Familial Focal Segmental Glomerulosclerosis Associated With an *ACTN4* Mutation and Paternal Germline Mosaicism

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Mutations in the *ACTN4* gene cause focal segmental glomerulosclerosis (FSGS), which shows autosomal dominant inheritance (Online Mendelian Inheritance in Man No. 603278, FSGS1). Most patients with a diagnosis of FSGS1 show a mild to moderate degree of proteinuria during adolescence or later, and some patients gradually progress to end-stage renal disease. Here, we report a familial case of FSGS1 in which 2 affected siblings showed unusual clinical, pathological, and genetic features. Both patients presented with full-blown rapidly progressing nephrotic syndrome in early childhood. Renal pathological findings were of an FSGS collapsing variant and FSGS not otherwise specified. A novel *ACTN4* mutation, p.Ser262Phe, was detected in the patients, and their father was found to have a germline mosaicism for the mutation. In addition, these siblings also had a heterozygous p.Thr5Met substitution in *NPHS1*, which encodes nephrin, although the functional significance of this substitution is unclear. This is the third clinical report of FSGS1 and the first case report of germline mosaicism confirmed in patients with hereditary podocyte disorders. FSGS1 may have widely variable clinical and pathological phenotypes and therefore should be considered in young children with full-blown and rapidly progressing nephrotic syndrome. The possibility of germline mosaicism makes interpretation of molecular diagnoses and genetic counseling more difficult.
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INDEX WORDS: Focal segmental glomerulosclerosis; α-actinin 4; *ACTN4* gene; germline mosaicism; collapsing glomerulopathy; nephrin; *NPHS1* gene.

The *ACTN4* gene encodes α-actinin-4, an actin-filament cross-linking protein. Mutations of this gene can cause focal segmental glomerulosclerosis (FSGS) that shows autosomal dominant inheritance (FSGS1; Online Mendelian Inheritance in Man No. 603278). To date, 5 familial or sporadic cases of FSGS1 were reported.[1,2] Most affected individuals showed nephrotic- or non–nephrotic-range proteinuria at the onset of adolescence or adulthood that slowly progressed to end-stage renal disease (ESRD).[1,2]

The collapsing variant of FSGS, or collapsing glomerulopathy (CG), a distinct morphological variant of FSGS, is pathologically characterized by segmental or global collapse of the glomerular basement membrane with hypertrophy and hyperplasia of podocytes, and it is clinically associated with rapid deterioration in renal function.[3] CG may be idiopathic or may occur secondary to a variety of conditions.[4] However, none of the patients with *ACTN4* mutations who underwent renal biopsies were found to have CG.[1,2]

This report describes 2 siblings with an *ACTN4* mutation who presented with full-blown nephrotic syndrome in early childhood, 1 of whom rapidly developed ESRD. Renal biopsies showed CG in 1 patient and FSGS not otherwise specified in the other. Their father, who was clinically silent, had germline mosaicism of the mutation.

CASE REPORTS

A 3-year-old boy was referred to our hospital because of new-onset nephrotic syndrome. Serum creatinine level was 0.5 mg/dL (44 μmol/L; creatinine clearance, 72 mL/min/1.73 m^2 [1.20 mL/s/1.73 m^2]), and albumin level was 1.6 g/dL (16 g/L). Urinalysis showed heavy proteinuria (protein, 9.6 g/d) without hematuria. His disease did not respond to initial steroid treatment. A kidney biopsy was performed, and the specimen contained 126 glomeruli, 20 of which showed typical collapsing lesions (Fig 1A). Segmental and

