

Deciphering the Genetic Basis of Focal Segmental Glomerulosclerosis Using Next-Generation Sequencing

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Abstract

Introduction:

Focal Segmental Glomerulosclerosis (FSGS) is a heterogeneous glomerular disorder characterized by segmental sclerosis of one or more glomeruli and prominent proteinuria. Podocyte injury underlies the pathogenesis and compromises the renal filtration barrier. FSGS can be classified into primary, genetic, and secondary forms, with secondary causes including maladaptive, infection-related, and drug-induced mechanisms. These subtypes differ in etiology, clinical course, treatment response, and prognosis.

Methods

Whole exome sequencing was conducted on patients with biopsy-proven FSGS to identify the underlying molecular etiopathogenesis utilizing the Illumina NextSeq2000 system. Bioinformatic analyses were performed on the SOPHIA DDM platform. Sanger sequencing was conducted to validate the detected variant in the proband and to conduct segregation analysis in her family members.

Results

Among the 50 FSGS patients, no clinically relevant variants were identified by NGS in 25 individuals. Known pathogenic variants in *SDCCAG8*, *NPHS2*, and *COQ8B* were confirmed by Sanger sequencing. Three novel variants in *MAPKBP1*, *ARHGAP24*, and *PODXL* were identified and validated through familial segregation analysis. Variants in seven probands were confirmed by Sanger sequencing, while 12 novel VUS variants failed to segregate with disease in family studies.

Conclusion

This study highlights the value of genetic analysis in understanding its molecular basis. Integration of WES with detailed clinical evaluation allowed the identification of multiple variants, improving insight into FSGS pathogenesis. Limitations include the lack of functional validation for novel variants and the inability to perform segregation analyses in some families.

Introduction

Focal Segmental Glomerulosclerosis (FSGS) represents a histopathological lesion defined by sclerosis affecting a portion of at least one glomerulus, the essential filtration structure of the kidney (1). These clinicopathological syndromes are defined by podocyte injury and prominent proteinuria. Podocytes, which are terminally differentiated visceral epithelial cells of the glomerulus, are critical for sustaining the integrity of the renal filtration barrier (2). Although FSGS was previously regarded as a distinct disease, growing evidence indicates that it represents a heterogeneous disorder with variable etiologies, clinical courses, and therapeutic responses. Based on etiology, FSGS is commonly divided into primary, genetic, and secondary subtypes. The secondary group includes maladaptive, infection-related, and medication-induced forms. Despite some overlapping clinical and histological findings, these subtypes vary significantly in their pathogenesis, management approaches, and prognostic outcomes (3).

During the past 30 years, the incidence and prevalence of FSGS-associated kidney failure have risen considerably. The distribution of the disease shows significant geographic and ethnic variability, with a higher susceptibility reported among Black populations compared with White or Asian groups (4). The incidence of FSGS is estimated to range between 1.4 and 21 cases per million population (5). Among adults presenting with nephrotic syndrome, approximately 40% are attributed to FSGS, and nearly 50% of these patients develop end-stage renal disease (ESRD) within 3–8 years after diagnosis (6).

A subset of FSGS cases is caused by rare pathogenic variants in several genes with high penetrance. These genetic forms may exhibit either autosomal recessive or autosomal dominant modes of inheritance (7). Recessive mutations are generally associated with early-onset disease, while dominant forms tend to present during adolescence or adulthood. Many of the implicated genes encode podocyte proteins involved in maintaining slit diaphragm integrity, regulating actin cytoskeleton dynamics, supporting nuclear function, mediating cellular metabolic processes, and facilitating adhesion to the glomerular basement membrane (8).

Despite extensive research, the underlying mechanisms of FSGS are not yet fully elucidated due to its heterogeneous nature and varied clinicopathological characteristics (6). Growing evidence from genomic and molecular studies suggests that genetic dysregulation plays a critical role in the onset and progression of the disease (5). In addition, limited early diagnostic tools often delay the recognition of FSGS in affected patients. A deeper understanding of the molecular basis of this disorder could contribute to the development of more accurate diagnostic methods and targeted therapeutic strategies (9).

Whole-exome sequencing (WES) has emerged as a powerful tool for investigating the genetic basis of complex renal disorders such as FSGS (10). Although WES has markedly improved the detection of genetic variants in FSGS, it remains insufficient to fully capture the disease's complex genetic architecture, and existing studies remain relatively limited in number and scope, underscoring the need for further comprehensive, integrative investigations. Consistent with this, a recent cohort study reported a detection rate of 11.3% in biopsy-proven FSGS patients, underscoring the clinical relevance of genetic testing in guiding patient management and avoiding unnecessary immunosuppressive therapy (11). Beyond classical podocyte-associated genes, emerging evidence indicates that novel genes involved in RNA processing (e.g., XPO5) and cytoskeletal regulation (e.g., ANLN) may contribute to FSGS pathogenesis, suggesting that the disease extends beyond a purely structural podocyte disorder (12, 13). In this context, the present study aimed to comprehensively investigate the genetic architecture of biopsy-proven FSGS using whole-exome sequencing, with a particular focus on identifying both known and novel variants and assessing their clinical significance through validation and segregation analyses.

