Clinical characteristics and mutation analysis of three Chinese children with autosomal recessive polycystic kidney disease

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Background: **There are few studies on the genotypes and phenotypes of autosomal recessive polycystic kidney disease in Chinese patients.**

Methods: ***PKHD1*** **mutations in three children were detected with PCR and direct sequencing, and their clinical data were retrospectively reviewed.**

Results: **All of the children had bilateral enlarged polycystic kidneys, congenital hepatic fibrosis and intrahepatic bile duct dilatation. One of three children had classical multiple small cysts throughout the kidneys, and the other two children had bilateral multiple renal cysts of various sizes. Two children had abnormally shaped livers, portal hypertension and splenomegaly. Two heterozygous mutations (p.T36M, and p.P137S) were detected in Patient 1 and two were detected in Patient 2 (p.L2658X and p.V836A). One heterozygous mutation (p.L1425R) was detected in Patient 3.**

Conclusions: **The study shows that renal and liver phenotypes of the Chinese children varied. Five mutations were identified in the three children, three of which were novel mutations.**

World J Pediatr 2014;10(3):271-274

Key words: **cirrhosis;**
mutation;
polycystic kidney

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doi: 10.1007/s12519-014-0503-z

Introduction

Autosomal recessive polycystic kidney disease (ARPKD) (OMIM_263200) is a severe and complex genetic kidney and liver disease. To date, polycystic kidney and hepatic disease 1 (*PKHD1*) is the only identified causative gene for ARPKD, and its longest open reading frame encodes the 4074 amino acid fibrocystin.[1] The typical manifestations of ARPKD are nonobstructive fusiform dilation of the bilateral renal collecting ducts, abnormal development of bile ductules and congenital hepatic fibrosis (CHF). But the broad spectrum of clinical features makes it difficult to confirm diagnosis of ARPKD. Genetic diagnosis of ARPKD based on mutation analysis may assist in making a correct diagnosis. Although the incidence of ARPKD is estimated to be 1 in 20,000 live births, and the carrier frequency is approximately 1 in 70, studies on the genotypes and phenotypes of Chinese ARPKD patients has only been conducted in three ARPKD patients to date. Therefore, with the aim of collecting more data on the genotypes and phenotypes of ARPKD in Chinese patients, we analyzed the clinical characteristics and results of mutation analysis of the *PKHD1* gene in three Chinese children with ARPKD.

Methods

We retrospectively reviewed clinical data of ARPKD patients in the Hereditary Kidney Disease Database of Peking University First Hospital from 2009 to 2013. Patients were diagnosed according to the current Clinical Diagnostic Criteria for ARPKD modified from Zerres.[2,3] Genomic DNA was extracted from blood from the peripheral vein. All encoding exons and exon–intron boundaries in the *PKHD1* gene (NM_138694) were amplified by PCR and direct sequencing was performed.[1,4] The study was approved by the Ethics Committee of Peking University First Hospital. All of the patients and their parents were informed and signed informed consents.

Brief report

Results

Case presentations

Patient 1

Patient 1 was a 7-year-old boy who presented with hepatosplenomegaly. His blood pressure was normal. Laboratory evaluation showed leucopenia, thrombocytopenia and mild proteinuria. Serum creatinine levels, creatinine clearance and liver synthetic function including alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase and alkaline phosphatase were normal. Abdominal ultrasound showed bilateral enlargement of the kidneys, loss of corticomedullary differentiation and polycystic kidneys. Abdominal CT showed multiple small kidney cysts, tiny stones, hepatosplenomegaly and portal hypertension. Abdominal MRI showed enlarged kidneys, multiple tiny bilateral kidney cysts, intrahepatic bile duct dilatation, hepatic fibrosis, splenomegaly and an abnormally shaped liver with a disproportionately increased ratio of the left lobe (Fig. A&B). The parental abdominal ultrasounds were normal.

