RENAL FAILURE
2025, VOL. 47, NO. 1, 2479184
https://doi.org/10.1080/0886022X.2025.2479184

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CLINICAL STUDY



# Etiology of nephrotic syndrome: insights from univariate and multivariate Mendelian randomization study

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**ABSTRACT**

Nephrotic syndrome (NS) is a common cause of chronic glomerular disease. However, the precise way in which one or more risk exposure traits of renal injury lead to NS remains unclear. In this study, we systematically examined the causal relationships between NS and various exposure traits, including traits related to chronic hepatitis B/C infection, COVID-19 (hospitalized), general allergy status, herbal tea intake, immunoglobulin E, childhood obesity, and the human leukocyte antigen (HLA)-II histocompatibility DM α/DP β1/DQ α2 chain, *via* multivariate Mendelian randomization (MVMR). A previously reported exposure trait, ulcerative colitis, was also included to analyze the independent effect of each significant exposure on the risk of developing NS. In the univariable MR analysis, immunoglobulin E (OR = 5.62, 95% CI = 2.91–10.84, $p=2.67\times10^{-7}$) and the HLA-II histocompatibility DQ α2 chain (OR = 0.70, 95% CI = 0.63–0.80, $p=2.83\times10^{-7}$) were shown to have effect estimates consistent with a greater risk of developing NS. The reverse MR analysis showed no evidence of causal effect from NS to histocompatibility DQ α2 chain ($p=0.76$). In MVMR, only the HLA-II histocompatibility DQ α2 chain retained a robust effect (OR = 0.71, 95% CI = 0.61–0.82; $p=9.39\times10^{-6}$), and the estimate for immunoglobulin E was weakened (OR = 1.04, 95% CI = 0.60–2.13; $p=0.92$). With two independent ulcerative colitis resources used for validation, ulcerative colitis was not significantly associated with NS. This study provides genetic evidence that the HLA-II histocompatibility DQ α2 chain has a predominant causal effect on the risk of developing NS. HLA-II histocompatibility-mediated immune abnormalities may lead to subtypes of NS and its pathological changes.

**ARTICLE HISTORY**
Received 8 December 2024
Revised 20 February 2025
Accepted 9 March 2025

**KEYWORDS**
Multivariate Mendelian randomization; nephrotic syndrome; steroid-sensitive nephrotic syndrome; human leukocyte antigen

## Introduction

Nephrotic syndrome (NS) is a common cause of chronic kidney disease (CKD). Early and effective disease management is crucial for preventing the progression of NS to CKD and reducing the incidence of end-stage kidney disease. It is characterized by severe proteinuria, a urinary protein/creatinine ratio ≥200 mg/mmol or a urine dipstick showing 3+ protein, a serum albumin concentration < 30 g/L, and edema [1]. Patients with NS can develop life-threatening complications, including infection and thrombosis, hypovolemia, and acute kidney injury (AKI) [2]. Although NS is not a single disease, severe proteinuria is a defining factor of NS. To date, the etiology and pathogenesis of the severe proteinuria remain obscure. Even after transplantation, patients with focal glomerulosclerosis (FSGS), a specific type of NS, may still experience recurrent severe proteinuria. The mechanism of recurrent focal FSGS in renal allografts is still unclear. Therefore, exploring the relationships between exposure factors and NS remains highly important from the perspective of disease prevention.

A considerable amount of epidemiological and experimental evidence has confirmed that various systemic diseases, such as pathogen infections, human allergic status, nutritional disorders, and immune circulating factors, play vital roles in the initiation and progression of NS [3–6]. Increasing evidence supports a relationship between hepatitis B or C infection and diverse extrahepatic manifestations, such as NS and polymyositis [7–8]. Moreover, the nutritional status (obesity and low birth weight) of patients is closely related to NS, but there is no unified conclusion [9–10]. Recently, a two-sample Mendelian randomization (MR) study

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/0886022X.2025.2479184.

revealed potential genetic relationships between ulcerative colitis status and the risk of developing NS [11]. However, these relationships may involve multiple factors, such as medication side effects, allergic status, immune system abnormalities, inflammatory mediators and bacterial toxins. MR studies with more systemic exposure traits and rigorous pleiotropy robustness tests are needed.

