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Clinical, Pathological, and Genetic Characteristics in Patients with Focal Segmental Glomerulosclerosis

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Key Points

- We investigated the association between focal segmental glomerulosclerosis histologic variants (Columbia classification) and monogenic variant detection rates.
- The perihilar variants had the strongest association with detection of monogenic variants.
- The tip variants had the weakest association with detection of monogenic variants.

Abstract

Background Approximately 30% of children with steroid-resistant nephrotic syndrome (SRNS) have causative monogenic variants. SRNS represents glomerular disease resulting from various etiologies, which lead to similar patterns of glomerular damage. Patients with SRNS mainly exhibit focal segmental glomerulosclerosis (FSGS). There is limited information regarding associations between histologic variants of FSGS (diagnosed using on the Columbia classification) and monogenic variant detection rates or clinical characteristics. Here, we report FSGS characteristics in a large population of affected patients.

Methods This retrospective study included 119 patients with FSGS, diagnosed using the Columbia classification; all had been referred to our hospital for genetic testing from 2016 to 2021. We conducted comprehensive gene screening of all patients using a targeted next-generation sequencing panel that included 62 podocyte-related genes. Data regarding patients' clinical characteristics and pathologic findings were obtained from referring clinicians. We analyzed the associations of histologic variants with clinical characteristics, kidney survival, and gene variant detection rates.

Results The distribution of histologic variants according to the Columbia classification was 45% (n=53) FSGS not otherwise specified, 21% (n=25) cellular, 15% (n=18) perihilar, 13% (n=16) collapsing, and 6% (n=7) tip. The median age at end stage kidney disease onset was 37 years; there were no differences in onset age among variants. We detected monogenic disease-causing variants involving 12 of the screened podocyte-related genes in 34% (40 of 119) of patients. The most common genes were *WT1* (23%), *INF2* (20%), *TRPC6* (20%), and *ACTN4* (10%). The perihilar and tip variants had the strongest and weakest associations with detection of monogenic variants (83% and 0%, respectively; P<0.001).

Conclusions We revealed the distributions of histologic variants of genetic FSGS and nongenetic FSGS in a large patient population. Detailed data concerning gene variants and pathologic findings are important for understanding the etiology of FSGS.

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Introduction

Nephrotic syndrome is characterized by heavy proteinuria, hypoalbuminemia, edema, and hyperlipidemia. It is the most common glomerular disease of childhood, with an estimated incidence of approximately 2–6.5 per 100,000 children (1,2). Approximately 80% of patients with pediatric nephrotic syndrome respond to steroid therapy, whereas the remaining 20% are resistant to steroid therapy. Most patients with steroid-resistant nephrotic syndrome (SRNS) exhibit focal segmental glomerulosclerosis (FSGS)—a common glomerular lesion in adults and children. Patients with FSGS exhibit rapid progression to ESKD (3,4).

SRNS is a clinically and genetically heterogeneous disorder. Thus far, more than 60 podocyte-related gene variants have been identified in patients with monogenic forms of SRNS/FSGS (5). Recent large cohort studies indicate that almost 30% of patients with childhood-onset SRNS have monogenic causative variants (6,7).

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FSGS is characterized by the presence of sclerosis in portions of at least one glomerulus in an individual kidney biopsy specimen. The etiology of FSGS is regarded as primary, secondary, genetic, or unknown. FSGS is classified using the Columbia classification system, which defines five morphologic variants of FSGS lesions on the basis of a light microscopy examination (8). However, classification of FSGS histologic variants on the basis of light microscopy findings alone cannot reliably differentiate among primary, secondary, and genetic FSGS (9). The results of some studies have suggested that histologic classification findings can be used to predict patient outcomes (10). To our knowledge, there have been no reports regarding associations of FSGS histologic variants with monogenic variant detection rates or other clinical characteristics on the basis of gene variant information. Here, we report the characteristics of FSGS in a large number of affected patients, along with their genetic backgrounds.

Materials and Methods

Study Participants

Individuals with FSGS provided informed consent for analysis of their clinical data and blood samples in this retrospective study. The study protocol was approved by the Institutional Review Board of Kobe University Graduate School of Medicine (IRB approval number: 301). Patients were eligible for inclusion if they were diagnosed with primary FSGS, confirmed *via* renal biopsy by the local hospital pathologist, between January 2016 and June 2021. Secondary FSGS caused by infections or drug-induced and other conditions causing known secondary FSGS were excluded. Clinical eligibility criteria included the presence of proteinuria. Participants were excluded if their biopsies indicated the presence of another primary disease (*e.g.*, Alport syndrome or IgA nephropathy). Using targeted next-generation sequencing (NGS), 62 podocyte-related genes were screened in 119 patients with FSGS; clinical characteristics of these patients were retrospectively reviewed.