Patient 2

Patient 2 was a 4-year-old boy who presented with polycystic kidneys and CHF. Laboratory evaluation showed that serum creatinine levels, liver synthetic function and urinalysis were normal. Abdominal ultrasound showed enlarged kidneys, increased echoes in the medulla, loss of corticomedullary differentiation, normal liver size and diffused low liver echoes. Enhanced abdominal CT showed enlarged kidneys, multiple renal cysts, intrahepatic bile duct dilatation and normal spleen (Figure 1C and D). The blood pressure was normal. The parents had no renal cysts or suggestion of another disease associated with the kidney cysts.

Patient 3

Patient 3 was a 2-year-old boy who presented with polycystic kidneys and CHF. Urinalysis showed the presence of a mild amount of protein in the urine. Abdominal ultrasound showed enlarged kidneys, multiple renal cysts and periportal fibrosis. Abdominal MRI showed enlarged kidneys, multiple bilateral kidney cysts of different sizes, hepatosplenomegaly, intrahepatic bile duct dilatation and an abnormally shaped liver with a disproportionately increased ratio of the left lobe (Fig. E&F). Parental ultrasounds were negative for liver diseases or kidney cysts.

Mutation analysis of the *PKHD1* gene

The molecular genetic results of the children are shown in the Table. *PKHD1* is a large gene variably assembled into a number of alternatively spliced transcripts that produce alternative isoform products. The sequences of 66 encoding exons (E2–E67) in the *PKHD1* gene were analyzed by BioEdit and SeqMan in DNAStar software. Every variant was checked against mutation databases, including Ensemble Database, Human Genome Mutation Database, and *PKHD1* Specific Mutation Database (http://www.humgen.rwth-aachen.de), to confirm whether the variant was previously published. Mutation analysis revealed 11 variants in the study, of which, seven were previously described and four were novel (p.P137S, p.V836A and p.Q1574H and p.L2658X). Two of the seven previously described variants (p.T36M and

