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Autosomal Dominant Tubulointerstitial Kidney Disease—Uromodulin Misclassified as Focal Segmental Glomerulosclerosis or Hereditary Glomerular Disease

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Introduction: Focal segmental glomerulosclerosis (FSGS) is a histopathologically defined kidney lesion. FSGS can be observed with various underlying causes, including highly penetrant monogenic renal disease. We recently identified pathogenic variants of *UMOD*, a gene encoding the tubular protein uromodulin, in 8 families with suspected glomerular disease.

Methods: To validate pathogenic variants of *UMOD*, we reviewed the clinical and pathology reports of members of 8 families identified to have variants of *UMOD*. Clinical, laboratory, and pathologic data were collected, and genetic confirmation for *UMOD* was performed by Sanger sequencing.

Results: Biopsy-proven cases of FSGS were verified in 21% (7 of 34) of patients with *UMOD* variants. The *UMOD* variants seen in 7 families were mutations previously reported in autosomal dominant tubulointerstitial kidney disease-uromodulin (ADTKD-UMOD). For one family with 3 generations affected, we identified p.R79G in a noncanonical transcript variant of *UMOD* co-segregating with disease. Consistent with ADTKD, most patients in our study presented with autosomal dominant inheritance, subnephrotic range proteinuria, minimal hematuria, and renal impairment. Kidney biopsies showed histologic features of glomerular injury consistent with secondary FSGS, including focal sclerosis and partial podocyte foot process effacement.

Conclusion: Our study demonstrates that with the use of standard clinical testing and kidney biopsy, clinicians were unable to make the diagnosis of ADTKD-UMOD; patients were often labeled with a clinical diagnosis of FSGS. We show that genetic testing can establish the diagnosis of ADTKD-UMOD with secondary FSGS. Genetic testing in individuals with FSGS histology should not be limited to genes that directly impair podocyte function.

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FSGS is the most common pattern of histologically defined glomerular injury to cause end-stage kidney disease in the United States.[1] Glomerular diseases including FSGS are the third most common cause of end-stage renal disease preceded only by diabetes and hypertension.[2] FSGS can have primary (idiopathic), secondary, and genetic causes.[3] Idiopathic causes of FSGS often present clinically with the nephrotic syndrome (heavy proteinuria, hypoalbuminemia, and peripheral edema and sometimes dyslipidemia and a prothrombotic state). The most characteristic feature on electron microscopy is widespread podocyte foot process effacement. There are many secondary causes of FSGS that are usually a reflection of a response to insults such as viral infections, medication-induced

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changes, and adaptive responses to reduced nephron mass.[4] Secondary FSGS usually presents clinically with less severity and sometimes an insidious onset with subnephrotic range proteinuria. Often, patients diagnosed with FSGS have no identifiable cause following an extensive workup for secondary causes. Identifying familial causes of FSGS has been an intense area of research. Over the past 2 decades, mutations in numerous genes have been identified that lead to progressive chronic kidney disease (CKD) and adult-onset FSGS through Mendelian or mitochondrial inheritance patterns.[3,5]

The genetic variants encoding proteins associated with FSGS are known to localize to compartments within the glomeruli. Many proteins encoded by genes found to be mutated in FSGS are essential for podocyte structure and/or function.[6] Most monogenic glomerular diseases, such as *INF2, ACTN4, TRPC6, ARHGAP24, ANLN, PODXL,* and *WT1*, are thought to have direct effects on podocytes.[3] By contrast, several groups have reported pathogenic variants of *COL4A3*/*A4*/*A5* in patients with FSGS.[7–9] Malone *et al*.[10] reported patients with novel variants of *COL4A3* or *COL4A4* from a cohort of 70 families diagnosed as hereditary FSGS. Defects in the glomerular basement membrane as a result of *COL4A* mutations were shown to have a pattern of secondary FSGS on the kidney biopsy. In addition, there is evidence for tubular disorders leading to FSGS, such as *CLCN5* and *SLC12A1,* which are mutated in Dent disease and type 1 Bartter syndrome, respectively.[11,12] More recently, Snoek *et al*.[13] identified a variant of *HNF1B* in an adult patient who presented with a picture of FSGS. PAX2 localizes to glomerular parietal epithelial cells and has been associated with FSGS, but how PAX2 mutations affect podocytes or contribute to FSGS remains unclear.[14–16] These studies suggest that podocyte injury and FSGS may be initiated by alterations in kidney cells, such as tubular cells that reside outside of the glomeruli.

UMOD encodes uromodulin, formerly known as Tamm-Horsfall glycoprotein, which is the most abundant protein in normal urine.[17] There are more than 100 mutations that have been identified in *UMOD*.[18] Pathogenic variants have been found to be concentrated in exons 3 and 4, often affecting cysteine residues.[19] Mutations have been thought to contribute to improper folding of uromodulin, leading the unfolded protein response, endoplasmic reticulum stress, and apoptosis.[20] *UMOD* variants can cause ADTKD, also known as ADTKD-UMOD. ADTKD-UMOD, previously referred to as medullary cystic kidney disease 2 and familial juvenile hyperuricemic nephropathy, is characterized by a family history with autosomal dominant inheritance, bland urine sediment with minimal proteinuria, and a slow progressing kidney disease with end-stage renal disease developing between the ages of 20 and 70.[12,17,21] Hyperuricemia, early-onset gout, and renal cysts are sometimes present. ADTKD-UMOD is a difficult condition to diagnose, requiring a high clinical suspicion and confirmation by genetic testing.