Details regarding family history of disease and other clinical features were obtained from the referring clinician or the patient's hospital records. The eGFR was calculated for patients aged >18 years using the creatinine-based eGFR formula for Japanese adults (11) and for patients aged 3 months to 18 years using the creatinine-based eGFR formula for Japanese children (12–14). For patients aged <3 months, the eGFR was calculated using the original Schwartz Equation (15) as follows: $k\times$body length (cm)/serum Cr level (mg/dl). For this study, the k value was set to 0.45. Levels of serum albumin and urinary protein and the eGFR were assessed at the same time as genetic testing was performed.

Variant Classification of FSGS

For all patients, FSGS was classified using the Columbia classification proposed by D'Agati *et al.* (8) in 2004: FSGS not otherwise specified (NOS), tip, perihilar, cellular, and collapsing variants. Renal biopsy classification for most patients was performed by a local pathologist. Renal biopsy classification for other patients was performed by a pathologist at another institution.

A recent study examined the abilities of renal pathologists to classify FSGS using the Columbia classification proposed by D'Agati *et al*. (8). The authors of the study concluded that the morphologic forms of FSGS defined by the Columbia system can be consistently identified by multiple observers, indicating a low probability of confusion between forms (16). Accordingly, we used the local hospital data as recorded when a single type of FSGS was present in a single specimen. However, difficulties may arise in the interpretation of lesions that exhibit features of more than one Columbia type of FSGS in a single tissue specimen. Six biopsy findings showed one or more variant. In such instances, the renal biopsy findings of patients with unclear classification results were reviewed by an additional pathologist before data analysis. These histologic evaluations were blinded to clinical and follow-up data.

Glomerular Size

Glomerular size was measured only in patients with a perihilar variant. Five control samples were collected from pediatric patients with minimal changes disease. The glomerular diameter was calculated as the mean value of the maximal diameter and the maximal chord perpendicular to it for each glomerulus. In a previous study, this method highly correlated with the glomerular area (r=0.98) (17). One to three glomeruli were randomly measured per biopsy, and the average of these measurements was used.

Genetic Analysis

Genomic DNA was extracted from peripheral blood using the Quick Gene Mini 80 system (Wako Pure Chemical Industries, Ltd., Tokyo, Japan). The extracted genomic DNA was used for targeted sequencing and Sanger sequencing. Targeted sequencing using NGS was conducted in the analysis of genes responsible for inherited renal disease. The genes included in the NGS panel are listed in Supplemental Table 1. The sample library for NGS analysis was prepared using SureSelect XT2 custom capture library 0.5–2.9 Mb (Agilent Technologies, Santa Clara, CA) in accordance with the manufacturer's instructions. Briefly, 150 ng of genomic DNA was used for a restriction reaction and then hybridized using SureSelect XT Low Input reagents. All indexed DNA samples were amplified by PCR and sequenced using the MiSeq platform (Illumina, San Diego, CA). The results were analyzed using SureCall v3.0 software (Agilent Technologies).

Confirmation of the Pathogenicity

Candidate variants were considered disease-causing variants when they met at least one of the following criteria: previous identification as a disease-causing variant in a published paper, predicted truncation (*i.e.*, nonsense, obligatory splice site, or frameshift variants), and/or, for all novel missense variants, the results of in silico testing with MutationTaster (http://mutationtaster.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/), or SIFT (http://sift.jcvi.org/) indicated pathogenicity. In addition to these three criteria, we confirmed the absence of contradictions between familial segregation and patient symptoms.

Statistical Analyses

The results are shown as medians and interquartile ranges (IQR). As applicable, the independent-samples chi-squared test or Fisher's exact test was used to compare categorical variables. The Mann–Whitney *U* test was used to compare median differences between two groups. The Kruskal–Wallis test was used to compare median differences among three or more groups. Multivariate logistic regression analysis was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) after controlling for potential confounders. Kaplan–Meier curves were generated for time to reach ESKD. We compared curves among groups using the log-rank test. Statistical analysis was performed using R v4.0.3 (2021-05-18; The R Foundation for Statistical Computing, Vienna, Austria). For all statistical analyses, $P<0.05$ was considered statistically significant.

