

BMJ Open Relationships between the clinical phenotypes and genetic variants associated with the immunological mechanism in childhood idiopathic nephrotic syndrome: protocol for a prospective observational single-centre cohort study

Han Chan,^{1,2} Hao Lee,³ Xia Yang,³ Jingzhi Wang,³ Xueying Yang,³ Chun Gan,³ Han Xiao,³ Qianqian Li,³ Jia Jiao,¹ Daoqi Wu,¹ Gaofu Zhang,^{1,2} Mo Wang,^{1,2} Haiping Yang,^{1,2} Qiu Li^{1,2}

To cite: Chan H, Lee H, Yang X, *et al.* Relationships between the clinical phenotypes and genetic variants associated with the immunological mechanism in childhood idiopathic nephrotic syndrome: protocol for a prospective observational single-centre cohort study. *BMJ Open* 2019;**9**:e028717. doi:10.1136/bmjopen-2018-028717

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2018-028717>).

Received 20 December 2018

Revised 23 July 2019

Accepted 24 July 2019



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For numbered affiliations see end of article.

Correspondence to

Dr Qiu Li; liqiu809@126.com

Dr Haiping Yang;
oyhp0708@163.com

ABSTRACT

Introduction Idiopathic nephrotic syndrome (INS) is the most common glomerulopathy that results in childhood chronic kidney disease in China, but the relationships between different clinical phenotypes and immunological genetic variants observed in patients with INS are ambiguous and have not been well studied. A cohort study combined with whole exome sequencing might further identify the effects of immunological genetic variants on clinical phenotypes and treatment outcomes.

Methods and analysis We describe a 3 year prospective observational single-centre cohort study to be conducted in the Children's Hospital of Chongqing Medical University in China. This study will recruit and investigate 336 patients with childhood-onset INS presenting with different clinical phenotypes. Whole exome sequencing will be conducted when patients progress to a confirmed clinical phenotype during follow-up. Relevant clinical and epidemiological data, as well as conventional specimens, will be collected at study entry and 1 month, 3 months, 6 months, 1 year, 2 years and 3 years after disease onset. After this cohort is generated, the immunological genetic variants of steroid-sensitive nephrotic syndrome without frequent relapse, steroid-resistant nephrotic syndrome and steroid-dependent/frequent relapse nephrotic syndrome will be evaluated.

Ethics and dissemination The study protocol is approved by Ethics Committee of Children's Hospital of Chongqing Medical University (reference number 2018–140). The results will be disseminated through peer-reviewed journals and conference presentations.

Trial registration number ChiCTR1800019795

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is the most common glomerulopathy that results in childhood chronic kidney disease in China. It

Strengths and limitations of this study

- This prospective cohort study is the first international study to prospectively investigate the genetic causes and risk variants associated with the immunological mechanisms of childhood idiopathic nephrotic syndrome (INS) in China.
- This study has the potential to provide patients with INS with an opportunity for further stratification and personalised medicine.
- The study will first confirm the role of variations in antigen presentation governed by the *HLA-II* polymorphism in steroid dependency and frequent relapses in patients with steroid-sensitive nephrotic syndrome.
- The findings will not be generalisable to all patients with childhood-onset INS in China and must be validated in multicentre studies with larger sample sizes.

is characterised by a combination of massive proteinuria, hypoalbuminaemia, oedema and hyperlipidaemia. Oral glucocorticoid has been recognised as a first-line treatment for INS. Glucocorticoid sensitivity occurs in 77.6% to 90.0% of INS patients during their initial treatment (steroid-sensitive nephrotic syndrome, SSNS), and the remaining 10% to 22.4% of patients are resistant to steroids (steroid-resistant nephrotic syndrome, SRNS). However, 80% to 90% of the initial responders relapse during the later treatment, and approximately half of these relapsers have a higher risk than other subsets of developing refractory nephrotic syndrome. Refractory nephrotic

syndrome can be divided into two subtypes: SRNS and steroid-dependent nephrotic syndrome/frequent relapse nephrotic syndrome (SDNS/FRNS).

