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Homozygous *WNT9B* variants in two families with bilateral renal agenesis/hypoplasia/dysplasia

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Abstract

WNT9B plays a key role in the development of the mammalian urogenital system. It is essential for the induction of mesonephric and metanephric tubules, the regulation of renal tubule morphogenesis, and the regulation of renal progenitor cell expansion and differentiation. To our knowledge, *WNT9B* has not been associated with renal defects in humans; however, *WNT9B*$^{-/-}$ mice have renal agenesis/hypoplasia and reproductive tract abnormalities. We report four individuals from two unrelated consanguineous families with bilateral renal agenesis/hypoplasia/dysplasia and homozygous variants in *WNT9B.* The proband from Family 1 has bilateral renal cystic dysplasia and chronic kidney disease. He has two deceased siblings who presented with bilateral renal hypoplasia/agenesis. The three affected family members were homozygous for a missense variant in *WNT9B* (NM_003396.2: c.949G>A/p.(Gly317Arg)). The proband from Family 2 has renal hypoplasia/dysplasia, chronic kidney disease, and is homozygous for a nonsense variant in *WNT9B* (NM_003396.2: c.11dupC/p.(Pro5Alafs*52)). Two of her siblings

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data are not publicly available due to privacy or ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. Specifically, the data from Family 1 can be accessed by request through the Genomics4RD platform.

died in the neonatal period, one confirmed to be in the context of oligohydramnios. The proband's unaffected brother is also homozygous for the nonsense variant in *WNT9B*, suggesting non-penetrance. We propose a novel association of *WNT9B* and renal anomalies in humans. Further study is needed to delineate the contribution of *WNT9B* to genitourinary anomalies in humans.

Keywords

WNT9B ; Congenital anomaly of the kidneys and urinary tract (CAKUT); renal agenesis/hypoplasia/dysplasia; WNT/β-catenin signaling pathway

INTRODUCTION

Congenital anomalies of the kidney and urinary tract (CAKUT) refers to a broad spectrum of malformations affecting the kidneys and all parts of the urinary tract due to abnormal embryonic development. Bilateral renal agenesis represents the most severe form of CAKUT and is lethal. Bilateral renal hypoplasia/dysplasia often leads to renal failure *in utero,* resulting in oligohydramnios and secondary pulmonary hypoplasia, arthrogryposis and facial deformation caused by the lack of amniotic fluid (also known as the Potter sequence). The identification of genetic etiologies for CAKUT has been challenging due to genetic and phenotypic heterogeneity, incomplete penetrance and the difficulty of testing tissues following a fetal demise (Nicolaou, Renkema, Bongers, Giles, & Knoers, 2015). Syndromic and non-syndromic genetic etiologies that have been associated with CAKUT include copy number variants, most commonly 22q11.2 and 17q12 deletions, and pathogenic variants in various genes, such as *PAX2*, *HNF1B, ITGA8,* and Fraser syndrome-related genes (Nicolaou et al., 2015; Sanna-Cherchi et al., 2012; Sanna-Cherchi, Westland, Ghiggeri, & Gharavi, 2018; Vivante, Kohl, Hwang, Dworschak, & Hildebrandt, 2014). These genes are involved in regulating renal developmental processes such as initiation of nephrogenesis, ureter budding and nephron segmentation (Blake & Rosenblum, 2014; Schedl, 2007; Vivante et al., 2014).

The WNT/β-catenin signaling pathway plays a key role in patterning during embryogenesis, including the development of the mammalian urogenital system (Park, Valerius, & McMahon, 2007). *WNT9B* encodes a ligand that binds to the Frizzled receptor and subsequently activates the canonical Wnt/β-catenin pathway (Clevers, 2006). WNT9B's activation of β-catenin has been shown to be essential for the induction of mesonephric and metanephric tubules in mice (Carroll, Park, Hayashi, Majumdar, & McMahon, 2005; Karner et al., 2009). The canonical Wnt/β-catenin signaling pathway is also involved in the regulation of renal progenitor cell expansion and differentiation, and ultimately the final number of nephrons in the kidney (Karner et al., 2011; Park et al., 2007; Ramalingam et al., 2018). WNT9B also activates a number of non-canonical signaling pathways that are independent of β-catenin, such as the planar cell polarity pathway (Clevers, 2006). The planar cell polarity pathway determines the polarization of cells perpendicular to their apical-basal axis which affects the plane of each cell division, an important process for organogenesis (Karner, Wharton, & Carroll, 2006). WNT9B's signaling through the noncanonical planar cell polarity pathway has been shown to regulate

