Genotype-phenotype correlation in patients with TSC2-PKD1 contiguous gene deletion syndrome

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Abstract

PKDTS is a progressive hereditary disease that leads to serious clinical symptoms and death. PKDTS cases are reported less frequently. Therefore, there are few studies on the correlation between genotype and phenotype. Similar studies on whether the missing fragments are concentrated in the thermogene are rare. Given the important value of diagnosing PKDTS, it is necessary to develop a diagnostic process. We firstly reported the clinical date of PKDTS patients in China ,also retrieved the case reports of PKDTS published in the past 22 years and summarized the clinical manifestations and genetic characteristics of these patients. Many PKDTS patients have the following symptoms: under 20 years of age, hemangiofibroma, multiple renal cysts, and mental retardation. We did not have find a relationship between clinical phenotype and genotype. The gene deletion of TSC2 and PKD1 is not a hotspot mutation. More reports with detailed clinical descriptions of PKDTS patients and Chinese patients show phenotypic heterogeneity. The gene deletion of TSC2 and PKD1 expanded the mutation database. Moreover, mTOR inhibitors are recommended for treatment. In addition, combining the advantages of exon sequencing and MLPA, we firstly developed a diagnostic process for the disease, which were helpful in detecting new PKDTS.

Abstract

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Keywords : TSC2-PKD1 contiguous gene deletion syndrome, sequencing, MLPA, diagnostic process.

INTRODUCTION

TSC2 and PKD1 are located adjacent to chromosome 16p13.3 and are separated by only a few nucleotides. Mutations in TSC2 and PKD1 genes lead to tuberous sclerosis complex (TSC) and autosomal dominant polycystic kidney disease (ADPKD), which is known as TSC2-PKD1 contiguous gene deletion syndrome (PKDTS)(Oyazato et al., 2011). .The deletion of large gene fragments spanning TSC2 and PKD1 leads to tuberous sclerosis and adult polycystic kidney disease (European Chromosome 16 Tuberous Sclerosis, 1993), which was first described in 1994(Brook-Carter et al., 1994).

TSC is an autosomal dominant neuroscience syndrome that is characterized by typical skin lesions, seizures and mental retardation. The incidence of TSC is approximately 1:5000-10000(Oyazato et al., 2011). TSC2 gene mutation often lead to tuberous sclerosis (Hinton et al., 2014) and multiple renal cysts. ADPKD is the most common single-gene disorder that causes end-stage renal disease (ESRD) (Torres et al., 2007). ADPKD is mainly manifested as multiple renal cysts and an increase in the size of the kidney, and it has an incidence of approximately 1:400-4000(Torres et al., 2007), affecting 12.5 million people worldwide (Chapman et al., 2015). The PKD1 gene has been associated with autosomal dominant polycystic kidney disease (Torres et al., 2007). Because TSC2 or PKD1 single gene mutation alone also result in multiple cysts, more reports with detailed clinical description of patients would be helpful to understand the genotype-phenotype correlation in patients with TSC2-PKD1 contiguous gene deletion syndrome. Meantime, there has been less discussion about the therapeutic effect of the disease in the past.

In this study, we reported 5 cases of PKDTS in Chinese Han nationality, and searched for patients with the disease reported in other literatures, eventually including 13 patients, and analyzed the potential association between clinical phenotypes and gene mutation sites.

MATERIALS AND METHODS

2.1 Ethical compliance

Ethical approval for the study was granted through the Chinese PLA General Hospital Medical Ethics Committee (2012–001). All patients in this study signed a consent form indicating that they had been fully informed about the nature of the interview, as well as the likely uses of their data.

2.2 Targeted NGS panel

Target region capture and next-generation human gene analysis technology were performed for ciliopathy-associated gene region and bioinformatics analyses. We used a kidney disease-associated gene analysis panel including TSC2 and PKD1 gene for sequencing.

Exome sequencing were performed as previously described(Zhang et al., 2012). Exome capture was carried out using the Agilent Sure Select Human All Exon V6 Kit (in solution) according to the manufacturer's

protocols; Read mapping and variant detection and functional annotation of genetic variants were also followed. Candidate genes were subsequently verified by Sanger sequencing or MLPA.