Methods

Study population and Patients Selection

Our study consisted of 50 patients who were admitted to Istanbul Faculty of Medicine, Istanbul University between 2024 and 2026. All patients underwent a kidney biopsy and were diagnosed with FSGS based on histopathological evaluation. The patients' age was ≥ 18 years in the study, without restriction by sex. Individuals with secondary causes of FSGS were excluded, including patients with obstructive uropathy, congenital anomalies of the kidney and urinary tract, HIV infection, parvovirus B19 infection, or those receiving medications known to be associated with FSGS, such as pamidronate and etc. The study protocol was approved by the Ethics Committee of Istanbul Faculty of Medicine (approval number: 2553224; May 9, 2024). Written informed consent was obtained from all participants prior to their inclusion in the study. Physical examination findings, clinical history, and laboratory data of the patients were systematically documented using standardized assessment forms. Furthermore, a detailed pedigree including at least three generations was established based on family history. The laboratory characteristics of the all patients are shown in Table 1.

Table 1
The laboratory characteristics of the all patients

Patients No	Total Protein/Creatin (Spot urination) (mg/g) < 200	Creatin (Serum)(mg/dL) 0.7–1.4	Albumin (Serum)(g/dL) 3.2–5.5	Total Protein (Serum)(g/dL) 6–8	Protein (Complete Urine) (mg/dL)
1	10655.74	1.71	6.3	3.89	3+
2	1264.24	1.99	3.93	8.9	1+
3	4339.53	7.01	3.59	6.1	2+
4	22065.28	1.57	2.6	5	3+
5	4230.26	0.51	3.23	6.1	2+
6	1810.47	2.85	4.12	6.7	2+
7	2640.85	1.18	3.93	7.5	3+
8	4748.60	0.8	3.53	6.1	3+
9	877.68	0.79	6.9	4.34	1+
10	2905.98	4.69	7.3	4.34	1+
11	7241.72	6.91	3.63	5.93	3+
12	210.24	1.23	4.54	6.8	Negative
13	6443.80	1.2	3.67	6.4	3+
14	8854.77	2.77	3.67	5.58	3+
15	6503.4	0.56	4.28	7.58	3+
16	3531.87	6.71	3.66	6.5	3+
17	-	-	-	-	-
18	426.75	1.91	4	6.9	1+
19	4204.63	2.76	4.03	6.91	3+
20	1271.17	1.77	4.65	7.05	2+
21	3000.97	0.63	3.95	5.87	2+
22	5892.41	0.65	2.98	5.5	3+
23	-	10.45	4.11	6.9	Negative
24	13186.41	5.11	3.48	5.5	3+
25	1798.67	4.43	4.67	7.6	Trace
26	96.55	0.68	4.95	7.08	Negative
27	2183.66	7.42	3.89	6.4	2+
28	71.24	0.52	4.54	6.57	Negative
29	1990.2	0.59	4	6.5	2+
30	-	-	-	-	-

Patients No	Total Protein/Creatin (Spot urination) (mg/g) < 200	Creatin (Serum)(mg/dL) 0.7–1.4	Albumin (Serum)(g/dL) 3.2–5.5	Total Protein (Serum)(g/dL) 6–8	Protein (Complete Urine) (mg/dL)
31	3369.53	0.74	4.74	7.46	3+
32	1588.46	2.67	44	70.9	Negative
33	-	-	-	-	-
34	-	0.79	4.88	6.77	Negative
35	2715.78	2.81	4.13	7	3+
36	5296.85	0.99	2.93	5.72	3+
37	597.66	0.93	4.88	7.77	3+
38	-	-	-	-	-
39	1336.08	7.67	4.56	7	3+
40	17331.39	4.89	1.94	4	3+
41	-	9.20	36.35	69.93	2+
42	893.87	0.5	4.48	7.57	3+
43	400	2.26	4.77	7.4	Negative
44	-	-	-	-	-
45	1533.41	2.33	4.48	7.19	3+
46	1199.32	1.25	4.8	8.19	2+
47	114.4	1.39	4.34	6.49	Negative
48	182.12	1.39	4.94	7.63	Negative
49	15482.76	1.56	3.92	6.45	3+
50	-	0.66	32.91	-	3+

Sample preparations

Genomic DNA was isolated from peripheral blood samples using the PureLink® Genomic DNA Mini Kit in accordance with the manufacturer's protocol. The quality and concentration of the extracted DNA were evaluated using the Qubit dsDNA Broad Range Quantitation Assay Kit on a Qubit Fluorometer.

Whole-Exome Sequencing

Wet-laboratory procedures for next-generation sequencing (NGS) were carried out using the Twist Human Core Exome Kit. This kit allows the capture and sequencing of approximately 43 Mb of the human genome, including protein-coding regions and clinically significant intergenic regions. Sequencing was performed on the Illumina NovaSeq 6000 platform following the manufacturer's recommended protocols.

Data Analysis

Sequence alignment and variant analysis were performed using the SOPHiA DDM platform. Raw sequencing reads were aligned to the current human reference genome (GRCh38). Following alignment, quality control and filtering procedures were applied according to the platform's standard parameters. Variant visualization and inspection of coverage and sequencing quality were conducted using the Integrated Genomics Viewer (IGV). A minimum coverage threshold of 100× was applied for variant evaluation.