renal tubule morphogenesis, such as tubule diameter and shape (Karner et al., 2009). In keeping with *WNT9B*'s importance in renal development, *WNT9B*$^{-/-}$ mice display renal agenesis/hypoplasia and/or cystic dysplasia of the kidneys with neonatal lethality (Carroll et al., 2005; Karner et al., 2009). *WNT9B*$^{-/-}$ mice were also reported to lack reproductive ducts at birth, which correlate with the epididymis and vas deferens in males, and most of the fallopian tubes, uterus, and upper vagina in females, suggesting that *WNT9B* is involved in the posterior extension of the Müllerian duct and the development of a functional reproductive tract in mice (Carroll et al., 2005). Mice with hypomorphic *WNT9B* knockdown alleles showed some rescue of the phenotype, as they had only renal cystic dysplasia and increased survival compared to *WNT9B*$^{-/-}$ mice, surviving to postnatal day 30 (Karner et al., 2009).

To our knowledge, *WNT9B* has not been conclusively associated with disease in humans. *WNT9B* is proposed as a candidate gene for Mayer-Rokitansky-Kuster-Hauser syndrome (Müllerian aplasia- OMIM %277000). Two heterozygous variants in *WNT9B* of unknown phase were reported in a woman with isolated Müllerian agenesis (Wang et al., 2014) and Waschk *et al.* reported five candidate heterozygous variants in *WNT9B* in five additional women with isolated Müllerian agenesis (Table 1) (Waschk et al., 2016). Chen *et al* also recently reported a heterozygous candidate variant in *WNT9B* in a woman with isolated Müllerian agenesis (Table 1) (Chen et al., 2021). No functional assays were performed in either of these reports to determine if these candidate variants impaired *WNT9B* function. Here, we report four individuals, from two unrelated families, with bilateral renal agenesis/hypoplasia/dysplasia with homozygous variants in *WNT9B,* which represent the first reported association of *WNT9B* and renal defects in humans.

METHODS

The study was approved by the Children's Hospital of Eastern Ontario Research Ethics Board and the institutional review board of the Boston Children's Hospital. Informed consent was obtained from the two families. Duo exome sequencing of the proband and mother from Family 1 was performed in a clinical laboratory. The raw genomic data of these two family members were repatriated and processed through the Care4Rare Canada research bioinformatic pipeline. The generated exome variant file was reanalyzed by two analysts looking for variants in known and novel genes that could explain the proband's renal anomalies. Segregation of the *WNT9B* variant in the two affected deceased siblings, the unaffected brother, and the father was done by Sanger sequencing (primers available on request) at the Children's Hospital of Eastern Ontario's Research Institute (Ottawa, Canada). DNA from the two affected fetal siblings was extracted from paraffin-embedded tissues from previous fetal autopsies using a QIAGEN commercial kit by a clinical molecular oncology laboratory at The Ottawa Hospital (Ottawa, Canada) (see (Sarnecka et al., 2019) for details of the protocol).

Exome sequencing as a singleton of the proband from Family 2 was performed on a research basis at Boston Children's Hospital (Boston, MA, USA) (see (van der Ven et al., 2018) and (Braun et al., 2016) for technical details). Segregation of the *WNT9B* variant in the proband,

the unaffected sibling, and the proband's parents was done by Sanger sequencing (primers available on request) at the Boston Children's Hospital (Boston, MA, USA).