2.3. Sanger sequencing

After gene point mutation sites were detected using NGS, they were validated using Sanger sequencing. In addition, blood samples provided by the patients' family members were also validated by Sanger sequencing. When reference sequences were found, the reference sequences and raw data were analyzed using Mutation Surveyor software (https://softgenetics.com/mutationSurveyor.php).

2.4. Multiplex Ligation-Dependent Probe Amplification (MLPA)

For detection of exon deletion or duplication, MLPA is optional. The SALSA MLPA kit is commercially available from MRC-Holland (Amsterdam, the Netherlands).

2.5.Clinical presentation and genetic analysis of PKDTS

To study the correlation between genotypes and phenotypes, we searched for cases reported between September 1997 and March 2019 with both tuberous sclerosis and autosomal dominant polycystic kidney disease that were confirmed by genetic testing. The clinical manifestations are also described in detail. Then we added data from these patients to our patient database to compare genomic distortion with clinical features.

3. RESULTS

Clinical presentation and genetic analysis of 5 Chinese patients.

Clinical history and genetic analysis of case 1

A 22-year-old Chinese Han male patient man presented with facial erythema and angiofibromas with malar distribution starting at the age of four (Fig. 1a). He developed seizures at the age of 6 years and was administered oral antiepileptic drugs (sodium valproate, carbamazepine). Kidney function, blood pressure and microalbuminuria were within the normal ranges at that time. During his junior year of high school, the patient's ability to learn and remember was less than that of his peers, although his verbal abilities were normal. The patient was hospitalized for sudden syncope at age 17. Brain MRI of the right side of the lateral ventricle space showed bilateral lateral ventricular wall tubercle sclerosis and bilateral frontal lobe parietal lobe hydrocephalus (Fig. 1b), so lateral ventricle tumor resection surgery was performed. Biopsy of the right forehead of his brain during craniotomy revealed subependymal giant cell astrocytoma (WHO I) (Fig.1e). The shape of his kidney did not reveal obvious abnormalities on ultrasound, but the serum creatinine levels reached 130 μmol/L.

At 21 years of age, gross hematuria occurred, and ultrasound examination revealed a progressive increase in kidney size: the left kidney measured 80 mm × 289 mm, and the right kidney measured 107 mm × 200 mm. There were many bilateral cysts; the largest cysts in the left kidney measured 66 mm × 102 mm, and the largest cysts in the right kidney measured 42 mm × 48 mm. Thus, the patient was diagnosed with ADPKD, which was confirmed by CT scan (Fig.1c,d). CT scans of the abdomen revealed bilateral kidney cysts (Fig.1c) and hepatic angiomyolipomas (Fig.1d). Renal function significantly decreased due to the increased cysts: urea nitrogen 29.98 mmol/L, creatinine 818.8 μmol/L, and uric acid 549 μmol/L. Thus, the patient was treated with hemodialysis. The clinical symptoms of such patients typically include both severely affected polycystic kidneys and tuberous sclerosis of the brain. However, another typical sign of TSC, lymphangioleiomyomatosis of the lungs, was absent in this patient. The screening of family members for kidney and hepatic cysts was negative. Neither his parents nor his two older sisters had tuberous sclerosis or decreased renal function. Family diagram of case 1 shown in figure. 2a.

A targeted NGS panel was used for genetic analysis. The mutations in the family were identified by exome sequencing of the patient with polycystic kidney disease and tuberous sclerosis, involving the TSC2 and PKD1 genes; subsequent mutations were identified by MLPA. The MLPA analysis result of case 1 are shown in Figure 1 of the Supplement.

In addition, the first PKDTS patient in the world with a PKD1 mutation was found. The chromosomal mutation sites of the PKD1 and UMOD genes are chr16-2158014h and chr16-20352618, respectively, and the nucleotide positions are c.6935C> T and c.1372G >T, respectively. Sequencing results revealed that the pathogenicity of the mutation of the PKD1 gene in Case 1 was unknown after analysis by software such as REVE, SIFT, PolyPhen, and MutationTaster. The incidence of mutations in the PKD1 gene in normal individuals is 0.0006.