Genetic differences contribute to phenotypic diversity in humans and pathogens, including variations in susceptibility to diseases, especially polygenic systemic disorders [12]. Advancements in genomic analysis techniques have enabled the identification and scoring of single-nucleotide polymorphisms (SNPs) to elucidate the genetics of individual disease susceptibility [13]. Multivariate MR (MVMR) is an emerging randomization method based on Mendelian inheritance laws. This approach combines the Mendelian law of segregation with the instrumental variable (IV) approach and can effectively avoid interference between variables and improve the reliability of the experiment. In MVMR, SNPs can be used as IVs in the presence of unknown confounding factors and overcome the limitations of conventional epidemiological studies [14–15].

In this study, we performed comprehensive and systematic analyses across all age groups, specifically focusing on pediatric steroid-sensitive nephrotic syndrome (SSNS), to determine which environmental and immune factors have predominant causal effects on the risk of developing NS. First, we conducted a genome-wide association study (GWAS) to identify IVs associated with each risk trait by using the UK Biobank (UKB), GWAS catalog, Integrative Epidemiology Unit (IEU) GWAS database and FinnGen database. We then conducted MR analyses, including MVMR, to elucidate which traits are of fundamental relevance to the risk of developing NS.

## Materials and methods

### Study design

Before the study was performed, we conducted a thorough and systematic analysis of the available GWAS data (Figure 1). Thus, we identified 10 exposure traits with strong IVs, including chronic hepatitis B/C infection, COVID-19 (hospitalized), general allergy status, herbal tea intake, immunoglobulin E, childhood obesity, and the human leukocyte antigen (HLA)-II histocompatibility DM α/DP β1/DQ α2 chain. Univariable MR analysis was conducted to evaluate whether genetically predicted pathogen infections, humoral allergic status, nutritional disorders, and immune-related circulating factors are significantly associated with the risk of developing NS. MVMR analysis was subsequently performed to evaluate the independent effects of these significant risk traits. The genetic IVs utilized in the analysis adhered to the three core assumptions of MR [16] (Figure 1): (1) genetic variation should be significantly associated with exposure (e.g., pathogen infections, humoral allergic state, nutritional disorders, and immune circulating factors); (2) genetic variation should be free from any associations with confounding factors; and (3) genetic variation must be associated with the risk of developing NS only through the above exposure traits. The reporting of this study strictly follows the most recent guidelines provided by the Strengthening the Reporting of Observational Studies in Epidemiology-Mendelian Randomization framework. (Table S1: STROBE-MR checklist).

### Data sources

The genetic variation data were obtained from published meta-analyses of GWASs and public databases, such as the UKB, GWAS catalog, IEU GWAS database and FinnGen database. We conducted a comprehensive analysis of the associations among pathogen infections, the humoral allergic state, nutritional disorders, and circulating immune factors. To ensure the IV candidates met the three core MR assumptions, SNPs significantly associated with exposure risk traits were selected using a genome-wide significance threshold of $p < 5 \times 10^{-8}$. The excluded potential risk traits are listed in Table S2. Finally, our study assessed 10 exposures in public databases based on their genetics, including traits associated with the following variables: chronic hepatitis B infection ($N = 351{,}885$), chronic hepatitis C infection ($N = 352{,}013$), COVID-19 (hospitalized) ($N = 1{,}887{,}658$), general allergy ($N = 484{,}598$), herbal tea intake ($N = 64{,}949$), immunoglobulin E ($N = 3{,}301$), childhood obesity ($N = 13{,}848$), human leukocyte antigen (HLA)-II histocompatibility DM α chain ($N = 3{,}301$), HLA-II histocompatibility DP β1 chain ($N = 3{,}301$), and HLA-II histocompatibility DQ α2 chain ($N = 3{,}301$). Summary statistics for NS were obtained from a GWAS study of the European cohort, comprising 526 NS patients and 342,005 controls [17]. Similarly, summary statistics for steroid-sensitive nephrotic syndrome (SSNS) were derived from 132 childhood SSNS patients and 2,000 controls within the European population [18]. The diagnosis of NS is based primarily on the Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. Given that a published MR study suggested an association between ulcerative colitis and NS, in the MVMR stage, summary statistics for ulcerative colitis obtained from the IEU GWAS were included. Table 1 provides a summary of the characteristics of the GWAS consortia utilized for each exposure trait.

