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, account for 85% and 15% of the ADPKD cases, respectively.

Methods: In this study, the mutation analysis of polycystic kidney disease (PKD) genes was performed 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 for abnormal splicing and assess its potential pathogenicity.

Results: A novel atypical splicing mutation that 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. The RT-PCR revealed the abnormal splicing of exon 6, which thus caused truncating mutation. These findings suggested that the atypical splice site alteration, IVS6+5G>C, in the PKD2 gene was the potential pathogenic mutation leading to ADPKD in this Chinese family.

Conclusion: The data available in this study provided strong evidence that IVS6+5G>C is the potential pathogenic mutation for ADPKD. In addition, our findings emphasised 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 high heterogeneity, featuring distinct kidney enlargement and finally, progressive renal insufficiency. The development of cysts is always in the bilateral kidneys, but they could also be present in other organs such as the liver and pancreas.¹ About 1.5 million individuals in China develop ADPKD, and the prevalence of this disease is estimated to vary from 1% to 2% worldwide.² Approximately 10% of patients with end-stage renal disease (ESRD) have 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 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 the ADPKD cases, respectively.¹⁰ However, a higher incidence of PKD2 (ranging from 26% to 36%) has been reported in a recent study.¹¹ In addition, the gene GANAB has also been identified to cause ADPKD.¹² Kidney progression of ADPKD is highly heterogeneous, partly resulting from different genetic factors (gene locus effect, modifier genes...
and even the unknown pathogenic genes) and environmental backgrounds.\cite{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.\cite{13,14,16}. In addition, significant intrafamilial kidney disease variability in ADPKD indicates that a potential modifier effect exists.\cite{17-19}

In this study, we report a novel atypical splicing variant in PKD2, which is presumed to cause ADPKD, in a Chinese family with several patients. To provide 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 managed at West China Hospital of Sichuan University was enrolled in this study [Figure 1]. In the family tree, four individuals were diagnosed as patients with suspected ADPKD by ultrasound examination according to Ravine’s criteria.\cite{20} The proband II-3, who was affected by polycystic kidney disease (PKD), had many cysts in the bilateral kidneys and liver, but her blood pressure, serum urea nitrogen, creatinine and uric acid concentrations were normal. To pinpoint the clinical diagnosis, targeted clinical exome sequencing was used to identify the potential genetic alterations because of the high heterogeneity of PKD. Blood samples were extracted from the proband, available members of her family, including I-1, II-2, II-5 and III1–3, and from the normal control.

In this study, we used a custom capture array (NimbleGen, Roche, Basel, Switzerland) targeting 191 genes, which were related to the main types of hereditary urinary system diseases. Briefly, the genomic DNA was extracted from peripheral blood using a genomic DNA isolation kit (Qiagen, Hilden, Germany), and the genomic DNA was then hybridised with the capture array to enrich the targeted exonic DNA, which was then sequenced by the Illumina HiSeq2500 platform (Illumina, San Diego, CA, USA) following the standard instructions.\cite{21} Raw image files were processed by the Bcl2Fastq for base calling and producing raw data. Low-quality sequences (quality score <20) were filtered out. Single nucleotide polymorphism 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 six family members [Figure 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 the assessment tool MutationTaster software (https://www.mutationtaster.org/).

RNA expression studies were conducted using primers located in the flanking of PKD2 exon 6 as described previously,\cite{22} and the data about primers are presented in Table 1. Total RNA was isolated from the peripheral blood using TransZol Up (TransGen Biotech, Beijing, China) and cDNA was produced by reverse transcription using the PrimeScript RT reagent kit based on the manufacturer’s instructions. As the amount of blood specimen was limited, real-time reverse transcription-polymerase chain reaction (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 (reference number: 2016(166)). Written informed consent was obtained from each participant.

![Figure 1: Diagram shows the family pedigree.](image)
RESULTS

The family history was further studied after the proband (II-3), a 51-year-old woman, was diagnosed with suspected ADPKD [Table 2]. The family members (I-1, II-2, II-5 and III1–3) were advised to undergo relevant examination, and the results indicated that the proband II-2 and II-5 had the same disease, and their serum creatinine levels were all normal without obvious symptoms and signs. Both the ultrasound examination and renal function of I-1 and III1–3 were normal. The proband’s father (I-2) died 8 years ago because of cerebral haemorrhage; he had been diagnosed with PKD on ultrasound examination but his renal function was normal. The proband’s mother (I-1) remains healthy to date. Thus, we deduced that the three sisters’ suspected ADPKD was inherited from their father [Figure 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. However, the unique variant was not found in any unaffected family member, including I-1 and III1–3 [Figure 2]. Meanwhile, MutationTaster prediction demonstrated that this variation was a disease-causing mutation. In addition, RT-PCR analysis was performed [Figure 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) [Figure 3a]. To some extent, it was likely that patients (II-2 and II-3) with PKD2 mutations had a lower full-length mRNA level 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) [Figure 3b]. This variant, IVS6+5G>C, was predicted to generate a truncated type of polycystin-2 with

