A Case of Familial Kidney Disease

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Case Presentation
L.N. was born in 1981 in the Los Angeles area, where she continues to live. As a child, she had a history of seizures but was otherwise healthy. Proteinuria was noted at 15 yr of age. Quantification of urine protein and blood chemistries from that time are not known. She underwent kidney biopsy at age 16.

Light microscopic examination of the biopsy revealed global or segmental glomerulosclerosis involving 10 of 12 glomeruli (Figure 1). Moderate atrophy involving 60% of the tubulointerstitial compartment was also apparent, and the arteries and arterioles exhibited mild subintimal sclerosis. Immunofluorescence microscopy showed segmentally prominent IgM and C3 deposition in the glomerular tufts but no IgA or IgG. Electron microscopy showed extenssive effacement of glomerular visceral epithelial cell (podocyte) foot processes, along with podocyte degenerative changes, including microvillous degeneration and cell swelling (Figure 1). There were no significant abnormalities of the glomerular basement membranes, and electron-dense deposits were not observed in the mesangium or along the peripheral capillary loops.

L.N.’s kidney disease did not respond to glucocorticoid therapy. Kidney disease progressed to stage 5 chronic kidney disease, and hemodialysis was initiated at age 18. Bilateral nephrectomy was performed at the time of dialysis initiation because of "malignant hypertension." Histologic examination of the nephrectomy specimen revealed end-stage histopathology, including widespread tubular atrophy and interstitial fibrosis, severe vascular sclerosis, and extensive global glomerulosclerosis involving >80% of the glomeruli (Figure 2).

After 2 yr on hemodialysis treatment for ESRD, L.N. received a cadaveric renal transplant; 18 mo later, her transplant failed and dialysis was resumed. The explanted allograft revealed features of advanced chronic allograft nephropathy, including extensive tubular atrophy and interstitial fibrosis, along with focal global glomerulosclerosis and prominent mononuclear inflammatory cell infiltrates in the areas of scarring. Transplant arteriopathy was also noted (Figure 3).

L.N. has a strong family history of kidney disease (Figure 4). Her mother, K.N., had a 13-yr history of proteinuria and severe hypertension before kidney biopsy was performed at age 16.

Light microscopic examination of the kidney biopsy from K.N. revealed global or segmental glomerulosclerosis involving seven of 13 glomeruli (Figure 5). Two of the segmentally sclerosed glomeruli also showed prominent hyalinosis, foam cells, and protein reabsorption granules. There was also moderate atrophy involving approximately 50% of the tubulointerstitium, with numerous protein reabsorption granules present in viable tubular epithelium. Mild arterial and arteriolar subintimal sclerosis was also noted. Immunofluorescence microscopy revealed prominent focal segmental IgM, C3, and C1q deposition in two of four glomeruli examined, without IgA or IgG. Electron microscopy of one preserved glomerulus revealed extensive podocyte foot process effacement and microvillous change but no deposits suggestive of immune complexes. K.N. quickly progressed to end-stage kidney failure. Hemodialysis was started at 32 yr of age. Soon after dialysis initiation, she received a cadaveric kidney transplant. By her report, the allograft is functioning well.

L.N.’s younger sister (B.C.) was noted to have proteinuria at age 20 during her first pregnancy. Her urine albumin-to-creatinine ratio was quantified at 8970 mg/g. The proteinuria failed to resolve after delivery. B.C.’s kidney biopsy showed FSGS with hyalinosis at the vascular pole involving three of 10 glomeruli (Figure 6). There was also very focal and mild atrophy and fibrosis of the tubulointerstitium, with numerous protein reabsorption granules present in tubular epithelium. No significant arterial or arteriolar sclerosis was noted. Immunofluorescence microscopy revealed prominent focal segmental IgM, C3, and C1q deposition in two of five glomeruli examined, associated with glomerular scarring. There was also mild diffuse granular deposition of IgM in the mesangial areas. There was no deposition of IgA or IgG in the glomeruli. Electron microscopy of one preserved glomerulus revealed segmental areas of capillary wall collapse and basement membrane wrinkling, as well as segmentally prominent podocyte foot process effacement and microvillous change. No deposits suggestive of immune complexes were noted; however, prominent electron-dense aggregated material was seen along the cytoplasmic margins of podocyte cell bodies (Figure 6). At 23 yr of age, B.C.’s kidney disease progressed to ESRD. She received a cadaveric transplant at age 24. The transplanted kidney is functioning well.
A younger brother, F.N., had “fluctuating BP” and moderate proteinuria (393 mg albumin per gram creatinine) when he was evaluated. He has been lost to follow-up. Because of the strong family history of kidney disease, the proband and her family were referred to a genetic counselor.

