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CASE REPORT

Compound heterozygosity of a de novo submicroscopic deletion and an inherited frameshift pathogenic variant in the *PKHD1* gene in a fetus with bilaterally enlarged and echogenic kidneys, enlarged abdomen and oligohydramnios

Takuya Sakyu[1,2] | **Samantha R. Stover**[2,3,4] | **Yue Wang**[1,2] | **Patricia Ward**[1,2] | **Manisha Gandhi**[3,4] | **Michael C. Braun**[3,5,6,4] | **Ignatia B. Van den Veyver**[2,3,4] | **Weimin Bi**[1,2]

[1]Baylor Genetics, Houston, Texas, USA

[2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA

[3]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas, USA

[4]Texas Children's Hospital, Houston, Texas, USA

[5]Division of Pediatrics Nephrology, Baylor College of Medicine, Houston, Texas, USA

[6]Department of Pediatrics, Baylor College of Medicine, Houston, Texas, USA

Correspondence
Weimin Bi, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX, USA.
Email: wbi@bcm.edu

Present address
Takuya Sakyu, H.U. Group Research Institute GK, Tokyo, Japan

Samantha R. Stover, Department of Obstetrics and Gynecology, Division of Maternal Fetal Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

Abstract

We present a fetus with bilaterally enlarged and echogenic kidneys. Prenatal testing detected compound heterozygosity for a 0.676 Mb de novo deletion and an inherited pathogenic variant in *PKHD1*. This is the first case of autosomal recessive polycystic kidney disease (ARPKD) with a prenatally detected disease-causing *PKHD1* deletion.

KEYWORDS

22q11.21 microdeletion/duplication, ARPKD, autosomal recessive disorder, copy number variants, *PKHD1*, prenatal CMA

1 | INTRODUCTION

The polycystic kidney and hepatic disease 1 (*PKHD1*) gene is mainly expressed in the kidney and is known as a relatively large ~500 Kb gene with 67 exons encoding a 4074 amino acid protein called fibrocystin or polyductin.[1] Loss-of-function of *PKHD1* causes autosomal recessive polycystic kidney disease (ARPKD, MIM:263200), which is characterized by bilaterally enlarged, diffusely echogenic kidneys.[2] ARPKD belongs to a group of congenital hepatorenal fibrocystic syndromes and is a cause of significant renal and liver-related morbidity and mortality in children. The proposed incidence of ARPKD is 1:20,000 and the majority of cases are identified late during pregnancy or in the neonatal period.[3]

In the context of prenatal cases, ultrasonography (US) may demonstrate bilaterally echogenic, enlarged kidneys, oligohydramnios or anhydramnios, or an empty urinary bladder in severe cases of ARPKD. If severely affected, they have pulmonary hypoplasia and high mortality due to pulmonary insufficiency, or multiple intrauterine compression anomalies of lethal Potter sequence. To confirm a suspected diagnosis of ARPKD after US evaluation, molecular genetic testing including single gene testing, a multigene panel or more comprehensive genomic testing such as chromosomal microarray analysis (CMA), next generation sequencing (NGS) panel, exome, and whole genome sequencing (WES and WGS) are considered to look for pathogenic variants of *PKHD1* or other genes. ARPKD causative variants have been identified along the entire length of the *PKHD1* gene, and multiple variant types have been described as pathogenic.[4] To date, >2500 pathogenic variants have been identified in PKHD1 and recorded in ClinVar.[5] While most reported pathogenic variants are single nucleotide variants (SNV), exonic deletions/duplications of the *PKHD1* gene are also registered. However, to the best of our knowledge, there is no report of a prenatally detected causative deletion of the *PKHD1* gene.

We report here compound heterozygosity in the *PKHD1* gene for a de novo deletion and an inherited frameshift variant in a fetus.