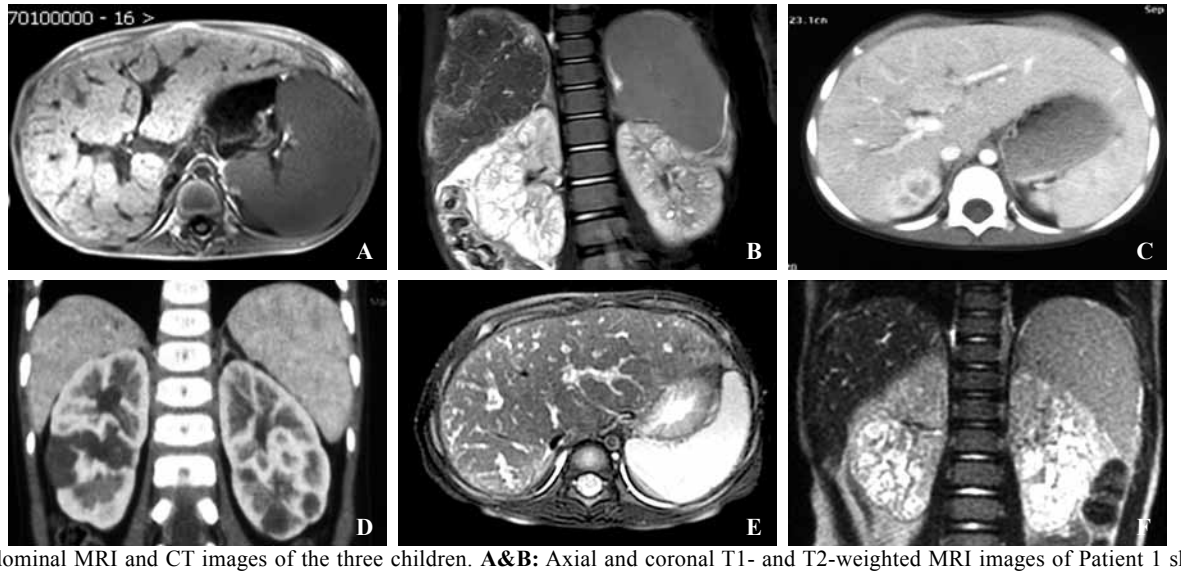

Fig. Abdominal MRI and CT images of the three children. **A&B:** Axial and coronal T1- and T2-weighted MRI images of Patient 1 showing enlarged kidneys, multiple tiny bilateral kidney cysts, intrahepatic bile duct dilatation and hepatosplenomegaly; **C&D:** Axial and coronal enhanced CT images of Patient 2 showing enlarged kidneys, multiple renal cysts and intrahepatic bile duct dilatation; **E&F**: Axial and coronal T2-weighted MRI images of Patient 3 showing enlarged kidneys, multiple bilateral kidney cysts, intrahepatic bile duct dilatation and hepatosplenomegaly.

p.L1425R) were pathogenic mutations.

For the three novel missense variants, the following methods were used to assess pathogenicity: (a) testing variant in 100 control chromosomes; (b) evaluating pathogenicity using PolyPhen and SIFT databases; (c) multiple sequence alignment in UCSC by comparing the conservation in five species: Pan troglodytes (chimp), Mus musculus (mouse), Canis lupis familiaris (dog), and Gallus gallus (chicken); (d) identifying their locations in the region of fibrocystin in the ExPASy database. Combining the above methods, two of the three novel missense variants (P137S and V836A) were classified as probably pathogenic variants, and the remaining variant (p.Q1574H) was classified as a neutral variant.

Five *PKHD1* mutations were identified in the three children in the study, which included three novel and two previously described mutations. Two compound heterozygote mutations were identified in Patient 1: p.T36M from his father and p.P137S from his mother. Two compound heterozygote mutations were identified in Patient 2: p.L2658X from his mother and p.V836A from his father. Only one compound heterozygote mutation from the father (p.L1425R) was identified in Patient 3.

Discussion

In this study, the three children were from independent families and had bilateral enlarged polycystic kidneys, CHF and intrahepatic bile duct dilatation fulfilling the Clinical Diagnostic Criteria for ARPKD,[2,3] however, their renal and liver phenotypes varied. With respect to cystic kidney disease, Patient 1 had classical multiple small cysts throughout the kidneys, but the remaining two children had bilateral multiple renal cysts of various sizes similar to a report of a 15-year-old Chinese ARPKD boy.[11] In a North American study,[2] ultrasound findings revealed echogenic kidneys with gross cysts in 50% of patients. Moreover, in the present study, the cystic kidney disease of the children involved both the renal cortex and medulla. A study by Guay-Woodford et al[12] reported that renal abnormalities involving both the renal cortex and medulla were observed in 63% of patients, the entire medulla only in 13%, and parts of the medulla only in 24%. In addition, Patient 1&3 were found to show mild proteinuria, and multiple tiny stones were detected in Patient 1. A recent study indicated that nephrocalcinosis and proteinuria were frequently observed in their ARPKD patients.[13]

The chief pathological characteristic of ARPKD liver disease is ductal plate malformation with associated periportal fibrosis owing to bile duct dysplasia. In the present study, all children presented with liver diseases, including CHF and intrahepatic bile duct dilatation. But Guay-Woodford et al. found that the presenting symptoms were related to liver disease in 26% of ARPKD patients who were older children, adolescents, or even adults.[12] In addition, Patient 1&3 had portal hypertension, splenomegaly and abnormally shaped livers with a disproportionately increased ratio of the left lobe that were not detected in Patient 2. An evaluation of liver disease in ARPKD patients showed portal hypertension and splenomegaly in 65% of patients, and a disproportionately enlarged left liver lobe in 69% of patients.[12] In the present study, liver synthetic function of two of the children was normal. The finding was consistent with a study that indicated that liver enzyme levels were normal in the majority of patients, and only mildly elevated in alkaline phosphatase and gamma-glutamyl transferase in the minority of ARPKD patients.[12]