Compliance with Ethical Standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Kobe University Graduate School of Medicine (IRB approval number: 301) and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Results

Background Characteristics

In total, 119 unrelated patients (76 men/boys and 43 women/girls) were included (Table 1). Their median age at disease onset was 7 years (range 1 day to 38 years); their median age at biopsy was 10 years (range 5 months to 50 years). At the time of genetic diagnosis, the median eGFR was 102.8 ml/min per 1.73 m^2 (IQR 71.7 ml/min per 1.73 m^2). The median urine protein level was 3.1 g/g Cr (IQR 6.2 g/g Cr), and the median serum albumin level was 3.1 g/dl (IQR 1.4 g/dl). Of the 119 patients with FSGS, 53 (45%) were diagnosed with the NOS variant, 25 (21%) with the cellular variant, 18 (15%) with the perihilar variant, 16 (13%) with the collapsing variant, and seven (6%) with the tip variant. Eleven patients had a family history of proteinuria, whereas 16 patients had a family history of renal failure. Nine patients had experienced complete remission. Almost half (56/111 patients) showed edema. For edema, the total number was different due to missing data. There were no significant differences in sex, age, family history of proteinuria or renal failure, remission rate, or clinical characteristics at the time of genetic testing among patients with any of the five FSGS variants. Features of edema were significantly different among patients according to the FSGS variant type ($P<0.001$).

Variant Characteristics According to Histologic Subtype

We detected disease-causing gene variants in 40 of the 119 patients (34%; Table 2). Notably, there were no familial relationships among patients in this study. In the nine patients who harbored autosomal recessive disease-causing variants, three homozygous and six compound heterozygous variants were found. The most common variants were detected in *WT1*, *INF2*, *TRPC6*, *ACTN4*, and *ADCK4* (nine, eight, eight, four, and three patients, respectively).

We detected a disease-causing gene variant in 83% of patients with the perihilar variant, 30% of patients with the NOS variant, 24% of patients with the cellular variant, 19% of patients with the collapsing variant, and 0% of patients with the tip variant (Figure 1). There were significant differences in disease-causing gene variants among the five FSGS variants ($P<0.001$). Disease-causing gene variants in patients who had the perihilar variant of FSGS were as follows: 40% were variants in *WT1*, 40% were variants in *TRPC6*, 13% were variants in *ACTN4*, and 7% were

Table 1. Clinical characteristics according to FSGS histologic subtype

Characteristic	Total	Not Otherwise Specified	Cellular	Perihilar	Collapsing	Tip	*P* Value
Total number of cases, *n* (%)	119 (100)	53 (45)	25 (21)	18 (15)	16 (13)	7 (6)	—
Age at onset, yr	7 (9)	7 (8)	8 (9)	8 (7.5)	5.5 (7.5)	8 (10)	0.69
Age group at onset, yr, *n* (%)							
<1	4 (34)	3 (6)	0 (0)	1 (6)	0 (0)	0 (0)	—
1–5	36 (30)	13 (25)	9 (36)	4 (22)	8 (50)	2 (29)	—
6–12	56 (47)	28 (53)	11 (44)	10 (56)	5 (31)	2 (29)	—
13–17	14 (12)	7 (13)	2 (8)	2 (11)	1 (6)	2 (29)	—
≥18	9 (8)	2 (4)	3 (12)	1 (6)	2 (13)	1 (14)	—
Age at biopsy, yr	10 (9)	10.5 (9)	10 (9)	11 (8.3)	5.5 (8.3)	13 (9.5)	0.22
Men, *n* (%)	76 (64)	33 (62)	15 (60)	9 (50)	14 (88)	5 (71)	0.20
Edema, *n*/*N* (%)	56/111 (50)	24/51 (47)	17/23 (74)	1/17 (6)	10/15 (67)	4/5 (80)	<0.001
Extra renal symptom, *n* (%)	12 (10)	5 (9)	2 (8)	3 (17)	1 (6)	1 (14)	0.78
Family history, *n* (%)	25 (21)	11 (21)	6 (24)	6 (33)	2 (13)	0 (0)	0.42
Remission, *n* (%)	9 (8)	4 (8)	4 (16)	1 (6)	0 (0)	0 (0)	0.47
Age at gene test, yr	12 (13)	13 (14)	9 (10)	12 (8.8)	7 (9.5)	13 (10.5)	0.19
Serum albumin, g/dl	3.1 (1.4)	3.3 (1.2)	2.7 (1.6)	3 (1.1)	2.4 (1.4)	3 (1.1)	0.30
Urine protein/creatinine ratio, g/gCr	3.1 (6.2)	2.2 (3.8)	4.0 (10)	3.5 (5.5)	4.1 (29.5)	5.1 (3.5)	0.10
eGFR, ml/min per 1.73 m^2	102.8 (71.7)	102 (69.9)	97.1 (77.9)	84.8 (58.2)	91.5 (43.3)	132 (69.9)	0.49

FSGS, focal segmental glomerulosclerosis.

