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ORIGINAL ARTICLE

Genetic testing in focal segmental glomerulosclerosis: in whom and when?

Ana María Tato[1,*], Noa Carrera[2,*], Maria García-Murias[2], Amir Shabaka[1], Ana Ávila[3], María Teresa Mora Mora[4], Cristina Rabasco[5], Karina Soto[6], Francisco Jose de la Prada Alvarez[7], Loreto Fernández-Lorente[8], Antolina Rodríguez-Moreno[9], Ana Huerta[10], Carmen Mon[11], Clara García-Carro[12], Fayna González Cabrera[13], Juan Antonio Martín Navarro[14], Ana Romera[15], Eduardo Gutiérrez[16], Javier Villacorta[17], Alberto de Lorenzo[18], Beatriz Avilés[19], Miguel Angel Garca-González[2,20] and Gema Fernández-Juárez[21,22]; on behalf of the GLOSEN group

[1]Department of Nephrology, Hospital Universitario Fundación Alcorcón, Alcorcón, Spain, [2]Laboratorio de Nefroloxía (No. 11), Grupo de Xenética e Bioloxía do Desenvolvemento das Enfermidades Renais, Instituto de investigación sanitaria de Santiago de Compostela – IDIS, Santiago de Compostela, Spain, [3]Department of Nephrology, Hospital Universitario Doctor Peset, Valencia, Spain, [4]Department of Nephrology, Hospital Universitario Juan Ramón Jiménez, Huelva, Spain, [5]Department of Nephrology, Hospital Universitario Reina Sofía, Córdoba, Spain, [6]Department of Nephrology, Hospital Fernando Fonseca, Lisbon, Portugal, [7]Department of Nephrology, Hospital Universitario Virgen Macarena, Sevilla, Spain, [8]Department of Nephrology, Hospital Virgen del Camino, Pamplona, Spain, [9]Department of Nephrology, Hospital Universitario Clínico San Carlos, Madrid, Spain, [10]Department of Nephrology, Hospital Universitario Puerta de Hierro, Majadahonda, Spain, [11]Department of Nephrology, Hospital Universitario Severo Ochoa, Leganés, Spain, [12]Department of Nephrology, Hospital Vall d'Hebrón, Barcelona, Spain, [13]Department of Nephrology, Hospital Universitario de Gran Canaria Doctor Negrín, Gran Canaria, Spain, [14]Department of Nephrology, Hospital Universitario Infanta Leonor, Madrid, Spain, [15]Department of Nephrology, Hospital de Ciudad Real, Ciudad Real, Spain, [16]Department of Nephrology, Hospital Universitario Doce de Octubre, Madrid, Spain, [17]Department of Nephrology, Hospital Universitario Ramón y Cajal, Madrid, Spain, [18]Department of Nephrology, Hospital HM San Chinarro, Madrid, Spain, [19]Department of Nephrology, Hospital Costa del Sol, Marbella, Spain, [20]Fundación Pública Galega de Medicina Xenómica-SERGAS, Complexo Hospitalario de Santiago de Compostela, Santiago de Compostela, Spain, [21]Department of Nephrology, Hospital Universitario La Paz, Madrid, Spain and [22]Instituto de Investigación de la Paz (IdIPAZ), Madrid, Spain

*A.M.T. and N.C. contributed equally to this work.
Correspondence to: Miguel A. García-González; E-mail: Miguel.garcia.gonzalez@sergas.es, Gema Fernández-Juárez; E-mail: gema.fernandezjuarez@salud.madrid.org

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ABSTRACT

Background. Genetic causes are increasingly recognized in patients with focal segmental glomerulosclerosis (FSGS), but it remains unclear which patients should undergo genetic study. Our objective was to determine the frequency and distribution of genetic variants in steroid-resistant nephrotic syndrome FSGS (SRNS-FSGS) and in FSGS of undetermined cause (FSGS-UC).
Methods. We performed targeted exome sequencing of 84 genes associated with glomerulopathy in patients with adult-onset SRNS-FSGS or FSGS-UC after ruling out secondary causes.
Results. Seventy-six patients met the study criteria; 24 presented with SRNS-FSGS and 52 with FSGS-UC. We detected FSGS-related disease-causing variants in 27/76 patients (35.5%). There were no differences between genetic and non-genetic causes in age, proteinuria, glomerular filtration rate, serum albumin, body mass index, hypertension, diabetes or family history. Hematuria was more prevalent among patients with genetic causes. We found 19 pathogenic variants in *COL4A3*–5 genes in 16 (29.3%) patients. *NPHS2* mutations were identified in 6 (16.2%) patients. The remaining cases had variants affecting *INF2*, *OCRL*, *ACTN4* genes or *APOL1* high-risk alleles. FSGS-related genetic variants were more common in SRNS-FSGS than in FSGS-UC (41.7% vs 32.7%). Four SRNS-FSGS patients presented with *NPHS2* disease-causing variants. *COL4A* variants were the most prevalent finding in FSGS-UC patients, with 12 patients carrying disease-causing variants in these genes.
Conclusions. FSGS-related variants were detected in a substantial number of patients with SRNS-FSGS or FSGS-UC, regardless of age of onset of disease or the patient's family history. In our experience, genetic testing should be performed in routine clinical practice for the diagnosis of this group of patients.

LAY SUMMARY

Prevalence of genetic causes in patients with a histological diagnosis of focal segmental glomerulosclerosis (FSGS) is probably underestimated due to the diagnostic challenges in its diagnosis. The criteria for genetic testing in adult patients with FSGS remains unclear. In this study, we found that there is a high prevalence of genetic forms in FSGS with steroid-resistant nephrotic syndrome (41.7%) and FSGS of undetermined cause (32.7%). The most common genetic variants in adult-onset FSGS steroid-resistant nephrotic syndrome were *NPHS2* gene mutations, whereas in FSGS of undetermined cause the most frequent genetic mutations were in *COL4A* genes. The age of onset of disease and the presence of a positive family history did not discriminate between genetic and non-genetic forms of FSGS. In conclusion, genetic testing should be performed in patients with FSGS and steroid-resistant nephrotic syndrome or FSGS of undetermined cause, regardless of the age of onset or family history of kidney disease.

Keywords: FSGS, hereditary diseases, nephrotic syndrome, podocytopathy, steroid-resistant

INTRODUCTION

Focal segmental glomerulosclerosis (FSGS) is a histological lesion seen on light microscopy, characterized by the presence of sclerosis in parts (segmental) of at least one glomerulus (focal). A primary disease or an adaptive phenomenon that results in podocyte injury and depletion may evolve into this histological pattern. According to the etiology, FSGS lesion has been classified into immunological, genetic and secondary forms. The latest version of the KDIGO guidelines includes FSGS of undetermined cause (FSGS-UC) to define patients with this histological pattern but whose etiology is unknown [1]. FSGS-UC is essentially a diagnosis of exclusion in patients in which primary, secondary or genetics causes have been ruled out.

