DGKE Variants Cause a Glomerular Microangiopathy That Mimics Membranoproliferative GN


Departments of *Pediatric Nephrology and Rheumatology and †Medical Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey; ‡Nephrogenetics Laboratory, Department of Pediatric Nephrology, Hacettepe University Faculty of Medicine, Ankara, Turkey; Departments of ‖Internal Medicine and ‖‖Immunology, University of Texas Southwestern Medical Center, Dallas, Texas; Departments of §Pediatric Nephrology and §§Pathology, Gazi University Faculty of Medicine, Ankara, Turkey; ‡‡Howard Hughes Medical Institute, Chevy Chase, Maryland; ‡‡‖Department of Pediatrics, University of Michigan, Ann Arbor, Michigan; §§§College of Medicine, The Ohio State University, Columbus, Ohio; Departments of ¶¶Histopathology and ¶¶Pediatric Nephrology, The Sydney Children’s Hospitals Network, Westmead, Sydney, Australia; ‡‡‡Department of Medicine, The University of Chicago, Chicago, Illinois; ‡¶¶The Research Institute at Nationwide Children’s Hospital, Columbus, Ohio; ‡¶¶¶Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah; §§§Louisiana State University, New Orleans, Louisiana; and ‡¶¶¶¶Eugene McDermott Center for Growth and Development, University of Texas Southwestern Medical Center, Dallas, Texas

ABSTRACT

Renal microangiopathies and membranoproliferative GN (MPGN) can manifest similar clinical presentations and histology, suggesting the possibility of a common underlying mechanism in some cases. Here, we performed homozygosity mapping and whole exome sequencing in a Turkish consanguineous family and identified DGKE gene variants as the cause of a membranoproliferative-like glomerular microangiopathy. Furthermore, we identified two additional DGKE variants in a cohort of 142 unrelated patients diagnosed with membranoproliferative GN. This gene encodes the diacylglycerol kinase DGKe, which is an intracellular lipid kinase that phosphorylates diacylglycerol to phosphatidic acid. Immunofluorescence confocal microscopy demonstrated that mouse and rat Dgke colocalizes with the podocyte marker WT1 but not with the endothelial marker CD31. Patch-clamp experiments in human embryonic kidney (HEK293) cells showed that DGKe variants affect the intracellular concentration of diacylglycerol. Taken together, these results not only identify a genetic cause of a glomerular microangiopathy but also suggest that the phosphatidylinositol cycle, which requires DGKE, is critical to the normal function of podocytes.

Received September 11, 2012. Accepted November 27, 2012.

F.O., B.L., and A.R. contributed equally to this work.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Massimo Attanasio, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas TX, 75390, or Dr. Fatih Ozaltin, Departments of Pediatric Nephrology and Rheumatology, Hacettepe University Faculty of Medicine, 06100, Sihyve, Ankara, Turkey. Email: massimo.attanasio@utsouthwestern.edu or fozaltin@hacettepe.edu.tr