In this study, we describe the association of *UMOD* variants in a cohort of patients with suspected hereditary glomerulonephritis or familial FSGS. We characterize 8 families previously identified to have pathogenic variants of *UMOD* by exome sequencing.[5] We confirm by Sanger sequencing that all members had variants of *UMOD*. Our results underline the importance of genetic testing in diagnosing ADTKD-UMOD correctly. Furthermore, we demonstrated that pathogenic *UMOD* variants well-established to cause tubular injury can have a phenotypic presentation of FSGS.

METHODS

Samples and Clinical Data From Human Subjects

Institutional Review Board approval was obtained from the Beth Israel Deaconess Medical Center (Boston, MA). Using a subset of patient data and samples from our previous study,[5] 8 families with *UMOD* mutations were analyzed for clinical correlation. All families included in this study had an autosomal dominant inheritance pattern. Clinical information was obtained from questionnaires, telephone interviews, electronic mail communications, physician reports, and kidney biopsy data. Inclusion criterion for this study was *UMOD* mutation–confirmed Sanger sequencing in our cohort of patients with suspected FSGS and/or FSGS reported on kidney biopsy. The biopsy reports reviewed contained the description of FSGS and/or podocyte foot process effacement but with no features of immune deposition or proliferative diseases. Human genomic DNA was isolated with standard methods using the Oragene self-collection kit (DNA Genotek, Kanata, Ontario, Canada) for saliva samples and the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) for blood samples.

Sanger Sequencing

The disease-causing variants of *UMOD* were verified using Sanger sequencing. Exon-flanking primers were used to sequence both strands of all affected and randomly selected unaffected patients. HotStarTaq DNA Polymerase (Qiagen) and a C100 Thermal Cycler (Bio-Rad, Hercules, CA) was used to generate polymerase chain reaction products that were sequenced by GENEWIZ. Primer sequences are listed in Table 1.

Sequence chromatograms were analyzed using SnapGene Viewer 4.1.

Statistics and Circle Pedigree

Fisher's exact test was used to access the significance of the enrichment of rare variant between case and control families; the test was performed by the *exact2x2* library of R (Version 3.5.1; R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org). The circle pedigrees were plotted by in-house Python and JavaScript scripts based on D3.js.[22] The source code is freely available from author's github at https://github.com/wavefancy/CircularPedigreeTree.

RESULTS

Mutations in *UMOD* Are Associated With Familial CKD of Seemingly Glomerular Origin

In our recent exome sequencing analysis of 662 exomes of individuals with suspected or biopsy-proven FSGS and 622 control participants, we identified rare coding variants of *UMOD*, a gene encoding the tubular protein uromodulin, in 8 families but in only 1 control ($P < 0.003$; odds ratio: 12.8; 95% confidence interval: 2.0–281).[5] *UMOD* variants were the likely cause of disease in 5.4% of families in which variants of known kidney disease genes were identified, and could explain 2% of families with FSGS we studied. Co-segregation of the rare variants associated with the disease was confirmed using Sanger sequencing. All affected members of these families inherited the suspicious *UMOD* variant (highlighted in red), whereas most unaffected members (highlighted in green) did not (Figure 1). *UMOD* variants were found in 9 members of these families who did not have overt kidney disease. Most of these individuals were from the youngest generations of these families, consistent with incomplete, age-related, penetrance. Our clinical data are not complete enough to define this age-related penetrance quantitatively. The variants of 3 families were in-frame deletions, whereas the presumed pathogenic variants of 5 of the families were point mutations (Figure 2 and Table 2). The in-frame deletions VCPEG (93–97) for families FGCM, FGCO, and FGGR were known pathogenic variants for ADTKD-UMOD.[19] The mutated residues at positions p.C106F, p.W202S, and p.C315F have been previously demonstrated to be associated with medullary cystic kidney disease 2 and familial juvenile hyperuricemic nephropathy (Figure 3a).[23–25] The *UMOD* variants were present in exons 3 and 4, regions frequently reported to have mutations. In addition, we identified an unreported point mutation, p.R79G, for family FGLV found in a noncanonical variant of uromodulin (ENST00000574195.1) with a 161 amino acid protein encoded by 3 of 4 exons (Figure 3b, Table 2). There was limited information for the *in silico* prediction scores for p.R79G in the noncanonical transcript variant with only a low CADD_PHRED (combined annotation-dependent depletion) score of 0.566 (Table 3). Nonetheless, the Sanger sequencing confirmed *UMOD* variants co-segregated with disease within our cohort of families providing further evidence that the *UMOD* variants are pathogenic mutations (Figure 1). The combination of large family sizes, majority of variants of affected individuals previously reported to be associated with kidney diseases, and multiple *in silico* predictions for the protein-damaging point mutations (Polyphen-2,[26] SIFT,[27] M-CAP,[28] LRT,[29] META_SVM,[30] GERP++ RS[31]; Table 3) support that the variants identified in these case families are pathogenic.