2 | CLINICAL REPORT

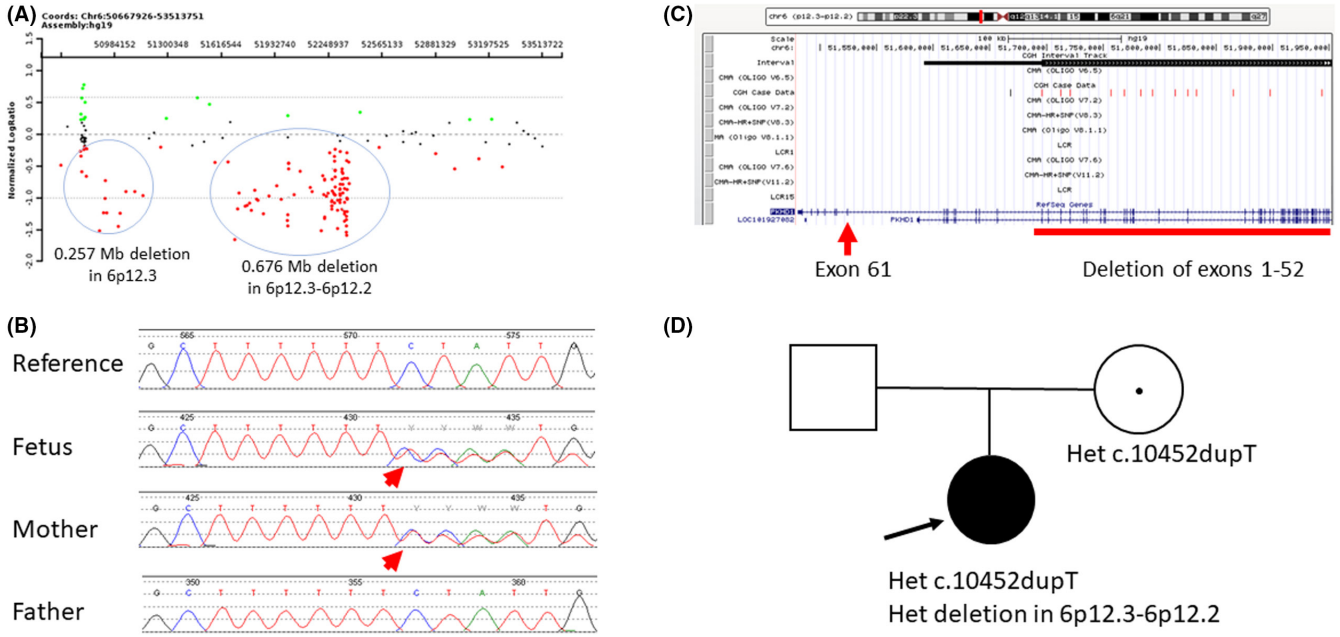
The fetus was at a gestational age of 20 weeks and 1 day with abnormal ultrasound findings of bilaterally enlarged and echogenic kidneys, enlarged abdomen, and oligohydramnios. Both parents are asymptomatic and of North European descent. The couple had unexplained infertility, and the pregnancy was achieved via in vitro fertilization (IVF). The mother was a known ARPKD carrier with a heterozygous c.10452dupT (p.Leu3485Serfs*18) pathogenic variant in the *PKHD1* gene detected by expanded carrier screening (GeneAware™). A frameshift was predicted 18 amino acids downstream. This variant was previously reported as pathogenic in a 3-week-old infant who died from ARPKD.[6] The father also underwent expanded carrier screening utilizing the same panel and was negative for all conditions analyzed.

First trimester screen and maternal serum alpha-fetoprotein (MSAFP) were negative. The fetal anatomy scan was completed using ultrasound (Voluson E8, GE healthcare systems, Inc). A ventricular septal defect (VSD) was noted with no other obvious intracardiac abnormalities. Both the right and left kidneys were markedly enlarged and slightly echogenic (Figure S1), and the abdominal circumference was also enlarged (>95th percentile). The amniotic fluid volume was subjectively reduced. The remainder of the anatomy scan was normal, though images of the spine were suboptimal and the nose/lip was not visible.