Most children with SRNS will progress to end-stage renal disease (ESRD) within 5 to 10 years after diagnosis.¹ Although steroid treatment remains the mainstay therapy for patients with SDNS/FRNS, prolonged glucocorticoid treatment is associated with significant morbidity, as many immunosuppressive agents are used to reduce the glucocorticoid dosage and side effects.² According to genomic studies conducted in the last 20 years, most cases of refractory nephrotic syndrome are likely due to genetic variants that cause structural and functional defects in the glomerular visceral epithelial cell, and the clinical and pathological phenotypes of each subgroup are tightly linked to the genetic phenotypes.^{3–5}

Immunosuppressive agents plus steroids therapy are the second-line therapy for refractory nephrotic syndrome when patients with INS fail to respond to steroid treatments. Previous studies have reported that human immune dysfunction has a close connexion with INS, especially systemic abnormalities of T cell function, and previous studies provide evidence that minimal changes in INS symptoms may be due to a circulating factor released by activated T cells.⁶ In addition, patients with Hodgkin's lymphoma have been shown to develop nephrotic syndrome as part of a paraneoplastic process in the malignancy that is known to affect B cell function.⁷ The proportion of memory B cells, has been shown to be increased in paediatric patients with SSNS to a greater extent than other B cell subsets, and the recovery of the percentages of B cells and memory B cells predicts the occurrence of a relapse after rituximab treatment.^{8–10} To date, the immunological aetiopathogenesis of INS remains unknown, and the identification of genetic variants associated with the humoral immune system in patients with INS remains to be confirmed.

The average incidence of INS is 4.7 (range 1.15 to 16.9) per 100 000 persons in studies published from 1946 to 2014, and Asians (particularly South Asians) are reported to have a higher incidence of INS than other ethnic groups.¹¹ According to a published national consensus in 2014, children with INS accounted for 20.0% of the total number of hospitalised children with urinary system diseases in China.^{12 13} China has a large, genetically diverse population base and a higher prevalence of the absolute number of patients with INS. With the development of high-throughput sequencing, extensive phenotyping combined with whole exome sequencing (WES) represents an effective tool for the early identification of INS aetiology, yielding an evidence-based algorithm for clinical management. A prospective, observational cohort study to establish the associations between different clinical phenotypes and immunological genetic variants in patients with INS is realistic and is urgently needed.

This cohort study is funded by the China National Natural Science Foundation (grant number 81770713) and China National Clinical Research Center Foundation

(grant number YBXM-2019–002) to describe the genetic causes and risk variants associated with the humoral immune system, thus further identifying their effects on clinical phenotypes and treatment outcomes. In this manuscript, we provide an overview of the design, methods and scope of this cohort study.

METHODS AND ANALYSIS

Study design, setting and participants

This study is a prospective, observational, single-centre cohort study based on the Strengthening the Reporting of Observational Studies in Epidemiology reporting guidelines. It will be performed at the Children's Hospital of Chongqing Medical University, Chongqing, China. The study will begin on 1 February 2019 and will last for 3 years. Patients will be freely permitted to quit the study at any time. The patients included in the study must meet the inclusion criteria: (1) An onset age from birth to 18 years. (2) A diagnosis of INS according to the following criteria: ① nephrotic proteinuria: proteinuria ≥ 50 mg/kg or morning urine protein/creatinine (mg/mmol) ≥ 200.0 in 24 hours, ② hypoproteinaemia: serum albumin less than 25 g/L, ③ hyperlipidaemia: serum cholesterol higher than 5.7 mmol/L and ④ different degrees of oedema. ① and ② of the above four items are necessary for diagnosis. (3) Patients must not have received any form of hormone or immunosuppressive therapy prior to inclusion in the study and have a clear and detailed history of drug treatment. Patients with SSNS or FRNS/SDNS should respond to prednisone or prednisolone within 4 weeks. Patients with SRNS exhibit persistent proteinuria despite adequate prednisone treatment for at least 4 weeks and require further immunosuppressant treatment. (4) Patients with chronic kidney disease caused by factors such as congenital nephrotic syndrome or secondary nephrotic syndrome (allergic purpura, lupus, hepatitis B, diabetes, hereditary nephritis, severe infection, tumour and drugs) will be excluded. (5) Patients must not present positive results for the following four items: ① three centrifugal microscopic examinations of urine red blood cells within 2 weeks with ≥ 10 /high-power field and confirmed glomerular haematuria; ② repeated or persistent hypertension \geq three times measured at different time points, defined as systolic blood pressure and/or diastolic blood pressure equal or greater than the 95th percentile for children and adolescents of the same sex, age and height, except when due to glucocorticoid use and other reasons; ③ abnormal renal function when insufficient blood volume has been ruled out; ④ continuous hypocomplementaemia. (6) Patients with a positive genetic test associated with podocytopathies will be excluded. A flowchart of the study design is shown in figure 1.

