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Monogenic Causes Identified in 23.68% of Children with Steroid-Resistant Nephrotic Syndrome: A Single-Centre Study

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Keywords
Steroid-resistant nephrotic syndrome · Focal segmental glomerulosclerosis · Monogenic causes

Abstract
Introduction: Steroid-resistant nephrotic syndrome (SRNS) is the second most common cause of end-stage kidney disease in children, mostly associated with focal segmental glomerulosclerosis (FSGS). Advances in genomic science have enabled the identification of causative variants in 20–30% of SRNS patients. ***Methods:*** We used whole exome sequencing to explore the genetic causes of SRNS in children. Totally, 101 patients with SRNS and 13 patients with nephrotic proteinuria and FSGS were retrospectively enrolled in our hospital between 2018 and 2022. For the known monogenic causes analysis, we generated a known SRNS gene list of 71 genes through reviewing the OMIM database and literature. ***Results:*** Causative variants were identified in 23.68% of our cohort, and the most frequently mutated genes in our cohort were WT1 (7/27), NPHS1 (3/27), ADCK4 (3/27), and ANLN (2/27). Five patients carried variants in phenocopy genes, including MYH9, MAFB, TTC21B, AGRN, and FAT4. The variant detection rate was the highest in the two subtype groups with congenital nephrotic syndrome and syndromic SRNS. In total, 68.75% of variants we identified were novel and have not been previously reported in the literature. ***Conclusion:*** Comprehensive genetic analysis is key to realizing the clinical benefits of a genetic diagnosis. We suggest that all children with SRNS undergo genetic testing, especially those with early-onset and extrarenal phenotypes.

Introduction

Nephrotic syndrome (NS) is the most common glomerular disease in children and adults, characterized by massive proteinuria (nephrotic range), hypoalbuminemia, and generalized oedema [1]. Based on the response to glucocorticoid therapy, NS is classified into steroid-sensitive nephrotic syndrome or steroid-resistant nephrotic syndrome (SRNS), with the latter related to an increased risk of developing chronic kidney diseases (CKD) or end-stage kidney disease (ESKD) [2, 3]. SRNS is highly phenotypically and genetically heterogeneous, which can either be a part of syndromic diseases or present as isolated proteinuria or fully developed NS. Focal segmental glomerulosclerosis (FSGS) is the most frequent histopathologic finding in SRNS [4].

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Table 1. Clinical characteristics of the 114 patients with SRNS/FSGS

Characteristic	All patients (*N* = 114)
Sex (male/female)	68/46
Family history of nephropathy	9
Age at onset, *n* (%)	
0–3 months	4 (3.51)
4 months–1 year	5 (4.39)
1–5 years	81 (71.05)
6–12 years	24 (21.05)
Form	
Isolated SRNS	92 (80.70)
Syndromic SRNS	9 (7.89)
Proteinuria with FSGS	13 (11.40)
Renal biopsy	
FSGS	51 (44.74)
MesPGN	19 (16.67)
MCN	11 (9.65)
MN	2 (1.75)
Other	22 (19.30)
Unavailable	9 (7.89)
Follow-up	
Duration of follow-up	4.25 years (IQR 2.73–7)
ESRD at last observation	13
Time to ESRD	4.08 years (IQR 3.58–5.33)
Loss of follow-up	10

FSGS, focal segmental glomerulosclerosis; MesPGN, mesangial proliferative glomerulonephritis; MCN, minimal change nephrotic syndrome; MN, membranous nephropathy; ESKD, end-stage kidney disease.

Comprehensive genetic screening has identified variants of genes essential for podocyte function in about 20–30% of children with SRNS [5–8]. More than 70 genes to date are known to cause SRNS and/or FSGS [9–11]. It is necessary to identify the genetic causes of SRNS because patients diagnosed could benefit significantly from discontinuation of ineffective immunosuppressive therapy. Additionally, identification of a variant in genes such as COQ2, COQ6, and ADCK4, which encode proteins in the CoQ10 biosynthesis pathway, may suggest a potential treatment (CoQ10 therapy) indicated for some rare monogenic nephropathies [12].