Clinical Discussion: Dr. Martin Pollak
The proband and her family came to our attention several years ago because of my laboratory’s interest in the genetics of FSGS. We enrolled the proband and members of her family into our study and collected blood samples for DNA extraction. We performed mutational analysis of the α-actinin-4 gene (ACTN4). As shown in Figure 7, patient B.N. has a nucleotide substitution leading to a nonconservative amino acid substitution (originally reported by Kaplan et al. [1]). This variant was present in all of the affected members of the family as shown in the pedigree diagram, including the brother who has moderate proteinuria and was lost to follow-up (Figure 4).

This patient and her family’s presentation to our laboratory occurred at an advanced stage in the evaluation. In the initial presentation of a patient with a familial, multigenerational kidney disease, mutations in the ACTN4 gene would be a relatively unlikely underlying cause. Before obtaining histologic and radiologic information, we would entertain a number of possible genetic causes, including polycystic kidney disease, medullary cystic kidney disease, and familial IgA nephropathy.
has come as a result of genetic studies in both humans and animal models. Congenital nephrotic syndrome (CNS) falls on one extreme of the phenotypic spectrum of these diseases. Mutations in the slit-diaphragm protein nephrin, encoded by NPHS1, lead to a syndrome characterized by severe neonatal nephropathy, a condition that is typically lethal without nephrectomy and renal transplantation (7). Nephrin has structural and signaling functions (Figure 8). In Finland, where this disease is relatively common, two specific nephrin mutations cause essentially all CNS; however, a large number of independent NPHS1 mutations that lead to CNS have now been described (8). Mutations in NPHS2, which encodes the podocin protein, can lead to podocytopathies with a wide range in age of presentation (9). As with NPHS1, disease is recessive, and two NPHS2 mutations are required for disease to appear. Because both NPHS1- and NPHS2-mediated diseases are recessive, this inherited disease often presents as sporadic rather than familial disease: On average, only one in four siblings of an affected child will share the same two disease-causing alleles. Whereas NPHS1 mutations always lead to very early-onset disease, there is a great deal of variability in the age of presentation with NPHS2 mutations (10). Disease may be neonatal or may present in adulthood. One NPHS2 variant, R229Q, is present in approximately 3% of the general population and, when inherited together with a second mutant allele, can lead to adult-onset disease (11). NPHS2-mediated disease leads to nephrotic-range proteinuria and often the full nephrotic syndrome. It is resistant to therapy with glucocorticoids and typically leads to the FSGS pattern of injury and to progressive renal insufficiency. NPHS2 mutations underlie a significant fraction of pediatric steroid-resistant nephrotic syndrome (20 to 30%) but a much smaller percentage of adult-onset disease (10–12).

Podocin, like nephrin, is a podocyte-integral membrane protein (Figure 8). Podocin interacts directly with nephrin, as well as the cation channel TRPC6. Recently, the TRPC6 gene was identified as a “podocytopathy” gene (13). Mutations in TRPC6 cause an autosomal dominant disease that is characterized by presentation in adulthood, proteinuria (usually subnephrotic), and kidney failure. The TRPC6 channel interacts directly with nephrin and podocin in vitro (14). The in vivo relevance of these interactions remains to be clarified. Recent studies suggest that TRPC6, perhaps together with podocin, forms part of a complex that senses and responds to changes in mechanical forces (15,16) (Figure 8).

