Mild Recessive Mutations in Six Fraser Syndrome–Related Genes Cause Isolated Congenital Anomalies of the Kidney and Urinary Tract

Stefan Kohl,* Daw-Yang Hwang,*† Gabriel C. Dworschak,**† Alina C. Hilger,†‡ Pawaree Saisawat,§ Asaf Vivante,* Natasa Stajic,†† Radovan Bogdanovic,††† Heiko M. Reutter,††‡ Elijah O. Kehinde,*† Velibor Tasic,†† and Friedhelm Hildebrandt*‡‡

*Department of Medicine, Boston Children’s Hospital, Harvard Medical School, Boston, Massachusetts; †Division of Nephrology, Department of Medicine, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan; ‡Institute of Human Genetics, and **Department of Neonatology, Children’s Hospital, University of Bonn, Bonn, Germany; †Department of Pediatrics, University of Michigan, Ann Arbor, Michigan; ‡Medical Faculty, University of Belgrade, Belgrade, Serbia; ††Institute of Mother and Child Healthcare of Serbia, Belgrade, Serbia; †‡Department of Surgery, Kuwait University, Safat, Kuwait; ‡‡Department of Pediatric Nephrology, University Children’s Hospital, Skopje, Macedonia; and §§Howard Hughes Medical Institute, Chevy Chase, Maryland

ABSTRACT

Congenital anomalies of the kidney and urinary tract (CAKUT) account for approximately 40% of children with ESRD in the United States. Hitherto, mutations in 23 genes have been described as causing autosomal dominant isolated CAKUT in humans. However, >90% of cases of isolated CAKUT still remain without a molecular diagnosis. Here, we hypothesized that genes mutated in recessive mouse models with the specific CAKUT phenotype of unilateral renal agenesis may also be mutated in humans with isolated CAKUT. We applied next-generation sequencing technology for targeted exon sequencing of 12 recessive murine candidate genes in 574 individuals with isolated CAKUT from 590 families. In 15 of 590 families, we identified recessive mutations in the genes FRAS1, FREM2, GRIP1, FREM1, ITGA8, and GREM1, all of which function in the interaction of the ureteric bud and the metanephric mesenchyme. We show that isolated CAKUT may be caused partially by mutations in recessive genes. Our results also indicate that biallelic missense mutations in the Fraser/MOTA/BNAR spectrum genes cause isolated CAKUT, whereas truncating mutations are found in the multigorgan form of Fraser syndrome. The newly identified recessive biallelic mutations in these six genes represent the molecular cause of isolated CAKUT in 2.5% of the 590 affected families in this study.


Congenital anomalies of the kidney and urinary tract (CAKUT) are one of the most frequent congenital abnormalities in humans, taking a high toll on affected individuals, their families, and health care. The estimated occurrence of CAKUT is 3–6 per 1000 live births.¹ They represent the most frequent cause of CKD and ESRD in children in the United States, accounting for >40% of all cases.² CAKUT comprise a broad spectrum of structural malformations and functional anomalies, including unilateral renal agenesis, renal hypoplasia, ureteropelvic junction obstruction, and vesicoureteral reflux. The clinically distinct CAKUT phenotypes have in common a disturbed embryonic codevelopment of tissues derived from the ureteric bud and the metanephric mesenchyme.³ Although >200 different forms of syndromic CAKUT have been described,⁴ isolated CAKUT account for the majority of cases.⁵-six Twenty-three autosomal dominant dominant genes have been identified to cause isolated CAKUT, with TNXB, WNT4, and DSTYK being the most recent ones.⁷–¹° According to the Mouse Genome Informatics database (http://www.informatics.jax.org), 1768 monogenic mouse models for CAKUT have been described, many of which are recessive and do not have a human disease correlate.

