MKS3-Related Ciliopathy with Features of Autosomal Recessive Polycystic Kidney Disease, Nephronophthisis, and Joubert Syndrome

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Objectives To describe 3 children with mutations in a Meckel syndrome gene (MKS3), with features of autosomal recessive polycystic kidney disease (ARPKD), nephronophthisis, and Joubert syndrome (JS).

Studydesign Biochemical evaluations, magnetic resonance and ultrasound imaging, electroretinograms, IQ testing, and sequence analysis of the PKHD1 and MKS3 genes were performed. Functional consequences of the MKS3 mutations were evaluated by cDNA sequencing and transfection studies with constructs of meckelin, the protein product of MKS3.

Results These 3 children with MKS3 mutations had features typical of ARPKD, that is, enlarged, diffusely microcystic kidneys and early-onset severe hypertension. They also exhibited early-onset chronic anemia, a feature of nephronophthisis, and speech and oculomotor apraxia, suggestive of JS. Magnetic resonance imaging of the brain, originally interpreted as normal, revealed midbrain and cerebellar abnormalities in the spectrum of the “molar tooth sign” that characterizes JS.

Conclusions These findings expand the phenotypes associated with MKS3 mutations. MKS3-related ciliopathies should be considered in patients with an ARPKD-like phenotype, especially in the presence of speech and oculomotor apraxia. In such patients, careful expert evaluation of the brain images can be beneficial because the brain malformations can be subtle. (J Pediatr 2009;155:386-92).

Human ciliopathies, caused by defects in proteins that reside on the cilia or its basal body, include disorders of both the respiratory (motile) and primary (immotile) cilia. Primary cilia are important organelles that sense extracellular chemical and mechanical stimuli such as fluid flow within kidney tubules and bile ducts.

Autosomal recessive polycystic kidney disease (ARPKD) is caused by mutations in PKHD1. This gene encodes fibrocystin/polyductin, a protein that functions in the primary cilia. Affected individuals have fusiform dilatations of the renal collecting ducts, leading to progressive renal insufficiency, and ductal plate malformation of the liver resulting in congenital hepatic fibrosis (CHF) and biliary macrocysts, often referred to as Caroli’s syndrome. Most patients with ARPKD present perinatally with enlarged, diffusely microcystic kidneys, and approximately 30% die of pulmonary hypoplasia. CHF invariably accompanies ARPKD. Although mild in early childhood, in most patients it eventually leads to portal hypertension (PH), often complicated by esophageal varices and hypersplenism. MKS3 mutations have not been previously associated with ARPKD.

Classic Meckel syndrome (MKS) is a severe, typically perinatally-lethal ciliopathy characterized by cystic dysplastic kidneys and CHF; polydactyly occurs in 55% of affected fetuses, and some patients present with Dandy-Walker malformation or hydrocephalus rather than the classic encephalocele. Five MKS causative genes, MKS1, MKS3, CEP290, RPGRIP1L, and CC2D2A, have been identified. The MKS3 gene, which encodes meckelin, has displayed frameshift, missense, and splice site mutations in several patients with classic MKS who died in the perinatal period with occipital encephalocele, cystic dysplastic kidneys, and postaxial polydactyly. Compared with patients having MKS1 mutations, those with MKS3 mutations are less likely to have polydactyly and encephalocele and more likely to have milder central nervous system (CNS) phenotypes with considerable variability. Three children with the “molar tooth sign” (MTS) on MRI (a midbrain and cerebellum malformation...
characteristic of Joubert syndrome and related disorders [JSRD]), displayed mutations in MKS3, and 2 fetuses with Meckel-like phenotypes had splice site mutations. Recently, MKS3 mutations have been reported in COACH (cerebellar vermian hypoplasia, oligophrenia, ataxia, coloboma, and hepatic fibrosis) syndrome, a distinct JSRD subgroup with core features of JS plus CHF.