Although FSGS is considered a podocyte disorder, genetic forms of FSGS result from pathogenic mutations in genes related to interaction between the podocyte and the basement membrane [2, 3]. The clinical presentation of genetic FSGS is extremely variable, from adult-onset mild disease to perinatal nephrotic syndrome. The causative gene determines the age of onset. Thus, disease-causing mutations in genes related to podocyte cytoskeleton or slit diaphragm (*NPHS1*, *NPHS2*, *LAMB2*…) are predominantly found in childhood [4–7], while pathogenic genetic variants associated to type 4A collagen (*COL4A*) genes represent most cases in adulthood [8, 9]. In the adult population, a genetic cause has been established in 8%–26% of cases, although it is possible that this number is underestimated [8–10]. The clinical and histological characteristics that seem to better predict the genetic etiology are the absence of response to immunosuppressive medication, the absence of diffuse podocyte foot process effacement in the renal biopsy, and normal serum albumin despite nephrotic proteinuria [3, 11, 12]. Nevertheless diffuse foot process effacement by itself may not be able to differentiate primary FSGS from genetic forms of FSGS [13]. Genetic testing is recommended for early-onset forms, especially those resistant to steroids. However, in the adult population, establishing the criteria for a genetic study continues to be a challenge [14]. The recommendations of the KDIGO guidelines for genetic testing also includes family history, features suggestive of a syndromic disease, aiding in diagnosis, limiting immunosuppression exposure, determining the risk of recurrent disease in kidney transplantation and for risk assessment in living kidney donor candidates, and prenatal diagnoses. Despite the guideline recommendations, the decision to perform a genetic test, in routine clinical practice remains complex. As in other hereditary nephropathies, most of the genetic FSGS do not have an obvious family history, FSGS-UC forms rarely receive immunosuppression, and lastly the optimum approach is to perform the etiological diagnosis at the beginning of the evaluation and not in the pre-transplant study [15, 16].

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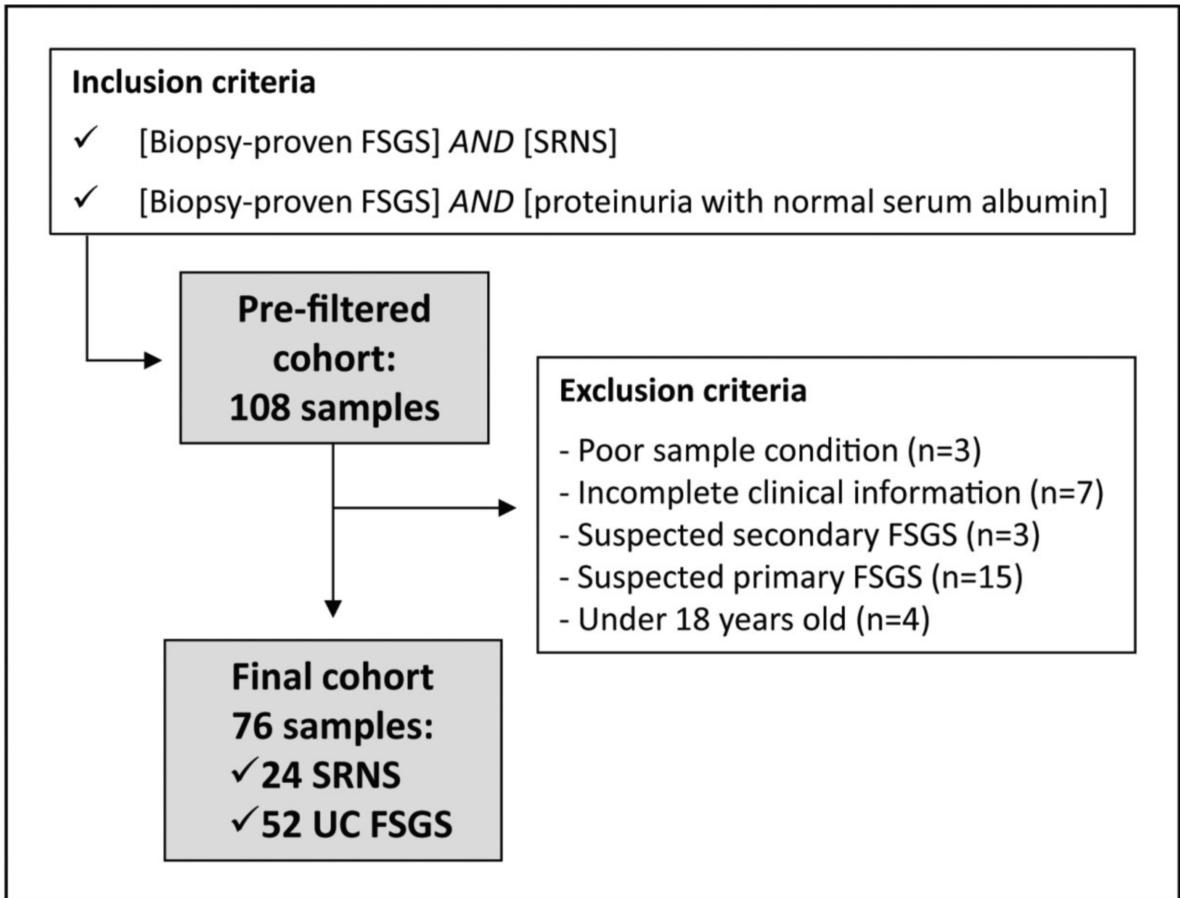

Figure 1: Screening flowchart. Patient flow through the study. Screening population included all adult patients with biopsy-proven FSGS and SRNS or FSGS-UC. Reasons for screening failures were classified as follows: poor sample condition ($n = 3$), incomplete clinical information ($n = 7$), suspected secondary FSGS ($n = 3$), suspected primary FSGS ($n = 15$) and under 18 years old at the time of biopsy ($n = 4$).

In this study, we report the prevalence of genetic variants in adult-onset FSGS according to clinical presentation, particularly, adult-onset steroid-resistant nephrotic syndrome (SRNS) and adult-onset FSGS-UC.

MATERIALS AND METHODS

Patients

This study was a retrospective multicenter cohort study performed in 18 Spanish and Portuguese hospitals. Patients were eligible if they had biopsy-proven FSGS and SRNS or FSGS-UC. SRNS-FSGS was defined as protein excretion higher than 0.3 g per 24 h after 16 weeks of prednisone treatment. FSGS-UC patients were defined as those with proteinuria of any range with normal serum albumin levels in whom a secondary cause of FSGS had been ruled out. Low birth weight, morbid obesity, any cause of reduction of renal mass, reflux nephropathy, sickle cell disease, any advanced kidney disease with substantial loss of nephrons, sleep apnea, cyanotic congenital heart disease, renal artery stenosis, malignant hypertension, cholesterol emboli, viral infections such as human immunodeficiency virus, parvovirus B19, cytomegalovirus or hepatitis C virus, hemophagocytic syndrome, and medications such as ledipasvir, sofosbuvir, mammalian target of rapamycin (mTOR) inhibitors (prior to biopsy), calcineurin inhibitors (prior to biopsy), anthracyclines, heroin, lithium, interferon, anabolic steroids and pamidronate, were considered possible causes of secondary FSGS.