Western Blot and real-time PCR (RT-PCR) analysis was conducted to assess WNT9B expression in the following control cells: lymphoblasts, fibroblasts, SHY-5Y cells, and induced pluripotent stem cells. For RT-PCR, cells were snap-frozen, and then total RNA was isolated using the Qiagen RNeasy minikit according to the manufacturer's instructions. Approximately 7 μg total RNA was reverse transcribed in a 20-μl reaction mixture using the iScript Advanced cDNA Synthesis Kit (Bio-Rad). A total of six sets of *WNT9B* primers were designed spanning the gene (sequences available upon request). PCRs were run across a gradient of annealing temperatures in all cell types. For Western blot, cells were lysed in RIPA buffer and briefly sonicated. Approximately 100 μg of total protein was subjected to SDS-PAGE and transferred to PVDF membranes. Membranes were blocked in 5% non-fat dry milk in TBS, then incubated with antibody against WNT9B (Invitrogen PA5-19419; 4μg/mL). After washing, blots were probed with donkey anti-goat peroxidase (1:1000), then developed using chemiluminescence reagents (ECL, Bio-Rad).

RESULTS

Clinical report of Family 1

The proband is a 16-month-old male with bilateral renal cystic dysplasia. Prenatal ultrasounds identified bilateral hydronephrosis and renal pelvis dilatation, with mild to moderate oligohydramnios. Fetal MRI did not identify pulmonary hypoplasia. The pregnancy and delivery were otherwise unremarkable. Postnatal renal ultrasounds showed bilateral cystic dilatation of the renal pelvis, hydronephrosis, hypoplasia of renal parenchyma in bilateral upper poles, and bilateral multiple small parenchymal cysts. Voiding cystourethrogram showed grade II right vesicoureteral reflux and grade IV-V left vesicoureteral reflux. The proband was put on trimethoprim prophylaxis. Kidney growth was normal on subsequent ultrasounds. At 16 months of age, he was diagnosed with mild chronic kidney disease (stage 2); his estimated glomerular filtration rate was decreased at 63 mL/min/1.73m^2 (normal: > 90 mL/min/1.73m^2), and his creatinine plasma level was mildly elevated (42 umol/L, upper limit of normal for age: 34 umol/L). His arterial blood pressure was 97/53 (blood pressure percentiles are 86% systolic and 90% diastolic based on the 2017 AAP clinical Practice Guideline (Flynn et al., 2017)). The proband's psychomotor development was normal. At 16 months of age, his weight was 10.8 kg (58% on WHO growth chart), and his height was 78.6 cm (25% on WHO growth chart). Dysmorphology examination revealed mildly distinctive ears with bilateral squared off helices. The rest of the examination was normal, including normal male external genitalia.

The family history was significant for parental consanguinity (the specific degree of relatedness was unknown to the parents) and two previous pregnancies which presented with an oligohydramnios sequence (Figure 1a). The couple's first pregnancy was medically terminated at 20 weeks of gestation, after the identification of bilateral severe renal hypoplasia and oligohydramnios. The autopsy reported bilateral severe renal hypoplasia/dysplasia (Figure 2) and Potter sequence, normal male external genitalia, and a bilateral sandal-toe-gap. The fetal karyotype was 46,XY. No further genetic testing was performed.

The couple's second pregnancy was again complicated by bilateral renal agenesis and oligohydramnios. The pregnancy was medically terminated at 21 weeks of gestation; autopsy reported bilateral renal agenesis with Potter sequence and normal female internal and external genitalia. The fetal karyotype was 46,XX. A fetal chromosomal microarray identified a maternally inherited 1.5 Mb 2q24.1q24.2 duplication of unknown significance (chr2:158,925,958-160,430,127 (GRCh37/hg19)), which was interpreted clinically as being likely benign. The microarray also revealed five long contiguous stretches of homozygosity greater than 10 Mb in size, estimated to encompass approximately 3.9% of the autosome genome, which is consistent with the known parental consanguinity and suggests that parents are second cousins. A CAKUT panel of 51 genes performed in a commercial laboratory on DNA from the second affected fetus was non-diagnostic. Both parents and the unaffected brother had a normal renal ultrasound.

Results of genome-wide sequencing in Family 1

Analysis of the proband's exome sequencing data identified a homozygous missense variant in *WNT9B*: NM_003396.2:c.949G>A/p.(Gly317Arg); the exome sequencing was done as a duo with the proband's mother and she was heterozygous for this variant. *In silico* analysis programs predicted the change of this conserved residue to impact protein structure and function (CADD 32). This missense variant was rare in gnomAD v2.1.1, with only one allele identified (1/249030 alleles, allele frequency of 0.0004%) (Karczewski et al., 2020). No other candidate genes or variants were identified by exome analysis to explain the proband's renal defect. Sanger sequencing was then performed on DNA extracted from tissues of the two previously affected fetuses, and both were homozygous for the *WNT9B* variant. Segregation testing in the unaffected father and brother showed that they were heterozygous for the variant.

