



Case Report

Whole-exome analysis of a child with polycystic kidney disease and ventriculomegaly

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ABSTRACT. Autosomal recessive polycystic kidney disease (ARPKD) is an inherited ciliopathy leading to progressive kidney and liver disease. Biallelic mutations in the *PKHD1* gene underlie this condition. We describe a child with bilaterally enlarged cystic kidneys, portal hypertension, and cerebral ventriculomegaly. Molecular genetic investigations using whole-exome sequencing and confirmation using Sanger sequencing revealed a homozygous pathogenic mutation in *PKHD1* underlying the clinical phenotype of ARPKD. Whole-exome data analysis was used to search for additional rare variants in additional ciliopathy genes that may have contributed to the unusual brain phenotype. Aside from a rare hypomorphic allele in *MKSI*, no other pathogenic variants were detected.

We conclude that the homozygous pathogenic mutation in *PKHD1* underlies the ciliopathy phenotype in this patient.

Key words: Autosomal recessive polycystic kidney disease; Ciliopathy; *PKHD1*; *MKSI*; Ventriculomegaly

INTRODUCTION

Autosomal recessive polycystic kidney disease (ARPKD) is a ciliopathy syndrome that is associated with bilaterally enlarged kidneys, often presenting *in utero* or neonatally (Adeva et al., 2006). It is also associated with congenital hepatic fibrosis and biliary dysgenesis. Children develop systemic hypertension, progressive renal failure, and portal hypertension. Neurological features are not usually associated with this disease.

MATERIAL AND METHODS

Informed consent was obtained from the parents of an affected child who was referred to the Center of Pediatric Nephrology and Transplantation, Cairo University. This study was approved by the Institutional Review Board at Cairo University Children's Hospital.

Genomic DNA was extracted from blood samples collected in EDTA tubes using the QIAGEN Blood and Cell Culture DNA kit according to manufacturer instructions (Qiagen, Valencia, CA, USA). Whole exome sequencing was performed using genomic DNA by AROS Applied Biotechnology AS, Denmark. The exome sequencing reads were processed and analyzed using a comprehensive bioinformatic workflow to identify rare but real variants. The quality of sequencing reads was firstly checked with FastQC (Andrews, 2014). Poly-N tails were trimmed off from reads with an in-house Perl script. The 13 bp on the left ends of all reads were clipped off with Seqtk (<https://github.com/lh3/seqtk>) to remove biased sequencing reads that caused by random hexamer priming (Hansen et al., 2010). Low quality bases ($Q \leq 20$) and standard Illumina (Illumina, Inc., San Diego, CA, USA) paired-end sequencing adaptors on 3' ends of reads were trimmed off using Trim-galore (Krueger, 2014). The high quality reads were then mapped to the human reference genome hg19 with Burrows-Wheeler Aligner (Li and Durbin, 2010). The alignments were then refined with tools of GATK (McKenna and Hanna, 2010). Genome variants of all of the samples were then called simultaneously according to GATK Best Practice recommendations (DePristo et al., 2011), including recalibration. Non-synonymous exonic variants were subsequently filtered by quality and the minor-allele frequency (MAF) as observed in other exome projects (1000 Genomes 2011 release and ESP5400), variants with $MAF > 0.05$ were excluded). Annovar (Wang et al., 2010) was used for annotations and prediction of functional consequences. Potential pathogenic variants (including *PKDHI*, *BBS10* and *MKSI*) were confirmed by Sanger sequencing and segregation analysis was performed using parental DNA samples. The ARPKD database (<http://www.humgen.rwth-aachen.de/index.php>) was also accessed to determine novelty of variants.

CASE REPORT

We report a female patient who presented at 2.5 years of age. Her mother had noted a long history of polyuria and complained that the child had delayed developmental milestones;

in particular, walking was delayed until 2 years of age. The patient was the second child of a consanguineous marriage and there were no other affected family members (Figure 1A).

Physical examination revealed prominent macrocephaly, bilateral enlarged and palpable kidneys, and evidence of systemic hypertension (blood pressure 150/90). At this stage, there were no clinical signs of portal hypertension. Fundal examination did not reveal any retinopathy or retinitis pigmentosa. The patient did not have evidence of polydactyly. Biochemical evaluation revealed significant renal impairment (serum creatinine 3.4 mg/dL; estimated glomerular filtration rate $12 \text{ mL} \cdot \text{min}^{-1} \cdot 1.73 \text{ m}^{-2}$) with a urine-specific gravity of 1009.