Whole exome sequencing (WES) has been widely applied to identify monogenetic causes of SRNS. Although it has limitations such as high costs and difficulty in handling enormous amounts of data, WES is a more efficient and unbiased approach to exploring new candidate genes compared to Sanger and panel sequencing [9]. The proportion of disease-causing variants in a single SRNS gene varied widely, depending on the characteristics of the population tested, including their ethnicity and nationality [4, 8, 13, 14]. As novel pathogenic genes are being discovered, the genetic variant profiles are changing. In this study, we sought to further investigate the monogenic causes of SRNS in our single centre using WES.

Materials and Methods

Human Participants

The study was approved by the Institutional Review Board of the Affiliated Children's Hospital of Nanjing Medical University. From January 2018 to December 2022, patients were enrolled based on the fulfilment of one of the following criteria: (1) onset of symptoms before 18 years and a clinical diagnosis of SRNS; (2) nephrotic range proteinuria with renal biopsy of FSGS; or (3) congenital onset. Exclusion criteria included (1) patients with age of onset beyond 18 years; (2) secondary causes of NS (infections such as syphilis, hepatitis B, and hepatitis C, as well as systemic diseases); and (3) diagnosis of Alport syndrome. NS was defined as proteinuria ≥50 mg/kg per 24 h with hypoalbuminemia or oedema, and steroid resistance was defined as persistent proteinuria after 4 weeks of daily 2 mg/kg prednisone treatment.

A total of 114 unrelated patients participated in the study. After receiving informed consent from their family members, blood samples and comprehensive clinical data were collected and analysed.

WES and Variant Calling

Genomic DNA was extracted from peripheral blood following standard procedures. WES was performed on an Illumina HiSeq 2000 (Bio-Rad, Hercules, CA, USA) using 2 × 100-bp paired-end reads. Variants with allele frequencies higher than 1% were filtered out. The minor allele frequency (MAF) was annotated using databases Genome Aggregation Database (gnomAD), dbSNP, 1000 Genomes MAF (Chinese), ExAC, and an in-house MAF database.

Known SRNS Gene Analysis

We generated a list of 71 known disease-causing genes through discussion, combined with the genotype and phenotype according to OMIM and literature review (online suppl. Table S1; for all online suppl. material, see https://doi.org/10.1159/000534853). The candidate variants were validated by Sanger sequencing, and the diagnostic variants were defined as "pathogenic" or "likely pathogenic" according to the American College of Medical Genetics and Genomics (ACMG) guidelines and included the variants of uncertain significance (VUS). The functional significance of unpublished variants was evaluated by SIFT [15], PolyPhen 2 [16], and Mutation Taster [17].

Statistics

Throughout the manuscript, data are given as medians (interquartile ranges) for continuous variables or percentages for categorical variables, respectively, analysed by the *t* test or χ^2 test. The statistical analysis was performed through SPSS version 26.0 (SPSS, Armonk, NY, USA).