Clinical history and genetic analysis of case 2

An 18-year-old Chinese Han male patient developed intermittent seizures at the age of 4. Brain MRI shows abnormal signals in bilateral cerebral hemisphere cortex and lateral ventricle, taking nodular sclerosis into consideration. Electroencephalogram showed moderate abnormal EEG with visible epileptic spikes. Cardiac ultrasound findings in the intracardiac space showed nodular sclerosis. The seizures continued for three years, and he was administered valproate, lamotriginea and substitution. Recently, this patient received sirolimus for epilepsy . At the age of four, he was diagnosed with faicial angiofibromas and confetti skin lesions on the skin were discovered. When the patient was 15 years old, ultrasound showed abnormal kidney morphology and increased volume. The size of the left kidney was 18.6cm×8.5cm×7.6cm, and the size of the right kidney was approximately 19.2cm×9.0cm×10.2cm(Fig. 1f). The renal capsule was irregularly tuberous. Diffuse cystic structures of varying sizes were observed. In these cystic structures, the left kidney was approximately 5.8cm×4.7cm×5.2cm, and the right kidney was approximately 5.3cm×4.7cm×4.7cm. At 18 years old, the urine protein was 1.8 g/d, and the serum creatinine was 135.3 μmmol/L, both of which exceeded the normal values. The patient developed normally before the disease and developed more slowly than normal after the disease.

Multiple PCR proliferation along with DHPLC analysis was used for the second patient. The mutations were identified by exome sequencing of the patient with polycystic kidney disease and tuberous sclerosis, and subsequent mutations were identified by MLPA. The MLPA analysis result of case 2 are shown in Figure 2 of the Supplement. No TSC2 and PKD1 deletions were found in his parents and a brother, although the patient carried a large spontaneous de novo deletion. A family diagram of case 2 is shown in Fig. 2b.

Clinical history and genetic analysis of case 3

The patient had a renal cyst at 6 months of birth. CT showed that the kidneys were significantly enlarged in size, and the demarcation of the cortex and medulla was unclear. The renal parenchyma showed diffuse cystic shadows of varying sizes, with clear borders and a diameter of approximately 5 mm-27 mm. No enhancement was seen in the enhanced scan (Fig. 1h, i). Her parents did have not clinically detected signs and symptoms of TSC and ADPKD, and no genetic testing was performed on them. The MLPA analysis result of case 3 are shown in Figure 3 of the Supplement. The family diagram of case 3 is shown in Fig. 2c.

Clinical history and genetic analysis of case 4

The patient was of low intelligence and had angiofibroma on the back at 8 years of age with low vision. Gross hematuria and proteinuria were found at the age of 20, and pain in the right waist was observed. Other tests results showed creatinine 299.5 μmol/L; glomerular filtration rate 24.50 mL/min/1.73 m2; urine routine: occult blood 3+; total urine protein 0.71 g/d; and ultrasound: left kidney 187 mm×104 mm×91 mm and right kidney 197 mm×100 mm×90 mm. The normal structure of the kidney disappeared, and multiple cystic echoes of varying sizes appeared. The larger left side was approximately 30 mm×26 mm, and the larger right side was approximately 29 mm×22 mm. Patient CT results showed bilateral polycystic kidney disease (Fig. 2 j), and chest CT showed multiple inflammatory nodules in both lungs. Brain CT was not examined, and the patient took compound α-ketoacid tablets and other medications. The MLPA analysis result of case 4 are shown in Figure 4 of the Supplement.His parents do not have clinically detected signs and symptoms of TSC and ADPKD, and no genetic testing was performed on them. A family diagram of case 4 is shown in Fig. 2d.

Clinical history and genetic analysis of case 5

Three months after the birth of the patient, the parents noticed that the child had an unstable head and squinting left eyes. Four months after birth, the child developed involuntary convulsions, blinked, and lost consciousness, accompanied by nystagmus, and MRI of the skull showed high-density shadows of the left occipital lobe, localized capillary tumors, and congenital atypical cerebral nodular sclerosis. This patient was clinically diagnosed with epilepsy. Six months after the patient was born, her speech development lagged. Family history: The patient has an older brother who is healthy. Physical examination showed mental retardation, unresponsiveness, poor expression, a 1.5 cm×1.1 cm confetti skin lesion on the lower jaw, and a 3 cm×2 cm angiofibroma on the posterior neck. The patient had been treated with sodium valproate, and the symptoms of epilepsy were controlled. The MLPA analysis result of case 5 are shown in Figure 5 of the Supplement. Her parents did not have clinically detected signs and symptoms of TSC and ADPKD, and no genetic testing was performed on them. A family diagram of case 5 is shown in Fig. 2e.