The term membranoproliferative glomerulonephritis (MPGN) defines a heterogeneous group of kidney diseases that frequently lead to kidney failure. The distinction of MPGN in three subtypes, based on different pathologic pictures, was recently replaced by a classification that accounts for the pathogenetic mechanisms underlying the diverse observed glomerular lesions. According to the new classification, MPGN is considered to fall in two etiological categories, in which either deposition of Ig or uncontrolled Ig-independent complement activation induces glomerular proliferative/exudative processes that result in mesangial expansion, hyperlobulated glomeruli, and, in the reparative phase,
Figure 1. Representation of the pedigrees described in this study and of the DGKE mutations in relation to the gene exon structure and protein domains. (A) Pedigrees of families UT-062, HU-314, and HU-500. Squares represent males, circles represent females. Black filled symbols indicate the affected status. Double-horizontal bars indicate consanguinity. (B) Sequence alignment across different species of the GPRIN1 gene in correspondence of the two sequence variants detected by exome sequencing in the affected individuals of family UT-062. The reference amino acids are represented in red. Dots on the bottom line represent medium (:) and low (.) evolutionary conservation. The absence of a dot indicates absence of evolutionary conservation. Numbers indicate the position of the last represented amino acid in
splitting (double contour or “tram track” appearance) of the glomerular basement membrane, thereby causing injury of podocytes, glomerular capillary- basement membrane, thereby causing track tide polymorphism array. We identi-
ing the 250K Affymetrix single nucleo-	al Material for a detailed description of Figure 2, and Table 1; see the Supplemen-
tal Table 2). We identified a total of 30 coding single nucleotide variations
within the three shared homozygous in-
tervals, three of which were not anno-
tated in the dbSNP database (build 131). Two of these variants were predicted
to result in nonsynonymous changes in two residues of the gene G protein–
regulated inducer of neurite outgrowth (GPRIN1) (p.Glu233Val and p.Gly236Val)
that are poorly conserved in the evolution (Figure 1B and Supplemental Figure 2).
Most importantly, this gene is exclusively expressed in neurons and no transcripts
have been detected in the kidney,11
excluding a causative role for the disease
in this family. Furthermore, six loss-of-
function variants (two frame shift and
four missense mutations predicted to be
deletious by SIFT [sorting tolerant
 tolerant intolerant] analysis12) have been
found in GPRIN1 in 628 individuals se-
quenced in the 1000 Genomes Project13
and in the 6530 samples of the National
Heart, Lung, and Blood Institute Exome
Sequencing (NES) Project,14 indicating
that this gene is weakly subject to evolu-
tional pressure. The third identified sin-
gle nucleotide variation was a transition
from C to T (c.127C>T) in the first cod-
ing exon of DGKE (NM_003647.2), a
gene that encodes the e isoform of di-
acylglycerol kinase,15 and creates a stop
codon at glutamate 43 (p.Gln43X) that
results in a predicted peptide missing all
the functional domains (Figure 1C). The
mutation was confirmed by Sanger se-
quencing and segregated in the pedigree
with an autosomal recessive pattern.
Because we performed homozygosity mapping,
only missense variants within the
homozygous identical by descent regions
were tested for segregation. To support
the causative role of DGKE mutations,
we used exon sequencing to screen a
worldwide cohort of 142 unrelated indi-
viduals diagnosed with MPGN. We
found a homozygous deletion, c.610delA
(p.Thr204GlnfsX6), in two affected sib-
lings from an unrelated consanguineous
Turkish family (HU-314) (Figure 1, A
and C and Figure 2). The mutation causes
a shift in the mRNA reading frame and is
predicted to result in the creation of a stop
codon at position 210 of the aberrant tran-
script. We reproduced the two mutations
by site-directed mutagenesis in DGKE
cDNAs cloned in myc-tagging expression
vectors. After overexpression in HEK293T
cells, our Western blot analysis detected
a truncated protein of the predicted size
obtained from the mutant c.610delA
but not from the c.127C>T mutant (Fig-
ure 3A), suggesting that the truncated
Peptide is rapidly degraded. A third mutation (c.889-2A>G) that abolishes the obligatory acceptor splice site of intron 5 was found in the affected siblings of a family (HU-500) of Lebanese origin from Australia (Figure 1, A and C and Figure 2). We sequenced the DGKE cDNA of one of the affected siblings (V-1) and of the carrier healthy siblings (V-4) and found that intron 6 was retained in the presence of the homozygous change but not in the wild type (Figure 1D, Supplemental Figure 3), confirming that the mutation results in an abnormal transcript predicted to create a premature stop codon (amino acid 350, p.Trp350X). Interestingly, we could not amplify the mutated allele from the heterozygote carrier (Figure 1D), which suggested that the number of mutated transcripts is negligible due to nonsense-mediated decay. This hypothesis was confirmed by quantitative real-time PCR (Figure 1E).

Diacylglycerol kinases (DGKs) are intracellular lipid kinases that are devoted to phosphorylate diacylglycerol (DAG) to phosphatidic acid18 (Supplemental Figure 4). We determined by immunofluorescence confocal microscopy that Dgkα is expressed in adult mouse and rat kidneys and colocalizes with the podocyte marker WT119 but not with the endothelial marker CD31 (Supplemental Figure 5A). Saturation of the antibody with molar excess of an unrelated Dgkα peptide proved the specificity of the immune reaction (Supplemental Table 1.

