Clinical and Pathological Heterogeneity in FSGS due to \textit{INF2} Mutations

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\section*{INTRODUCTION}

Mutations in inverted-formin-2 (\textit{INF2}) are a frequent cause of inherited focal and segmental glomerulosclerosis (FSGS), accounting for approximately 9\% to 17\% of familial cases.\textsuperscript{1,3} More than 50 mutations in the \textit{INF2} gene have been described with R218Q being the most common. This missense mutation leads to progressive kidney disease mediated by podocyte injury. \textit{INF2} contains a C terminal diaphanous autoregulatory domain and an N terminal diaphanous inhibitory domain necessary for interaction with regulatory binding proteins to modulate actin assembly. All pathogenic \textit{INF2} mutations identified to date are localized in the diaphanous inhibitory domain and the presence of an R218Q mutation (c.653G\textgt;A, pArg218Gln) in the cleavage-dependent region of diaphanous inhibitory domain leads to the loss of the autoinhibitory activity. This results in disruption of \textit{INF2} with dynein light chain 1 and prevents appropriate nephrin localization to the slit diaphragm increasing nephrin degradation.\textsuperscript{4,5} \textit{INF2} mutations also play a major causal role in Charcot-Marie-Tooth neuropathy among patients with FSGS. Among patients with the hereditary form of Charcot-Marie-Tooth neuropathy due to \textit{INF2} mutations, FSGS is found in nearly all cases. However, in patients with FSGS and \textit{INF2} mutations, Charcot-Marie-Tooth is less commonly found.\textsuperscript{11–12}

\textit{INF2}-related kidney disease has been described mostly among children and adolescents, and usually presents with proteinuria. However, the disease can range from early onset with relatively rapid progression to kidney failure to a more insidious course and onset at older age.\textsuperscript{53} Here, we report the case of a 25-year-old female who presented with nephrotic range proteinuria and no known family history of kidney disease. Her renal biopsy findings revealed C3 deposition, thin glomerular basement membranes, and features of collapsing glomerulopathy. Serologic and biochemical evaluation failed to identify an etiology. Ultimately, she was found to have an R218Q mutation in \textit{INF2}. Herein, we describe her clinical presentation and renal pathology, and review the reported phenotypes and renal biopsy findings of patients with \textit{INF2} mutations.

\section*{CASE PRESENTATION}

A 25-year-old female presented to our nephrology clinic for a second opinion in June 2017. In January 2016 she was noted to have positive urine dipstick and a urine protein-to-creatinine ratio of 5.7 g/g. Her serum creatinine was 1.1 mg/dl with an estimated glomerular filtration rate of 60 ml/min per 1.73 m\textsuperscript{2}.

Past medical history was significant for cochlear atresia requiring cochlear implant in childhood. Tourrette’s syndrome was diagnosed at the age of 4 years, after presenting with involuntary motor movement. Her neurologic examination revealed no signs of peripheral neuropathy or pes cavus. At the age of 24 years she was diagnosed with hypertension and started on hydrochlorothiazide. Other medications included levonorgestrel-ethynyl estradiol, citalopram, and occasional use of ibuprofen. She did not use tobacco, alcohol, or recreational drugs. Family history was remarkable for 2 cerebrovascular ischemic events and a patent foramen ovale in her mother, venous thromboembolism in 2 of her mother’s siblings, and hypertension and coronary artery disease in her father.
reported no family history of kidney disease or relatives with proteinuria or hematuria.

**RESULTS**

A kidney biopsy was performed in April 2016 (Figure 1) and revealed focal cortical scars with isolated collapsing glomerular lesions, focal global sclerosis in 18%, and segmental sclerosis in 2.7% of glomeruli. The immunofluorescence microscopy revealed moderate C3 deposition in the mesangium. The electron microscopy showed preserved interdigitating foot processes of the glomerular visceral epithelial cells in most capillaries and several ill-defined intramembranous and mesangial electron-dense deposits. Morphometric analysis revealed thin glomerular basement membranes with harmonic mean thickness of 220 nm. The estimated interstitial fibrosis and tubular atrophy in the sample was 20%.

