



CLINICAL RESEARCH ARTICLE

Mutational spectrum and novel candidate genes in Chinese children with sporadic steroid-resistant nephrotic syndrome

Jianguo Li¹, Lijun Wang², Ling Wan³, Tiantian Lin³, Wentao Zhao⁴, Hang Cui⁴, Huarong Li³, Li Cao³, Jianxin Wu⁵ and Ting Zhang⁶

BACKGROUND: Approximately 10–20% of children with idiopathic nephrotic syndrome (NS) fail to respond to steroid therapy. NS is divided into steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS). Over 45 recessive and dominant genes have been found to be associated with SRNS and/or focal segmental glomerulosclerosis (FSGS).

METHODS: Targeted sequencing of 339 candidate genes, expressed in glomerular filtration barrier or located in the signaling pathway of podocyte function, were sequenced by NGS in a cohort of total 89 Chinese Han children (29 sporadic SRNS, 33 sporadic SSNS, and 27 healthy).

RESULTS: Two variants (*WT1* p.R441X and *NPHS2* p.G149V) were screened out as pathogenic mutations and 14 variants were likely pathogenic. Mutations of *KIRREL2* (SRNS vs SSNS: 24.1% vs 3.0%, adjusted OR = 10.11, 95% CI: 1.56–198.66, $P = 0.039$) were significantly associated with the risk of pediatric sporadic SRNS. Besides, three pathogenic or likely pathogenic variants were identified in *HP* gene.

CONCLUSION: Two pathogenic mutations and 14 likely pathogenic mutations were discovered through targeted sequencing of 339 candidate genes. Two genes, *HP* and *KIRREL2*, as candidate genes, were first proposed to be associated with the risk of pediatric sporadic SRNS.

Pediatric Research (2019) 85:816–821; <https://doi.org/10.1038/s41390-019-0321-z>

INTRODUCTION

Idiopathic nephrotic syndrome (NS) is a common glomerular disease in children, occurring in 16 per 100,000 children¹, and is characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia, and edema at different levels. The first-line therapy for children with idiopathic NS is steroid treatment. Most children with idiopathic NS show a favorable long-term outcome when treated with glucocorticosteroid, although a number of them may experience steroid-dependent relapses². Approximately 10–20% of children with idiopathic NS fail to respond to steroid therapy. Moreover, 30–40% of these patients will progress to end-stage renal disease (ESRD) within 10 years upon disease onset, and dialysis or renal transplantation will be the only treatments they can benefit from³. According to the patients' responses to steroid therapy, NS is divided into steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS). Focal segmental glomerulosclerosis (FSGS) is the major disease subtype observed in patients with SRNS. Molecular genetic studies have revealed that a considerable proportion of patients with SRNS carried some gene mutations, leading to profound podocyte dysfunction, and these patients were usually also resistant to immunosuppressive agents⁴.

Currently, over 45 genes have been reported to be associated with SRNS and/or FSGS with autosomal dominant (AD), autosomal recessive (AR), X-linked dominant (XD), X-linked recessive (XR), or maternal modes of inheritance^{5,6}, such as *WT1* (AD), *NPHS1* (AR), *NPHS2* (AR), *WDR73* (AR), *XPO5* (AR), *NXF5* (XR), *COL4A5* (XD), and so on^{7–10}. Almost all the patients with SRNS harboring pathogenic

mutations would eventually suffer from renal failure. The incidence of genetic mutations in children with sporadic SRNS is estimated to be 20% in Caucasian, and the frequently mutated genes were *NPHS2* and *WT1*¹¹. However, the prevalence of *NPHS2* mutation is quite low in Asians^{12,13}. Thus it suggests that the mutational spectrum varies significantly among different races and ethnicities. There are only few reports about mutation profiles of children with SRNS in China.

In this study, 339 genes expressed in glomerular filtration barrier or located in the signaling pathway of podocyte function were chosen to be interrogated by next-generation sequencing (NGS) in Chinese Han children with sporadic SRNS or SSNS. The aim of this study is to discover novel pathogenic mutations in Chinese Han children with sporadic SRNS.

METHODS

Patients

Twenty-nine sporadic SRNS Chinese Han children, as well as 33 sporadic SSNS Chinese Han children worked as the disease control group, were recruited from Capital Institute of Pediatrics between 2011 and 2015. The inclusion criteria are: (1) non-consanguineous marriages with primary NS and (2) no family history of renal diseases. Twenty-seven healthy Chinese Han children without family history of renal diseases or abnormal urinalyses checked by imaging examination were also included in this study.

