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A new atypical splice mutation in *PKD2* leading to autosomal dominant polycystic kidney disease in a Chinese family

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ABSTRACT

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is a very common hereditary renal disorder. Mutations in PKD1 and PKD2, identified as disease-causing genes, cause about 85% and 15% of ADPKD cases, respectively.

Methods: In this study, the mutation analysis of PKD genes was implemented in a Chinese family with suspected ADPKD using targeted clinical exome sequencing (CES). The candidate pathogenic variants were further tested by using Sanger sequencing and validated for co-segregation. In addition, reverse transcription-polymerase chain reaction (RT-PCR) was performed to test abnormal splicing and assess its potential pathogenicity.

Results: A novel atypical splicing mutation which belongs to unclassified variants (UCVs), IVS6+5G>C, was identified in three family members by CES and was shown to co-segregate only with the affected individuals. RT-PCR reveals the abnormal splicing of exon 6, thus to cause truncating mutation. These findings suggest that the atypical splice site alteration, IVS6+5G>C, in the PKD2 gene is the potential pathogenic mutation leading to ADPKD in the Chinese family.

Conclusion: The data available in this study provided strong evidence that IVS6+5G>C is the potential pathogenic mutation for ADPKD. Meantime, this case also emphasizes the significance of functional analysis of UCVs and genotype-phenotype correlation in ADPKD.

Keywords: atypical splice mutation, autosomal dominant polycystic kidney disease, genetic test, PKD genes

INTRODUCTION

Autosomal-dominant polycystic kidney disease (ADPKD) is a common hereditary disorder with highly heterogeneity, featuring distinct kidney enlargement and finally progressive renal insufficiency. The development of cysts is always found in the bilateral kidneys, but could also present in other organs, such as liver and pancreas.[1] ADPKD develops about 1.5 million individuals in China, and the prevalence of this disease is estimated to vary from 1% to 2% worldwide.[2] Approximately 10% of cases with end-stage renal disease (ESRD) were ADPKD, which is the fourth most common cause of kidney failure worldwide.[3]

Two mapped genes, PKD1 and PKD2, encoding polycystin-1 (PC-1) and polycystin-2 (PC-2) respectively, are identified to cause ADPKD.[4,5] The pathogenic variants of PKD1 and PKD2 lead to the dysfunction of the corresponding protein products, giving rise to aberrant cellular signalling pathways with increased or disorganised fluid secretion and cell growth that brings about fluid accumulation and cyst formation.[6-9]

Variants in the genes PKD1 and PKD2 have been reported to account for approximately 85% and 15% of individuals with ADPKD, respectively.[10] However, a higher incidence of PKD2 (ranging from 26 to 36%) has been reported in a recent study.[11] Additionally，the gene GANAB has also been identified to cause ADPKD recently.[12] Kidney progression of ADPKD is highly heterogeneous, partly result from different genetic factors (gene locus effect, modifier genes, even the unknown pathogenic genes) and environmental backgrounds.[13-16] After adjusting for age, individuals with PKD1 mutations have larger kidneys and earlier age of onset of ESRD than those with PKD2 mutations.[13,14,16] In addition, significant intra-familial kidney disease variability in ADPKD indicates a potential modifier effect exist.[17-19]

In this study, we report a novel atypical splicing variate in PKD2, which is presumed to cause ADPKD, in a Chinese family with several patients. To afford more evidence for its

pathogenicity, the variant was also tested in the normal control. Coupled with this, the importance of functional analysis of unclassified variants (UCVs) to distinguish the likely pathogenic mutation from the polymorphisms for clinical experience is also discussed.

METHODS

A large Chinese family was enrolled from our hospital (Fig. 1). In the family tree, 4 individuals were diagnosed as patients with suspected ADPDK by ultrasound examination according to Ravine's criteria.[20] The proband II-3, who was affected by polycystic kidney disease, had many cysts in the bilateral kidneys and liver, but her blood pressure, serum urea nitrogen, creatinine and uric acid concentration were normal. In order to pinpoint the clinical diagnosis, the targeted clinical exome sequencing was used to identify the potential genetic alterations because of the highly heterogeneity of polycystic kidney disease. Blood samples were extracted from the proband, available members of her family including I-1, II-2, II-5, III1-3, and from the normal control.