In this study, we hypothesized that targeted genes in recessive mouse models with CAKUT may also be mutated in humans with isolated CAKUT. Candidate genes were selected based on re-
cessive murine models for CAKUT with the distinct CAKUT phenotype of unilateral renal agenesis in homozygous null animals. We reckoned that mouse models with unilateral renal agenesis represented the most promising CAKUT candidate genes, because unilateral renal agenesis is a severe and specific CAKUT phenotype. To test this hypothesis, we performed bar-coded next-generation sequencing (NGS)–based exon sequencing of these candidate genes as previously described by our group.11,12

We analyzed the coding sequences of 12 recessive murine candidate genes (Supplemental Table 1) in 672 individuals from 590 families with isolated CAKUT (Supplemental Table 2). Mutations in 17 known CAKUT-causing genes were excluded before this study (see Concise Methods). For individuals with renal hypoplasia (n=101), we also excluded the presence of an HNF1B deletion by quantitative PCR.

In 15 of 672 individuals from 590 families (2.5%) with isolated CAKUT, we identified 21 different mutations in six different recessive genes (Table 1). These genes were FRAS1, Frem2, GRIP1, Frem1, ITGA8, and GREM1. The most frequently mutated gene in this study was FRAS1, accounting for six unrelated individuals with isolated CAKUT. In the six murine “single kidney” genes BAG6, CTNNB1, DACT1, ILK, LIN7C, and LRP4, there were no biallelic mutations present in humans with isolated CAKUT. We considered recessive alleles as likely disease causing if they either were protein-truncating (n=2) or missense mutations affecting an evolutionary conserved amino acid residue and with a minor allele frequency of <1% in 13,000 control chromosomes of the National Heart Lung and Blood Institute Exome Sequencing Project (n=17). All detected alleles were confirmed by Sanger sequencing in genomic DNA of the affected individuals.

In the genes FRAS1, Frem2, and GRIP1, which cause Fraser syndrome if mutated (Online Mendelian Inheritance in Man [OMIM] 219000), we detected recessive missense mutations in 11 unrelated individuals with isolated CAKUT (n=6, 4, and 1, respectively) (Table 1). Mutations in FRAS1, Frem2, or GRIP1 were detected in individuals with different isolated CAKUT phenotypes (Table 1). Ten of 11 individuals had no extrarenal manifestations characteristic for Fraser syndrome (e.g., cryptophthalmos, syndactyly, or genital malformations). One individual, A3455-21, had CAKUT and anal atresia, which is a diagnostic criterion of Fraser syndrome (Table 1). However, this was not sufficient to make the clinical diagnosis.13 Interestingly, we here discovered recessive missense mutations in FRAS1, Frem2, and GRIP1 as novel causes of isolated CAKUT, whereas biallelic truncating mutations are known to cause Fraser syndrome with multiorgan involvement (Supplemental Table 3).

In addition to the Fraser syndrome genes, we detected recessive mutations in the gene FREM1 that cause the closely Fraser-related phenotypes Manitoba Oculotrichoanal (MOTA) syndrome14 (OMIM 248450) or Bifid Nose with or without Anorectal and Renal Anomalies (BNAR) syndrome15 (OMIM 608980) in an allele-dependent manner. In this study, we identified two unrelated individuals with isolated CAKUT with the same homozygous mutation in FREM1 (FREM1 p.A1627S) (Table 1). Individual A688-21 had isolated CAKUT, whereas individual A3369-21 had CAKUT with bilateral syndactyly of toes II-III, which is a feature of MOTA syndrome. However, MOTA/BNAR-characteristic facial dysmorphism was absent from both individuals. Similarly to the detected mutations in the Fraser genes, our data support that biallelic missense mutations in FREM1 cause isolated CAKUT, whereas truncating mutations are only reported in individuals with the severe multiorgan phenotype of MOTA/BNAR syndrome (Supplemental Table 3).

