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ORIGINAL ARTICLE

Basic Study

Screening analysis of candidate gene mutations in a kindred with polycystic liver disease

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Abstract

AIM: To find potential mutable sites by detecting mutations of the candidate gene in a kindred with polycystic liver disease (PCLD).

METHODS: First, we chose a kindred with PCLD and obtained five venous blood samples of this kindred after the family members signed the informed consent form. In the kindred two cases were diagnosed with PCLD, and the left three cases were normal individuals. All the blood samples were preserved at -85 ℃. Second, we extracted the genomic DNA from the venous blood samples of the kindred using a QIAamp DNA Mini Kit and then performed long-range polymerase chain reaction (PCR) with different primers. The exons of *PKD1* were all sequenced with the forward and reverse primers to ensure the accuracy of the results. Next, we purified the PCR products and directly sequenced them using Big Dye Terminator Chemistry version 3.1. The sequencing reaction was conducted with BiomekFX (Beckman). Finally, we analyzed the results.

RESULTS: A total of 42 normal exons were identified in detecting mutations of the *PKD1* gene. A synonymous mutation occurred in exon 5. The mutation was a homozygous T in the proband and was C in the reference sequence. This mutation was located in the third codon and did not change the amino acid encoded by the codon. Missense mutations occurred in exons 11 and 35. These mutations were located in the second codon; they changed the amino acid sequence and existed in the dbSNP library. A nonsense mutation occurred in exon 15. The mutation was a heterozygous CT in the proband and was C in the reference

sequence. This mutation was located in the first codon and resulted in a termination codon. This mutation had an obvious influence on the encoded protein and changed the length of the protein from 4303 to 2246 amino acids. This was a new mutation that was not present in the dbSNP library.

CONCLUSION: The nonsense mutation of exon 15 existed in the proband and in the third individual. Additionally, the proband was heterozygous for this mutation, so the mutable site was a pathogenic mutation.

Key words: Gene mutation; Polycystic liver disease; Kindred

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Core tip: This study explored the mutable sites in the *PKD1* gene and found the potential mutable sites by detecting mutations of the candidate gene in a kindred with polycystic liver disease (PCLD). We analysed five blood samples obtained from the kindred by using long-range polymerase chain reaction and direct sequencing with different primers. We found synonymous mutations in exons 5, 11, 15, and 35 of the *PKD1* gene. Only the mutation in exon 15 was not in the dbSNP library, and it was a heterozygous CT in the proband and was C in the reference sequence. This mutation was located in the first codon and resulted in a termination codon. This nonsense mutation existed also in the third individual, so the mutable site was a pathogenic mutation.

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INTRODUCTION

Polycystic liver disease is a type of autosomal dominant polycystic liver disease. It can be divided into polycystic liver disease associated with autosomal dominant polycystic kidney disease (ADPKD) and the independent type of polycystic liver disease. Currently, four genes have been identified to be associated with the onset of this disease: the polycystic kidney disease genes *PKD1* and *PKD2* and the independent-type polycystic liver genes *PRKCSH* and *SEC63*. Polycystic liver disease often has a familial history. Simple polycystic liver disease is rare, and patients with polycystic liver disease account for more than half of the cases of ADPKD. The disease is common in women over 40 years old, and cysts can grow during pregnancy or from taking estrogen drugs. The patients with polycystic liver disease associated with ADPKD often have kidney damage. Renal complications are important factors that cause the death of patients. In the present study, we used long-range polymerase chain reaction (PCR) and direct sequencing with different sequencing primers for screening mutations in a kindred with polycystic liver disease, with aims to provide a theoretical basis to conduct genetic research of polycystic liver disease, assist in prenatal diagnosis in the future and clarify the patterns and characteristics of polycystic liver disease gene mutations.

MATERIALS AND METHODS

Ethics statement

This study was approved by the medical Ethics Committee of the Shandong Cancer Hospital. We collected clinical data from the members of the polycystic liver disease pedigrees after they signed the informed consent form. Among 5 peripheral blood samples obtained, 2 were collected from patients (Ⅲ1, Ⅳ3) diagnosed with polycystic liver disease and 3 from healthy subjects (Ⅲ2, Ⅳ4, Ⅳ5). We stored the blood samples at -85 ℃ in an ultra-low temperature freezer.

Clinical data

The clinical data of three generations of a kindred with polycystic liver disease are shown in Figure 1. The proband (Ⅲ1) was a 44-year-old woman who was diagnosed with polycystic liver disease associated with ADPKD and had undergone an operation (Figures 2-4). Case Ⅲ2 was the husband of the proband who was 46 years old and did not have the disease. Case Ⅳ3 was the older daughter of the proband who was 24 years old and also had polycystic liver disease. Case Ⅳ4 was the youngest daughter of the proband who did not have the disease. Case Ⅳ5 was the proband's nephew who did not have the disease. The proband's mother died from polycystic liver disease, and her two uncles both died from polycystic kidney disease.

DNA extraction

We extracted the genomic DNA using the QIAamp DNA Extraction Kit (QIAGEN) from the peripheral venous blood samples of the members of the AKPLD kindred. The QIAamp DNA technique allows to extract genomic, mitochondrial, bacterial, parasitic and viral DNA from human tissue samples and can be used directly for PCR and marker detection. The method ensures obtaining highly purified DNA.