Clinical report of Family 2

The proband is an 8-year-old girl who presented with renal hypoplasia/dysplasia and chronic renal insufficiency. Renal ultrasound at 6 years of age revealed diffuse increase in echogenicity of the right kidney and a cortical cyst of 1.4cm × 1.1cm. The left kidney was hypoplastic and diffusely echogenic, and there was mild dilatation of the renal pelvis. The proband has stage 4 chronic kidney disease. At 8 years of age, her creatinine level was 157 umol/L (upper limit of normal: 115 umol/L), her blood urea nitrogen level was 14.3 mmol/L (upper limit of normal: 6.4 mmol/L), and her albumin level was 33 g/L (lower limit of normal: 40.2 g/L). A blood pressure measured at 8 years of age was 115/59 (blood pressure percentiles are 95% systolic and 50% diastolic based on the 2017 AAP clinical Practice Guideline (Flynn et al., 2017)). At 8 years and 2 months of age, her weight was 19.6 kg (3% on WHO growth chart), and her height was 117 cm (3% on WHO growth chart). A pelvic ultrasound reported normal uterus and ovaries, and external examination showed normal female genitalia. The proband also has congenital hearing loss, for which she received cochlear implant, and congenital hip dysplasia that required surgical repair.

The family history was significant for parental consanguinity (parents are first cousins) and two male siblings who died in the neonatal period (Figure 1b), neither of whom had imaging or autopsy performed. The first sibling died within on the first day of life. Oligohydramnios

had been identified in that pregnancy at 32 weeks, and an emergency Caesarian section was performed at 36 weeks of gestation for fetal distress. No renal assessment was reported to the family. The second sibling died at 20 days of life from an unidentified etiology. That pregnancy was reportedly unremarkable, with no fetal malformations or oligohydramnios identified. The proband also has a 9-year-old brother who is reported to have congenital hearing loss. He is otherwise healthy and had a normal renal ultrasound. Both parents have had a normal renal ultrasound.

Results of genome-wide sequencing in Family 2

Analysis of the proband's exome sequencing data identified a homozygous nonsense variant in *WNT9B* (NM_003396.2: c.11dupC/p.(Pro5Alafs*52)). This nonsense variant is located in exon 1 of the gene, and is therefore predicted to cause nonsense-mediated mRNA decay. This variant was rare in gnomAD v2.1.1 (2/36870 alleles, allele frequency of 0.005%) (Karczewski et al., 2020). Sanger sequencing confirmed that the variant was homozygous in the proband and showed that each parent was heterozygous for the variant. The proband's unaffected brother was also homozygous for the variant in *WNT9B*. DNA from the two siblings who died in the neonatal period was not available for analysis.

In addition, the proband and her 9-year-old brother also carried a homozygous nonsense variant in *MYO15A* (NM_016239.3:c.10043delT/p.(Leu3348Argfs*105)), which causes hearing loss. This variant has not been observed in the gnomAD population database (Karczewski et al., 2020) or in affected individuals in the literature or ClinVar. The variant is predicted to cause nonsense-mediated mRNA decay in a gene for which loss-of-function is a known mechanism of disease. Biallelic loss-of-function variants in *MYO15A* are associated with Deafness- autosomal recessive 3 (OMIM #600316). Parents are heterozygous for this variant. This variant was classified as pathogenic based on ACMG criteria (Richards et al., 2015) and was therefore determined to be the genetic basis of the congenital hearing loss identified in the proband and her brother.

Expression levels of WNT9B protein

Western Blot and RT-PCR did not detect any *WNT9B* expression in any of the control lymphoblasts, fibroblasts, SHY-5Y cells, or induced pluripotent stem cells. Therefore, we were unfortunately unable to assess *WNT9B* expression or perform any functional analysis on patient cells.