Clinical data, biopsy reports and laboratory data for the 2 years following biopsy were carefully reviewed. Those patients that presented a potential secondary or primary cause of FSGS (i.e. abrupt onset, response to immunosuppression) were excluded from the study.

Relevant medical data and family history information was collected. Patients with first- to third-degree relatives with proteinuria and/or renal failure were defined as familial cases; otherwise, they were defined as sporadic cases.

We excluded patients under 18 years at the time of biopsy and patients with FSGS due to a secondary cause (Fig. 1). Laboratory data at the time of kidney biopsy and the following 2 years were collected, as well as the biopsy reports. The study was approved by local ethics committees, and all participants provided written informed consent.

Genetics

DNA was extracted from peripheral blood (leukocytes) received from each patient with the commercial kits GenEXTM Genomic Kit (GeneAllTM, Seoul, Korea) and QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), following manufacturer's guidelines. Quality and concentration of the obtained DNA was checked with Nanodrop (ThermoScientific, Rochester, NY, USA).

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We employed targeted next-generation sequencing (NGS) technology to study of the main genes known to cause FSGS. This subset of genes is part of a custom panel of genes designed for targeting the main genes associated with glomerular pathologies (Supplementary data, Table S1). The exons, splice sites and flanking regions of these candidate genes were captured and amplified using the "TruSeq DNA Library Prep for Enrichment" kit (Illumina, San Diego, CA, USA) with the "xGen hybridization capture of DNA libraries" kit (IDT, Integrated DNA technologies, www.idtdna.com), and sequenced on the Next Seq 500 Illumina platform (Illumina, San Diego, CA, USA). All DNA handling and sequencing procedures were completed at NefroCHUS or the Galician Public Foundation for Genomic Medicine (FPGMX).

Raw sequencing reads were processed according to GATK best practice guidelines [17, 18]. Reads were aligned to the human reference genome (GRCh37) using bwa version 0.7.17-r1188. Low-quality reads were removed from the primary data set using fastpversion0.20 [19]. Variants were called using GATK version 4.1.9 [17, 18], Pindel version 0.2.5b9 [20] and ExomeDepth version 1.1.15 [21], following their corresponding best practice guidelines. Variants were annotated with an in-house annotation pipeline merging SnpEff's functional gene annotation with ANNOVAR annotations to retrieve population frequencies (1000 Genomes Project, gnomAD and an in-house database among others), functional prediction scores (SIFT, CADD, etc.) or clinical information (ClinVar, OMIM, etc.).

Variants were classified independently by two geneticists specialized in hereditary kidney diseases according to the American College of Medical Genetics (ACMG) guidelines and recent amendments [22]. Disease-causing variants were defined as those that were classified as "pathogenic" or "likely pathogenic" and that were explicative of the patient's nephropathy. A bibliographic search of each diagnostic variant was carried out using different search tools (PubMed, ClinVar and the Human Gene Mutation Database) to determine whether they had been previously reported. Segregation analysis was carried out by Sanger sequencing.

Statistical analysis

Statistical significance was determined by the two-sample *t*-test (two-tailed), Fisher or χ^2-tests (two-tailed) and Mann–Whitney U tests using SPSS version 21 (IBM, Armonk, NY, USA). $P < .05$ was considered to be statistically significant.

Comparison of our genetic findings with other comparable published works

A bibliographic search was carried out to identify studies that met the following criteria: (i) cohort of patients clinically diagnosed with FSGS with inclusion criteria similar to ours; (ii) genetic study performed by NGS based on glomerular candidate gene panels.

RESULTS

Baseline characteristics

A total of 108 patients from 104 families were assessed for eligibility, of whom 32 patients (29.6%) were excluded from the study. Main reasons for screening failures were not meeting the inclusion criteria ($n = 22$), and inadequate clinical, laboratory or biopsy data ($n = 10$). The final study cohort in this study consisted of 76 adult patients biopsied between 1991 and 2019 (Fig. 1).

Baseline characteristics of the genetically studied cohort are shown in Table 1. Overall, mean age was 40.8 ± 12.1 years, 52 participants (68.4%) were male and 93.4% were Caucasian. Of the 76 patients included in the study, 52 belonged to the FSGS-UC group (68.4%), and 24 to the SRNS-FSGS group (31.6%). Electron microscopy examination was available only in 9 (11.8%) patients. The mean estimated glomerular filtration rate (eGFR) at the time of biopsy was 77 ± 34 mL/min/1.73 m^2. Median 24-h urine protein excretion was 4.1 g/day (IQR 1.6–5.0 g/day). More than half of the patients (56.6%) were hypertensive, and 7 patients (9.2%) were diabetic. Mean body mass index (BMI) was 26.7 ± 4.9 kg/m^2. No patients presented with histological lesions of diabetes, hypertension or any other superimposed kidney disease on renal biopsy. Fifty-five (72.4%) patients were on angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker prior to their renal biopsy. Family history of any kind of chronic kidney disease was present in 34 (44.7%) patients.

Most of the patients received some type of immunosuppressive treatment, without response. Since this is a retrospective study, with patients treated in 18 different centers over almost 30 years, the immunosuppression regimens were heterogeneous. Immunosuppression was more prevalent in the SRNS-FSGS group than in the FSGS-UC group (100% vs 27%). All nephrotic patients received steroids, 66.6% calcineurin inhibitors, 29.2% mycophenolate mofetil, 2.9% mTOR inhibitors and 16.6% rituximab.

In the non-nephrotic group, 26.9% of patients received steroids, 15.4% calcineurin inhibitors and 9.6% mycophenolate mofetil. Nine (17.3%) patients were treated with two or more immunosuppressive agents. Seventeen patients in the genetic cause group had been under immunosuppressive treatment. None of them had a complete remission and in seven patients a partial remission was achieved.

Genetic findings in the global FSGS cohort

The detection rate of disease-causing mutations in the global cohort was 35.5% (27 of 76 patients, Fig. 2). The median time from kidney biopsy to the genetic diagnosis was 5.8 (2.8–10.6) years. Of these patients, 11 (40.8%) had an autosomal dominant disease, 9 (33.3%) an autosomal recessive disease (compound heterozygous variants), 6 (2.2%) an X-linked disease and 1 (3.7%) had two pathogenic mutations—one in *COL4A4* and another in *ACTN4*. Table 2 and Supplementary data, Table S2 show a comprehensive list of the genetic findings. Patients with variants of uncertain significance (VUS) where the segregation analysis was not possible or lacking segregation were considered negative for genetic testing.

Ten relatives of six probands were received to carry out a study of carriers for examining co-segregation (probands IDs: 17; 37; 66; 75; 79; and 81; Supplementary data, Table S3). For family FSGS_MAD21 (proband 37), the co-segregation study rules out that the variant identified in the *COL4A3* gene, initially classified as VUS, is related to the pathology, but it does not discard the implication of *APOL1* risk haplotypes. For the other five families, the study of carriers in relatives supports the involvement of the proposed variant in the pathology.