Table 1. Clinical features of the patients with DGKE mutations

<table>
<thead>
<tr>
<th>Family and Individual</th>
<th>Consanguinity</th>
<th>Nucleotide Alteration</th>
<th>Segregation</th>
<th>Age at Onset (yr)</th>
<th>Proteinuria at Onsetb</th>
<th>Serum Albumin at Onset (g·dl⁻¹)</th>
<th>Serum Creatinine at Onset (mg·dl⁻¹)</th>
<th>Age at ESRF (yr) and Outcome</th>
<th>Histology</th>
<th>Last Proteinuriab</th>
<th>Last Serum Albumin (g·dl⁻¹)</th>
<th>Last Serum Creatinine (mg·dl⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT-032</td>
<td></td>
<td></td>
<td>H, M, P</td>
<td>5</td>
<td>4+</td>
<td>2.5</td>
<td>3</td>
<td>8 (transplanted at 20 yr)</td>
<td>MPGN</td>
<td>2+</td>
<td>4</td>
<td>1.46</td>
</tr>
<tr>
<td>V-3</td>
<td></td>
<td>c.127C&gt;T (p.Gln43X)</td>
<td></td>
<td>2</td>
<td>4+</td>
<td>2.2</td>
<td>0.6</td>
<td>Died at 4 yr</td>
<td>MPGN</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>V-4</td>
<td></td>
<td>c.127C&gt;T (p.Gln43X)</td>
<td></td>
<td>4</td>
<td>4+</td>
<td>2.5</td>
<td>0.6</td>
<td>None at 30 yr</td>
<td>MPGN</td>
<td>4+</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>V-6</td>
<td></td>
<td>c.127C&gt;T (p.Gln43X)</td>
<td></td>
<td>0.8</td>
<td>4+</td>
<td>2.4</td>
<td>0.6</td>
<td>None at 19 yr</td>
<td>MPGN</td>
<td>Trace</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>HU-314</td>
<td></td>
<td></td>
<td>H, M, P</td>
<td>17</td>
<td>4+</td>
<td>2.5</td>
<td>1.9</td>
<td>3+</td>
<td>MPGN</td>
<td>3+</td>
<td>3.2</td>
<td>4</td>
</tr>
<tr>
<td>IV-1</td>
<td>Y</td>
<td>c.delA 604-610</td>
<td>H, M, P</td>
<td>8</td>
<td>2+</td>
<td>4.8</td>
<td>0.68</td>
<td>None at 19 yr</td>
<td>MPGN</td>
<td>4+</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>IV-2</td>
<td>Y</td>
<td>c.delA 604-610 (p.Thr204GlnfsX6)</td>
<td>H, M, P</td>
<td>0.8</td>
<td>4+</td>
<td>4.8</td>
<td>0.68</td>
<td>None at 19 yr</td>
<td>MPGN</td>
<td>Trace</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>HU-500</td>
<td></td>
<td></td>
<td>H, M, P</td>
<td>1.5</td>
<td>4+</td>
<td>2.8</td>
<td>0.4</td>
<td>None at 12 yr</td>
<td>MPGN</td>
<td>1+</td>
<td>3.9</td>
<td>0.3</td>
</tr>
<tr>
<td>V-1</td>
<td>Y</td>
<td>c.889-2A&gt;G (p.Trp350X)</td>
<td></td>
<td>1.5</td>
<td>4+</td>
<td>1.7</td>
<td>0.77</td>
<td>None at 2 yr</td>
<td>MPGN</td>
<td>3+</td>
<td>3.4</td>
<td>0.4</td>
</tr>
<tr>
<td>V-2</td>
<td>Y</td>
<td>c.889-2A&gt;G (p.Trp350X)</td>
<td></td>
<td>1.5</td>
<td>4+</td>
<td>2.4</td>
<td>0.34</td>
<td>None at 2 yr</td>
<td>MPGN</td>
<td>4+</td>
<td>2.8</td>
<td>0.3</td>
</tr>
<tr>
<td>V-3</td>
<td>Y</td>
<td>c.889-2A&gt;G (p.Trp350X)</td>
<td></td>
<td>0.8</td>
<td>4+</td>
<td>4.8</td>
<td>0.68</td>
<td>None at 19 yr</td>
<td>MPGN</td>
<td>Trace</td>
<td>4</td>
<td>0.9</td>
</tr>
</tbody>
</table>

ESRF, end stage renal failure; H, homozygous in affected individual; M, heterozygous mutation identified in the mother; P, heterozygous mutation identified in the father; N/A, not available.

aNucleotide positions are numbered according to DGKE (NM_003647.2). All mutations were absent in 166 healthy Turkish children.
bAssessed by urine dipstick.