Primer design

The *PKD1* gene contains 46 exons, but the first 30 exons have repeat regions in the genome. The traditional method in which a single exon is amplified and sequenced cannot separate true genetic regions and repeat sequences. Therefore, the repeat regions were amplified twice by using nested PCR, but this was too troublesome. We used the method proposed by

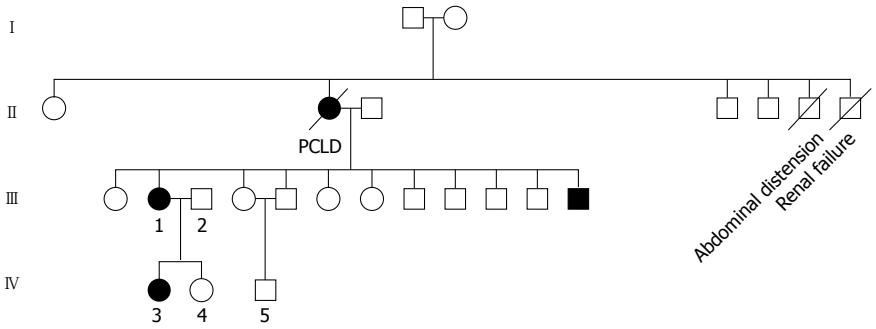

Figure 1 Diagram showing the members of the kindred with polycystic liver disease. PCLD: Polycystic liver disease.

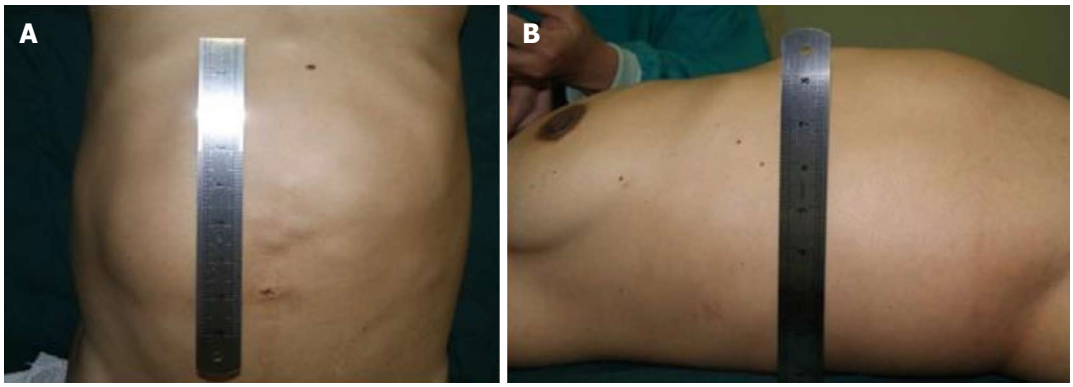

Figure 2 Images showing the proband.

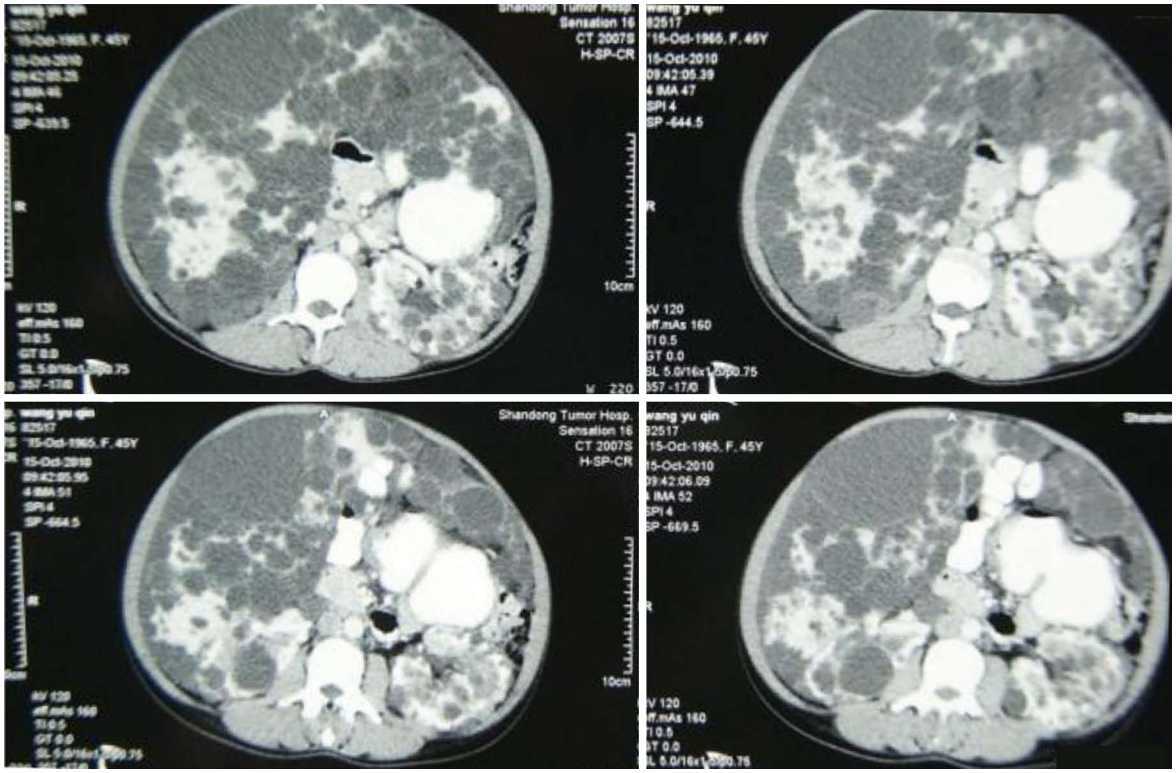

Figure 3 Computed tomography images.

