

Broadening the Ciliopathy Spectrum: Motile Cilia Dyskinesia, and Nephronophthisis Associated With a Previously Unreported Homozygous Mutation in the *INVS/NPHP2* Gene

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Nephronophthisis associated ciliopathies (NPHP-AC) are a group of phenotypically related conditions that include Joubert syndrome, Meckel syndrome, nephronophthisis (NPHP), and Senior-Loken syndrome. We report on a male fetus with prenatal ultrasound findings at 24 weeks of gestation of anhydramnios, large and echogenic kidneys and *situs inversus totalis*. Histopathology revealed nephronophthisis and tracheal mucosa electron microscopy revealed ciliary dysgenesis. DNA analysis of the NPHP genes showed a previously unreported homozygous mutation, p.Arg603* (c.1078+1G>A), in the *INVS/NPHP2* gene. This mutation is thought to abolish the splice donor site for exon 8, which likely disrupts the normal splicing of the *INVS/NPHP2* gene. © 2013 Wiley Periodicals, Inc.

Key words: *INVS/NPHP2*; nephronophthisis; ciliopathy; *situs inversus*; splicing

INTRODUCTION

Nephronophthisis-associated ciliopathies (NPHP-AC) are a group of phenotypically related conditions that include Joubert syndrome, Meckel syndrome, nephronophthisis (NPHP) and Senior-Loken syndrome. These associated conditions are thought to be united by a common pathogenesis of dysfunction of the primary cilium/basal body complex [Otto et al., 2011]. These conditions have a shared broad phenotype with autosomal recessive inheritance. Many of the phenotypes tend to cluster around the type of cell affected in a particular organ. Renal failure that can begin in early childhood and progress into adolescence in NPHP is thought to result from kidney tubular basement

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membrane disintegration, tubular atrophy, and multiple cyst formations [Chaki et al., 2011; Otto et al., 2011].

Nephronophthisis is due to homozygous or compound heterozygous mutations of *INVS/NPHP2* typically diagnosed at a year of age, with the presence of hypertension and a concomitant or subsequent rather rapid onset of renal failure. The *INVS/NPHP2* gene encodes the protein inversin, which has multiple ankyrin domains and two IQ calmodulin-binding domains [Schön et al., 2002; Otto et al., 2003, Otto et al., 2008]. On ultrasonography, the kidneys are usually

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hyperechogenic (cystic), with histopathological findings of focal tubule dilatation and diffuse moderate interstitial fibrosis [Otto et al., 2003]. Other manifestations include abnormal left-right axis, cardiac abnormalities such as septal defects, *situs inversus*, and hepatic involvement.

It has been suggested that there is an interaction between inversin, β -tubulin and the protein nephrocystin whose gene, when mutated causes NPHP1 [Chaki et al., 2011]. The co-localization of these proteins to the primary cilia, which are involved in left-right axis determination, explains the overlapping phenotypes that have been seen to date.

Reports of *INVS/NPHP2* mutations consistently result in dysplastic kidneys with histologic changes of tubular basement membrane disruption, tubulointerstitial nephropathy, and corticomedullary cysts [Bellavia et al., 2010]. But not all individuals with *INVS/NPHP2* mutations have extra-renal manifestations. The variability of the clinical manifestations raises the possibility of the presence of additional modifier genes [Tory et al., 2009; Chaki et al., 2011]. In humans, the incidence of *situs inversus* is much lower, than the up to 90% observed in the *inv/inv* mouse. The possibility exists that in humans, as opposed to the *inv/inv* mouse, there may be other modifiers of the left-right axis that are still unknown, or that the absent *situs* might be related to specific mutations in the *INVS/NPHP2* gene.

CLINICAL REPORT

The mother was 20-year-old primigravida woman of French Canadian/First Nation descent and the father was 21 years old and of European descent. The couple was seen initially regarding fetal ultrasound findings of anhydramnios, large and echogenic kidneys, hepatic septated cyst, and *situs inversus totalis*. The couple was counselled and decided to terminate the pregnancy at 24 weeks gestation.