There were also secondary focal and segmental sclerotic glomeruli, which could be seen in the advanced stage of any glomerulopathy.
We also detected DGKε by Western blot in human immortalized podocytes and rat kidneys and testes (Supplemental Figure 5, C and D), where the enzyme was previously reported to be expressed. DGKε is the smallest of the known mammalian DGKs and lacks extra-enzymatic regulatory domains, a characteristic that suggests that this isoform is constitutively active. Another distinctive feature of DGKε is its marked substrate selectivity for DAG that is acylated with arachidonic acid at the sn-2 position (sn-2-arachidonoil-DAG). The selectivity for arachidonoil-DAG results in the enrichment with arachidonic acid of the phospholipids that participate to the phosphatidylinositol (PI) cycle and this effect is considered to be the main function of the enzyme.

TRPC6 is a calcium-permeable cation channel expressed in the foot processes of podocytes, and is known to be directly activated by DAG through a protein kinase C–independent mechanism. To demonstrate that lack of DGKε affects DAG production in podocytes, we tested the effect of DGKE mutations on intracellular DAG concentration, taking advantage of the fact that currents through TRPC6 channels are directly regulated by DAG levels and that, as a consequence, their registration can be used as readout of intracellular DAG abundance.

We measured TRPC6 activity by ruptured whole-cell patch-clamp electrophysiological analysis in HEK293T cells cotransfected with TRPC6, M3 muscarinic receptor, and full-length or c.610delA mutant human DGKE. No detectable peptide was obtained from the c.127C>T mutant (Figure 3A). TRPC6 currents activated by the M3 receptor agonist carbachol were significantly reduced in cells transfected with wild-type DGKE mutations in intracellular DAG concentration, taking advantage of the fact that currents through TRPC6 channels are directly regulated by DAG levels and that, as a consequence, their registration can be used as readout of intracellular DAG abundance.

We measured TRPC6 activity by ruptured whole-cell patch-clamp electrophysiological analysis in HEK293T cells cotransfected with TRPC6, M3 muscarinic receptor, and full-length or c.610delA mutant human DGKE. No detectable peptide was obtained from the c.127C>T mutant (Figure 3A). TRPC6 currents activated by the M3 receptor agonist carbachol were significantly reduced in cells transfected with wild-type DGKE (Figure 3, B–D), supporting the evidence that the enzyme metabolizes and decreases cellular DAG content. Consistent with the finding that the c.610delA mutation is loss of function, TRPC6 currents in cells expressing the mutant were not different from mock-transfected cells. Because HEK293 cells constitutively express DGKε, we also measured the activity of TRPC6 by electrophysiological analysis after

Figure 5B). We also detected DGKε by Western blot in human immortalized podocytes and rat kidneys and testes (Supplemental Figure 5, C and D), where the enzyme was previously reported to be expressed. DGKε is the smallest of the known mammalian DGKs and lacks extra-enzymatic regulatory domains, a characteristic that suggests that this isoform is constitutively active. Another distinctive feature of DGKε is its marked substrate selectivity for DAG that is acylated with arachidonic acid at the sn-2 position (sn-2-arachidonoil-DAG). The selectivity for arachidonoil-DAG results in the enrichment with arachidonic acid of the phospholipids that participate to the phosphatidylinositol (PI) cycle and this effect is considered to be the main function of the enzyme.

TRPC6 is a calcium-permeable cation channel expressed in the foot processes of podocytes, and is known to be directly activated by DAG through a protein kinase C–independent mechanism. To demonstrate that lack of DGKε affects DAG production in podocytes, we tested the effect of DGKE mutations in intracellular DAG concentration, taking advantage of the fact that currents through TRPC6 channels are directly regulated by DAG levels and that, as a consequence, their registration can be used as readout of intracellular DAG abundance.

We measured TRPC6 activity by ruptured whole-cell patch-clamp electrophysiological analysis in HEK293T cells cotransfected with TRPC6, M3 muscarinic receptor, and full-length or c.610delA mutant human DGKE. No detectable peptide was obtained from the c.127C>T mutant (Figure 3A). TRPC6 currents activated by the M3 receptor agonist carbachol were significantly reduced in cells transfected with wild-type DGKE (Figure 3, B–D), supporting the evidence that the enzyme metabolizes and decreases cellular DAG content. Consistent with the finding that the c.610delA mutation is loss of function, TRPC6 currents in cells expressing the mutant were not different from mock-transfected cells. Because HEK293 cells constitutively express DGKε, we also measured the activity of TRPC6 by electrophysiological analysis after