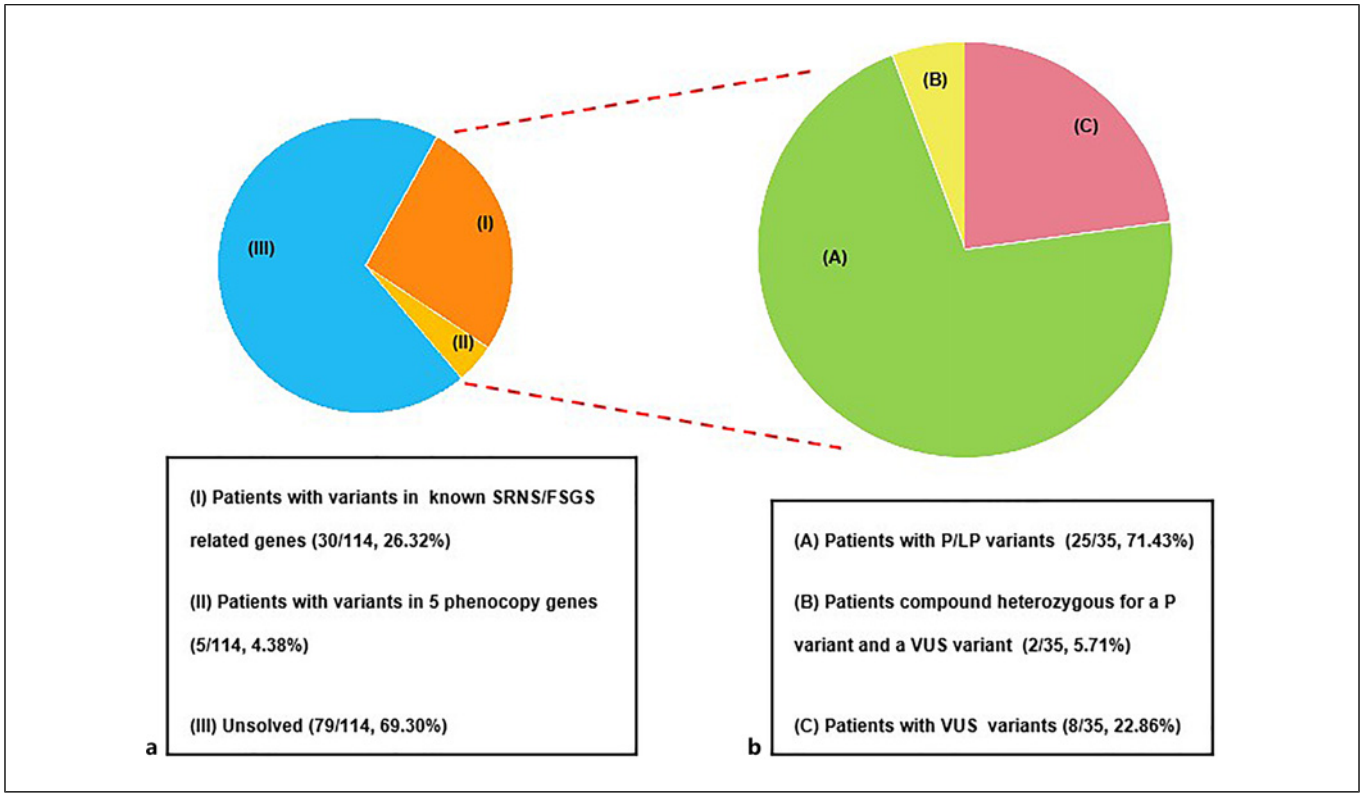

Fig. 1. Molecular diagnostic yield in our cohort and pathogenicity of variants in 35 patients with SRNS/FSGS. **a** Number and percentage of 114 patients in which variants in the known SRNS/FSGS-related genes (26.32%) or in phenocopy genes (4.38%) were detected by WES. **b** Pathogenicity of variants identified in 35 patients according to the ACMG/AMP guideline. 71.43% of patients carried P/LP level variants. 5.71% of patients were found to be compound heterozygous for a P variant and a VUS variant, and 22.86% had VUS. VUS, variant of uncertain significance; LP, likely pathogenic; P, pathogenic.

Results

Characteristics of Cohort

A total of 114 unrelated index patients (68 males, 46 females) with a clinical diagnosis of SRNS or nephrotic proteinuria with FSGS were enrolled in this study. Their age of onset ranged from 9 days to 12.96 years with a median age of 2.96 years (IQR 1.94–5.19). The main demographic and clinical characteristics of the patients are summarized in Table 1.