Summary of clinical and genetic characteristics

Based on reports from previous literature, we developed two tables(Table 1, Table 2) of the clinical characteristics of PKDTS (Oyazato et al., 2011, Kacerovska et al., 2009) . The clinical features and genomic aberrations of 5 new Chinese patients are summarized in Table 1. The clinical data of PKDTS patients reported in the literature are summarized in Table 2.

To study the potential associations between various aspects of clinical phenotype and extent of deletion in TSC2, PKD1, and other genomic regions, we studied 5 newly discovered Chinese patients and searched for PKDTS cases reported over the past 22 years that had been genetically tested and described their clinical manifestations in detail.

We have summarized the basic situation of this disease. Patients are younger, most of them are younger than 20 years old, and there is basically no difference in gender between men and women.

Our study found that many patients had angiofibromas, multiple renal cyst seizures and intellectual disability. In addition, few patients had ungual fibromas, intraoral fibromas and liver cysts.

We also found that the TSC/PKD1 deleted exon region did not focus on a specific region, and there were no hotspot mutations.

Finally, we found no genotype-phenotype correlations for this disease, as have been previously reported for tuberous sclerosis complex and autosomal dominant polycystic kidney disease symptoms.

4. DISCUSSION

We report 5 patients with tuberous sclerosis who primarily showed skin or nervous system symptoms, which are frequently thought to be associated with the tuberous sclerosis itself, even if there are bilateral kidney cysts or decreased renal function. Kidney cysts may be found in both TSC and ADPKD, and an estimated 2% to 3% of patients with TSC have a contiguous deletion of the TSC2-PKD1 gene, causing early renal insufficiency(Lim et al., 2014, Dixon et al., 2011, Siroky et al., 2011, Back et al., 2015, Santos et al., 2017). The ADPKD-associated PKD1 gene deletion is easily overlooked. Our findings provide new insights into ADPKD and TSC screening and mark a possibly meaningful step towards improved diagnosis and treatment of patients with ADPKD or TSC.

MLPA is a novel diagnostic tool for genetic screening, which is gradually becoming the principal method for the detection of exon deletion and duplication (Schouten et al., 2002).Therefore, deletions and duplications detected by exome sequencing should always be confirmed by MLPA and other methods (Laass et al., 2004, Kozlowski et al., 2007, Yu et al., 2015).

Compared with other studies, the combination of exome sequencing technology and MLPA was used for the first time to screen and diagnose TSC2-PKD1 continuous gene deletion syndrome, which provides a new method for the diagnosis and accurate treatment of the disease. The diagnosis of inherited disease is established by genetic testing using the MLPA technique .To examine this patient, we used next-generation sequencing,

also known as high-throughput sequencing, which can be applied to de novo sequencing, whole-genome re-sequencing, and targeted resequencing in genomics research. With its advantages of a high resolution, high throughput, high efficiency and high sensitivity, this technology can accurately detect single nucleotide substitutions, insertion deletions of bases, deletion of DNA fragments, and copy number variations. In this case, the patient's genetic testing results showed a heterozygous deletion of the TSC2 and PKD1 genes.

We describe the large genomic deletions on chromosome 16p13.3 from the case 1 patient as novel mutations underlying TSC2-PKD1 contiguous deletion syndrome in this family because the TSC2 and PKD1 genes were deleted from the maternal chromosome due to a de novo, spontaneous genetic mutation. Genomic rearrangements involve gross alterations of chromosomes or large chromosomal regions and can take the form of deletions, inversions or translocations(Boehm et al., 2007).