Study procedures

The researchers include statisticians and clinicians. The clinicians will be responsible for the diagnosis and treatment of the patients but will not participate in the statistical analyses, particularly the bioinformatics

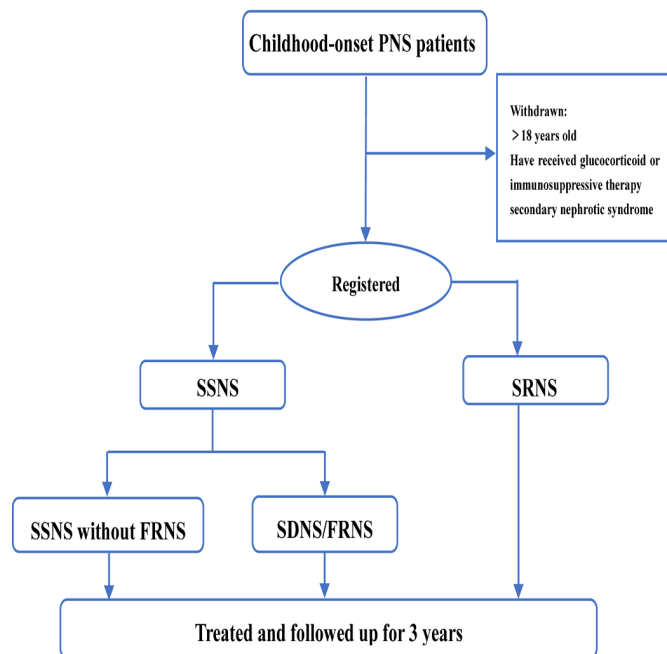


Figure 1 Flowchart of the study design. PNS, primary nephrotic syndrome; SDNS, steroid-dependent nephritic syndrome; FRNS, frequent relapse nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; SSNS, steroid-sensitive nephrotic syndrome.

analysis of the patients' complete exon sequencing data. Relevant clinical and epidemiological data will be prospectively collected from the enrolled patients using

Table 2 Blood test parameters

Blood lipid			
Liver function	Blood lipid levels	Immune function	
TBIL (μmol/L)	TC (mmol/L)	IgG (g/L)	CD3 ⁺ (%)
DBIL (μmol/L)	TG (mmol/L)	IgA (g/L)	CD3 ⁺ CD8 ⁺ (%)
IBIL (μmol/L)	HDL (mmol/L)	IgM (g/L)	CD3 ⁺ CD4 ⁺ (%)
TP (μmol/L)	LDL (mmol/L)	IgE (IU/mL)	CD3 ⁺ CD4 ⁺ CD8 ⁺ (%)
ALB (g/L)		C3 (g/L)	CD3 ⁺ CD4 ⁺ CD8 ⁺ (%)
ALT (g/L)		C4 (g/L)	NK (%)
BUN (mmol/L)			CD19 ⁺ (%)
CREA (μmol/L)			CD4/CD8
URCA (μmol/L)			

TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TP, total protein; ALB, albumin; ALT, alanine aminotransferase; BUN, urea nitrogen; CREA, creatinine; URCA, uric acid; TC, total cholesterol; TG, total glyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; IgE, immunoglobulin E; C3, complement C3; C4, complement C4.