Table 1. Primer sequences for *UMOD* used in this study

Family	Forward primer	Reverse primer
FGCM, FGCO, FGGR	TGCCCACCACATTGACACAT	TTCTGTCCACAGGATGGTGC
FGDC, FGJF	ACTCACAGTGCCATCCATCC	AACCCTGAAGCTGGGCTTTT
FGJD	AGCCTCTTGCCGGCTTTAAT	GAGTGTCACCTGGCGTACTG
FGIT	GGATGAGGACTGTGGGGAGA	GGATGGATGGCACTGTGAGT

Nonclassical Clinical Presentation for UMOD-Associated Kidney Disease

The identification of known variants of *UMOD* in our cohort with suspected FSGS, hereditary glomerulonephritis, and/or nephrotic syndrome raised the possibility that the *UMOD* variants contributed to secondary FSGS. In our study cohort, patients with *UMOD* gene mutation did not have the usual clinical characteristics associated with *UMOD* nephropathy, such as reports of gout, documented hyperuricemia, or medullary cysts on renal ultrasonography.[19] Analysis of patient demographics revealed a male-to-female ratio of 1 to 1 for affected individuals with *UMOD* variants (17 male and 17 female individuals, Table 4). The age for development of end-stage renal disease ranged from 21 to 68 years. The age of diagnosis for the affected individuals was usually by the time they had reached end-stage renal disease in their third to sixth decade of life but earlier in some individuals (ages 20, 29, and 16 for FGIT11, FGIT234, and FGJD11, respectively). Consistent with the diagnosis of ADTKD-UMOD, urine sediment was typically bland with minimal or no proteinuria (Table 4). In most affected individuals, the amount of albuminuria was < 1 g/d.

Kidney Biopsy From a UMOD Cys106Phe Individual

Because *UMOD* encodes uromodulin, a protein localized to the thick ascending limb of the Loop of Henle,

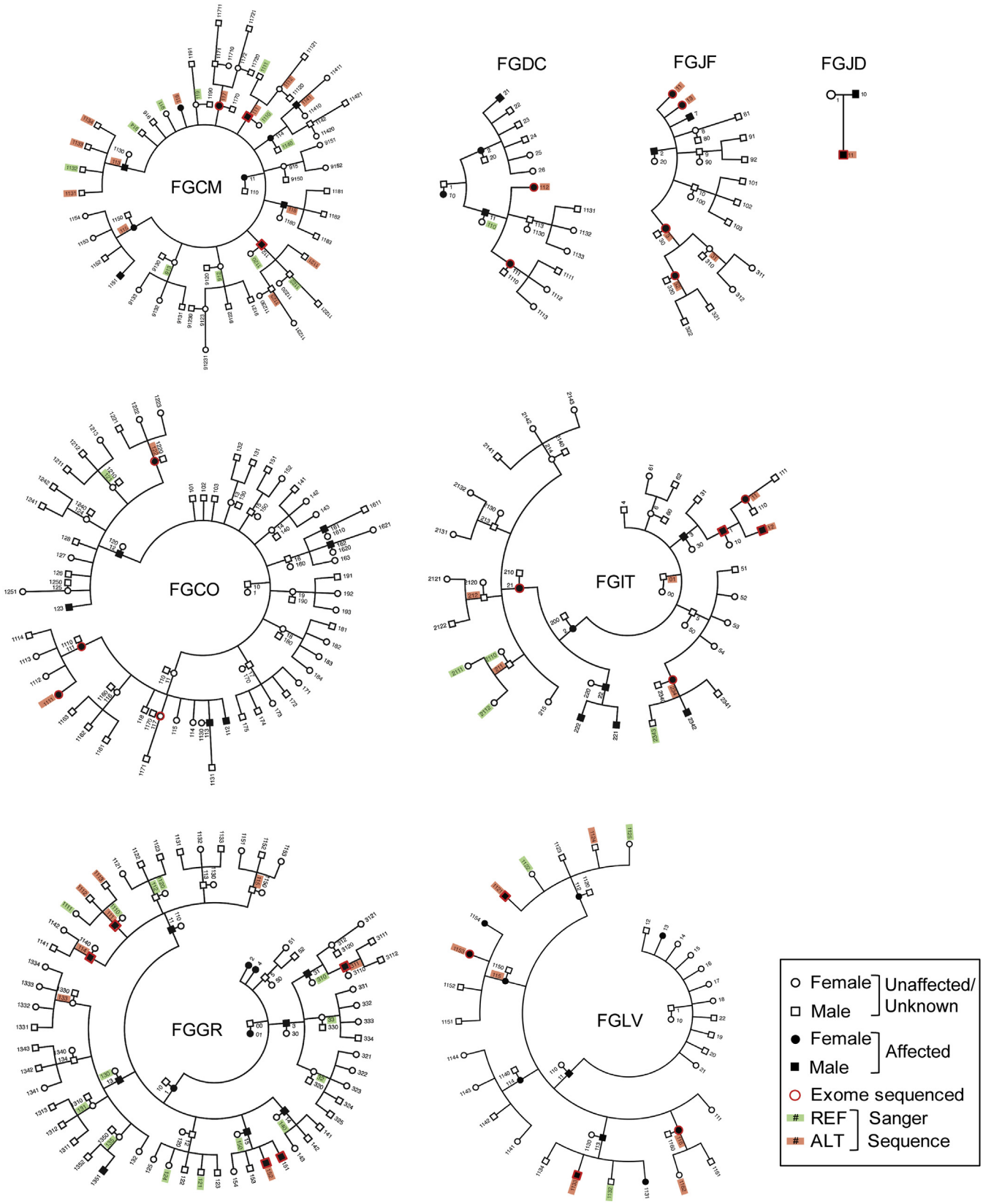

Figure 1. Pedigrees for 8 families with uromodulin (*UMOD*) variants. Pedigrees for families FGCM, FGIT, FGCO, FGLV, FGGR, FGDC, FGJF, and FGJD. Affected individuals with impaired kidney function are indicated in filled circles (female) and squares (male), and unfilled circles and squares for family members not on renal replacement therapy, but with otherwise unknown or unaffected status. Individuals who had exome sequencing of samples are indicated with a red outline. Sanger sequencing showing wild-type (REF, reference alleles) sequence and mutation (ALT, alternative alleles) are highlighted in green and red, respectively.

