

Analysis of renal lesions in Chinese tuberous sclerosis complex patients with different conditions of TSC1/2 genetic mutations

Wenda Wang

Peking Union Medical College Hospital

Yang Zhao

Peking Union Medical College Hospital

Xu Wang

Peking Union Medical College Hospital

Zhan Wang

Peking Union Medical College Hospital

Guoyang Zheng

Peking Union Medical College Hospital

Yi Cai

Xiangya Hospital Central South University

Hua Fan

Peking Union Medical College Hospital

Hanzhong Li

Peking Union Medical College Hospital

Yushi Zhang (✉ 15120002969@163.com)

Peking Union Medical College Hospital

Research

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Abstract

Objective The renal manifestations of tuberous sclerosis complex (TSC) are complicated and various. We'd like to report the information of genetic mutations in TSC patients with renal lesions, and to discuss the relationship between features of renal lesions and genetic mutations, including mutant genes and mutant types. **Methods** TSC patients with renal lesions who came to Urology Department of our hospital from January 1st, 2015 to January 1st, 2020 were retrospectively analyzed. TSC patients who received next generation sequencing (NGS) of TSC1/2 and imaging examinations were screened out. When familial TSC patients were confirmed, only the probands were included. The patients who had imaging evaluation in our hospital before any treatment for TSC renal angiomyolipomas (AMLs) were also selected for further analysis of relationship between genetic mutations and AML sizes. **Results** 70 clinically or genetically diagnosed TSC patients with renal lesions were included. The average age was 29.3 ± 8.3 years old. Male-female ratio was 1:1.5. 15 patients (21.4%) were probands of TSC families (3 TSC1, 10 TSC2, and 2 NMI). 67 patients (95.8%) had bilateral renal AMLs with one patient had a pathological diagnosis of epithelioid AML (TSC2 mutation). One patient had multiple renal cysts (TSC2 mutation), one had renal cell carcinomas (RCCs) (TSC1 mutation) and one had Wilms tumors (TSC1 mutation). Among the 70 included patients, 4 patients had TSC1 mutations, 51 had TSC2 mutations, and 15 had no mutation identified (NMI). There was no statistically significant difference between TSC2 mutations and NMI groups (11.4 ± 5.7 vs. 8.0 ± 5.6 cm, $P = 0.077$) when considering AML sizes. There was also no statistically significant difference among AML sizes of patients with TSC2 mutation types of nonsense, missense, frameshift, slipping, and fragment deletion ($P = 0.712$). And no statistically significant difference was found between maximum diameters in familial and sporadic patients, either (11.4 ± 5.8 vs. 10.5 ± 5.8 , $P = 0.663$). **Conclusions** The conditions of TSC genetic mutations will affect type and severity of renal lesions. Other focuses such as protein structure and function need to be studied for renal manifestations. Except for patients with TSC1 and TSC2 genetic mutations, patients with NMI and familial patients are also needed more attention for the pathogenesis of them is still unknown.

Introduction

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterized by hamartomas in organs including the brain, kidney, lung, skin, and heart^[1]. The birth incidence of TSC has been estimated to be approximately 1 in 6000^[2]. Renal lesions are the most common cause of death in adult TSC patients. These renal diseases of TSC may occur in early childhood with progress throughout adulthood^[3]. The most common kidney manifestation of TSC is angiomyolipoma (AML), which occurs in 70–90% TSC patients^[4]. The other kinds of lesions include renal cysts and renal cell carcinomas (RCCs).

75–90% of patients who meet TSC standard clinical criteria present TSC1 or TSC2 mutations^[5], and about 60–70% of TSC cases are sporadic^[6–7]. 10–15% of patients have no TSC1 or TSC2 mutations (also known as no mutation identified, NMI), although these patients could be clinically diagnosed. In the previous studies, researchers found that patients with TSC2 mutations present severer clinical features than others^[8–11]. However, the studies focus on the relationship between TSC genetic mutations and TSC renal lesions are relatively few. Here we report the information of genetic mutations in TSC patients with renal lesions, and discuss the relationship between renal lesions and TSC mutations, including mutant genes and mutant types.