An abdominal ultrasound scan (USS) confirmed bilateral enlarged hyperechogenic kidneys with poor corticomedullary differentiation and scattered small cysts, the largest being 0.8 cm in diameter. The liver and spleen appeared normal. An abdominal USS was performed on both parents and demonstrated no cystic change within the kidneys. An abdominal computed tomography (CT) scan of the patient confirmed bilateral enlarged cystic kidneys (Figure 1B), whilst CT imaging of the child's brain revealed bilateral and almost symmetrical dilatation of both lateral ventricles (Figure 1C) as well as dilatation of the 3rd and 4th ventricles.

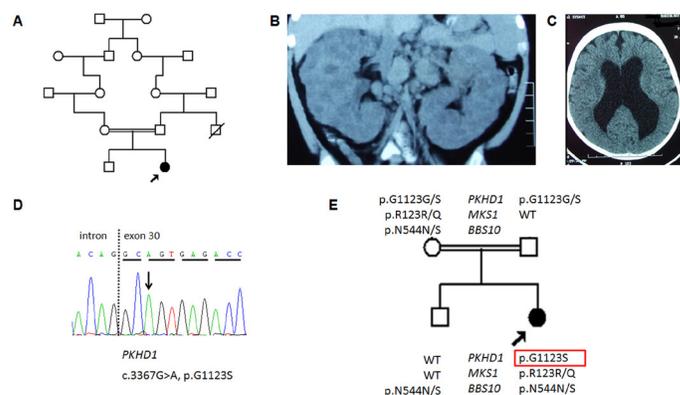


Figure 1. Family structure, imaging and mutational analysis of the affected patient and relatives. **A.** Pedigree diagram showing consanguinity. The arrow indicates the proband. **B.** Abdominal computed tomography (CT) scan shows bilateral enlarged cystic kidneys (at 2 years and 10 months of age). **C.** Brain CT shows ventriculomegaly (at 2 years and 7 months of age). **D.** Sanger sequencing confirmation of homozygous *PKHD1* allele. **E.** Segregation of the *PKHD1* allele and additional rare ciliopathy variants.

The patient was started on supportive therapy for her chronic kidney disease that unfortunately progressed, leading to end-stage renal disease (ESRD) at the age of 3 years and 5 months. ESRD was treated with renal replacement therapy (hemodialysis) and complicated by resistant hypertension requiring treatment with five antihypertensive agents. In addition, 1 year later, she developed portal hypertension and hepatosplenomegaly, presumed to be secondary to congenital hepatic fibrosis. The clinical diagnosis was consistent with an autosomal recessive ciliopathy phenotype given the renal, hepatic, and cerebral (ventriculomegaly) phenotypes. We undertook a molecular genetic analysis of the affected child and her unaffected parents to determine the precise cause.

Whole-exome data analysis on the affected child revealed a homozygous missense mutation c.3367G>A, p.G1123S in *PKHD1* (Figure 1D) within an 18.3-Mb region of homozygosity on chromosome 6. This *PKHD1* missense mutation has previously been reported in patients with ARPKD (Sharp et al., 2005). However, the association of ventriculomegaly and

PKHD1 mutation is novel. We analyzed the whole-exome data set to determine if potential disease-causing variants in other ciliopathy genes were present. Rare known ciliopathy gene variants (MAF <1%) are shown in Table 1, including variants in *MKSI* and *BBS10*, which were confirmed using segregation analysis (Figure 1E). There were no variants or additional alleles that would account for ventriculomegaly phenotype, aside from a hypomorphic heterozygous allele (p.R123Q) in *MKSI* whose significance is undetermined.

Table 1. Rare variants in ciliopathy syndrome genes detected by whole-exome sequencing of the proband.

Ciliopathy Gene	Variant	%MAF 1000g2012	%MAF ESP6500	dbSNP135	Poly-Phen2 output: score	Reference
<i>PKHD1</i>	Homozygous c.3367G>A; p.G1123S	n/a	0.0154	rs142107837	Probably damaging: 0.999	Sharp et al., 2005
<i>MKSI</i>	Heterozygous c.368G>A; p.R123Q	n/a	0.025	rs202112856	Possibly damaging: 0.883	Leitch et al., 2008
<i>BBS10</i>	Heterozygous c.1631A>G; p.N544S	0.41	0.6689	rs34737974	Benign: 0.0	Pereiro et al., 2011

MAF = minor allele frequency.

DISCUSSION

ARPKD is a ciliopathy syndrome with clinical and pathological features that include renal collecting duct dilatation, biliary dysgenesis, and portal fibrosis. The age of presentation is extremely variable. The condition may present *in utero* with enlarged echogenic kidneys and/or oligohydramnios. Perinatal complications, including pulmonary hypoplasia, lead to a high neonatal death rate (Sharp et al., 2005). Those surviving the neonatal period usually reach ESRD in infancy, early childhood or adolescence. In contrast, some affected patients have been diagnosed as adults with hypertension, chronic kidney disease, and portal hypertension (Busher et al., 2004). Mutations in a single gene, *PKHD1*, underlie this condition, which encodes a large protein known as polyductin or fibrocystin. Typically, two truncating mutations in *PKHD1* present with severe perinatal phenotypes, whereas missense mutations produce milder disease (Bergmann et al., 2003; Furu et al., 2003; Rossetti et al., 2003; Sharp et al., 2005).