In addition to Fraser spectrum genes, we detected homozygous recessive mutations in ITGA8 and GREM1, which have not previously been implicated in human CAKUT. In individual A876-21 with a left duplicated collecting system and left high-grade vesicoureteral reflux, we detected a homozygous obligatory splice-site mutation in ITGA8 (Table 1). The nucleotide change ITGA8 c.2982+2T>C is predicted to abrogate the splice donor site of exon 28 (5’ splicing site) by five publically available splice-site prediction algorithms (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer, and Human Splicing Finder).

In individual A3573-21, we detected a homozygous missense mutation in the BMP4-antagonist GREM1 (Table 1). GREM1 p.P35A segregated from the heterozygous parents to the affected child with unilateral renal agenesis. The unaffected sibling A3579-22 was heterozygous for the mutation (Table 1).

In this study, we identified mutations in six recessive murine “single kidney” candidate genes in 672 individuals from 590 families as novel single-gene causes of isolated CAKUT. These six recessive genes account for mutations in 2.5% of 590 families with isolated CAKUT. Together with heterozygous mutations in 17 known autosomal dominant CAKUT-causing genes, which account for another 6.3%,16 we are now able to molecularly “solve” almost 10% of cases with isolated CAKUT in our cohort.

We detected recessive biallelic missense mutations in the four Fraser spectrum genes in individuals with isolated CAKUT. The proteins encoded by the Fraser genes FRAS1, Frem2, and FREM1 form a tertiary protein complex lining the extracellular epithelial-mesenchymal interface.17,18 Loss of function of any of these proteins disrupts the Fraser-protein complex and leads to the severe multigang developmental phenotype of Fraser syndrome in humans and mice (Supplemental Table 1).14,19–21 In this study, 11 of 13 individuals with recessive mutation in the Fraser genes had no extrarenal phenotype. We observed almost exclusively biallelic missense mutations in individuals with isolated CAKUT, whereas individuals with Fraser syndrome generally are reported to have protein-truncating alleles (Supplemental Table 3). This may represent an example of allelism in a recessive disease, which has been described and discussed more extensively in nephronophthisis, a rare form of cystic kidney disease.22 Hence, we propose the
Table 1. Recessive mutations in 6 murine candidate genes detected in 15 unrelated individuals with isolated CAKUT