The potential pathogenicity of identified variants was assessed using several in silico prediction tools, including MutationTaster, PolyPhen-2, and SIFT, together with Combined Annotation-Dependent Depletion (CADD) scores(14–16). Population allele frequencies were obtained from the Genome Aggregation Database (gnomAD) (<https://gnomad.broadinstitute.org>)(17). Clinical relevance and previously reported associations of the detected variants were evaluated through ClinVar and an extensive review of the literature(18). Variant classification was ultimately performed in accordance with the guidelines of the American College of Medical Genetics and Genomics (ACMG).

Sanger Sequencing

Sanger sequencing was performed to validate variants identified by next-generation sequencing that were suspected to be disease-related, including pathogenic, likely pathogenic, and variants of uncertain significance (VUS). In addition, segregation analysis was conducted in available family members. Raw sequencing data were analyzed using ABI Sequencing Analysis v5.4 software, and sequence interpretation was carried out using SeqScape v3.0 software. Chromatogram data obtained from Sanger sequencing were analyzed using Chromas software, and the electropherogram peak profiles were visually inspected to confirm the identified variants.

Results

Among the 50 patients included in our study, in 25 individuals no clinically significant variants that are related with FSGS were identified by NGS. known Pathogenic variants were detected in three patients in *SDCCAG8* (c.784G > T), *NPHS2* (c.538G > A) and *COQ8B* (c.1199 dupA) genes, and these findings were confirmed by Sanger sequencing in the affected individuals. In three additional patients, novel variants were identified in the *MAPKBP1*, *ARHGAP24* and *PODXL* genes, these variants subsequently confirmed through segregation analysis within their family members. In seven patients, the detected variants were validated by Sanger sequencing in the probands; however, segregation analysis could not be performed due to the unavailability of family members. Additionally, variants detected in 12 patients, including potential novel candidate genes and variants, variants of uncertain significance (VUS), underwent family segregation analysis; however, the segregation findings did not support these variants as pathogenic/ likely pathogenic contributors to FSGS. The list of all identified gene variants showed in Table 2.

Table 2
List of Identified Gene variants

Patients NO	Gene	Location	Mutation Type	HGVSc	HGVSp	Validation in proband	Family Segregation
1	COL4A5	chrX Exon 15	Missense (VUS)	c.844G > C	p.(Gly282Arg)	Yes	Yes
2	SDCCAG8	chr1 Exon 8	Nonsense (Pathogenic)	c.784G > T	p.(Glu262*)	Yes	No
5	ROBO2	chr3 Exon 25	Missense (VUS)	c.3971G > A	p.(Gly1324Glu)	Yes	Yes
5	STOX1	chr10 Exon 3	Missense (VUS)	c.2357T > C	p.(Met786Thr)	Yes	Yes
8	NPHS2	chr1 Exon 5	Missense (Pathogenic)	c.538G > A	p.(Val180Met)	Yes	No
10	CD2AP	chr6 Exon 3	Missense (VUS)	c.274G > C	p.(Ala92Pro)	Yes	No
12	COQ8B	chr19 Exon 13	Frameshift (Likely Pathogenic)	c.1199dupA	p. (His400Glnfs*11)	Yes	No
13	MAPKBP1	chr15 Exon23	Missense (VUS)	c.2635C > T	p.(Pro879Ser)	Yes	Yes
16	LAMA5	chr20 Exon 48	Missense (VUS)	c.6404C > T	p.(Pro2135Leu)	Yes	No
17	GAPVD1	chr9 Exon 14	Missense (VUS)	c.2275C > T	p.(Arg759Cys)	Yes	Yes
20	TRIM8	chr10 Exon 6	Missense (VUS)	c.1354G > A	p.(Ala452Thr)	Yes	No
26	CFHR5	chr1 Exon 4	Frameshift (Likely Pathogenic)	c.568dup	p. (Tyr190Leufs*8)	Yes	Yes
27	CFHR5	chr1 Exon 3	Missense (VUS)	c.417A > G	p.(Ile139Met)	Yes	Yes
27	TBC1D8B	chrX Exon 16	Missense (VUS)	c.2659A > G	p.(Ile887Val)	Yes	Yes
28	ARHGAP32	chr11 Exon 22	Missense (VUS)	c.4783C > T	p.(Arg1595Cys)	Yes	Yes
29	ARHGAP32	chr11 Exon 22	Missense (VUS)	c.5192C > T	p.(Pro1731Leu)	Yes	Yes

Patients NO	Gene	Location	Mutation Type	HGVSc	HGVSp	Validation in proband	Family Segregation
30	<i>PODXL</i>	chr7 Exon 2	splice_donor_+1 (Pathogenic)	c.706 + 1G > A	P.?	Yes	Yes
32	<i>TLN1</i>	chr9 Exon 38	Missense (VUS)	c.4993A > G	p.(Ile1665Val)	Yes	Yes
38	<i>ALDH16A1</i>	chr19 Exon 13	Nonsense (Pathogenic)	c.1699C > T	p.(Arg567*)	Yes	No
40	<i>TRPC6</i>	chr11 Exon 2	Missense (VUS)	c.200G > A	p.(Arg67Gln)	Yes	Yes
41	<i>COL4A3</i>	chr2 Exon 48	Intronic (VUS)	c.4462 + 9C > T	p.(=)	Yes	Yes
41	<i>SMARCAL1</i>	chr2 Exon 3	Nonsense (Likely Pathogenic)	c.217C > T	p.(Gln73*)	Yes	Yes
43	<i>WT1</i>	chr11 Exon 1	Missense (VUS)	c.386C > T	p.(pro129Leu)	Yes	Yes
44	<i>ARHGAP24</i>	chr4 Exon 9	Missense (VUS)	c.1496G > A	p.(Arg499Gln)	Yes	No
46	<i>ANLN</i>	chr7 Exon 6	Missense (VUS)	c.1216G > C	p.(Ala406Pro)	Yes	No
48	<i>R3HDML</i>	chr20 Exon 4	Missense (VUS)	c.517G > A	p.(Val173Met)	Yes	No
50	<i>ARHGAP24</i>	chr4 Exon 9	Missense (VUS)	c.1684A > C	p.(Ile562Leu)	Yes	Yes