Table. *PKHD1* variants identified in the children

Patient No.	Gender	Age at diagnosis	Exon	cDNA change	Amino acid change	Allele frequency	Conserved in five species†	PolyPhen prediction (score)	SIFT Prediction (score)	ExPASy	References
1	Male	7 y	3	c.107C>T	p.T36M*						5-8
			6	c.409 C>T	p.P137S*	0/100	Yes	Probably damaging (1.000)	Tolerated (0.07)	IPT/TIG 2	Novel
			24	c.2489A>G	p.N830S						7-9
			38	c.4722 A>T	p.Q1574H	0/100	Yes, absent in mouse	Probably damaging (0.999)	Tolerated (0.57)	IPT/TIG 11	Novel
2	Male	4 y	22	c.2278C>T	p.R760C						5-10
			24	c.2507 T>C	p.V836A*	0/100	Yes, absent in chicken	Probably damaging (0.991)	Damaging (0.03)	Non- conservative region	Novel
			50	c.7973 T>A	p.L2658X*						Novel
			66	c.11696A>G	p.Q3899R						5-8
			67	c.12143A>G	p.Q4048R						7-10
3	Male	2 y	22	c.2278C>T	p.R760C						5-10
			32	c.3785C>T	p.A1262V						5-10
			32	c.4274T>C	p.L1425R*						11
			66	c.11696A>G	p.Q3899R						5-8
			67	c.12143A>G	p.Q4048R						6-10

*: described as a pathogenic variant; †: conserved in five species: human, chimp, mouse, dog and chicken.

In the present study, five compound heterozygote mutations (p.P137S, p.V836A, p.L1425R, p.L2658X and p.T36M) were identified in the three children. Three of these mutations, p.P137S, p.V836A and p.L2658X, were novel. According to some previous studies that truncating mutations and a large amount of missense variants in the *PKHD1*gene were identified in their ARPKD patients,[4,14] we chose direct sequencing to detect the *PKHD1* mutations in this study. The *PKHD1* mutation detection rate in this study is close to that reported in the literature.[15,16] However, in the current study, Patient 3 was detected with only one heterozygous mutation. Some previous studies have also reported that almost one third of ARPKD families were identified as having only one heterozygous *PKHD1* mutation.[12,14] The reason for this finding might be due to the mutation location being in 3' and 5' UTRs, deep intronic, noncoding exon regions of the gene, or it might be caused by another causative gene mutation. In addition, in the present study, consistent with previous studies,[8,17] every family had their own specific mutations, and patients who survived beyond the newborn period and had normal kidney function were not found without two truncating mutations.

In conclusion, the results of this study showed varying renal and liver phenotypes of three ARPKD children. Only one child had classical multiple small cysts throughout their kidneys. In addition to CHF and intrahepatic bile duct dilatation, portal hypertension, splenomegaly and an abnormally shaped liver with a disproportionately increased ratio of the left lobe were detected in two children. Five mutations were identified in the three children, three of which were novel.

Acknowledgments

We thank Dr. Hui-Jie Xiao, Yong Yao and Fang-Rui Ding for the acquisition and analysis of data in this study.

Funding: This study was supported by grants from the National 12th Five Years Science and Technology Support Project (2012BAI03B02), the National Nature Science Foundation (81070545) and the Beijing Nature Science Foundation (7102148).
Ethical approval: The study was approved by the Ethical Committee of Peking University First Hospital.
Competing interest: None.
Contributors: Shu-Ping Liu, Jie Ding and Fang Wang designed the study and drafted the article. All authors collected and analyzed the data, revised the paper and approved the final manuscript.

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Received April 2, 2014
Accepted after revision May 23, 2014