¹Department of Rheumatology and Immunology, Children's Hospital Affiliated to Capital Institute of Pediatrics, YaBao Road 2, 100020 Beijing, China; ²The Intensive Care Unit 2, Children's Hospital of Hebei Province, 050031 Shijiazhuang, Hebei Province, China; ³Department of Nephrology, Children's Hospital Affiliated to Capital Institute of Pediatrics, YaBao Road 2, 100020 Beijing, China; ⁴Vishuo MedTech Ltd, 100070 Beijing, China; ⁵Department of Biochemistry, Capital Institute of Pediatrics, YaBao Road 2, 100020 Beijing, China and ⁶Molecular Immunology Laboratory, Capital Institute of Pediatrics, YaBao Road 2, 100020 Beijing, China
Correspondence: Jianguo Li (jianguoli_18@hotmail.com)

Received: 17 April 2018 Revised: 30 October 2018 Accepted: 21 December 2018

Published online: 2 February 2019

The procedures were approved by the Ethics Committee of Capital Institute of Pediatrics. After receiving informed consent from, blood samples and comprehensive clinical data were collected and analyzed. NS was defined by the level of urinary protein excretion (≥ 50 mg/kg per 24 h) and the presence of hypoalbuminemia. Steroid resistance was defined by persistent proteinuria after 4 weeks of daily 1.5–2 mg/kg prednisone treatment (maximum dose 60 mg/day).

Targeted NGS and mutational analysis

Genomic DNA was extracted from patients' peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Shanghai, China) according to the manufacturer's protocol. A set of probes were developed to capture the whole exons of 339 target genes (Table S1) (Roche). A portion of these genes have been demonstrated to express at glomerular filtration barrier including the fenestrated endothelial cell layer, the glomerular basement membrane (GBM), and the epithelial podocyte layer. The other portion of these genes may be involved in the signaling pathways of podocyte predicted by GeneCards, STRING, KEGG and other databases. Targeted sequencing was performed on the HiSeq 2500 platform (Illumina).

Low-quality reads, duplicate reads, and potential adaptor contaminations were removed from the primary data and reads were quality-trimmed. The unique reads were mapped to the human genome (hg19/GRCh37) using the BWA software (Burrows Wheeler Aligner <http://sourceforge.net/projects/biobwa/>). GATK software (<https://software.broadinstitute.org/gatk/>) was used to detect single-nucleotide mutations, small insertions, and deletions. The mutation effects on protein function were evaluated using SIFT (<http://sift.jcvi.org/>), PolyPhen-2 HDIV (<http://genetics.bwh.harvard.edu/pph2/>), and PolyPhen-2 HVAR (<http://genetics.bwh.harvard.edu/pph2/>).

Mutations will be filtered out from further analysis if meeting any of the following criteria:

1. minor allele frequency $>0.5\%$ as shown in the public database, such as 1000 Genomes Project (1000 G) (<http://www.1000genomes.org/>), Exome sequencing project (ESP6500) (<http://evs.gs.washington.edu/EVS/>), and Exome Aggregation Consortium (ExAC) (<http://exac.broadinstitute.org/>).
2. mutations in introns except the splicing site (± 10 bp).
3. synonymous mutations (not caused changes of amino acid sequence).
4. at least two scores within the listed range: SIFT (score > 0.05), PolyPhen-2 HDIV (score ≤ 0.452), or PolyPhen-2 HVAR (score ≤ 0.446).
5. mutations also detected in the healthy Chinese Han children.

All rare mutations were validated by Sanger sequencing. Direct sequencing of the purified PCR products was performed using ABI 310 Genetic Analyzer (Applied Biosystems). Sequence analysis was carried out using the Sequence Pilot software¹⁴.

Statistical analysis

Median, standard deviations, and range values were calculated for continuous variables. Chi-square (χ^2) test, Fisher test, and *t* test of univariate analysis were performed to examine the relationship between diseases (SRNS, SSNS) and patients' clinical pathological features. Associations between SRNS and patients' gene mutations were further evaluated by multivariate logistic regression analysis. Adjusted odds ratio (OR) and 95% confidence interval (95% CI) were given. *P* values <0.05 (two-sided) were considered as statistically significant. All statistical analyses were performed with R 3.2.3 (<http://www.r-project.org/>).