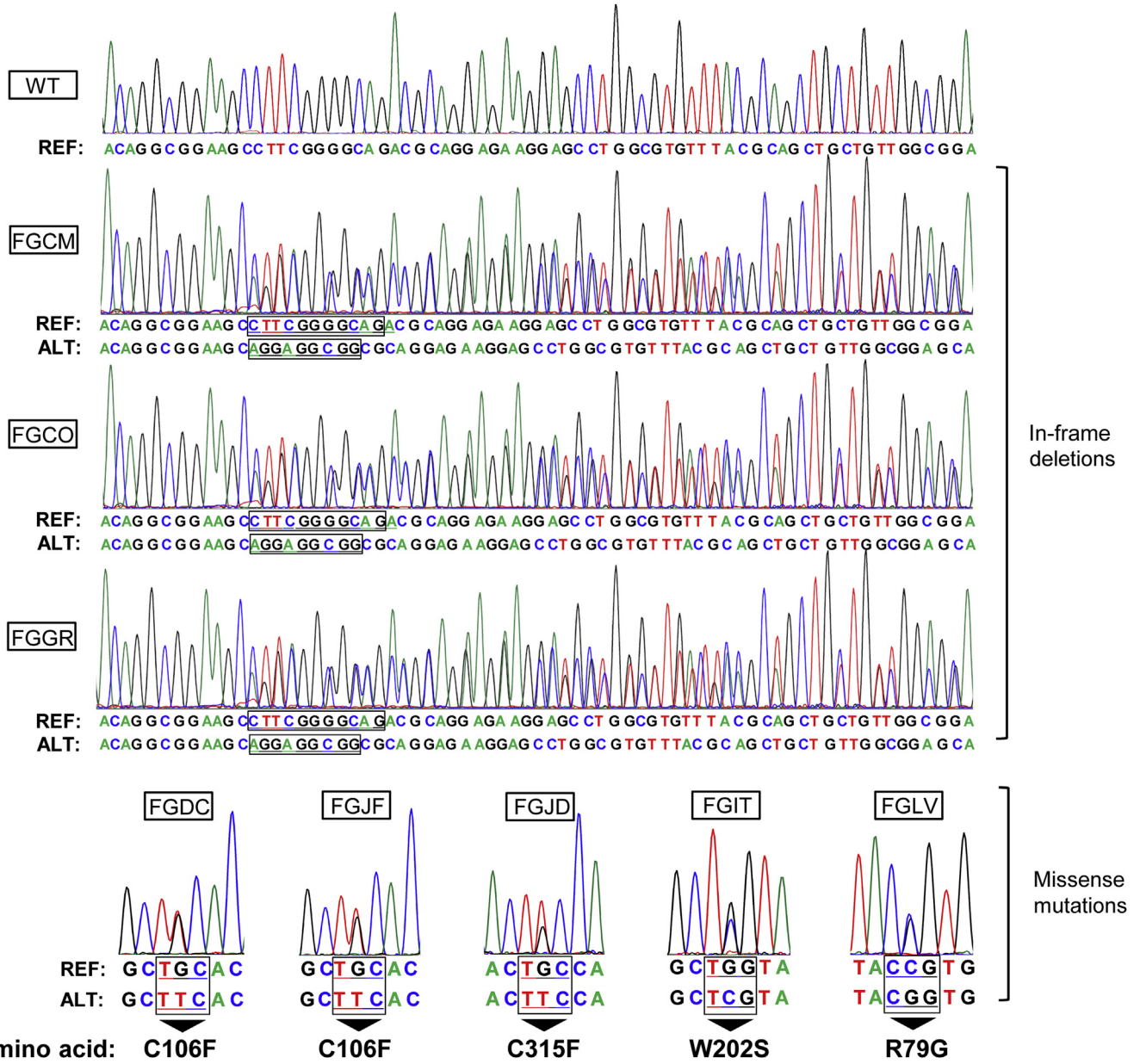

Figure 2. Uromodulin (*UMOD*) variants in 8 families. Chromatograms of unaffected wild-type (WT) and *UMOD* variants for representative affecteds for FGCM, FGCO, and FGGR (in-frame deletions), and FGDC, FGJF, FGJD, and FGIT (missense mutation; codon for mutation underlined). ALT, alternative; C, cysteine; F, phenylalanine; G, glycine; R, arginine; REF, reference; S, serine; W, tryptophan.

we reasoned that glomerular changes observed in the setting of defective uromodulin is most likely a secondary process. A representative biopsy from patient FGJF32 is shown in Figure 4. Light microscopy showed segmental sclerosis, with one glomerulus noted as being globally sclerosed and another that was unremarkable. There was severe and widespread tubular atrophy and interstitial fibrosis (data not shown), consistent with a chronic process as seen with both (primary) FSGS and ADTKD-UMOD. Electron microscopy showed localized segmental fusion of foot processes and microvillous transformation of visceral epithelial cells.