a case report form (tables 1–3). The patients will be evaluated at the hospital on study entry and at 1 month, 3 months, 6 months, 1 year, 2 years and 3 years after disease onset. When a patient develops FRNS/SDNS or SRNS, renal biopsy, including immunofluorescence staining and transmission electron microscopy, will be recommended. If the patient's parents refuse the renal biopsy,

Table 1 Baseline values and frequency of collection of follow-up data for the included patients

Characteristic	0 months	1 month	3 months	6 months	1 year	2 years	3 years
Age (years)							
Gender (male/female)							
BMI index (kg/m ²)							
Blood pressure (mmHg)							
Clinical manifestation onset							
Premature birth history							
Passive smoking history							
Family history							
Cumulative cost of treatment							
Quality of life score (points)							
Time to first remission (days)							
Time to first relapse (days)							
Predisposing causes during relapse							
Average relapse frequency							
eGFR (mL/min•1.73m ²)							
Urinary protein/creatinine (mg/mmol)							
24 hours urinary protein level/weight (mg/kg)							
Renal pathological biopsy type							
Repeat renal biopsy and type							

BMI, body mass index; eGFR, estimated glomerular filtration rate.

Table 3 Follow-up table for patients receiving immunosuppressants

Baseline	0 months	1 month	3 months	6 months	1 year	2 years	3 years
Total prednisone dose (daily/alternate day therapy) (weeks)							
Immunosuppressant therapy (types)							
Time at which the first immunosuppressant was added (weeks)							
Relapse frequency after treatment with the first immunosuppressant (n)							
Glucocorticoid dose administered with the first immunosuppressant (mg/d)							
Total number of glucocorticoid-related adverse events after treatment with the first immunosuppressant (n)							
Time to remission after adding this immunosuppressive agent (days)							
Time to relapse after adding this immunosuppressive agent (weeks)							
Relapse frequency after adding this immunosuppressive agent (weeks)							
Number of adverse events associated with the immunosuppressant (n)							
Blood concentration of immunosuppressants (µg/mL)							

immunosuppressive agents will be reasonably selected according to conventional experience with immunosuppressant administration and evidence-based practices.

Specimen collection and laboratory analysis

Blood and urine are the main specimens of interest and will be collected at study entry and 1 month, 3 months, 6 months, 1 year, 2 years and 3 years after disease onset. All collected specimens will be transferred to a reference laboratory without delay. Blood tests will include assessments of liver function, renal function, immunological function, blood lipid levels and the estimated glomerular filtration rate (eGFR). The urine analysis will include urinary protein/creatinine levels and 24 hours urinary protein levels/weight. A renal biopsy will be performed when a patient has progressed to SRNS or SDNS/FRNS. The renal biopsies will be evaluated using light microscopy, electron microscopy and immunofluorescence staining. Genomic DNA will be extracted from peripheral blood using a standard protocol, and whole exome sequencing will be conducted when patients progress to a confirmed clinical phenotype (SSNS without FR, SDNS/FRNS and SRNS) during follow-up.

The inspection methods used in the study will follow standard inspection procedures. eGFR will be evaluated using the Schwartz formula, with constant K (approximately 0.45 for infants within 1 year of age, 0.33 for infants born at less than 2.5 kg and within 1 year of age, 0.55 for children and adolescent girls and 0.7 for adolescent boys), L for length (cm) and Scr for serum creatinine (mg/dL). The following procedures will be used for whole exome sequencing:

- ▶ The genomic DNA will be extracted from the samples using the conventional Cetyltrimethylammonium bromide (CTAB) method, and the quality of sample DNA will be detected.¹⁴ DNA

samples without degradation or RNA contamination that result in an OD260/OD280 ratio from 1.8 to 2.0 and a concentration greater than 1.5 µg will be used to establish the database.

