

Identification of a Novel Intragenic Deletion of the *PKHD1* Gene in a Patient with Autosomal Recessive Polycystic Kidney Disease

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Abstract

Background

Autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations in the *PKHD1* gene. In the present study, we describe a severe case of ARPKD carrying a point mutation and a novel four-exon deletion of *PKHD1* gene.

Materials and Methods

The *PKHD1*, *PKD1* and *PKD2* genes were analyzed using next-generation sequencing, whereas the *PKHD1* gene exon deletions/duplications were screened using multiplex ligation-dependent probe amplification.

Results

The c.2279G>A (p.Arg760His) mutation and a deletion encompassing exons 24-27 of *PKHD1* gene were detected in compound heterozygosity in the affected neonate. The complete documentation of the genetic basis of the disease offered the possibility of a targeted prenatal diagnosis in the following pregnancy of the couple.

Conclusion

Given that the molecular analysis of ARPKD is mainly based on sequencing techniques, the *PKHD1* gene exon deletion/duplication screening should be performed as a complementary assay in patients suspected to have ARPKD in the absence of two pathogenic mutations.

Key Words: Genetic diagnosis, Next-generation sequencing, *PKHD1*, Polycystic kidney disease.

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1- INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD, OMIM: 263200) constitutes a rare genetic disorder with an estimated incidence of 1:20,000 (1). Patients are characterized by enlarged kidneys with dilatation of the collecting ducts and interlobular liver fibrosis accompanied by biliary duct hyperplasia (2). The clinical manifestations of the disease arise either during the perinatal period or in early childhood, while 30% of affected children die within the first year of life (3). The ultrasound screening of the most severely affected fetuses usually detects enlarged echogenic kidneys that lead to oligodramnios pulmonary hypoplasia (4). ARPKD is caused by mutations in *PKHD1* gene, located on the short arm of chromosome 6 (6p12.2) (5).

PKHD1 gene, which consists of 86 exons, encodes the 4074-amino-acid protein polyductin/fibrocytin (6). The protein is mainly expressed in the basal body of the primary cilium and the bile duct epithelial cells (7). Pathological mutations are spread all over *PKHD1* gene, a fact that complicates the molecular genetic analysis. Specifically, more than 800 genetic variants have been identified in ARPKD patients, which are mostly compound heterozygotes.

The development of next-generation sequencing has offered a more efficient screening of *PKHD1* gene and a time/cost-effective molecular diagnosis of ARPKD. In our present report, we describe a severe case of ARPKD that was due to the compound heterozygosity of a point mutation and a novel four-exon deletion of *PKHD1* gene.

2- CASE PRESENTATION

The subject of the current study was a male neonate born naturally at the 37th week of pregnancy. The neonate, weighing 2610 g, presented with severe respiratory

problems during delivery and required respiratory system support with self-expanding pouch and cardiac massage so as to obtain normal respiratory function and heart rate. Due to abdominal distension and flatulence, ultrasound analysis of upper and lower abdomen was performed revealing unequal, enlarged kidneys (9.5 and 9.8 cm) with punctuate crystals and small vesicles.

The laboratory test results (Glc: 149 mg/dl, Urea: 45 mg/dl, Cr: 1.85 mg/dl, Na: 140 mmol/l, K: 3.9 mmol/l, Ca: 7.4 mg/dl, P: 5.8 mg/dl, Mg: 1.48 mg/dl), the prolonged oliguria and the arterial hypertension (106/54 mmHg) forced the clinicians to prescribe captopril and furosemide. Forty-eight hours after birth, the neonate presented a sudden drop of transcutaneous oxygen saturation accompanied by cyanosis and bradycardia, facts that led to his intubation. The neonate was transferred to the Neonatal Intensive Care Unit of the 2nd Department of Pediatrics of 'P. & A. Kyriakou' Children's Hospital of Athens. The new ultrasound analysis verified the presence of enlarged echogenic polycystic kidneys. Given the above findings, the molecular analysis of *PKHD1*, *PKD1* and *PKD2* genes was performed in Genesis Genoma Lab.

The continuous deterioration of infant's health resulted in his death 20 days after his admission to 'P. & A. Kyriakou' Children's Hospital of Athens. The aforementioned molecular analysis revealed the genetic basis of the disease, giving the opportunity of prenatal diagnosis through chorionic villus sampling (CVS) in the following pregnancy of the couple. The study protocol was in accordance to the Helsinki declaration, while written informed consent was obtained from the parents of the proband and for the publication of the current report.

3- METHODS

Genomic DNA was extracted from the peripheral blood sample (QIAamp DNA Blood Mini Kit, Qiagen, Hilden, Germany) of the affected proband and the entire coding regions of *PKHD1* (NM_138694.3), *PKD1* (NM_001009944.2) and *PKD2* (NM_000297) genes were amplified by polymerase chain reaction (PCR). The resulting library was loaded on an Illumina NextSeq clamshell-style cartridge for paired-end sequencing. The data were analyzed using state-of-the-art bioinformatics software.