Interestingly, most ARPKD families show concordant phenotypes. However, unknown genetic modifiers may dramatically change phenotypes, even within the same family. Intrafamilial variability ranging from neonatal death to survival to childhood and striking differences in kidney and liver disease severity have previously been noted (Bergmann et al., 2005). The transcription factor HNF1-beta (encoded by *TCF2*) may play a role in regulating *PKHD1* transcription (Hiesberger et al., 2004). In addition, in the *cpk* murine model of ARPKD, *Kif12* was identified as a possible modifier gene (Mrug et al., 2005). Yet-to-be-defined genetic modifiers are also likely to be altering the disease severity in the PCK rat model of ARPKD (O'Meara et al., 2012). In our patient, we did not identify any variants in *TCF2* or *KIF12*.

The gene product of *PKHD1* (polyductin/fibrocystin) is expressed in the human adult kidney, liver, and pancreas, and was shown to localize to the primary cilia of murine inner medullary collecting duct cells (Menezes et al., 2004). ARPKD is therefore recognized as a ciliopathy. However, to our knowledge, central nervous system phenotypes have not been

reported in association with *PKHD1* mutations.

A previous case report has documented the combination of ventriculomegaly and cystic kidneys (Reuss et al., 1989). Molecular genetic analysis was not undertaken. Six cases in two related families were reported. The first case was a stillborn fetus with ventriculomegaly and was found to have normal sized but echo-dense kidneys with histological findings of corticomedullary cysts. Three subsequent pregnancies from the same parents resulted in similar antenatal findings. The brain showed focal hyperplasia of the choroid plexus but no obstructive cause for the hydrocephalus (Reuss et al., 1989). A related branch of the family produced two additional fetuses of similar appearance, one of whom had polydactyly. This feature, together with an absence of hepatic fibrosis in these cases, points away from ARPKD as a diagnosis and suggests another ciliopathy syndrome such as Meckel syndrome (MKS).

MKS is a severe, often lethal ciliopathy characterized by occipital meningoencephalocele, cystic kidney disease, liver fibrosis, and polydactyly (Kyttälä et al., 2006). Brain phenotypes may include hydrocephalus. Mutations in *MKSI*, *TMEM216*, *TMEM67*, and other ciliopathy syndrome genes may cause MKS phenotypes. *MKSI* is expressed in the brain, kidney, liver, and digits, and gene knockdown leads to a loss of primary cilia (Dawe et al., 2007).

Aside from MKS, heterozygous variants in *MKSI* have been reported in other ciliopathies. A single heterozygous missense variant in *MKSI* (p.D286G) has been found in patients with nephronophthisis (Otto et al., 2011) and Bardet-Biedl syndrome (BBS) (Leitch et al., 2008). Another BBS patient had the heterozygous p.D286G change in *MKSI* in association with a *RPGRIP1L* A229T allele (Khanna et al., 2009). The *MKSI* variant we have identified (p.R123Q) has previously been noted as an additional hypomorphic allele in two individuals with BBS (without neural tube defects). In these cases, the first was in association with a homozygous pathogenic mutation in *BBS10* and the second was in addition to a single heterozygous mutation in *BBS10* (Leitch et al., 2008). The concept of oligogenicity and triallelism within ciliopathy syndromes is well recognized and has been reported previously (Hoefele et al., 2007; Leitch et al., 2008; Davis et al., 2011). However, the finding of *PKHD1* mutations together with modifier alleles is unusual. In a Moroccan family in whom one affected member (fetus 6) exhibited features that resembled both ARPKD and MKS a genetic analysis revealed homozygosity across *PKHD1* together with a heterozygous *CEP290* p.R205X allele (Baala et al., 2007).

BBS is a ciliopathy syndrome where oligogenicity has previously been reported (Beales et al., 2003). The heterozygous *BBS10* rare variant we identified in the proband is, however, predicted to be benign using *in silico* analyses (Table 1), and was also present in the unaffected sibling (Figure 1). It is unlikely that this allele contributed to the clinical phenotype.

We have described a patient with clinical features diagnostic of ARPKD in combination with ventriculomegaly and hydrocephalus. Genetic analysis revealed a known pathogenic homozygous mutation in *PKHD1* in association with a rare but hypomorphic heterozygous *MKSI* allele. We conclude this patient has genetically proven ARPKD with an additional neurological phenotype, which remains unexplained.

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