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CLINICAL RESEARCH

Steroid-Resistant Nephrotic Syndrome Is Associated With a Unique Genetic Profile in a Highly Admixed Pediatric Population

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Introduction: The profile of genetic and nongenetic factors associated with progression to kidney failure (KF) in steroid-resistant nephrotic syndrome (SRNS) is largely unknown in admixed populations.

Methods: A total of 101 pediatric patients with primary SRNS were genetically assessed targeting Mendelian causes and *APOL1* status with a 62-NS-gene panel or whole exome sequencing, as well as genetic ancestry. Variant pathogenicity was evaluated using the American College Medical of Genetics and Genomics (ACMG) criteria.

Results: Focal segmental glomerulosclerosis (FSGS) was diagnosed in 54% of patients whereas familial disease was reported by 13%. The global genetic ancestry was 65% European, 22% African, 10.5% Native American, and 2% East-Asian, while 96% of cases presented with the first 3 components. *APOL1* high-risk genotypes were identified in 8% of families and causative Mendelian variants in 12%: *NPHS1* = 3, *NPHS2* = 3, *PLCE1* = 2, *WT1* = 2, *COQ2* = 1, and *CUBN* = 1. Two novel causative variants arose in the Native American background. The percentage of African genetic ancestry did not associate with the number of *APOL1* risk alleles. Forty-four percent of all patients progressed to KF. Mendelian forms and *APOL1* high-risk genotypes were associated with faster progression to KF. Cox regression analyses revealed that higher non-European genetic ancestry, self-declared non-White ethnicity, age of onset <1 year or ≥ 9 years, and non-minimal change disease (MCD) histology associated with higher risk of KF, independently of genetic findings.

Conclusion: Mendelian variants and *APOL1* high-risk genotype compose a unique causative genetic profile associated with pediatric SRNS in this highly admixed population, accounting for approximately 20% of families. This ancestry pattern is consistent with the identification of *APOL1* high-risk genotypes in children with low proportion of African genetic ancestry. Self-declared ethnicity, age of manifestation and histology were independently associated with the risk of KF.

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Most cases of nephrotic syndrome (NS) in pediatric patients are classified as idiopathic (INS). This group of disorders is classically defined by edema, hypoalbuminemia, and proteinuria, after ruling out secondary causes.[1] The current classification of INS is mainly based on steroid responsiveness. Patients who do not achieve remission in 4 weeks of their first steroid course are classified as steroid-resistant, comprising 10% to 20% of INS cases. Approximately 50% of patients aged <18 years with SRNS progress to kidney failure (KF) in 5 years, representing the second most frequent cause of chronic KF in pediatric populations.[2,3] In the largest study of pediatric NS performed to date in Brazil, SRNS or congenital NS (CNS) accounted for 19.2% of INS cases, 34.5% of which progressed to KF.[4]

Whereas minimal change disease (MCD) is typically associated with pediatric steroid-sensitive INS (SSNS), focal and segmental glomerulosclerosis (FSGS) is the predominant histological pattern in SRNS children.[2,5] Collapsing glomerulopathy (CG), in turn, currently considered a distinct entity from FSGS by most authors,[6] is characterized by massive proteinuria and fast progression to KF. Children with SRNS onset before age of 4 years may also present diffuse mesangial sclerosis, often associated with Mendelian causes.[7–9]

SRNS-causative variants have initially been identified in *WT1*, *NPHS1*, and *NPHS2*, later followed by variants in a series of genes that encode constituents of cytoskeleton, mitochondria, nucleus, lysosome, trafficking vesicles and glomerular basement membrane.[8–12] In addition to Mendelian causes, *APOL1* high-risk genotype has been recognized as a crucial genetic susceptibility factor to SRNS, fundamentally affecting African American and admixed populations.[13–15]

Mendelian forms have been reported in 8.4% to 43.5% of cases in pediatric or pediatric/young-adult SRNS populations worldwide.[8–11,16–21] Moreover, *APOL1* high-risk genotypes were present in 46% of African American children with NS in the CkiD and Neptune cohorts, whereas a frequency of 6% was found in a pediatric Brazilian NS cohort with admixed ancestry.[13,14,16] In spite of the cohort differences in these studies, such as ages of disease onset, rates of parental consanguinity, frequencies of familial disease history, ancestries and genetic testing strategies, they revealed higher rates of known Mendelian causes when NS onset occurred within the first year of life. In fact, Mendelian forms accounted for approximately 70% of CNS cases, followed by a progressive reduction with increase in age.[8–10] *APOL1* high-risk genotypes, in turn, were detected more often in older children with NS.[13,14] Of note, Mendelian forms, and more recently *APOL1* high-risk genotypes, were associated with worse kidney function outcomes, progressing more frequently and more rapidly to KF.[10,14] In contrast, NS recurrence after kidney transplantation (KT) was almost absent in both of these groups.[8–10,14,16]

Most published SRNS cohorts are based on western developed countries, including patients who predominantly were self-declared as White. Mendelian causes were more often observed in populations with higher inbreeding rate. Patients from South America, however, including Brazil, are poorly represented in such cohorts.[8–10] In contrast to the available studies, Brazil has a highly genetically admixed population and a low rate of parental consanguinity.[22] In this context, we have previously investigated the prevalence and impact of *APOL1*-associated NS in a Brazilian pediatric SRNS population.[14]

Interestingly and unlike in the United States, though considering the limited number of *APOL1* high-risk genotype patients, we found no correlation between non-White self-declared ethnicity and *APOL1* high-risk genotypes. These findings led us to explore and characterize the genetic signatures of SRNS or CNS in a Brazilian cohort, with a uniquely admixed genetic profile. To accomplish this, we performed a comprehensive genetic evaluation, aiming to characterize the prevalence of Mendelian and *APOL1* high-risk genotype-related forms, as well as the impact of such variants, the genetic background, and nongenetic factors on the course of kidney function.

METHODS

Study Design

Our cohort included 101 patients from 98 families, 89 of whom were included in the previous study that addressed their *APOL1* status.[14] Of these patients, 73 were followed-up with at Instituto da Criança e do Adolescente, University of São Paulo Medical Center (Supplementary Figure S1), whereas 28 were referred from other Brazilian centers. Inclusion criteria comprised SRNS and/or INS with FSGS, or familial INS, associated with NS onset before the age of 18 years.

SRNS was defined as absence of steroid responsiveness after receiving 60 mg/m^2/d of steroids for 4 weeks followed by 4 additional weeks on alternate days.[2,3,23] Resistance to steroids was presumed for patients with CNS and those who manifested the disease within the first year of life and did not receive this therapy. Patients with diagnosis other than INS, such as C3 nephropathy, IgA nephropathy, lupus nephritis, membranous nephropathy or Alport syndrome were excluded from the study.

Clinical, laboratory, and histological data were retrospectively and prospectively collected from NS onset to the last follow-up visit. This information encompassed all relevant data related to diagnosis and classification of NS. Patient records included sex (defined by biological characteristics), age at diagnosis, glomerular histology-based diagnosis when kidney biopsy was performed, self-reported ethnicity, parental consanguinity, family history of NS or KF, follow-up duration, response to steroids, use and response to calcineurin inhibitors, estimated course of glomerular filtration rate, time to KF, and NS recurrence following KT.

Because this study evaluated *APOL1* risk alleles in a genetically admixed population, we analyzed data on both self-reported ethnicity and genetic ancestry according to Flanagin *et al*.[24] Self-reported ethnicity was provided by the patients' guardians at their first appointment or assessment: the term "non-White" was applied to patients who self-reported as Black, Pardo, Asian or Native Indian, whereas "White" was applied to those who self-reported as White. According to the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística-IBGE),[25] "Pardo" is a classification that encompasses admixture of 2 or more ethnicities among Black, Native Indian, and White populations. "Ancestry," on the other hand, was classified according to molecular genetics analysis based on the single nucleotide polymorphism array described later as African, European, Native American, and East Asian.