Collagen 4A3–5 variants were the most frequent molecular diagnoses, with 19 pathogenic or probably pathogenic mutations identified in 16 (59.3%) patients (Fig. 2 and Table 2). There were six patients with *COL4A5* pathogenic or likely pathogenic variants (NM_033380.3: p.G1030S;

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Table 1: The clinical picture: baseline characteristics of the global cohort and according to biopsy criteria.

N (%)	Global cohort N = 76	SRNS-FSGS N = 24 (31.6)	FSGS-UC N = 52 (68.4)
Genetic finding, %	35.5	41.7	32.6
Age (years), mean ± SD	40.4 ± 12.1	38.4 ± 11.5	40.8 ± 12.5
Male sex, %	68.4	66.6	69.2
BMI (kg/m^2), mean ± SD	26.7 ± 4.9	26.3 ± 4.9	26.9 ± 4.9
Hypertension, %	56.6	37.7	65.4
Diabetes mellitus, %	9.2	12.5	7.7
Family history, %	44.7	33.3	50
RAS blockade, %	72.4	66.7	75.0
Immunosuppression, %	48.70	100	26.9
Glucocorticoids, %	48.70	100.0	26.9
CNI, %	31.60	66.7	15.4
MMF, %	15.80	29.1	9.6
mTor inhibitors, %	1.30	4.2	0
Rituximab, %	5.20	16.6	0
eGFR CKD-EPI (mL/min), mean ± SD	77.6 ± 34. 0	73.1 ± 37.2	79.5 ± 32.6
Serum albumin (g/dL), mean ± SD	3.6 ± 0.8	2.8 ± 0.7	4.0 ± 0.6
Proteinuria (g/day), mean ± SD	4.1 ± 3.5	7.1 ± 4.4	2.7 ± 1.3
Hematuria, %	60.5	70.8	55.8

All data refer to the time of kidney biopsy.
RAS: renin–angiotensin system; eGFR CKD-EPI: estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration; CNI: calcineurin inhibitor; MMF: mycophenolate mofetil.

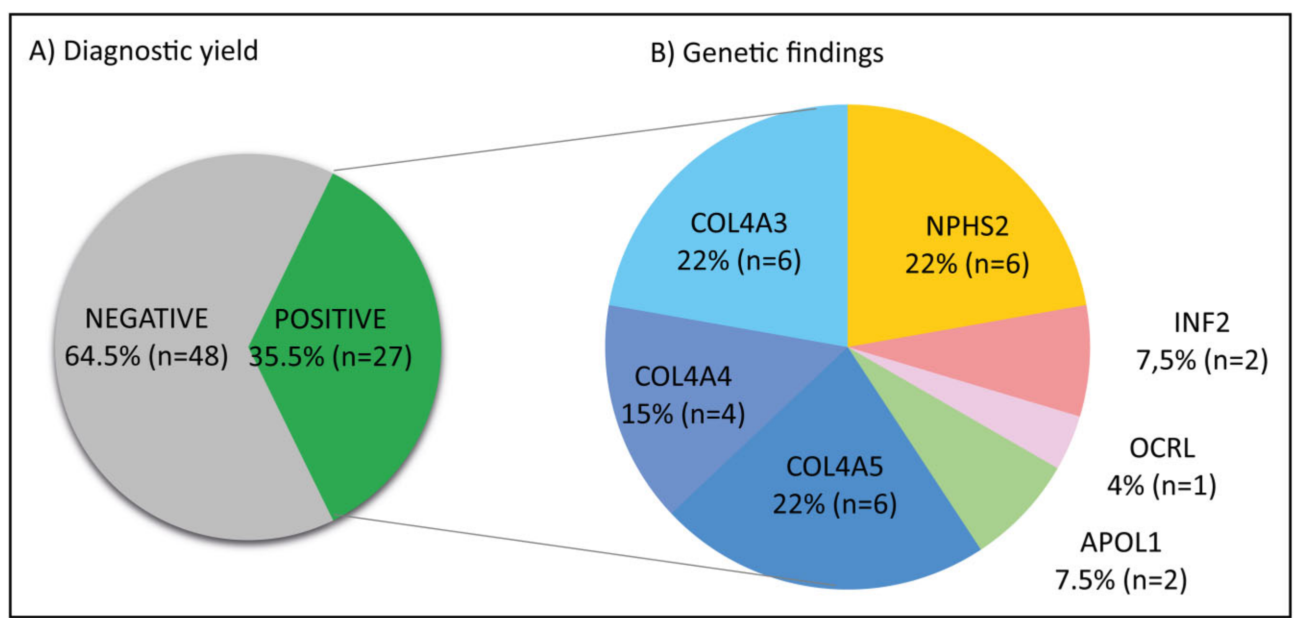

Figure 2: Diagnostic yield and genetic findings. Section (**A**) shows the diagnostic yield obtained. Section (**B**) summarizes the genetic findings, grouped by gene. One patient had a dual diagnosis (see Table 2).

c.1032 + 4A > C; c.465 + 4dup; p.C1527S; p.G600V), four patients with *COL4A4* pathogenic or likely pathogenic variants (NM_000092: p.Y268Ter; p.G1163D; p.C1588W; p.G252D), one of them with an additional pathogenic or likely pathogenic in a second candidate gene (*ACTN4*, NM_004924.6: p.R251W), and six patients with *COL4A3* pathogenic or likely pathogenic mutations (NM_000091: p.G1015E; p.L1474P; p.G130R; p.R1661C; p.G640R; p.G1155S; p.G1251S), three of them in heterozygous state and three in compound heterozygous state. Compound heterozygote pathogenic or likely pathogenic variants identified in *NPHS2* gene (NM_014625: p.R238S; p.M187I; p.A288T; p.R286TfsTer17; p.E310K; p.H325Y; p.R229Q) accounted for six (22.2%) cases. In the remaining cases we found pathogenic or likely pathogenic variants affecting several other candidate genes (*INF2*, NM_022489: p.I115N, p.F68L; *OCRL*, NM_000276.4: p.C679R). Two patients of African ancestry presented the two *APOL1* high-risk alleles, G1 and G2 [23, 24].

At the time of the biopsy, there were no differences between patients with a genetic diagnosis and patients with a negative result in terms of age, proteinuria, GFR, serum albumin, BMI,

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Table 2: List of diagnostic variants.