A custom capture array (NimbleGen, Roche) targeting 191 genes, which were related with the main types of hereditary urinary system diseases, was used in this study. Briefly, the genomic DNA was extracted from peripheral blood using a Qiagen genomic DNA isolation kit (Qiagen, Hilden, Germany), and the genomic DNA was then hybridized with the capture array to enrich the targeted exonic DNA, which was then sequenced by the Illumina hiseq2500 platform following the standard instructions.[21] Raw image files were carried out by the Bcl2Fastq for base calling and producing the raw data. Low quality sequences (the quality score < 20) were filtered out. SNPs and indels in the sequence were identified using Samtools and Pindel. The potential pathogenic mutations identified were validated by Sanger sequencing analyses in the proband, her 6 family members (Fig. 1) and the normal control. Segregation was performed by sequence analysis of the related mutations in the family

members, and the significance of the atypical splicing mutation was predicted using assessment tool: Mutation Taster software.

RNA expression studies were conducted using primers located in the flanking of PKD2 exon 6 as described previously[22] and the data about primers is expressed in Table 1. Total RNA was isolated from the peripheral blood using TransZol Up (TransGen Biotech, China) and cDNA was produced by reverse transcription using the Primescript RT reagent kit based on the manufacturer's instruction. Because of the amount of blood specimen was limited, the real-time RT-PCR was not further performed.

The study was conducted in conformity with the declaration of Helsinki and was approved by the ethics committee of West China Hospital of Sichuan University. Written informed consent was obtained from each participant.

RESULTS

The family history was further studied after the proband (II-3), a 51-year-old women, was diagnosed with suspected ADPKD (Table 2). The family members (I-1, II-2, II-5 and III1-3) were advised to do relevant examination, and the results indicated that the proband, II-2 and II-5 had the same disease and their serum creatinine were all normal without obvious symptoms and signs. I-1 and III1-3 were normal both in ultrasound examination and renal function. The proband’s father (I-2) died 8 years ago because of cerebral haemorrhage, but was diagnosed with polycystic kidney disease by ultrasound examination and his renal function is normal. The proband’s mother (I-1) is healthy to date. So we supposed these three sisters' suspected ADPKD was inherited from their father, and the family tree drawing was revealed in the Fig. 1.

Using targeted DNA-HiSeq, only one atypical splicing change (IVS6+5G>C) of PKD2 was detected in the proband. Segregation analysis confirmed that the patients II-2 and

II-5 were also heterozygous for this variate. But the unique variant was not found in any unaffected family members including I-1, III1-3 (Fig. 2). Meanwhile, MutationTaster prediction demonstrated that this variation was disease-causing mutation. In addition, RT-PCR analysis was preformed (Fig. 3), and agarose-gel electrophoresis demonstrated two distinct PCR products with cDNA of the proband and the normal control: an exactly spliced exon 6 fragment (375 bp) and a smaller sized PCR product (146 bp) (Fig. 3A). To some extent, it was likely that patients (II-2 and II-3) with PKD2 mutations had a lower full-length mRNA levels versus the normal control and other family members without ADPKD. Sequencing of the lower fragment revealed that exon 6 was skipped (PKD2 c.1320_1548del229) (Fig. 3B). This variant, IVS6+5G>C, is predicted to generate a truncated type of PC2 with dysfunction that lacks the C-terminal domain and five normal transmembrane domains.[22]

DISCUSSION

Targeted next-generation sequencing (NGS) for PKD1 and PKD2 genes has been available and could be as a useful method for improving diagnosis of ADPKD. This approach is especially helpful in the clinical assessment of the risk for younger individuals with doubtful imaging results and in subjects with de novo mutations of PKD genes. Recently, comprehensive test of mutations in PKD1 and PKD2 by two studies have reported that atypical splice mutations bear responsibility for 3.5% to 5% of ADPKD.[23] Among the UCVs cases, it may be difficult to identify the splicing defects, given that the certain silent coding sequence missense mutations can disrupt pre-mRNA processing, with evident influence on the structure of gene product.[24] Furthermore, aberrant splicing could also occur, with missense changes influencing 5’ and 3’ splice sites rather than the classical GT and AG dinucleotides.[25]