Study Approval

The clinical research protocol was approved by the Ethics Committee of Instituto da Criança e do Adolescente of University of São Paulo, under the number 2.296.618. This study was conducted under the ethical guidelines and regulations of clinical research as well as the principles of the Declaration of Helsinki. All guardians signed an informed consent form, and patients aged $>$ 7 years signed an informed assent form, both covering clinical data and genetic testing.

Molecular Genetics Analyses

DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). Samples were submitted to sequencing of 62 NS-associated genes using 2 customized targeted-gene panels (Figure 1a) or to whole exome sequencing (Supplementary Methods: Molecular genetics analysis, section 1A).

The generated read sequences were aligned to human reference genome GRCh37/hg19 using the Burrows-Wheeler Aligner (BWA-MEM aligner, GNU General Public License version 0.7.17, MIT License, Cambridge, MA) and screened for duplicate reads with the biobambam tool. Variant calling was performed with Freebayes and the resulting variant call formats were annotated with the ANNOVAR software (http://annovar.openbioinformatics.org).[26]

Assessment of Variant Pathogenicity

The resulting data were filtered focusing on variants with at least 10 reads at low frequency ($\leq$0.1%) in controls on gnomAD (https://gnomad.broadinstitute.org/), AbraOM (https://abraom.ib.usp.br/)[27] and SELAdb (http://intranet.fm.usp.br/sela/).[28] The variants were subsequently ranked according to their pathogenicity potential, first considering stop-gain, splice-site disrupting and frameshift ones, because such variants are related to expected loss of function. Prediction of pathogenicity of missense variants was based on multiple *in silico* programs, including Polyphen2, Mutation Assessor, SIFT, and PROVEN. The assessed variants had their sequencing reads visually inspected using Integrative Genomics Viewer. Sanger sequencing was performed to confirm the potential causative variants, to proceed the segregation analysis when parental DNA was available, and to confirm *APOL1* G1 and/or G2 variants. It was also used to analyze *APOL1* G1 and/or G2 in 1 patient in whom a *WT1* pathogenic variant was found in a panel for sexual development disorders.

Potentially causative variants were assessed for pathogenicity by applying the ACMG guidelines for interpretations of sequence variants.[29] Variant of uncertain significance (VUS) associated with high pathogenicity scores predicted by *in silico* programs and phenotypes strongly correlated with the mutated gene were submitted to additional molecular mechanics evaluation to strengthen the assessment of pathogenicity. In these cases, we performed primary sequence alignment to evaluate the conservation of mutated amino acids, followed by macromolecular mechanics *in silico* and structural analyses. Using structural information acquired through high resolution experimental x-ray diffraction method for crystallographic analyses, this computational tool provides key elements that can predict the impact of a variant associated with

a **Genes included in the 42- and 20-gene panels**

Nextera rapid capture *(Illumina*, San Diego, CA):

ACTN4; ADCK4; ARHGDIA; NPHS2; CD2AP; COQ2; CRB2; CUBN; DGKE; EMP2; EYA1; COQ6; FAT1; INF2; ITGA3; ITGB4; KANK1; KANK2; KANK4; LAMB2; LMX1B; MYO1E; NPHS1; PAX2; PDSS2; PLCE1; PODXL; PTPRO; SCARB2; SMARCAL1; TRPC6; TTC21B; WDR73; WT1; XPO5; APOL1; ARHGAP24; MEFV; NXF5; NUP107; NUP205; NUP93

Ampliseq *(Illumina*, San Diego, CA):

NEIL1; COL4A3; COL4A4; COL4A5; FN1; ANLN; MYH9; MAGI2; TNS2; DLC1; CDK20; ITSN1; ITSN2; CFH; CFI; CD46; C3; CFHR5; ADAMTS13; MCP1

b **Characterization of patient population**

	Number of patients=100
Geographic origin:	
University of São Paulo Center	73 (73%)
Other Brazilian centers	27 (27%)
Age of nephrotic syndrome onset	2.9 years (1.5-6.8)
Sex:	
Male	60 (60%)
Female	40 (40%)
Self-declared ethnicity:	
White	61 (61%)
Pardo	34 (34%)
Black	3 (3%)
Asian	1 (1%)
Unknown	1 (1%)
Family history:	
Yes	13 (13%)
No	86 (86%)
Unknown	1 (1%)
Parental consanguinity:	
Yes	6 (6%)
No	94 (94%)
Response to steroids:	
Sensitive	9 (9%)
Steroid resistance	81 (81%)
Presumed steroid resistance	10 (10%)
Response to calcineurin inhibitor:	
Complete remission	36 (53%)
Partial remission	2 (3%)
No remission	30 (44%)
Renal histology:	
FSGS	54 (54%)
MCD	19 (19%)
CG	12 (12%)
DMS	6 (6%)
Mesangioproliferative glomerulonephritis	2 (2%)
Crescentic pauci-imune GN	1 (1%)
No biopsy performed	6 (6%)
Follow-up time	5.6 years (2.4-9.3)
KF	44 (44%)
Kidney transplant	29 (29%)
NS recurrence	9/29 (31%)

FSGS: focal and segmental glomerulosclerosis, MCD: minimal change disease, CG: collapsing glomerulopathy, KF: kidney failure

c **Mendelian and *APOL1* high-risk genotype frequencies and distribution**

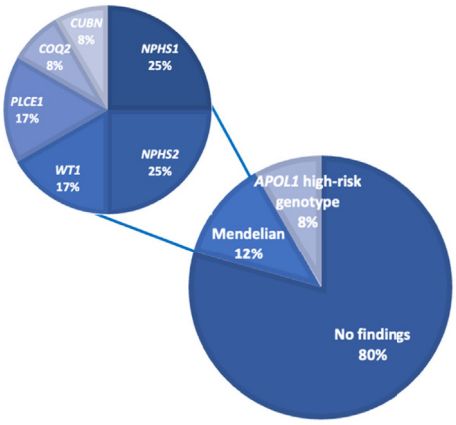


Figure 1. (a) Genes included in the nephrotic syndrome (NS) panel. (b) Demographic, clinical, laboratory, and histological characterization of the patient population. (c) Distribution of Mendelian and *APOL1* high-risk genotype-related pediatric forms of NS among the evaluated Brazilian steroid-resistant nephrotic syndrome (SRNS) or congenital nephrotic syndrome (CNS)/focal segmental glomerulosclerosis (FSGS) families. CG, collapsing glomerulopathy; FSGS, focal and segmental glomerulosclerosis; GN, glomerulonephritis; KF, kidney failure; MCD, minimal change disease.

specific mutated residue(s) on the corresponding protein structure, and potentially, on the protein function. Based on this information, it may allow predicting the pathogenic potential of the analyzed variant (Supplementary Methods: Molecular mechanics evaluation, section 1B, Supplementary References).

ACMG-based pathogenic and likely pathogenic variants, as well as VUSs with pathogenicity supported by molecular mechanics simulations, were considered causative for Mendelian forms of SRNS or CNS if consistent with the expected pattern of inheritance. Segregation was evaluated in all pedigrees with available constitution and information.

Analyses of Genetic Ancestry

To ascertain the individual genetic ancestry, all 101 patients of the cohort were genotyped with a specifically designed high-density single nucleotide polymorphism array (Infinium Global Screening Array v3.0, Illumina, San Diego, CA). This procedure also allowed inferring the genetic ancestral origin of the identified causative Mendelian variants (Supplementary Methods: Analysis of genetic ancestry, section 1C, Supplementary References).

Genotype-Phenotype Correlations

Search for genotype-phenotype correlations included analyses of Mendelian variants associated with NS as well as the *APOL1* genotype status, using as

phenotypic features age of NS onset, extrarenal manifestations, histological diagnosis, self-declared ethnicity, family history of NS and/or KF, parental consanguinity, rate of progression to KF, time to reach KF, and NS recurrence following KT.