| Table 1. Clinical evaluation of family members. |
|-----------------------------------------------|
| Patient code   | Age (yr) | 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          |
| F: female, M: male |

| Table 2. PKD2 gene primers used in qualitative RT-PCR. |
|--------------------------------------------------------|
| Gene   | Primers | Sequence (5′–3′)  | Primers |
| PKD2   | Forward | TCTCAAGTAAAGACCCAAAC | E5–E7   |
|        | Reverse | GATGCTCAAAGTGGGAAAA  |         |
| GAPDH  | Forward | TGACCCCAACTGCTTAGC   | E7–E8   |
|        | Reverse | GCCATGACGTGTCATGAG    |         |

RT-PCR: reverse transcription-polymerase chain reaction

Figure 2: Diagram shows the variation identified of the proband and family members by Sanger sequencing method.
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Figure 3: Transcript analysis. (a) Presence of PKD2 mRNA splice variants in a 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 peripheral blood leukocytes of the proband (II-3) and her family members (II-2 and III-3). GAPDH internal control expression is shown below. (b) Location and predicted impact of PKD2 IVS6+5G>C in the proband. Upper panel shows the sequence of the 146-bp fragment in (a), where the conserved 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.

DISCUSSION

Targeted next-generation sequencing for PKD1 and PKD2 genes has been available and could be a useful method for improving diagnosis of ADPKD. This approach is especially helpful in the clinical assessment of 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 shown that atypical splice mutations bear responsibility for 3.5%–5% of ADPKD. Among the UCV cases, it may be difficult to identify the splicing defects, given that certain silent coding sequence missense mutations can disrupt pre-mRNA processing, with evident influence on the structure of gene product. Furthermore, aberrant splicing could also occur, with missense changes influencing 5′ and 3′ splice sites rather than the classical GT and AG dinucleotides.

Sequencing of both PKD1 and PKD2 genes in this Chinese family failed to detect a definite pathogenic mutation, but identified an atypical splice variant in PKD2, IVS6+5G>C, and it was shown to co-segregate only with affected subjects. In addition, this mutation near a donor site in PKD2 exon 6 caused the accumulation of a higher level of aberrant PKD2 del_Ex6 transcript, accompanied by a decline in the expression level of full-length mRNA of PKD2, as compared to the normal control. This change predicted that the variation altered the conserved donor splice site of intron 6 and possibly used the last donor splice site in intron 5 [Figure 4]. Tan et al. also reported that lower PKD2 del_Ex6 transcript levels were found in normal controls, but considerably higher level was found in a patient whose mutation was in the 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 the proband’s ADPKD phenotype. This conclusion was also supported by co-segregate analysis and bioinformatic analysis, which suggested that this variant is predicted to change the normal splicing. However, there remains a possibility that there are mutations that 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 caused the disease. In this case, whole-genome sequencing may be a better approach than the targeted exome approach.

Kidney progression of ADPKD is highly heterogeneous, and more recently, three studies have concluded that gender and the types and locations of mutations might affect renal disease severity. In the first study, 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 study, patients with PKD1 protein-truncating mutations had the most severe kidney disease followed by those with PKD1 small in-frame deletion/insertion (IF indels), PKD1 nontruncating mutations and PKD2 mutations.\[27\] In the third study, gender was an important determinant of renal prognosis in individuals with PKD2 mutations; women had the best renal outcome, and many would 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 have milder kidney disease versus those with other mutation forms (adjusted for the gender effect).\[13\] In the present study, although the proband and her two sisters were above 50 years old and had been diagnosed with ADPKD for more than 20 years, their renal function was normal. Furthermore, the proband’s father had normal kidney function even when he was seriously ill because of cerebral haemorrhage at the age of 70 years. Taken together, the patients in this family are expected to have a better renal prognosis.

Autosomal dominant polycystic kidney disease is a common inherited PKD featuring the formation of fluid-filled cysts. Hundreds of different variants have been found, but to date, the pathogenesis of most has remained elusive, and this has led to considerable uncertainty or difficulty for clinical practice. Thus, it is critical to determine whether an observed variant is pathogenic or not. To ascertain this, any candidate mutation must be carefully investigated, particularly when no other family member could be chosen for co-segregation analysis. Sometimes, the functional analysis of the mutation is also very important, particularly for UCVs, as in the case of this family.