Table 2. Variant characteristics according to FSGS histologic subtype

Characteristic	Total	Not Otherwise Specified	Cellular	Perihilar	Collapsing	Tip	*P* Value
Variant identified rate	40/119 (34%)	16/53 (30%)	6/25 (24%)	15/18 (83%)	3/16 (19%)	0/7 (0%)	<0.001
Variants (*n* cases)	*WT1* (9), *INF2* (8), *TRPC6* (8), *ACTN4* (4), *ADCK4* (3), *NUP107* (2), *COL4A5* (1), *LAMA5* (1), *LAMB2* (1), *PAX2* (1), *SMARCAL1* (1), *TTC21B* (1)	*INF2* (5), *WT1* (3), *ADCK4* (2), *ACTN4* (2), *COL4A5* (1), *LAMB2* (1), *NUP107* (1), *TTC21B* (1)	*INF2* (2), *ADCK4* (1), *LAMA5* (1), *SMARCAL1* (1), *TRPC6* (1)	*WT1* (6), *TRPC6* (6), *ACTN4* (2), *PAX2* (1)	*INF2* (1), *NUP107* (1), *TRPC6* (1)		

FSGS, focal segmental glomerulosclerosis.

variants in *PAX2*. All variants manifested autosomal dominant inheritance.

When patients were stratified according to causative variant detection status, patients with variants in the analyzed genes had a higher frequency of family history of proteinuria or renal failure ($P=0.02$) than did patients without variants in the analyzed genes (Table 3). Patients with variants had a lower frequency of edema ($P<0.001$) than did patients without variants. At the time of genetic testing, patients with variants had a high serum albumin level ($P=0.03$) and low eGFR ($P=0.01$) compared with patients who lacked variants.

To determine the relationships of genetic variants with patient characteristics, five parameters (age, sex, family history of proteinuria or renal failure, edema, and Columbia classification) were entered in multivariate logistic regression analysis. Patients with variants had a significantly higher frequency of family history of proteinuria or renal failure than did patients without variants (OR=1.23; 95% CI, 1.02 to 1.48; $P=0.03$). Patients with variants were less likely to show edema (OR=0.73; 95% CI, 0.62 to 0.86; $P<0.001$) than were patients without variants. Compared with patients who exhibited the tip variant of FSGS, the risk of genetic disease was greater in patients who had the cellular, collapsing, and NOS variants of FSGS (the risk was similar among these three groups of patients). The risk was highest in patients who had the perihilar variant of FSGS (OR=1.64; 95% CI, 1.07 to 2.5; $P=0.02$; Table 4). When patients stratified according to nephrotic range hypoalbuminemia status (where nephrotic range hypoalbuminemia was defined as serum albumin level <2.5 mg/dl), a causative gene variant was detected in only six patients with low albumin. There were two patients each with the *TRPC6* and *ACTN4* variants, and one patient each with the *SMARCAL1* and *WT1* variants (Supplemental Table 2). All variant data are shown in Supplemental Table 3.

Renal Prognosis According to Histologic Subtype

Fifteen patients had progressed to stage 5 CKD and begun RRT by the time of genetic testing. According to FSGS variant type, six patients had the NOS variant, four had the cellular variant, two each had the perihilar and collapsing variants, and one had the tip variant.

The eGFR rate was >90 ml/min per 1.73 m^2 in 64 of 102 (63%) evaluable patients, 60–89 ml/min per 1.73 m^2 in 17 (17%) patients, 30–59 ml/min per 1.73 m^2 in 16 (16%) patients, and <30 ml/min per 1.73 m^2 in five (5%) patients (Supplemental Table 4). The median age at ESKD onset was 37 years (Figure 2A). Kaplan–Meier analysis of renal survival revealed no significant differences among the patients with any of the five FSGS variants in terms of the median age at progression to ESKD (Figure 2B). This age also did not significantly differ between groups in which the genetic variant was detected (33 years, $n=40$) and not detected (37 years, $n=79$; $P=0.3$, log-rank test; Figure 2C). There was no significant difference in the median age at progression to ESKD when patients were stratified according to nephrotic range (NA, $n=36$) and non-nephrotic range serum albumin levels (38 years, $n=73$; $P=0.9$, log-rank test; Figure 2D).

Glomerular Size in Patients with Perihilar Variant

Eight patients (five men, three women) with perihilar variants samples were examined. Median age at biopsy was 8.5 years (range 6–18 years). The diameter range was 115.8–191.7 μm, with a median value of 150.0 μm. When the glomerular diameter in patients with the perihilar variant was compared with that of controls, there was no significant difference in the glomerular size (Supplemental Table 5).

Discussion

To our knowledge, this is the first study to show an association between the presence of genetic FSGS and specific variants according to the Columbia classification. The results will be very insightful for clinicians and pathologists.