Statistical Analyses

Fisher exact test or Chi-square test was used to compare frequencies between or among groups as appropriate. Parametric data of 2 groups were compared using Student's *t* test, whereas nonparametric data were compared between 2 groups using the Mann-Whitney U test and among more than 2 groups employing the Kruskal-Wallis test. Comparative analyses of kidney survival among groups were assessed using the Kaplan-Meier curve analysis with log-rank test, followed by Cox regression with Firth' penalized maximum likelihood bias reduction to predict the probability of survival in a multivariable model. All tests were performed using the software SPSS version 24.0 (IBM, NY), except for Cox regression, which was carried out using R 4.1. (https://cran.r-project.org/bin/windows/base/).

RESULTS

Patients With MCD Did Not Progress to KF

Following the genetic analysis of the 101 patients, 1 of them was diagnosed with Alport syndrome. He had been referred from another center to this study because he manifested NS at 1 year of age. After this result he was screened and hearing loss was detected when he was aged 11.7 years.[8] Based on the inclusion criteria, the patient with Alport syndrome was excluded from all performed analyses (Supplementary Clinical Data, section S3).

Patients manifested NS at a median age of 2.9 (1.5–6.8) years (Figure 1b). Sixty-one children were self-declared White, 38 were non-White children (1 of whom was Asian), and in 1 this information was missing. Parental consanguinity was reported in 6% of cases, whereas family history of NS and/or FSGS was reported in 13%. FSGS was the most frequently detected kidney histology (54%), whereas CG was diagnosed in 12%, MCD in 19%, and diffuse mesangial sclerosis in 6%.

During the follow-up period of 5.6 (2.4–9.3) years, 44% of patients progressed to KF at 2.5 (1.0–5.2) years and 29% received KT (Figure 1b). Patients who progressed to KF manifested NS at older ages (4.1 [1.5–10.4] vs. 2.6 [1.5–4.0] years, $P = 0.029$), displayed the histological diagnosis of CG more often (11/12 vs. 1/12, $P < 0.001$), and self-declared as non-White more frequently (65% vs. 35%, $P = 0.001$) (Supplementary Table S1). Patients with MCD histology and/or who reached complete remission with calcineurin inhibitors did not progress to KF during a follow-up period of 9.2 ± 1.0 and 10.1 ± 0.9 years, respectively. Of note, multivariable Cox regression analysis revealed that non-MCD histology was a risk factor for progression to KF, independently of the genetic profile (odds ratio: 15.72, 95% confidence interval: 2.11–2007.7, $P = 0.002$) (Supplementary Table S2). Importantly, no significant differences in progression to KF were observed between monogenic and non-monogenic patients when analyzing only the FSGS cases ($P = 0.684$) or the non-MCD ones ($P = 0.311$).

Self-Declared Non-White Patients Presented With Higher Risk of Progression to KF

The global genetic ancestry of all patients was 65% European, 22% African, 10.5% Native American and 2% East-Asian. Among them, 9 displayed >85% European ancestry, one displayed 99% East-Asian ancestry, and none displayed >62% African ancestry (Supplementary Figure S2).

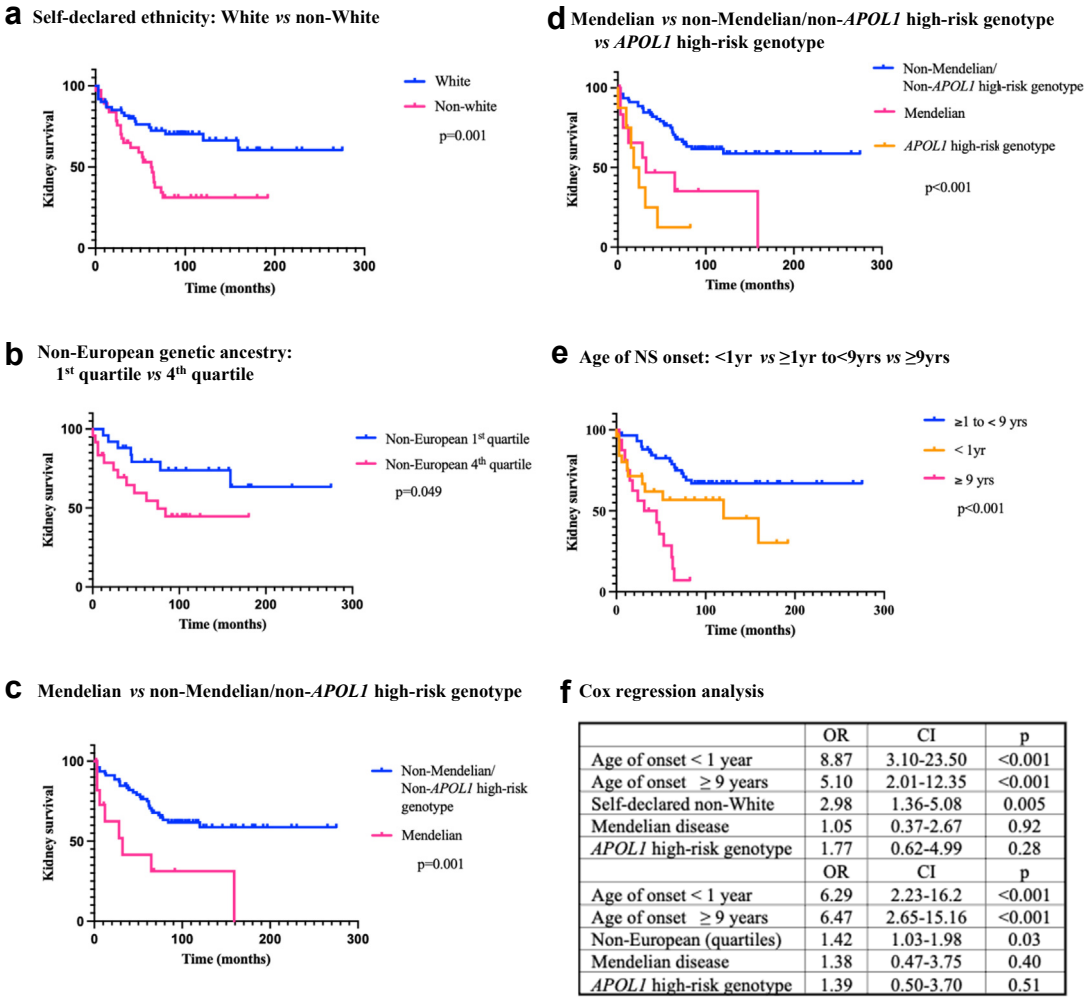
As expected, our analysis revealed higher African ancestry in self-declared non-White patients than in self-declared White patients (29% vs. 15%, $P < 0.001$; Supplementary Figure S3) as well as a higher Native American ancestry (12% vs. 10%, $P = 0.038$; Supplementary Figure S3). In consistency with the African ancestry representation and the observed poorer course of kidney function in self-declared non-White patients (Supplementary Table S1, Figure 2a), patients with higher percentage of non-European genetic ancestry displayed a faster progression to KF ($P = 0.049$; Figure 2b).

APOL1 High-Risk Genotypes Account for a Significant Amount of Identified Genetically Related NS Cases

APOL1 high-risk genotypes were identified in 8% of families (Figure 1c), reaching a frequency similar to the one previously observed in another cohort of Brazilian NS children submitted to KT (8.4%).[16] The component of African genetic ancestry was 28% (14.2–42.4) in *APOL1* high-risk genotype patients, and did not differ among the 3 mentioned groups either (Table 1, Supplementary Figure S3). Our *APOL1* high-risk genotype patients presented with a higher age of NS onset than children with Mendelian causes or without identified genetic basis, and displayed CG or FSGS histology, progression to KF (7/8 patients), and no disease recurrence following KT (4/4 cases) (Tables 1 and 2).

