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RESEARCH ARTICLE

Identification of Two Novel Mutations in *PKHD1* Gene from Two Families with Polycystic Kidney Disease by Whole Exome Sequencing

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Abstract: ***Background*:** Polycystic kidney disease (PKD) is an autosomal recessive disorder resulting from mutations in the *PKHD1* gene on chromosome 6 (6p12), a large gene spanning 470 kb of genomic DNA.

***Objective*:** The aim of the present study was to report newly identified mutations in the *PKHD1* gene in two Iranian families with PKD.

***Materials and Methods*:** Genetic alterations of a 3-month-old boy and a 27-year-old girl with PKD were evaluated using whole-exome sequencing. The PCR direct sequencing was performed to analyse the co-segregation of the variants with the disease in the family. Finally, the molecular function of the identified novel mutations was evaluated by *in silico* study.

***Results*:** In the 3 month-old boy, a novel homozygous frameshift mutation was detected in the *PKHD1* gene, which can cause PKD. Moreover, we identified three novel heterozygous missense mutations in *ATIC*, *VPS13B*, and *TP53RK* genes. In the 27-year-old woman, with two recurrent abortions history and two infant mortalities at early weeks due to metabolic and/or renal disease, we detected a novel missense mutation on *PKHD1* gene and a novel mutation in *ETFDH* gene.

***Conclusion*:** In general, we have identified two novel mutations in the *PKHD1* gene. These molecular findings can help accurately correlate genotype and phenotype in families with such disease in order to reduce patient births through preoperative genetic diagnosis or better management of disorders.

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1. INTRODUCTION

Polycystic kidney disease (PKD) is one of the most prevalent hereditary autosomal recessive disorders in children, including neonates and young people, with 1/6000 to 1/55000 frequency. In the most severe cases, it can be diagnosed prenatally by the presence of enlarged, echogenic kidneys and oligohydramnios [1, 2]. However, in the milder forms, clinical manifestations are usually detected in the neonatal and childhood period.

Various mutations in *PKHD1* gene, located on chromosome 6 (6p12) with 75 exons, are associated with this disease. The largest protein product of this gene is called the FPC/polyductin complex (FPC). It is a single-membrane spanning protein whose absence leads to abnormal ciliogenesis in the kidneys [3, 4]. Until now, more than 300 mutations have been identified in different regions of this gene. The most common mutation in *PKHD1* gene is a missense mutation in exon 3 (NM_138694.3: p.T36M c.107C>T). With the exception of this mutation, which accounts for approximately one-fifth of the mutations that cause the disease, no other mutation hotspot has been found in this gene. Most of the mutations appear to be unique to one genealogy [4].

Symptoms of PKD are usually present in infancy and have been recognized by clinical presentation in adulthood [5, 6]. The most common manifestations of this disease in infants are a history of oligohydramnios enlargement of kidneys and pulmonary hypoplasia. Moreover, other organs be-

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sides the kidney are also involved in patients with PKD [4, 7]. Although the relationship between genotype and phenotype in this disease is not fully understood, the presence of truncating mutations usually results in a severe form of disease and death in the fetus [4, 8].

In the present study, we identified two novel frameshift and missense mutations in *PKHD1* gene in two different Iranian families with PKD by whole-exome sequencing.

2. MATERIALS AND METHODS

2.1. Patients Presentation

In the present study, two Iranian patients with polycystic kidney disease were referred to Aria Gene Medical Genetics Laboratory (Qom-Iran) in June 2019. The first patient was a 3-month-old boy (the third child of the family) with PKD (IV-1), which was offspring of consanguineous marriages (Fig. **1A**). Another child of the family was male and suffered from polycystic kidney disease (IV-2). The second patient was a 27-year-old (III-6) woman with polycystic kidney disease and two recurrent abortions history (IV-1 and IV-2), and two infant mortalities (IV-3 and IV-4) at early weeks (Fig. **1B**). The studied probands showed that various symptoms include potter facies, hypertension, lung hypoplasia, periportal fibrosis, portal hypertension, hepatomegaly, hepatic cysts, pancreatic cysts, bile duct dilatation and proliferation, splenomegaly, esophageal varices, enlarged kidneys, cystic kidneys, renal failure, increased echogenicity of entire parenchyma, loss of corticomedullary differentiation, interstitial fibrosis, dehydration, enlarged kidney splenectomy, and cystic kidneys. According to the ethical standards of the Declaration of Helsinki, the patients and their parents were informed about the study, and informed consent was signed. The study was performed with the approval of the Institutional Review Board (IRB), and informed consent was obtained from patients, or authorized representative/guardian, and before genetic testing. The pedigree of the patients was drawn (Cyrillic 2.1 software) to determine the inheritance pattern of the disease.