Methods

Participants

We retrospectively analyzed TSC patients with renal lesions who came to Urology Department of Peking Union Medical College Hospital (PUMCH) from January 1st, 2015 to January 1st, 2020. The diagnosis of TSC was made based on the clinical diagnostic criteria of the 2012 international tuberous sclerosis complex consensus conference^[4] or genetic diagnosis of TSC1/2 gene. TSC patients who received next generation sequencing (NGS) of TSC1/2 genes and imaging examinations were screened out for analysis of genetic and clinical features. When familial TSC patients were confirmed, only the probands were analyzed. The patients who had imaging evaluation in our hospital before any treatment for TSC renal AMLs were also selected for further analysis of relation between genetic mutations and AML sizes. We recorded the maximum diameter at the biggest cross-section of the largest lesion in each patient. Our study was approved by the Ethics Committee of PUMCH. Written informed consent was obtained from all the subjects for genetic tests and clinical information analysis.

NGS and mutation analysis

Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The genomic DNA was fragmented into 200 ~ 250 bp fragments and was purified by Agencourt AMPure XP kit (BGI-Shenzhen, Shenzhen, China). After modification of DNA fragments, ligation-mediated polymerase chain reaction (PCR) and purification were conducted, following the hybridization reaction using the customized gene fragment-capturing chips (Roche NimbleGen, Madison, WI). Amplification with the highfidelity DNA polymerase and high-throughput sequencing of the qualified DNA samples were carried out for continuous bidirectional sequencing of 90 cycles. The Illumina basecalling software (V. 1.7, Illumina) was used to deal with the original image data, and the Burrows-Wheeler Aligner software (BGI-Shenzhen, Shenzhen, China) was used for sequence alignments of qualified raw reads, which had been conducted using sequencing quality assessment. The bam data were used for reads coverage in the target region and sequencing depth computation, single-nucleotide polymorphism (SNP) and insertion-deletion calling, and copy number variation detection. First, SNPs and insertion-deletions were called using SOAPsnp software (BGI-Shenzhen, Shenzhen, China) and Samtools pileup software (BGI-Shenzhen, Shenzhen, China), respectively. Second, if an SNP frequency was more than 0.05 in any of the 4 databases (dbSNP, HapMap, 1000 Genomes Project, and BGI local database), it would be regarded as a polymorphism, not a causative mutation. Lastly, all of the mutations were retrieved from the Leiden Open Variation Database (LOVD) databases to be labeled as already reported or novel.

The sequences of the Homo sapiens hamartin and tuberin proteins were obtained from the National Center for Biotechnology Information database. The mutations in the TSC1 or TSC2 gene were compared with those in the Tuberous Sclerosis Database. The possible impact of the identified mutations on protein function, as a result of an amino acid substitution, was examined using available online tools SIFT and PolyPhen-2.

After probable causative mutations were found, participants and their affected family members underwent Sanger sequencing or quantitative PCR to verify the mutations. The PCR cycling protocol consisted of an initial denaturation at 95 °C for 3 minutes, followed by 35 cycles, at 95 °C for 40 seconds, at 55 °C for 30 seconds, and at 72 °C for 30 seconds, with a final extension at 72 °C for 10 minutes.

Statistical analysis

All statistical analyses were performed using SPSS19.0 software (SPSS Inc., USA). Data were expressed as means \pm standard deviation (mean \pm SD) or n (%) as appropriate. Student's unpaired t test or Tukey test was used to

determine differentiation state of continuous variables between different groups. A P value of less than 0.05 was considered statistically significant.