The Rate of Mendelian Causes Is Lower Than Observed in Previous Multicentric Studies

We identified 13 patients with causative Mendelian variants (Figure 1c), encompassing 12% of all families and 14% of families with SRNS or presumed SRNS cases. Interestingly, the detection rate of Mendelian



f Cox regression analysis

	OR	CI	p
Age of onset < 1 year	8.87	3.10-23.50	<0.001
Age of onset ≥ 9 years	5.10	2.01-12.35	<0.001
Self-declared non-White	2.98	1.36-5.08	0.005
Mendelian disease	1.05	0.37-2.67	0.92
APOL1 high-risk genotype	1.77	0.62-4.99	0.28
	OR	CI	p
Age of onset < 1 year	6.29	2.23-16.2	<0.001
Age of onset ≥ 9 years	6.47	2.65-15.16	<0.001
Non-European (quartiles)	1.42	1.03-1.98	0.03
Mendelian disease	1.38	0.47-3.75	0.40
APOL1 high-risk genotype	1.39	0.50-3.70	0.51

Figure 2. (a) Renal survival was lower in patients who self-declared being non-White, and (b) when genetic ancestry was addressed, patients with higher percentages of non-European ancestry reached kidney failure faster than patients with higher percentages of European ancestry. Renal survival after NS onset was lower in patients (c) with Mendelian causes, (d) *APOL1* high-risk genotypes, (e) NS onset before 1 year or at or after 9 years of age. (f) Cox regression multivariable analysis revealed that age of NS onset before 1 year or at or after 9 years of age increase the risk to reach kidney failure. NS, nephrotic syndrome.

forms was 11% for the in-center prevalent cases, a rate numerically but not significantly lower than the one observed in cases referred from external centers (18.5%, $P = 0.318$). Importantly, patients with Mendelian causes progressed faster to KF than the ones with non-Mendelian and non-*APOL1* high-risk genotype-related forms ($P = 0.001$, Figure 2c) and no disease recurrence was observed in all 4 patients submitted to KT (Table 2). None of the identified Mendelian variants was identified in the 9 patients with steroid-dependent NS included for FSGS (7 cases) or familial NS (2 cases) or in 36 patients who achieved complete remission after using calcineurin inhibitors.

Analysis of Pathogenicity: ACMG Criteria and Molecular Mechanics Simulation

Comprehensive analysis of pathogenicity yielded 19 variants considered causative (Table 3): 2 in *NPHS1*, 1 in *NPHS2*, and 2 in *CUBN* were classified as pathogenic, whereas 3 in *NPHS1*, 3 in *NPHS2*, and 2 in *WT1* were classified as likely pathogenic according to ACMG-based pathogenicity criteria. The *NPHS2* p.(Arg229Gln) variant is pathogenic in the identified genetic scenario. Two variants in *COQ2* and 2 in *PLCE1* classified as VUSs had their pathogenicity supported by molecular mechanics simulations. An additional VUS, *PLCE1* c.3582+5G>T, was assessed for potential splice-site disruption according to Jian *et al.*[30] This variant reached an ADA score of 0.9984 and Random Forests of 0.87. Because both scores exceeded the cutoff of 0.6, they strongly supported disruption of the splice site and, consequently, its pathogenicity.

COQ2 is a membrane-embedded molecule with 9 transmembrane helices and an extramembrane cap domain that surrounds a central cavity containing the

Table 1. Frequencies of *APOL1* no risk allele, 1 risk allele and high-risk genotype in self-declared White patients and non-White patients, and percentages of genetic global ancestry (median) and progression to kidney failure in the three *APOL1* genotype status

APOL1 genotype status	*APOL1* 0 risk allele $n = 84$	*APOL1* 1 risk allele $n = 8$	*APOL1* high-risk genotype $n = 8$	*P*
Self-declared ethnicity[a]				
White	53/82 (65%)	5/8 (62.5%)	3/8 (37.5%)	0.319
Non-White	30/82 (35%)	3/8 (37.5%)	5/8 (62.5%)	
Percent genetic ancestry for each *APOL1* genotype group				
European	67% (55–76)	66% (55–76)	58% (49–73)	0.56
African	23% (11–28)	17% (13–30)	28% (14–42)	0.41
Native American	10% (6–13)	12% (7–16)	10% (8–16)	0.62
KF among all patients	33/84 (39%)	4/8 (50%)	7/8 (87.5%)	0.030
KF excluding patients with Mendelian forms	25/70 (36%)	4/8 (50%)	7/8 (87.5%)	0.017

KF, kidney failure.
[a]Total number of patients = 98. One patient with missing data and the Asian patient were excluded from this analysis.

active site.[31,32] The results of molecular mechanics simulations show that the p.(Phe383Leu) and p.(Pro142Ala) variants determine a disruption of several molecular contacts, so that the total potential energy of these molecules becomes greater than in its native version. This instability increases the volume of the active site and may change its transprenylation activity, supporting pathogenicity for both variants (Figure 3a, b, and c; Supplementary Molecular Mechanics Simulation, section 2A). Molecular mechanics analyses also supported pathogenicity for the PLCE1 mutations p.Glu2088 and p.Leu1233 (Supplementary Molecular Mechanics Simulation, section 2B, Supplementary Figure S4).

Table 2. Comparative analyses of demographic and clinical data, family history, self-declared ethnicity, histology, and disease recurrence following kidney transplantation among Mendelian forms versus *APOL1* high-risk genotype forms versus non-*APOL1* high-risk genotype/non-Mendelian forms

Genotype status	Mendelian $n = 13/100$ (13%)	*APOL1* high-risk genotype $n = 8/100$ (8%)	Non-Mendelian/ non-*APOL1* high-risk genotype $n = 79/100$ (79%)	*P*
Age of onset (yr)	0.4 (0.3–3.5)	11 (10.0–14.5)	2.9 (1.7–5.0)	<0.001
KF	8/13 (61%)	7/13 (87.5%)	29/79 (37%)	0.009
Family history of NS or kidney disease	4/13 (33%)	1/13 (12.5%)	8/79 (10%)	0.085
Self-declared ethnicity[a]				
White	10/13 (83%)	3/8 (37.5%)	48/79 (61.5%)	
Non-White	2/13 (17%)	5/8 (62.5%)	30/79 (38.5%)	0.112
Histology[b]				
FSGS	6/11 (54.5%)	3/8 (37.5%)	45/75 (60%)	
MCD			19/75 (25%)	<0.001
DMS	3/11 (27%)		3/75 (4%)	
CG	2/11 (18%)	5/8 (62.5%)	5/75 (7%)	
Other			3/75 (4%)	
NS recurrence	0/4	0/4	10/21 (48%)	0.055

CG, collapsing glomerulopathy; DMS, diffuse mesangial sclerosis; FSGS: focal segmental glomerulosclerosis; KF, kidney failure; MCD, minimal change disease; NS, nephrotic syndrome.
[a]Total number of patients = 98. One patient with missing data, and the only Asian patient was excluded from this analysis.
[b]Patients submitted to kidney biopsy = 94/100.

The Identified Mendelian Profile Has Marked Particularities

Like other studies,[8–10] monogenic causative variants were detected in 60% of patients with CNS and 64% of children with NS onset before 1 year of age (Table 4). Among these cases, causative variants were observed in homozygosity (25%), heterozygosity (17%), or compound heterozygosity (58%). In all homozygous patients, the genetic variant was found in a genomic region of runs of homozygosity, and the inbreeding coefficient from runs of homozygosity indicates some level of inbreeding (Supplementary Table S3).