### Selection criteria for IVs

To align the candidate IVs with the three core MR assumptions, SNPs significantly associated with the exposure ($p < 5 \times 10^{-8}$) were initially selected from the GWAS. A conventional linkage disequilibrium (LD) pruning process was applied, using an $r^2$ threshold of <0.001 and a window size of 10,000 kb. To maintain allele alignment, SNPs with inconsistent alleles and ambiguous palindromic configurations were either removed or appropriately adjusted. After the initial filtering process, the SNPs associated with potential confounding factors were removed *via* PhenoScanner V2 (http://www.phenoscanner.medschl.cam.ac.uk/) [19]. The F-statistic formula was used to assess the robustness of the IVs:

$$F = \frac{\beta^2}{SE^2}$$

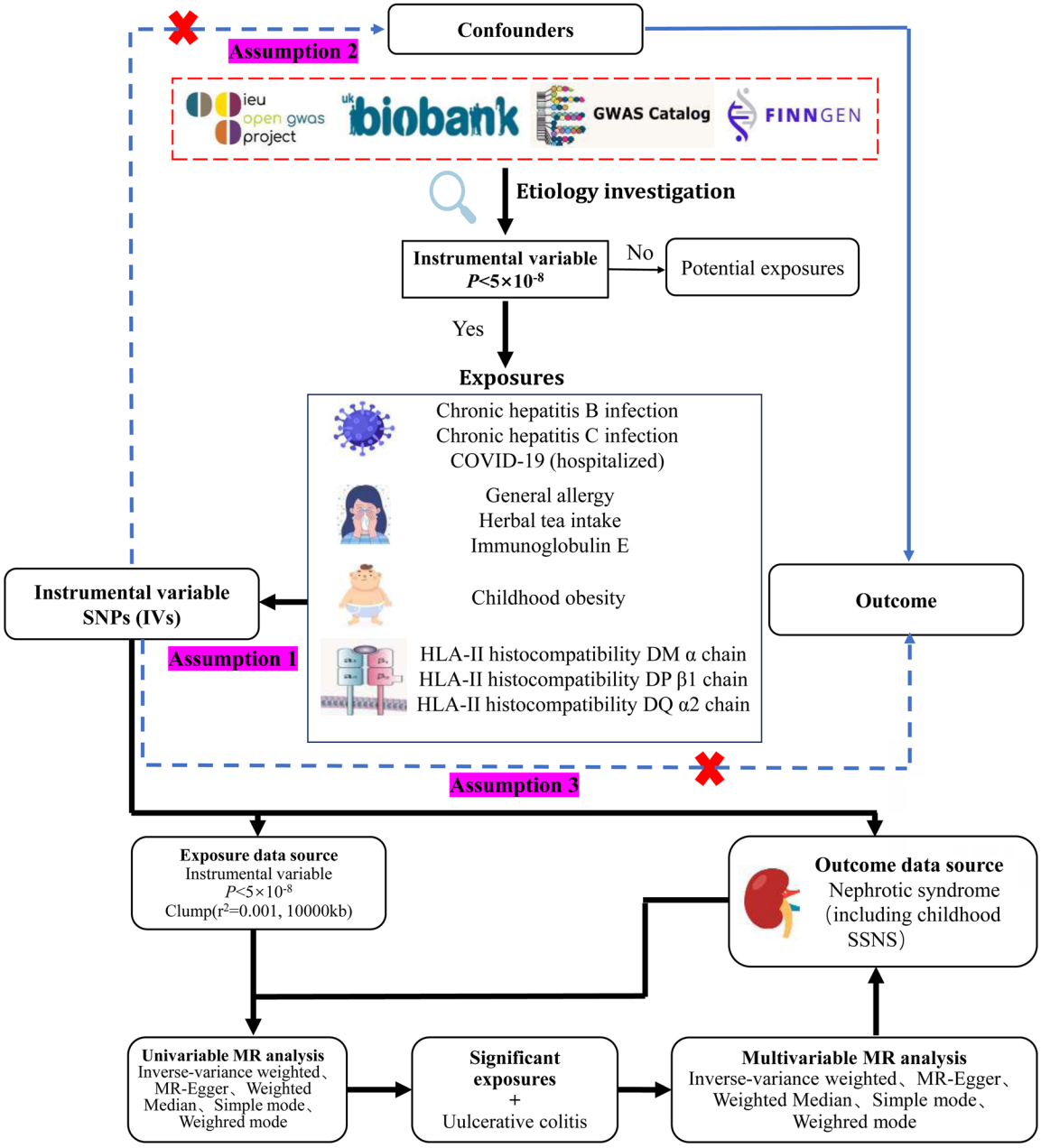


**Figure 1.** Schematic diagram of the MR design and flowchart of the univariable and MVMR analyses. The continuous lines represent the relationships that held in the MR analysis. The dashed lines depict the associations that should not be present to satisfy the second and third assumptions. The continuous bold lines represent the progression of the study. SNPs: single-nucleotide polymorphisms; COVID-19: Coronavirus Disease 2019; SSNS: steroid-sensitive nephrotic syndrome.

$$R^2 = \frac{F}{N-2+F}$$

IVs with an F-statistic well above 10 are considered robust, reducing the likelihood of weak instrument bias [20].

### *Statistical analysis*

To identify significant exposure traits suitable for MVMR analysis, we first applied five statistical methods to explore the causal relationship between exposure traits and NS. Among these, inverse-variance weighting (IVW) served as the primary approach, computing the weighted average of the Wald estimates for each selected SNP [21]. This method provides the most reliable results when the IVs are free from pleiotropy. Heterogeneity and pleiotropy were quantified using the Cochran Q test, $I^2$ statistic, and MR-Egger intercept. If a $p$ value less than 0.05 is observed in the heterogeneity