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], Samantha Stover[2], Yue Wang[1], Patricia Ward[1], Manisha Gandhi[2], Michael Braun[2], Ignatia B Van den Veyver[2], and Weimin Bi[1]

[1]Baylor Genetics
[2]Baylor College of Medicine

September 14, 2022

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.

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

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

1. Baylor Genetics, Houston, TX
2. Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX
3. Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX
4. Division of Pediatrics Nephrology, Baylor College of Medicine, Houston, Tx
5. Department of Pediatrics, Baylor College of Medicine, Houston, TX
6. Texas Children's Hospital, Houston, TX

+ Current address: H.U. Group Research Institute GK, Tokyo, Japan ++ Current address: Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville, TN TS(Takuya.Sakyu@hugp.com),SRS(samstover16@gmail.com) YW(ywang@baylorgenetics.com),PW(pward@baylorgenetics.com),MG(manishag@bcm.edu), MCB(mcbraun@texaschildrens.org),IBCDV(iveyver@bcm.edu), WB(wbi@bcm.edu)

Running title: Prenatal detection of a submicroscopic deletion associated with ARPKD

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

***Corresponding Author:**

Weimin Bi, PhD

Department of Molecular and Human Genetics

Baylor College of Medicine

One Baylor Plaza

Houston, TX, 77030-3411

Email: wbi@bcm.edu

ORCID ID is 0000-0002-1330-7031

Data availability statement : All the data are provided within this manuscript.

Funding statement : There is no funding for this study

Conflict of interest disclosure : 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.

Ethics approval statement : This study follows the clinical ethics

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

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.

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 our best 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^{TM}). 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 screen 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, Chicago, IL, USA). 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 (Supplemental Figure 1), 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 were 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 (Supplemental figure 2a). The sizes and location as well as the affected genes of these losses are illustrated in Supplemental figure 2b. 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 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 (Supplemental figure 2c 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 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]. 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 detection of both SNVs and CNVs on a single platform[14], and therefore 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 an one in four risk of an 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 analysis and summary, writing- original draft, review & editing.

Samantha R Stover: Genetic counseling of the family and writing – review & editing.

Patricia Ward: Genetic counseling and writing – review & editing.

Yue Wang: Data curation, writing – review & editing.

Manisha Gandhi: providing clinical data and writing – review & editing.

Michael C Braun: Providing clinical data and writing – review & editing

Ignatia B Van den Veyver: Writing – review & editing

Weimin Bi: Conceptualization, data curation, data analysis, Writing – review & editing.

ACKNOWLEDGEMENTS

The authors would like to thank the patient family for granting permission for publication of this study.

FUNDING INFORMATION

There is no relevant funding statement

DATA AVAILABILITY STATEMENT

All the data are provided within this manuscript.

CONSENT

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

REFERENCES

1. Ward CJ, Hogan MC, Rossetti S, et al. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet* . Mar 2002;30(3):259-69. doi:10.1038/ng833

2. Sweeney WE, Avner ED. Polycystic Kidney Disease, Autosomal Recessive. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. *GeneReviews((R))* . 1993.

3. Bergmann C, Senderek J, Windelen E, et al. Clinical consequences of PKHD1 mutations in 164 patients with autosomal-recessive polycystic kidney disease (ARPKD). *Kidney Int* . Mar 2005;67(3):829-48. doi:10.1111/j.1523-1755.2005.00148.x

4. Hartung EA, Guay-Woodford LM. Autosomal recessive polycystic kidney disease: a hepatorenal fibrocystic disorder with pleiotropic effects.*Pediatrics* . Sep 2014;134(3):e833-45. doi:10.1542/peds.2013-3646

5. Landrum MJ, Lee JM, Benson M, et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res* . Jan 4 2018;46(D1):D1062-D1067. doi:10.1093/nar/gkx1153

6. Bergmann C, Senderek J, Schneider F, et al. PKHD1 mutations in families requesting prenatal diagnosis for autosomal recessive polycystic kidney disease (ARPKD). *Hum Mutat* . May 2004;23(5):487-95. doi:10.1002/humu.20019

7. Breman A, Pursley AN, Hixson P, et al. Prenatal chromosomal microarray analysis in a diagnostic laboratory; experience with >1000 cases and review of the literature. *Prenat Diagn* . Apr 2012;32(4):351-61. doi:10.1002/pd.3861

8. Yuan B, Wang L, Liu P, et al. CNVs cause autosomal recessive genetic diseases with or without involvement of SNV/indels. *Genet Med* . Oct 2020;22(10):1633-1641. doi:10.1038/s41436-020-0864-8