All 3 patients with CNS with identified Mendelian NS harbored *NPHS1* variants in homozygosity or compound heterozygosity (Table 3). Patient 2 was included in the performed analyses despite the short follow-up time, because our analytical model considers the influence of different follow-up time periods. A fourth patient with CNS presented with 2 *NPHS1* VUSs in compound heterozygosity. One of them is associated with a splice-site change with significant pathogenicity score by *in silico* analysis (*NPHS1*: NM_004646: c.1930+5G>A, ADA score = 1, RF 0.966), whereas molecular mechanics simulations were not able to strongly support a causative role for the other VUS in *NPHS1*, c.716C>T p.(Pro239Leu) (Supplementary Figure S5). Although this variant could potentially affect the interaction between nephrin and NEPH1, a variant with a similar potential effect (*NPHS1* p.[Leu237Pro]) has been analyzed and placed within an uncertain interpretation range of pathogenicity by 2 previous studies.[49,50] Based on these data and the intermediate level of certainty of our data on *NPHS1* p.(Pro239Leu), we did not consider this variant causative, also placing it in the uncertain interpretation range. Moreover, at 2.9 years of age the patient did not present with edema, his serum albumin was 3.45 mg/dl, and estimated glomerular filtration rate was 60 ml/min per 1.73 m^2. In this scenario, this case was not considered a Mendelian form of the disease, though pathogenicity of *NPHS1* p.(Pro239Leu) cannot be excluded. The transference of this patient to the Mendelian group, however, would not change the conclusion on the kidney survival comparison between Mendelian versus non-Mendelian/non-*APOL1* high-risk genotype patients (faster progression to KF in the Mendelian group, $P = 0.005$). The conclusions associated with the Cox regression analyses

Table 3. Variants identified, respective genes, mode of inheritance, segregation, ACMG criteria, gnomAD frequency, classification of pathogenicity, genetic local ancestry, references, age of NS onset, kidney histology, extrarenal manifestations, immunosuppression, time to KF, and time of follow-up

Gene/Inheritance	Pt	Variant/Zygosity	Segregation Analysis	ACMG Criteria	gnomAD	Classif	Inferred Genetic Ancestry	Previously Reported Region/ Countries	Ref	Age of NS onset	Histology	Extrarenal manifestation	IS/Response	Time to KF	Follow up (time)
NPHS1 NM_004646.4 AR	1	c.2132G>A p.(Arg711His)/ Het c.1137T>A p.(Ile446Asn)/ Het	Father Het Mother Het	PM2, PM5, PP2, PP3 PM1, PM2, PP2, PP3, PP5	- 1/0/ 250,602	LP LP	EUR EUR	Maori/New Zealand England/ India	33 34–36	17 d	Not done		None	17 mo	Peritoneal dialysis
	2	c.3286+1G>A/Hom	Not available	PVS1, PM2, PP3	-	P	NAM (ROH)	New	New	10 d	DMS	-	None	-	-
	3	c.514_516delCCA p.(Thr172del)/Het c.2728T>C p.(Ser910Pro)/ Het	Mother Het Father Het	PM1, PM2, PM4, PP3, PP5 PM2, PP2, PP3, PP5	2/0/ 251,116 1/0/ 251,340	P LP	EUR AFR	Netherlands, USA USA, Spain, African American	34,35,37,38 39–41	2 mo	FSGS	-	None	-	Unilateral nephrectomy
NPHS2 NM_014625.4 AR	4	c.506T>C p.(Leu169Pro)/ Hom	Mother Het (Father not available)	PM2, PP2, PP3, PP4, PP5	4/0/ 220,918	LP LP	EUR (ROH)	Italy, Spain	42,43	10 yr	FSGS	Hypothyroidism	Steroid/ Tacro Proteinuria reduction	5.6 yr	Hemodialysis
	5	c.914T>C p.(Leu305Pro)/ Het c.686G>A p.(Arg229Gln)/ Het	Mother Het Father Het	PM1, PM2, PP2, PP3	- 3%	LP	AFR EUR	Greek/Cyprus Africa, Brazil, Spain	44,45 46,47	5.7 yr	FSGS	-	Steroid/Cyclosp/ Rituximab Proteinuria reduction	-	CKD stage 1 (5.6yr)
	6	c.738+1G>C/Het c.928G>A p.(Glu310Lys)/ Het	Father Het Mother Het	PVS1, PM2, PP3, PM1, PM2, PM5, PP2, PP3	-	LP LP	AFR EUR	New Frrance	New 46,48	1.4 yr	Not done	-	Steroid/Cyclosp No remission	2.8 yr	KT age 6.9 yr No recurrence
PLCE1 NM_016341.4 AR	7	c.3698 T>C p.(Leu1233Pro)/Hom	Mother Het Father Het	PM2, PP3, PP4	-	VUS	NAM (ROH)	New	New	5 mo	CG	-	None	2 mo	KT, age 4.2 y No recurrence
	8/ 9	c.4458+5G>T/Het c.6262_6264delGAG p.(Glu2088del)/Het	Mother Het Father Het	PM2, BP4, PP3, PP4 PM2, PM4, PP3	-	VUS VUS	EUR EUR	New New	New New	5 mo 5 mo	DMS DMS	Rectal prolapse Congenital foot deformities Rectal prolapse	Steroid/No remission Steroid/No remission	1 mo 1 mo	KT, age 3 yr No recurrence Peritoneal/ Hemodialysis
COQ2 NM_001358921.2 AR	10	c.1147T>C p.(Phe383Leu)/ Het c.424C>G p.(Pro142Ala)/ Het	Mother Het (Father not available)	PM2, PP3, PP4 PM2, PP3, PP4	- 0/2/ 225,164	VUS VUS	EUR or NAM EUR or NAM	Brazil New	16 New	10 mo	COQ2 CG		Steroid/Cyclosp No remission	10 mo	Peritoneal dialysis
WT1 NM_024426.6 AD	11	c.1447+5G>A/Het	Mother Het[a]	PS3, PM2, BP4	-	LP	EUR	USA/Canada	51	11 mo	FSGS	Streak ovaries	Steroid/ Cyclosp/ Mycophen Mofetil No remission	13 yr	KT, age 14.8 yr No recurrence
	12	c.1447+4C>T/Het	Probably *de novo*[b]	PS3, PM2, BP4	-	LP	EUR	France	52	6.8 yr	FSGS	Hypospadia	None	-	CKD strage 3 (7.6 yr)
CUBN NM_001081.4 AR	13	c.7968_7969insTTATA p.(2657fs)/Het c.3672+1G>C/Het	Mother Het Father Het	PVS1, PM2, PP3 PVS1, PM2, PP3	- -	P P	EUR EUR	New New	New New	5 mo	FSGS	-	None	-	CKD stage 1 (3.5 yr)

AD, autosomal dominant; AFR, African; AR, autosomal recessive; CG, collapsing glomerulopathy; CKD, chronic kidney disease; Classif, classification; Cyclosp, cyclosporine; DMS, diffuse mesangial sclerosis; EUR, European; FSGS, focal segmental glomerulosclerosis; gnomAD, the genome aggregation database; Het, heterozygous; Hom, homozygous; IS, immunosuppression; KF, kidney failure; KT, kidney transplantation; LP, likely pathogenic; NAM, Native American; NS, nephrotic syndrome; P, pathogenic; Pt, patient; Ref, references; ROH, runs of homozygosity; Tacro, tacrolimus; VUS, variant of uncertain significance.

[a]Mother with FSGS.

[b]Absent in mother and father.