3 | MOLECULAR REPORT

Placental biopsy was performed followed by clinical prenatal CMA on the retrieved sample using a customized array (OLIGO V8.3) that has exon-by-exon coverage of over 1700 disease genes or candidate disease gene.[7] CMA revealed four copy number losses, consistent with heterozygous deletions, at 4q12, 6p12.3, 6p12.3p12.2, and 22q11.21 (Figure S2A). The sizes and location as well as the affected genes of these losses are illustrated in Figure S2B. The 6p12.3p12.2 deletion is ~0.676 Mb in size and encompasses exon 1 through 52 of the *PKHD1* gene (Figure 1A). Parental CMA did not detect this deletion. The identification of a heterozygous deletion involving the *PKHD1* gene and the ultrasound finding of bilateral enlarged and echogenic kidneys suggested that the fetus may have polycystic kidney disease caused by *PKHD1* pathogenic variants. The mother was known to carry a heterozygous c.10452dupT (p.L3485Sfs*18) pathogenic variant in the *PKHD1* gene. Thus, targeted Sanger sequencing was performed, which confirmed that the fetus was heterozygous for this familial variant (Figure 1B). The maternally inherited SNV is located in exon 61, which is outside the de novo deletion of exons 1–52 (Figure 1C). As illustrated in Figure 1D, the fetus had a de novo deletion and the familial variant. The *PKHD1* deletion in this case was previously described among a large series of disease-causing copy number variants (CNVs) associated with autosomal recessive genetic conditions.[8]

SNP array was then performed to determine the configuration of the two *PKHD1* variants in the fetus. The



(E)

Chr	Position	Mother			Father			Fetus		
		Genotype	B Allele Freq	Log R Ratio	Genotype	B Allele Freq	Log R Ratio	Genotype	B Allele Freq	Log R Ratio
6	51684250	AA	0.0007	0.057	BB	1	0.0462	AA	0.0317	-0.4979
6	52193550	AA	0	0.1222	BB	1	0.1715	AA	0.0234	-0.1917

FIGURE 1 Molecular findings in *PKHD1* in the fetus. (A) Clinical prenatal chromosomal microarray plot showing two separate deletions at 6p12. The two deletions are indicated with blue circles. The deletion at 6p12.3-p12.2 involves *PKHD1*. (B) Sanger sequencing chromatogram showing the maternally inherited c.10452dupT variant in the fetus. The extra single nucleotide T was indicated with a red arrow. (C) Locations of the inherited variant (red arrow) and the de novo deletion of exons 1–52 (red bar) in *PKHD1*. (D) Pedigree of the fetus' family. The mother is a carrier of ARPKD with a heterozygous c.10452dupT variant. The fetus inherited this variant and also carried a de novo deletion. (E) Trio SNP array analysis showed two informative SNPs in the deleted region, indicating the intact copy of chromosome 6 in the fetus is of maternal in origin.

genotypes of SNPs in the region of copy number loss in chromosome 6 involving *PKHD1* were examined. Two informative SNPs were identified, for which the mother showed a genotype of "AA" and the father showed a genotype of "BB" while the fetus showed 0% for B-allele frequency, consistent with a genotype of "A" for the non-deleted allele (Figure 1E). This result indicated that the non-deleted copy of *PKHD1* was inherited from the mother, while the other copy of chromosome 6 with a deletion was paternal in origin. Therefore, the de novo deletion was on the parentally inherited allele while the frameshift variant was on the maternally inherited allele (*in trans* configuration).

The deletion in 22q11.21 is ~0.692 Mb in the central 22q11.2 region between low copy repeats LCR22B and LCR22D, encompassing the distal portion of the common ~3 Mb DiGeorge/Velocardiofacial syndrome (DGS/VCFS) deletion (Figure 2A). It does not involve the minimal critical region associated with DGS/VCFS that includes *TBX1* and thus is not expected to lead to the classic DGS/VCFS phenotype. This atypical deletion of 22q11.21 has been reported in patients with a variable phenotype that is mildly suggestive of DGS/VCFS as well as in an asymptomatic parent.[9] CMA detected a paternal duplication at 22q11.21, involving the same region that was deleted in the fetus. Atypical nested 22q11.2 duplications are a possible risk factor for neurodevelopmental phenotypes, particularly for autism spectrum disorder, speech and language delay, and behavioral abnormalities. Incomplete penetrance and highly variable clinical expressivity have been documented.[10] The ~1.180 Mb deletion at 4q12 and ~0.257 Mb deletion at 6p12.3 contain no RefSeq genes and therefore were interpreted as likely benign variants (Figure S2C and Figure 1A). Parental CMA did not detect these two losses in either parent indicating that these changes are de novo events.