Sequencing of both PKD1 and PKD2 genes in the patients of this ADPKD family failed to detect a definite pathogenic mutation, but identify an atypical splice variant in PKD2, IVS6+5G>C, and was shown to co-segregate only with affected subjects. In addition, this mutation nearby a donor site in the PKD2 exon 6 caused the accumulation of higher level of aberrant PKD2 del_Ex6 transcript accompanied by a decline in the expression level of full-length mRNA of PKD2 compared with the normal control. This change predicted to that the variation altered conserved donor splice site of intron 6 and possible used of the last donor splice site in intron 5 (Fig. 4). Tan et al[22] also reported that lower PKD2 del_Ex6 transcript levels were found in the normal controls, but considerably higher level in the patient whose mutation was in PKD2 gene (c.1320G>T). In the present study, no other PKD gene variants were detected in the proband, strongly indicating that this atypical splice mutation was responsible for her ADPKD phenotype. This conclusion was also supported by co-segregate analysis, and bioinformatic analysis which suggested that this variate is predicted to change the normal splicing. However, the possibility that there are mutations which are either intronic (and would not be picked up by this targeted exome approach) or modifiers in another gene that interact with the splice variant, which are causing the disease still exists. In this case, the whole-genome sequencing maybe a better approach than the targeted exome approach.

Kidney progression of ADPKD is highly heterogeneous and more recently, three studies have concluded that the types and locations of mutations, and gender might affect renal disease severity. In the first report, individuals with variants in the 3' half of PKD1 likely had milder kidney disease than those with variants in the 5' half of the gene.[26] In the second report, patients with PKD1 protein truncating (PT) mutations had the most severe kidney disease followed by those with PKD1 small in–frame deletion/insertion (IF indels), PKD1 nontruncating (NT) mutations, and PKD2 mutations;[27] in the third, gender was an

important determinant of renal prognosis in the individuals with PKD2 mutations and women have the best renal outcome, and many will die of older age without progression to ESRD. In addition, the study also revealed that the location of PKD2 variants did not affect the age of onset of ESRD. However, individuals with splice site mutations most likely to have milder kidney disease versus those with other mutation forms (adjusted for the gender effect).[15] In the current study, the proband and her two sisters have been diagnosed with ADPKD for more than 20 years, and they all more than 50 years old, but their renal function is normal. What is more, the proband's father also had a normal kidney function even when he was seriously ill because of cerebral haemorrhage in 70 years old. Taken the above together, the patients in this family will have a better renal prognosis.

ADPKD is a common inherited polycystic kidney disease featuring the formation of fluid-filled cysts, hundreds of different variants have been found, but to date the pathogenesis of most of them has remained elusive, which has generated a huge uncertainty or difficulty for clinical practice. Thus, it is very urgent to determine whether an observed variate is pathogenic or not. To make sure of that, any candidate mutation requires to be carefully investigated, particularly when no other family member could be chosen for co-segregation analysis. Sometimes the functional analysis of the mutation was also very important, particularly for UCVs, just like this case.

In conclusion, the present study has found a unique atypical splice site alteration, IVS6+5G>C, of the *PKD2* gene in a Chinese family, which leads to aberrantly spliced exon 6 that generates a truncated protein forecasted to have void function. This unique splice mutation is also present in normal controls but lower levels. All data available suggests that it might be the pathogenic mutation leading to ADPKD in this family.