Monogenic Profile of SRNS

We performed WES in 114 unrelated patients and totally identified 48 candidate variants in 35 patients. As shown in Figure 1a, 26.32% (30/114) had variants in 14 known SRNS/FSGS-related genes and 4.38% (5/114) had variants in five phenocopy genes, including MYH9, MAFB, TTC21B, AGRN, and FAT4. The overall detection rate of causal variants in our SRNS cohort was 23.68% (27/114), including 25 patients with pathogenic (P) or likely pathogenic (LP) variants and 2 patients carrying compound heterozygous variants with VUS/P level in MYO1E and LAMB2, respectively (shown in Fig. 1b; online suppl. Table S2). In another 8 patients, 11 VUS were reported (shown in Fig. 1b). In particular, 55.56% (15/27) of them were diagnosed with autosomal dominant disease and 44.44% (12/27) with autosomal recessive disease. The most frequently mutated genes in our cohort were WT1 (7/27), NPHS1 (3/27), ADCK4 (3/27), and ANLN (2/27), comprising 55.56% of all causative variants identified (Table 2). NPHS2, which dominated other reports, was absent in the present study. Among all variants identified (including VUS), 33 out of the 48 variants (68.75%) were novel variants that have not been reported in the literature. A detailed description of all the 48 variants is listed in online supplementary Tables S2 and S3.

Genotype-Phenotype Correlations

In the cohort, syndromic SRNS accounted for 7.89% of the patients (9/114), and SRNS occurred as an isolated kidney disease in 80.70% of the patients (92/114). We

Table 2. Mutated genes in 27 patients with a known monogenic cause in the cohort

Gene	Inheritance	Patients, *n*
Known SRNS/FSGS genes		
WT1	AD	7
NPHS1	AR	3
ADCK4	AR	3
ANLN	AD	2
TRPC6	AD	1
PAX2	AD	1
PLCE1	AR	1
ACTN4	AD	1
MYO1E	AR	1
LAMB2	AR	1
LMX1B	AD	1
Phenocopy genes		
MYH9	AD	1
MAFB	AD	1
FAT4	AR	1
TTC21B	AR	1
AGRN	AR	1

SRNS, steroid-resistant nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; AD, autosomal dominant; AR, autosomal recessive.

compared the detection rates between different forms and showed that the rate of syndromic SRNS was significantly higher than that of isolated ones (88.89% vs. 10.86%, $p < 0.05$). Proteinuria with FSGS had a detection rate of 61.53%. Among patients with a causative variant, neurological abnormalities including intellectual disability and facial dysmorphism were the most common extrarenal manifestation, followed by abnormality of genitalia (Table 3).

The distribution and frequencies of variants in SRNS/FSGS genes differed depending on the age of onset. The highest proportion of genetic variants was found in the congenital group (4/4 or 100.00% of the patients). Variants in only NPHS1 and WT1 were identified in this group. In the infantile-onset group (from 4 months to 1 year), three out of the 5 patients (60.00%) had variants in PLCE1, LAMB2, and ADCK4. Furthermore, variants were detected in 18.52% of the early childhood-onset (1–5 years) and 16.67% of the late childhood-onset groups (6–12 years).

Kidney biopsy results were available for 106 patients (4 patients with congenital nephrotic syndrome (CNS) were not biopsied, and the results of 4 patients were not available), including 51 cases of FSGS, 19 cases of mesangial proliferative glomerulonephritis, and 11 patients with minimal change nephropathy. Causative variants were identified in 15 of the 51 patients with FSGS (29.41%), while none of the patients with MsPGN either minimal change nephropathy had a genetic diagnosis.

WT1 was the most common causative gene (7/27), leading to the diagnoses of Frasier syndrome in patients 1, 4, and 5, Denys-Drash syndrome in patients 6 and 7, and SRNS in patients 2 and 3 (online suppl. Table S2). The variants were all located in exons 8 and 9 and the intronic region of WT1, except for one truncating mutation in exon 10. Three unrelated cases were found to be caused by the same WT1 variants, c.1447 + 5(IVS9)G>A, one presenting with isolated SRNS, and two presenting with Frasier syndrome. In addition, we identified two novel variants c.1450delT (p.C484Vfs*16) and c.1367G>C (p.C456S) in WT1. The most common histopathological finding in WT1 disease was FSGS (4/7). Three cases developed into ESKD, one developed into CKD stage 4, and two developed into CKD stage 1 during the period of follow-up period.

NPHS1 was the most commonly mutated gene in the subtype of CNS in our cohort (3/4), with six different causal variants identified in 3 patients. Among them, c.1740G>C (p.W580C), c.3213delG (p.L1072Ffs*71), and c.616(exon6)C>A (p.P206T) were three novel variants. Besides, patient 9, who carried compound heterozygous variants c.3213delG (p.L1072Ffs*71) and c.2663G>A (p.R888K), also presented with epilepsy except for CNS.