DISCUSSION

We present four individuals from two unrelated consanguineous families affected by bilateral renal agenesis/hypoplasia/dysplasia with homozygous rare variants in *WNT9B.* Multiple lines of evidence suggest that the alteration of *WNT9B* is associated with the renal defects in these individuals. First, the observed renal anomalies in the four individuals are similar to the phenotypes described in *WNT9B* knockout mice and in mice with hypomorphic *WNT9B* knockdown alleles. The identification of this overlapping renal dysgenesis phenotype in our patients suggests that the identified *WNT9B* variants are also associated with renal anomalies in humans. Second, the characteristics of the variants

identified in *WNT9B* are convincing for pathogenicity. The affected residue G317 in Family 1 is highly conserved across the Wnt proteins, and required for *WNT9B* disulfide bonding (Janda, Waghray, Levin, Thomas, & Garcia, 2012). In addition, it is rare in population databases, *in silico* models predict that this missense change is deleterious, and we have demonstrated concordant segregation in six family members. The nonsense variant in Family 2 is predicted to introduce a premature stop codon and lead to nonsense-mediated mRNA decay. This is compatible with a loss-of-function mechanism of disease, suggested by the observed phenotype in *WNT9B*$^{-/-}$ mice. We were unfortunately unable to detect the WNT9B protein in control lymphoblasts or fibroblasts, and therefore could not perform functional assays to determine if our variants lead to loss of *WNT9B* function. *WNT9B* has been shown to be expressed in mice embryonic kidneys (Carroll et al., 2005), but the Genotype-Tissue Expression project reports that *WNT9B* is only lowly expressed in most adult human tissues except the kidneys (GTEx Consortium, 2013), which is presumably why we were unable to detect its expression in the tissues we tested. Third, all of the renal phenotypes presented here, ranging from lethal bilateral renal agenesis to renal dysplasia and mild renal insufficiency, are along the same spectrum in the four confirmed affected individuals from two unrelated families. Although there was only one confirmed affected child in Family 2, it is likely that there was at least one additional affected sibling given the report of oligohydramnios and neonatal death, though we cannot confirm this.

Given the above, it was surprising that the unaffected brother in Family 2 was also homozygous for the nonsense mutation in *WNT9B*. This presumably speaks to the underlying complexity of the genetics of CAKUT, which have been historically difficult to elucidate. This result could be explained by incomplete penetrance, which is well described in CAKUT disorders (Nicolaou et al., 2015). Factors potentially influencing reduced penetrance in CAKUT include polymorphisms in other genes involved in the different stages of renal development and *in utero* environment, such as maternal weight, maternal hyperglycemia and factors leading to low birth weight (Nicolaou et al., 2015). Reduced penetrance is less common in autosomal recessive disorders, but is reported in some, such as hereditary hemochromatosis, Gaucher disease, and Schimke immunoosseous dysplasia (Cooper, Krawczak, Polychronakos, Tyler-Smith, & Kehrer-Sawatzki, 2013). Environmental exposure, patient gender and single nucleotide variants or variation in gene dosage in interacting genes were suggested to have an effect on these phenotypes (Cooper et al., 2013). The effect of modifier genes, environmental factors, and epigenetic influences may explain the variable expressivity and non-penetrance observed in the two reported families.

WNT9B$^{-/-}$ female mice were reported to have Müllerian agenesis and *WNT9B*$^{-/-}$ male mice lacked the epididymis and vas deferens; therefore it is noteworthy that the four affected individuals in this report do not have any known anomalies of the reproductive tract. The difference between the null mouse Müllerian phenotypes and our patient phenotypes could be explained by a difference in *WNT9B*'s role in the urogenital system development between humans and mice, domain specific mutations, or the effect of a variable degree of loss of *WNT9B* function, though the variant in Family 2 is presumably a null variant. As prior reports have suggested *WNT9B* as a candidate gene for human Müllerian agenesis (Chen et al., 2021; Wang et al., 2014; Waschk et al., 2016), we looked further at the reported variants in these papers (Table 1). The five heterozygous candidate variants for Müllerian