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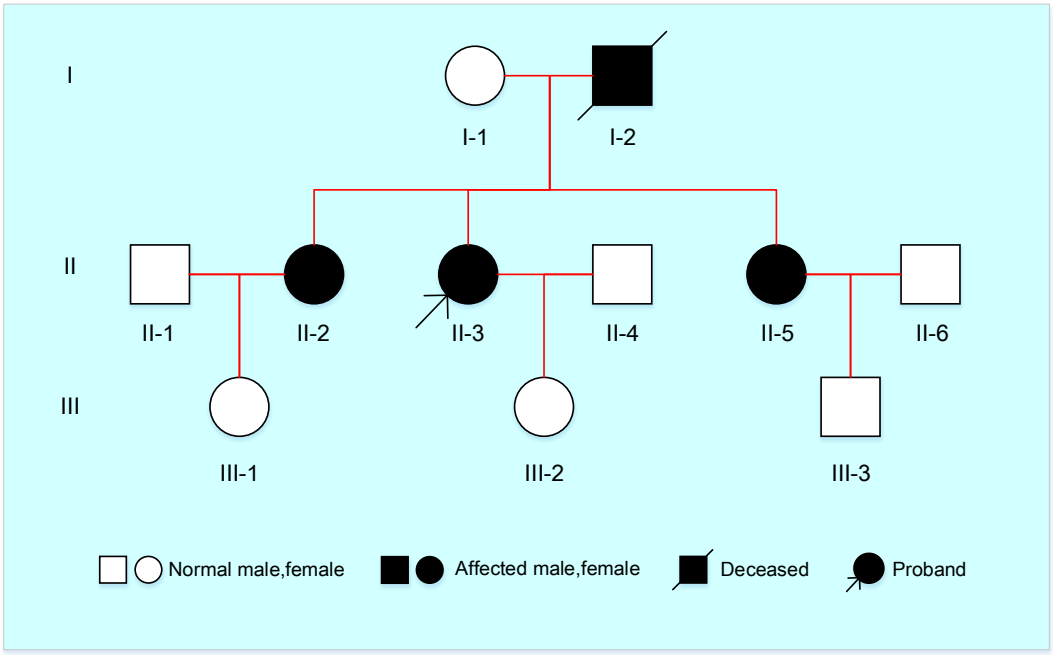

Fig 1. Family pedigree. The arrow indicates the proband. The box with a stripe: deceased family member.