Segregation analysis that was performed in 12 of the 50 patients by evaluating available family members. The detected variants (Table 2) were first confirmed by Sanger sequencing in these patients; however, segregation analysis did not demonstrate a significant association with the disease, and therefore these variants were not classified as pathogenic or likely pathogenic. In addition, for seven patients in whom the variants were validated only in the probands, segregation analysis in family members is required to further clarify the potential relationship between the detected variants and the disease.

In th patient 13 (a 55_year_old Turkish man) a novel homozygous c.2635C > T (Depth:362, VF: %100) missense variant of the *MAPKBP1* gene was identified in the proband. This variant was confirmed in the proband by Sanger sequence analysis, and it was not detected in the other healthy family members. According to ACMG, it is classified as Variant of Uncertain Significance (VUS) variation (PM2, BP4). The pedigree of the patient, the IGV view of the identified variant, and the Sanger sequencing electropherogram are shown in Fig. 1.

In th patient 30 (a 47_year_old Turkish woman) A novel heterozygous c.706 + 1G > A (Depth:110, VF: %59.1) splice site variant of the *PODXL* gene was identified in the proband. This variant was confirmed in the proband by Sanger sequence analysis, and it was also detected in the other two affected family members but not in the healthy family member. According to ACMG, it is classified as pathogenic variation (PVS1, PM2, PP3). The pedigree of the patient, the IGV view of the identified variant, and the Sanger sequencing electropherogram are shown in Fig. 2. Urinalysis and blood biochemical evaluation of individual IV-1 showed no evidence of proteinuria.

In th patient 50 (a 32_year_old Turkish man) A novel heterozygous c.1684A > C (Depth:493, VF: %46.2) missense variant of the *ARHGAP24* gene was identified in the proband. This variant was confirmed in the proband by Sanger sequence analysis, and it was not detected in the healthy family member. According to ACMG, it is classified as pathogenic variation (PM2, PP3). The pedigree of the patient, the IGV view of the identified variant, and the Sanger sequencing electropherogram are shown in Fig. 3.

Discussion

FSGS represents a complex and heterogeneous glomerular disorder and remains one of the major causes of nephrotic syndrome and progressive kidney disease worldwide (19). The underlying mechanisms of FSGS are highly diverse and involve structural and functional injury to podocytes, which ultimately disrupts the integrity of the glomerular filtration barrier and leads to proteinuria and progressive renal damage (20). Increasing evidence suggests that genetic factors play a crucial role in the development of FSGS, particularly in patients with early-onset disease or familial clustering (21).

In recent years, next-generation sequencing technologies, including targeted gene panels and whole-exome sequencing, have substantially improved the identification of genetic causes of FSGS (22). These approaches have enabled the discovery of numerous variants affecting genes involved in podocyte structure and function, highlighting the critical role of podocyte injury in the pathogenesis of the disease (23). However, the genetic architecture of FSGS remains highly heterogeneous, and ongoing studies continue to identify new candidate genes associated with the disorder. Consequently, restricting genetic investigations to a limited number of well-known genes may reduce diagnostic yield and overlook potentially relevant pathogenic variants in affected individuals (24).

In this study, WES performed in 50 cases did not reveal any pathogenic, likely pathogenic, or VUS variants, nor CNVs, in known OMIM genes or candidate genes potentially related to FSGS in 25 patients. The absence of detectable variants in half of the cohort suggests that the underlying genetic factors may reside in genomic regions not covered by the current panel or may remain undetected due to technical limitations of WES. Approaches such as whole-genome sequencing targeting deep intronic and regulatory regions, together with transcriptome analyses like RNA sequencing (RNA-seq), may help further elucidate the molecular mechanisms contributing to FSGS (25, 26).

Validation of variants identified by WES and segregation analysis in family members are essential to clarify their clinical significance. In this study, potentially disease-associated variants were detected in seven cases (patients 10, 16, 20, 38, 44, 46, and 48); however, Sanger sequencing could not be performed due to the lack of available family samples. Therefore, segregation analyses will be necessary in future studies to more definitively establish the clinical relevance of these variants in FSGS.

Following WES, variants detected in 12 patients (patients 1, 5, 17, 18, 26, 27, 28, 29, 32, 40, 41, and 43) were further evaluated by Sanger sequencing. The results indicated that these variants were benign and were not considered to have a pathogenic role in the development of FSGS.