Q test of the IVW method, it indicates the presence of heterogeneity. In such cases, the WM method or the MR–Egger method may provide more reliable causal estimates. Leave-one-out analysis was conducted to exclude each SNP to assess its individual influence on the results. The asymmetry of the funnel plot was examined to improve the reliability of the conclusions, and the

Table 1. Characteristics of the GWAS consortia used for each exposure trait.

| Exposure/Outcome | Database | ID numbers | Numbers of SNPs | Ethnicity | Sample sizes |
|---|---|---|---|---|---|
| **Infections** | | | | | |
| Chronic hepatitis B infection | IEU | ebi-a-GCST90018804 | 19,079,722 | European | 351,885 |
| Chronic hepatitis C infection | GWAS catalog | GCST90018805 | 19,074,546 | European | 352,013 |
| COVID-19 (hospitalized) | IEU | ebi-a-GCST011081 | 8,107,040 | European | 1,887,658 |
| **Anaphylactic disease** | | | | | |
| General allergy | GWAS catalog | GCST90038661 | 9,587,836 | European | 484,598 |
| Herbal tea intake | IEU | ukb-b-13344 | 9,851,867 | European | 64,949 |
| Immunoglobulin E | GWAS catalog | GCST90241471 | 10,534,735 | European | 3,301 |
| **Nutritional disorders** | | | | | |
| Childhood obesity | IEU | ieu-a-1096 | 2,442,739 | European | 13,848 |
| **Human leukocyte antigen** | | | | | |
| HLA-II histocompatibility DM α chain | GWAS catalog | GCST90241448 | 10,534,735 | European | 3,301 |
| HLA-II histocompatibility DP β1 chain | GWAS catalog | GCST90241449 | 10,534,735 | European | 3,301 |
| HLA-II histocompatibility DQ α2 chain | UKB | prot-a-1347 | 10,534,735 | European | 3,301 |
| **Previously reported** | | | | | |
| Ulcerative colitis | | | | | |
| Ulcerative colitis resource 1 | IEU | ebi-a-GCST90038684 | 9,587,836 | European | 484,598 |
| Ulcerative colitis resource 2 | FinnGen | ULCEROTH | 16,380,459 | European | 214,620 |
| **Nephrotic syndrome** | GWAS catalog | GCST90018884 | 24,196,233 | European | 342,531 |
| **Steroid-sensitive nephrotic syndrome** | GWAS catalog | GCST009711 | 5,389,455 | European | 2132 |