Ying-Cai Tan to synthesize all the *PKD1* amplification and sequencing primers (Tables 1 and 2)[1]. The bidirectional sequencing of the *PKD1* exons ensured the accuracy of the results.

PCR amplification

The PCR reaction setup is shown in Table 3. Because the amplified products were too long, we added dimethyl sulfoxide (DMSO) in the system to improve

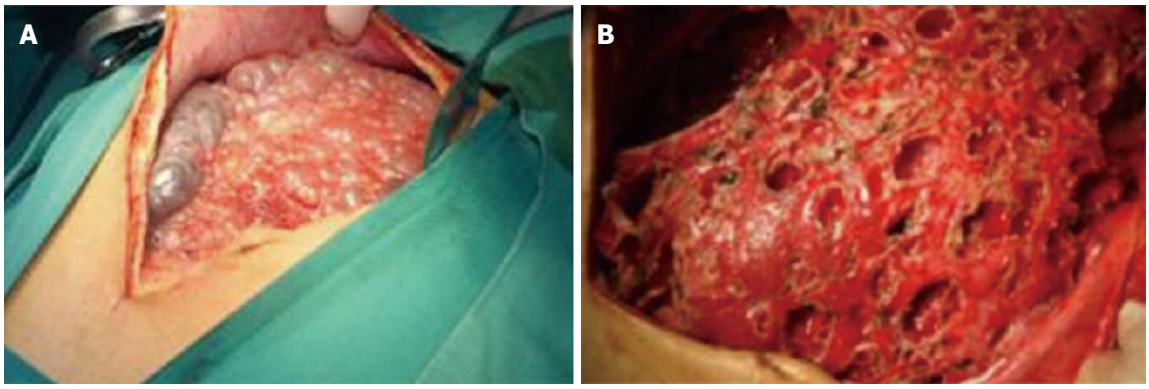

Figure 4 Images showing the findings in the operation.

Table 1 Primers for amplification of PKD1 b long-range polymerase chain reaction

Fragment	Size (bp)	Exon	Forward primer	Reverse primer	Temperature (℃)
Exon 1	2278	1	5′-CGCAGCCTTACCATCCACCT-3′	5′-TCATCGCCCCTTCCTAAGCA-3′	64
Exons 2-7	4041	2-7	5′-CCCCGAGTAGCTGGAACTACAGTTACACACT-3′	5′-CGTCCTGCTGTGCCAGAGGCG-3′	70
Exons 8-12	3893	8-12	5′-ACGTCTGCGAGCTGCAGCCC-3′	5′-CTGCAGGGACAGGCGTCAGTGA-3′	70
Exons 13-15	4391	13-15	5′-TGGAGGGAGGGACGCCAATC-3′	5′-GTCAACGTGGGCCTCCAAGT-3′	65
Exons 15-21	5253	15-21	5′-ATCCCTGGGGGTCCTACCATCTCTTA-3′	5′-ACACAGGACAGAACGGCTGAGGCTA-3′	68
Exons 22-26	3276	22-26	5′-ATGCTTAGTGAGGAGGCTGTGGGGGTCCA-3′	5′-GCTTAAAGGGGAATGGCTTAAACCCG-3′	70
Exons 27-34	3916	27-34	5′-CGGGTCACCGGTTGTGGCA-3′	5′-ATGAGGCTCTTTCCACAGACAACAGAGGTT-3′	70
Exons 35-41	2632	35-41	5′-CAAGAGGCTCAAGAAACTGCCCG-3′	5′-GGGCTGTGGAAGCCGCCTA-3′	68
Exons 42-46	2370	42-46	5′-GAGTAGTTCTCCAGGAGTGCCG-3′	5′-ATTCTGCCTGGCCCTCGGCCTT-3′	63

the efficiency. The PCR conditions were as follows: (1) fragment one: 94 ℃ for 4 min; followed by 35 cycles of 94 ℃ for 40 s, 62 ℃ for 1 min, and 65 ℃ for 150 s, with a final extension step at 65 ℃ for 10 min; (2) fragments two and three: Touchdown protocol composed of an initial step of 94 ℃ for 2 min; followed by 14 cycles of 94 ℃ for 30 s, 74 ℃ for 60 s, with a decrease of 0.5 ℃ per cycle, and 65 ℃ for 4 min; followed by 30 cycles of 94 ℃ for 30 s, 65 ℃ for 60 s, and 65 ℃ for 4 min, with a final extension step of 65 ℃ for 10 min; (3) fragment four: 94 ℃ for 2 min, followed by 35 cycles of 94 ℃ for 20 s and 65 ℃ for 5.5 min, with a final extension step at 65 ℃ for 10 min; (4) fragment five: 94 ℃ for 2 min, followed by 35 cycles of 94 ℃ for 30 s, 68 ℃ for 30 s, and 65 ℃ for 5 min, with a final extension step at 65 ℃ for 10 min; and (5) fragments six to nine: 94 ℃ for 2 min; followed by 14 cycles of 94 ℃ for 30 s, 66 ℃ for 30 s with a decrease of 0.5 ℃ per cycle, and 65 ℃ for 3.5 min; followed by 30 cycles of 94 ℃ for 30 s, 59 ℃ for 30 s, and 65 ℃ for 3.5 min, with a final extension step of 65 ℃ for 10 min.