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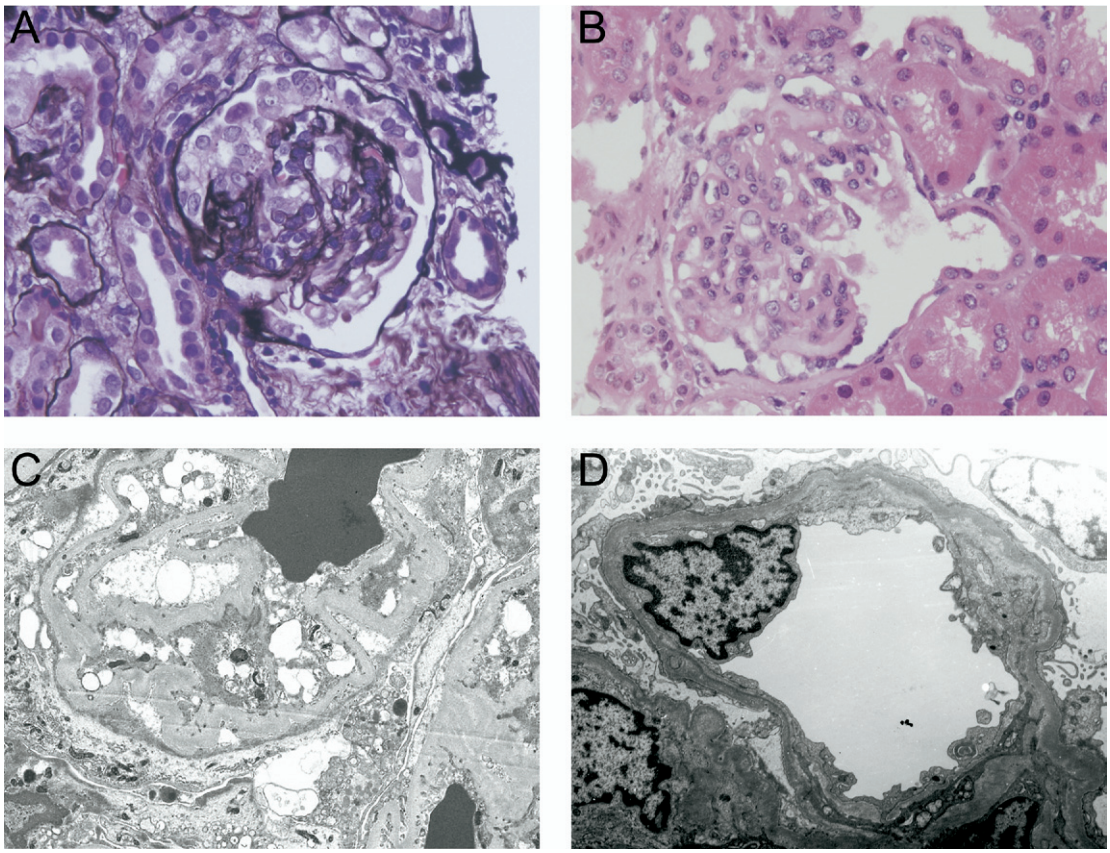

Figure 1. Renal pathological findings of (A and C) the patient and (B and D) his elder sister. (A) A glomerulus with global glomerular capillary collapse with podocyte hypertrophy and hyperplasia. (Periodic acid–methenamine silver stain; original magnification ×400.) (B) A glomerulus with classic segmental sclerosis without glomerular capillary collapse. (Hematoxylin and eosin stain; original magnification ×400.) (C, D) Glomerular basement membranes appear to be well preserved, and podocyte foot processes are fully effaced in both panels. (Original magnification: [C] ×2,500, [D] ×4,000). (E-G) Immunohistochemical study of α-actinin-4 expression in glomeruli of the patient. α-Actinin-4 expression was (E) restricted in podocytes in glomeruli with minor abnormalities, (F) focally decreased in glomeruli with segmental sclerosis, and (G) markedly decreased in hyperplastic epithelial cells. The primary antibody was 1:250-diluted rabbit polyclonal anti-actinin-4 antibody,[5] a gift from Dr K. Honda (National Cancer Institute, Tokyo, Japan), and antigen-antibody binding was visualized by using a Histofine kit including antigoat antirabbit immunoglobulin G (Nichirei, Tokyo, Japan) and liquid DAB+ Substrate Chromogen system (Dako, Denmark, Glostrup, Denmark).

global sclerosis were noted in 18 and 14 glomeruli, respectively. Electron microscopy showed a well-preserved glomerular basement membrane and fully effaced podocyte foot processes (Fig 1C). Immunohistochemical study, performed after the genetic diagnosis, showed that α-actinin-4 expression was restricted in podocytes in glomeruli with minor abnormalities (Fig 1E), focally decreased in glomeruli with segmental sclerosis (Fig 1F), and markedly decreased in hyperplastic epithelial cells (Fig 1G). After 11 months, serum creatinine level increased to 1.9 mg/dL (71 μmol/L; creatinine clearance, 26 mL/min/1.73 m^2 [0.43 mL/s/1.73 m^2]).

The patient's elder sister incidentally was found to have proteinuria at the age of 3.7 years and developed full-blown nephrotic syndrome at age 4. A biopsy performed at another hospital showed segmental sclerosis in 3 of 10 glomeruli without collapsing lesions (Fig 1B). Podocyte foot processes were fully effaced (Fig 1D). We did not have renal biopsy tissue from this patient and therefore were able to interpret only the available histological slides (light microscopy and electron microscopy) provided by another hospital. Her disease did not respond to treatment (steroid, cyclosporine, cyclophosphamide, and mycophenolate) and rapidly progressed to ESRD. She received a kidney transplant from her mother at age 5.7 years, but died on postoperative day 9 of sepsis. Urinalyses of their parents and an elder brother showed no abnormality.

As part of a routine genetic study for steroid-resistant FSGS in our hospital, mutational analyses of *NPHS2* (whole exons), *ACTN4* (exon 8), and *WT1* (exons 8 and 9) were performed in this family. A heterozygous sequence alteration of c.785C→T (p.Ser262Phe) in *ACTN4* was detected in both affected siblings (Fig 2A). This alteration results in loss of a *Drd*I restriction enzyme site, and *Drd*I restriction fragment