UKB: UK Biobank; IEU: Integrative Epidemiology Unit; FinnGen: Finnish Genome Research Project; GWAS catalog: Genome-Wide Association Studies Catalog; COVID-19: Coronavirus Disease 2019.

MR-PRESSO method was employed to detect and remove outliers caused by horizontal pleiotropy.

MVMR was carried out to determine the independent effect of significant exposure traits on the risk of developing NS [22–23]. Based on univariable MR estimates, the MVMR method provides low bias and robust causal inference under varying levels of pleiotropy. In this study, a Bonferroni-corrected threshold of $p < 0.025$ (0.05/2) was used to denote statistical significance in the MVMR analysis. The linkage disequilibrium score regression (LDSC) method was used to determine the heritability of significant exposures and NS, as well as the genetic correlation between them [24]. All analyses were performed using the TwoSampleMR (version 0.5.10), Mendelian randomization (version 0.8.0), and MRPRESSO (version 1.0) packages in R Software 4.3.2 (https://www.R-project.org) [25].

## Results

### GWAS of the exposure traits

In the GWAS, we identified many independent SNPs associated with each exposure trait at $p < 5 \times 10^{-8}$: 6672 SNPs associated with chronic hepatitis B infection, 294 associated with chronic hepatitis C infection, 714 associated with COVID-19 (hospitalized), 62 associated with general allergies, 111 associated with herbal tea intake, 62 associated with immunoglobulin E, 229 associated with childhood obesity, 217 associated with the HLA-II histocompatibility DM α chain, 217 associated with the HLA-II histocompatibility DM α chain, and 9440 associated with the HLA-II histocompatibility DQ α2 chain (Figure 2). A considerable number (426 SNPs) of SNPs used in each of the exposure-related genetic IVs showed significant associations with other exposure traits in conventional GWASs ($p < 5 \times 10^{-8}$) (Figure 3A). By exploring the relationships of the genetic IVs with each exposure trait, we identified widespread associations (Figure 3B). For example, in addition to its association with chronic hepatitis B infection, genetic IVs for chronic hepatitis B infection showed strong positive associations with childhood obesity and the HLA-II histocompatibility DM α chain.

### Univariable MR analysis of the exposure traits related to the risk of developing NS

After the above screening process, we selected 5, 3, 5, 29, 19, 1, 6, 2, 2 and 4 SNPs as the IVs of chronic hepatitis B infection, chronic hepatitis C infection, COVID-19 (hospitalized), general allergy, herbal tea intake, immunoglobulin E, childhood obesity, HLA-II histocompatibility DM α chain, HLA-II histocompatibility DP β1 chain, and HLA-II histocompatibility DQ α2 chain. The F statistics of all of the SNPs were more significant than 10 (range, 31.122–358.599), indicating that the results were unlikely to be affected by weak IVs (Table S3). On individual assessment through conventional MR, we found that immunoglobulin E and the HLA-II histocompatibility DQ α2 chain had effect estimates consistent with a greater risk of developing NS (Figure 4 and Table S4). Immunoglobulin E had an odds ratio (OR) of 5.62 (95% CI: 2.91–10.84; $p = 2.67 \times 10^{-7}$) for NS and an odds ratio (OR 0.70, 95% CI: 0.63–0.80, and $p = 2.83 \times 10^{-7}$) for the histocompatibility DQ α2 chain. We performed an additional reverse MR analysis to explore reverse causality. The reverse MR analysis did not identify a causal effect from NS on immunoglobulin E (OR 0.99, 95% CI: 0.97–1.01, and $p = 0.36$) or the histocompatibility DQ α2 chain (OR 0.98, 95% CI: 0.97–1.00, and $p = 0.76$). Univariable MR analysis revealed no significant causal relationships between the remaining eight exposure traits and the risk of developing NS (all $p$ values > 0.05). All MR