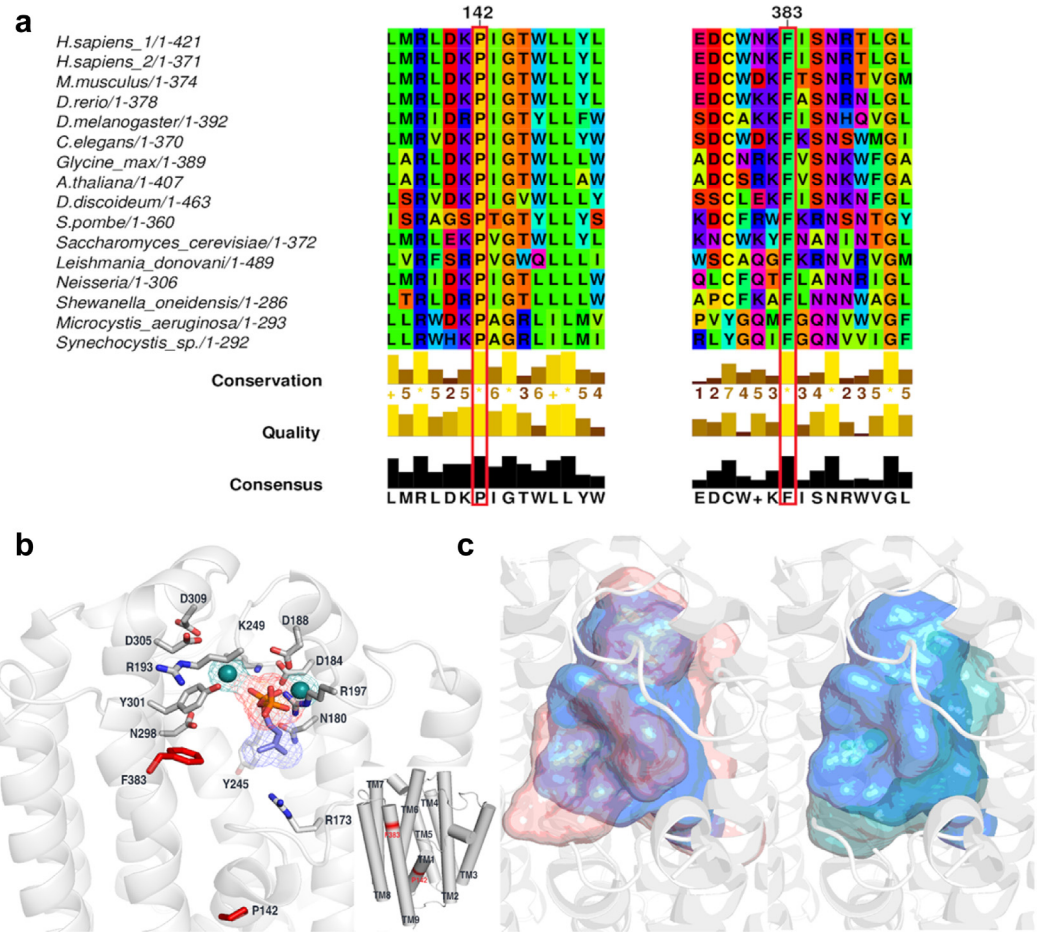


Figure 3. (a) Conservation of COQ2 p.Pro142 and p.Phe383. (b) Structure of p-hydroxibenzoato profenil transferase. COQ2 p.Pro142 and p.Phe383 are close to the active site, possibly changing its volume and, consequently, its transprenylation activity (mutated residues colored in red). (c) Surface volume of the wild type model in blue, p.Pro142 in red, and p.Phe383 in green.

would not be impacted either by the swap of this patient from the non-Mendelian/non-*APOL1* high-risk genotype group to the Mendelian group.

NPHS2 variants in homozygosity or compound heterozygosity accounted for the disease in 3 children with SRNS (Table 3). Although 1 of these patients progressed to KF at 4.2 years of age and received KT with no recurrence, the other 2 presented with reduction of proteinuria with calcineurin inhibitor (Table 3; Supplementary Clinical Data, section 3).

Three new variants in *PLCE1* were found in 3 children from 2 families, in homozygosity or compound heterozygosity (Table 3). All 3 patients developed NS at 5 months of age and were started on peritoneal dialysis by 2 months of disease progression (Table 3; Supplementary Clinical Data, section 3).

The 2 patients with *WT1* splice-site variants had a 46 XY karyotype and presented disorder of sexual development, in agreement with previous descriptions (Table 3; Supplementary Clinical Data, section 3).[51–53]

One of our patients displayed *COQ2* variants in compound heterozygosity (Table 3). This child manifested renal histology of CG, including numerous and dysmorphic mitochondria, a morphological profile fully consistent with COQ2 glomerulopathy. Despite the initiation of CoQ10 replacement just after kidney biopsy, no NS improvement was observed. In this setting, he rapidly progressed to KF.

Patient 13 presented with nephrotic range proteinuria (UP/C 2.56) and edema at the age of 5 months. Histology revealed FSGS. Serologies were negative, the patient did not receive immunosuppression, and he was started on angiotensin-converting enzyme inhibitor. Edema resolved and UP/C became fixed around 1.2 in the following 3.5 years, a finding consistent with partial remission. We therefore hypothesized that there was another factor related to FSGS at disease manifestation (an additional/environmental condition), which likely resolved with time. The fixed proteinuria in the nonnephritic range observed in the follow-up, in turn, is consistent with the typical pathogenic pattern associated with *CUBN* variants. Considering that the *CUBN* variants contributed to the NS presentation, the patient was included in the analyzed Mendelian group.

Table 4. Distribution of clinical and genetic findings according to age of nephrotic syndrome onset

Age of nephrotic syndrome onset	<1 yr $n = 14$	1–8.9 yrs $n = 68$	≥9 yrs $n = 18$	P
Sex				
Male	10 (71%)	42 (62%)	8 (44%)	0.264
Female	4 (29%)	26 (38%)	10 (56%)	
Self-declared ethnicity[a]				
White	12 (92%)	42 (63%)	7 (39%)	
Non-White	1 (8%)	25 (37%)	11 (61%)	0.010
Percent genetic ancestry for each age group				
European	68% (54–73)	68% (57–79)	60% (51–72)	0.495
African	20% (17–28)	21% (11–28)	25.9% (14–38)	0.353
Native American	13% (7–15)	10% (6–13)	10% (8–13)	0.800
East-Asian	0 (0–2)	0 (0–2)	0% (0–1)	0.028
Histology				
FSGS	4 (31%)	39 (62%)	11 (61%)	
MCD	0	19 (30%)	0	<0.001
DMS	6 (46%)	0	0	
CG	3 (23%)	2 (3%)	7 (39%)	
Other	0	3 (5%)		
Mendelian	9 (64%)	3 (4%)	1 (6%)	<0.001
APOL1 high-risk genotype	0	0	8 (44%)	<0.001
KF	9 (64%)	19 (28%)	16 (89%)	<0.001

CG, collapsing glomerulopathy; DMS, diffuse mesangial sclerosis; FSGS, focal segmental glomerulosclerosis: KF, kidney failure; MCD, minimal change disease; NS, nephrotic syndrome.
[a]One patient with missing data and the only Asian patient were excluded from this analysis.

We have also found 4 novel variants of potential autosomal dominant inheritance classified as VUSs; however, they were not considered causative based on the reasons outlined in Supplementary Data of Patients with VUSs (Section 4, and Supplementary Table S4).

The Novel Causative Variants Display a Unique Profile, Including Native American Origin

Among the identified causative variants, 8 (42%) are novel: *NPHS1* (NM_004646.4): c.3286+1G>A; *NPHS2* (NM_014625.4): c.738+1G>A; *CUBN* (NM_001081.4): c.7968_7969insTTATA p.2657fs; *CUBN* (NM_001081.4): c.3672+1G>A; *PLCE1* (NM_016341.4): c.3582+5G>T; *PLCE1* (NM_016341.4): c.6262_6264delGAG p.Glu2088_2088del; *PLCE1* (NM_016341.4): c.3698 T>C p.(Leu1233Pro); and *COQ2* (NM_001358921.2): c.424C>G p.(Pro142Ala).

Importantly, 7 of these variants are absent and 1 is found at a very low frequency in gnomAD (Table 3). By analyzing the ancestry of the specific DNA regions that encompass these novel variants, we showed that 2 arose in the Native American genetic background, 4 in the European, 1 in the African, and 1 could not be distinguished between European and Native American backgrounds. Among the 11 previously reported variants, 8 arose in the European genetic background, 2 in the African, and 1 could not be distinguished between European and Native American backgrounds.