Variables	SRNS	SSNS	<i>P</i> value
Age of onset (months, mean \pm SE)	50.9 \pm 45.5	46.4 \pm 25.6	0.637
Male	15	26	0.048
Female	14	7	
Urine protein (mg/kg/day, mean \pm SE)	162.2 \pm 64.6	—	—
Serum albumin (g/L, mean \pm SE)	14.7 \pm 4.8	—	—
Remission time (days, mean \pm SE)	—	9.2 \pm 4.7	—

SRNS steroid-resistant nephrotic syndrome, SSNS steroid-sensitive nephrotic syndrome

RESULTS

Clinical characteristics

The clinical characteristics of 29 sporadic SRNS children and 33 sporadic SSNS children are summarized in Tables S2 and S3. For 29 SRNS patients, the mean age of disease onset was 50.9 (SE: ± 45.5) months. Renal biopsy revealed FSGS in 8 patients, mesangial proliferative glomerulonephritis (MsPGN) in 6 patients, and minimal-change nephrotic syndrome (MCNS) in 2 patients. There were 5 patients with chronic renal failure (CRF) and 3 patients with acute renal failure (ARF). The mean level of urine protein and serum albumin were 162.2 (SE: ± 64.6) (mg/kg/d) and 14.7 (SE: ± 4.8) (g/L), respectively. For 33 SSNS children who worked as the disease control group, patients' age of disease onset ranged from 13 to 100 months, with a mean at the age of 46.4 (SE: ± 25.6) months, and the mean time of remission was 9.2 (SE: ± 4.7) days (Table 1). All patients with SRNS were treated with glucocorticoids combined with immunosuppressor, and high level of urine protein was reduced in 13 patients but not to the normal level (Table S2).

Identification and classification of variants in NS

After a multistep filtration as illustrated in Fig. 1, 143 variants from the SRNS group and 135 variants from the SSNS group were selected as the candidate variants for subsequent analysis. After cross-checking against 143 variants from SSNS group, 128 SRNS-specific mutations were identified in 87 out of the 339 target genes. These mutations include 124 missense, 1 nonsense, 1 splice sites, 1 small deletion, and 1 small insertion. And 83 out of the 128 mutations (64.8%) are novel mutations, which have not been reported in the literatures. A detailed description of all these 128 mutations is listed in Table S4. These 128 exclusive mutations were further classified into three groups based on available evidence (Table 2).

Two variants that met pathogenic criteria were regarded as pathogenic mutations for SRNS (Table 3). Notably, the truncating mutation in *WT1* (p.R441X), previously reported as a pathogenic mutation causing Denys-Drash syndrome and Wilms' tumor^{15–17}, was found in patient I-3 with CRF. Furthermore, in patient I-26 with FSGS, we found a novel homozygous variant (p.G149V) in *NPHS2*, which is the most frequently mutated gene in SRNS.

Fourteen variants classified as likely pathogenic mutations were identified in 11 genes from 10 patients (Table 3). Among these likely pathogenic mutations, 11 were nonsynonymous variants that occurred in well-known SRNS-associated genes. Two variants p.R802W (novel) and p.R1464H, located in *MYH9* gene that was known to cause Macrothrombocytopenia with sensorineural deafness, Epstein syndrome, Sebastian syndrome, and Fechtner syndrome, were found in a female patient with FSGS and ARF and

a male patient with MCNS, respectively. A variant (p.E1109K) in *LAMB2* causing AR Pierson syndrome was identified in two female patients with CRF and MsPGN, respectively. Furthermore, two novel homozygous variants in *HP* and *RHOXF2B* were detected in two patients. A female patient diagnosed as FSGS harbored a splicing site variant (c.1516+1G>A) in *CD44* gene.

Analysis of the candidate genes related with the risk of SRNS
To evaluate the risk of genes mutated in SRNS patients in disease development, we collapsed information on all rare variants within a gene into a single dichotomous variable for each subject by indicating whether or not the subject has any rare variants within this gene. Fisher test was applied to compare the mutation frequency between the SRNS and the SSNS groups. The results demonstrated that children with SRNS had a higher chance to obtain mutations in *KIRREL2* than in children with SSNS (SRNS vs SSNS: 24.1% vs 3.0%, OR = 10.18, 95% CI: 1.13–456.32, $P = 0.021$), and the mutations of *ITGA2* were more likely to occur in SRNS

children than in SSNS children (SRNS vs SSNS: 13.3% vs 0%, $P = 0.043$) (Fig. 2). However, the logistic multivariate analysis results indicated that only *KIRREL2* mutations were more frequently observed to be significant in NS patients who were steroid-resistant (SRNS vs SSNS: 24.1% vs 3.0%, adjusted OR = 10.11, 95% CI: 1.56–198.66, $P = 0.039$) (Table 4).