- ▶ After constructing the library, we will use Qubit for the preliminary quantitative analysis and then use an Agilent 2100 instrument to detect the insert size of the library. The effective concentration of the library will be accurately quantified using Quantitative-real time polymerase chain reaction (Q-PCR) to ensure the quality of the library.
- ▶ The original images of the data obtained using an Illumina NextSeq 500 instrument will be transformed into the original sequences by performing a base recognition analysis.¹⁵
- ▶ The results will be compared with the human genome using the Burrows-Wheeler Aligner. Single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels) will be detected with GATK software.¹⁶ Additionally, several databases (such as dbSNP, 1000g, ESP6500, HGMD, OMIM, GO, KEGG, etc) associated with ANNOVAR software will be used to annotate the mutation results, focusing on patients with HLA-class I and HLA-class II allele distribution, HLA-related humoral immune gene frequencies and SNPs among the clinical genotypes.¹⁷ The immunological signaling pathways included in the study are presented in table 4.

Treatment

This study strictly adheres to the Improving Global Outcomes Glomerulonephritis Work Group guidelines for the use of immunosuppressants and evidence-based practices to explain treatment options to the parents of patients and jointly choose to use immunosuppressants^{18 19}; it does not involve randomised allocation and

Table 4 Immunological signalling pathways analysed in the study

Path ID	KEGG Pathway
hsa04640	Haematopoietic cell lineage
hsa04610	Complement and coagulation cascades
hsa04611	Platelet activation
hsa04620	Toll-like receptor signalling pathway
hsa04624	Toll and Imd signalling pathway
hsa04621	NOD-like receptor signalling pathway
hsa04622	RIG-I-like receptor signalling pathway
hsa04623	Cytosolic DNA-sensing pathway
hsa04625	C-type lectin receptor signalling pathway
hsa04650	Natural killer cell-mediated cytotoxicity
hsa04612	Antigen processing and presentation
hsa04660	T cell receptor signalling pathway
hsa04658	Th1 and Th2 cell differentiation
hsa04659	Th17 cell differentiation
hsa04657	IL-17 signalling pathway
hsa04662	B cell receptor signalling pathway
hsa04664	Fc epsilon RI signalling pathway
hsa04666	Fc gamma R-mediated phagocytosis
hsa04670	Leucocyte transendothelial migration
hsa04672	Intestinal immune network for IgA production
hsa04062	Chemokine signalling pathway

IgA, immunoglobulin A.

blinding methods. The therapeutic schemes for immunosuppressants are as follows: (1) cyclophosphamide: oral: 2 to 3 mg/(kg·d) for 8 weeks, intravenous therapy: 8 to 12 mg/(kg·d) 2 days every 2 weeks, total dose of ≤ 168 mg/kg or 500 mg/m² once a month for 6 months; (2) cyclosporine A: 4 to 6 mg/(kg·d), once every 12 hours,

maintaining a blood concentration of 80 to 120 ng/mL for 12 to 24 months and monitoring serum concentrations; (3) tacrolimus: 0.05 to 0.15 mg/(kg·d) once every 12 hours, maintaining a blood concentration of 5 to 10 µg/L for 12 to 24 months and monitoring serum concentrations; (4) mycophenolate mofetil: 20 to 30 mg/(kg·d) once every 12 hours, with a maximum dose of less than 1 g each treatment, for at least 6 months, decreasing to 10 mg/(kg·d) after proteinuria remission is observed for 3 to 6 months and maintaining this treatment for 12 to 24 months; (5) rituximab: 375 mg/(m²·times) once a week for 1 to 4 weeks; (6) levamisole: 2.5 mg/(kg·d), qod, for 12 to 24 months. The recommended immunosuppressants for patients with FRNS/SDNS and SRNS are listed in table 5.

Clinical outcomes

Primary outcome

The primary efficacy outcome is the analysis of the genetic variants associated with the differences in immunological mechanisms between patients with SSNS without FR and patients with SRNS or FRNS/SDNS.