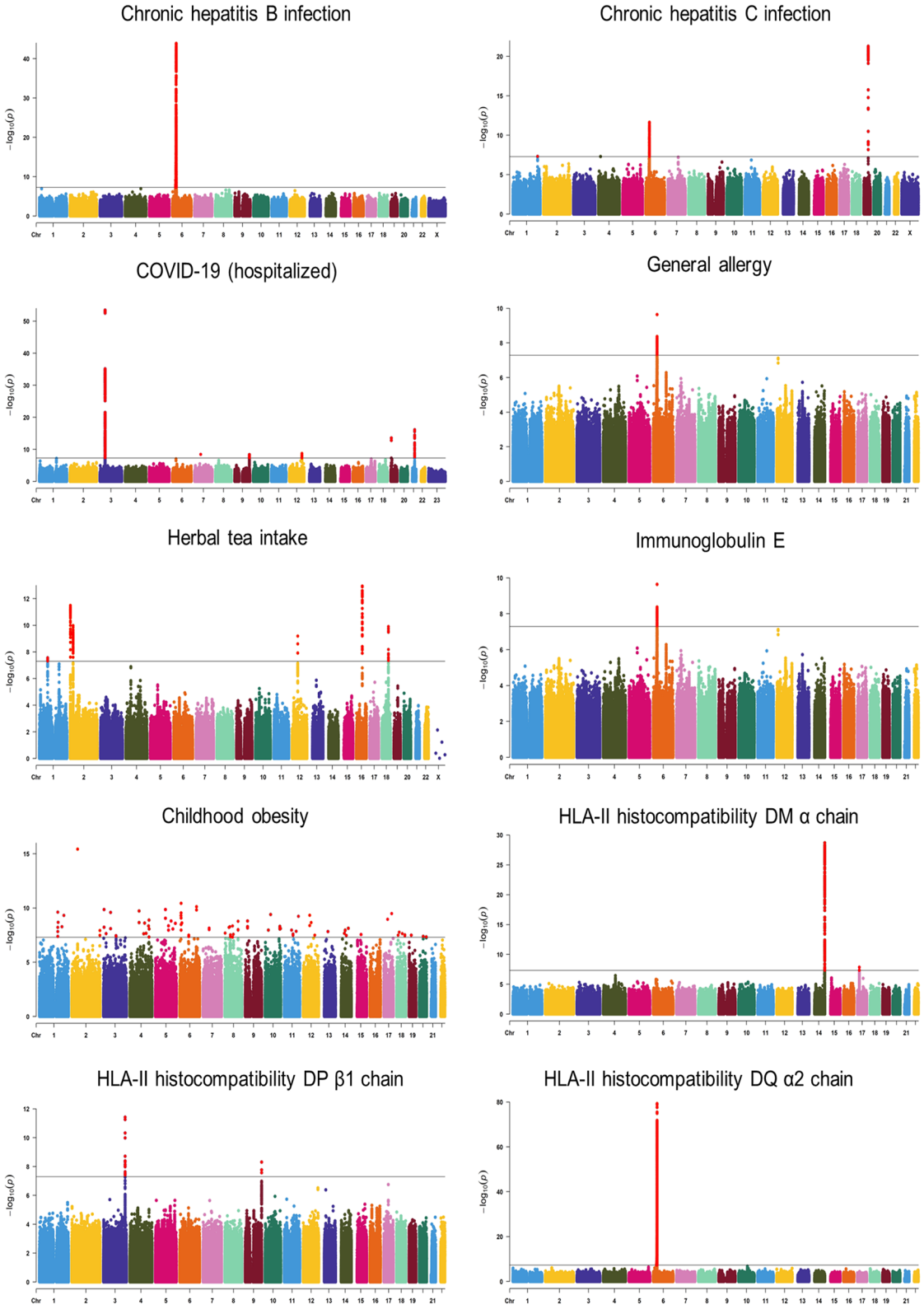


**Figure 2.** Manhattan plots showing findings from the GWAS of exposure-related traits. The vertically distributed dotted lines represent SNP loci linked across different chromosomal positions. The horizontal dotted line illustrates the Y-axis value conventionally used to denote a SNP that reached statistical significance in the GWAS, i.e., at $p < 5 \times 10^{-8}$.

analyses (IVW, MR–Egger, WM, simple mode, and weighted mode) yielded the same results. The number of effective IVs for chronic hepatitis C infection, immunoglobulin E, the HLA-II histocompatibility DM α chain, and the HLA-II histocompatibility DP β1 chain was less than 4. Comprehensive comparisons of MR-PRESSO ($p$ value for Global Test > 0.05), the Cochran Q test ($p$ value > 0.05), $I^2$ statistics ($I^2$ = 0.000), and the MR–Egger test (Egger intercept = –0.0005, $p$ value >