The long-range PCR amplification products were analyzed by 2% agarose gel electrophoresis. In Figure 5, PCR amplification products for fragments 1 to 9 are shown from the left to the right. The number and size of the fragments are shown in Table 1.

DNA sequencing

The DNA was sequenced by the DNA dideoxy sequencing method using a Biomek FX Beckman fluid moving workstation. We purified the PCR amplification products using the Zymoclean Gel DNA Recovery Kit (D4001), quantified the DNA with a NanoDrop 8000 spectrophotometer and sequenced the DNA with an ABI 3730XL automated DNA sequencer.

A total of 42 normal exons were identified in detecting mutations of the *PKD1* gene. A synonymous mutation occurred in exon 5. The mutation was a homozygous T in the proband, which was C in the reference sequence. It was located in the third codon and did not change the amino acid encoded by the codon. Missense mutations occurred in exons 11 and 35. These mutations were located in the second codon; they changed the amino acid sequence and existed in the dbSNP library. A nonsense mutation occurred in exon 15. This mutation was a heterozygous CT in the proband, which was C in the reference sequence. It was located in the first codon and resulted in a termination codon. This was a new mutation that was not deposited in the dbSNP library.

DISCUSSION

Polycystic liver disease can be divided into two types according to its clinical manifestations: polycystic liver disease related to polycystic kidney disease and the independent type of polycystic liver disease[2]. At present, etiological studies have delved deep into the levels of molecules and genes, and four genes have