For the detection of possible deletions/duplications along *PKHD1* gene, multiplex ligation-dependent probe amplification (MLPA) was performed. For the above analysis, two different probemixes were used (MLPA P341 *PKHD1* mix 1 & MLPA P342 *PKHD1* mix 2, MRC-Holland, Amsterdam, Netherlands) according to the manufacturer's instructions. The multiplex PCR products were separated on a capillary sequencer ABI3500, Genetic Analyzer, Applied Biosystems, USA), and the respective data were analyzed with Coffalyser.Net (MRC-Holland, Amsterdam, Netherlands).

4- RESULTS

The heterozygous presence of the missense mutation c.2279G>A (p.Arg760His, p.R760H) of *PKHD1* gene (NM_138694.3) was revealed in the

affected neonate after the next-generation sequencing analysis. The above genetic variant, which causes substitution of the amino acid arginine by histidine at position 760, is considered to be pathogenic according to international literature (8), mutation databases (HGMD, ClinVar, dbSNP) and *in silico* tools used for the prediction of the disease-causing potential of DNA variants (Mutation taster, Polyphen). No mutations were detected in the *PKD1* and *PKD2* genes.

For full documentation of the genetic basis of the disease, the presence of a second mutation would be necessary. The second mutation could either be a genetic variant in deep intronic or regulatory regions of the gene, or a deletion/duplication in the coding region of *PKHD1* gene. In order to investigate the potential presence of a *PKHD1* gene exon deletion/duplication, MLPA analysis was performed. The above screening revealed a heterozygous deletion encompassing exons 24, 25, 26 and 27 of *PKHD1* gene (**Figure.1**), which completed the genetic diagnosis of ARPKD.

The molecular screening of the parents using bidirectional Sanger sequencing and MLPA analysis revealed that the c.2279G>A (p.Arg760His) mutation was of paternal origin, while the deletion of exons 24-27 was of maternal origin. In the following pregnancy, CVS was performed at the 12th week of gestation and the molecular testing of the parental mutations detected only the c.2279G>A (p.Arg760His) mutation of *PKHD1* gene.