Nephronophthisis is generally characterized by chronic tubulointerstitial nephritis that progresses to renal failure. Renal ultrasound shows kidneys of normal or small size with increased echogenicity. In contrast to ARPKD, in which the microscopic cystic dilatations of the collecting ducts appear throughout the cortex and the medulla and result in enlarged kidneys from birth, the renal cysts in nephronophthisis typically localize at the corticomedullary junction and occur after renal failure develops. Blood pressure in juvenile nephronophthisis is typically normal before the onset of renal failure. To date, mutations in 8 different genes (NPHP1, 3, 4, 5, 6, 7, 8, and 9) have been identified in juvenile nephronophthisis. MKS3 mutations have not been associated with NPHP. Approximately 10% to 20% of patients with nephronophthisis have extrarenal organ involvement that resembles other disorders.

In this report, we present detailed findings of 3 children who were ascertained by clinical characteristics of ARPKD and JSRD, but who exhibited pathogenic MKS3 mutations. These cases illustrate an overlap among ARPKD, nephronophthisis, and JSRD and extend the clinical spectrum of MKS3-related ciliopathies.

### Methods

The patients and their families were evaluated at the NIH Clinical Center under the intramural protocol number 03-HG-0264 approved by the National Human Genome Research Institute (NHGRI) (www.clinicaltrials.gov, trial NCT00068224). Written informed consent was obtained. Ultrasonographic studies were performed by a single technologist (K.T.D.) using 5- and 7-MHz transducers (AVI Sequoia Inc, Mountain View, California). DNA sequencing, all coding exons were amplified from genomic DNA by use of specific intronic primers (primer sequences and conditions are available upon request). Bidirectional sequencing of PCR products was performed using BigDye v3.1 (Applied Biosystems, Foster City, California) terminator chemistry, and reactions were purified with BigDye X Terminator (Applied Biosystems) technology. Extension fragments were separated by capillary electrophoresis on a 3730xl DNA Analyzer (Applied Biosystems). Sequence variants were identified using the software package, Variant Reporter (Applied Biosystems). To test its functional consequences, the C615R mutation was introduced into a pCMV-HA vector, containing the full-length MKS3 wild-type insert, using the Quik-Change Site-Directed Mutagenesis Kit (Stratagene Inc, Cedar Creek, Texas) To localize wild-type and mutant mecklin within cells, we transfected 90% confluent IMCD3 cells with 1 μg of plasmid DNA (pCMV-HA-MKS3[C615R] constructs) using FuGENE6 transfection reagent(Roche Inc, Basel, Switzerland) After 72 hours of incubation at 37°C/5%CO2, cells were fixed in ice-cold methanol for 5 minutes and blocked in 1 × PBST, 10% normal goat serum, 1% BSA. Cells were immunostained for the ER marker GRP94 (1:200 rat monoclonal 9G10, Abcam Ltd, Cambridge, United Kingdom), endogenous mecklin (1:100 affinity purified rabbit polyclonal), and the HA epitope (1:100 mouse monoclonal HA-7, Sigma Ltd, St. Louis, Missouri) by standard procedures. Primary antibodies were detected by application of Alexa Fluor fluorescent conjugates (Invitrogen Inc, Carlsbad, California) as follows: goat anti-rat IgG-633, goat anti-rabbit IgG-568 and goat anti-mouse IgG-488, respectively, diluted ×500 in diluent buffer (1 × PBST, 1% BSA). DNA was stained with DAPI (2 μg/mL). Confocal images were obtained using a Nikon Eclipse TE2000-E confocal imaging systems, controlled by EZ-C1 3.50 (Nikon Ltd, Tokyo, Japan) software. Z-stacks were taken every 0.5 μm, and cells were assessed for immunostaining in the apical region (within the first 2.5 μm of the cell, above the nucleus) or in the mid-cell regions (below the apical region but above the last 1 μm of the cell). Images were processed in Metamorph, and figures were assembled using Adobe Photoshop CS3 (Adobe Systems Inc, San Jose, California).