Because PKD1 gene mutations with dominant inheritance can cause the polycystic kidney phenotype and TSC2-encoded protein helps polysystin-1 in cell membrane localization, and the carboxy terminal tail of polycystin-1 regulates the localization of TSC2 to repress mTOR, the complete deletion of adjacent PKD1-TSC2 genes can produce a synergistic effect, thereby increasing polycystic kidney symptoms (Ong et al., 1999, Dere et al., 2010) The case 1 patient initially showed a slight decrease in renal function, followed by the rapid development of renal cysts and progression to uremia within 4 years, which may have been related to deletions of the PKD1 and TSC2 genes and could also be explained by the long-term use of antiepileptic drugs.

Exome sequencing has enabled increasingly accurate and cost-effective methods for mutation detection in patients with inherited kidney disorders(Renkema et al., 2014). Using exome sequencing in the early stage of disease for young patients where the clinical diagnosis is not evident is the best approach (Rejeb et al., 2017). Currently, the exome sequencing is now widely used in many hospitals to help clinicians determine the etiology of many rare diseases. However, the exome sequencing analysis fn chromosomal deletion or duplication is not the technique of choice; rather, array-CGH analysis or MLPA is preferred (which is more convenient with a genetic testing kit). Exome sequencing is the technique of choice for point mutations or very small deletions/duplications of an unknown gene, even if the percentage of success is still far from 100% in patients with unknown syndromes. Therefore, MLPA and Sanger sequencing will be performed only if a causative mutation is identified by exome sequencing to validate these mutations, especially for TSC2-PKD1 contiguous gene deletion syndrome.

In summary, based on the important value of diagnosing PKDTS, our clinical experience, the important role of MLPA in diagnosing exon fragment deletions, and the advantages of next-generation sequencing, we designed a diagnosis process for patients with symptoms or signs of tuberous sclerosis (Fig. 3).

The UMOD gene is transcribed in the kidney and encodes the Tamm Horsfall protein, a GPI-anchored glycoprotein, which is the most abundant protein in normal urine. Mutations in the UMOD lead to myeloid cystic nephropathy 2 and familial juvenile hyperuricemia nephropathy (Hart et al., 2002). In this case, the mutation of the UMOD gene was found by the gene sequencing, hyperuricemia was also found in the patients with clinical manifestations, and the serum uric acid reached 549 μmol/L. Therefore, the increase of uric acid may be related to the mutation of the UMOD gene and decreased renal function caused by end-stage renal disease.

There is a mutation site in PKD1 of case 1 in this study that has not been reported in other articles. The incidence of mutations in the PKD1 gene in normal individuals is low (0.0006), and the pathogenicity of the mutation of the PKD1 gene in case 1 was unknown after analysis using many software programs, suggesting that this mutation does not rule out its pathogenicity. Many studies have shown that the PKD1 mutation may lead to autosomal dominant polycystic kidney disease. In our study, a CT scan of the kidneys of the abdomen revealed bilateral renal cysts with renal failure, which is considered related to the deletion of PKD1 and TSC2 genes, but it does not rule out that the mutation in PKD1 exacerbates symptoms such as polycystic kidney disease. This study reported for the first time that the PKD1 gene was deleted in one single strand of DNA and the presence of mutations in another single strand of DNA in patients with

PKDTS. This mutation enriched the database resources of PKDTS.

On the basis of 5 newly discovered Chinese patients,we also examined 13 patients who had been genetically tested and described in detail their clinical manifestations to study their genotypic-phenotypic correlation. We found that the disease can cause damage to various systems of the eye, skin, nerves, liver, kidneys, heart, etc. The gene deletion of PKDTS is not concentrated on specific exons, and the disease has no hotspot pattern. There was no significant correlation between gene deletion sites and deletion degree and clinical manifestations(Oyazato et al., 2011). In summary, PKDTS showed phenotypic heterogeneity.In summary, PKDTS showed phenotypic heterogeneity.

In terms of clinical manifestations, these patients rarely have ungual fibromas , Intraoral fibromas and liver cysts. The conclusion that there are fewer hepatic cysts is the same as that of Oyazato Y(Oyazato et al., 2011).

From our research, many patients with PKDTS have the following symptoms: under 20 years of age, hemangiofibroma, multiple renal cysts, and mental retardation.When some patients have the above symptoms and characteristics, it is necessary to focus on screening for PKDTS using the above diagnostic process.