Results

In total, 70 clinically or genetically diagnosed TSC patients with renal lesions were retrospectively analyzed during January 1st, 2015 to January 1st, 2020 in PUMCH. The average age was 29.3 ± 8.3 years old. There were more female patients with a male-female ratio of 1:1.5. 15 patients (21.4%) were probands of TSC families (3 TSC1, 10 TSC2, and 2 NMI). When all the familial TSC patients were included, there were 95 patients in total. Among the 70 patients for analysis, 67 patients (95.8%) had bilateral renal AMLs with one patient had a pathological diagnosis of epithelioid AML. The patient received surgical resection for rapid progress. The renal lesions were multiple renal cysts in one patient, RCCs in one patient and Wilms tumors in one patient, respectively (Table 1).

Table 1
Demographic characteristics of 70 patients.

Age (yr, M \pm SD) (range)	29.3 \pm 8.3 (8 ~ 45)
Gender	
Male	28/70 (40.0%)
Female	42/70 (60.0%)
Familial TSC	15/70 (21.4%)
Renal lesions	
AML	67/70 (95.8%)
Epithelioid AML	1/70 (1.4%)
Multiple renal cysts	1/70 (1.4%)
Renal cell carcinomas	1/70 (1.4%)
Wilms tumor	1/70 (1.4%)

Among the 70 TSC patients, 4 patients had TSC1 gene mutation. The patient with RCCs had nonsense mutation of TSC1 gene at c.2227C > T. The patient with Wilms tumors had fragment deletion of TSC1. 51 patients had TSC2 gene mutation. One patient with fragment deletion of TSC2 had the renal lesions of multiple renal cysts. No TSC1 or TSC2 gene mutation were detected in 15 patients with clinical diagnosis (Table 2). The details were summarized in Tables 3 and 4.

Table 2
TSC1 and TSC2 gene mutations in 70 patients.

TSC1	4/70 (5.7%)
Nucleotide mutation	
Nonsense	3/4 (75.0%)
Fragment mutation	1/4 (25.0%)
TSC2	51/70 (72.9%)
Nucleotide mutation	
Nonsense	18/51 (35.3%)
Missense	7/51 (13.7%)
Frameshift	15/51 (29.4%)
Slipping	4/51 (7.9%)
Fragment mutation	7/51 (13.7%)
NMI	15/70 (21.4%)

Table 3
TSC1 gene mutation data.

Site	Mutation type	Protein change	Lesions	Familial or not	Pathogenicity
Nucleotide mutation					
c.733C > T	Nonsense	p.Arg245Ter	AML	Yes	Pathogenic
c.2227C > T	Nonsense	p.Gln743Ter	RCC	No	Pathogenic
c.1372C > T	Nonsense	p.Arg458Ter	AML	Yes	Pathogenic
Fragment mutation					
EX9_12DEL	-	-	Nephroblastoma	No	Likely pathogenic

Table 4
TSC2 gene mutation data.