<table>
<thead>
<tr>
<th>Gene</th>
<th>Family - Individual</th>
<th>Sex</th>
<th>Geographic Origin</th>
<th>Renal Phenotype</th>
<th>Extrarenal Phenotype</th>
<th>Nucleotide Change (Zygosity)</th>
<th>Exon</th>
<th>Amino Acid Change</th>
<th>Segregation/State of Alleles</th>
<th>Mm</th>
<th>Gg</th>
<th>Xt</th>
<th>Dr</th>
<th>EVS</th>
<th>SIFT</th>
<th>MT</th>
<th>PP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRAS1</td>
<td>A1250-21</td>
<td>Male</td>
<td>Kuwait</td>
<td>Hypospadias</td>
<td>None</td>
<td>c.4579C&gt;T (H)</td>
<td>34</td>
<td>R1527W</td>
<td>Hom</td>
<td>R</td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>24 of 12,416 Deleterious Disease causing</td>
<td>0.724</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAS1</td>
<td>A1402-21</td>
<td>Male</td>
<td>Arabia</td>
<td>Right duplex</td>
<td>None</td>
<td>c.4579C&gt;T (h)</td>
<td>34</td>
<td>R1527W</td>
<td>trans()</td>
<td>R</td>
<td>R</td>
<td>P</td>
<td>R</td>
<td>24 of 12,416 Deleterious Disease causing</td>
<td>0.724</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAS1</td>
<td>A2381-21</td>
<td>Female</td>
<td>Germany</td>
<td>Left agenesis</td>
<td>None</td>
<td>c.7867C&gt;T (H)</td>
<td>55</td>
<td>R2623*</td>
<td>Paternal inheritance</td>
<td>0 of 13,000 N/A N/A N/A</td>
<td>0.999</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAS1</td>
<td>A3455-21</td>
<td>Male</td>
<td>Macedonia</td>
<td>Right agenesis</td>
<td>Anal atresia</td>
<td>c.4579C&gt;T (H)</td>
<td>34</td>
<td>R1527W</td>
<td>Maternal inheritance</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>0 of 13,000 Deleterious Disease causing</td>
<td>0.724</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAS1</td>
<td>A3975-21</td>
<td>Male</td>
<td>Germany</td>
<td>Right ectopic medullary cystic kidney disease</td>
<td>None</td>
<td>c.4159_4161delinsTTA (h)</td>
<td>31</td>
<td>A1387L</td>
<td>trans\in() 1000Gc</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>13 of 12,487 N/A N/A N/A</td>
<td>0.999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREM2</td>
<td>A1023-21(d)</td>
<td>Male</td>
<td>India</td>
<td>Right agenesis</td>
<td>None</td>
<td>c.9806G&gt;A (h)</td>
<td>64</td>
<td>R3269Q</td>
<td>trans\in() 1000Gc</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>88 of 12,570 Deleterious Disease causing</td>
<td>0.999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREM2</td>
<td>A1417-21</td>
<td>Male</td>
<td>UK</td>
<td>VUR</td>
<td>None</td>
<td>c.9806G&gt;A (H)</td>
<td>64</td>
<td>R3269Q</td>
<td>Hom</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>88 of 12,570 Deleterious Disease causing</td>
<td>0.999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREM2</td>
<td>A1548-21</td>
<td>Male</td>
<td>India</td>
<td>Right agenesis</td>
<td>None</td>
<td>c.4031G&gt;A (H)</td>
<td>1</td>
<td>R1344H</td>
<td>trans\in() 1000Gc</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>25 of 12,981 Deleterious Disease causing</td>
<td>0.085</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREM2</td>
<td>A1232-21</td>
<td>Male</td>
<td>Kuwait</td>
<td>PUV, right VUR, left UPJO, CKD</td>
<td>None</td>
<td>c.649C&gt;T (H)</td>
<td>1</td>
<td>R217C</td>
<td>trans()</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>0 of 13,000 Deleterious Disease causing</td>
<td>0.836</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FREM2</td>
<td>A1548-21</td>
<td>Male</td>
<td>India</td>
<td>B UPJO</td>
<td>CM</td>
<td>c.4820A&gt;G (H)</td>