All ADCK4 variants were identified in 3 patients diagnosed with proteinuria with FSGS. All 3 patients carried compound heterozygous variants and had a same variant c.748(exon9)G>C (p.D250H). All 3 patients received CoQ10 supplementation, and their renal function remained at CKD stage 1.

Phenocopies

In addition to the known SRNS-related genes, we identified phenocopy gene variants in 5 patients (online suppl. Table S3). They were patient 31 with a MYH9 variant (usually associated with macrothrombocytopenia and granulocyte inclusions with or without nephritis or sensorineural hearing loss [18]), patient 32 with an MAFB variant (usually associated with multicentric carpotarsal osteolysis syndrome [19]), patient 33 with a TTC21B variant (usually associated with nephronophthisis [20]), patient 34 with an AGRN variant (usually associated with myasthenic syndrome [21]), and patient 35 with a FAT4 variant (usually associated with Van Maldergem syndrome 2 [22]).

Patient 32

The patient presented at age two with oedema, massive proteinuria, and hypoalbuminemia. She was refractory to steroid therapy and developed renal dysfunction immediately,

Table 3. Extrarenal manifestations of 8 patients with a causative variant

Patient	Gene	Extrarenal manifestations
1	WT1	Male pseudohermaphroditism
4	WT1	Male pseudohermaphroditism
5	WT1	Male pseudohermaphroditism
6	WT1	Clitoral hypertrophy
9	NPHS1	Epilepsy
25	ACTN4	Intellectual disability Patent ductus arteriosus Facial dysmorphism (ocular hypertelorism, thick lip)
32	MAFB	Intellectual disability Facial dysmorphism (strabismus, small jaw) Claw hands
34	AGRN	Bilateral ptosis
35	FAT4	Autism Intellectual disability

Table 4. Characteristics of genetic variants in large cohort studies in Asian population

Year	2022	2020	2020	2019	2017
Region	China	Korea	Japan	China	China
Method	NGS	TES, WES	WES	TGS, WES	Targeted NGS
Detection rate, *n* (%)	106/283 (37.46)	127/29 (143.64)	69 of 230 (30.00)	94/281 (33.45)	34/120 (28.33)
Common genes with disease-causing variants, *n* (%)	WT1 32 (30.19) NPHS1 18 (16.98) NPHS2 15 (14.15) ADCK4 13 (12.26) TRPC6 6 (5.67)	WT1 30 (23.62) COQ6 12 (9.45) NPHS1 11 (8.66) NUP107 9 (7.09) COQ8B 8 (6.30)	WT1 17 (24.64) NPHS1 8 (11.59) INF2 8 (11.59) TRPC6 7 (10.14) LAMB2 6 (8.70)	ADCK4 16 (17.02) WT1 15 (15.96) NPHS1 8 (8.51) INF2 6 (6.38) PLCE1 6 (6.38)	ADCK4 8 (23.53) NPHS1 7 (20.59) WT1 7 (20.59) NPHS2 4 (11.76)

NGS, next generation sequencing; WES, whole exome sequencing; TES, targeted exome sequencing; TGS, target gene sequencing.

1 month after diagnosis. Then she had a renal biopsy that demonstrated diffuse sclerosing glomerulonephritis. Patient 32 was a test-tube baby. She exhibited intellectual disability (unable to speak at the age of two), facial dysmorphism (strabismus and small jaw), and claw hand. Molecular diagnostics demonstrated a well-described heterozygous variant c.212C>T (p.P71L) in the MAFB gene. Her parents and twin sister were healthy and have had no abnormalities in their genetic tests.

Patient 34

The patient presented with bilateral ptosis at birth, and the neostigmine test could not improve it. He underwent a surgery for congenital ptosis at the age of three. When the patient was 5 years old, he was diagnosed of SRNS, and the percutaneous renal biopsy revealed IgM nephropathy. Therefore, he received concomitant immunosuppressive therapy with tacrolimus. Genetic analysis revealed two novel compound heterozygous variants in the AGRN gene: c.125A>C (p.E42A) and c.3473A>G (p.K1158R).