As carrying pathogenic or likely pathogenic mutations, three genes (*HP*, *RHOXF2B*, *CD44*) were highly speculated to be associated with SRNS. We calculated the RVIS scores for each candidate gene in order to assess how well these genes tolerate functional variations (<http://chgv.org/GenicIntolerance/>). RVIS is a score that measures the ratio of any functional variation defined as missense, nonsense, and splice acceptor/donor variants and synonymous (neutral) variation residing in a single gene. The score is designed to rank genes in terms of whether they have any common functional genetic variation relative to the genome wide expectation, given the amount of apparently neutral variations a certain gene has. *HP* and *ITGA2* may be intolerant, but *KIRREL2* is probably more tolerant to functional variants in the general population (Table 5).

DISCUSSION

In the present study, targeted sequencing of 339 candidate genes were sequenced by NGS in a cohort of total 89 Chinese Han children (29 sporadic SRNS, 33 sporadic SSNS, and 27 healthy), which is the most number of genes interrogated in a single study to date. The mean age of disease onset was 50.9 (SE: ± 45.5) months in the cohort of 29 SRNS patients (15 males, 14 females) and 46.4 (SE: ± 25.6) months in the cohort of 33 SSNS children (26 males, 7 females) as the disease control group.

We identified 128 exclusive mutations from 87 genes in all of the 29 sporadic SRNS children, including 124 missense, 1 nonsense, 1 splice sites, 1 small deletion, and 1 small insertion. And 84 out of the 128 variants (64.8%) were novel mutations. Among these 128 mutations, 15 mutations identified from 12 children with SRNS were located in well-known SRNS-related genes. The mutation rate is 40%, which was higher compared to the mutation rates described in previously studies with cohorts of Chinese SRNS children (28.3%¹⁸ and 31.7%¹⁹). Based on our selective criteria, 3 pathogenic mutations and 14 likely pathogenic mutations were achieved.

The *HP* gene encodes an acute phase-reactant tetrameric haptoglobin²⁰. The major function of haptoglobin is to bind free plasma hemoglobin (Hb) in order to form a stable Hp–Hb complex. It prevents the Hb-induced oxidative tissue damage and the loss of iron in the kidneys²¹. Mutations in *HP* may cause ahaptoglobinemia or hypohaptoglobinemia²². Furthermore, an evidence yielded by a latest research revealed that the level of haptoglobin in the serum was markedly elevated in SRNS patients compared to SSNS patients²³. But the relationship between the

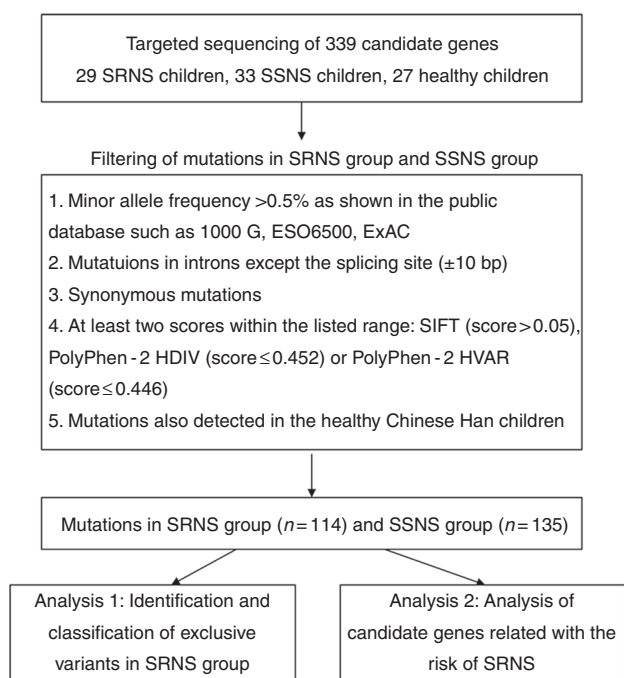


Fig. 1 Schematic visualization of the analytical workflow for this study. First, candidate mutations in the steroid-resistant nephrotic syndrome (SRNS) group and the steroid-sensitive nephrotic syndrome group were selected by a multistep filtration. Second, two kinds of analysis were performed to reveal genetic characteristics of Chinese children with SRNS

Table 2. Criteria for classifying variants

Definition	Description
Pathogenic	Null variants (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multiexon deletion) in well-established SRNS genes Previously reported pathogenic variants for SRNS Novel nonsynonymous variants of well-established SRNS genes in a homozygous state
Likely pathogenic	Nonsynonymous variants of well-established SRNS genes in a heterozygous state Novel or rare null variants Novel nonsynonymous variants in a homozygous state
Uncertain significance	Criteria listed above are not met
SRNS steroid-resistant nephrotic syndrome	

Table 3. Variants classified as pathogenic and likely pathogenic mutations in SRNS children and patients' disease characteristics