2.2. Genomic DNA Extraction and Whole-Exome Sequencing

The peripheral blood sample (5 mL) were taken from studied patients and family members, and added to a tube contains with EDTA as the anticoagulant. Extraction of genomic DNA was performed using DNA purification kit, according to the manufacturer's instructions. The quantity and quality of extracted genomic DNA were investigated using a nanodrop instrument and electrophoresis on 1% agarose, respectively. The whole-exome sequencing was employed to enrich all genomic coding regions and some important other genomic regions. The next-generation sequencing was performed to sequence approximately 100 million reads on Illumina Sequencer (Illumina, San Diego, CA, USA). Bioinformatics analysis of the sequencing results was performed using international databases. Genetic variants such as point mutations and indels were identified using SAMtools and annotated by ANNOVAR software. The candidate genes were considered a variant that fulfilled the following criteria: (i) missense, nonsense, frameshift, and splice-site variants, (ii) absent or rare (frequency below 1%) in the two databases (db SNP, 1000 G), and (iii) homozygous variants in the patient [9].

2.3. Confirmation of Whole Exome Sequencing Results by Sanger Sequencing

The target exons containing mutations of *PKHD1* gene were typically amplified using 10 pmole of primer, 0.2 UTaq DNA polymerase, 200 µM of each dNTPs, 0.67 µl of 50 mM $MgCl_2$, 60 ng DNA and 2.5 µl of PCR buffer in 25 µl of PCR reactions. The PCR conditions included an initial denaturation step for 3 min at 95°C, 30 sec at 95°C, 30 sec at 60°C with a 1°C at 72°C for 35 cycles, and finally 10 min at 72°C. The PCR products were separated on 2% agarose

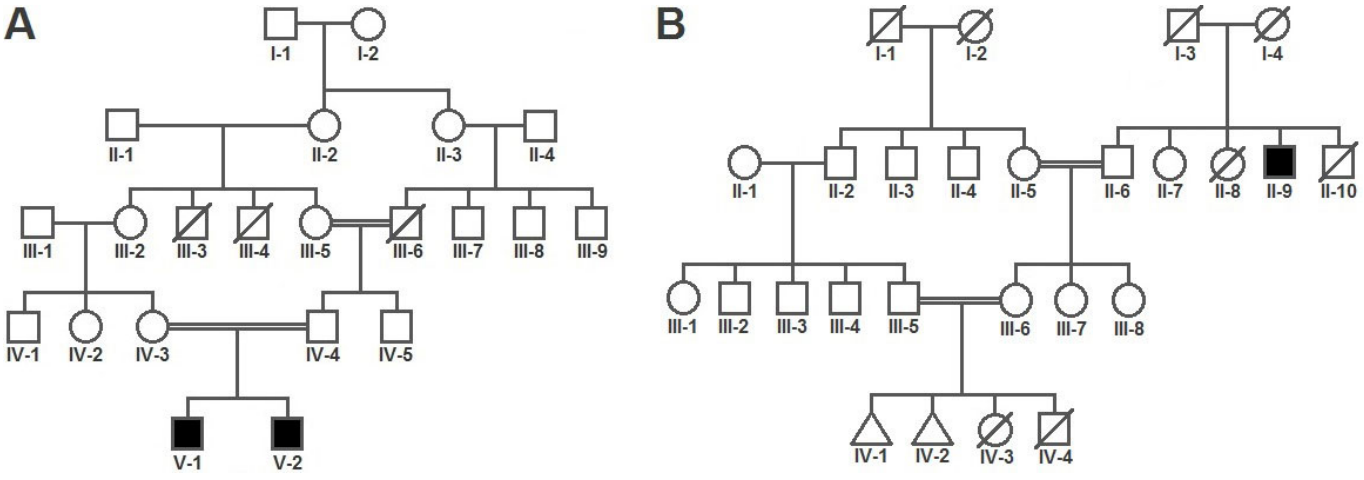

Fig. (1). Pedigree analysis and molecular evaluation of studied patients with ARPKD. A 3 months-old boy with a novel homozygous frameshift mutation in *PKHD1* gene (**A**). A 27 years-old woman with a novel heterozygous mutation in *PKHD1* gene (**B**).

gels and visualized Gel Green® stained. Subsequently, to confirm the identified mutation, the PCR products were subjected to direct sequencing. Then, the PCR products were sequenced on an ABI 3130 automated sequencer (Applied Bio systems, Forster City, CA, USA). Sequence data searches were performed in non-redundant nucleic and protein databases BLAST (http://www.ncbi.nlm.nih.gov/BLAST) [10].

3. RESULTS

In the first patient (a 3-month-old boy with polycystic kidney disease), a novel homozygous frameshift mutation was detected in the *PKHD1* gene (NM_138694: exon 7: p.H154fs c.460delC), which can cause PKD. In addition, we identified three novel heterozygous missense mutations in *ATIC* (NM_004044: exon 6: p.R171K c.G512A), *VPS13B* (NM_017890: exon 61: p.C3916Y c.G11747A), and *TP53RK* (NM_033550: exon2: p.G253R c.G757A) genes. These mutations were not previously reported, and thus classified as a variation of unknown significance (VUS). In this patient, the four identified mutations in *PKHD1* gene were in the homozygous state, whereas their parents were in the heterozygous state.