Mutations in ACTN4, the gene that encodes the cytoskeletal and actin-binding protein α-actinin-4, cause an autosomal dominant form of kidney disease that is characterized by subnephrotic proteinuria, focal podocyte foot process effacement, and FSGS (1,17). This form of disease is similar to that seen with TRPC6 mutations, both histologically and phenotypically. Both forms of disease are rare. Although both TRPC6 and α-actinin-4 are very widely expressed, the diseases that are associated with mutations in these proteins are kidney specific. In the glomerulus, TRPC6 localizes to the slit-diaphragm complex (14). By contrast, α-actinin-4 seems to co-localize with actin filaments in the central core of the foot processes (18). In both cases, disease seems to be biologically as well as genetically dominant and

Figure 6. (Left) Photomicrograph of periodic acid-Schiff–stained kidney biopsy from L.N.’s sister, B.C., showing segmental glomerulosclerosis. Electron micrograph showing podocyte foot process effacement and aggregated electron-dense material along the cell membrane of the podocyte cell body.

Figure 7. DNA sequence tracing. The (heterozygous) T703C mutation leads to a serine-to-proline substitution at amino acid residue 262.

In relatively small families, it may not be possible to distinguish autosomal dominant disease from recessive disease with incomplete penetrance or X-linked disorders. The pattern of multigenerational inheritance of renal dysfunction in Fabry disease, for example, can be hard to distinguish from a dominant disorder, unless the family is large enough for the absence of male-to-male transmission to be clear (2,3). Similarly, defects in the mitochondrial genome can lead to late-onset renal disease and a pattern of inheritance that can be hard to recognize (4–6).

The histologic evaluation of the proband and her mother showed FSGS. This is of course a very nonspecific lesion that can be seen as a secondary finding in a large number of primary renal insults. In this family, however, the absence of significant hematuria, absence of anatomic abnormalities, and lack of other systemic illness all suggest that this is a primary glomerular disorder. The focal podocyte foot process effacement seen on electron microscopy here suggests a primary lesion in podocyte structure or function.

ACTN4-associated FSGS is a rare form of kidney disease. I would place this disorder within the group of inherited diseases that are probably best termed “inherited podocytopathies.” I prefer this phrase to the more convenient “familial FSGS,” which describes a fairly nonspecific and biologically downstream histologic lesion.

Molecular Genetics

In the past decade, we have gained a much greater appreciation of the role of the podocyte in glomerular disease. Much of this
"gain of function." At least some of the disease-causing TRPC6 mutations increase the activity of the channel (13,14). Disease-causing α-actinin-4 mutations lead to increased actin affinity and other cellular abnormalities (1,17,19,20).

Mutations in NPHS1, NPHS2, ACTN4, and TRPC6 account for only a fraction of inherited glomerular disease. Mutations in the WT1 and LAMB2 genes cause kidney disease as components of syndromes but can also cause isolated congenital nephrotic syndrome (21,22). Together, mutations in WT1, LAMB2, NPHS1, and NPHS2 explain the majority of neonatal disease (23). The recent identification of phospholipase Cε (PLCE1) mutations in families with childhood-onset nephrotic syndrome further complicates the classification of these diseases (24). Mutations in both PLCE1 alleles cause a form of nephrotic syndrome that seems (sometimes) to respond to immunosuppressive therapy. PLCE1-associated disease seems to be due, at least in part, to a problem in glomerular development.

Most but not all inherited FSGS is steroid resistant (25). Additional loci for steroid-responsive nephrotic syndrome as well as steroid-responsive nephrotic syndrome/FSGS have been described, and in all likelihood, there are many more yet to be located (26–28) (M.R.P. et al., unpublished observations).

There is some correlation between the specific genetic lesion and the phenotype within these inherited podocytopathies (Table 1). Defects in both alleles for genes that encode critical slit-diaphragm proteins (podocin, nephrin) are earlier in onset and more often associated with the nephrotic syndrome than the dominant forms of disease caused by heterozygosity for dominant mutations in TRPC6 and ACTN4. Barisoni et al. (29) suggested a taxonomy of podocyte disorders on the basis of a combination of histopathologic appearance and etiology (including genetic alterations).