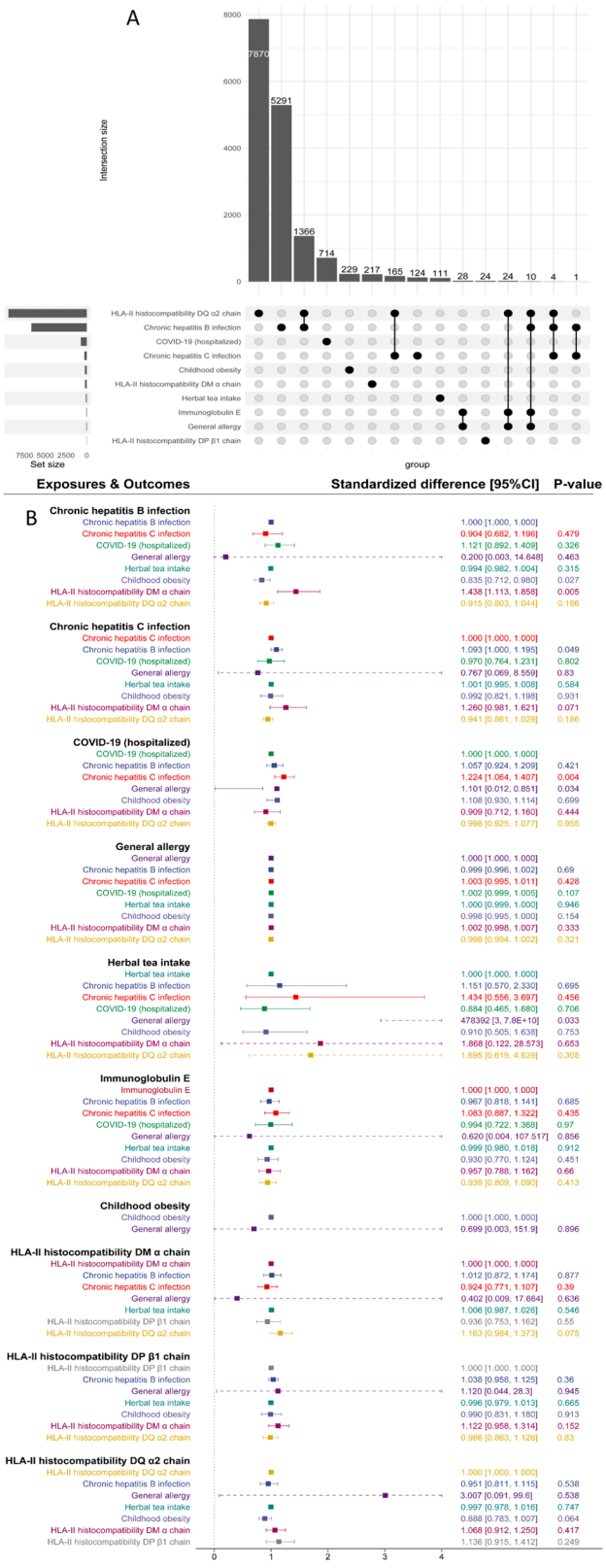


**Figure 3.** Characteristics of genetic IVs utilized as exposure-related traits. (A) UpSet plot of SNPs. An UpSet plot is a visualization tool used to represent the intersections of multiple sets, often as an alternative to a Venn diagram. (B) Associations between genetic IVs and exposure-related traits *via* the IVW approach. CI: confidence interval.

0.05) also revealed that IVs had no heterogeneity or horizontal pleiotropy in our study (Table S5). Except for one IV for immunoglobulin E, the MR-PRESSO outlier test and leave-one-out analysis revealed that all of the above results were unaffected by outliers. Leave-one-out analysis diagrams, scatter plots, and funnel plots are all shown in Figure S6.

## *MVMR of the exposure traits in connection with the risk of developing NS*

Both the HLA-II histocompatibility DQ α2 chain and immunoglobulin E are integral serum immune factors in the human body. To study the relationship between the serum levels of these immune factors and the risk of developing NS, we screened 41 SNPs (Table S7) as IVs of MVMR to analyze how significant exposure traits (immunoglobulin E and the HLA-II histocompatibility DQ α2 chain) independently affect the risk of developing NS. The MVMR-IVW estimates revealed that the HLA-II histocompatibility DQ α2 chain (OR = 0.71, 95% CI = 0.61–0.82, $p = 9.39 \times 10^{-6}$) was significantly associated with the risk of developing NS, whereas the immunoglobulin E chain (OR = 1.04, 95% CI = 0.60–2.13, $p = 0.92$) suggested no significant causal relationships with the risk of developing NS (Figure 5). Owing to insufficient IVs, many methods could not be