Site	Mutation type	Protein change	Lesions	Familial or not	Pathogenicity
Nucleotide mutation					
c.3581G > A	Nonsense	p.Trp1194Ter	AML	No	Pathogenic
c.3685C > T	Nonsense	p.Gln1229Ter	AML	Yes	Pathogenic
c.2194C > T	Nonsense	p.Gln732Ter	AML	No	Pathogenic
c.1874C > G	Nonsense	p.Ser625Ter	AML	No	Pathogenic
c.1513C > T	Nonsense	p.Arg505Ter	AML	No	Pathogenic
c.658C > T	Nonsense	p.Gln220Ter	AML	No	Pathogenic
c.2194C > T	Nonsense	p.Gln732Ter	AML	No	Pathogenic
c.2590C > T	Nonsense	p.Gln864Ter	AML	No	Pathogenic
c.3685C > T	Nonsense	p.Gln1229Ter	AML	No	Pathogenic
c.3412C > T	Nonsense	p.Arg1138Ter	AML	No	Likely pathogenic
c.3412C > T	Nonsense	p.Arg1138Ter	AML	No	Pathogenic
c.4129C > T	Nonsense	p.Gln1377Ter	AML	No	Likely pathogenic
c.4129C > T	Nonsense	p.Gln1377Ter	AML	No	Likely pathogenic
c.3412C > T	Nonsense	p.Arg1138Ter	AML	No	Pathogenic
c.3750C > G	Nonsense	p.Tyr1250Ter	AML	No	Pathogenic
c.4255C > T	Nonsense	p.Gln1419Ter	AML	No	Pathogenic
c.1507C > T	Nonsense	p.Gln503Ter	AML	No	Likely pathogenic
c.1108C > T	Nonsense	p.Gln370Ter	AML	No	Likely pathogenic
c.1831C > T	Missense	p.Arg611Trp	AML	No	Pathogenic
c.3475C > T	Missense	p.Arg1159Trp	AML	No	VUS
c.5126 C > T	Missense	p.Pro1709Leu	AML	No	Pathogenic
c.5024C > T	Missense	p.Pro1675Leu	AML	No	Pathogenic
c.1831C > T	Missense	p.Arg611Trp	AML	No	Pathogenic
c.2032G > A	Missense	p.Ala678Thr	AML	No	Likely pathogenic

Site	Mutation type	Protein change	Lesions	Familial or not	Pathogenicity
c.856A > G	Missense	p.Met286Val	AML	No	Likely pathogenic
c.2233_2234del	Frameshift	p.Lys745AspfsX16	AML	Yes	Pathogenic
c.1852del	Frameshift	p.Leu618CysfsX80	AML	No	Pathogenic
c.1047dup	Frameshift	p.Arg350Ter	AML	Yes	Pathogenic
c.4544_4547del	Frameshift	p.Asn1515SerfsX60	AML	Yes	Pathogenic
c.1762_1763del	Frameshift	p.Glu588Terfs	AML	No	Likely pathogenic
c.788_789insC	Frameshift	p.Leu263LeufsX75	AML	Yes	Likely pathogenic
c.4006_4007insC	Frameshift	p.Ser1336SerfsX78	AML	No	Likely pathogenic
c.203_204 insA	Frameshift	p.Ala68AlafsX7	AML	Yes	Likely pathogenic
c.3683_3684insG	Frameshift	p.Leu1228LeufsX6	AML	No	Likely pathogenic
c.2738_2739 insT	Frameshift	p.Thr913ThrfsX2	AML	No	Likely pathogenic
c.2319 delA	Frameshift	p.Leu773LeufsX56	AML	No	Likely pathogenic
c.3601_3602insGGCCC	Frameshift	p.Thr1203GlyfsX9	AML	No	Likely pathogenic
c.4926delC	Frameshift	p.Asn1643ThrfsX29	AML	No	Likely pathogenic
c.788_789insC	Frameshift	p.Leu263LeufsX75	AML	No	Likely pathogenic
c.1201_1202insA	Frameshift	p.His401GlnfsX9	AML	Yes	Likely pathogenic
c.976-1G > A	Splicing	-	AML	No	Pathogenic
c.1444-1G > C	Splicing	-	AML	No	Likely pathogenic
c.1947-1G > C	Splicing	-	AML	No	Likely pathogenic
c.2098-2A > G	Splicing	-	AML	No	Pathogenic
Fragment mutation					
chr16:2112430–2136922	-	-	AML	No	Pathogenic

Site	Mutation type	Protein change	Lesions	Familial or not	Pathogenicity
chr16:2120398–2121999	-	-	AML	No	Likely pathogenic
c.5027_5068 + 32del	Splicing	p.Leu1676_Asp1690delinsHis	AML	No	Likely pathogenic
chr16:2098173–2138668	-	-	Multiple renal cysts	Yes	Pathogenic
EX2_16 DEL	-	-	AML	No	Likely pathogenic
EX22_24 DEL	-	-	AML	No	Likely pathogenic
EX2_42 DEL	-	-	AML	Yes	Likely pathogenic