In our study, pathogenic variants previously reported in association with FSGS were identified in three patients, including variants in *SDCCAG8* c.784G > T p.(Glu262*), *NPHS2* c.538G > A p.(Val180Met), and *COQ8B* c.1199dupA p.(His400Glnfs*11). The c.784G > T p.(Glu262)* variant in *SDCCAG8* has been described by Halbritter et al. (2013) in compound heterozygosity with c.696T > G p.(Y232*) (27). The c.538G > A p.(Val180Met) variant detected in *NPHS2* was first reported by Boute et al.

(2000) as a homozygous missense variant in a family with steroid-resistant nephrotic syndrome and has also been observed in other family as a compound heterozygosity with the R138Q variant (28). The c.1199dupA p.(His400Glnfs11) frameshift variant identified in *COQ8B* was initially described by Ashraf et al. (2013) in a Turkish patient with nephrotic syndrome type 9 born to consanguineous parents. This single-base insertion leads to a frameshift and a premature stop codon, resulting in early protein truncation (29). The identification of these variants in our study group further supports the role of monogenic defects in a subset of FSGS patients and emphasizes the clinical value of genetic testing in the diagnostic evaluation of glomerular diseases.

In patients 13, 30, and 50, in WES analyses results, we identified novel VUS variants in the *MAPKBP1* c.2635C > T p.(Pro879Ser), *PODXL*, and *ARHGAP24* genes, respectively, which have not been previously reported. The *MAPKBP1* gene has been associated with nephronophthisis-20 (OMIM #617271), a disorder inherited in an autosomal recessive manner. Nephronophthisis-20 is a form of tubulointerstitial nephritis characterized by progressive renal fibrosis that ultimately leads to ESKD. In a study by Macia et al. (2017), two patients with Nephronophthisis type 20 born to consanguineous Turkish parents were reported to harbor homozygous pathogenic variants in the *MAPKBP1* gene. One patient carried the c.1318C > T p.(Arg440)* variant in exon 12 and presented with facial dysmorphism as an extrarenal feature. Another patient was found to have a homozygous splice-site mutation c.2444-1G > A p.(Leu814fs40*), with short stature reported as the main extrarenal manifestation (30). Our identified novel homozygous c.2635C > T p.(Pro879Ser) missense variant in exon 23 of the *MAPKBP1* gene (NM_014994.2) has not been previously reported in the ClinVar or HGMD databases and is also absent from the population database gnomAD. Data obtained from in silico tools, the Franklin, and the Varsome databases suggest that the identified variant may have a pathogenic role in FSGS.

The *PODXL* gene (OMIM#602632), encodes podocalyxin, a major component of the polyanionic glycocalyx on the apical surface of podocytes. This protein plays a crucial role in maintaining the structural integrity of the glomerular filtration barrier and the proper function of podocyte foot processes (31). Although no disorder caused by pathogenic variants in the *PODXL* gene has been formally listed in OMIM, accumulating evidence suggests a potential role in hereditary kidney disease. Podocalyxin, encoded by *PODXL*, has recently been implicated in both recessive and dominant forms of familial nephropathy and may contribute to the development of FSGS (32). In 2019, Lin et al. identified a heterozygous nonsense variant (c.976C > T; p.Arg326Ter) in a Chinese family with OD inheritance, characterized by proteinuria and progression to end-stage renal disease (33). Our identified novel heterozygous c.706 + 1G > A splice site variant of the *PODXL* gene has not been previously reported in the ClinVar and HGMD databases and is also absent from the population database gnomAD. Data obtained from in silico tools, the Franklin, and the Varsome databases suggest that the identified variant may have a pathogenic role in FSGS.

The *ARHGAP24* gene (OMIM#610586) encodes a Rac-specific Rho-GTPase-activating protein involved in signaling pathways that regulate cell migration, differentiation, and proliferation (34). Although no disorder caused by pathogenic variants in this gene has been formally listed in OMIM, evidence from the literature suggests that heterozygous pathogenic variants in *ARHGAP24* may be associated with autosomal dominant FSGS. In line with this, Atsushi et al. (2022) identified two novel missense variants in *ARHGAP24* (c.1217G > T, p.Ser406Ile and c.2234T > C, p.Ile745Thr) in patients diagnosed with FSGS (35). Our identified novel heterozygous missense variant c.1684A > C p.(Ile562Leu) in exon 9 of the *ARHGAP24* gene (NM_001025616.2) has not been previously reported in the ClinVar and HGMD databases. According to the population database gnomAD, this variant is extremely rare, being reported in only 1 out of 1,613,906 alleles in the heterozygous state. Data obtained from in silico tools, the Franklin, and the Varsome databases suggest that the identified variant may have a pathogenic role in FSGS.

In conclusion, our study is the first study to investigate the genetic etiology of FSGS in the Turkish population and emphasizes the importance of genetic investigation in elucidating the molecular basis of FSGS. The combination of WES with comprehensive clinical characterization enabled the detection of multiple gene variants related to FSGS, contributing to a better understanding of its underlying genetic mechanisms and plays an important role in differentiating FSGS subtypes and informing appropriate therapeutic strategies. The limitation of our study is the lack of functional validation for the

identified novel variants, and the inability to perform segregation analysis in the families of some other probands that conformed other novel variants in them.

Declarations

Statement of Ethics

The study protocol was approved by the Ethics Committee of Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey (approval number: 2553224; May 9, 2024). Written informed consent was obtained from all participants prior to their inclusion in the study.