agenesis reported by Waschk *et al* (Waschk et al., 2016), have allele counts of 1, 348, 55, 0 and 12 in gnomAD v2.1.1 (Karczewski et al., 2020), suggesting that at least three of them are too common to cause this rare anomaly on their own. In addition, the variants were not segregated in unaffected family members to our knowledge, and therefore they may be inherited. The remaining rare, though unsegregated, variants were in *WNT9B*'s exons 3 and 4, and the missense variant identified in Family 1 of this report was in exon 4, therefore unlikely to be related to a domain specific effect. In the Wang *et al* paper (Wang et al., 2014), the phase of the two variants was unknown. One variant was rare but in the 3' untranslated region and not predicted to impact splicing based on SpliceAI score (Jaganathan et al., 2019), and the other was a missense variant that is now known to have 8 allele counts in gnomAD v2.1.1 (Karczewski et al., 2020), making these unlikely to cause this rare phenotype. Moreover, the heterozygous nonsense variant recently reported by Chen *et al* is not expressed in *WNT9B*'s canonical transcript (NM_003396.2) and is in the last exon of the NM_001320458.2 transcript, removing only four amino acids from the WNT9B protein and not predicted to result in nonsense-mediated mRNA decay (Chen et al., 2021). This variant has two allele counts in gnomAD v2.1.1 (Karczewski et al., 2020) and was not segregated in family members. The above evidence does not suggest that this variant is the genetic basis for the Müllerian agenesis present in the affected individual. Also, in our reported families, both sets of parents of the affected individuals, who are heterozygous carriers of a presumed pathogenic *WNT9B* variant, had normal fertility and the woman were able to bear children, thus suggesting that they had normal Müllerian structures. Therefore, it is not clear that heterozygous variants in *WNT9B* are sufficient to cause reproductive tract anomalies in humans.

In conclusion, we report the first association of *WNT9B* and renal anomalies in humans. These findings are consistent with the renal phenotype observed in null mice. Given the observed nonpenetrance in a family member of Family 2, we recognize that the phenotype-genotype relationship is more complex than simple autosomal recessive inheritance. Ongoing work is needed to further delineate the contribution of *WNT9B* to both renal anomalies and genitourinary anomalies in humans.

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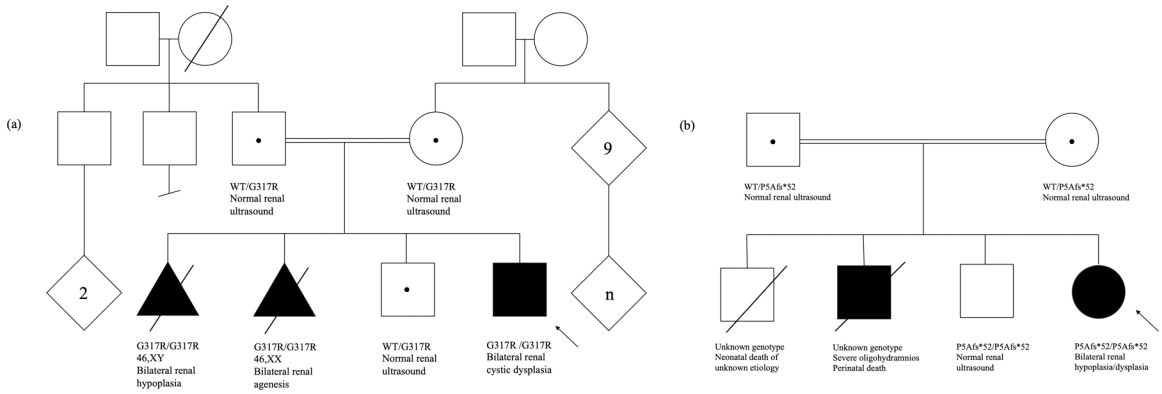

FIGURE 1.

(a) Pedigree of Family 1 showing parental consanguinity, information on phenotype and genotype of the six family members. Black symbols represent affected individuals. Symbols containing a dot represent a heterozygous carrier status. Variants in *WNT9B* (NM_003396.2); WT: Wild type; G317R: c.949G>A/p.Gly317Arg. (b) Pedigree of Family 2 showing parental consanguinity, and the available information about the phenotype and genotype of the six family members. Variants in *WNT9B* (NM_003396.2); WT: Wild type; P5Afs*52: c.11dupC; p.Pro5Alafs*52.