The results of laboratory values and evaluation for secondary causes of FSGS are shown in Table 1. A second renal biopsy (not shown) was performed by her local providers in February 2017. Her total
**Table 1.** Basic metabolic parameters during time of follow-up and additional testing  

| Chemistry, Blood | Initial evaluation | 2017 | 2019 | 2021 |
|------------------|--------------------|------|------|------|
| Sodium (mEq/l)   | 141                | 143  | 143  | 144  |
| Potassium (mEq/l)| 3.6                | 4.2  | 4.4  | 4.1  |
| HCO₃⁻ (mEq/l)    | 26                 | 23   | 26   | 22   |
| BUN (mg/dl)      | 13                 | 14   | 15   | 10   |
| Creatinine (mg/dl)| 1.1              | 1.1  | 1.5  | 1.3  |
| eGFR (mL/min/1.73 m²) | 60               | 60   | 47   | 54   |
| Calcium (mg/dl)  | 9.2                | 9.2  | 8.9  | 9.2  |
| Phosphorus (mg/dl)| 3.6               | 3.6  | 3.6  | 3.3  |
| Albumin (g/dl)   | 4.2                | 4.2  | 4.2  | 4.2  |
| LDL (mg/dl)      | 96                 | 68   |      |      |
| Triglycerides (mg/dl)| 423            |      |      |      |
| Protein to creatinine ratio, Urine (g/g) | 5.7 | 2.9 | 1.7 | 1.7 |

Viral serologies Results  
Hepatitis B antigen Negative  
Hepatitis B core ab Negative  
Hep C ab Negative  

Immunologic Results  
Serum electrophoresis No monoclonal band seen  
IgG (mg/dl) 373  
IgA (mg/dl) 99  
IgM (mg/dl) 19  
Urine electrophoresis No monoclonal band seen  
Complement 3 (mg/dl) 138  
Complement 4 (mg/dl) 30  
ADAMST13 activity (%) 101  
ANA Negative  
ANCA Negative  
Rheumatoid factor Negative  
Anti-Smith Negative  
Scleroderma antibody Negative  
Anti-RNP Negative  
RNA polymerase III ab Negative  
Methyl(malonlic acid (nmol/l)) 149  

Hypercoagulability work up Results  
Prothrombin mutation Negative  
Factor V Leiden Mutation Negative  
Cardiolipin antibodies (IgG, IgM) Negative  
Beta-2-glycoprotein IgG Negative  

ANA, antinuclear antibodies; ANCA, antineutrophil cytoplasmatic antibodies; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration; HCO₃⁻, bicarbonate; LDL, low density lipoprotein; RNP, ribonucleoprotein.

protein-to-creatinine ratio was 3.8 g/g and her serum creatinine was 1.2 mg/dl. The biopsy revealed similar findings of collapsing lesions, C3 glomerular deposition, and thin glomerular basement membrane. The degree of global glomerulosclerosis had progressed to 43% and the tubular atrophy and interstitial fibrosis to 20%.

Genetic testing was performed using the Natera kidney gene panel (Supplementary Methods). A heterozygous autosomal dominant missense mutation in INF2 (c.548G>A, pArg183His) were identified. Additional variants of uncertain significance included a heterozygous autosomal recessive synonymous mutation in gene coding PLG. Pathogenic variants of COL4A3-A5 were not detected.

The same INF2 missense mutation (c.653G>A, pArg218Gln) was identified in her father (aged 61 years). The missense mutation in BCSIL and synonymous mutation in PLG were also identified. Further clinical assessment revealed low grade proteinuria (albumin-to-creatinine ratio ~200–250 mg/g), a serum creatinine of 1.1 mg/dl and estimated glomerular filtration rate of 71 ml/min per 1.73 m². He is currently treated with lisinopril 10 mg daily for hypertension and was recently found to have diabetes.

**DISCUSSION**

FSGS can result from direct podocyte injury (autoimmune or genetic), during repair of inflammatory injury (IgA nephropathy, lupus nephritis, or antineutrophil cytoplasmatic antibodies-associated vasculitis), or from secondary structural and functional adaptations (nephrectomy, obesity, reflux nephropathy, sickle cell disease, infections, or drugs).

Numerous proteins encoding genes within the podocyte have been identified to cause FSGS (Supplementary Table S1 and Figure S1). Mutations in COL4A genes are also found in patients presenting with FSGS and thin basement membrane abnormalities. The association between several missense mutations in INF2 and FSGS among 2 large families with clinical manifestations of proteinuria of unknown cause (>250 mg albumin per gram of creatinine), end stage kidney disease (ESKD) of unknown cause, or biopsy-proven FSGS was initially reported in 2010. One family (FG-JN) had the R218Q mutation in 10 members with a diagnosis between ages 22 and 45 years. Two additional family members were subsequently found to have the same mutation and ESKD followed by kidney transplantation developed in 3 out of 12 family members. Two additional families with the R218Q mutation were identified over the following 2 years by the same group (Table 2). Family FG-LW (n = 3) presented with proteinuria in their 20s and they all developed ESKD within 2 years.

