dominant neurocutaneous disorder affecting multiple organs. Tuberous sclerosis complex is caused by mutation in either one of the two disease-causing genes, TSC1 or TSC2, encoding for hamartin and tuberin, respectively. TSC2/PKD1 contiguous gene deletion syndrome is a very rare condition due to deletion involving both TSC2 and PKD1 genes. Tuberous sclerosis complex cannot be easily diagnosed since there is no pathognomonic feature, although there are consensus diagnostic criteria for that. Mutation analysis is useful and plays important roles. We report here two novel gross deletions of TSC2 gene in Malay patients with tuberous sclerosis complex and TSC2/PKD1 contiguous gene deletion syndrome, respectively.

Key words: Tuberous sclerosis complex – TSC2 – PKDTS – TSC2/PKD1 Contiguous gene deletion syndrome

INTRODUCTION

Tuberous sclerosis complex (TSC; OMIM #191100 and #613254) is an autosomal dominant neurocutaneous disorder affecting multiple organs. The most common affected organs include brain, heart, skin, eyes, kidneys, lungs and liver. It is characterized by a broad phenotypic spectrum including seizures, mental retardation, renal dysfunction, dermatological abnormalities and tumors. The clinical manifestations of TSC vary individually. Some patients may suffer from chronic symptoms while others may have mild symptoms and can even appear asymptomatic (1). TSC is caused by mutation in either one of the two disease-causing genes, TSC1 (tuberous sclerosis complex 1; chromosome 9q34; Gene ID 7248; RefSeq NC_000009.11) or TSC2 (tuberous sclerosis complex 2; chromosome 16p13.3; Gene ID 7249; RefSeq NC_000016.9), encoding for hamartin and tuberin, respectively (2,3).

TSC2/PKD1 contiguous gene deletion syndrome (PKDTS; OMIM #600273) was first reported after the discovery of a patient with deletion involving both TSC2 and PKD1 (polycystic kidney disease 1) genes (chromosome 16p13.3; Gene
ID 5310; RefSeq NC_000016.9). Subsequently, similar finding has been reported in a number of TSC patients who had presented during early infancy with severe polycystic kidneys (4).

TSC cannot be easily diagnosed since there is no pathognomonic feature (1), although there are consensus diagnostic criteria for that (5,6). Mutation analysis is useful and plays several important roles. First, the new diagnostic criteria recognize that the identification of either a TSC1 or TSC2 pathogenic mutation in DNA from normal tissue is sufficient to make a definite diagnosis of TSC, (5) which is relevant especially in young patients in whom many clinical features have yet to develop. Second, mutation analysis of the patient’s family members may also provide reassurance that other members of the family do not carry the mutation, except in the rare case of mosaicism among unaffected family members (7). Third, mutation analysis enables prenatal genetic diagnosis in families with either a child or a parent with a known mutation (8). We report here two novel gross deletions of TSC2 gene in Malay patients with TSC and PKDTS, respectively.

**PATIENTS AND METHODS**

Patients were diagnosed based on the diagnostic criteria for TSC (5,9). Informed consent was obtained from the patient’s parents prior to blood taking.

**PATIENT 1**

Three-generation family pedigree of Patient 1 is shown in Figure 1. Patient 1 is a Malay boy, aged 5 years old, who was born at term gestation weighing 2.8 kg. Cardiac masses were incidentally found during an antepartum fetal ultrasound. The postnatal echocardiography demonstrated bilateral ventricular cardiac rhabdomyoma. Hypopigmented patches were found over his trunk and chest during newborn examination. During the subsequent investigations and follow-up, he showed many features of TSC which included seven major features: facial angiofibroma, forehead plaque, hypomelanotic macules, cortical tuber, subependymal nodule, multiple retinal nodular hamartomas and cardiac rhabdomyoma. These were accompanied by two minor features, cerebral white matter radial ‘migration tracts’ and multiple renal cysts. He developed seizures at 9 months. Electroencephalography (EEG) recording showed frequent inter-ictal discharges over the right posterior temporal regions which were more prominent during sleep. Despite sodium valproate therapy, he experienced brief breakthrough seizures once a month. He walked unaided at 2 years old. At 5 years old, he was able to speak in short sentences. His parents were non-consanguineous. He has 5 healthy siblings. His mother, who is currently 40 years old, is taking two anticonvulsants for epilepsy that has started since 14 years old. She received laser therapy for facial angiofibroma in the past but did not have other cutaneous features of TSC. There was no renal ultrasound or neuroimaging available for her. His father is healthy. Neither the father nor the siblings underwent any genetic testing for TSC.