- ▶ SSNS without FR: Adequate treatment with 2 mg/(kg·d) or 60 mg/(m²·d) prednisone results in proteinuria remission after 4 weeks of treatment, except in patients with SDNS/FRNS, FRNS and secondary SRNS.
- ▶ SRNS: Persistent proteinuria is observed despite adequate prednisone treatment for at least 4 weeks. SRNS is divided into initial SRNS and secondary SRNS, the latter is diagnosed when proteinuria is still positive 4 weeks after one or more remissions following the initial corticosteroid treatment.
- ▶ SDNS/FRNS: Two consecutive relapses during the reduction of corticosteroid therapy or within 2 weeks of discontinuation of corticosteroid therapy and more than two relapses within a 6 month period or relapse

Table 5 The recommended immunosuppressants for patients with FRNS/SDNS and SRNS

FRNS/SDNS	SRNS	
Drugs selection	Pathologic biopsy	Therapeutic strategies
Cyclophosphamide	Minimal change disease	1. Tacrolimus or cyclophosphamide 2. Rituximab 3. Cyclosporine A 4. MMF
Tacrolimus or cyclosporine A	Focal segmental glomerulosclerosis	1. Tacrolimus or cyclosporine A 2. Cyclophosphamide 3. MMF or rituximab
MMF	Mesangial proliferative glomerulonephritis	1. Cyclophosphamide 2. Tacrolimus or cyclosporine A
Rituximab	Membranous nephropathy	Tacrolimus or cyclosporine A
Levamisole	Membranoproliferative glomerulonephritis	1. Cyclophosphamide 2. Tacrolimus or cyclosporine A 3. MMF

FRNS/SDNS, frequent relapse/steroid-dependent nephrotic syndrome; MMF, mycophenolate mofetil; SRNS, steroid-resistant nephrotic syndrome.

for more than 4 of 12 months during the course of the disease.

Secondary outcomes

The secondary endpoints are divided into five items:

1. Rates of SSNS without FR, SRNS and SDNS/FRNS after 3 years of treatments.
2. Treatment outcomes after 3 years, including clinical cure, complete remission, partial remission and no remission.
3. Estimated glomerular filtration rate.
4. Glucocorticoid-associated toxicity.
5. Immunosuppression-associated toxicity.

Sample size calculation

The sample size calculation was based on the primary efficacy endpoint, and previously published studies showed that 20% to 30% of patients with INS ultimately develop SSNS without FR, 40% progress to SDNS/FRNS and 20% to 30% progress to SRNS.^{4 20} According to Zhou *et al*,²¹ HLA-DR9 is present in 71.4% of patients with FRNS, compared with 27.37% of patients with SSNS without FR. Adeyemo *et al*²² confirmed that high-risk Apolipoprotein L1 (APOL1) variants are present in 16.9% of patients with SSNS without FR and 40% of patients with SRNS. Based on the comparison of two proportions in two samples with a two-sided equality formula²³ using at a two-sided significance level of $\alpha=0.05$ and a power of 0.9, 304 patients (76 with SSNS without FR, 152 with SDNS/FRNS and 76 with SRNS) are required. Three hundred and thirty-six patients will be enrolled to account for a 10% dropout rate in each group. The procedure used to calculate the sample size is shown in online supplement 1.

$n_A = \kappa n_B$ and

$$n_B = f(x) = \left(\frac{P_A(1-P_A)}{\kappa} + P_B(1-P_B) \right) \left(\frac{Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}}{P_A - P_B} \right)^2$$

$\kappa = n_A / n_B$ is the matching ratio.

Data analysis

R Project 3.1.1 and IBM SPSS 22.0 software (IBM Corporation, New York) will be used to analyse the data. Results will be reported as ORs with 95% CIs. Statistical analyses will be two-tailed; the level of significance will be set at 0.05. The primary outcome will be determined using X^2 or Fisher's exact tests. All p values will be corrected with the Bonferroni correction for multiple comparisons of genetic variants. The squared correlation coefficient (r^2) and the Lewontin standardised disequilibrium coefficient (D'), both of which are measures of linkage disequilibrium, will be estimated using Haploview 4.2 software (Daly Lab at the Broad Institute, Cambridge, Massachusetts, USA).²⁴ Haplotype analyses are inferred with a standard expectation-maximisation algorithm and a partition-ligation approach using Haploview 4.2 software.²⁵