Returning to the proband and her family, what should we do in the clinic in the evaluation and treatment of a patient with FSGS or nephrotic syndrome with or without a positive family history? What is the role of genetic testing in the clinical care of patients with FSGS (or with proteinuria and renal insufficiency in the absence of a histologic diagnosis)? Most, although not all, inherited podocyte disease does not respond to immunosuppression. If, for example, we can explain a patient’s disease on the basis of two podocin mutations, then we can avoid prolonged treatments with toxic therapies that will not be effective. In the absence of a positive family history, NPHS2 mutations still must be considered. As mentioned previously, disease-causing NPHS2 mutations are much more likely to be the cause of disease in children than in adults. From a practical point of view, genetic testing should not change the initial evaluation of a child or an adult with nephrotic- or near-nephrotic-range proteinuria; however, in a child with new-onset nephrotic syndrome that does not show an early response to steroids, NPHS2 testing does seem warranted. Mutations in other podocytopathy genes are much less likely to be a cause of disease, and it is hard to make the case for routine testing of any gene other than NPHS2. Techniques that are used for making molecular diagnoses can be applied to fetal DNA samples for prenatal diagnosis. This is certainly reasonable in at-risk pregnancies in families with known NPHS2, NPHS1, WT1, and other early-onset recessive diseases (30–32).

Although NPHS2 testing is now available as a commercial genetic test in CLIA-approved laboratories, I strongly encourage the physicians of patients with possible inherited podocyte defects also to enroll their patients in one of the several ongoing research studies being conducted. Information about our Table 1. Summary of inherited podocyte disorder genes

<table>
<thead>
<tr>
<th>Histology</th>
<th>Phenotype</th>
<th>Gene</th>
<th>Gene Product</th>
<th>Inheritance</th>
<th>Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS</td>
<td>Moderate proteinuria, progressive CKD</td>
<td>ACTN4</td>
<td>α-Actinin-4</td>
<td>Autosomal dominant</td>
<td>Adult</td>
</tr>
<tr>
<td>FSGS</td>
<td>Moderate proteinuria, progressive CKD</td>
<td>TRPC6</td>
<td>TRPC6 cation channel</td>
<td>Autosomal dominant</td>
<td>Adult</td>
</tr>
<tr>
<td>Diffuse podocyte foot process effacement</td>
<td>Congenital nephrosis</td>
<td>NPHS1</td>
<td>Nephrin</td>
<td>Autosomal recessive</td>
<td>Neonatal</td>
</tr>
<tr>
<td>FSGS/MCD</td>
<td>Steroid-resistant nephrotic syndrome or subnephrotic proteinuria and progressive CKD</td>
<td>NPHS2</td>
<td>Podocin</td>
<td>Autosomal recessive</td>
<td>Highly variable: Neonatal to adult</td>
</tr>
<tr>
<td>Diffuse mesangial sclerosis/FSGS</td>
<td>Nephrotic syndrome</td>
<td>PLCE1</td>
<td>Phospholipase epsilon 1</td>
<td>Autosomal recessive</td>
<td>Childhood</td>
</tr>
</tbody>
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*Nonsyndromic inherited podocytopathies and responsible genes. Several other genes that typically cause podocyte abnormalities together with extraglomerular abnormalities when defective can also rarely be responsible for podocyte limited disease (e.g., WT1, LAMB2) (23). CKD, chronic kidney disease; MCD, minimal-change disease.
own research study is available at http://www.fsgs.bwh.harvard.edu.

A few other clinical points are worth mentioning. As with any nephrologic disease, precise phenotyping is critical. A patient with, say, FSGS secondary to renal dysplasia is quite a different disease from FSGS secondary to TRPC6 mutations. Histologic evidence of FSGS together with abnormalities in other organ systems greatly alters the previous probability of any particular gene as the culprit; therefore, we should be careful to exclude secondary FSGS and podocyte injury from, for example, Fabry disease or Nail-Patella syndrome before looking for NPHS2 mutations.