### Results

Patient 1 (PKD-238) is an 8 year-old boy diagnosed with ARPKD at week 19 of gestation, when routine prenatal ultrasound showed diffusely hyperechoic and enlarged fetal kidneys with loss of corticomедullary differentiation. The amount of amniotic fluid was initially normal but progressively decreased. Fetal growth was normal, and no other anomalies were detected. The patient was delivered vaginally at 36 weeks without complications. Urine output was normal. Serum creatinine peaked at 1.6 mg/dL on the third day of life, decreased to 0.4 mg/dL at 1 month, and remained normal for the first 6 years of life. Severe hypertension, diagnosed on the first day of life, was difficult to manage with 2 antihypertensives, and left ventricular hypertrophy developed. This resolved after 2 years of age, when the hypertension became easier to control. Hyponatremia required oral sodium chloride supplements until 7 months of age. Marked polyuria and polydipsia were present. A normocytic normochromic anemia appeared at 1 month and persisted despite iron supplementation. Liver enzymes and liver ultrasound were unremarkable at birth. By age 5 years, liver enzymes were elevated; ALT and AST ranged between 100 and 250 U/L and AP between 450 and 500 U/L. Synthetic function of the liver remained intact. A liver biopsy at age 7 years showed CHF (Figure 1, F). Central hypotonia and motor delays were noted after 6 months. At 12 months, a diagnosis of global developmental delay and speech apraxia prompted an extensive evaluation, which included a normal eye examination. An MRI of the brain performed at 14 months of age was reported as normal, resulting in
persistence of the diagnosis of ARPKD, with probable cerebral palsy. Head size, weight, and height remained at the 50th percentile.

On evaluation at the NIH Clinical Center at age 8 years, ultrasound revealed enlarged hypechoic kidneys with small macrocysts in the cortex and medulla (Figure 1, C). Other
laboratory findings are presented in Table I. The liver was diffusely hypechoic and inhomogeneous on ultrasound, without cysts. The spleen was mildly enlarged at 11.5 cm. Ophthalmologic examination showed mild, slow torsional nystagmus when fixating clockwise. The retina, optic nerve, and anterior chamber were normal. Snellen visual acuity was 20/50 OD and 20/40 OS. On the Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV), the verbal comprehension index was 63 (1st percentile). His perceptual reasoning index was 61 (1st centile). A full-scale IQ could not be derived because he did not complete the test. Sequencing of the \textit{PKHD1} gene revealed no pathogenic variations. Given the milder CNS phenotype and frequent absence of polydactyly in reported patients with the milder CNS phenotype and frequent absence of polydactyly and neonatal course were uneventful. At 12 months he was diagnosed with oculomotor apraxia associated with central hypotonia and mild developmental delay. He made progress with speech and physical therapy. At age 3, a screening ultrasound, prompted by the diagnosis of polycystic kidney disease in his younger sister (patient 2), showed enlarged hypechoic kidneys; at the same visit, hypertension was diagnosed. Two antihypertensive medications achieved borderline blood pressure control. Renal function was normal. A brain MRI performed at age 22 months was interpreted as normal. He began to have decline in glomerular function at age 5.5 and underwent kidney transplantation at age 8 years. The extracted native kidneys exhibited moderate chronic interstitial nephritis, glomerulosclerosis, tubular atrophy, and nephrocalcinosis as well as cysts, mostly accumulated at the corticomedullary junction but also scattered throughout the cortex and medulla (Figure 1, H and I). He had polyuria and polydipsia and chronic normochromic normocytic anemia since early infancy. His growth was normal. He developed chronic elevation of liver enzymes and splenomegaly and had an episode of cholangitis at age 5 years. A liver biopsy performed at 5.5 years showed CHF (Figure 1, G). Eye evaluation at NIH revealed slight slowing of the saccades, without significant evidence of OMA. His vision was 20/20 OU. The optic discs and retina were normal. On ERG, mixed responses were slightly subnormal but cone responses were normal. The full-scale IQ of patient 3 was 76, with index scores of 81, 71, 94, and 75, respectively.

Sequencing of \textit{PKHD1} in patients 2 and 3 was negative. Similarly, testing for the common \textit{NPH1} gene deletion and for \textit{NPHP2} mutations was negative. Sequencing of the \textit{MKS3} gene showed that both siblings were homozygous and their parents were heterozygous for the missense mutation c.1843 T>C, p.Cys615Arg in exon 18 (Figure 2, B). On retrospective review, subtle findings within the spectrum of the MTS were noted on patient 3’s brain MRI performed at 22 months (Figure 1, M).

To document the consequences of the splice mutation, we amplified and sequenced the RT PCR product of RNA extracted from the transformed peripheral blood lymphocytes of patient 1 and demonstrated that the c.224-2 A>T mutation resulted in skipping of exon 2 (Figure 3, A and B; available at www.jpeds.com) with a subsequent frameshift.