Table 2 Primers for sequencing of *PKD1* exons

Primer name	Sequence	Genomic position (starting from the 5′ end)	Distance to exon-intron junction	Exons covered
PKD1-exon1-F	5′-GCGTCGCTCAGCAGCAGGT-3′	2185830	-140 Ex1	1
PKD1-exon1-R	5′-GCCCGCGTCCTGCTTCCC-3′	2185383	93 Ex1	
PKD1-exon2-3-F	5′-GGGATGCTGGCAATGTGTGGGAT-3′	2169468	-89 Ex2	2-3
PKD1-exon2-3-R	5′-GGACCAACTGGGAGGGCAGAA-3′	2169038	77 Ex3	
PKD1-exon4-F	5′-GGCGGTGCTGTCAGGGTG-3′	2168948	-102 Ex4	4
PKD1-exon4-R	5′-CCAGAGAGGCCTTCCTGAGC-3′	2168582	95 Ex4	
PKD1-exon5A-F	5′-GAACAGCATGGGAGCCTGTGAGT-3′	2168561	-98 Ex5	5
PKD1-exon5A-R	5′-AGCCGGCCCAGCGGCATC-3′	2168033	Within an exon	
PKD1-exon5B-F	5′-GCCTGTCCCTCTGCTCCG-3′	2168262	Within an exon	5
PKD1-exon5B-R	5′-GTGTCAACGGTCAGTGTGGGC-3′	2167696	96 Ex5	
PKD1-exon6-F	5′-GTGTCTGCTGCCCACTCCC-3′	2167787	-124 Ex6	6
PKD1-exon6-R	5′-CTCCTTCCTCCTGAGACTCCC-3′	2167397	93 Ex6	
PKD1-exon7-F	5′-GCTGCTGTGAGGGTGGGAGGA-3′	2167120	-66 Ex7	7
PKD1-exon7-R	5′-TCCACCGCGGGCGCTCGGCA-3′	2166763	71 Ex7	
PKD1-exon8-F	5′-CTGGGCTGAGGAGGAGGG-3′	2166760	-115 Ex8	8
PKD1-exon8-R	5′-GGGCACAAGCAACATTAAGGCCC-3′	2166413	117 Ex8	
PKD1-exon9-F	5′-CCTCTTCCTGGGAAGTTCGGGT-3′	2166206	-87 Ex9	9
PKD1-exon9-R	5′-ACTCTGGTGGCCACAGGACCA-3′	2165895	98 Ex9	
PKD1-exon10-F	5′-GCAGGCAGTTGGGCATCTCTG-3′	2165697	-71 Ex10	10
PKD1-exon10-R	5′-GACCCTGGGCAGCAGACAG-3′	2165309	70 Ex10	
PKD1-exon11A-F	5′-GTGTGGCTGACGAAGCGGG-3′	2165035	-109 Ex11	11
PKD1-exon11A-R	5′-CCGTGGCGTTGGCACCAG-3′	2164455	Within an exon	
PKD1-exon11B-F	5′-CGCTATGAGGTCCGGGCAG-3′	2164641	Within an exon	11
PKD1-exon11B-R	5′-CCCTCACTGGGAAGCCAGG-3′	2164077	93 Ex11	
PKD1-exon12-F	5′-GGACTCTCCAGCCCGACG-3′	2163387	-94 Ex12	12
PKD1-exon12-R	5′-GCAGAGGTGAAGGTGGAGC-3′	2163088	74 Ex12	
PKD1-exon13-14-F	5′-GTGGAGGGAGGGACGCCAA-3′	2163037	-73 Ex13	13-14
PKD1-exon13-14-R	5′-GTCACAGTGAGGGCTGTTGGG-3′	2162230	111 Ex14	
PKD1-exon15A-F	5′-TTCTGCCGAGCGGGTGGG-3′	2161938	-64 Ex15	15
PKD1-exon15A-R	5′-CATGTCGAAGGTCCACGTGATGT-3′	2161427	Within an exon	
PKD1-exon15B-F	5′-GACATGAGCCTGGCCGTGG-3′	2161516	Within an exon	15
PKD1-exon15B-R	5′-CCACCTCTGGCTCCACGCA-3′	2161027	Within an exon	
PKD1-exon15C-F	5′-CACGCGGAGCGGCACGTT-3′	2161121	Within an exon	15
PKD1-exon15C-R	5′-GGTGACCTCCGGACCCTC-3′	2160626	Within an exon	
PKD1-exon15D-F	5′-TCTGCTGTGGGCCGTGGG-3′	2160706	Within an exon	15
PKD1-exon15D-R	5′-CTGTACCGTGTGGTTGGTGGG-3′	2160215	Within an exon	
PKD1-exon15E-F	5′-ACAGCATCTTCGTCTATGTCCTG-3′	2160303	Within an exon	15
PKD1-exon15E-R	5′-GGTTCCCTGCCGTCATGGTG-3′	2159812	Within an exon	
PKD1-exon15F-F	5′-GGGCTGAGCTGGGAGACCT-3′	2159899	Within an exon	15
PKD1-exon15F-R	5′-GACAGCTGAGCCGGCAGC-3′	2159417	Within an exon	
PKD1-exon15G-F	5′-CTGTGGGCCAGCAGCAAGGT-3′	2159494	Within an exon	15
PKD1-exon15G-R	5′-CGTGCGGTTCTCACTGCCCA-3′	2159012	Within an exon	
PKD1-exon15H-F	5′-GACGTCACCTACACGCCCG-3′	2159095	Within an exon	15
PKD1-exon15H-R	5′-CCTCCCAGCGGTACTCAGTCT-3′	2158603	Within an exon	
PKD1-exon15I-F	5′-GATGCGGCGATCACAGCGCA-3′	2158688	Within an exon	15
PKD1-exon15I-R	5′-GGCCAGCCCTGGTGGCAA-3′	2158164	89 Ex15	
PKD1-exon16-F	5′-GGCCCGTCCTCAGTGCCT-3′	2158167	-134 Ex16	16
PKD1-exon16-R	5′-GCGGCCTCCACCAGCACTA-3′	2157790	94 Ex16	
PKD1-exon17-18-F	5′-GAAACCTGGAGTTTGGGAGCAGC-3′	2157047	-98 Ex17	17-18
PKD1-exon17-18-R	5′-TGACGTCACAGAGTCGGG-3′	2156344	55 Ex18	
PKD1-exon19-20-F	5′-GCACGGGTGAGTGCAGGC-3′	2156404	-99 Ex19	19-20
PKD1-exon19-20-R	5′-CCGGGATGAGCCCTCTGCAA-3′	2155768	98 Ex20	
PKD1-exon21-F	5′-AGTCGTGGGCATCTGCTGGC-3′	2155550	-75 Ex21	21
PKD1-exon21-R	5′-CAAGCTGCCCGTCTGCCCT-3′	2155240	83 Ex21	
PKD1-exon22-F	5′-CAGGTGAGGACCCGTGTAGAGA-3′	2154725	-82 Ex22	22
PKD1-exon22-R	5′-GGGAGGAGGGAGGCAGAG-3′	2154431	68 Ex22	
PKD1-exon23A-F	5′-GCACCTCGCTCTCTGCCC-3′	2154024	-128 Ex23	23
PKD1-exon23A-R	5′-GCCACCTTGGTGGAGACGG-3′	2153512	Within an exon	
PKD1-exon23B-F	5′-GGCTGCCACTTCTCCATCCC-3′	2153651	Within an exon	23
PKD1-exon23B-R	5′-GACACCCATGGAAGCCCTACG-3′	2153183	84 Ex23	
PKD1-exon24-F	5′-CGTGGCAGAGGGTGGGCT-3′	2153064	-93 Ex24	24
PKD1-exon24-R	5′-CTCGCTGCCTGCCGTCCC-3′	2152721	94 Ex24	
PKD1-exon25-26-F	5′-GGCTCTGAGACTGCGACATCCAA-3′	2152702	-68 Ex25	25-26
PKD1-exon25-26-R	5′-CTTGTTCTGACGCCTGCGACG-3′	2151964	98 Ex26	
PKD1-exon27-28-F	5′-GCTGAGATGACTTGCCTGGGATG-3′	2150644	-77 Ex27	27-28
PKD1-exon27-28-R	5′-ACTGCAGGAGGCCACGGG-3′	2150094	73 Ex28	