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 at lower levels. The available data suggest that it might be the pathogenic mutation leading to ADPKD in this family.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES
1. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. Lancet 2007;369:1287-301.
2. Xue C, Zhou CC, Wu M, Mei CL. The clinical manifestation and management of autosomal dominant polycystic kidney disease in China. Kidney Dis (Basel) 2016;2:111-9.
3. Wilson PD. Polycystic kidney disease. N Engl J Med 2004;350:151‑64.
4. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. Cell 1994;77:881‑94.
5. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, et al. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 1996;272:1339‑42.
6. Bycroft M, Bateman A, Clarke J, Hamill SJ, Sandford R, Thomas RL, et al. The structure of a PKD domain from polycystin‑1: Implications for polycystic kidney disease. EMBO J 1999;18:297‑305.
7. Distefano G, Boca M, Rowe I, Wodarczyk C, Ma L, Piontek KB, et al. Polycystin-1 regulates extracellular signal-regulated kinase-dependent phosphorylation of tuberin to control cell size through mTOR and its downstream effectors S6K and 4EBP1. Mol Cell Biol 2009;29:2539‑71.
8. Wegierski T, Steff D, Kopp C, Tauber R, Buchholz B, Nitschke R, et al. TRPP2 channels regulate apoptosis through the Ca2+concentration in the endoplasmic reticulum. EMBO J 2009;28:490‑9.
9. Stekrova J, Reiterova J, Svobodova S, Kebrdlova V, Lnenicka P, Merta M, et al. New mutations in the PKD1 gene in Czech population

Figure 4: Diagram shows (a) normal splicing of intron 6 and (b) abnormal splicing at a donor splice acceptor site in exon 6 due to IVS6+5G>C mutation in the PKD2 gene in the patients of this family.
with autosomal dominant polycystic kidney disease. BMC Med Genet 2009;10:78.

10. Peters DJ, Sandkuijl LA. Genetic heterogeneity of polycystic kidney disease in Europe. Contrib Nephrol 1992;97:128-39.

11. Pei Y, Watnick T. Diagnosis and screening of autosomal dominant polycystic kidney disease. Adv Chronic Kidney Dis 2010;17:140-52.

12. Aldridge M, Patel C, Mallett A, Trnka P. Antenatally diagnosed ADPKD. Kidney Int Rep 2018;3:1214-7.

13. Hateboer N, v Dijk MA, Bogdanova N, Coto E, Saggar-Malik AK, San Millan JL, et al. Comparison of phenotypes of polycystic kidney disease types 1 and 2. European PKD1-PKD2 Study Group. Lancet 1999;353:103-7.

14. Dicks E, Ravani P, Langman D, Davidson WS, Pei Y, Parfrey PS. Incident renal events and risk factors in autosomal dominant polycystic kidney disease: A population and family-based cohort followed for 22 years. Clin J Am Soc Nephrol 2006;1:710-7.

15. Magistroni R, He N, Wang K, Andrew R, Johnson A, Gabow P, et al. Genotype-renal function correlation in type 2 autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2003;14:1164-74.

16. Harris PC, Bae KT, Rossetti S, Torres VE, Grantham JJ, Chapman AB, et al. Cyst number but not the rate of cystic growth is associated with the mutated gene in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2006;17:3013-9.

17. Persu A, Duyme M, Pirson Y, Lens XM, Messiah T, Breuning MH, et al. Comparison between siblings and twins supports a role for modifier genes in ADPKD. Kidney Int 2004;66:2132-6.

18. Paterson AD, Magistroni R, He N, Wang K, Johnson A, Fain PR, et al. Progressive loss of renal function is an age-dependent heritable trait in type 1 autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2005;16:755-62.

19. Fain PR, McFann KK, Taylor MRG, Tison M, Johnson AM, Reed B, et al. Modifier genes play a significant role in the phenotypic expression of PKD1. Kidney Int 2005;67:1256-67.

20. Pei Y, Obaji J, Dupuis A, Paterson AD, Magistroni R, Dicks E, et al. Unified criteria for ultrasonographic diagnosis of ADPKD. J Am Soc Nephrol 2009;20:205-12.

21. Hoefele J, Mayer K, Scholz M, Klein HG. Novel PKD1 and PKD2 mutations in autosomal dominant polycystic kidney disease (ADPKD). Nephrol Dial Transplant 2011;26:2181-8.

22. Tan YC, Blumenfeld J, Michaela A, Donahue S, Balina M, Parker T, et al. aberrant PKD2 splicing due to a presumed novel missense mutation in autosomal-dominant polycystic kidney disease. Clin Genet 2011;80:287-92.

23. Rossetti S, Consugar MB, Chapman AB, Torres VE, Guay-Woodford LM, Grantham JJ, et al. Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2007;18:2143-60.

24. Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: A web resource to identify exonic splicing enhancers. Nucleic Acids Res 2003;31:3568-71.

25. Krawczak M, Reiss J, Cooper DN. The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: Causes and consequences. Hum Genet 1992;90:41-54.

26. Rossetti S, Burton S, Strmecki L, Pond GR, San Millán JL, Zerres K, et al. The position of the polycystic kidney disease 1 (PKD1) gene mutation correlates with the severity of renal disease. J Am Soc Nephrol 2002;13:1230-7.

27. Hwang YH, Conklin J, Chan W, Roslin NM, Liu J, He N, et al. Refining genotype-phenotype correlation in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2016;27:1861-8.