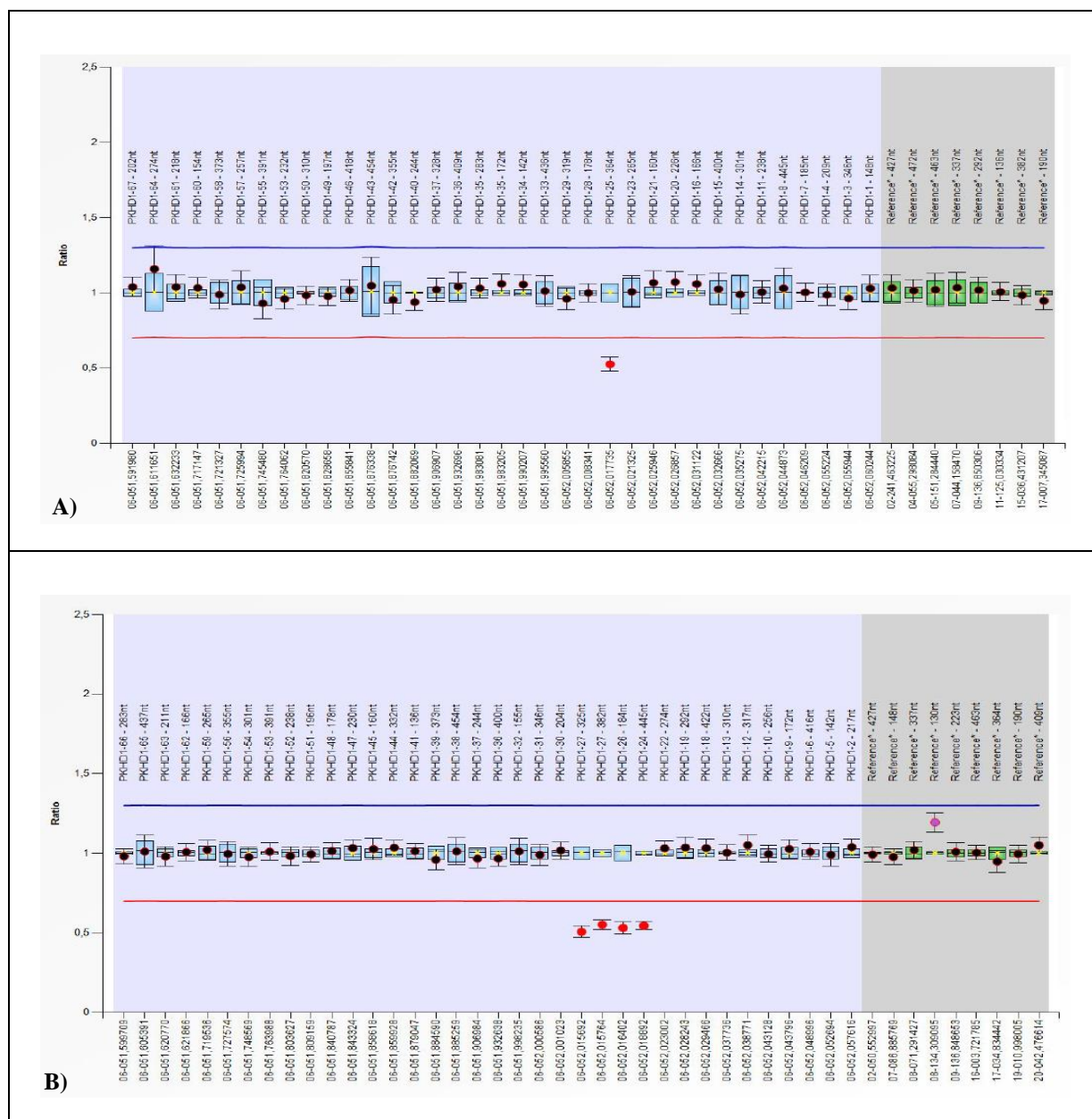


Fig.1: Multiplex ligation-dependent probe amplification (MLPA) analysis of the affected proband using A. MLPA P341 *PKHD1* probemix 1 and B. MLPA P342 *PKHD1* mix 2 (MRC-Holland, Amsterdam, Netherlands).

5- DISCUSSION

The routine diagnostic approach for the determination of the genetic basis of ARPKD is *PKHD1* gene mutation analysis using either Sanger sequencing or next-generation sequencing. However, no *PKHD1* gene mutations have been identified in 13-20% of ARPKD patients

(9-11). This noteworthy rate could be attributed to the presence of mutations in deep intronic and/or regulatory regions of the *PKHD1* gene, intragenic *PKHD1* exon deletions/duplications or mutations in the *PKD1* or *PKD2* genes. In the last case, we refer to autosomal dominant polycystic kidney disease (PKD) patients with severe early symptoms (~20% of patients), who

are phenotypically indistinguishable from ARPKD patients (12). In the current study, we described a severe case of ARPKD carrying the missense mutation c.2279G>A (p.Arg760His, p.R760H) and a deletion of exons 24-27 of *PKHD1* gene in double heterozygosity. The aforementioned point mutation has already been described in an ARPKD patient (8). As concerns the four-exon deletion, it is reported for the first time according to our knowledge. However, similar single- and multi-exon deletions/duplications have already been detected in ARPKD patients (13-18). The development of MLPA technique has allowed the complete screening of *PKHD1* exon deletions and duplications. The deleterious effects of the detected genetic variants could be understood by the early onset, the severe symptoms and the immediate fatal outcome of our patient.

The fact that c.2279G>A (R760H) mutation of the *PKHD1* gene has been identified in compound heterozygosity with c.4870C>T (R1624W) mutation in an ARPKD patient (8) with a later onset of the disease is indicative of the increased pathogenicity of the observed four-exon deletion. The *PKHD1* gene encodes the protein fibrocystin, a large transmembrane receptor-like protein (6). Fibrocystin, which is mainly present in fetal and adult kidney cells, controls the intracellular calcium homeostasis and participates in the regulation of cell adhesion, repulsion, differentiation and proliferation (19).

The deletion of exons encoding nonrepetitive parts of fibrocystin is expected to affect the structure and consequently the function of the protein. The observed deletion is localized in the middle of its coding region, a fact that enforces the possible negative effects on fibrocystin function. Our finding supports previous reports (13, 14) demonstrating the significance of *PKHD1* gene copy number variation testing for ARPKD patients who

need their mutation profile for prognosis or family planning. The Sanger sequencing analysis or the mutation scanning of *PKHD1* gene has achieved a mutation detection rate of 82% (9, 14, 20). According to these reports, two mutations are expected to be detected in nearly 67% (82x82%) of ARPKD patients, only one mutation is expected to be identified in nearly 29% (2x82x18%) of ARPKD patients and no mutations in the remaining 4% of patients. Therefore, nearly 1/3 of the patients have no complete molecular diagnosis of ARPKD, suggesting the application of *PKHD1* gene exon deletion/duplication screening.

The genetic diagnosis of the affected proband of the current report offered the opportunity of targeted prenatal testing of the parental mutations in the following pregnancy of the couple. Since the embryo was carrying the paternal point mutation, there was a 50% chance to carry the maternal *PKHD1* gene exon deletion too, namely to be affected by Autosomal Dominant PKD (ADPKD). The application of the respective MLPA analysis, completing the prenatal genotyping of the embryo, averted the probability of a pregnancy termination.

6- CONCLUSION

In conclusion, this is the first report of a deletion encompassing exons 24-27 of *PKHD1* gene. The *PKHD1* gene exon deletion/duplication screening is strongly recommended in genetic and prenatal diagnosis cases, where either one heterozygous mutation or no mutations of the *PKHD1* gene have been identified through sequencing techniques.

7- CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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