Conflict of Interest Statement

None declared

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References

1. Esmaeilzadeh Aghjeh M, Suer I, Dirim AB, Kaya M, Ozturk S (2025) Advances in focal segmental glomerulosclerosis research: genetic causes to non-coding RNAs. *Mol Biol Rep* 52(1):384
2. Liu F, Chen J, Luo C, Meng X (2022) Pathogenic role of MicroRNA dysregulation in podocytopathies. *Front Physiol* 13:948094
3. De Vriese AS, Sethi S, Nath KA, Glasscock RJ, Fervenza FC (2018) Differentiating primary, genetic, and secondary FSGS in adults: a clinicopathologic approach. *J Am Soc Nephrol* 29(3):759–774
4. Bensink ME, Goldschmidt D, Zhou Z-Y, Wang K, Lieblich R, Bunke CM (2024) Kidney failure attributed to focal segmental glomerulosclerosis: A USRDS retrospective cohort study of epidemiology, treatment modalities, and economic burden. *Kidney Med* 6(2):100760
5. D'Agati VD, Kaskel FJ, Falk RJ (2011) Focal segmental glomerulosclerosis. *N Engl J Med* 365(25):2398–2411
6. Podestà MA, Ponticelli C (2020) Autoimmunity in focal segmental glomerulosclerosis: A long-standing yet elusive association. *Front Med* 7:604961
7. Pollak MR, Familial FSGS (2014) *Adv Chronic Kidney Dis* 21(5):422–425
8. Lepori N, Zand L, Sethi S, Fernandez-Juarez G, Fervenza FC (2018) Clinical and pathological phenotype of genetic causes of focal segmental glomerulosclerosis in adults. *Clin kidney J* 11(2):179–190
9. Salwa-Żurawska W, Żurawski J, Woźniak A, Burchardt P (2012) Focal segmental glomerulosclerosis: a diagnostic problem. *Pol J Pathol* 63(1):49–57
10. Warejko JK, Tan W, Daga A, Schapiro D, Lawson JA, Shril S et al (2018) Whole Exome Sequencing of Patients with Steroid-Resistant Nephrotic Syndrome. *Clin J Am Soc Nephrol* 13(1):53–62
11. Isaranuwatthai S, Chanakul A, Ittiwut C, Ittiwut R, Srichomthong C, Shotelersuk V et al (2023) Pathogenic variant detection rate by whole exome sequencing in Thai patients with biopsy-proven focal segmental glomerulosclerosis. *Sci Rep* 13(1):805
12. Hussain HMJ, Cai Y, Weng Q, Tong J, Aftab A, Jin Y et al (2022) Mutation in XPO5 causes adult-onset autosomal dominant familial focal segmental glomerulosclerosis. *Hum Genomics* 16(1):57
13. Zhang J, Xu J, Xiao T, Xiong J, Zhang B (2026) Clinical and genetic diagnosis of a patient with focal segmental glomerulosclerosis due to a novel variant of the ANLN gene. *CEN Case Rep* 15(1):11

14. Adzhubei I, Jordan DM, Sunyaev SR (2013) Predicting functional effect of human missense mutations using PolyPhen-2. *Curr protocols Hum Genet* 76(1):7 1–7. 41
15. Kumar P, Henikoff S, Ng PC (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* 4(7):1073–1081
16. Schwarz JM, Cooper DN, Schuelke M, Seelow D (2014) MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 11(4):361–362
17. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q et al (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* 581(7809):434–443
18. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S et al (2016) ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res* 44(D1):D862–D8
19. Pollak MR (2008) Focal segmental glomerulosclerosis: recent advances. *Curr Opin Nephrol Hypertens* 17(2):138–142
20. Rosenberg AZ, Kopp JB (2017) Focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol* 12(3):502–517
21. Sadowski CE, Lovric S, Ashraf S, Pabst WL, Gee HY, Kohl S et al (2015) A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J Am Soc Nephrol* 26(6):1279–1289
22. Torra R, Barros X, Díaz-Encarnación M, Fayos L, Furlano M, Pilco M et al (2025) When should the nephrologist think about genetics in patients with glomerular diseases? *Clin kidney J* 18(3):sfaf044
23. Bonilla M, Efe O, Selvaskandan H, Lerma EV, Wiegley N (2024) A review of focal segmental glomerulosclerosis classification with a focus on genetic associations. *Kidney Med* 6(6):100826
24. Shane Klomp L, Levchenko E, Westland R (2024) Developmental causes of focal segmental glomerulosclerosis. *Glomerular Dis* 4(1):95–104
25. Kim HS, Kim M, Suh J-S, Lee Y (2025) Case Report: Whole genome sequencing identifies a novel deep intronic COL4A5 variant of uncertain significance in X-linked Alport syndrome. *Front Pead* 13:1639471
26. Zhi X, Ai Q, Sheng W, Yu Y, Shu J, Yu C et al (2022) Identification of a Novel Deep Intronic Variant by Whole Genome Sequencing Combined With RNA Sequencing in a Chinese Patient With Menkes Disease. *Front Genet* 13:852764
27. Halbritter J, Porath JD, Diaz KA, Braun DA, Kohl S, Chaki M et al (2013) Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. *Hum Genet* 132(8):865–884
28. Boute N, Gribouval O, Roselli S, Benessy F, Lee H, Fuchshuber A et al (2000) NPHS2, encoding the glomerular protein podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome. *Nat Genet* 24(4):349–354
29. Ashraf S, Gee HY, Woerner S, Xie LX, Vega-Warner V, Lovric S et al (2013) ADCK4 mutations promote steroid-resistant nephrotic syndrome through CoQ 10 biosynthesis disruption. *J Clin Investig* 123(12):5179–5189
30. Macia MS, Halbritter J, Delous M, Bredrup C, Gutter A, Filhol E et al (2017) Mutations in MAPKBP1 cause juvenile or late-onset cilia-independent nephronophthisis. *Am J Hum Genet* 100(2):323–333
31. Kershaw DB, Beck SG, Wharram BL, Wiggins JE, Goyal M, Thomas PE, Wiggins RC (1997) Molecular cloning and characterization of human podocalyxin-like protein: orthologous relationship to rabbit PCLP1 and rat podocalyxin. *J Biol Chem* 272(25):15708–15714
32. Anna A, Monika G (2018) Splicing mutations in human genetic disorders: examples, detection, and confirmation. *J Appl Genet* 59(3):253–268
33. Lin F-J, Yao L, Hu X-Q, Bian F, Ji G, Jiang G-R et al (2019) First identification of PODXL nonsense mutations in autosomal dominant focal segmental glomerulosclerosis. *Clin Sci* 133(1):9–21
34. Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dilthey A et al (2012) Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. *Nat Genet* 44(5):502–510
35. Kondo A, Kitakado H, Masuda C, Ishiko S, Aoto Y, Sakakibara N et al (2022) Investigation of the Pathogenicity of Novel Missense Variants in the ARHGAP24 Gene by Quantitative Analysis of the Active Rac1: Two Cases of Proteinuria With Renal Dysfunction: FR-PO447. *J Am Soc Nephrol* 33(11S):445