Autopsy confirmed *situs inversus totalis* with the morphological right atrium appendage and ventricle on the left side and morphological left structures on the right. The left sided right ventricle was non apex forming, 1.15 cm from tricuspid valve hinge to apex, compared to the right sided left ventricle, 1.9 cm from mitral valve hinge to apex (Fig. 1). The kidneys were large and cystic displaying the infantile form of NPHP (Figs. 2 and 3) with multiple cysts. There were also pancreatic and hepatic cysts (Fig. 4). Electron microscopy of tracheal mucosa showed ciliary dysgenesis (Figs. 5 and 6), and upon review of at least 100 cilia as suggested by the Shoemark et al. [2012] 10% contained a central microtubule pair, 40% contained a single microtubule, and 50% did not contain any central microtubules.

Investigations included a normal aCGH, and DNA analysis for the NPHP genes (Prevention Genetic) showed a previously unreported homozygous mutation c.1078+1G>A in the *INVS/NPHP2* gene (Fig. 7). This mutation is thought to abolish the splice donor site for exon 8, which likely disrupts the normal splicing of the *INVS/NPHP2* gene. Given the presence of ciliary dysgenesis in this case we also performed mutation analysis of the genes *DNAH5*, *DNAH11*, *DNAI1*, *DNAI2*, *DNAL1*, *CCDC39*, *CCDC40*, *LRRC50*, *DNAAF1*, *KTU1/DNAAF2*, *TXNDC3*, *RSPH4A*, *RSPH9*, *RPGR*, and *OFD1*, known to be associated with primary ciliary dyskinesia (PCD) and no mutation was detected.

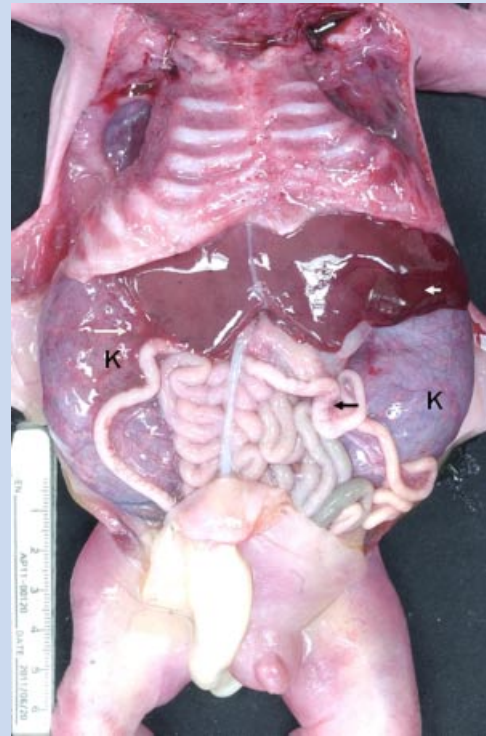


FIG. 1. Fetal autopsy showing *situs inversus* and enlarged kidneys [K]. White arrows show the left-sided liver and the black arrow points to the left-sided appendix.

DISCUSSION

The *INVS* gene encodes a protein containing multiple ankyrin domains and two IQ calmodulin-binding domains. The encoded protein may function in renal tubular development and function, and in left-right axis determination. This protein interacts with



FIG. 2. Enlarged cystic kidneys with a combined weight of 93 g [expected 5.7 ± 1.6 g].