A significant percentage of patients who have FSGS histology and receive kidney transplants develop recurrent disease. This fraction is significantly lower in individuals with familial disease (33). In some cases of recurrent proteinuria in patients with podocyte gene defects, the cause seems to be an immune reaction to the previously “missing” antigen, now presented to the patient in the allograft (34,35).

It is impossible for any clinician to keep the details of all inherited kidney diseases at his or her fingertips. The list of well-defined inherited renal phenotypes and causal genes continues to grow. Physicians should be aware of Internet-based resources, such as OMIM (Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov) and GeneTests (a publicly funded resource; http://www.genetests.org), which can help to guide the evaluation of unusual and/or familial disease.

**Pathology Discussion**

**Dr. Mariam Alexander**

FSGS is a common pattern of glomerular injury, defined by segmental sclerotic lesions involving only a subpopulation of glomeruli that occurs in several settings. It is often classified as “primary FSGS” when the underlying cause is unknown. “Secondary FSGS” is most often seen as a structural and functional adaptation that is attributable to a host of causes, which include morbid obesity, unilateral renal agenesis, and scarring from previous glomerulopathies. Several morphologic variants of FSGS as observed by light microscopy have been defined for primary FSGS. These include classic FSGS (the most common form), as well as the collapsing variant, tip variant, perihilar variant, and cellular variant (36). More generalized features of chronic damage, including global sclerosis, tubular atrophy, and interstitial fibrosis, become increasingly apparent as the disease progresses. These nonspecific histologic changes are generally proportional to the degree and extent of glomerular involvement. Although the appearance of the glomerulus on light microscopy in FSGS differs among the histologic variants, they all share ultrastructural findings of podocyte alterations. These include foot process effacement, microvillous change, and cytoplasmic vacuolization.

**Dr. Joel Henderson**

As Dr. Alexander discussed, the findings at the light microscopic level in the initial biopsy (Figure 1) correspond to a commonly seen pattern of glomerular injury known as FSGS. This overused and misunderstood descriptive phrase is best thought of as describing a pattern of injury and does not suggest a specific disease mechanism. Almost any lesion of the kidney may set in motion progressive changes that, on biopsy, resemble this pattern by light microscopy. In concert with the clinical history, ultrastructural studies are necessary to determine whether this pattern of injury is directly attributable to a lesion that arises in the glomerular visceral epithelial cell or podocyte (e.g., “primary FSGS”; rarely, inherited podocytopathies) or the kidney damage is the result of an initial loss of functional renal mass from any cause, which precipitates progressive glomerular damage (i.e., “secondary FSGS”).

The key distinguishing feature between these two possibilities is the presence of the characteristic ultrastructural features of podocyte injury in otherwise preserved glomeruli, which would point to a lesion of the podocyte. In this particular case, there is clear evidence of podocyte injury in the initial biopsy, present in glomeruli that appear preserved at the light microscopic level (Figures 1 and 2). Nevertheless, even the clear presence of podocyte injury is not sufficient to ascribe a specific cause to the glomerulosclerosis and kidney damage. Genetic studies are needed to identify the small subset of cases in which a known “FSGS-causing” mutation is responsible.