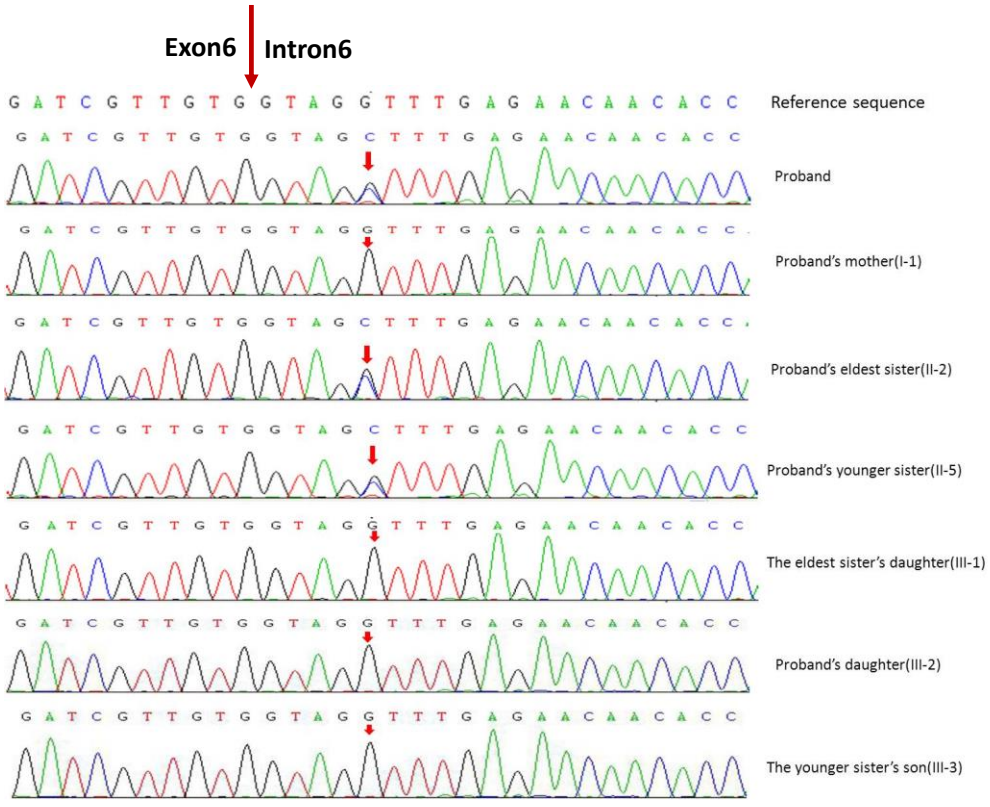

Fig 2. Variation identified of the proband and family members by Sanger sequencing method.

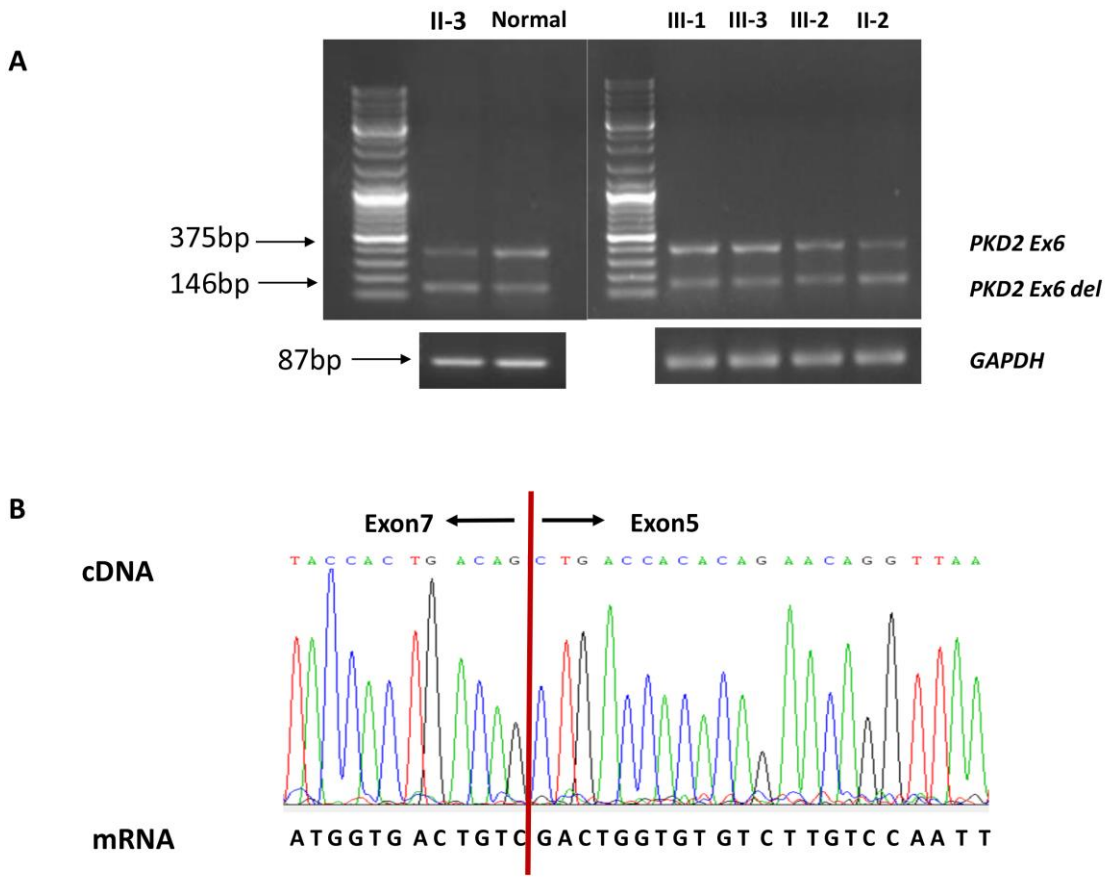

Fig 3. Transcript analysis. A: Presence of PKD2 mRNA splice variants in normal individual, the proband (II-3) and her family members. Pan Primers (Ex6F/Ex6R) generate normal (top 375 bp) and alternate spliced (bottom 146 bp) exon 6 transcripts, respectively, in PBLs of the proband (II-3) and her family members (II-2 and III1-3). GAPGH internal control expression is shown below. **B:** Location and predicted impact of the PKD2 IVS6+5G>C in the proband. Upper panel shows the sequence of the 146 bp fragment in A the conversed donor splice site of intron 6 is altered. Use of the distal donor splice site in intron 5 generates a transcript with 229 fewer nucleotides.