The Columbia classification was published in 2004 to organize better the multiple histologic variants of FSGS (8). In a previous study performed in Japan (18), disease was classified in 201 patients with a diagnosis of FSGS; pathologic variants comprised NOS in 60%, perihilar in 15%,

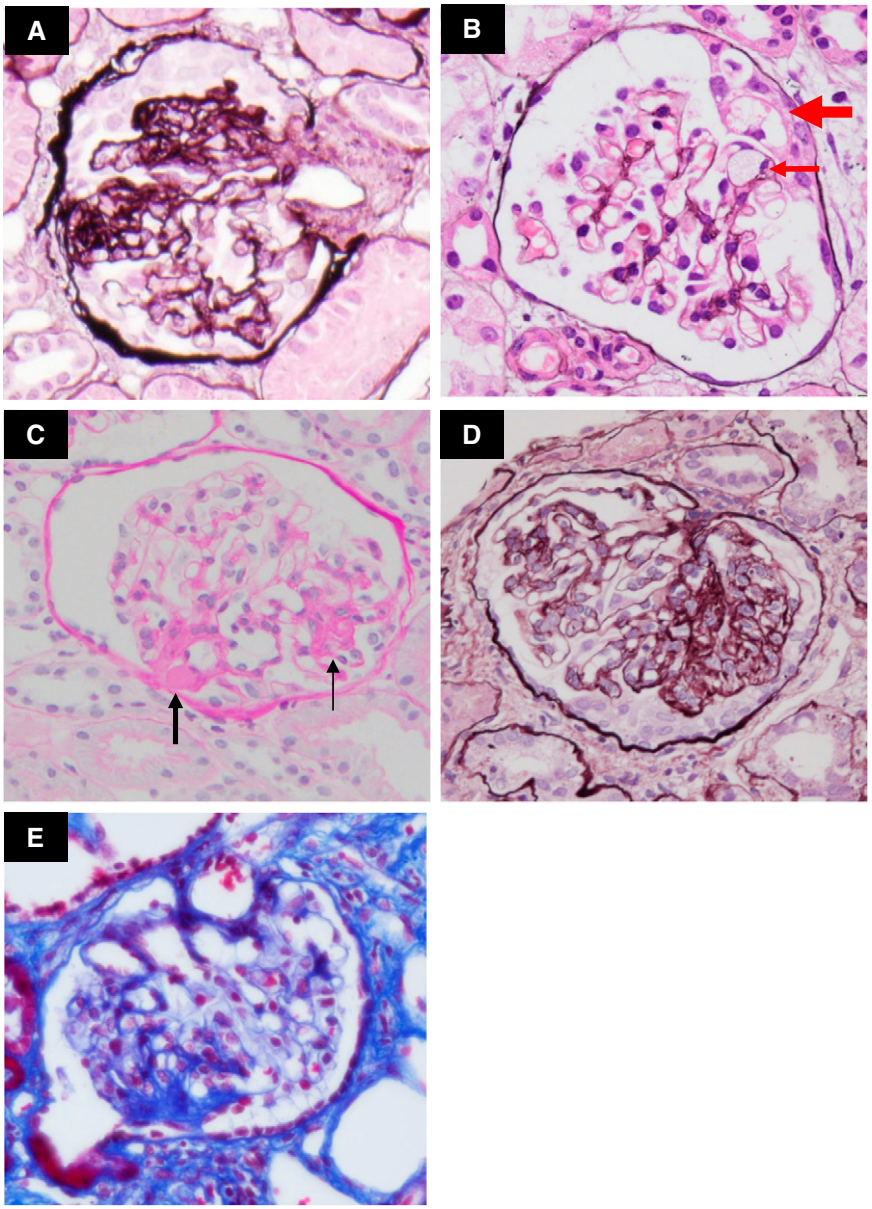

Figure 1. | **Major histologic variants.** (A) Variants not otherwise specified (NOS) showed an increase in the mesangial matrix and segmental obliteration of the capillary lumina (periodic acid silver-methenamine, ×400). Patient with *INF2* variant (no. 10). (B) The cellular variant displays segmental endocapillary hypercellularity occluding lumina, with foam cells (thin red arrow) and podocyte hypertrophy (thick red arrow; periodic acid silver-methenamine, ×400; ID 63). (C) The perihilar variant shows segmental sclerosis (thin arrow) and perihilar hyalinosis (thick arrow) at the glomerular hilus (periodic acid–Schiff, ×200). Patient with *TRPC6* variant (no. 28). (D) The collapsing variant showed segmental collapse and overlying podocyte hypertrophy and hyperplasia (periodic acid silver-methenamine, ×400). Patient with *TRPC6* variant (no. 35). (E) The tip variant exhibits sclerotic lesion at the tip of the glomerulus near the beginning of the proximal tubule (Masson's trichrome, ×400; ID 407).

cellular in 10%, tip in 9%, and collapsing in 7%. In most studies, including ours, the NOS variant was the most frequent among patients with variants. The second most frequent variant was the cellular variant in the present study, which was variable in previous studies (0%–29%) (10,19–22). This proportional bias may be associated with differences in the inclusion criteria from each study and distribution of the children.