ID	Sex	Age at biopsy (years)	Potential causal gene	Potential causing variant					
				cDNA and protein change	State (inheritance)	Type of variant	ACMG class[a]	dbSNP ID	Ref. (PMID)
SRNS-FSGS patients (based on biopsy)									
9	M	19	*NPHS2*	c.862G > A (p.A288T)	Het (AR/AD)	Missense	LP	rs1490010141	20 947 785
				c.686G > A (p.R229Q)	Het (AR/AD)	Missense	VUS-LP	rs61747728	30 241 959, 24 509 478
48	M	34	*NPHS2*	c.855_856del (p.R286TfsTer17)	Het (AR/AD)	Frameshift	P	rs749740335	24 742 477
				c.686G > A (p.R229Q)	Het (AR/AD)	Missense	VUS-LP	rs61747728	30 241 959, 24 509 478
95	M	30	*NPHS2*	c.973C > T (p.H325Y)	Het (AR/AD)	Missense	LP	rs551511369	19 406 966
				c.686G > A (p.R229Q)	Het (AR/AD)	Missense	VUS-LP	rs61747728	30 241 959, 24 509 478
99	F	43	*NPHS2*	c.928G > A (p.E310K)	Het (AR/AD)	Missense	P	NA	19 145 239
				c.686G > A (p.R229Q)	Het (AR/AD)	Missense	VUS-LP	rs61747728	30 241 959, 24 509 478
4	F	28	*COL4A3*	c.3044G > A (p.G1015E)	Het (AR/AD)	Missense	P	rs121912826	11 961 012
				c.4421T > C (p.L1474P)	Het (AR/AD)	Missense	HYPOM[b]	rs200302125	26 346 198
31	M	34	*COL4A3*	c.3044G > A (p.G1015E)	Het (AR/AD)	Missense	P	rs121912826	11 961 012
				c.4421T > C (p.L1474P)	Het (AR/AD)	Missense	HYPOM[b]	rs200302125	26 346 198
36	M	48	*COL4A4*	c.801_802del (p.Y268Ter)	Het (AR/AD)	Nonsense	P	NA	NA
			ACTN4	c.751C > T (p.R251W)	Het (AD)	Missense	VUS-LP	rs898478281	NA
3	M	56	*COL4A5*	c.3088G > A (p.G1030S)	Hemiz (XLD)	Missense	P	rs104886210	27 627 812
63	M	29	*APOL1*	G1: c.1024A > G, c.1152T > G (p.S342G, p.I384M)	Het (Risk Fact.)	Missense	Risk factor	G1 (rs73885319, rs60910145)	25 168 832
				G2: c.1164_1169del (p.N388_Y389del)	Het (Risk Fact.)	In-frame deletion	Risk factor	G2 (rs71785313)	25 168 832
17	M	36	*OCRL*	c.2035T > C (p.C679R)	Hemiz. (XLR)	Missense	LP	NA	NA
FSGS-UC patients (based on biopsy)									
65	M	23	*NPHS2*	c.928G > A (p.E310K)	Het (AR/AD)	Missense	P	NA	19 145 239
				c.686G > A (p.R229Q)	Het (AR/AD)	Missense	VUS-LP	rs61747728	30 241 959, 24 509 478
69	F	34	*NPHS2*	c.714G > T (p.R238S)	Het (AR/AD)	Missense	P	rs748812981	15 253 708
				c.561G > A (p.M187I)	Het (AR/AD)	Missense	LP	NA	20 947 785
66	F	39	*INF2*	c.344T > A (p.I115N)	Het (AD)	Missense	LP	NA	NA
79	F	59	*INF2*	c.202T > C (p.F68L)	Het (AD)	Missense	LP	NA	NA
6	F	59	*COL4A3*	c.1918G > A (p.G640R)	Het (AR/AD)	Missense	P	rs200672668	11 134 255
55	M	51	*COL4A3*	c.388G > C (p.G130R)	Het (AR/AD)	Missense	P	NA	NA
				c.4981C > T (p.R1661C)	Het (AR/AD)	Missense	LP	rs201697532	25 229 338
64	M	54	*COL4A3*	c.3463G > A (p.G1155S)	Het (AR/AD)	Missense	P	rs774583962	NA
84	M	27	*COL4A3*	c.3751G > A (p.G1251S)	Het (AR/AD)	Missense	P	NA	28 632 965
5	M	45	*COL4A4*	c.3488G > A (p.G1163D)	Het (AR/AD)	Missense	LP	NA	NA

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Table 2: Continued

ID	Sex	Age at biopsy (years)	Potential causal gene	Potential causing variant: cDNA and protein change	State (inheritance)	Type of variant	ACMG class[a]	dbSNP ID	Ref. (PMID)
				SRNS-FSGS patients (based on biopsy)					
19	M	nk	*COL4A4*	c.4764T > G (p.C1588W)	Het (AR/AD)	Missense	LP	NA	24052634
75	M	39	*COL4A4*	c.755G > A (p.G252D)	Het (AR/AD)	Missense	P	NA	33532864
21	M	36	*COL4A5*	(c.1032 + 4A > C)	Hemiz (XLD)	Likely splicing effect	LP	NA	NA
56	M	46	*COL4A5*	(c.465 + 4dup)	Hemiz (XLD)	Likely splicing effect	LP	NA	NA
59	M	50	*COL4A5*	(c.465 + 4dup)	Hemiz (XLD)	Likely splicing effect	LP	NA	NA
73	M	40	*COL4A5*	c.4579T > A (p.C1527S)	Hemiz (XLD)	Missense	LP	rs755766520	NA
81	F	47	*COL4A5*	c.1799G > T (p.G600V)	Het (XLD)	Missense	P	NA	NA
37	M	18	*APOL1*	G1: c.1024A > G, c.1152T > G (p.S342G, p.I384M)	Het (risk fact.)	Missense	Risk factor	G1 (rs73885319, rs60910145)	25168832
				G2: c.1164_1169del (p.N388_Y389del)	Het (risk fact.)	In-frame deletion	Risk factor	G2 (rs71785313)	25168832

Variants classified in May 2023.

[a]See ref. [22] (https://pubmed.ncbi.nlm.nih.gov/25741868/).

[b]This variant can be considered hypomorphic (44). In association with another variant of *COL4A3*, as is the case in both patients, it may be associated with autosomal recessive Alport syndrome and kidney failure.

Het: heterozygous; Hemiz: hemizygous; AD: autosomal dominant; AR: autosomal recessive; nk: not known; P: pathogenic; LP: likely pathogenic; HYPOM: hypomorphic.

hypertension, diabetes, electron microscopy findings or positive family history (Table 3). Hematuria was more prevalent among patients with an identified genetic cause ($P = .008$; Table 3). There was no difference in eGFR decline between genetic and idiopathic groups during the follow-up period. Patients with *COL4A3–5* mutations were older at the age of clinical diagnosis (44 vs 31 years, $P = .015$) than those with pathogenic variants in *NPHS2* gene (Fig. 3A). Patients who combined *COL4A3* or *COL4A4* with other diagnostic variants were a median of 10 years younger, although the difference was not statistically significant. Four out of 16 (25%) patients with *COL4A* variants presented with chronic kidney failure but all *NPHS2* patients showed eGFR above 60 mL/min/1.73 m^2 (mean eGFR 69 vs 117 mL/min/1.73 m^2, $P = .005$) (Fig. 3B). Furthermore, proteinuria was lower in patients with *COL4A* variants compared with *NPHS2* variants (2.7 vs 6.8 g/day, $P = .021$). Interestingly, we found no differences in prevalence of hematuria between *COL4A* and non-*COL4A* patients.