Several recent reports have identified INF2 mutations in familial and sporadic cases of FSGS (Table 2). In a screening study of 109 patients with FSGS or minimal change disease and 6 patients with ESKD of unknown cause, the R218Q mutation was identified in 2 brothers with ESKD and subsequently identified in their father who developed ESKD at the age of 57 years.

There is significant heterogeneity in the clinical course of patients with R218Q INF2 mutations.
Reported phenotypes (Table 2) suggest that ESKD develops in the third decade and occurred in 14 of 27 patients. Additional environmental and genetic factors likely affect the R218Q mutation phenotype. Our patient’s father has only mild kidney disease at the age of 61 years, consistent with numerous members of family FG-JN (Table 2).

The kidney biopsies in our case also revealed diverse and somewhat unexpected pathologic findings, including abnormalities of the complement system and basement membranes. To determine whether these findings were unique to our case or more generally found in patients with INF2 mutations, we reviewed the pathology reports of all available cases and directly examined the tissue of a member from the FG-JN family. Similar to our case, C3 mesangial deposition was identified in this individual and reported in a member of the family FG-LW. In addition, the pathology reports of 5 out of 10 individuals with other INF2 mutations (S186P and E220K) described C3 deposition and focal thinning of the glomerular basement membrane. In the literature, C3 mesangial deposition and focal thinning of the glomerular basement membrane are a major cause of autosomal dominant focal segmental glomerulosclerosis. These findings along with our case suggest that mutations in INF2 may contribute to the development of thin basement membranes, complement deposition, and collapsing and sclerosing features in the glomeruli.

This report provides a detailed description of the clinical course and renal pathological findings in a patient with R218Q INF2 mutation and summarizes similar findings in other patients with INF2-related FSGS. Future studies will be needed to provide additional insights into factors affecting disease expression and allow for the development of individualized therapies (Table 3).

DISCLOSURE

MRP is listed as an inventor of issued patent US9499967B2 related to INF2 mutational analysis. All the other authors have nothing to disclose.

PATIENT CONSENT

The patient and her family provided consent to publish this case study.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary References.

Supplementary Methods.

Figure S1. Graphical representation of identified mutations associated with FSGS.

Table S1. Identified mutations associated with FSGS.

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Table 2. Clinical phenotype of the patients with R218Q INF2 mutation

| Family (N) | Race | Ethnicity | Age of diagnosis (Yr) | Biopsy findings | ESKD (N) | Age at ESKD (Yr) | Transplantation (N) | Current age of patients with no ESKD (Yr) |
|-----------|------|-----------|-----------------------|-----------------|----------|-----------------|-------------------|-----------------------------------------|
| Brown et al. | White | Non-Hispanic | 20–42 | FSGS, C3 deposition | 3 | 28–32 | 3 | Not reported | 31–66 |
| FG-JN (N = 12) | White | Non-Hispanic | 24–26 | FSGS, C3 deposition | 3 | 24–27 | 3 | Not reported | NA |
| FG-LW (N = 3) | White | Asian | No reported | FSGS | 1 | 26 | 1 | Not reported | NA |
| Boyer, et al. | White | European | 10–24 | FSGS | 1 | 30 | Not reported | Not reported | Not reported |
| J (N = 1) | White | European | 15 | FSGS | NA | NA | NA | No ESKD at age 28 |
| Gbadegesin et al. | White | Canadian | 16–48 | FSGS | Not reported | Not reported | Not reported | Not reported |
| Safarikova, et al. | White | European | Not reported | FSGS | 3 | Not reported | Not reported | Not reported |
| No 133 (N = 4) | White | European | Not reported | FSGS | 3 | 27–57 | Not reported | Not reported |

ESKD, end stage kidney disease; FSGS, focal and segmental glomerulosclerosis; NA, not applicable.

*Five out of 10 individuals with INF2 mutations (S186P and E220K) exhibit C3 mesangial deposition.

*One patient with INF2 R177H mutation exhibit C3 mesangial deposition.

*As of April 2022.

Table 3. Teaching points

1. FSGS is a morphologic pattern associated with podocyte injury and clinically manifests with proteinuria.

2. INF2 mutations are seen in 9%-17% of cases of FSGS.

3. Patients with R218Q INF2 mutations have a variable phenotype ranging from early onset of kidney failure (mid 20s) to only minor kidney disease at older ages (mid 40-60s).

4. Patients with INF2 mutations and FSGS may also have pathologic findings that include C3 deposition, thin basement membranes, and collapsing glomerulopathy.

FSGS, focal and segmental glomerulosclerosis.
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