Is there any morphologic finding that alone might help to distinguish between genetic and other causes of podocyte injury or that might even allow for the distinction between the various known genetic causes of podocyte injury in autosomal dominant or adult-onset disease? To date, no such morphologic features have been clearly defined. Detailed studies that might shed more light on this possibility have not yet been possible because of the extremely limited availability of biopsy material from patients with known genetic lesions. Such studies are important, because any specific morphologic features might hint at a disease mechanism for further study and ultimately point toward a target for treatment or prevention. One ultrastructural characteristic of familial forms of FSGS is that foot process effacement and other ultrastructural features of podocyte injury do not always appear diffuse. This may reflect relatively mild severity of the cellular lesion associated with these genetic alterations, as well as differences in the extent of disease “progression” from cell to cell. In the case of ACTN4-associated disease, studies in animal models and in vitro suggest that aggregation of cytoskeletal proteins, promoted by the presence of mutant α-actinin-4, may play a central role in precipitating podocyte damage (19). These aggregated proteins are easily seen at the ultrastructural level. Human biopsy material from patients with proven ACTN4 mutations is very limited, but similar cytoplasmic aggregates are seen in this and other cases (Figure 1C). Aggregated microfilaments are often seen at the basal aspect of effaced foot processes in minimal-change disease, but this aggregated material does not appear elsewhere in the podocyte, particularly in the major processes and central cell body, as it does in ACTN4-associated disease. Aggregated proteins and corresponding electron densities in the podocyte are not a characteristic feature of other kidney diseases that cause nephrotic syndrome. These findings underscore the need for the nephrologist and the renal pathologist to
be ever mindful of the value of this material to the kidney disease researcher (Figure 8).

Questions
Dr. Jeremy Duffield (Assistant Professor of Medicine, Harvard Medical School and Brigham and Women’s Hospital, Boston, MA): I wonder whether FSGS is analogous to autosomal dominant polycystic kidney disease, whereby to have disease, one needs to have a spontaneous mutation in the other allele.

Dr. Pollak: I don’t think we need to invoke this mechanism. We are convinced that the mutations that we see in ACTN4 in association with disease are dominant at a cellular level. This is different from what has been observed in autosomal dominant polycystic kidney disease and many dominantly inherited cancer syndromes in which one loss-of-function allele is inherited and a spontaneous mutation in the second allele leads to complete loss of function in a cell or cells, leading to disease. However, we have not looked specifically at this question, so I cannot rule out that it may occur.

Dr. Edgar Milford (Associate Professor of Medicine, Harvard Medical School and Brigham and Women’s Hospital): Could you discuss the evidence for allelic exclusion as it pertains to familial FSGS?

Dr. Pollak: We see evidence of RNA expression from both the mutant and wild-type α-actinin-4 alleles in leukocytes from patients who are heterozygous for ACTN4 mutations. We have not looked to see whether this is true in podocytes in ACTN4-mediated FSGS because this is difficult to do technically.

Dr. Barry Brenner (Professor of Medicine, Harvard Medical School and Brigham and Women’s Hospital): First, because you know the genotype from family members, could you screen children by harvesting podocytes from their urine? Second, in families with podocin-associated disease, were members without proteinuria screened? Is R229Q present in individuals without significant disease?

Dr. Pollak: Yes, once we know the specific mutation or mutations segregating in a family, it is easy to genotype additional family members using small DNA samples, such as from urinary podocytes or even cheek cells. This is much simpler than identifying the putative disease-causing mutations in the initial probands from a family.

Regarding podocin-associated disease, we occasionally observe only mild proteinuria or no proteinuria in family members with two mutant NPHS2 alleles, typically in children. NPHS2-associated disease is highly variable in clinical presentation—mutations can cause neonatal nephropathy as well as adult-onset FSGS (11,23). NPHS2 R229Q is present in approximately 7% of the general population in the heterozygous state, corresponding to an allele frequency of approximately 3.5% (11); however, without a second mutant allele, the presence of this R229Q variant does not cause overt disease. There does seem to be a slightly increased risk for microalbuminuria in individuals who are heterozygous for this R229Q variant (37).

Dr. Theodore Steinman (Professor of Medicine, Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA). The index case presented with seizures. Was this an incidental finding?

Dr. Pollak: No other family members experienced seizures. I do not believe that this is directly related to the kidney disease. We have not seen seizure disorders with any significant frequency in the ACTN4-mutant families whom we have studied.

Acknowledgments
Members of family described in this article are enrolled in a study approved by the institutional review board at Brigham and Women’s Hospital and funded by the National Institutes of Health (DK54931). The original molecular genetic analysis of the family discussed here appeared in reference (1).

Disclosures
None.

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