Patient	Gene	Mutations	Status	rs IDs	Classification	Renal biopsy	Renal function
I-1 (F)	<i>NPHS1</i> (AR)	c.G928A p.D310N	het	—	Likely pathogenic	ND	CRF
	<i>PLCE1</i> (AR)	c.C5522T p.P1841L	het	rs192586920	Likely pathogenic		
	<i>SMARCAL1</i> (AR)	c.T1274C p.L425P	het	—	Likely pathogenic		
I-3 (M)	<i>WT1</i> (AD)	c.C1321T p.R441X	het	rs121907909	Pathogenic	ND	CRF
I-4 (F)	<i>ITGB4</i> (AR)	c.G4943A p.R1648Q	het	rs200991558	Likely pathogenic	MsPGN	N
I-6 (M)	<i>NPHS1</i> (AR)	c.C2309T p.P770L	het	rs200976626	Likely pathogenic	ND	N
I-7 (M)	<i>COL4A4</i> (AR)	c.A899G p.E300G	het	—	Likely pathogenic	MsPGN	N
	<i>ITGB4</i> (AR)	c.G3385A p.A1129T	het	rs201321412	Likely pathogenic		
I-8 (F)	<i>LAMB2</i> (AR)	c.G3325A p.E1109K	het	rs79677861	Likely pathogenic	ND	CRF
I-14 (F)	<i>LAMB2</i> (AR)	c.G3325A p.E1109K	het	rs79677861	Likely pathogenic	MsPGN	N
	<i>HP</i>	c.G960C p.K320N	hom	—	Likely pathogenic		
I-20 (F)	<i>MYO1E</i> (AR)	c.G620A p.R207Q	het	—	Likely pathogenic	ND	N
I-23 (M)	<i>MYH9</i> (AD)	c.G4391A p.R1464H	het	rs199968414	Likely pathogenic	MCNS	N
	<i>RHOXF2B</i>	c.C398T p.A133V	het	—	Likely pathogenic		
I-25 (F)	<i>CD44</i>	c.1516+1G>A Splice	het	rs141953333	Likely pathogenic	FSGS	N
I-26 (M)	<i>NPHS2</i> (AR)	c.G446T p.G149V	hom	—	Pathogenic	FSGS	N
I-27 (F)	<i>MYH9</i> (AD)	c.C2404T p.R802W	het	—	Likely pathogenic	FSGS	ARF

F female, M male, AD autosomal dominant, AR autosomal recessive, XD X-linked dominant, FSGS focal segmental glomerulosclerosis, MCNS minimal-change nephrotic syndrome, MsPGN mesangial proliferative glomerulonephritis, ND not determined, CRF chronic renal failure, ARF acute renal failure, N normal, het heterozygous, hom homozygous, SRNS steroid-resistant nephrotic syndrome

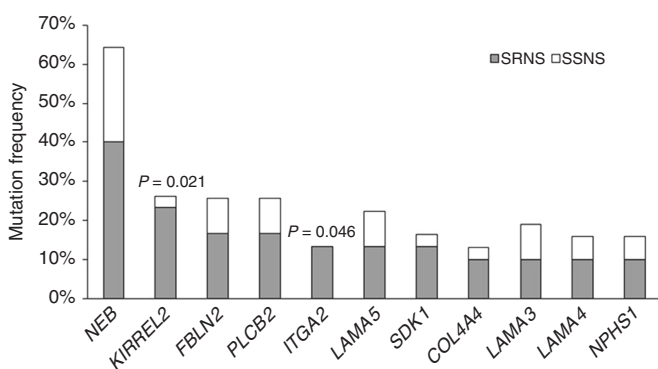


Fig. 2 Percentage of patients with mutations in a given gene. Histograms indicate fractions (in percentage) of patients carrying mutations in the corresponding genes identified from steroid-resistant nephrotic syndrome group and steroid-sensitive nephrotic syndrome group, respectively

mutations in *HP* and the occurrence of SRNS remains inconclusive. The novel homozygous mutation in *HP* p.K320N was found in patient I-14 with MsPGN but showing a normal renal function. And the *HP* p.R227P mutation was detected in patient I-27 with FSGS and acute renal failure. Even though no mutations in gene *HP* were identified from the SSNS group, the statistical analysis implicated that there was no significant link between *HP* gene and sporadic SRNS ($P = 0.222$). The reason is probably that the sample size in this study cohort is relative small.