<td>1</td>
<td>D1607G</td>
<td>trans()</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>0 of 13,000 Deleterious Disease causing</td>
<td>0.983</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Gene</th>
<th>Family - Individual</th>
<th>Sex</th>
<th>Geographic Origin</th>
<th>Renal Phenotype</th>
<th>Extrarenal Phenotype</th>
<th>Nucleotide Change (Zygosity)</th>
<th>Exon</th>
<th>Amino Acid Change</th>
<th>Segregation/State of Alleles</th>
<th>Mm</th>
<th>Gg</th>
<th>Xt</th>
<th>Dr</th>
<th>EVS</th>
<th>SIFT</th>
<th>MT</th>
<th>PP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FREM2</td>
<td>A358-21</td>
<td>Female</td>
<td>Kuwait</td>
<td>B VUR, CKD</td>
<td>None</td>
<td>c.7211T&gt;C (H)</td>
<td>13</td>
<td>I2404T</td>
<td>Hom</td>
<td>I</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>0 of 13,000</td>
<td>Deleterious</td>
<td>Disease causing</td>
<td>0.075</td>
</tr>
<tr>
<td>GRIP1</td>
<td>A3390-21</td>
<td>Female</td>
<td>Macedonia</td>
<td>Right duplex</td>
<td>None</td>
<td>c.1846G&gt;A (H)</td>
<td>16</td>
<td>G616R</td>
<td>Paternal inheritance</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>0 of 13,000</td>
<td>Deleterious</td>
<td>Disease causing</td>
<td>0.606</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c.2750G&gt;T (H)</td>
<td>22</td>
<td>R917L</td>
<td>Maternal inheritance</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>0 of 13,000</td>
<td>Tolerated</td>
<td>Disease causing</td>
<td>0.087</td>
</tr>
<tr>
<td>FREM1</td>
<td>A3369-21</td>
<td>Male</td>
<td>Macedonia</td>
<td>B VUR III&lt;sup&gt;a&lt;/sup&gt;</td>
<td>B SD II/III</td>
<td>c.4879G&gt;T (H)</td>
<td>28</td>
<td>A1627S</td>
<td>Hom</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>1 of 12,307</td>
<td>Deleterious</td>
<td>Disease causing</td>
<td>0.893</td>
</tr>
<tr>
<td>FREM1</td>
<td>A688-21</td>
<td>Male</td>
<td>Macedonia</td>
<td>Right VUR</td>
<td>None</td>
<td>c.4879G&gt;T (H)</td>
<td>28</td>
<td>A1627S</td>
<td>Hom</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>1 of 12,307</td>
<td>Deleterious</td>
<td>Disease causing</td>
<td>0.893</td>
</tr>
<tr>
<td>ITGA8</td>
<td>A876-21</td>
<td>Female</td>
<td>Macedonia</td>
<td>Left duplex, left VUR III&lt;sup&gt;b&lt;/sup&gt;</td>
<td>None</td>
<td>c.2982+2T&gt;C (H)</td>
<td>28</td>
<td>—</td>
<td>Hom</td>
<td>0 of 13,000</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GREM1</td>
<td>A3573-21</td>
<td>Female</td>
<td>Macedonia</td>
<td>Left agenesis</td>
<td>None</td>
<td>c.103C&gt;G (H)</td>
<td>2c</td>
<td>P35A</td>
<td>Paternal inheritance, matenal inheritance</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>P</td>
<td>34 of 12,952</td>
<td>Tolerated</td>
<td>Disease causing</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Mm, <i>mus musculus</i>; Gg, <i>gallus gallus</i>; Xt, <i>xenopus tropicalis</i>; Dr, <i>danio rerio</i>; EVS, allele frequency in EVS database; SIFT, sorting intolerant from tolerant prediction class; MT, mutation taster prediction class; PP2, PolyPhen2 homvar score; FRAS1 (NM_025074.6); H, homozygous; Hom, homozygous; h, heterozygous; trans, alleles are in-trans, meaning they are on different chromosomes; Agenesis, renal agenesis; N/A, not applicable; trans, in-trans, alleles are in-trans because of uneven distribution (24 of 12,416 versus 0 of 13,000) of counts in the National Heart Lung and Blood Institute Exome Sequencing Project cohort of 6500 control individuals (http://evs.gs.washington.edu/EVS/).