### Table I. Laboratory findings at NIH evaluation

<table>
<thead>
<tr>
<th>Age at evaluation (Y)/sex</th>
<th>Normal Range</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
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<tbody>
<tr>
<td>Hb (g/dL)</td>
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<td>11.3*</td>
<td>10.5*</td>
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<tr>
<td>Htc (%)</td>
<td>35–45</td>
<td>32.2*</td>
<td>31.7*</td>
<td>34.5*</td>
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<td>MCV (fL)</td>
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<td>80.2</td>
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<td>79.9</td>
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<td>Serum creatinine (mg/dL)</td>
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<td>1.3*</td>
<td>1.64*</td>
<td>Posttransplant</td>
</tr>
<tr>
<td>Serum cystatin C (mg/L)</td>
<td>0.55–1.03</td>
<td>2*</td>
<td>2.06*</td>
<td>Posttransplant</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>90–170</td>
<td>40*</td>
<td>25*</td>
<td>Posttransplant</td>
</tr>
<tr>
<td>(ml/min/1.73 m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>6–41</td>
<td>135*</td>
<td>413*</td>
<td>66*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>9–34</td>
<td>158*</td>
<td>290*</td>
<td>61*</td>
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<tr>
<td>AP (U/L)</td>
<td>37–520</td>
<td>383</td>
<td>651*</td>
<td>376</td>
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<td>179*</td>
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<td>105*</td>
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<td>Prothrombin time (s)</td>
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<td>14.3</td>
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<td>Albumin (g/dL)</td>
<td>3.7–4.7</td>
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<td>3.9</td>
<td>4.1</td>
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</table>

Hb, hemoglobin; Hto, hematocrit; MCV, mean corpuscular volume; ALT, alanine amino transferase; AP, aspartate amino transferase; GGT, glutamyl transpeptidase; AST, aspartate amino transferase; AP, alkaline phosphatase.

*Abnormal values.
in exon 3, resulting in premature truncation after 79 amino acids. To assess subcellular localization of wild-type and mutant (p.Cys615Arg) meckelin, we used immunofluorescent confocal microscopy of the polarized, ciliated IMCD3 cell-line. Both endogenous and transfected wild-type HA epitope-tagged meckelin are folded in the ER and transported efficiently to the apical cell surface and primary cilia (Figure 2, top panel). Very little of the wild-type protein is found at mid-cell z-slices in the confocal images. However, the HA-tagged meckelin containing the C615R mutation is partially retained at the mid-cell regions (Figure 2, bottom panel), possibly in lysosomes, although it can also be transported to the apical cell surface. There was no colocalization of this mutant protein with primary cilia (data not shown).

**Discussion**

These 3 children with MKS3 mutations are unique in that they represent an overlap among ARPKD, nephronophthisis,
and JSRD (Table II). They share the following clinical features: (1) enlarged kidneys with diffuse microcystic changes (Figure 1, C through E) resulting in progressive renal failure in childhood (ARPKD); (2) early-onset severe hypertension (ARPKD); (3) chronic normocytic normochromic anemia (nephronophthisis); (4) urinary concentration defect resulting in polyuria and polydipsia (ARPKD and nephronophthisis); (5) congenital hepatic fibrosis and portal hypertension (ARPKD and a subset of nephronophthisis); (6) OMA and speech apraxia associated with mild midbrain and cerebellum abnormalities within the spectrum of the MTS (JSRD) (Figure 1, L and M). Features typical of ARPKD include increased kidney size with preserved reniform contours reflecting diffuse microcystic changes of the medulla extending to the cortex (Figure 1, B through E) and the presence of severe hypertension beginning years before the decline in glomerular function. Transient neonatal hyponatremia, as seen in patient 1, is another feature typical of ARPKD. However, the presence of CNS abnormalities conflicts with the diagnosis of ARPKD, in which the pathology is limited to the kidneys and the liver. Furthermore, chronic anemia is a classic feature of nephronophthisis, which does not manifest early-onset hypertension before end-stage renal disease and is typically associated with small or normal-sized kidneys (Table II).