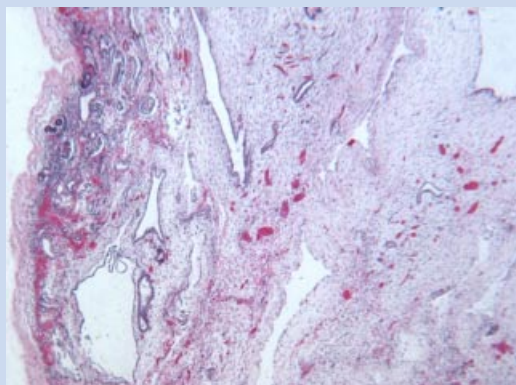


FIG. 3. Kidney showing increased mesenchymal tissue and cysts predominantly in the renal cortices; hematoxylin; and eosin stain (40 \times).

nephrocystin and constitutes a connection between primary ciliary function and left-right axis determination.

Although laterality disorders have been observed in 90% of the *inv/inv* mouse [Bergmann et al., 2008] it has been rarely reported in NPHP and to date there have been only 4 due to mutations in *INVS/NPHP2* gene (Table I). Otto et al. [2003] reported a patient born to a consanguineous Turkish family who had *situs inversus*, ventricular

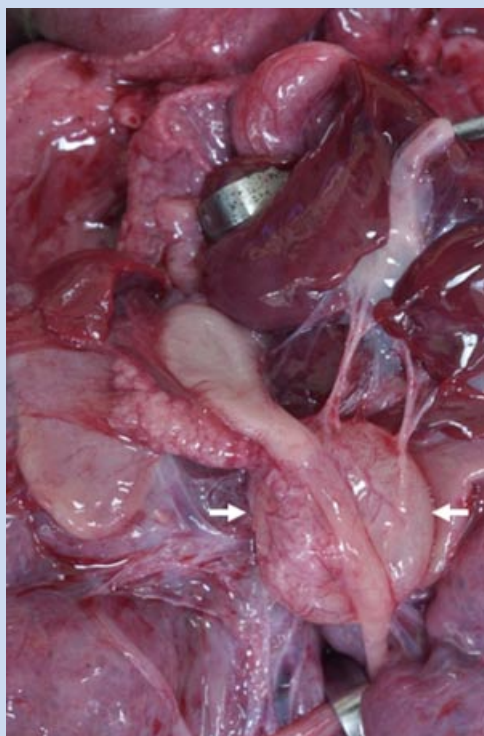


FIG. 4. Cyst present in the head of the pancreas (white arrows).

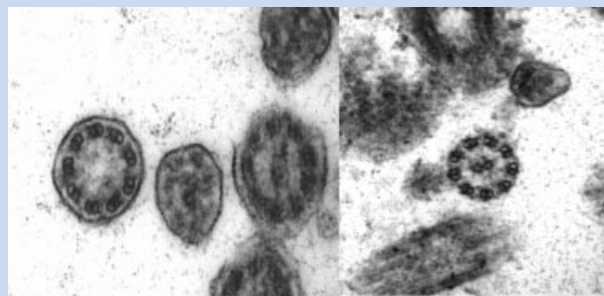


FIG. 5. Left: Electron microscopy of tracheal mucosa. Left, patient showing absence of central microtubule doublets in cilia; Right: normal 23-week gestation fetus showing cilia with central microtubule doublets.

septal defect, and recurrent bronchitis due to a c.1807C>T mutation in the *INVS/NPHP2* gene. The second case, from France, had *situs inversus*, aortic and pulmonary valve stenosis and recurrent bronchitis due to a c.1194T>G mutation in the *INVS/NPHP2* gene [Tory et al., 2009] and a third case, from Belgium, was interesting for the discordance of *situs inversus*, in one of the two affected sibs, both with NPHP, who had the same c.2695C>T mutation [Bellavia et al., 2010] (Table I). Our case is only the fourth report of *situs inversus* associated with a new mutation in the *INVS/NPHP2* gene and adds to the variability of clinical manifestations seen in individuals with *INVS/NPHP2* mutations. The possibility exists that in humans, as opposed to the *inv/inv* mouse, there may be other still unknown genetic modifiers of left-right axis and the loss of *situs* might be related to specific mutations in the *INVS* gene.