Figures

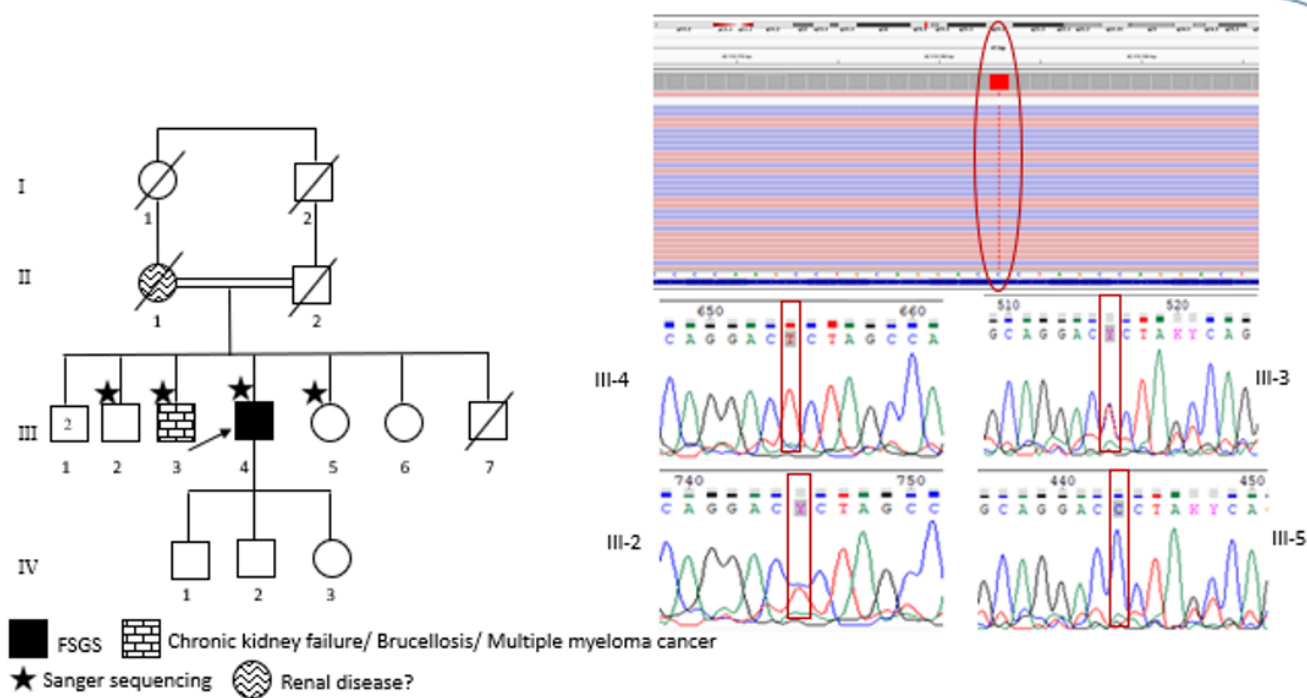


Figure 1
The pedigree of the patient 13, IGV view of the identified variant and Sanger sequencing electropherograms.