All secondary outcomes will be evaluated descriptively using appropriate statistical methods based on the underlying distribution of the data. Categorical variables will

be analysed using X^2 or Fisher's exact tests for comparisons among groups (ordinal categorical variables will be confirmed by Mann-Whitney U and Kruskal-Wallis H tests). Continuous variables will be reported as means \pm SD for normally distributed data and as medians with IQRs when the values are not normally distributed. A Kaplan-Meier analysis and Cox proportional hazards regression analysis will be used to analyse factors that influence the treatment outcomes after 3 years and decrease the glomerular filtration rate. Age, gender, body mass index, blood pressure, onset of clinical manifestations, premature birth history, passive smoking history, average relapse frequency, eGFR, urinary protein/creatinine levels, 24 hours urinary protein level/weight, renal pathological biopsy type and therapeutic intervention factors will be selected as risk factors in the multivariate analysis.

Patient and public involvement

Patients were not involved in the development of the research question or the design of this study. No patients involved in the recruitment to and conduct of the study. We aim to publish the study results as open access, which will be readily available to patients and the public.

Ethics and dissemination

The protocol and informed consent forms are in full compliance with human subject research protection regulations. Prior to the study, all personnel will be trained, and before patients are entered into the cohort, personnel will explain the research purpose to them and obtain their informed consent. The findings of this study will be published in a peer-reviewed journal and via conference presentations. According to the policy of the International Committee of Medical Journal Editors, the study has been registered in the Chinese Clinical Trial Registry, China (chictr.org.cn, ChiCTR1800019795).

DISCUSSION

Glomerulopathies are an important cause of childhood chronic kidney disease (CKD) in China, particularly INS. The incidence of CKD in children is increasing annually, and it is receiving increasing attention. The increased risk of vascular disease in children with CKD leads to a significant increase in the mortality rate. In addition, the disease itself generates considerable medical expenses and a substantial burden on the family and society.

To date, several lines of evidence have indicated that the immune system plays a potentially crucial pathogenic role in non-genetic INS.⁵ WES has been adopted in many cohort studies because of its lower cost, the lower amounts of DNA required, the possibility of comparatively more rapid turnaround and its greater sequencing depth.²⁶⁻²⁸ In this prospective cohort study, we chose WES to evaluate the presence of genetic variants in immune-related genes in patients with INS divided into three subgroups (SSNS without FR, SRNS and FRNS/SDNS). The SSNS without FR group will serve as the general control group. The study will adopt rigorous inclusive and exclusive criteria.

The patients included in the study will all be definitively diagnosed with INS in the absence of congenital nephrotic syndrome, which often has an onset occurring at less than 1 year of age. Refractory nephrotic syndrome is recognised as SDNS, SRNS or FRNS based on published literature in the study.^{29–31} In addition, unlike adults,¹⁷ the study will adopt proteinuria ≥ 50 mg/kg as the diagnostic criterion for childhood INS in clinical practice to adjust for weight, an important confounding factor.^{12 32 33}

Ethnic differences and genetic background may be linked to the clinical phenotypes of SSNS and SRNS. As a result of the rapid development of next-generation genome sequencing technology, the number of studies focusing on the genetic causes of SSNS and SRNS have increased in the past 5 years. The incidence of INS is higher among Asian children than among Caucasians, and Asian children have a high risk of becoming to SSNS.^{34 35} To date, numerous case reports of familial SSNS have been published.^{36 37} SSNS is characterised by relapse and remission and tends to improve with age. However, because variability in the phenotype of SSNS has rarely been reported, no single gene has been identified as a cause of monogenic SSNS. In 2014, Gbadegesin *et al*³⁸ conducted an exome array study and identified HLA-DQA1 and PLCG2 missense coding variants as candidate loci for SSNS. Recently, Adeyemo *et al*²² confirmed that HLA-DQA1 is a risk locus for SSNS in African-American children, consistent with its role in determining the SSNS risk in children with European, Asian and African ancestries; additionally, the authors found that high-risk APOL1 variants are not associated with SSNS but were significantly associated with SRNS. However, these studies were limited by the small patient cohort and the exclusive use of SNP panels to evaluate admixture and population stratification. In 2018, Jia *et al*³⁹ performed a genome-wide association study with a replication study in a Japanese population and showed that the HLA-DR/DQ region was associated with childhood SSNS.