The maximum diameters of AMLs in patients who had imaging evaluation in our hospital before any treatment were analyzed according to TSC gene mutations. All the patients presented bilateral involvement. For no patients with TSC1 mutation had pre-treatment imaging records, the AML maximum diameters were compared between patients with TSC2 mutation (TSC2 group) and those with NMI (NMI group). There was no statistically significant difference between two groups (11.4 ± 5.7 vs. 8.0 ± 5.6 cm, $P = 0.077$), and the maximum diameter of AML in one patient could also be as long as 22.0 cm in NMI group (Table 5). When considering mutation types, there was also no statistically significant difference among different TSC2 mutation types of nonsense, missense, frameshift, slipping, and fragment deletion ($P = 0.712$) (Table 6). No statistically significant difference was found between AML maximum diameters in familial and sporadic patients, either (11.4 ± 5.8 vs. 10.5 ± 5.8 cm, $P = 0.663$) (Table 7).

Table 5
Comparison of AML maximum diameters between TSC2 group and NMI group.

	TSC2 group	NMI group	P
AML maximum diameter (cm)	11.4 ± 5.7	8.0 ± 5.6	0.077
N, range (cm)	42, 3.0 ~ 26.0	12, 2.5 ~ 22.0	-

Table 5
Comparison of AML maximum diameters between TSC2 group and negative group.

	TSC2 group	NMI group	P
AML maximum diameter (cm)	11.4 ± 5.7	8.0 ± 5.6	0.077
N, range (cm)	42, 3.0 ~ 26.0	12, 2.5 ~ 22.0	-

Table 6
Comparison of AML maximum diameters among different TSC2 mutation types.

	Nonsense	Missense	Frameshift	Slipping	Fragment mutation	P
AML maximum diameter (cm)	10.6 ± 5.1	11.1 ± 7.1	13.3 ± 7.3	10.1 ± 4.4	10.1 ± 3.6	0.712
N, range (cm)	16, 3.8 ~ 20.4	4, 3.0 ~ 20.2	13, 3.3 ~ 26.6	3, 5.8 ~ 14.6	6, 5.8 ~ 16.2	-

Table 7
Comparison of AML maximum diameters between familial and sporadic TSC patients.

	Familial TSC	Sporadic TSC	P
AML maximum diameter (cm)	11.4 ± 5.8	10.5 ± 5.9	0.663
N, range (cm)	11, 3.3 ~ 26.6	43, 2.5 ~ 22.0	

Discussion

TSC is an autosomal dominant genetic disease, which can also occur due to a sporadic germline mutation^[12]. In 1997, the TSC1 gene on chromosome 9q34 was first discovered^[7], while the TSC2 gene on chromosome 16p13.3 was discovered in 1993^[13]. The frequency of TSC2 mutations is reported higher than that in TSC1, and when considering both familial and sporadic conditions, TSC2 mutations are found in about 60% and TSC1 mutations in about 19% of TSC patients^[14]. However, TSC1 or TSC2 mutations cannot be shown by conventional genetic testing in 10 ~ 25% TSC patients^[4]. Renal lesions in TSC patients mainly include AMLs and multiple renal cysts, and RCCs are relatively rare. AMLs are the most common renal features in TSC patients. About 80% of TSC patients develop AMLs, which are significant causes of death. The risk of spontaneous bleeding of AML is related to its volume, and approximately 25 ~ 50% of AML patients with diameter > 3 ~ 4 cm will experience hemorrhage^[15-16]. Except for AMLs, renal cysts are also relatively common TSC renal lesions. PKD1 gene is proximal to TSC2 gene on chromosome 16, which can lead to TSC/PKD contiguous gene syndrome with development of polycystic kidney disease (PKD)^[17]. The patient who developed bilateral multiple renal cysts in our study also had fragment deletion on TSC2 gene, with his daughter having the same mutation and presenting the same renal lesions. The reasons for TSC patients developing multiple, bilateral RCCs remains unknown, and no other driver mutations have been identified in TSC-associated RCCs^[3]. There was also a patient with TSC1 gene mutation had bilateral Wilms tumors in our study. Wilms tumor is the most common malignant renal tumor in children. Wilms tumor has a high degree of genetic heterogeneity, and the related genes include WT1 (chromosome 11p13), WTX (chromosome Xq11.1), CTNMB1 (chromosome 3p22.1) and TP53 (chromosome 17p13.1)^[18]. Spreafico et al reported a girl with TSC2 mutation who developed a unilateral Wilms tumor. However, the girl also was found to have mutations of WT1 and WTX genes^[19]. According to the existing studies, it is likely that the occurrence of Wilms tumor is coincidental and the conditions of TSC are not associated with an increased risk of Wilms tumor^[18].