Comparison between positive vs negative genetic patients with SRNS-FSGS or FSGS-UC based on KDIGO guidelines inclusion criteria

The presence of pathogenic or likely pathogenic genetic variants related to FSGS was more common in the SRNS-FSGS group (41.7% vs 32.7%), although this difference was not statistically significant (Table 3). The distribution of genetic findings among 10 patients with SRNS-FSGS was: four patients (40%) with two *NPHS2* variants (compound heterozygotes), one patient with the two high risk haplotypes of *APOL1* gene in heterozygosity (G1 and G2 haplotypes), one patient with a hemizygous *OCRL* variant and four patients (40%) with variants in the collagen 4A genes: 1 patient (male) presented with a *COL4A5* variant, two patients with two compound heterozygous variants at *COL4A3*, and another patient with one *COL4A4* variant and another *ACTN4* variant (Fig. 3A). *COL4A3–5* variants were the most prevalent finding in the FSGS-UC group, with 12 patients carrying one or two variants in these genes (76.4%). In particular, there were four patients with *COL4A3* variants (one heterozygotes, three compound heterozygous), three with a *COL4A4* variant (all heterozygote) and five with a *COL4A5* variant (four hemizygous males and one heterozygous female).

We stratified our cohort according to the KDIGO guidelines for indications for genetic diagnosis (patients with family history of kidney disease, syndromic features, early-onset or disease resistant to immunosuppressive therapy). All patients with SRNS-FSGS are included in these recommendations, and FSGS-UC patients were stratified according to having or not indications for genetic diagnosis. In accordance with these recommendations, 71% of patients with FSGS-UC would be candidates for genetic testing. However, we did not find differences between the prevalence of genetic causes among those who presented some criteria for genetic testing (29.7%) compared with those who did not (29.7 vs 40%, $P = .474$).

Table 3 shows clinical characteristics in patients with positive vs negative genetic testing results in SRNS-FSGS and FSGS-UC groups. No significant clinical differences were found between FSGS-UC and SRNS-FSGS patients. There were four cases with SRNS-FSGS among Alport patients, and two patients with NPHS2 variants presented with FSGS-UC. This reinforces the need to genetically diagnose those patients with such a disease profile, in order to properly classify them.

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Table 3: Baseline characteristics of the global cohort and according to biopsy criteria and genetic finding.

Biopsy criterion	Global cohort				SRNS-FSGS			FSGS-UC		
Genetic finding		NEG	POS	P-value	NEG	POS	P-value	NEG	POS	P-value
N (%)		49 (64.5)	27 (35.5)		14 (58.3)	10 (41.7)		35 (67.3)	17 (32.7)	
Age (years), mean ± SD	40.4 ± 12.1	40.3 ± 12.6	39.6 ± 11.5	.80	39.9 ± 12.2	36.4 ± 10.6	.47	40.5 ± 12.9	41.5 ± 11.9	.79
Male sex, %	68.4	65.3	74.1	.43	57.1	80	.39	68.6	70.6	.082
BMI (kg/m^2), mean ± SD	26.7 ± 4.9	27.0 ± 4.8	26.3 ± 5.1	.55	27.4 ± 5.3	24.8 ± 4.1	.21	26.8 ± 4.7	27.2 ± 5.7	.82
Hypertension, %	56.6	61.2	48.1	.27	50	20	.21	65.7	64.7	.94
Diabetes mellitus, %	9.2	10.2	7.4	.65	21	0	.24	5.7	11.8	.59
Family history, %	44.70	44.9	44.4	.97	21.4	50	.20	54.3	41.2	.38
RAS blockade, %	72.4	73.5	70.4	.78	64.3	70	.65	77.1	70.6	.43
Immunosuppression, %	48.70	45	52	.46	100	100		25.0	29.40	.41
eGFR CKD-EPI (mL/min), mean ± SD	77.6 ± 34.0	76.2 ± 33.3	80.0 ± 35.9	.67	77.8 ± 33.7	81.6 ± 42.1	.38	79.1 ± 32.9	74.3 ± 32.9	1
Serum albumin (g/dL), mean ± SD	3.6 ± 0.8	3.6 ± 0.9	3.6 ± 0.7	.78	2.5 ± 0.7	2.9 ± 0.6	.41	4.0 ± 0.6	3.9 ± 0.4	.65
Proteinuria (g/day), mean ± SD	4.1 ± 3.5	4.1 ± 3.2	4.2 ± 4.2	.60	7.1 ± 5.6	7.0 ± 5.6	.77	2.9 ± 1.8	2.3 ± 1.5	.25
Hematuria, %	60.5	49.0	81.5	.008	50	100	.019	48.6	70.6	.18

All data refer to the time of kidney biopsy.
P-values are derived from Fisher's two-tailed test, χ^2 two-tailed test or Mann–Whitney U tests for comparison of characteristics in patients with positive vs negative genetic test results. $P < .05$ was considered to be statistically significant.
RAS: renin–angiotensin system; eGFR CKD-EPI: estimated glomerular filtration rate by Chronic Kidney Disease Epidemiology Collaboration; CNI: calcineurin inhibitor; MMF: mycophenolate mofetil; NEG: no disease-causing variant identified; POS: disease-causing variant identified.

DISCUSSION

Genetic testing in FSGS has usually been reserved for familial cases, patients with specific phenotypes and children with SRNS-FSGS [5, 25–29]. However, our results support that adult-onset genetic FSGS is frequent in both SRNS-FSGS and FSGS-UC, even in the absence of a family history or resistance to immunosuppressive therapies.

In a large cohort of CKD patients, Groopman *et al.* [30], showed that 62% of patients with pathogenic mutations in *COL4A3*–5 did not have clinical diagnoses of the nephropathies classically associated with these genes, and 16% of patients had been diagnosed of FSGS. In a cohort of adult disease onset and a high likelihood for hereditary FSGS, Braunisch *et al.* [31] identified a monogenic cause in 29% of patients, after performing whole-exome sequencing.

In our cohort of SRNS-FSGS, we found candidate disease-causing variants in 41.7% of all patients, even though family history of CKD was not an inclusion criterion. Moreover, among the patients with SRNS-FSGS and positive genetic diagnosis, only 50% had a family history of renal disease. Therefore, we consider that the absence of family history should not preclude genetic study in FSGS presenting with SRNS. Variant penetrance, the presence of other genetic variants and environmental factors would explain the different phenotypes among relatives with the same pathogenic mutation.

To contextualize our results with what has been published to date, we set out to compare our findings with those of other studies comparable to ours—namely studies including FSGS cohorts with criteria similar to ours, and in which an NGS strategy based on glomerular candidate gene panels had been used. After reviewing the literature, we found two articles that met our criteria (Fig. 4). In the study by Gribouval *et al.* [8] where the spotlight was extended, considering sporadic SRNS and/or FSGS, pathogenic mutations were identified in 11.8% of patients, and an additional 10.4% of patients carried *APOL1* high-risk alleles. Gast *et al.* [9] analysed a cohort of 75 FSGS probands by means of a custom NGS panel of 39 candidate genes. They found definitely or probably pathogenic mutations in 15 of them (20%). In our series, only patients without secondary FSGS were included, which could explain the high percentage of disease-causing mutations.