In the second patient (a 27-years-old woman with polycystic kidney disease and two recurrent abortions history and two infant mortality at early weeks due to metabolic and/or renal disease), we detected a novel missense mutation (NM_138694.6: exon7: p.H2216R c.A6647G) in *PKHD1* gene and a novel mutation (MN_004453.3: p.Ser425AegFsTer3 c.1273-1276derTCAA) in *ETFDH* gene. These mutations were not previously reported and thus classified as a variation of unknown significance (VUS). In this patient and her husband, the two identified mutations in *PKHD1* and *ETFDH* genes were in the heterozygous state, which can cause recurrent abortions and infant mortality.

4. DISCUSSION

PKD is caused by mutations in the *PKHD1* gene, which extends over about 470 kb and includes at least 66 exons with a large number of alternatively spliced transcripts. The longest continuous open reading frame encodes a 4074 amino acid protein PKD-causing mutations that have been identified along the entire length of the *PKHD1* gene, and multiple mutation types are pathogenic [11]. Approximately, 750 pathogenic mutations have been cataloged in the PKD mutation database (URL), of which half are missense mutations. The most common mutation is a missense mutation in exon 3 (p.T36M c.C107T), which accounts for about 20% of all mutant alleles [12]. In a study on fetuses and neonates with PKD, it was found that after adjusting for gestational age, the extent of the dilatation of the collecting duct, but not portal fibrosis, significantly correlated with the presence of two truncating mutations [13]. In general, patients with two truncating mutations often have a severe phenotype and die in the perinatal stage [14]. However, there are exceptions, for example, it has been reported that a child homozygous for a large *PKHD1* deletion was able to survive well past the neonatal period [15]. In addition, patients with the same genotype may also have significantly different phenotypes, suggesting that genetic modifiers modulate disease expression [12].

The multi-domain transmembrane protein fibrocystin/polyductin (FPC) is the protein product encoded by *PKHD1* gene. This protein is a single transmembrane-spanning protein with a long extracellular N-terminus and short cytoplasmic tail [16]. The FPC undergoes notch-like proteolytic processes, resulting in the shedding of the extracellular domain into the tubular lumen. Meanwhile, the C-terminal domain translocates into the nucleus for transcription regulation [17]. During embryonic development, the *PKHD1* gene is widely expressed in neural tubes, bronchi, primordial guts, and early ureteric buds, mesonephric tubules, adrenal cortex, and immature hepatocytes, which suggests that it plays an important role in organ development and tubular morphogenesis. In adult tissues, the FPC is mainly expressed in kidneys (primarily in collecting ducts and thick ascending loops of Henle), and in ductal epithelial cells of the liver and in the pancreas [16, 18]. FPC is mainly localized to primary cilia and is currently believed to be the "sensation antenna" of kidney epithelial cells or bile duct cells [19]. Incorrect protein structure of FPC leads to loss of polarity in kidney tubular epithelial cells or biliary epithelial cells, and cysts are easily formed.

In the present study, we reported two newly identified mutations in the *PKHD1* gene by whole-exome sequencing in two different Iranian patients with polycystic kidney disease. We identified two mutations, (p.H154fs c.460delC) and (p.H2216R c.A6647G) within the coding sequence of *PKHD1* in the parents. The unreported mutations of the coding sequence of the PKHD1 gene were confirmed by the Sanger sequencing. According to the published data and Global Variome shared LOVD (https://databases.lovd.nl/shared/genes/PKHD1), these identified variants seem to be novel mutations. These results indicate the high efficiency of the whole-exome sequencing method in detecting various mutations. This is not only an economical means for detection, but also significantly reduces detection time, and gives reliable results. However, it is important to mention that repeat expansion diseases like fragile X, large deletion, duplication and copy number variations, such as Duchene muscular dystrophy, are not detectable by this method.

Since missense mutations are usually associated with a milder disease phenotype, the missense mutation in the second proband (III-6) is consistent with the mild disease phenotype in our study. On the other hand, some mutations that result in complete loss of protein are associated with intrauterine death; the frameshift mutation in the first proband (IV-1) is consistent with the severe disease phenotype. The autosomal recessive inheritance pattern indicates a higher incidence of disease in kinship marriages, given the high incidence rates of the disease in different generations of offspring marriage. Therefore, the genetic examination of patients helps to predict the likelihood of recurrence in families and allows for prenatal diagnosis and preimplantation ge-

netic diagnosis. The genomic regions besides exons of protein-coding genes and mutations in the upstream and downstream regulatory regions have not been investigated, which indicate the limitation of the present study.

CONCLUSION

In conclusion, next-generation sequencing technology and subsequent validation Sanger sequencing is a cost-effective and powerful tool to detect large genes, such as *PKHD1*. Both reported genetic variants in the *PKHD1* gene strongly supported previous studies, and suggested that this gene contains different hotspot regions. However, further studies will be required to clarify the genotype-phenotype correlations in patients with various mutations in the *PKHD1* gene.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was performed with the approval of the Institutional Review Board (IRB).

HUMAN AND ANIMAL RIGHTS

No animals were used in this studies that are the basis of this research. All procedures performed on humans were in accordance to the ethical standards of the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Informed consent was obtained from the patients.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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