Figure 2. Loss-of-function mutations in DGKE cause MPGN-like glomerular microangiopathy. (A) Kidney biopsy of patient UT-062 V-6. The represented glomerulus is hypertrophic and hypercellular. Focal capillary obliteration and thickening of the basement membrane can also be noticed (arrows) compared with patent capillaries (arrowheads). Hematoxylin and eosin. (B) Periodic acid–Schiff staining of a specimen from patient HU-314 IV-2 shows focal duplication of the glomerular basement membrane (arrowheads), causing thickening of the basement membranes in a hypertrophic glomerulus. (C) Image of the kidney biopsy from patient HU-500 V-1. The represented glomeruli are hypertrophic, hyperlobulated, and hypercellular (arrowhead) and present obliteration of the vascular spaces with endothelial cell swelling (arrows). Hematoxylin and eosin. (D) Transmission electron microscopy image of a glomerulus of patient UT-062 V-6. The capillary lumen is obliterated by the body of an endothelial cell (asterisk). Endothelial cytoplasmic rim is swollen (white arrow). Lamina rara interna is irregularly widened with flocculent material (black arrows). Foot processes on the epithelial side are partially effaced (arrowheads). (E) Electron micrograph of kidney biopsy of patient HU-500 V-1. Two swollen endothelial cells (asterisks) occlude a capillary lumen. The basement membrane is split (arrows) by the interposition of a mesangial cell. Uranyl acetate and lead citrate. (F) Electron micrograph of the kidney biopsy of patient HU-500 V-2. A swollen endothelial cell (asterisk) obstructs the lumen of a capillary. The basement membrane is split (white arrowhead) by the interposition of a mesangial cell (arrow). The podocyte foot processes are effaced (black arrowhead). Uranyl acetate and lead citrate. (G–J) Immunoperoxidase staining of biopsies from patient HU-500 V-1. Segmental deposition of IgM (G) and patchy deposition of IgG (H) are visible. Less intense stain was obtained with anti-C1q (I) and no C3 deposition was detected (J). (K and L) Immunofluorescence microscopy of biopsy from patient UT-062 V-6 showing week peripheral segmental deposition of IgM (K) and no intraglomerular deposits of C3 (L). Scale bar, 20 μm in A–C; 1 μm in E and F; 20 μm in G–L.
suppressing DGKe expression with targeting small interfering RNAs (siRNAs) or with nontargeting siRNAs as a control (Figure 3E). In accordance with the previous experiment, TRPC6 currents were increased when DGKe expression was minimal (Figure 3, F–H), indicating that DGKe is required to maintain adequate cellular levels of DAG.

In this study, we have identified mutations in the podocyte expressed gene DGKE as the cause of a glomerular microangiopathy with histologic signs of both MPGN and endothelial distress in three unrelated families, by combining whole exome sequencing and homozygosity mapping.16 Homozygosity mapping is based on the remarkable finding that, for an affected individual born from consanguineous parents, the probability of carrying an autosomal recessive deleterious mutation in a genomic segment that is homozygous by descent is nearly equal to one,16 which confers a very high statistical power to this technique. We have shown that DGKE is expressed in podocytes and controls the intracellular concentration of DAG, a component of the PI cycle that participates in multiple cellular functions and in the lipid-mediated intracellular signaling.24 Further experiments will be needed to verify if perturbation of this pathway might also affect the signaling between podocytes and endothelial cells, which would explain the vascular lesions observed in our patients. Three of the patients described in this work responded to immunosuppressive therapy (steroids and cyclosporine A or cyclophosphamide) with partial remission, alike a patient affected with diffuse mesangial sclerosis carrying a loss-of-function mutation in the gene PLCE1, which encodes for another enzyme of the PI cycle, (Supplemental Figure 4),25 suggesting that the components of this metabolic and signaling pathway are of particular importance in the physiology of podocytes.

**CONCISE METHODS**

**Genotyping, Homozygosity Mapping, and Sequencing**

DNA was extracted with standard procedures from peripheral blood and whole-genome genotyping was performed by 250K Nsp/I-SNP-chip Affymetrix hybridization array. Exome capture was obtained by using SureSelect Human All Exon 38 Mb reagent kit (Agilent). Captured exons were massively parallel sequenced using a Genome Analyzer IIx (Illumina). Single nucleotide variants and insertion/deletion calls were obtained using CLC Bio Genomic Workbench 4 platform. Sanger exon sequencing was performed in a
Worldwide cohort of 142 unrelated patients affected with MPGN.