The 2021 KDIGO guidelines proposes a new classification of FSGS that includes a new category, FSGS of undetermined cause [1]. It covers patients whose presentation is similar to secondary FSGS: proteinuria with normal serum albumin and in which a secondary etiology has not been identified. Our study explores the genetic etiology in this specific subgroup. In our cohort, 35.5% of the patients had an underlying pathological genetic cause that could be responsible for their clinical and histological picture.

Our results are consistent with other series which reported type 4 collagen mutations to be the most frequent disease-causing mutations in adult FSGS population [8, 9, 32–34].

In our study, this distribution is modified according to the clinical characteristics, with genetic variants related to collagen type 4 being the most frequent in FSGS-UC and those related to the slit diaphragm the most frequent in SRNS-FSGS. However, both variants were detected in both groups. *COL4A5* variants with X-linked inheritance have been attributed to be the driver in 85% of cases of Alport disease. Nevertheless, recent studies have highlighted the relevance of *COL4A4* and *COL4A3* mutations [35–42]. We found 71.4% of Alport disease patients to be secondary to *COL4A3* and *COL4A4* variants. Our cohort showed

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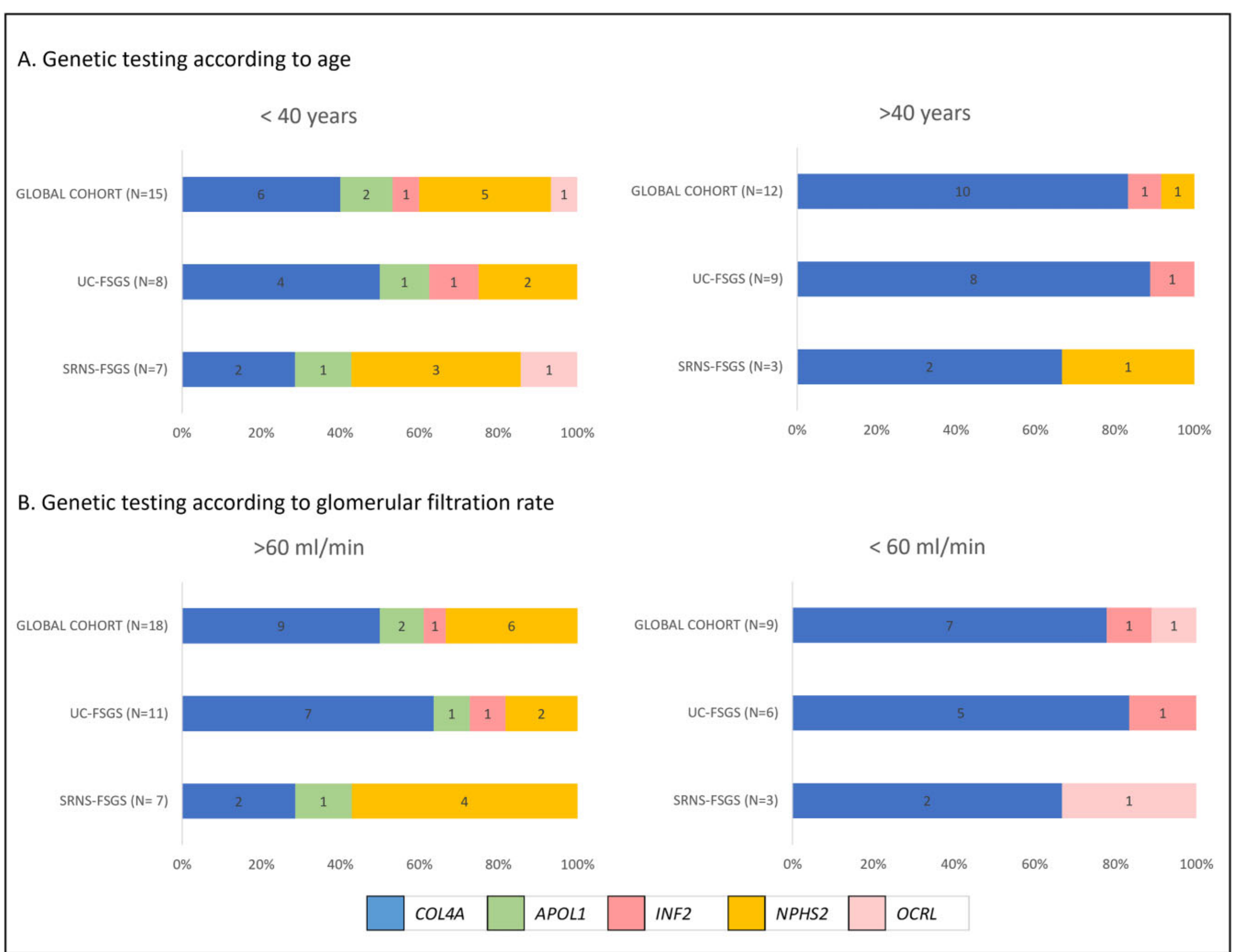

Figure 3: Genetic testing according to age (A) and glomerular filtration rate. (B) according to the inclusion criteria (whole cohort, FSGS-UC or SRNS-FSGS).

the wide phenotypic spectrum of *COL4A3*–5 associated FSGS, ranging from SRNS in early adulthood to non-nephrotic proteinuria in middle age. As described in other studies, hematuria was not always present [9, 37]. We found that all patients with monogenic *COL4A3* or -*A4* variants were hypertensive and none of them presented with nephrotic proteinuria. Of particular interest is that patients with compound heterozygous variants at *COL4A4* genes, or one heterozygous variant in one of these genes with an additional variant in another gene (*ACTN4*), seemed to have an earlier onset and three out of four presented with SRNS-FSGS.

The genetic approach could contribute significantly to shortening the diagnostic process, and allow focus on targeted treatments.

The clinical phenotype is not helpful in most cases. As mentioned above, not all patients with collagen 4 variants presented hematuria and nor did patients with the rest of the variants present the full spectrum of associated syndromes. Indiscriminate empirical treatment of FSGS with steroids should be avoided unless the suspicion of a primary FSGS is very high. In our series, among patients with a potential causative variant, five patients (50%) with SRNS-FSGS received additional immunosuppression, and 5 (29%) patients with non-nephrotic proteinuria had been under unnecessary immunosuppressive treatment.

FSGS reflects irreversible lesions in the kidney, therefore familial screening and presymptomatic care of affected relatives is of utmost importance. Genetic testing enables genetic counseling and preimplantation genetic diagnostics.

As a limitation of this study, our cohort consisted mainly of Caucasian adults, thus the genetic diagnostic yield or the genetic findings cannot be extrapolated to other populations. Patient inclusion in this study was based on clinical criteria, as electron microscopy was not available in many cases. We believe that changes in podocyte effacement or glomerular basement membrane might improve the efficiency of genetic testing indication. Although our selection criteria did not prioritize familial cases, 45% of patients reported a family history of kidney disease. Importantly, this variable did not differ between patients with and without pathogenic genetic variants.