SNP array genotyping data indicated that the allele with the 22q11.21 deletion was paternal in origin. For three SNPs in this region with "BB" genotype in the mother, "AA" genotype in the father, the fetus showed 100% for B allele frequency, indicating that the non-deleted copy of chromosome 22 was inherited from the mother and the

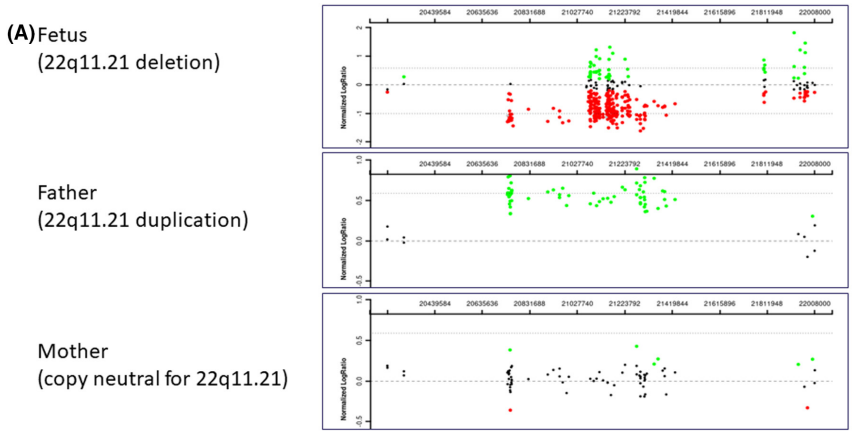


FIGURE 2 Chromosomal microarray plots showing the copy number status at 22q11.21 in this family. (A) The fetus had a de novo deletion at 22q11.21, the father had a duplication in the same region, while the mother showed copy neutral. (B) Trio SNP array data analysis revealed six informative SNPs at 22q11.21, indicating that the chromosome 22 with a deletion in the fetus was derived from the father.

(B)

Chr	Position	Mother			Father			Fetus		
		Genotype	B Allele Freq	Log R Ratio	Genotype	B Allele Freq	Log R Ratio	Genotype	B Allele Freq	Log R Ratio
22	21147293	AB	0.4783	0.0857	BB	1	0.1359	AA	0.02109	-0.3057
22	21460220	AB	0.5506	0.1134	BB	1	0.244	AA	0.01999	-0.4213
22	21431054	AB	0.496	-0.035	BB	0.9872	0.0816	AA	0.05092	-0.4794
22	21009596	BB	1	0.0542	AA	0	0.324	BB	0.9818	-0.5012
22	21427379	BB	1	-0.023	AA	0	0.2303	BB	0.98026	-0.3066

copy with a deletion was from the father. Another three SNPs had "AB" genotype in the mother, "BB" genotype in the father and 0% for B allele frequency in the fetus, further indicating that the deleted copy of chromosome 22 is paternal in origin (Figure 2B).

4 | DISCUSSION

CNVs can cause not only autosomal dominant and X-linked disorders, but also autosomal recessive conditions. While CNVs causing recessive disorders have been reported in postnatal cases, such CNVs are rarely detected and reported prenatally. We report here a prenatal case with kidney disease caused by a compound heterozygous partial gene deletion and a familial SNV, which exemplifies the importance of considering CNVs in prenatal diagnosis for autosomal recessive disorders in fetuses.

Autosomal recessive (AR) disorders, such as ARPKD, are caused by defects of both alleles of a gene located on an autosome. Individuals with an AR disorder often inherit disease-causing variants from asymptomatic carrier parents. AR disorders are caused by reduced or complete loss of function of the gene product, which are mainly attributed to single nucleotide variants (SNVs) and small insertions/deletions (indels). The other genetic changes include CNVs and rarely copy neutral events such as balanced chromosome translocations/inversions and uniparental disomy (UPD).[8,11,12] The recognition of the role of CNVs in AR disorders has improved with more and more CNVs being identified in genes associated with a wide spectrum of AR disorders. For example, different sized submicroscopic deletions of *VPS13B* have been reported in patients with Cohen syndrome (OMIM: 216550).[13] Genome-wide detection of small intragenic heterozygous CNVs still largely relies on CMA having increased coverage in disease genes.[8] CMA has been widely used for prenatal genetic testing; however, AR disease-causing CNVs have been rarely reported in prenatal cases. One contributing factor is that microarrays used for clinical prenatal testing often can detect large microdeletions/duplications, but do not have sufficient probes for detection of smaller changes in AR disease genes. The microarray used in this case has an enhanced coverage for a subset of AR disease genes, including *PKHD1*. The capability of detecting AR disease-causing CNVs would be greatly enhanced if a microarray has an enhanced coverage for all the AR disorder genes. In addition to CMA, WGS has been used for the detection of both SNVs and CNVs on a single platform,[14] and therefore, the future use of WGS for prenatal diagnosis may greatly facilitate prenatal detection of CNVs.