Patient 35

He developed oedema of both lower limbs and eyelids, massive proteinuria, and hypoproteinaemia at the age of 2. After 4 weeks of the standard dose of steroid treatment, he was diagnosed with SRNS. The child has autism spectrum disorder and is still nonverbal at age 2. His parents were healthy with negative genetic tests, and his grandmother had an intellectual disability. We identified two novel compound heterozygous variants, c.1418_c.1419 ins ACTGCTG (p. P473Pfs*31) and c.2200 T>C (p.S734P) in the FAT4 gene.

Table 5. Characteristics of genetic variants in large cohort studies in Western countries

Year	2018	2018	2018	2017	2016	2015	2014
Region	USA, Canada, New Zealand, UK, Nigeria, and Sri Lanka	European countries, the Middle East and Latin America	USA	UK	UK	Europe, the Middle East and Latin America	USA
Method	NGS	NGS	WES	NGS	WES	NA	TES
Detection rate, *n* (%)	40/181 (22.10)	373/1,554 (24.00)	74/300 (24.67)	44/209 (21.05)	49/187 (26.20)	277/1,174 (23.59)	526/1,783 (29.50)
Common genes with disease-causing variants, *n* (%)	INF2 12 (30.00) COL4A3 5 (12.50) WT1 4 (10.00)	NPHS2 156 (41.8) WT1 59 (15.7) NPHS1 47 (12.6) SMARCAL1 20 (5.4)	NPHS1 13 (17.57) PLCE1 11 (14.87) NPHS2 8 (10.81) SMARCAL1 8 (10.81)	NPHS1 12 (27.3) WT1 9 (20.45) NPHS2 7 (15.91) LMX1B 4 (9.09)	NPHS1 14 (28.6) NPHS2 12 (24.5) WT1 4 (8.16)	NPHS2 138 (49.82) WT1 48 (17.33) NPHS1 41 (14.80)	NPHS2 177 (33.65) NPHS1 131 (24.90) WT1 85 (16.16) PLCE1 37 (7.03)

NGS, next generation sequencing; WES, whole exome sequencing; TES, targeted exome sequencing; NA, not available.

Discussion

More than 70 disease-causing genes for SRNS/FSGS have been identified due to the advent of modern technologies such as genetic mapping and WES. In our cohort with 114 children, the detection rate was 23.68% (27/114) and WT1 was the most frequently mutated gene, followed by NPHS1, ADCK4, and ANLN. In contrast to previous studies, novel variants that have not been previously reported accounted for a high proportion of the variants found in our study, comprising 68.75%. Two patients carried compound heterozygous variants with VUS/P level in MYO1E and LAMB2, respectively. Although they carried a VUS variant in one allele, considering that their phenotypes were highly specific to SRNS, and no other causative genes related to kidney diseases were identified in WES analysis, we defined them as diagnostic variants.

The detection rates have been reported to be related to several factors, including the form of SRNS, age at onset, pathological phenotype, and ethnic background. In our study, patients with the syndromic type had significantly higher detection rates than those of isolated SRNS, while the detection rate of proteinuria combined with FSGS was as high as 61.53%, suggesting that genetic testing should be recommended for children with unexplained proteinuria combined with FSGS. The variant detection rate was the highest in children with CNS and decreased significantly with increasing age at the first manifestation during the first 5 years of life.