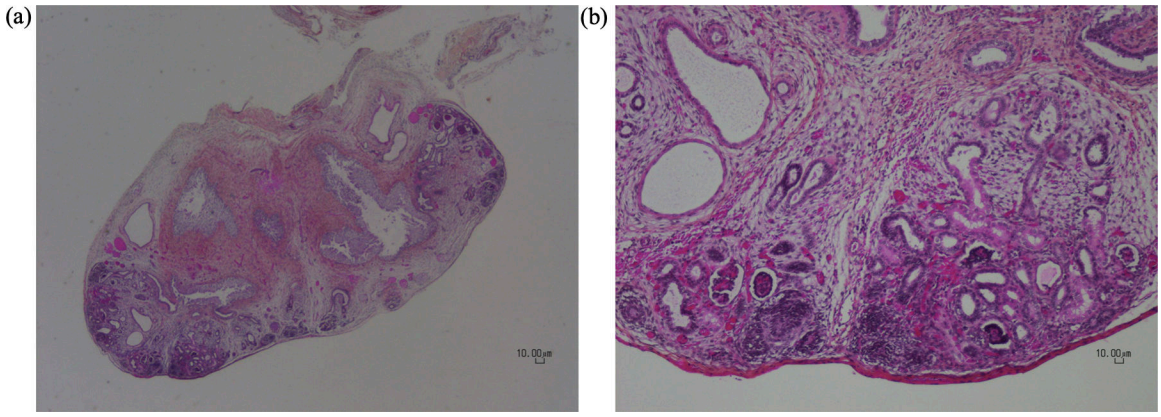

FIGURE 2.

Hematoxylin and Eosin staining of renal tissue from the autopsy of the affected male fetus from Family 1 at 21weeks of gestation showing dysplastic kidney. (a) 2x magnification. (b) 10x magnification showing rare dysplastic glomeruli with dilated tubular structures in a background of loose fibromyxoid tissues.

Table 1.

Comparison of homozygous variants in *WNT9B* in the two families with renal agenesis/hypoplasia/dysplasia in this report and previously published heterozygous variants in women with Müllerian agenesis

	This report		Wang *et al* 2014	Waschk *et al* 2016					Chen *et al* 2021
	Family 1	Family 2		1	2	3	4	5	
Variant (NM_003396.2)	c.949G>A	c.11dup	c.28G>T/ c.*158C>T	c.472C>G	c.665G>A	c.722G>A	c.974G>A	c.1029C>A	c.976C>T (NM_001320458.2)[¶]
Protein change	p.G317R	p.P5Afs*52	p.A10S/ −	p.Q158E	p.R222H	p.R241H	p.R325H	p.Cys343*	p.Q326*
Zygosity	Homo	Homo	Het/Het	Het	Het	Het	Het	Het	Het
Gene location	Exon 4/4	Exon 1/4	Exon 1/4/ 3'UTR	Exon 3/4	Exon 4/4	Exon 4/4	Exon 4/4	Exon 4/4	Exon 5/5[¶]
gnomAD v2.1.1 allele frequency (%)	0.0004	0.005[‡]	0.017/0	0	0.12	0.019	0.004	0.0004	0.002
Familial segregation results	Concordant[†]	Inconclusive[§]	Not done, unknown phase	Not done	Not done	Not done	Not done	Not done	Not done
CADD score	32	-	21/6	22	26	25	29	-	-

[†]The three affected individuals in Family 1 are homozygous, each parent is heterozygous and the unaffected sibling is not homozygous for the variant in *WNT9B*

[‡]The p.P5Afs*52 variant is covered in fewer than 50% of individuals in gnomAD v2.1.1 and allele frequency estimates may not be reliable. In gnomAD v3.1 (which consists of genome data) this variant is observed in 4 alleles (4/151500 alleles, allele frequency of 0.003%).

[§]Both the affected individual and the unaffected brother in Family 2 are homozygous for the variant in *WNT9B*. Each parent is heterozygous for the variant. The variant was not segregated in the two siblings from Family 2 who died in the neonatal period.

[¶]The p.Q326* variant falls outside of the NM_003396.2 canonical transcript, and is only expressed in the NM_001320458.2 transcript, which has 5 exons.