Thus far, monogenic variants have been detected in approximately 30% of patients with childhood-onset SRNS according to the results of large-cohort studies performed using NGS (6,7). Howie reviewed 252 articles regarding genetic causes of FSGS. Various clinical features were reported with minimal detail, although >80% of the studies did not use the Columbia classification (23). There have been few reports concerning the relationship between genetic FSGS and the Columbia classification. These are generally limited to a few small case studies. Several kidney specimens from patients with *ACTN4* mutations exhibited perihilar segmental sclerosing lesions (24). Perihilar lesions,

Table 3. Comparison of clinical and histologic phenotypes between patients according to variant detection status

Characteristic	Total	Patients with Variants	Patients without Variants	*P* Value
Total number of cases, *n* (%)	119	40 (34)	79 (66)	
Age at onset, yr	7 (9)	7.5 (8.3)	7 (9)	0.99
Age group at onset, yr, *n* (%)				
<1	4 (34)	4 (10)	0 (0)	
1–5	36 (30)	9 (23)	27 (34)	
6–12	56 (47)	19 (48)	37 (47)	
13–17	14 (12)	5 (13)	9 (11)	
≥18	9 (8)	3 (8)	6 (8)	
Age at biopsy, yr	10 (9)	10 (9.3)	9.5 (8.8)	0.27
Columbia classification, *n* (%)				
NOS	53	16 (40)	37 (47)	<0.001
Cellular	25	6 (15)	19 (24)	
Perihilar	18	15 (38)	3 (4)	
Collapsing	16	3 (8)	13 (16)	
Tip	7	0 (0)	7 (9)	
Men, *n* (%)	76 (64)	23 (58)	53 (67)	0.32
Edema, *n*/*N* (%)	56/111 (50)	6/37 (16)	50/74 (68)	<0.001
Extra renal symptom, *n* (%)	12 (10)	5 (13)	7 (9)	0.53
Family history, *n* (%)	25 (21)	14 (35)	11 (14)	0.02
Remission, *n* (%)	9 (8)	1 (3)	8 (10)	0.27
Age at gene test, yr	12 (13)	12 (11.5)	12 (12.5)	0.40
Serum albumin, g/dl	3.1 (1.4)	3.4 (1.4)	3 (1.4)	0.03
Urine protein/creatinine ratio, g/gCr	3.1 (6.2)	2.5 (4.45)	3.48 (6.23)	0.27
eGFR, ml/min per 1.73 m^2	102.8 (71.7)	84.8 (59.9)	108 (72.4)	0.01

characterized by hyalinosis and sclerosis involving the vascular pole, have been reported in primary forms of FSGS and are not etiologically specific; however, such lesions are common in patients with maladaptive FSGS. Maladaptive FSGS results from a mismatch between the glomerular load and glomerular capacity (25). Under these conditions (*e.g.*, prematurity, obesity), glomerular hypertrophy is often observed. In the present study, we did not detect any glomerular hypertrophy in eight patients with perihilar variants. Because the glomerular size increases with normal body growth, it is necessary to determine the existence of glomerular hypertrophy while taking into consideration the patient's age and body surface. More samples are needed to evaluate whether perihilar variants are caused by genetic variants that exhibit glomerular hypertrophy. Kidney biopsy revealed FSGS in six patients with *COQ6* variants, including a NOS variant in three patients and a collapsing variant in three patients (26). One patient with a *COQ2* variant showed collapsing glomerulopathy. The findings were consistent with the results in a murine model of collapsing glomerulopathy where the animals spontaneously develop proteinuria and renal disease associated with the presence of numerous dysmorphic mitochondria in podocytes (27). In the present study, the perihilar and tip variants had the strongest and weakest associations with detection of monogenic variants (83% and 0%, respectively; $P<0.001$). These relationships were detected among 119 patients with FSGS—the largest such patient population reported thus far. These results should be carefully considered in clinical practice. For patients with the perihilar variant of FSGS, genetic testing should be conducted as soon as possible to avoid ineffective treatment.