DISCUSSION

ADTKD-UMOD is an underrecognized genetic kidney disease.[32,33] To the best of our knowledge, no previous studies have reported *UMOD* variants causing FSGS. Our work highlights the possibility that some patients diagnosed with FSGS or hereditary glomerulonephritis may have an underlying *UMOD* mutation as the etiology for their suspected glomerular disease. The actual prevalence for ADTKD-UMOD is difficult to determine because the condition is often not suspected and underdiagnosed.[34] Recently, Gast *et al.*[33] examined patients with stages 3 to 5 CKD and found that ADTKD-UMOD was the most common form of inherited kidney

Table 2. Rare variant list for the *UMOD* gene

Family	POS_HG19	REF	ALT	HGVSp	HGVSc
FGJD	20359574	C	A	ENSP00000306279.4: p.Cys315Phe	ENST00000302509.4: c.944G>T
FGIT	20360018	C	G	ENSP00000306279.4: p.Trp202Ser	ENST00000302509.4: c.605G>C
FGDC FGJF	20360306	C	A	ENSP00000306279.4: p.Cys106Phe	ENST00000302509.4: c.317G>T
FGCM FGCO FGGR	20360333	CCTTCGGGGCAGA	CAGGAGGCGG	ENSP00000306279.4: p.Val93_Gly97delinsAlaAlaSerCys	ENST00000302509.4: c.278_289delinsCCGCCTCCT
FGLV	20361045	G	C	ENSP00000460845.1: p.Arg79Gly	ENST00000574195.1: c.235C>G
G48055 (control)	20348046	AG	CA	ENSP00000306279.4: p.Cys582Gly	ENST00000302509.4: c.1743_1744delinsTG

ALT, alternative nucleotide(s); HG19, human genome assembly from Genome Reference Consortium; HGVSc, the Human Genome Variation Society notation for coding sequence name; HGVSp, the Human Genome Variation Society notation for protein sequence name; POS, genomic position; REF, reference nucleotide(s).
Transcripts: Ensembl transcripts available at www.ensembl.org.

disease following autosomal dominant polycystic kidney disease. Groopman *et al.*[35] identified 66 distinct monogenic disorders in their 2 cohorts totaling 3315 patients with CKD, of which 3% was explained by mutations in *UMOD*. Similarly, affected members of the 8 families investigated in our study were initially assumed to have FSGS or hereditary glomerulonephritis. In retrospect, it is not surprising that some patients with ADTKD-UMOD are classified as FSGS because ADTKD-UMOD has no specific clinical or histopathological features by conventional kidney biopsy. Trimarchi *et al.*[36] showed a link between mucin-1 (*MUC1)* gene mutation for a case of secondary FSGS. As noted earlier, there is evidence for mutations in genes that cause tubular disease (e.g., *CLCN5*, *SLC12A1*, *HNF1B*) initially presenting with a clinical phenotype labeled as FSGS.[11,12,13] We expect mutations in *MUC1*, *UMOD,* and other genes encoding tubular proteins to be more frequently identified in many patients with CKD with seemingly glomerular origin.

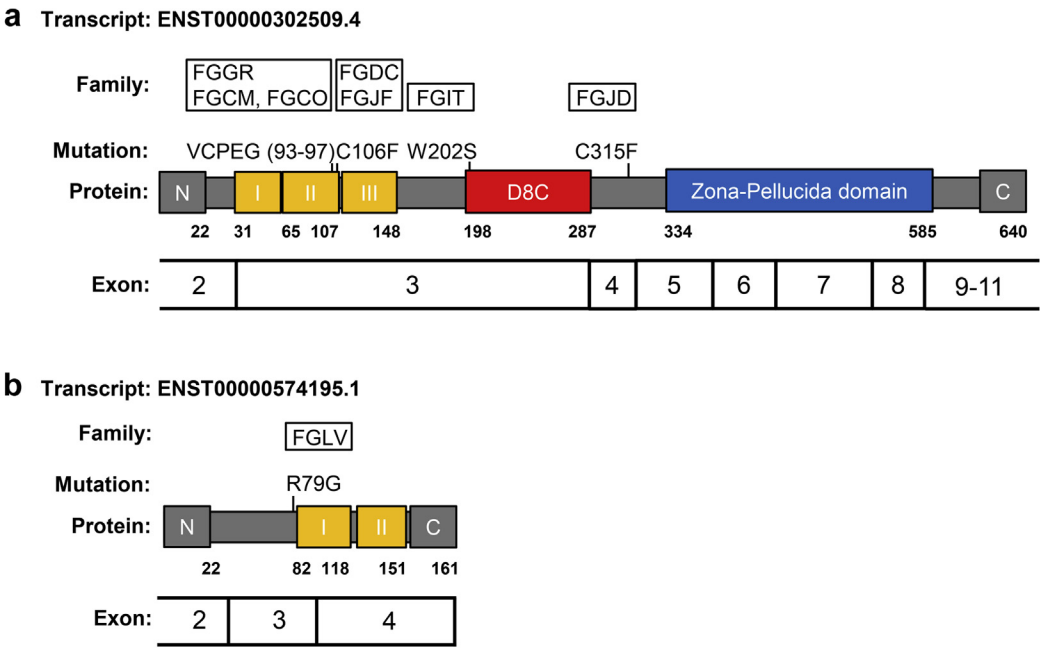

Figure 3. Schematic representation for the relative locations of uromodulin (*UMOD*) variants. (a) *UMOD* transcript ENST00000302509.4 displaying exons 1 to 11 of with the locations of 4 mutations. The location of in-frame deletions and missense substitutions are indicated by single-letter amino acid codes. The mutations in the present study were found in exons 3 and 4. An open reading frame predicts a 640–amino acid protein for uromodulin. The protein domains of UMOD include 3 epidermal growth factor-like domains denoted by I, II, and III, cysteine-rich region containing the domain of 8 cysteines (D8C). The in-frame deletions and the missense mutations found in the present study are indicated. (b) *UMOD* transcript ENST00000574195.1 displaying exons 1 to 4 for family FGLV with the location of one newly identified mutation. The open reading frame encoded by exons 1 to 3 predicts a 161–amino acid protein for uromodulin. The predicted protein domains for this transcript include 2 epidermal growth factor-like domains denoted by I and II.