The *KIRREL2* gene also named as Neph3 or filtrin encodes a protein with five Ig domains. *KIRREL2* belongs to a member of the NEPH family that interacts with podocin, a glomerular slit diaphragm protein²⁴. Interestingly, a recent study showed the evidence of Neph3 in preventing the glomerular protein leakage, as the decreased expression of Neph3 in renal biopsies are found to be associated with podocytopathy and protein-losing nephropathy²⁵. In addition, nonsynonymous variants in *KIRREL2* gene were also proven to cause the nephrotic syndrome in dogs²⁶. Moreover, a recent investigation indicated an association of the V353M variant in *KIRREL2* with the disease severity of primary hematuric glomerulopathies. Functional studies showed that Neph3 homodimerization and Neph3–Nephrin heterodimerization were severely compromised by this mutation. Thus the variant

Table 4. Correlation of genes mutations with the risk of SRNS

Variable	Patients		Univariate		Multivariate	
	SRNS	SSNS	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
<i>KIRREL2</i>	Wild type	22	32	Ref.	Ref.	
	Mutated	7	1	10.18 (1.13–456.32)	0.021	10.11 (1.56–198.66)
<i>ITGA2</i>	Wild type	25	33	Ref.	Ref.	
	Mutated	4	0	—	0.043	—

CI confidence interval, OR odds ratio, Ref. reference, SRNS steroid-resistant nephrotic syndrome, SSNS steroid-sensitive nephrotic syndrome

Table 5. The Residual Variation Intolerance Scores (RVIS) and percentiles extracted for the candidate genes in SRNS children patients

Gene	RVIS scores	RVIS percentiles
<i>KIRREL2</i>	0.67	84.7%
<i>CD44</i>	0.23	68.52%
<i>ITGA2</i>	−0.24	36.32%
<i>HP</i>	−0.8	12.24%
<i>RHOXF2B</i>	NA	NA

Genes are ranked in an ascending order of RVIS percentile
NA not available, SRNS steroid-resistant nephrotic syndrome

V353M acts as a negative modifier of primary glomerular hematuria²⁷. In this study, the statistical analysis revealed that children with sporadic SRNS had a higher chance to obtain mutations in *KIRREL2* than in children with sporadic SSNS (SRNS vs SSNS: 24.1% vs 3.0%, adjusted OR = 10.11, 95% CI: 1.56–198.66, *P* = 0.039). Further investigations will be necessary in order to inspect whether the mutations in gene *KIRREL2* are related to pediatric SRNS. The gene *ITGA2* encodes the alpha subunit of a transmembrane receptor for collagens and related proteins, named alpha-2 integrin, which forms a heterodimer with a beta subunit and mediates the adhesion of platelets and other cell types to the extracellular matrix²⁸. Alpha-2 integrin was shown to involve in cell adhesion and also participated in cell-surface-mediated signaling²⁹. Loss of alpha-2 integrin was associated with bleeding disorder platelet type 9 and antibodies against this protein were found in several immune disorders, including neonatal alloimmune thrombocytopenia^{30,31}. *ITGA2*-deficient mice had moderate level of proteinuria and showed enhanced glomerular and tubulointerstitial matrix deposition and fibrosis. The cell–matrix interaction via collagen receptors (*DDR1*, *ITGA2*, *COL4A3*) regulated GBM maturation and matrix accumulation, which were crucial for the maintenance of normal GBM architecture and function^{32,33}. Besides, the pediatric NS is caused by the abnormal structure and dysfunction of the glomerular filtration barriers, including the fenestrated endothelial cell layer, the GBM, the epithelial podocyte layer, and the mesangial cells. However, the mutations in *ITGA2* were found to be not significantly associated with the risk of SRNS by the logistic multivariate analysis in the present study. Future studies should concentrate on examining the role of *ITGA2* in renal disease, especially in pediatric SRNS.

The *MYH9* gene encodes the nonmuscle myosin heavy chain IIA (myosin II), a cytoskeletal contractile protein that is abundantly expressed in the kidney, mainly in the glomerulus and peritubular vessels³⁴. Mutations in *MYH9* gene are related to a number of rare autosomal-dominant disorders, such as May–Hegglin disease, Sebastian syndrome, Fechtner syndrome, and Epstein syndrome. Patients with these diseases often showed symptoms of

congenital macrothrombocytopenia, polymorphonuclear inclusion bodies, proteinuria, chronic kidney disease progressing toward ESRD, sensorineural deafness, and presenile cataracts³⁵. It has been reported that the p.E1841K mutation, one of common missense mutations in *MYH9*, resulted in the alteration of podocyte cytoskeletal structure and caused podocytes to be more susceptible to the injury of a damaging stimulus³⁶. In this study, two heterozygous single-nucleotide polymorphisms (SNPs), *MYH9* p.R1464H and *MYH9* p.R802W (novel), were detected in patient I-23 with MCNS and patient I-27 with FSGS and acute renal failure, respectively. Furthermore, the blood routine examination of patients I-23 and I-27 did not show platelet abnormalities. It suggests that these two SNPs may contribute to the induction of sporadic SRNS and are likely pathogenic. Unfortunately, the parental DNA was not available in this study and thus de novo mutations were not identified in these pediatric patients with SRNS.