Patients with SSNS may develop steroid dependency and/or frequent relapses (SDNS/FRNS), which are difficult to treat. Infections are often the origin of frequent relapses, treatment failure and significant morbidity.⁴⁰ According to the results from randomised clinical trials, levamisole prolongs the time to relapse and prevents recurrence in patients with FRNS.¹⁹ Classic HLA class II molecules are highly polymorphic heterodimeric transmembrane proteins encoded by the HLA-II gene on chromosome 6. The presentation of exogenous antigens in HLA class II molecules by professional antigen-presenting cells is a crucial step in the production of specific CD4⁺T cells in adaptive immune responses. Viral or bacterial infections are tightly correlated with these antigen processing and presentation pathways.^{41 42} In 2005, Bakr *et al*⁴³ suggested the HLA-DRBI*07011 allele confers a susceptibility to frequent relapse and a steroid-dependent or steroid-resistant course in patients with SSNS in Egypt. HLA polymorphisms shape the relative abundance of self-epitope-specific T cells that lead to protection or

autoimmunity, and in a Goodpasture disease mouse model, HLA-DR15/DR1 transgenic mice predominantly present CD4⁺Foxp3⁺ regulatory T cells expressing tolerogenic cytokines, which exert a protective effect.⁴⁴ Based on these two studies, part of the HLA-DR region may be associated with FRNS in Chinese patients.^{21 45} However, the small sample size and limited region from which the patients included in these studies were recruited remains a limitation that prohibits further analyses of variations that potentially explain FRNS/SDNS. Therefore, we based our study on the assumption that variations in antigen presentation governed by HLA-II polymorphisms exert substantial effects on steroid dependency and frequent relapses in patients with SSNS presenting with infections.

The study has several limitations that should be considered. First, only a single centre will be participating in this cohort study. Considering that Children's Hospital of Chongqing Medical University is the most highly regarded children's clinical medical centre in Western China and that the patients who are being treated for INS reside in various provinces and cities, we argue that the included patients will be an appropriately representative population. However, our findings will not be generalisable to all patients with INS in China. Second, we used the published incidence of high-risk variants to calculate the sample size, but the incidence of the variants and HLA allele distribution among Chinese patients with INS is not well known, which may lead to an inaccurate sample size calculation. If the sample size is insufficient to reveal risk variants or candidate loci, we will seek additional funds to expand the sample size.

Despite the aforementioned limitations, China has the largest and most ethnically diverse population in the world, and thus is the region with the most genetic diversity. To date, few cohort studies of patients with childhood-onset INS have been conducted in China. Based on clinical and genetic screens, we will conduct a prospective, observational, population cohort study in sufficient number of Chinese patients with INS. The results will support the clinical utility of whole exome sequencing as the most relevant tool for screening the rapidly evolving environment of monogenic SRNS and will provide patients with INS an opportunity for further stratification and personalised medicine.

Author affiliations

¹Department of Nephrology, Ministry of Education Key Laboratory of Child Development and Disorders; National Clinical Research Center for Child Health and Disorders (Chongqing); China International Science and Technology Cooperation base of Child development and Critical Disorders; Children's Hospital of Chongqing Medical University, Chongqing, China

²Chongqing Key Laboratory of Pediatrics, Chongqing, China

³China International Science and Technology Cooperation Base of Child Development and Critical Disorders, Chongqing, China

Contributors All the authors have accepted responsibility for the full content of this manuscript and approved its submission. QiuL proposed and will conduct the study, and HY and HC will be responsible for data collection and data extraction. HY, GZ and MW will be responsible for data analysis. HL, XuY, JW, XiY, CG, HX, QiaL, JJ, DW will be responsible for enrolling patients in the study and collecting specimens.

Funding This cohort study is funded by the China National Natural Science Foundation (grant number 81770713) and China National Clinical Research Centre Foundation (grant number YBXM-2019-002).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval Ethical approval was obtained from the Ethics Committee of Children's Hospital of Chongqing Medical University on 16 November 2018 (reference number 2018-140, 2018-11-16).

Provenance and peer review Not commissioned; externally peer reviewed.

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