Ivemark et al. [1959] reported two sibs with what they termed renal-hepatic-pancreatic dysplasia (RHPD, OMIM #208540). Further investigation ascribed the RHPD to a mutation in the

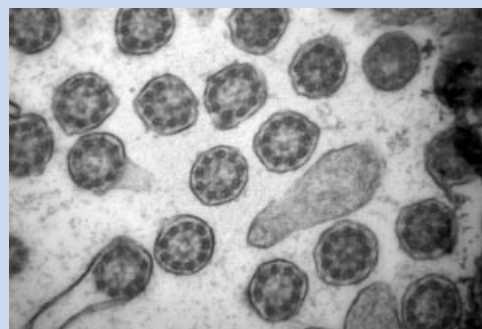


FIG. 6. Electron microscopy of tracheal mucosa from the proband showing abnormalities in multiple central microtubule doublets in cilia.

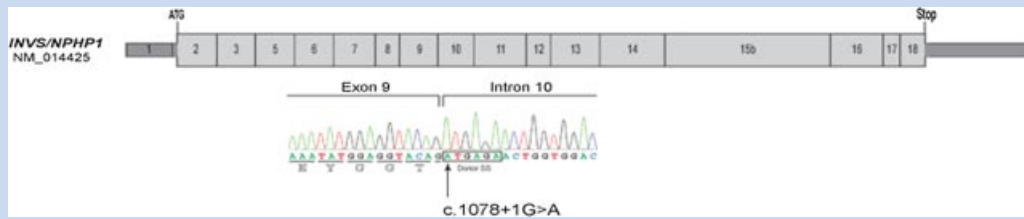


FIG. 7. Chromatogram of the *INVS/NPHP2* gene from the proband illustrating the c.1078+1G>A mutation.

TABLE I. Reported Cases with Mutations in the *INVS/NPHP2* Gene Associated with *situs inversus*

Case	Origin/consanguinity/age at diagnosis	NPHP2–Nuc seq	Findings
Otto et al. [2003]	Turkey/consanguineous/12 months	c.1807C>T-homozygous	ST; ventricular septal defect; HEK; no renal cysts; recurrent bronchitis
Tory et al. [2009]	French/non-consanguineous/18 months	c.1194T>G c.2695C>T	ST; aortic and pulmonary valve stenosis; HEK; no renal cysts; anemia; recurrent bronchitis
Bellavia et al. [2010]	Italy/non-consanguineous/renal failure at age 10 years	c.2695C>T-homozygous	ST with complete transposition of the large arteries; small kidneys with multiple small cysts; absence of retinitis pigmentosa
Present case	French-Canadian and European/non-consanguineous/prenatal	c.1078+1G>A-homozygous	ST; right atrium and appendage with left morphology and left atrium having right morphology with indeterminate appendage and reversed ventricles. Cystic and HEK displaying the infantile form of NPHP. There were also pancreatic and hepatic cysts. Electron microscopy of tracheal mucosa showed ciliary dysgenesis

ST, situs inversus; HEK, hyperechogenic enlarged kidneys.

NPHP3 gene [Bergmann et al., 2008]. Some of the affected patients with this condition have had enlarged polycystic kidneys, pancreatic cysts, dilated dysgenetic bile ducts, dilated pancreatic ducts, postaxial polydactyly, complete *situs inversus*, polysplenia, preauricular fistulas, and Dandy–Walker malformation, consistent with a ciliopathy and probably Joubert-like condition [Ivemark et al., 1959; Torra et al., 1996; Bergmann et al., 2008]. Our patient had pancreatic and hepatic cysts with normal brain anatomy and histopathology. Thus, it is possible that RHPD is genetically heterogeneous with one of the causative genes being the *INVS* gene. This is the first report of a PCD phenotype associated with NPHP and suggests that motile ciliary defects should be looked for in other ciliopathies.

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