TSC2 mutations are usually related to a more severe phenotype than that in TSC1 mutations^[20]. The ratio of TSC1 mutations in our study is lower than reported, and this may be due to more patients with TSC1 mutations having milder phenotypes and patients with TSC2 mutations being more likely to seek treatment. According to previous studies, patients with TSC2 mutations usually have larger AML sizes and higher risk for AML hemorrhage^[21]. TSC patients with NMI are also reported to have milder phenotypes than patients with TSC2 mutations^[22]. In our study, we compared AML maximum diameters between patients with TSC2 mutations and NMI, and found that the average of maximum diameters in patients with TSC2 mutations might have a higher trend. However, no statistically significant difference has been found when compared with that in NMI patients. The results may be limited by small samples of patients with NMI. But we also should recognize that the maximum diameter in patients with NMI can be as long as 22.0 cm.

21.4% of TSC patients were classified as NMI, and this ratio is generally consistent with those in previous studies^[23]. In previous studies, mosaicism and intronic mutations could be identified by NGS in patients who had no mutation identified after conventional molecular diagnostic analysis of TSC1 and TSC2 in the past^[5]. However, there is still a significant part of patients with NMI in our study with the method of NGS. Nevertheless, it is not possible in our opinion that there is another gene which will lead to typical TSC.

Here we also compare AML sizes among different kinds of mutation types. TSC gene mutations include nonsense mutations, missense mutations, small deletions or insertions, splice site changes and large deletions or rearrangements. Few studies did this work in the past^[24], and our results show that there is no direct relationship between mutation types and severity of renal phenotypes. However, further studies, such as those about protein structure and function, should be conducted in the future.

Typically, TSC1 mutations are more likely to be familial than TSC2 mutations^[25-26]. This phenotypic diversity can be partly explained by the poorer prognosis of patients carrying TSC2 mutations^[26]. In our study, three of four patients with TSC1 mutations had familial history, while ten of fifty-one patients with TSC2 were familial. Interestingly, two of fifteen patients with NMI also presented familial disease. This indicates that inherited changes of genes may participate in disease onset, and further studies are needed to find them out.

Conclusions

The conditions of TSC genetic mutations will affect type and severity of renal lesions. Other focuses such as protein structure and function need to be studied for renal manifestations. Except for patients with TSC1 and TSC2 genetic mutations, patients with NMI and familial patients are also needed more attention for the pathogenesis of them is still unknown.

Abbreviations

tuberous sclerosis complex (TSC)

angiomyolipoma (AML)

renal cell carcinoma (RCC)

no mutation identified (NMI)

next generation sequencing (NGS)

polymerase chain reaction (PCR)

polycystic kidney disease (PKD)

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Peking Union Medical College Hospital (Beijing, China).

Consent for publication

Consent for publication was obtained from all the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YZ and HL conceived and designed the study. YC and HF helped to evaluate the feasibility of the study, and guided the implementation. WW, YZ, XW, ZW and GZ performed the data collection. WW conducted data analysis and produced the manuscript.

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Not applicable.

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