9. Fernandez L, Nevado J, Santos F, et al. A deletion and a duplication in distal 22q11.2 deletion syndrome region. Clinical implications and review. *BMC Med Genet* . Jun 2 2009;10:48. doi:10.1186/1471-2350-10-48

10. Woodward KJ, Stampalia J, Vanyai H, et al. Atypical nested 22q11.2 duplications between LCR22B and LCR22D are associated with neurodevelopmental phenotypes including autism spectrum disorder with incomplete penetrance. *Mol Genet Genomic Med* . Feb 2019;7(2):e00507. doi:10.1002/mgg3.507

11. Trost B, Walker S, Wang Z, et al. A Comprehensive Workflow for Read Depth-Based Identification of Copy-Number Variation from Whole-Genome Sequence Data. *Am J Hum Genet* . Jan 4 2018;102(1):142-155. doi:10.1016/j.ajhg.2017.12.007

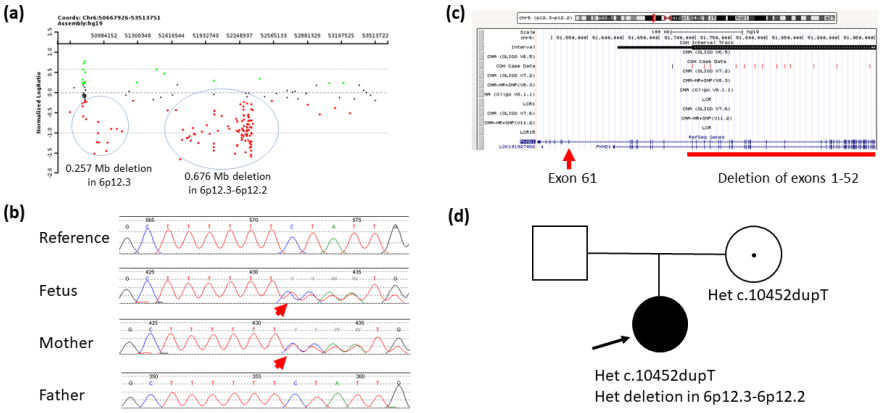
12. Yao R, Zhang C, Yu T, et al. Evaluation of three read-depth based CNV detection tools using whole-exome sequencing data. *Mol Cytogenet* . 2017;10:30. doi:10.1186/s13039-017-0333-5

13. Parri V, Katzaki E, Uliana V, et al. High frequency of COH1 intragenic deletions and duplications detected by MLPA in patients with Cohen syndrome. *Eur J Hum Genet* . Oct 2010;18(10):1133-40. doi:10.1038/ejhg.2010.59

14. Gross AM, Ajay SS, Rajan V, et al. Copy-number variants in clinical genome sequencing: deployment and interpretation for rare and undiagnosed disease. *Genet Med* . May 2019;21(5):1121-1130. doi:10.1038/s41436-018-0295-y

15. Antonarakis SE. Carrier screening for recessive disorders. *Nat Rev Genet* . Sep 2019;20(9):549-561. doi:10.1038/s41576-019-0134-2

16. Liu P, Gelowani V, Zhang F, et al. Mechanism, prevalence, and more severe neuropathy phenotype of the Charcot-Marie-Tooth type 1A triplication. *Am J Hum Genet* . Mar 6 2014;94(3):462-9. doi:10.1016/j.ajhg.2014.01.017

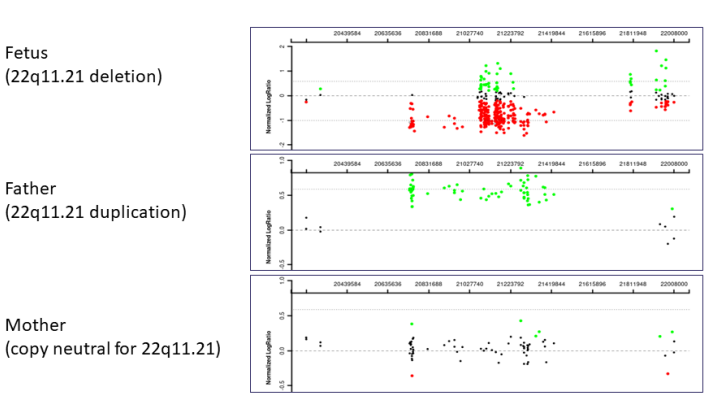


(e)

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 2

(a)



(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