**PATIENT 2**

Three-generation family pedigree of Patient 2 is shown in Figure 2. Patient 2 is a Malay girl, aged 1 year and 8 months, who presented at 2 months old with infantile spasms. The latter was difficult to control despite multiple anticonvulsants that included sodium valproate, phenobarbital and clonazepam. Her EEG showed a normal background activity with intermittent multifocal epileptiform discharges. Her brain computed tomography (CT) scan revealed cortical tuber over the left frontal lobe and multiple small subependymal nodules along both lateral ventricle walls as well as both Foramen Monroes. Subsequent brain magnetic resonance imaging (MRI) scan revealed left subependymal giant cell astrocytoma, multiple subependymal hamartomas, cortical, subcortical and white matters tubers (Fig. 3). She has normal echocardiogram finding. Clinically, she has multiple hypomelanotic macules and hypertension, which upon investigations was found to be related to polycystic kidneys. Her blood pressure was well controlled with nifedipine. She has normal renal function. Both her parents and her only other older siblings were clinically unaffected and no genetic testing was done on them.

Both patients were diagnosed as definite cases of TSC, based on the diagnostic criteria (5,9).
METHODS

Genomic DNA of the patients was extracted from whole blood using commercially available kit (QIAGEN). The DNA was then subjected to SALSA MLPA P124-C1 for TSC1 analysis and SALSA MLPA P046-C1 TSC2 for TSC2 analysis (MRC-Holland). Results obtained were analyzed in Coffalyser.Net software. Figure 4 described the positions of multiplex ligation-dependent probe amplification (MLPA) ligation sites of TSC2 exon 41 and PKD1 exon 46 that provide insights into tail-to-tail arrangement of the two genes.

RESULTS

Mutation analyses using MLPA identified two different gross deletions of TSC2 gene in each patient.

In Patient 1, a gross deletion involved a loss of six consecutive exons, starting from exon 26 until exon 31 of TSC2 gene. This mutation is designated as g.del_ex26-ex31; c.2970-3886del917bp;p.S990R-FsX36. The deletion spanned a coding region of 917 bp in length (Fig. 4). MLPA test done on the mother, however, could not identify any exonic deletion of TSC2 as was identified in the patient.

In Patient 2, a gross deletion of 10 consecutive last exons of TSC2 was identified. The deletion is designated as g.del_ex32-41; c.3887-5404del1516bp;p.A1295X, starting from exon 32 until exon 41 of TSC2 gene and spanned ~1.5 kb in length. Our MLPA analyses also found that the deletion is contiguous into the adjacent PKD1 gene, involving at least the gene’s exon 46 (Fig. 5).

DISCUSSION

We reported here two cases of novel gross deletions in the TSC2 gene of Malaysian patients with TSC, one involved exons 26–31 and another one involved exons 32–41 which is...
contiguous into adjacent PKD1. Both mutations have never been reported before (10).

We postulated that both TSC2 mutations are the cause of the TSC in the respective patient. TSC2 mutation found in Patient 1 that involves consecutive deletion of exons 26–31 is predicted to cause a shift from the wild-type reading frame, leading to a truncated tuberin at 36 amino acids downstream. Patient 2 showed a TSC2/PKD1 contiguous genes deletion.
The TSC2 mutation found in Patient 2 involves consecutive deletion of the last 10 exons of the gene and will truncate the tuberin at amino acid position 1295.

Despite presence of related clinical features, we could not, however, identify the same exonic deletions in the mother of Patient 1. This might be due to somatic mosaicism, which have been found in a fairly significant number of patients with clinically apparent TSC (11) and may hamper molecular diagnosis (12), especially that using MLPA (13).

From the perspective of the integrity of protein production, both mutations seem to lead to similar effect. Both are predicted to produce a truncated tuberin without the necessary GAP (GTPase activating protein) domain (14). GAP domain is believed to produce a truncated tuberin without the necessary cooperation during the study.

CONCLUSIONS

As a conclusion, we have identified two novel gross deletions of TSC2 gene as causes of TSC in two Malaysian patients. One of the cases is a novel description of TSC2/PKD1 contiguous deletion syndrome.

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Conflict of interest statement

None declared.

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