The Age of NS Manifestation is Associated With the Risk of Disease Progression

Multivariable Cox regression analysis revealed a higher risk of progression to KF in self-declared non-White patients, even when adjusted for genetic profiles (hazard ratio: 2.98, 95% confidence interval: 1.36–6.08, $P = 0.005$) (Figure 2f). Moreover, it identified age of NS onset <1 year or ≥9 years as a major risk factor for progression to KF (hazard ratio: 8.87, 95% confidence interval: 3.10–23.5, $P < 0.001$, hazard ratio: 5.1, 95% confidence interval: 2.01–12.35, $P < 0.001$, respectively; Figure 2e). Interestingly, children with NS onset before 1 year of age were predominantly in the self-declared White category (92%), had diffuse mesangial sclerosis as the main histological pattern (46%), and most often presented with Mendelian causes of NS (64%) (Table 4). Conversely, all cases with histology of MCD occurred in patients who manifested NS between ages 1 and 9 years. Children within this age range of NS onset presented with lower risk of progression to KF (Figure 2e and Table 4). Of note, patients who developed NS at or above 9 years of age more often self-declared being non-White and progressed more rapidly to KF than children who manifested NS at ages 1 to 9 years (Figure 2e and Table 4). Moreover, all patients aged ≥9 years presented with CG or FSGS as the histological diagnosis and all children with *APOL1* high-risk genotypes belonged to this age group (Table 4).

DISCUSSION

Most variants in genes associated with Mendelian NS have been described in highly inbreeding and/or predominantly Caucasian populations whereas *APOL1* high-risk genotypes have been associated with NS or FSGS in African Americans, in scenarios that underrepresent the Southern hemisphere and multiethnic admixed populations. The frequencies of Mendelian pediatric NS diverge among previous cohorts, depending on the factors previously outlined.[8–10,16–21] In our patient population, the low rate of consanguinity and familial cases are likely to mostly explain the 12% proportion of Mendelian causes, a lower rate than in most previous cohorts. Multicentric studies identified Mendelian frequencies within the 23.6% to 43.6% range,[8–10,16,17,19–21] whereas a single-center cohort with 72 NS prevalent consecutives cases detected 11.1% of monogenic causes.[18] It must be noted, however, that another Brazilian study including SRNS cases referred for KT, but not patients with CNS, found monogenic causes in only 8.4% of them.[16] In this

study, the *APOL1* high-risk genotype rate was 8%, similar to the frequency observed in our cohort. A British study evaluating Mendelian and *APOL1*-associated forms of NS in pediatric patients, in turn, reported 3 *APOL1* high-risk genotype-related cases in children with African ancestry among 187 patients, likely reflecting the low representation of this ethnic group in this population.[10] In contrast, in another study, 74% of African American children with FSGS were found to harbor *APOL1* high-risk genotypes, whereas no monogenic causes were identified by analyzing 20 NS genes.[13] Our data revealed that self-declared ethnicity and the percentage of African genetic ancestry did not differ among patients harboring *APOL1* high-risk genotypes, 1 *APOL1* risk allele, or no *APOL1* risk allele. Of note, 3 of 8 patients (37.5%) with *APOL1* high-risk genotype self-declared as being White, and revealed only 22% of African ancestry. The low number of *APOL1* high-risk genotype patients, however, limited the power of this analysis.

Other potential contributors to the low Mendelian rate detected in our study include the presence of 9 steroid-dependent NS cases, the low number of patients with NS onset at age <1 year, and the lack of assessment of copy number variations. Moreover, we applied a stringent approach to the pathogenicity classification of variants, and did not assess the potential impact of intronic variants. It is also possible that other genes associated with SRNS or INS not yet described are mutated particularly in Native American and/or African ancestry DNA, because these populations are underrepresented in international genome databases and previous NS genetic studies. Finally, we excluded the Alport syndrome patient from our analyzed population, whereas other casuistries have included such patients.[10,16,19,21] It must be noted, however, that the higher Mendelian rate identified in cases referred from external centers (18.5%) had an increasing effect on the overall frequency, likely reflecting a selection bias in referrals from external institutions.

Only 10% of our patients had European ancestry above 85%, whereas none reached this level of African ancestry. One patient had East-Asian ancestry, whereas the proportion of Native American ancestry was essentially uniform within the assessed population. In contrast, European and African ancestries displayed inversely proportioned distribution in our patient population. This finding is not only in accordance with the significant genetic admixture in the Brazilian population but also indicates that the admixture degree is very high. Our data revealed a genomic continental ancestry roughly like ones previously described in studies analyzing Brazilian populations.[54,55] These reports found predominant European ancestry (~70%) reaching higher percentages in the country's Southern region, followed by 20% African and 10% Native American. The significant ancestry variability among the patients, in fact, is consistent with a cohort with high genetic heterogeneity (Supplementary Figure S2). Interestingly, African ancestry was low even in our patients with *APOL1* high-risk genotypes.

Considering that data on genetic ancestry are not accessible in medical practice, ethno-racial self-declaration is still used as a proxy for genetic ancestry, despite a low correlation between them.[55,56] Association studies using genetic ancestry markers, however, became strategic in recent decades.[56–60] In our study, independently of *APOL1* high-risk genotype, patients with non-European ancestry or self-declared non-White patients progressed faster to KF, conditions found to be risk factors for this outcome. It must be noted that, although the mean genomic ancestry of the KF cases was 29.3% African, this component was still higher than in self-reported White patients. Although these observations raise fundamental questions, self-reported Black and Mixed-ethnicity individuals are more likely to have lower income and lower degree of education, factors that negatively impact health-related outcomes.[60,61]

In agreement with other pediatric studies on SRNS or FSGS,[13,14] our patients with *APOL1* high-risk genotypes developed NS at older ages and all but 1 progressed to KF. The kidney histological patterns associated with *APOL1* high-risk genotype, including only CG and FSGS, are consistent with the worse kidney outcome associated with this genotype status. Although we could not show that patients with only 1 *APOL1* risk allele display a faster progression or are at increased risk of KF, a trend of faster progression was observed in a study including a larger pediatric cohort.[14]

Consistent with currently available data,[8–10] variants in *NPHS1* accounted for most cases with NS onset within the first year of life in whom a Mendelian cause was identified and were responsible for all CNS cases in whom monogenic pathogenic variants were found. Variants in *NPHS2*, in turn, are the most common Mendelian cause of NS within the entire age spectrum, except in Japan and Korea.[8,17,19] Not surprisingly, therefore, *NPHS2* variants were detected in 3 of our 14 patients with Mendelian forms. Interestingly, 1 of such cases displayed compound heterozygosity in trans of p.(Arg229Gln) with the p.(Leu305Pro) variant in exon 8, predicted by ACMG as likely pathogenic. It is expected, as previously described,[48,62] that p.(Leu305Pro) may exert a dominant negative effect in this case, altering the dimerization and localization of p.(Arg229Gln) podocin. Interestingly, 2 of the 3 patients with causative variants in *NPHS2* were the only 2 cases who presented with significant reduction in

proteinuria, but not remission, after therapy with steroid and calcineurin inhibitors. Of note, *in vivo* and *in vitro* data suggest that p.(Arg229Gln) podocin (arg231g in mice) presented with reduced expression and increased proteasomal degradation.[62] Because calcineurin inhibitors can restore the expression of podocin and synaptopodin in puromycin-treated podocytes,[63] its beneficial effect on our patients may have followed this mechanism. Moreover, podocin may have its expression and localization modified in acquired and/or inflammatory diseases,[63] so its expression profile may be restored at some extent in response to immunosuppression.