Table 3. *In silico* prediction scores of clinical pathogenicity for *UMOD* variants

Family	Nucleotide change	GnomAD_ genomes_AF	CLIN_SIG	PolyPhen-2	SIFT	MetaSVM	M-CAP	LRT	GERP++_RS	CADD_ PHRED
FGJD	c.944G>T	0	NA	D	D	D	D	D	4.64	32
FGIT	c.605G>C	0	NA	D	D	D	D	D	5.13	32
FGDC FGJF	c.317G>T	0	Likely pathogenic	D	D	D	D	D	5.45	28
FGCM FGCO FGGR	c.278_289delins CCGCCTCCT	0	Pathogenic	NA	NA	NA	NA	NA	NA	20.4
FGLV	c.235C>G	0	NA	NA	NA	NA	NA	NA	NA	0.566
G48055 (control)	c.1743_1744 delinsTG	0	NA	NA	NA	NA	NA	NA	NA	NA

CLIN_SIG, Clinvar database annotation signature; D, deleterious; GnomAD_genomes_AF, variant frequency in GnomAD database; NA, not applicable.
Prediction tools for predicting deleteriousness of single nucleotide variants or insertion/deletion variants: PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Sorting Tolerant From Intolerant; M-CAP, Mendelian Clinically Applicable Pathogenicity; LRT, likelihood ratio test; GERP++RS, Genomic Evolutionary Rate Profiling rejected substitution; and CADD, Combined Annotation Dependent Depletion.

Most mutations in the *UMOD* gene identified in our study were previously reported to be pathogenic. The mutations p.C315F, p.W202S, and p.C106F are predicted to be pathogenic variants with a probably deleterious effect as predicted by numerous *in silico* prediction algorithms (Table 3). The GERP++_RS and CADD_PHRED scores were 4.64 and 32, respectively. Mutations p.W202S and p.C106F have been reported previously. p.C315F has not been reported, but a mutation at the same position, the C315R, was reported in affected families with glomerulocystic kidney disease, and C315Y were previously reported as pathogenic.[25,37] The position is in a conserved and functional important region of the *UMOD* gene.[14,38] p.Val93_Gly97delinsAlaAlaSerCys has been reported multiple times as pathogenic.[33,39,40] Interestingly, we identified a rare transcript (ENST00000574195.1) with mutation of p.R79G for family FGLV. This variant of the noncanonical transcript has not been reported in the GnomAD database, which sequenced 125,748 whole exomes and 15,708 whole genomes.[41] The mutated residue was present in the coding region for ENST00000574195.1, whereas the canonical transcript (ENST00000302509.4) did not have an amino acid change, as the nucleotide was present in an intron. The mutation is likely a pathogenic variant, as the variant co-segregates with the disease in a large family (FGLV). Nonetheless, future work will be needed to determine the expression and function of this truncated transcript.

A limitation of our study was the incomplete availability of clinical data of interest, including laboratory studies and diagnostic imaging. Certainly, a more comprehensive collection of uric acid levels and kidney ultrasounds may have provided evidence for ADTKD-UMOD. Future studies could analyze original patient samples for quantification of percentage of foot process effacement from electron micrographs and tissue staining for uromodulin to assess for abnormalities in protein localization or expression. If kidney biopsy samples were available for our affected family members, we predict uromodulin staining for patients with *UMOD* pathogenic variants would have shown a granular or endoplasmic staining pattern. Additional staining of tissue for uromodulin in patients with possible ADTKD-UMOD could help guide clinicians to consider genetic testing.

The high rate of *UMOD* variants found in patients given a histopathological diagnosis of FSGS or suspected glomerular disease was unexpected. It remains unclear if tubular injury in patients with *UMOD* precedes glomerular changes or if there is crosstalk between the tubular compartment and glomeruli.[42] Uromodulin is exclusively produced in the thick ascending limb of the Loop of Henle. Future work will need to investigate how genetic mutations in *UMOD*, a gene encoding the thick ascending limb protein uromodulin, cause injury to podocytes and how glomeruli adapt to injury originating from the tubules. Trudu *et al.*[43] demonstrated that *UMOD* gene variants may increase uromodulin expression to induce salt-sensitive hypertension leading to kidney damage. In the case of IgA nephropathy with FSGS, there may be immune-mediated mechanisms of podocyte injury leading to segmental sclerosis.[44] It is also possible that injured tubular-mediated production of proinflammatory and profibrotic cytokines can lead to podocyte cell death.[45] Future avenues of research will be to characterize podocyte injury induced by cellular mediators arising from the tubules, mesangial cells, or endothelial cells.

Will genetic testing alter disease management for patients with ADTKD-UMOD? If *UMOD* pathologic variants are identified in patients who were initially diagnosed with FSGS, such patients should be reclassified as ADTKD-UMOD with FSGS. Furthermore, potential therapies that can delay the progression of ADTKD-UMOD would be the appropriate therapy, rather than the immunosuppressive agents often used in FSGS. Family members who are potential allograft donors should undergo a genetic evaluation for *UMOD* to determine donor eligibility status and be made