PKD1-exon29-30-F	5′-CTCCGTGGGAGGTTGGGCA-3′	2150142	-70 Ex29	29-30
PKD1-exon29-30-R	5′-CGCCTTTCCCTCTGGCTGC-3′	2149542	103 Ex30	
PKD1-exon31-32F	5′-CGGGCTCTGTCCTGTCTGC-3′	2148079	-94 Ex31	31-32
PKD1-exon31-32R	5′-CCCAGCAAGGACACGCAGC-3′	2147642	87 Ex32	
PKD1-exon33-34-F	5′-GGAAGCCCAGGGTGTCCGT-3′	2147595	-91 Ex33	33-34
PKD1-exon33-34-R	5′-CAGCCCTGCCCTGGCACC-3′	2147034	115 Ex34	
PKD1-exon35-F	5′-CAAGAGGCTCAAGAAACTGCCCG-3′	2144309	-98 Ex35	35-36
PKD1-exon36-R	5′-GAGAAGTACAGGGCTTCCAGCAA-3′	2143714	98 Ex36	
PKD1-exon37-F	5′-CTCGGCTGGGAGCCACTG-3′	2143832	-93 Ex37	37
PKD1-exon37-R	5′-GCCTTCTGAGGTGAGGAAAGGG-3′	2143439	106 Ex37	
PKD1-exon38-F	5′-CCACACCTGCCGCAGCCAT-3′	2143190	-96 Ex38	38
PKD1-exon38-R	5′-CAAAGGTATCTACACATGTCCAC-3′	2142856	99 Ex38	
PKD1-exon39-F	5′-GCCAGCAGGGCAGTGGGA-3′	2142698	-105 Ex39	39
PKD1-exon39-R	5′-CAGCTAGGGAGCAGGGCTGA-3′	2142385	96 Ex39	
PKD1-exon40-F	5′-GTGGCGCCGAACCAGAGC-3′	2142289	-100 Ex40	40-41
PKD1-exon41-R	5′-GGGCTGTGGAAGCCGCCTA-3′	2141678	104 Ex41	
PKD1-exon42-F	5′-CCTCAGCCACGCCTGCACT-3′	2141678	-80 Ex42	42
PKD1-exon42-R	5′-GGGTGAGACGCTGCCGGG-3′	2141338	86 Ex42	
PKD1-exon43-F	5′-CAGCGTCCCTCCCGCCCT-3′	2141213	-38 Ex43	43-44
PKD1-exon44-R	5′-CAGGAAGACACGAGCTGCGG-3′	2140578	97 Ex44	
PKD1-exon45-F	5′-GCTGGCCATCCTGGTAGGTGA-3′	2140687	-96 Ex45	45
PKD1-exon45-R	5′-GGACTTTGTGGCGGAACTGGG-3′	2140180	106 Ex45	
PKD1-exon46-F	5′-GGAGAGGGACACGCCCTG-3′	2140264	-69 Ex46	46
PKD1-exon46-R	5′-ATTCTGCCTGGCCCTCGGCCTT-3′	2139635	Within 3′UTR	

The genomic primer positions were according to the February 2009 human reference sequence (GRCh37/hg19). Ex: Exon; F: Forward; R: Reverse; UTR: Untranslated region.

Table 3 Polymerase chain reaction reaction system setup

Polymerase chain reaction reaction system	Volume (μL)
LongAmp® Taq DNA polymerase	1
LongAmp Taq reaction buffer	5
Deoxynucleotide solution mix	3
Dimethyl sulfoxide	1.25
Forward primer (10 μmol/L)	1
Reverse primer (10 μmol/L)	1
DNA template	1
H_2O	11.75
Total volume	25

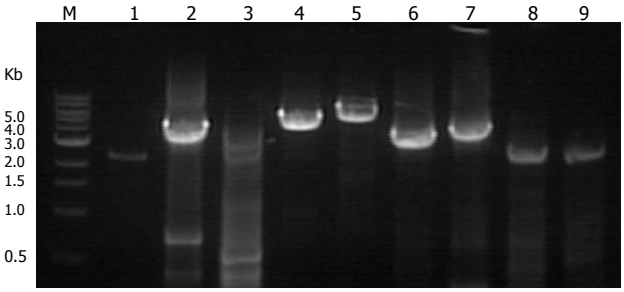

Figure 5 Images showing long-range polymerase chain reaction amplification.

been found to be clearly associated with the onset of this disease: *PKD1* and *PKD2* in polycystic kidney disease and *PRKCSH* and *SEC63*[3-6] in the independent type of polycystic liver disease. *PKD1* is located on the short arm of chromosome 16 (16p13.3)[7], and *PKD2* is located on the long arm of chromosome 4[8]; the incidence rate of mutations in these genes is approximately 1/500. The *PKD1* gene contains 46 exons, is transcribed into an mRNA of 14.1 kb in length and encodes a protein consisting of 4303 amino acids; this protein is a membrane protein of the receptor type. The *PKD2* gene contains 15 exons, is transcribed into an mRNA of 5.3 kb in length, and encodes a protein containing 968 amino acids. In total, 85% of the patients with polycystic kidney disease have the *PKD1* mutation(s), and 15% have the *PKD2* mutation(s). The mutations are evenly distributed in the two genes; there is no hotspot region of mutation. Some studies have shown that the location of the mutations may affect the severity of the clinical phenotype. Because the *PKD1* gene is larger and there are homologous regions in the genome, genetic testing can be troublesome. The main tool for detection is based on PCR amplification, followed by sequencing of the corresponding exon region. The non-repetitive regions of the *PKD2* and *PKD1* genes can be directly amplified and sequenced, but amplification of the repetitive regions of *PKD1* require nested PCR: primers for long-range PCR should be designed in the location where the homology of repetitive regions is not high so that a single exon could be amplified and sequenced. However, this method is not only cumbersome but also expensive. In this study, we used a method according to an article published this year that omitted the nested PCR step; thus, *PKD1* can be effectively detected. Independent polycystic liver disease is caused by a mutation of the *PRKCSH* gene on chromosome 19 (19p13.2-13.1)[9] or the *SEC63* gene on chromosome 6 (6q21-q23)[10]. *PRKCSH*[11] contains 18 exons, is transcribed into a 1.6 kb mRNA molecule and encodes a protein containing 527 amino acids; this protein is an endoplasmic reticulum protein. The *SEC63* gene has 21 exons, is transcribed into an mRNA of 3.4 kb in length and encodes a protein containing 760 amino acids. Mutations of these two genes may affect the