<sup>a</sup> A2381-22 was published in a previous study of our group reporting identical mutations. A2381-21 had bilateral renal agenesis with absent urinary bladder (termination of pregnancy).

<sup>b</sup> No parental DNA available. Alleles are most likely in trans because individuals of the 1000 Genomes Project show absence of linkage disequilibrium (Supplemental Table 6).

<sup>c</sup> No parental DNA available. Compound heterozygous state was confirmed by subcloning of genomic DNA and Sanger sequencing of mono-molecular clones.

<sup>d</sup> A1023-21 was published in a previous study of our group for the heterozygous FREM2 mutation p.T2338I.

<sup>e</sup> No parental DNA available. Compound heterozygous state was confirmed by subcloning of genomic DNA and Sanger sequencing of mono-molecular clones.

<sup>f</sup> No parental DNA available. Alleles are most likely in trans because individuals of the 1000 Genomes Project show absence of linkage disequilibrium (Supplemental Table 6).
identified six recessive monogenic causes of isolated CAKUT in humans, two of them as novel CAKUT genes. We thereby identified the first recessive isolated CAKUT genes in humans. The six genes found to be mutated encode for proteins that functionally converge on the GDNF-RET/BMP signaling pathways at the interface of the ureteric bud and the metanephric mesenchyme. We showed genetic evidence that mild mutation in the Fraser spectrum genes (FRAS1, FREM2, GRIP1, and FREM1) causes isolated CAKUT as the mildest phenotype of the clinical spectrum. These six genes contribute to 2.5% of all cases of isolated CAKUT; thus, up to 20% of individuals with CAKUT could now be molecularly “solved” by exon sequencing in 29 genes and copy number variation analysis.9,29–31 Rapidly developing sequencing technology will continue to facilitate identification of rare single-gene causes of human CAKUT and enables us to conduct large-scale mutation analyses providing families with a genetic diagnosis in an increasing number of cases.

CONCISE METHODS

Human Participants
After informed consent, we obtained clinical data, blood samples, and pedigrees from individuals with CAKUT. Approval for research on humans was obtained from the University of Michigan Institutional Review Board. Mutations in the following genes known to be mutated in isolated CAKUT in humans were excluded before this study: BMP4, BMP7, CDC51, CHD1L, EYA1, GATA3, HNF1B, PAX2, RET, ROBO2, SALL1, SIX1, SIX2, SIX5, SOX17, UMOD, and UPK3A. HNF1B deletions were excluded by quantitative PCR in individuals with the CAKUT phenotype of renal hypodysplasia.

Candidate Gene Selection
Candidate genes were selected from the Mouse Genome Informatics database based on the presence of the CAKUT phenotype of unilateral renal agenesis (phenotype ID MP: 0003604) in homozygotes. Eighty-one genotypes involving 39 genes matched this criterion. Twenty-eight genes were excluded because they either were known to be associated with isolated CAKUT (n=5), or were reported in mouse models with conditional/complex knock-out alleles, or were not purely autosomal recessive. FREM2 was included in this study because three of four Fraser spectrum genes (FRAS1, GRIP1, and FREM1) met the inclusion criteria. The selected candidate genes were BAG6, CTNNBIP1, DACT1, FRAS1, FREM1, FREM2, GREM1, GRIP1, ITK, ITGA8, LIN7C, and LRP4 (Supplemental Table 1).

Targeted Exon Sequencing
For 12 genes, 355 target-specific primer pairs were designed covering 273 coding exons (Supplemental Table 5). The amplicon size ranged from 150 to 290 bp. Targeted amplification and NGS was done as described previously by our group11,12 with the following alterations: 12 primer pairs were multiplexed in 48 pools to allow for amplification of 576 PCR products per sample simultaneously using the Fluidigm 48.48 Access Array IFC System. NGS was carried out using an Illumina HiSeq2000 instrument (1×150 bp single reads) or Illumina MiSeq V2 instrument (2×250 bp paired reads). Our results showed that 326 amplicons (91.8%) had a coverage >100X and 11 amplicons (3.0%) were covered <10X (Supplemental Table 3). All identified mutations were confirmed by Sanger sequencing in genomic DNA. Segregation analysis was performed if parental DNA was available. Alternatively, fragments of genomic DNA harboring two alleles were TA cloned into pGEM Easy-vector (Promega). Six clones were Sanger sequenced in order to confirm the compound heterozygous state.

Bioinformatics
NGS data alignment and variant detection were performed using CLC Genomics Workbench 4.9 software. Variant filtering was carried out as published previously by our group.11,12 Homozygous or ≥2 heterozygous alleles in one gene were considered for subsequent confirmation by Sanger sequencing (Supplemental Table 5).

ACKNOWLEDGMENTS
The authors thank the affected individuals, their families, and their physicians who contributed to this study.

This work was supported by grants from the National Institutes of Health (R01-DK045345 and R01-DK088767 to F.H.). F.H. is an investigator of the Howard Hughes Medical Institute, a Doris Duke Distinguished Clinical Scientist, and a Warren E. Grupe Professor.

DISCLOSURES
None.

REFERENCES
BRIEF COMMUNICATION


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1038/ki.2013.508/-/DCSupplemental.