Table 4. Phenotype information for 8 families with *UMOD* variants

Family	Individual	Sex	Age at diagnosis	Age of ESRD	Hematuria	Proteinuria (ACR or mg/d)	SCr (mg/dl)	Working diagnosis	Biopsy findings c/w FSGS
FGCM	111	M	U	58	Neg	Pos (1100)	U	Hereditary GN	U
FGCM	112	M	55	55	Neg	Pos	U	ESRD NYD	U
FGCM	113	M	25	40	U	Pos	U	Hereditary GN	U
FGCM	115	F	32	39	Pos	Pos	11.2	ESRD NYD	U
FGCM	117	F	U	50	U	Pos (278)	1.9	Hereditary GN	Global and segmental sclerosis
FGCM	118	M	34	U	U	Pos	U	Hereditary GN	U
FGCM	1141	M	U	57	U	U	U	ESRD NYD	U
FGCM	1151	M	U	36	Neg	U	U	ESRD NYD	U
FGCO	122	F	40	47	U	U	U	ESRD from HTN	U
FGCO	1111	F	41	42	Neg	U	1	Hereditary GN	U
FGGR	111	M	54	55	Pos	Pos (130.7)	1.6	Hereditary GN	U
FGGR	114	M	40	49	Neg	Pos (84.5)	1.5	FSGS	FSGS
FGGR	151	M	44	52	Neg	Pos (>303)	3.2	FSGS	FSGS
FGGR	152	M	U	50	Neg	Pos (>629)	U	NS NYD	U
FGGR	311	M	57	N/A	Neg	U	4	Hereditary GN	U
FGDC	111	F	U	45	U	Pos	U	FSGS	FSGS
FGDC	112	F	40	46	Neg	Pos	1.1	FSGS	FSGS
FGIT	1	M	U	45	Neg	Neg	U	Hereditary GN	U
FGIT	11	F	20	21	Neg	Pos (175 mg/d)	1.6	Hereditary TIN	Effaced FPs
FGIT	12	M	38	N/A	Neg	Pos (165.5)	2.4	CKD NYD	None
FGIT	21	F	37	68	Neg	Pos (72)	1.4	Hereditary GN	U
FGIT	22	M	36	45	Neg	Neg	1	Hereditary GN	U
FGIT	234	F	29	35	U	Neg	3.2	HTN related	None
FGJD	1	F	U	69	U	U	U	HTN related	U
FGJD	11	M	16	36	Neg	Neg	7.9	FSGS	FSGS[a]
FGJF	3	F	U	40	Neg	Neg	1.9	FSGS	U
FGJF	11	F	U	51	Neg	Neg	1.7	FSGS	FSGS
FGJF	13	F	U	40	Neg	Neg	U	FSGS	None
FGJF	32	F	34	36	Neg	Pos (700 mg/d)	2.5	FSGS	FSGS
FGLV	115	F	U	60	Pos	Pos (298.8)	3.5	Hereditary GN	U
FGLV	116	F	U	51	Neg	Neg	1.9	ESRD NYD	U
FGLV	1121	M	47	52	Neg	Pos	U	ESRD NYD	U
FGLV	1133	M	37	42	Pos	Neg	2.4	ESRD NYD	U
FGLV	1153	F	U	40	Neg	Pos (30.9)	1.2	ESRD NYD	U

ACR, albumin-to-creatinine ratio in mg/g; CKD, chronic kidney disease; c/w, consistent with; ESRD, end-stage renal disease; F, female; FPs, foot processes; FSGS, focal segmental glomerulosclerosis; GN, glomerulonephritis; IgAN, IgA nephropathy; M, male; Neg, negative; none, no findings consistent with FSGS; NS, nephrotic syndrome; NYD, not yet determined; Pos, positive; SCr, serum creatinine; TIN, tubulointerstitial nephritis; U, unknown.
[a]Insufficient material for native kidneys; based on biopsies of 2 allografts showing FSGS (suspected FSGS recurrence).

ineligible as a donor if they carry one of the *UMOD* variants. Genetic counseling can be of help in discussing interpretation and implications of test results with family members.

Last, Nafar *et al.*[46] have suggested that uromodulin can potentially be a biomarker for FSGS. It may be possible to avoid genetic testing of patients with a high suspicion for ADTKD-UMOD by reviewing, and if needed, reinvestigating patients with suspected ADTKD-UMOD with staining of kidney biopsy specimens for uromodulin. Immunohistochemistry may provide clues such as abnormalities in uromodulin staining, for example, coarsely granular cytoplasmic staining or perinuclear positivity in flattened tubular epithelial cells in the Loop of Henle epithelium for patients with *UMOD* mutations.[5,45,47]

It is well recognized that the term FSGS can been applied to a wide range of conditions, begging the question as to whether or not it has any useful meaning for diagnostics.[11] The etiology and pathogenesis in a patient with FSGS histology cannot be determined solely by assessing features of lesions by microscopy.[3] It is quite clear that the descriptor FSGS should be confined to use as a description of histology, rather than as a clinical disease designation. Genetic testing would avoid misdiagnosis of ADTKD-UMOD as a glomerular injury (e.g., FSGS) and establish the correct molecular and clinical diagnosis. We favor including *UMOD* in standard genetic testing panels used in the diagnosis of FSGS. The probability that a specific *UMOD* variant found is causally related to disease depends on multiple factors: presence or absence of other candidate variants, evidence of tubulo-interstitial disease, and presence or absence of nephrotic symptoms. Avoiding the term FSGS as a clinical diagnosis could prevent incomplete evaluation and encourage clinicians