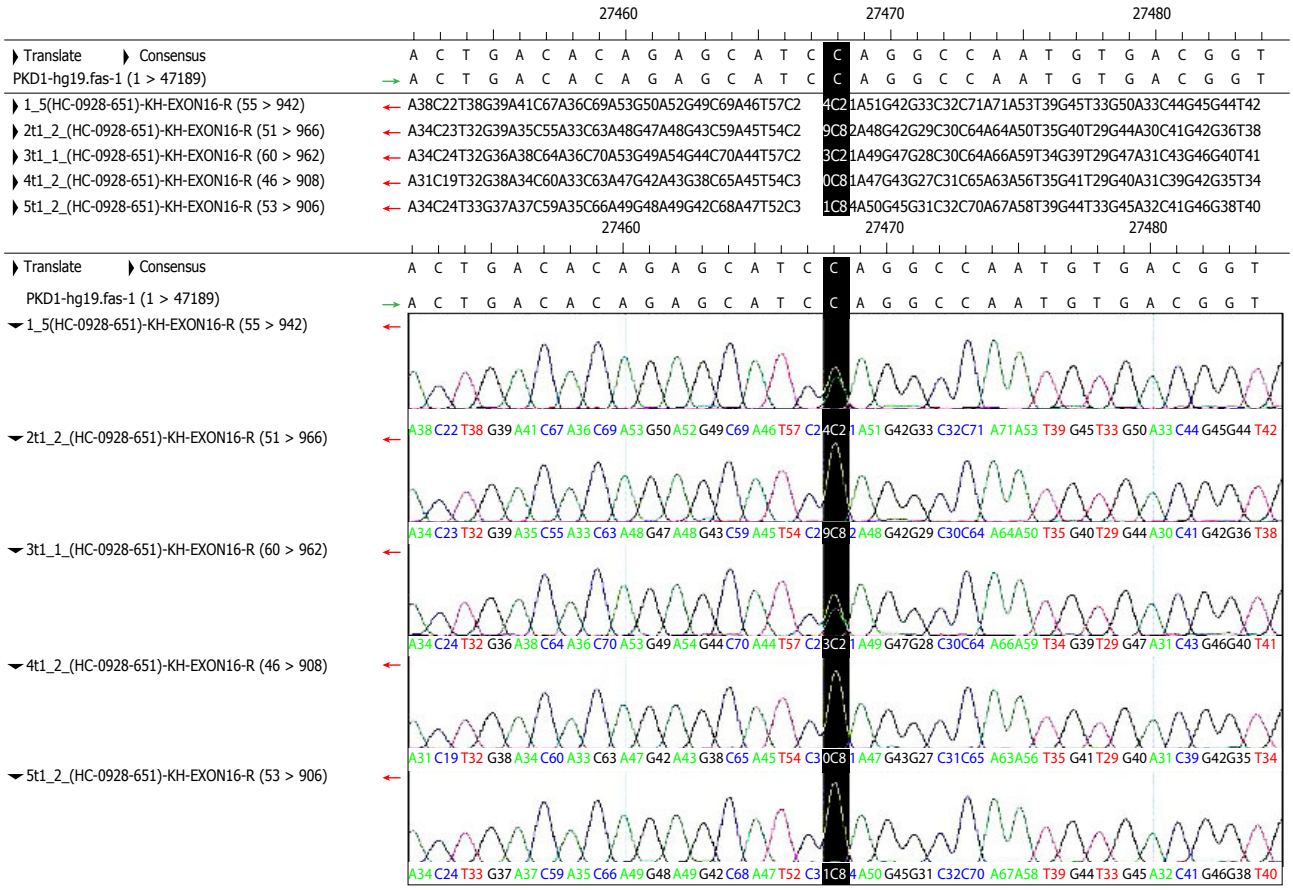

Figure 6 Sequencing peak chart of exon 15 from 5 individuals.

integrity of the membrane protein.

The possible pathogenic mechanisms of the mutations may be as follows: (1) Primary cilia are a core part of the signal transduction system. If there are mutations in the genes encoding the cilial proteins, this will change the cilial structure and disorder the reaction and transmission. These mutations will reduce the internal flow of Ca^{2+} and increase the concentration of cAMP[12], resulting in the hyperplasia of bile duct epithelial cells and the formation of a cyst[13]; (2) Liver cyst Sec proteins are involved in the synthesis of new proteins in the endoplasmic reticulum. The transport of translated and synthesized new proteins and the modification of the N-terminal glycosylation are mainly related to glucosidase Ⅱ. Mutated liver cyst proteins cannot be bound to glucosidase, which results in mature defects of newly synthesized proteins[14]; (3) The further expansion after the formation of small cysts may be related to the following factors[15]: the pressure of the cystic cavity, the reconstruction of the surrounding matrix, the formation of new blood vessels, regulating factors, and so on. In the epithelial cyst model, when the secretion of cystic fluid and intracapsular pressure increase, the proliferation of epithelial cells is significantly sped up[16]. After the surrounding tissue is invaded by hyperplastic epithelial cells, metalloproteinases can remodel the matrix. At present, the enzyme has been separated from the epithelial cells of patients with the independent type of polycystic liver disease. The formation of new blood vessels can maintain the development of the epithelium and endothelium of the cyst[17]. The factor produced by the liver cyst epithelium can activate the vascular endothelial growth factor signaling pathway and lead to endothelial cell proliferation and differentiation. After estrogen stimulation, insulin-like growth factor I acts on the intrahepatic bile duct epithelium, and the bile duct epithelial cells proliferate rapidly[18]. A clinical investigation showed that multiparas were more likely to have a liver cyst; the patients who had used oral contraceptives or postmenopausal hormone replacement therapy may have had more and larger liver cysts[19-22]; and (4) The "two hit" theory[23]: in animal models, obvious cystic degeneration was present only when the animals had damage to both *PKD* alleles. This showed that cyst phenotypic development required the "two hit" condition. The first hit was the bud mutation of the initiative PKD1 or PKD2. The second hit was a single somatic mutation which caused the hyperplasia of luminal epithelial cells. At present, this theory has been confirmed in polycystic liver disease related to polycystic kidney disease but not in the independent type of polycystic liver disease.