In summary, this study presents a clear mutation panorama of 339 genes based on the cohort of total 89 Chinese Han children (29 sporadic SRNS, 33 sporadic SSNS, and 27 healthy) and reveals the mutational spectrum and novel candidate genes in Chinese Han children with SRNS. Based on our selective criteria, 2 pathogenic mutations and 14 likely pathogenic mutations were discovered. And two candidate genes, *HP* and *KIRREL2*, were first proposed to be associated with the induction of pediatric sporadic SRNS. Our results demonstrated that screening for pathogenic mutations in children with idiopathic NS might be helpful for early disease diagnosis, and the unnecessary use of renal biopsy and steroid treatment for pediatric patients could be prevented.

ACKNOWLEDGEMENTS

We are indebted to the patients and families for participating in this study and to the clinical teams involved in recruitment and sample collection. This work was supported by the National Natural Science Foundation of China (81100504, 81570649).

ADDITIONAL INFORMATION

The online version of this article (<https://doi.org/10.1038/s41390-019-0321-z>) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

- Gipson, D. S. et al. Management of childhood onset nephrotic syndrome. *Pediatrics* **124**, 747–757 (2009).
- Hahn, D., Hodson, E. M., Willis, N. S. & Craig, J. C. Corticosteroid therapy for nephrotic syndrome in children. *Cochrane Database Syst. Rev.* **3**, CD001533 (2015).
- Mekahli, D. et al. Long-term outcome of idiopathic steroid resistant nephrotic syndrome: a multicenter study. *Pediatr. Nephrol.* **24**, 1525–1532 (2009).