Previous studies have indicated that there are differences in the distribution and frequencies of variants across different ethnic background and regions. The ethnic background might play a role in the incidence of these variants. We compared the characteristics of genetic variants in large studies that enrolled more than 100 patients over the past decade [4–8, 11, 13, 14, 23–26] (Tables 4, 5). The overall detection rate in these studies ranged from 21.05% to 43.64%. Several studies conducted in western countries showed that the three most commonly mutated genes include NPHS1, NPHS2, and WT1 [5, 6, 8, 13, 25]. However, an international cohort study evaluating 492 individuals from 181 families showed that abnormalities in INF2 (30.00%), COL4A3 (12.50%), and WT1 (10.00%) were most commonly identified ones [7]. In another study from the USA, NPHS1 (17.57%), PLCE1 (14.87%), and NPHS2 (10.81%) are the three major causative genes [4]. SMARCAL1 is also a commonly mutated gene in western countries with frequencies of 5.4% and 10.81% in two large studies [4, 8]. It has been suggested in several studies that the prevalence of NPHS2 variants is lower in Asian countries [11, 14], but a study in China showed that the NPHS2

variants were one of the most common causes, accounting for 14.15% of 106 patients with disease-causing variants [23]. In addition, the higher frequency of ADCK4 gene mutations was noted, especially in China, where the frequencies were 23.53%, 17.02%, and 12.26% in the three studies [13, 23, 26].

Glomerulopathy due to ADCK4 variants has been reported to be identified primarily in adolescents with rapid progression to ESKD [27]. In our cohort, the onset age of three children was below 3 years, and all of them carried 250-position variants, including three p.D250H and one p.D250N. These characteristics are similar to those of another multi-centre study in China [23]. More interestingly, the D250H variant has been found only in the Chinese population so far [23, 26].

ADCK4 interacts with components of the CoQ10 biosynthesis pathway, and patients with ADCK4 mutations have reduced cellular CoQ10 content. It has been suggested that oral administration of CoQ10 may reverse proteinuria and stabilize renal function if initiated early during the course of the disease [28]. Three patients in our study were treated with CoQ10 supplementation immediately after testing positive for ADCK4 gene variants, and their renal function remained at CKD stage 1.

Phenotyping plays an important role in the diagnosis of SRNS in children. Five cases in our study were genetically proven phenocopies. Among them, we identified two novel compound heterozygous variants of the FAT4 gene in patient 35. FAT4 variants cause Van Maldergem syndrome and Hennekam syndrome, both autosomal recessive disorders characterized by typical facial gestalt and mild to moderate intellectual disability [29]. Some of them with FAT4 variants showed renal hypoplasia. FAT4 gene has only been reported to be associated with SRNS in one study, with left kidney hypodysplasia present in the proband [30]. Patient 35 in our study showed a syndromic phenotype, with both SRNS and autism spectrum disorders, without renal dysplasia. Furthermore, we identified two novel compound heterozygous variants of the AGRN gene in patient 34, who showed bilateral ptosis immediately at birth and developed SRNS at the age of 5. The AGRN gene encodes the heparan sulphate proteoglycan which plays a central role in the formation and maintenance of the neuromuscular junction and directs key events in postsynaptic differentiation. It is also expressed in the basement membranes of the kidneys [31]. Bilateral ptosis in patient 34 was consistent with clinical features reported in previous studies [32]. However, it is the first time that variants in AGRN have been identified in SRNS patients.

In conclusion, the overall mutation detection rate in this cohort of 114 children with SRNS was 23.68%. The most frequently mutated genes included WT1, NPHS1, ADCK4, and ANLN. For the first time, we identified variants in the AGRN gene in children with SRNS. We suggest that all children with SRNS undergo genetic testing, especially those with early-onset and extrarenal phenotypes.

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Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of Children's Hospital of Nanjing Medical University, approval number [202306015-1]. Written informed consents were obtained from the participants' parent/legal guardian to participate in the study.

Conflict of Interest Statement

All authors declare no conflicts of interest.

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Author Contributions

Luyan Zhang, Bixia Zheng, and Aihua Zhang conceived the study. Luyan Zhang, Fei Zhao, Guixia Ding, and Ying Chen participated in data preparation and analyses. Luyan Zhang, Sanlong Zhao, Qiuxia Chen, Yugen Sha, and Ruochen Che contributed towards drafting and critically revising the manuscript. Songming Huang, Aihua Zhang, and Bixia Zheng reviewed the final manuscript and agreed to be accountable for all aspects of the work. All authors approved the final version of the manuscript.

Data Availability Statement

All data generated or analysed during this study are included in this article and its online supplementary material. Further enquiries can be directed to the corresponding author.

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