The disease was associated with *PLCE1* variants in 2 of our families (one of them with 2 affected members) and with *WT1* variants in another 2 families, findings consistent with the observation that variants in these genes are among the most frequent causes of Mendelian NS.[8,9] All 3 identified *PLCE1* variants are novel and had their pathogenicity supported by our comprehensive analysis.

Because CoQ2 belongs to the CoQ10 biogenesis pathway, pathogenic variants in *COQ2* directly affect mitochondrial function, and have been associated with SRNS.[64] One of our patients, who developed CG associated with dysmorphic mitochondria in podocytes, harbored two *COQ2* variants in the setting of histological and mitochondrial features typical of COQ2 nephropathy. In patients with this disorder, reduction of proteinuria has been described following CoQ10 replacement.[65] In our patient, however, this goal was not achieved, likely due to the delay in starting the treatment and, maybe, the functional impact of his variants.[64,65]

Pathogenic variants in *CUBN* have been described as phenocopies of SRNS. *CUBN* encodes cubilin, an uptake receptor involved in albumin reabsorption in the proximal tubule. Although causative variants located 5′ to or at exon 8 are associated with vitamin B12 intestinal malabsorption, causative mutations located 3′ to this exon are associated with tubular proteinuria and appear not to affect long-term kidney function.[66]

A notable finding of our study is that 25% of the identified novel variants arose in the Native American genetic background. To our knowledge, this is the first time that local genetic ancestry has been addressed in the analysis of Mendelian variants associated with NS. This approach is particularly important in admixed populations such as in Brazil, allowing the identification of causative variants of different ethnical origins playing a pathogenic role in distinct genetic backgrounds.

Consistent with previous studies, patients with SSNS displaying FSGS histology who presented with remission after treatment with calcineurin inhibitor were less likely to have Mendelian disease.[67] Moreover, none of the FSGS patients who achieved remission after steroid or calcineurin inhibitor therapy harbored an *APOL1* high-risk genotype. Patients with Mendelian forms, followed by *APOL1* high-risk genotype, progressed to KF faster than non-Mendelian/non-*APOL1* high-risk genotype children. This data support increased severity for NS cases associated with identified genetic causes. The patient with variants in *CUBN*, in turn, will probably have a benign course.[66] Notably, however, multivariable Cox analyses identified NS onset at age <1 year or ≥ 9 years of age, self-declared non-White ethnicity, and non-MCD histology as factors associated with increased risk of progression to KF, independently of the genetic profiles. Age of SRNS onset, therefore, is a risk factor to reach KF. Interestingly, whereas age <1 year comprised most identified Mendelian cases, age of ≥ 9 years included all patients with *APOL1* high-risk genotype, indicating that genetic and likely non-genetic factors contribute to disease severity in these age ranges. In contrast, patients with SRNS with disease onset within the 1 to 9-year age range were less likely to reach KF. It is important to highlight, therefore, the need for risk stratification in patients with SRNS, particularly with an age of onset of 1 to 9 years, where many patients do not progress to KF and respond to immunosuppression. Moreover, further investigation addressing other factors potentially associated with remission after using calcineurin inhibitors may contribute to KF risk stratification, including evaluation of edema severity, proteinuria, and albuminemia at disease presentation, as well as kidney histological patterns/markers.

MCD kidney histology was present in 26% of children with no Mendelian variants or *APOL1* high-risk genotypes and was not observed in any patient with *APOL1* high-risk genotype or causative Mendelian variant. These data indicate that SRNS associated with underlying genetic causes is almost never expressed as MCD. All patients with MCD did not reach KF, in line with a histology associated with relatively preserved renal function. In contrast, all but 1 patient with CG progressed to KF.

All transplanted patients with Mendelian causes or those with *APOL1* high-risk genotype did not exhibit disease recurrence in the graft, as opposed to 48% recurrence in non-Mendelian/non-*APOL1* high-risk genotype children. These findings agree with the current concept that recurrence is much less likely to occur in patients with SRNS with genetic causes or susceptibility.[10,14,16]

In conclusion, this study analyzed key features of a highly admixed Brazilian cohort of pediatric patients with SRNS or CNS or FSGS, a profile underrepresented

in the current NS literature. More than 20% of the cases were identified with genetic causes, including a lower rate of Mendelian forms compared to most previous cohorts but a significant representation of *APOL1* high-risk genotype. Patients with Mendelian and *APOL1* high-risk genotype forms had a faster decline in renal function and the Mendelian, *APOL1* high-risk genotype, and non-Mendelian/non *APOL1* high-risk genotype groups presented with different ages of NS onset and kidney histological patterns. Interestingly, NS onset at <1 year of life and at or after the age of 9 years associated with worse renal outcome. Higher genomic non-European ancestry was also a risk factor to KF. Of note, self-declared ethnicity and the percentage of African genetic ancestry did not differ among groups with different *APOL1* genotype status; however, it must be pointed out that the number of patients with *APOL1* high-risk genotype in the cohort was limited. The finding of 2 novel variants that originated in the Native American genetic background underscores the need of investigating pediatric NS in developing countries. Our study expands the genetic-clinical-histological associations in pediatric NS and opens the analysis of Mendelian variants to genetic ancestry investigation, contributing to a better and wider understanding of NS pathogenesis.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

AW designed the study, carried out the experiments, acquired, analyzed, and interpreted the data, drafted and critically reviewed the article. PDM de MN carried out the experiments, acquired, analyzed, and interpreted the data. KN analyzed and interpreted the genetic ancestry data. AML analyzed and interpreted the genomic data. EHW analyzed and interpreted the data. FMF analyzed the *in silico* molecular mechanics experiments. DMAM acquired the data. AMN carried out the experiments. MSG carried out the experiments. SAA acquired the data. TMC acquired the data. JSF acquired the data. VMSB acquired the data. MHV acquired the data. FH analyzed and interpreted the genomic and phenotype data. MGS analyzed and interpreted the genomic and phenotype data and critically reviewed the manuscript. LFO designed the study, analyzed, and interpreted the genomic and phenotype data, and critically reviewed the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Section 1: Supplementary Methods.

Section 1A: Molecular genetic analyses.

Section 1B: Molecular mechanics evaluation.

Section 1C: Analysis of genetic ancestry.

Section 2: Supplementary Molecular Mechanics Simulation.

Section 2A: Molecular mechanics simulations for COQ2.

Section 2B. Molecular mechanics simulations for PLCE1.

Section 3: Supplementary Clinical Data.

Section 4: Supplementary Data of Patients with Variants of Uncertain Significance.

Supplementary Reference.

Figure S1. Cohort and study overview.

Figure S2. Global genetic ancestry inference. The column represents the proportion of genetic ancestries inferred for each patient. The colors blue, red, green and orange correspond to African, European, Native American and East Asia ancestry, respectively.

Figure S3. Comparison of European, African and Native American ancestry between self-declared White and non-White categories, and among *APOL1* 0RA (risk allele), 1RA and high-risk genotypes.

Figure S4. PLCE1: superimposition of the PLCE1 model and the 2 mutated domains EF hand (red) and RA1 (magenta).

Figure S5. NPHS1: superimposition between the average NPHS1 wild-type (green shades) and p.Pro239Leu mutated (red shades) models.

Table S1. Comparative analyses of age of NS onset, self-declared ethnicity, kidney histology, and response to immunosuppression between patients who progressed and did not progress to kidney failure.

Table S2. Cox regression analysis: age of NS onset, self-declared non-White status, kidney histology, and progression to kidney failure.

Table S3. Runs of homozygosity inference (ROH), homozygosity-based inbreeding coefficient (FROH), and size (Mb) of ROH observed in specific genomic regions, for each NS related gene.

Table S4. Variants of uncertain significance identified, respective genes, mode of inheritance, segregation, ACMG criteria, gnomAD frequency, classification of pathogenicity, age of NS onset, kidney histology, immunosuppression, time to kidney failure, and follow-up time.

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