4. Giglio, S. et al. Heterogeneous genetic alterations in sporadic nephrotic syndrome associate with resistance to immunosuppression. *J. Am. Soc. Nephrol.* **26**, 230–236 (2015).
5. Joshi, S., Andersen, R., Jespersen, B. & Rittig, S. Genetics of steroid-resistant nephrotic syndrome: a review of mutation spectrum and suggested approach for genetic testing. *Acta Paediatr.* **102**, 844–856 (2013).
6. Brown, E. J., Pollak, M. R. & Barua, M. Genetic testing for nephrotic syndrome and FSGS in the era of next-generation sequencing. *Kidney Int.* **85**, 1030–1038 (2014).
7. Lovric, S., Ashraf, S., Tan, W. & Hildebrandt, F. Genetic testing in steroid-resistant nephrotic syndrome: when and how? *Nephrol. Dial. Transplant.* **31**, 1802–1813 (2016).
8. Tasic, V., Gucevi, Z. & Polenakovic, M. Steroid resistant nephrotic syndrome—genetic consideration. *Pril. (Makedon. Akad. Nauk. Umet. Odd. Med. Nauk.)* **36**, 5–12 (2015).
9. Becherucci, F. et al. Lessons from genetics: is it time to revise the therapeutic approach to children with steroid-resistant nephrotic syndrome? *J. Nephrol.* **29**, 543–550 (2016).
10. Ha, T.-S. Genetics of hereditary nephrotic syndrome: a clinical review. *Korean J. Pediatr.* **60**, 55–63 (2017).
11. Sadowski, C. E. et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. *J. Am. Soc. Nephrol.* **26**, 1279–1289 (2015).
12. Maruyama, K. et al. NPHS2 mutations in sporadic steroid-resistant nephrotic syndrome in Japanese children. *Pediatr. Nephrol.* **18**, 412–416 (2003).
13. Li, J. et al. WT1 gene mutations in Chinese children with early onset nephrotic syndrome. *Pediatr. Res.* **68**, 155–158 (2010).
14. Petrovski, S., Wang, Q., Heinzen, E. L., Allen, A. S. & Goldstein, D. B. Genic intolerance to functional variation and the interpretation of personal genomes. *PLoS Genet.* **9**, e1003709 (2013).
15. Little, M. H. et al. Zinc finger point mutations within the WT1 gene in Wilms tumor patients. *Proc. Natl. Acad. Sci. USA* **89**, 4791–4795 (1992).
16. Little, M. H. et al. Evidence that WT1 mutations in Denys-Drash syndrome patients may act in a dominant-negative fashion. *Hum. Mol. Genet.* **2**, 259–264 (1993).
17. Schumacher, V. et al. Correlation of germ-line mutations and two-hit inactivation of the WT1 gene with Wilms tumors of stromal-predominant histology. *Proc. Natl. Acad. Sci. USA* **94**, 3972–3977 (1997).
18. Wang, F. et al. Spectrum of mutations in Chinese children with steroid-resistant nephrotic syndrome. *Pediatr. Nephrol.* **32**, 1181–1192 (2017).
19. Wang, Y. et al. Mutation spectrum of genes associated with steroid-resistant nephrotic syndrome in Chinese children. *Gene* **625**, 15–20 (2017).
20. Haugen, T. H., Hanley, J. M. & Heath, E. C. Haptoglobin: a novel mode of biosynthesis of a liver secretory glycoprotein. *J. Biol. Chem.* **256**, 1055–1057 (1981).
21. Asleh, R. et al. Genetically determined heterogeneity in hemoglobin scavenging and susceptibility to diabetic cardiovascular disease. *Circ. Res.* **92**, 1193–1200 (2003).
22. Teye, K. et al. A-61C and C-101G Hp gene promoter polymorphisms are, respectively, associated with ahaptoglobinaemia and hypohaptoglobinaemia in Ghana. *Clin. Genet.* **64**, 439–443 (2003).
23. Wen, Q. et al. Proteomic profiling identifies haptoglobin as a potential serum biomarker for steroid-resistant nephrotic syndrome. *Am. J. Nephrol.* **36**, 105–113 (2012).
24. Sellin, L. et al. NEPH1 defines a novel family of podocin interacting proteins. *FASEB J.* **17**, 115–117 (2003).
25. Ihalmo, P. et al. Expression of filtrin in human glomerular diseases. *Nephrol. Dial. Transplant.* **22**, 1903–1909 (2007).
26. Littman, M. P., Wiley, C. A., Raducha, M. G. & Henthorn, P. S. Glomerulopathy and mutations in NPHS1 and KIRREL2 in soft-coated Wheaten Terrier dogs. *Mamm. Genome* **24**, 119–126 (2013).
27. Voskarides, K. et al. A functional variant in NEPH3 gene confers high risk of renal failure in primary hematuric glomerulopathies. Evidence for predisposition to microalbuminuria in the general population. *PLoS ONE* **12**, e0174274 (2017).
28. Inoue, O., Suzuki-Inoue, K., Dean, W. L., Frampton, J. & Watson, S. P. Integrin alpha2beta1 mediates outside-in regulation of platelet spreading on collagen through activation of Src kinases and PLCgamma2. *J. Cell Biol.* **160**, 769–780 (2003).
29. Kim, S.-H., Turnbull, J. & Guimond, S. Extracellular matrix and cell signalling: the dynamic cooperation of integrin, proteoglycan and growth factor receptor. *J. Endocrinol.* **209**, 139–151 (2011).
30. Lane-Serff, H., Sun, Y., Metcalfe, P. & Wright, G. J. Expression of recombinant ITGA2 and CD109 for the detection of human platelet antigen (HPA)-5 and -15 alloantibodies. *Br. J. Haematol.* **161**, 453–455 (2013).
31. Peterson, J. A. et al. New low-frequency platelet glycoprotein polymorphisms associated with neonatal alloimmune thrombocytopenia. *Transfusion* **50**, 324–333 (2010).
32. Girgert, R. et al. Integrin alpha2-deficient mice provide insights into specific functions of collagen receptors in the kidney. *Fibrogenesis Tissue Repair* **3**, 19 (2010).
33. Rubel, D. et al. Collagen receptors integrin alpha2beta1 and discoidin domain receptor 1 regulate maturation of the glomerular basement membrane and loss of integrin alpha2beta1 delays kidney fibrosis in COL4A3 knockout mice. *Matrix Biol.* **34**, 13–21 (2014).
34. Arrondel, C. et al. Expression of the nonmuscle myosin heavy chain IIA in the human kidney and screening for MYH9 mutations in Epstein and Fechtner syndromes. *J. Am. Soc. Nephrol.* **13**, 65–74 (2002).
35. Galeano, D., Zanolli, L., L'Imperio, V., Fatuzzo, P. & Granata, A. Renal diseases related to MYH9 disorders. *G. Ital. Nefrol.* **34**, 40–57 (2017).
36. Cechova, S. et al. MYH9 E1841K mutation augments proteinuria and podocyte injury and migration. *J. Am. Soc. Nephrol.* **29**, 155–167 (2018).