Table 4. Multivariate logistic regression analysis of risk factors in patients with variants

Risk Factor	Patients with Variants versus Patients without Variants Odds Ratio	95% Confidence Interval	*P* Value
Age	0.99	0.98 to 1.01	0.34
Men	0.99	0.84 to 1.15	0.86
Family history	1.23	1.02 to 1.48	0.03
Edema	0.73	0.62 to 0.86	<0.001
Columbia classification			
Cellular	1.12	0.76 to 1.66	0.57
Collapsing	1.03	0.68 to 1.54	0.90
Perihilar	1.64	1.07 to 2.5	0.02
NOS	1.15	0.79 to 1.67	0.48

The most common disease-causing gene in this study was *WT1*. Most detected genes (78%; including *WT1*) were autosomal dominant disease-causing genes. Because autosomal dominant diseases tend to progress slowly, we speculate that they exhibit FSGS histologic findings during podocyte injury repair. The Wilms' tumor 1 (*WT1*) gene encodes a transcription factor critical for kidney and gonadal embryogenesis. Variants in the *WT1* exon 8 or 9 DNA-binding site are associated with isolated diffuse mesangial sclerosis and early progression to ESKD known as Denys–Drash syndrome. However, variants involving other sites in *WT1* result in a mild phenotype (including FSGS) (28). Indeed, in this study, all *WT1* variants were outside the DNA-binding site and led to slower progression compared with previous findings in patients with Denys–Drash syndrome (29).