CNVs contribute to biallelic variations that cause AR disorders either combined with an SNV/indel on the other allele, or with a CNV on the other allele. Carriers of high-penetrant autosomal recessive alleles have one in four risks of affected fetus if the partner is also a carrier of a pathogenic allele in the same gene.[15] In this case, the mother was an ARPKD carrier and the father was negative for a pathogenic variant in *PKHD1*. When a heterozygous de novo deletion *PKHD1* was detected in the fetus, Sanger sequencing for the familial variant in *PKHD1* was initiated, which led to the quick finding of the inherited variant in the fetus. Therefore, the carrier screening results efficiently guided the subsequent prenatal testing that led to identification of the causative gene. This case also showed that carrier screening not only facilitates

identifying high risk pregnancies and targeted prenatal genetic testing, but it may also lead to a faster diagnosis and prevent unnecessary additional testing.

The fetus did not inherit the recurrent 22q11.21 microduplication from the father; instead, the fetus had a de novo microdeletion of this region on the paternally inherited chromosome 22. A previous study revealed that offspring of individuals with the Charcot–Marie–Tooth disease type 1A duplication may have a triplication of the same region that is generated through recombination between sister chromatids on the duplication bearing chromosome.[16] It is unknown whether the occurrence of 22q11.21 microdeletion in the fetus is an event associated with the father's duplication or the deletion in the fetus and the duplication in the father happened at the same region by coincidence. Further studies are needed to determine whether a microduplication flanked by low copy repeats may increase the risk of a microdeletion in offspring.

In summary, we present the first ARPKD case with compound heterozygosity of a prenatally detected de novo deletion and an inherited frameshift pathogenic variant in *PKHD1*. Our studies indicate that prenatal deletion/duplication analysis may contribute to the diagnosis of autosomal recessive disorders in addition to microdeletion/duplication syndromes.

AUTHOR CONTRIBUTIONS

Takuya Sakyu: Data curation; formal analysis; writing – original draft; writing – review and editing. **Samantha Stover:** Methodology; resources; writing – review and editing. **Yue Wang:** Data curation; writing – review and editing. **Patricia Ward:** Methodology; writing – review and editing. **Manisha Gandhi:** Resources; writing – review and editing. **Michael Braun:** Resources; writing – review and editing. **Ignatia B Van den Veyver:** Writing – review and editing. **Weimin Bi:** Conceptualization; data curation; formal analysis; writing – review and editing.

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CONFLICT OF INTEREST

Baylor College of Medicine (BCM) and H.U. Group Holdings Inc. have formed a joint venture with shared ownership and governance of Baylor Genetics (BG), which performs genetic testing and derives revenue. WB, YW, and PW are employees of BCM and derive support through a professional services agreement with BG.

DATA AVAILABILITY STATEMENT

All the data are provided within this manuscript.

ETHICAL APPROVAL

This study follows the clinical ethics.

CONSENT

The authors have obtained written informed consent from the patient for this publication.

PATIENT CONSENT STATEMENT

This study was performed under the patient consent.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

No material from other sources is included in this manuscript.

CLINICAL TRIAL REGISTRATION

There is no clinical trial registration.

ORCID

Takuya Sakyu https://orcid.org/0000-0002-0762-3951
Yue Wang https://orcid.org/0000-0002-9197-9810
Manisha Gandhi https://orcid.org/0000-0002-4193-1093
Ignatia B. Van den Veyver https://orcid.org/0000-0002-0651-5924
Weimin Bi https://orcid.org/0000-0002-1330-7031

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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