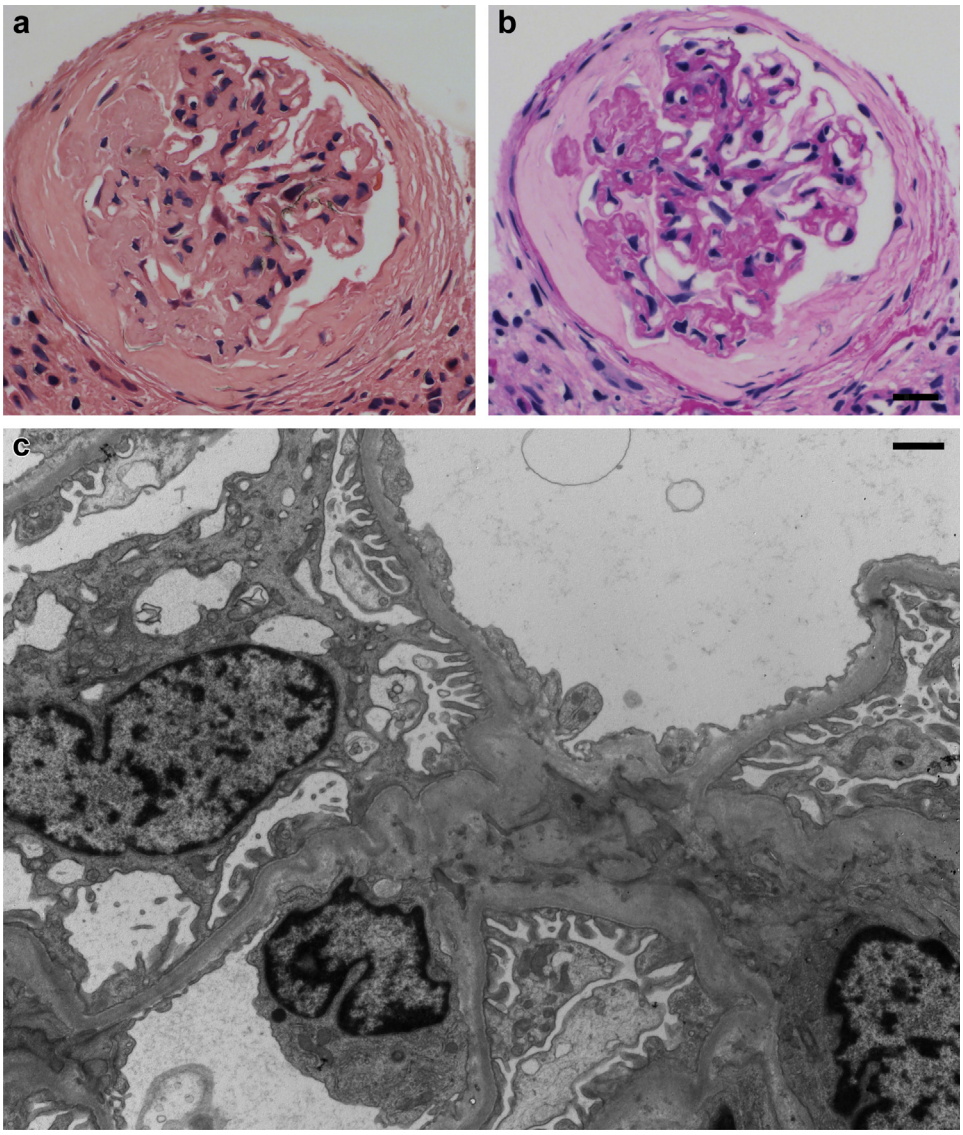

Figure 4. Family FGJF proband (FGJF32) biopsy showing focal segmental glomerulosclerosis (FSGS). Glomerulus stained with (a) periodic acid–Schiff and (b) hematoxylin and eosin showing focal segmental sclerosis. Localized podocyte foot process changes. Extensive tubular atrophy and interstitial fibrosis were present in other regions (not shown here). Bar = 30 μm. (c) Electron microscopy from FGJF32 showing segments of glomerular tufts with segmental collapse and sclerosis of capillary loops. Segmental fusion of foot processes and microvillous transformation of visceral epithelial cells. No evidence of immune complex deposition or inflammation or cell proliferation. Bar = 1 μm.

to consider genetic testing to establish a molecular diagnosis.

We note that one affected individual was suspected to have recurrent FSGS in a kidney allograft (Table 4). This label of *recurrent* disease in UMOD-associated ADTKD suggests that either the variant was not causally related to disease, or the process affecting the allograft was not *recurrence*. This highlights the occasional difficulties in making a genetic diagnosis consistent with all clinical findings.

Our study reinforces the need to carefully evaluate the clinical presentation, patient bloodwork, and kidney biopsy results before stopping with a diagnosis of *FSGS*. Should FSGS be thought of as something equivalent to interstitial fibrosis/tubular atrophy? During the secondary workup for causes of FSGS, genetic testing for mutations in *UMOD* may provide a higher yield for identifying the cause for FSGS. Patients with suspected *UMOD* and a family history with affected first-degree relatives or multiple affected relatives should have noninvasive genetic testing done before consideration of a kidney biopsy. Because kidney biopsies are seldom performed in the pediatric population and are an expensive procedure carrying risks, consideration of genetic testing in individuals and families, such as the ones analyzed in this study, may be highly informative. As genetic testing and exome sequencing are now readily available and

affordable, we anticipate that molecular testing for such cases will be more mainstreamed with foreseeable point of care testing in the near future. We propose genetic testing of *UMOD* in patients with any family history showing chronic changes on kidney biopsy even if clinical data show absence of hyperuricemia or gout. With unidentified causes of biopsy-proven FSGS and suspected secondary FSGS, we recommend taking a more thorough clinical history for gout, family history for gout or kidney disease, and checking urate levels. If there is a strong family history of FSGS of unclear etiology, one should consider genetic testing for *UMOD*.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

JC, MW, and MRP designed the research; JC and MSW performed the experiments and sequencing; MW performed computational analysis and contributed experimental data; JC, MSW, AB, and AK obtained and processed clinical and pathology data; and LH provided histopathology information and images. JC wrote the manuscript. JC, MW, and MRP edited the manuscript. All authors approved the final version of the manuscript.

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