In terms of family history, among these patients, including 13 search cases and 5 Chinese cases, only 1 patient whose mother had TSC and PKD symptoms was found(Longa et al., 1997). We speculate that PKDTS patients have a poor prognosis because of this disease, and many patients rarely get married.

For treatment, there are no other methods except conservative symptomatic treatment, such as dialysis, nephrectomy, anti-epilepsy, hypotension, and hemangioma resection. Tolvaptan is a treatment option and is the first FDA-approved drug treatment to slow kidney function decline in adults at risk of rapidly progressing ADPKD (Chebib et al., 2018). In addition, mTOR inhibitors (sirolimus or everolimus) are also recommended. First, the frequency of epileptic seizures was reduced by mTOR inhibitors in patients in our study after receiving mTOR inhibitors and other antiepileptic drugs. Furlano M et al. also had similar research reports (Furlano et al., 2017). Second, mTOR inhibitors have been approved as a first-line therapy for AMLs in TSC (Furlano et al., 2017, Bissler et al., 2016). Simultaneously, these drugs have shown some efficacy in reducing the progressive increase in kidney volume of ADPKD (Serra et al., 2010, Walz et al., 2010) .Third, PKDTS patients could have benefited from the administration of mTOR inhibitors at renal transplantation, lowering the risk of AMLs complications (Furlano et al., 2017, Bissler et al., 2008).

5.CONCLUSION

This is the first report of Chinese patients diagnosed with TSC2-PKD1 contiguous gene deletion syndrome, and we summarized in detail the clinical manifestations, gene deletion types, treatment and family history of PKDTS cases reported in the past 22 years. Patients with phenotypic heterogeneity are helpful in understanding this disease. In addition, the targeted NGS panel and diagnostic process were helpful in detecting new PKDTS cases.

DATA AVAILABILITY STATEMENT

All data that support the findings of this study are included in this manuscript and its supplementary information files.

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CONFLICTS OF INTEREST

None.

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FIGURE LEGENDS

Figure. 1 Photographic images and Clinical appearances of the patients.The clinical data of case 1 includes figure.a, figure.b, figure.c, , figure.d and figure.e; The clinical data of case 2 includes figure.f; The clinical data of case 3 includes figure.h and figure.i; The clinical data of case 5 includes figure.j. (**a**) Face of the case 1 at age 22 years. Facial erythema and angiofibromas with malar distribution (White arrows). (**b**) MRI scan of the brain of showed the right side of the lateral ventricle space, with bilateral lateral ventricular wall tubercle sclerosis and bilateral frontal lobe parietal lobe hydrocephalus. (**c** ,**d**) CT scans of the abdomen revealed bilateral kidney cysts (**c**) and hepatic angiomyolipomas (**d**). (**e**) Histological analysis of right forehead craniotomy of his brain showed subependymal giant cell astrocytoma (WHO I). (**f**)ultrasound showed abnormal kidney morphology and increased volume. (**h** ,**i**) CT showed the kidneys were significantly enlarged in size and the demarcation of the cortex and medulla was unclear. (**j**)Patient CT results show bilateral polycystic kidney disease .

Figure. 2 Pedigree of the Chinese Han families. Circles and squares represent females and males, respectively. Black arrow denotes the proband. Family diagrams of cases 1, 2, 3, 4 and 5 are respectively shown in figures .2a, 2b, 2c, 2d and 2e.

Figure. 3 The diagnosis process for patients with symptoms or signs of tuberous sclerosis.

Supplement

Figure 1 MLPA detection revealed deletions in heterozygosis in exon of the PKD1and TSC2 gene of case 1.

Figure 2 MLPA detection revealed deletions in heterozygosis in exon of the PKD1and TSC2 gene of case 2.

Figure 3 MLPA detection revealed deletions in heterozygosis in exon of the PKD1and TSC2 gene of case 3.

Figure 4 MLPA detection revealed deletions in heterozygosis in exon of the PKD1and TSC2 gene of case4.

Figure5 MLPA detection revealed deletions in heterozygosis in exon of the PKD1and TSC2 gene of case 5.

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