**Whole-Cell Patch-Clamp Electrophysiological Analyses**

HEK293T cells were transiently cotransfected with vectors expressing enhanced green fluorescent protein for the identification of the transfected cells, M3 muscarinic receptor, human TRPC6, and wild-type or mutant DGKs. Whole-cell patch-clamp recording was performed using an Axopatch 200B patch-clamp amplifier (Axon Instruments Inc., Foster City, CA) as previously described.26

**siRNA-Mediated DGKE Targeting**

A pool of four siRNAs targeting different sequences of DGKE mRNA was purchased from Thermo Scientific (SMART pool L-011493-00-0005, anti-human DGKE NM_003647) and used according to the manufacturer's indications.

**Immunofluorescence Confocal Microscopy**

The following antibodies were used: anti-DGKE (sc-98729 and sc-100372) and anti-CD31 (MAB1393; Millipore), anti-α-smooth actin (A5228; Sigma), antifibrinogen (4440-8004; AbD Serotech), and anti-C3b (Ab11871; Abcam). Specimens were fixed with paraformaldehyde and imbedded in optimal cutting temperature (OCT) compound. Tissue sections were permeabilized in 0.1% Triton X-100 in PBS, incubated in a solution of 0.1% sodium borohydride, blocked in a solution with bovine serum albumin, and incubated overnight at 4°C with primary antibody, followed by incubation with secondary antibody. For the competition assay, the primary antibody was coincubated in blocking solution with a competing peptide at a molar concentration 200 times higher than the antibody, for 1 hour at room temperature. The solution was centrifuged for 5 minutes, and the supernatant was recovered and used on tissue sections. Images were acquired using a Zeiss LSM 510 confocal microscope.

**Western Blot Analyses**

Equal amounts of protein were mixed with 4× Laemmli sample buffer (Bio-Rad 161-0737) and denatured at 95°C for 10 minutes. Samples were run on 10% polyacrylamide gels, transferred to polyvinylidene difluoride membranes (LC2002), blocked in 5% BSA, and probed with primary and secondary antibodies. Proteins were visualized with Lumion reagent (sc-2048; Santa Cruz).

**Site-Directed Mutagenesis**

Appropriate mutagenic oligonucleotide primers were designed flanked by unmodified nucleotide sequences and used to amplify full-length DGKE cDNA cloned into pCDNA3.1 (Supplemental Table 3). Mutant clones were selected by enzymatic digestion using the QuickChange mutagenesis kit (Agilent).

**Quantitative Real-Time PCR**

Total RNA was isolated using TRIzol (Invitrogen) and purified with the Qiagen RNeasy Mini Kit according to the manufacturer’s protocols. ThermoScript RT-PCR Kit (Invitrogen) was used to obtain first-strand RT. Real-time PCR was performed using iQ SYBR Green Supermix (Bio-Rad). The β-actin gene was used as a normalization.

An exhaustive description of all experiments and methods is available in the Supplemental Material.

**Acknowledgments**

We are grateful to the families for their participation to this study. We thank Carlos Arana, Yun Lian, and Georgina Konstantinou for their technical support and the primary physician of family HU-314, Professor Sukru Sindel, who provided the clinical details of the oldest sibling.

M.A. is supported by grants from the National Institutes of Health (NIH) (1R01DK090326-01A1, P30DK079328-04), a 2010 American Society of Nephrology Norman Siegel Research Award, and a Satellite Healthcare Foundation Norman Coplon extramural research award. F.O. was supported by a grant from the Scientific and Technological Research Council of Turkey (1085417). The Nephрогenetics Laboratory at the Hacettepe University Faculty of Medicine, Department of Pediatrics, was established by the Hacettepe University Infrastructure Project (Grant 06A 101 008). F.H. was supported by grants from the NIH (DK1069274, DK1068306, and RC4-DK090947), and is an investigator for the Howard Hughes Medical Institute, a Doris Duke Distinguished Clinical Scientist, and a Frederick G.L. Huetwell Professor. C.H. was supported by grants from the NIH (DK85726) and the University of Texas Southwestern O’Brien Kidney Research Core Center (P30DK79328). M.K.T. was supported by a grant from the NIH (CA095463).

**Disclosures**

None.

**References**


DGKE and MPGN-Like Nephropathy


This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2012090903/-/DCSupplemental.