Some studies have tested non-glomerular genes in patients with FSGS [31, 34]. The Toronto GN Registry cohort [33] reported CAKUT (congenital anomalies of the kidney and urinary tract) genes in 5% of patients with a histological diagnosis of FSGS. We restricted our study to 84 genes known to be causative of genetic FSGS.

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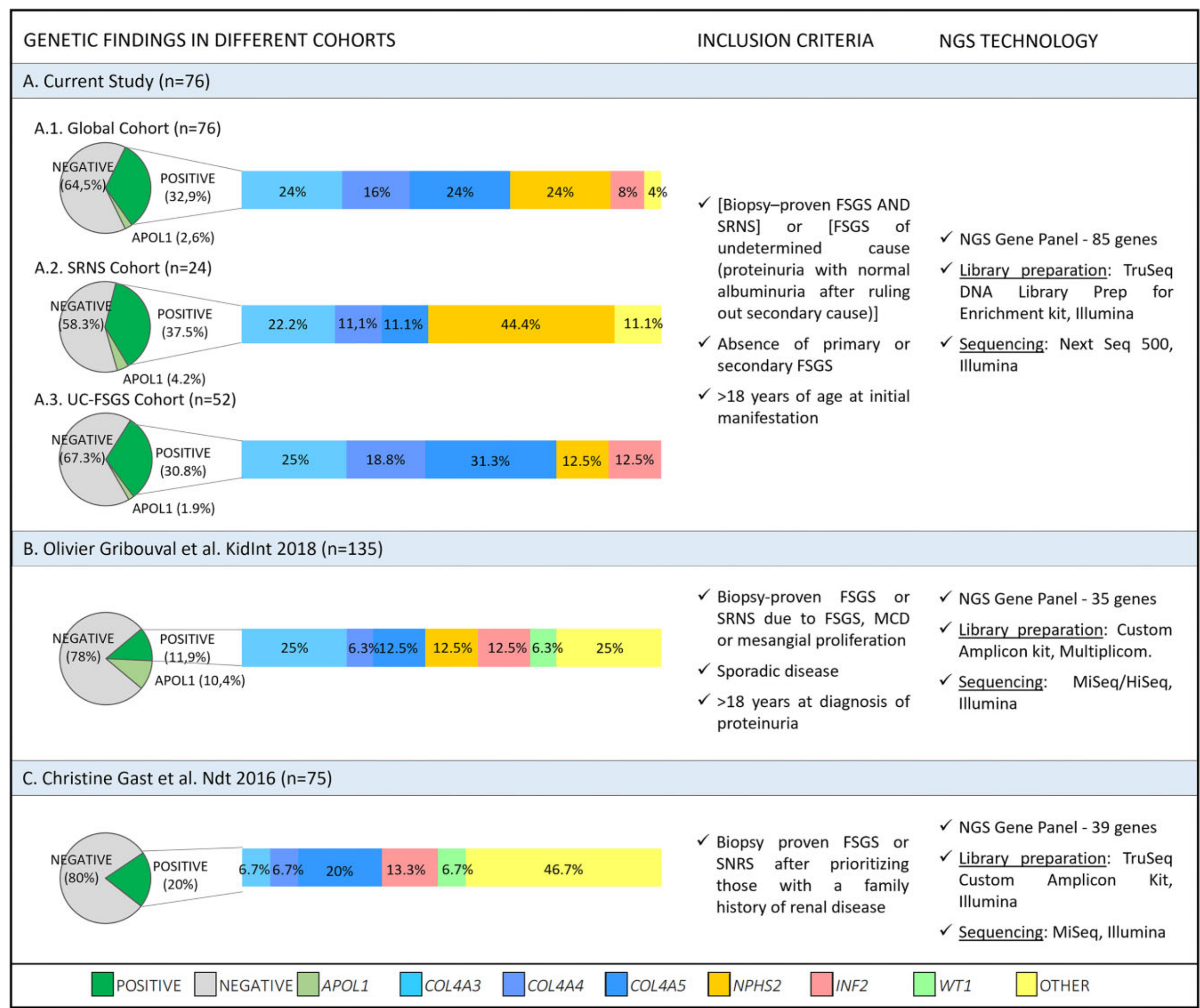

Figure 4: Comparison of cohorts. This figure shows schematically our study and that of two other comparable cohorts; particularly, we show for each study the inclusion criteria considered, the NGS strategy employed, and the genetic results obtained. Section (**A**) refers to our cohort. The genetic results are represented both for the global cohort and broken down into the two considered subgroups: the FSGS-UC cohort and the SRNS-FSGS cohort. Section (**B**) shows the results of Gribouval *et al.* [8]. Section (**C**) shows the results of Gast *et al.* [9].

It is possible that the variants detected are not the only cause of the disease and that additional metabolic, hypertensive or other factors were involved to develop the fully expressed phenotype. However, we believe that this does not diminish the importance of determining a possible genetic origin, which would aid in limiting unnecessary immunosuppression, studying other pathologies of the syndrome and an early detection of affected relatives.

Miao *et al.* [43] detected a monogenic variant in 33.3% patients with secondary FSGS with known causes. Our study excluded patients with secondary causes for FSGS, and selected those in whom underlying genetic findings were more likely to be present, therefore we cannot rule out a genetic cause in secondary FSGS.

Targeted NGS analysis does not exclude the presence of variants in unexamined regions (introns, regulatory regions or regions not accessible to hybridization), nor large deletions, insertions or inversions that cannot be detected by the technique used.

In conclusion, genetic testing is a minimally invasive diagnostic procedure that helps to determine the origin of the patient's disease, provides guidance on prognosis and future treatments, and enables family screening. Our experience supports the implementation of genetic testing in routine clinical practice for the diagnosis of patients with SRNS-FSGS or FSGS-UC, regardless of the patient's age at disease onset or family history of kidney disease.

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest to disclose.

AUTHORS' CONTRIBUTIONS

Research idea and study design: N.C., G.F.-J., M.A.G.-G., A.M.T. Data acquisition: A.A., B.A., G.F.-J., L.F.-L., C.G.-C., E.G., F.G.C., A.H., A.L., J.A.M.N., C.M., M.T.M.M., F.J.P.A., C.R., A.R.-M., A.R., A.S., K.S., A.M.T., J.V. Data analysis/interpretation: N.C., G.F.-J., M.A.G.-G., A.M.T., M.G.-M., A.S. Statistical analysis: A.M.T. Supervision or mentorship: G.F.-J., M.A.G.-G. Each author contributed significant intellectual content during the drafting or revision of the manuscript and agrees to be personally responsible for his or her own contributions and to ensure that questions concerning the accuracy or completeness of any part of the work, even those in which the author was not directly involved, are appropriately investigated and resolved, including with bibliographic documentation if appropriate.

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DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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