The main surgical treatment methods for polycystic liver disease are: (1) percutaneous liver cyst

puncture drainage and/or anhydrous alcohol injection treatment; (2) liver cyst opening the window to the top decompression; (3) liver resection or liver resection combined with cyst fenestration; (4) hepatic artery interventional embolization; and (5) liver transplantation. In recent years, the study of gene therapy has made remarkable achievements, especially in the treatment of genetic diseases. However, polycystic liver disease gene therapy research currently focuses on the *in vitro* study of cysts of liver cells; studies have not reported on these genes *in vivo*. At present, gene therapy is not yet fully mature[24-27].

According to the detection results, we found that the mutation[3] in exon 15 of the proband was a heterozygous CT mutation in the polycystic liver disease kindred. Through PCR amplification and DNA sequencing of this site in other individuals with this kindred, we found that the third individual with *AKPKD* also carried this nonsense mutation, which was not present in the remaining healthy individuals (Figure 6). The mutation was not included in the dbSNP library, but we discovered a record of it in the *PKD* mutation database. This mutation was located at the first position of the codon and changed the encoded amino acid into a termination codon; this obviously influenced the encoded protein. Therefore, the mutation was genetic with the disease in the kindred and was considered a pathogenic mutation. In the future, we can carry out an experiment to confirm the above results by developing an animal model. In addition, the characteristics of the clinical phenotype in this kindred showed that renal involvement mainly occurred in male patients, and female patients usually had the disease manifestation in the liver and kidney but primarily the liver. The results showed that hormones had a certain influence on the clinical phenotype, but the mechanism was not clear and requires further study. This study provided a new idea for investigating molecular genetic principles and mechanisms in polycystic liver disease and for operating polycystic liver disease screening, diagnosis and treatment in the future.

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COMMENTS

Background

Polycystic liver disease (PCLD) is an autosomal dominant polycystic liver disease. It can be divided into polycystic liver disease associated with polycystic kidney and the independent type polycystic liver disease. Now four genes have been found to be associated with the onset of this disease and there are polycystic kidney disease genes *PKD1* and *PKD2* and the independent type polycystic liver genes *PRKCSH* and *SEC63*. There have been many studies on the biological and gene therapy of PCLD recently.

Research frontiers

Recently the authors have made great achievements in the research of the genetic treatment, especially in the treatment of hereditary diseases. It has been a hotspot in the medical research. The gene therapy is the best treatment for hereditary diseases in theory. So the research of pathogenic genes provides a theoretical basis for the gene therapy.

Innovations and breakthroughs

The authors extracted the genomic DNA by using QIAamp DNA Extraction Kit from the peripheral venous blood samples of the members of the AKPLD family. The technique of QIAamp DNA can extract the genomic, mitochondrial, bacterial, parasitic and viral DNA from the human tissue samples and can be directly used for PCR and marker detection. The method can ensure obtaining high purity DNA. The five blood samples obtained from the family were analysed by using long-range polymerase chain reaction and direct sequencing with different primers.

Applications

A total of 42 normal exons were identified in detecting mutations of the *PKD1* gene. The synonymous mutation occurred in the exon 5. The mutation was homozygous T in the proband and was C in the reference sequence. It was located at the third codon and did not change the amino acid coded by this codon. The missense mutation occurred in the exons 11 and 35. They were located at the second codon, changed amino acid sequence and exited in the dbSNP library. The nonsense mutation occurred in the exon 15. The mutation was CT heterozygous in the proband and was C in the reference sequence. It was located at the first codon and resulted in a termination codon. It was a new mutation and was not deposited in the dbSNP library.

Terminology

Autosomal dominant inheritable disease is that the pathogenic genes are located in autosomes and a single gene mutation can cause the disease. The nonsense mutation is that the code which the base stands for becomes a termination codon due to the change of the base, and makes the peptide chain stop synthesis ahead of time.

Peer-review

This is a good retrospective study in which the authors analyzed the mutable sites in the polycystic liver disease gene and found the potential mutable sites by detecting mutations of the candidate gene in a family with PCLD. In the detection of the *PCLD* gene mutations, only 2158432(hg19) of the exon 15 was not in the dbSNP library and the proband was the heterozygosis. All were accord with the dominant mode of inheritance. By detecting other individuals' the same mutable sites. We found the third individual also have it. So the mutable site was a pathogenic mutations and was genetic.

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