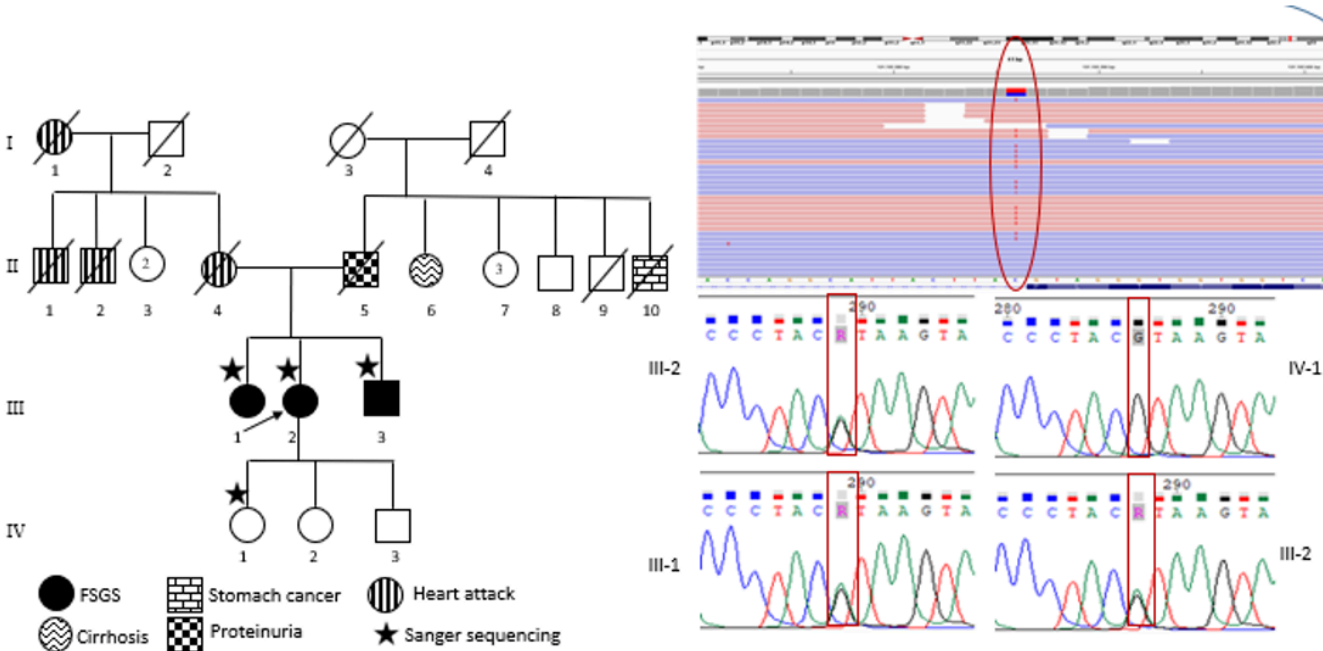


Figure 2

The pedigree of the patient 30, IGV view of the identified variant and Sanger sequencing electropherograms

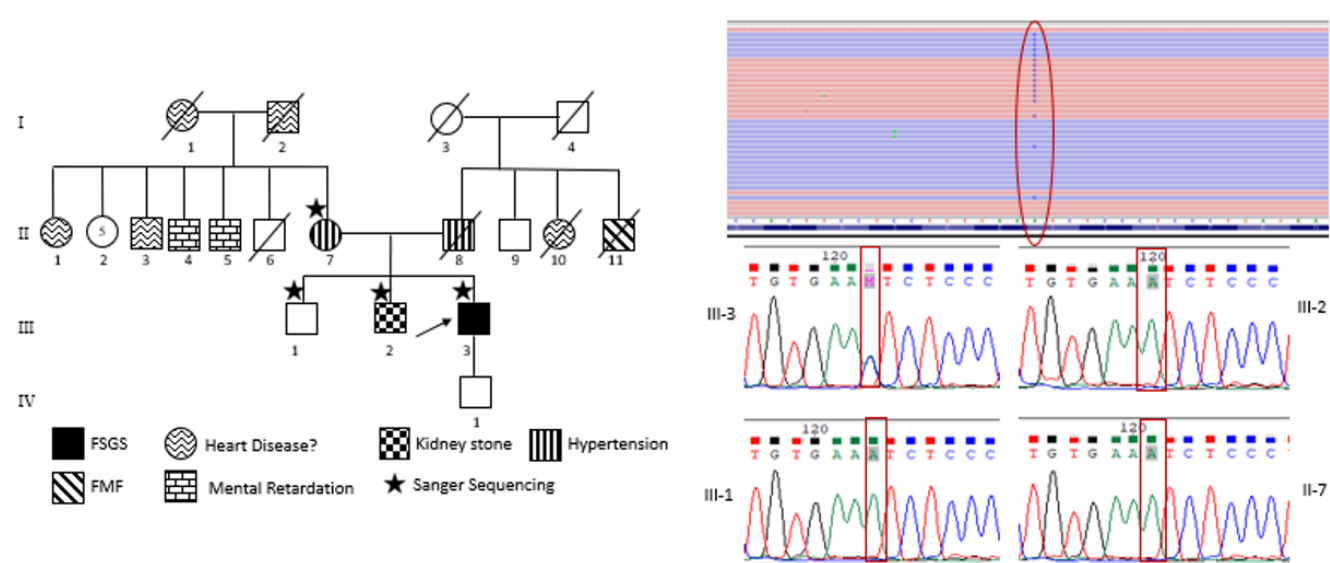


Figure 3

The pedigree of the patient 50, IGV view of the identified variant and Sanger sequencing electropherograms