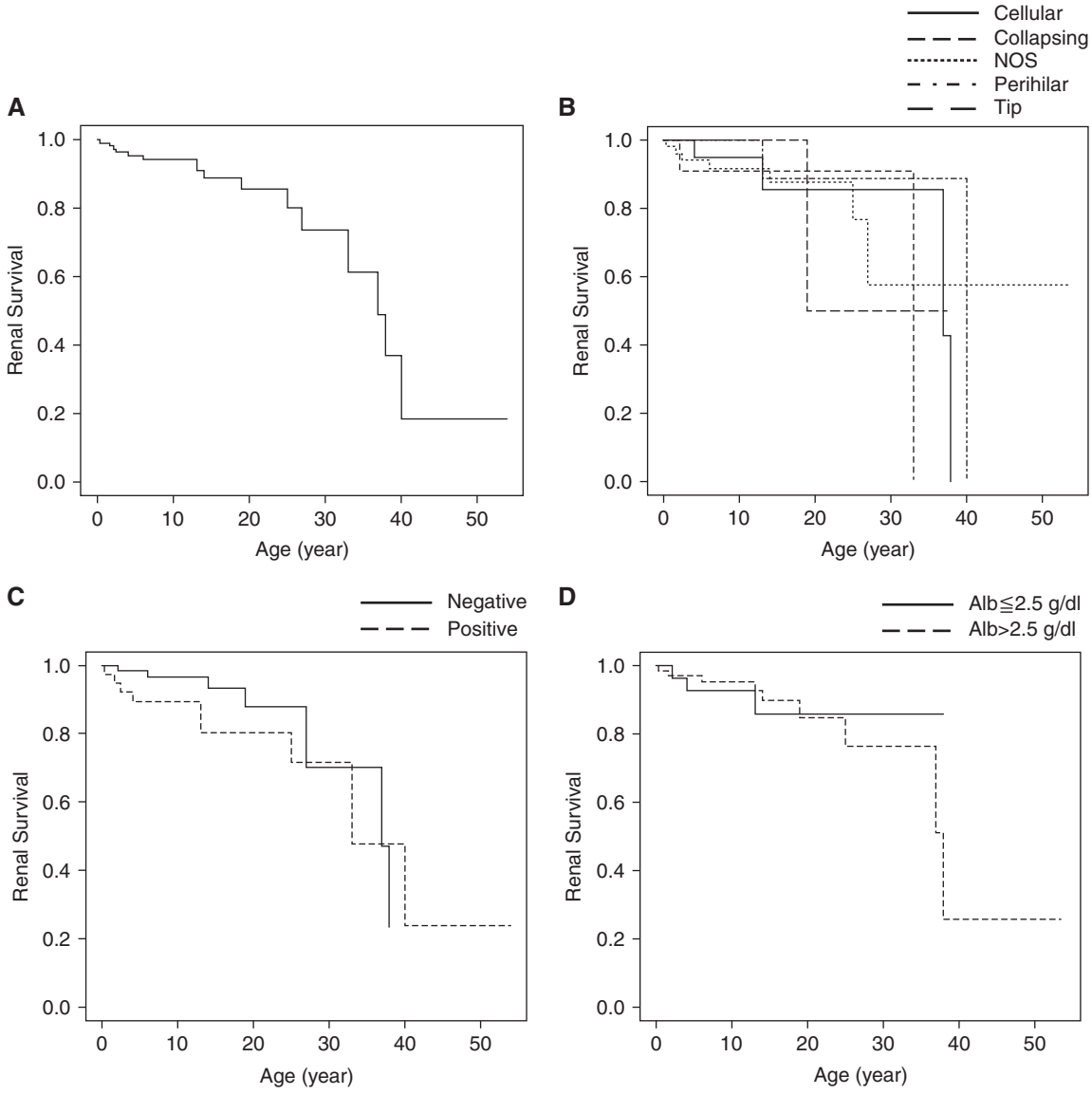

Figure 2. | **Kaplan–Meier survival analysis of the progression to ESKD.** (A) Median age at ESKD onset among all patients: 37 years. (B) Kaplan–Meier survival analysis of the progression to ESKD among patients with the five focal segmental glomerulosclerosis variants (P=0.9). Solid line indicates patients with the cellular variant (n=25; median age at ESKD onset: 37 years). Short dashed line indicates patients with the collapsing variant (n=16; median age at ESKD onset: 33 years). Dotted line indicates patients with the NOS variant (n=53; median age at ESKD onset: 40 years). Dot-dash line indicates patients with the perihilar variant (n=18; median age at ESKD onset: 40 years). Long dashed line indicates patients with the tip variant (n=7; median age at ESKD onset: 19 years). (C) Kaplan–Meier survival analysis of the progression to ESKD in patients with and without genetic variants (P=0.30). Solid line indicates patients without genetic variants (n=79; median age at ESKD onset: 37 years). Dashed line indicates patients with genetic variants (n=40; median age at ESKD onset: 33 years). (D) Kaplan–Meier survival analysis of the progression to ESKD in patients with and without nephrotic range serum albumin levels (P=0.90). Solid line indicates patients with low albumin levels (n=36; median age at ESKD onset: 38 years). Dashed line indicates patients with non-nephrotic albumin levels (n=74; median age at ESKD onset: 38 years).

In previous studies, univariate analyses indicated that the renal prognosis was good in patients with the tip variant of FSGS (10,20–22) and poor in patients with the collapsing variant (10,20,21,30). In contrast, we found no differences in renal prognosis among patients according to FSGS variant type. No genetic variant was associated with the tip variant of FSGS. We speculate that patients with the tip variant of FSGS have a good prognosis because this variant might result from immunologic mechanisms other than genetic causes, implying that immunosuppressants can provide effective treatment. In patients with the tip variant of FSGS, treatment should be focused on the use of immunosuppressants.

In previous reports of patients with SRNS, the likelihood of identifying a genetic variant was increased in patients with family history of nephrotic syndrome and in patients with extrarenal manifestations (31). In our study of 119 patients compared according to gene variant status, risk

factors for genetic disease were family history of proteinuria or renal failure, absence of edema, and the perihilar variant of FSGS. In our previous reports of Japanese patients with severe proteinuria, we found that patients with variants were approximately six-fold more likely to show an absence of edema (7). Thus, genetic testing might be considered in patients without edema. Compared with the tip variant of FSGS, the perihilar variant was strongly associated with genetic variant detection. In patients who are pathologically diagnosed with the perihilar variant of FSGS, genetic testing should be considered. Clinically, it is important to patients and clinicians whether the patient has genetic variants. The benefits of a definitive molecular diagnosis include avoiding unnecessary treatment, predicting prognosis, detecting potentially treatable variants, and providing precise genetic counseling. The Columbia classification can be useful for further diagnosis in patients with FSGS.

This study had several limitations. First, it was a cross-sectional retrospective study with a small study population, which limits the generalizability of the findings. Second, the examinations of pathologic findings largely relied on the expertise of attending clinicians and pathologists. Third, treatment details were not available for some patients, which limited our ability to make inferences regarding their disease characteristics. Finally, our study included only the Japanese population, and we did not consider *APOL1*-related FSGS. Validation studies in other cohorts are required.

In conclusion, we found that the FSGS genetic variant was associated with the Columbia classification among patients with proteinuria. These pathologic findings will aid in the diagnosis and determination of underlying disease etiology in patients with FSGS.

Disclosures

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Author Contributions

Y. Aoto, T. Horinouchi, S. Ishiko, K. Matsuoka, H. Nagase, R. Rossanti, N. Sakakibara, and T. Yamamura were responsible for data curation; Y. Araki, S. Fujinaga, Y. Gotoh, R. Hamada, Y. Hamasaki, S. Ito, K. Kamei, T. Okamoto, A. Takeda, and S. Tanaka were responsible for resources; S. Hara, H. Kaito, Y. Ogawa, H. Oguchi, K. Sakuraya, M. Yamamoto, and N. Yoshikawa were responsible for the investigation; K. Iijima was responsible for supervision; C. Nagano wrote the original draft of the manuscript; and K. Nozu reviewed and edited the manuscript.

Supplemental Material

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Supplemental Table 1. Genes targeted by next-generation sequencing analysis.

Supplemental Table 2. Comparison of genes between patients with low albumin levels and patients with normal albumin levels.

Supplemental Table 3. Variant genotypes of 40 patients with focal segmental glomerulosclerosis in whom disease-causing variants were identified.

Supplemental Table 4. Renal function stage according to focal segmental glomerulosclerosis histological subtype.

Supplemental Table 5. Comparison of glomerular diameter between patients with the perihilar variant and controls.

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