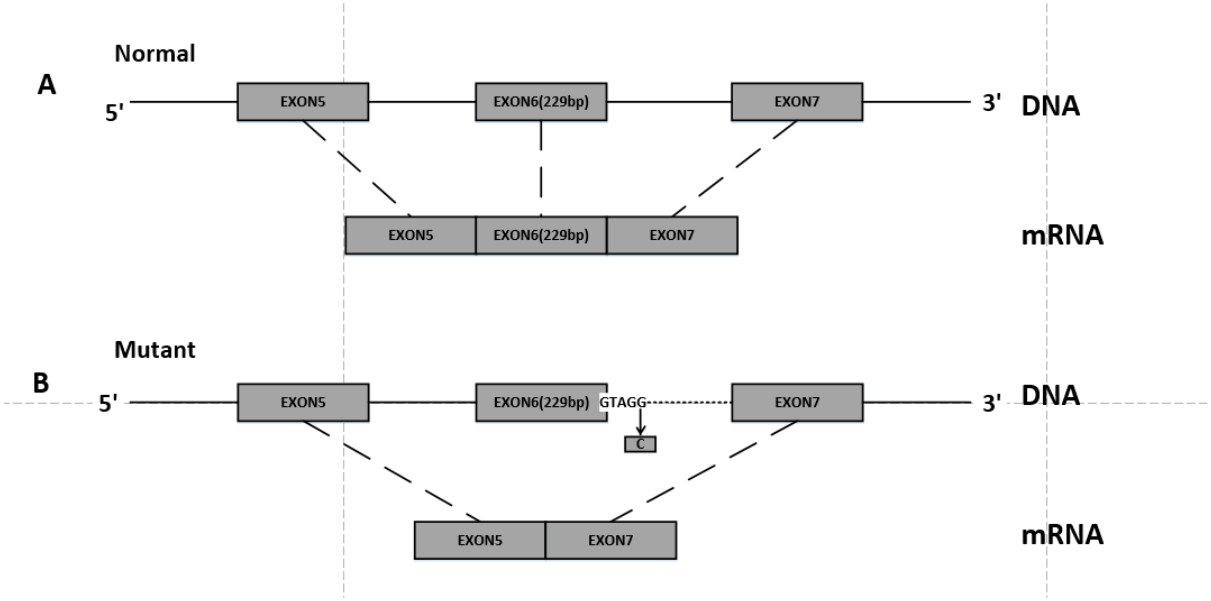

Fig 4. Diagram showing normal splicing of intron 6 (upper) and abnormal splicing at a donor splice acceptor site in exon 6 (lower) due to IVS6+5G>C mutation in the PKD2 gene in the patients of this family.

Table 1 Clinical evaluation of family members.

Patient code	Age on the day (years)	Gender	Polycystic kidney	Polycystic liver	serum creatinine
I-1	78	F	No	No	normal
I-2	Deceased	M	Yes	Yes	normal
II-2	53	F	Yes	Yes	normal
II-3	51	F	Yes	Yes	normal
II-5	47	F	Yes	Yes	normal
III-1	31	F	No	No	normal
III-2	27	F	No	No	normal
III-3	24	M	No	No	normal

Table 2. *PKD2* genes primers used in qualitative RT-PCR

Gene	Primers	Sequence (5'-3')	Primers
PKD2	Forward Reverse	TCTCAGTGTACAACGCCAACA GATGCTCAAAGTTGGGGAAA	E5-E7
GAPDH	Forward Reverse	TGCACCACCAACTGCTTAGC GGCATGGACTGTGGTCATGAG	E7-E8