Thus, our patients’ clinical findings constitute an overlap phenotype among ARPKD, nephronophthisis, and JSRD. The native kidney extracted from patient 3 had features of both ARPKD and nephronophthisis, that is, cysts distributed throughout the cortex and medulla of the kidney (Figure 1, H), moderate chronic interstitial nephritis, marked glomerulosclerosis, and tubular atrophy (Figure 1, H and I). In addition, the CNS abnormalities in these patients, specifically the speech apraxia in patient 1, the OMA in patients 2 and 3, and the MTS in patients 1 and 3 (Figure 1, L and M) are features of JSRD, which can be associated with nephronophthisis and/or CHF. We note that the wpk rat, homozygous for an MKS3 mutation, was proposed as a model for ARPKD before the identification of its CNS abnormalities. Our patients with MKS3 mutations appear to recapitulate aspects of that phenotype. Of note, the brain MRIs of patients 1 and 3 were performed and initially interpreted as “normal,” apparently because of the subtlety of the findings. An MKS3-related ciliopathy should be considered in patients carrying the diagnosis of “ARPKD,” especially in the presence of speech apraxia or OMA. In such patients, careful evaluation of brain MRI scans by those with expertise in identifying this specific spectrum of brain abnormalities may be beneficial.

Neither of the 2 MKS3 mutations we identified in these patients is previously reported. However, both mutations are likely to be pathogenic because of the following data. The cysteine at position 615 of meckelin (the protein encoded by MKS3) is completely conserved among human,chimp, dog, mouse, and rat. We did not detect the c.1843 T>C, p.Cys615Arg mutation in 210 control chromosomes. The splice mutation c.224-2 A>T disrupts the universal AG splice acceptor site. Finally, the p.Cys615Arg mutation alters the subcellular localization of meckelin (Figure 2). Meckelin is a ciliary protein associated with an extremely wide phenotypic spectrum, ranging from perinatally lethal Meckel syndrome to an ARPKD/nephronophthisis/JSRD-like phenotype. The p.Cys615Arg mutation is the first MKS3 mutation described that localizes to the intracellular loop of the meckelin protein (Figure 3, C); MKS3 mutations associated with MKS primarily localize to the extracellular domain, and mutations in JSRD patients are seen in the extracellular, transmembrane, and intracellular carboxy terminal domains. As more MKS3 mutations are identified and more ciliopathy patients are screened for defects in multiple ciliary proteins, we may discover the degree to which the clinical variability in MKS3-related ciliopathies is explained by the severity and location of the MKS3 mutations, in contrast to the contribution of modifier genes. One such example of the probable effect of strong modifiers was apparent in an MKS family with 5 affected fetuses displaying a wide variability of central nervous system involvement ranging from severe encephalocoele to completely normal, despite the fact that all 5 fetuses carried the same 2 MKS3 mutations.

This variability suggests that other genes influence the phenotype.

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### References


### Table II. Patients findings compared with features of various ciliopathies

<table>
<thead>
<tr>
<th></th>
<th>patient 1</th>
<th>patient 2</th>
<th>patient 3</th>
<th>ARPKD</th>
<th>MKS</th>
<th>JSRD</th>
<th>Juvenile NPHP</th>
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<tr>
<td>Molar tooth sign</td>
<td>+</td>
<td>+</td>
<td></td>
<td>±</td>
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<td>±</td>
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<td>Enlarged diffusely cystic kidneys</td>
<td>+</td>
<td>+</td>
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<td>±</td>
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<td>±</td>
<td>±</td>
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<td>Early onset severe hypertension</td>
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<td>Anemia</td>
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<td>Congenital hepatic fibrosis</td>
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</table>

*Unknown because patients with typical MKS do not survive beyond the perinatal period.*

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Figure 3. MKS3 mutations found in patients 1 through 3. Analysis of the c.224-2 A>T splice site mutation at the RNA level (A and B) is shown. Expected wild-type size (483 bp) is seen in the control, whereas in patient 1 the smaller band (394 bp) corresponds to the transcript lacking exon 2. A. Direct sequencing of the RT-PCR product from patient 1 shows skipping from exon 1 to exon 3, B. Localization of the p.Cys615Arg mutation (red sphere) to the intracellular loop of the meckelin protein, C, in comparison with the location of the mutations reported in MKS (blue diamonds) and JSRD patients (green diamonds) is shown. Modified from Khaddour et al.20.