used, and the remaining three MVMR analysis methods (MR–Egger, weighted median, and MR–LASSO) were not used. Therefore, we adjusted the threshold of IVs to $p < 5 \times 10^{-7}$. Despite the heterogeneity associated with the MVMR-IVW method ($p < 0.05$), the MVMR–Egger, MVMR-weighted median, and MR–LASSO methods revealed that the HLA-II histocompatibility DQ α2 chain, but not immunoglobulin E, was significantly associated with the risk of developing NS (Figure 5).

In addition, considering the confounding IVs from ulcerative colitis, we conducted MVMR analysis using independent ulcerative colitis resources from the IEU GWAS and FinnGen. The MVMR-IVW estimates revealed that the HLA-II histocompatibility DQ α2 chain (Model 1, OR = 0.72, 95% CI = 0.61–0.84, $p = 4.84 \times 10^{-5}$; Model 2, OR = 0.71, 95% CI = 0.56–0.89, $p = 0.003$) was still significantly associated with the risk of developing NS, and the ulcerative colitis status (Model 1, OR = 0.85, 95% CI = $8.71 \times 10^{-13}$–$8.28 \times 10^{11}$, $p = 0.99$; Model 2, OR = 0.98, 95% CI = 0.74–1.30, $p = 0.90$) was not significantly associated with the risk of developing NS.

## *MR analysis for subtypes of NS*

This study fully utilized publicly available genetic repositories associated with NS. SSNS is one of the main types of NS, and more than 90% of cases manifest as minimal change disease (MCD) in children. A GWAS dataset comprising 2132 European individuals (132 children with SSNS and 2,000 controls) was included. Univariable MR analysis revealed no significant causal relationships between the HLA-II histocompatibility DQ α2 chain and the risk of developing childhood SSNS (IVW, OR 0.92, 95% CI: 0.20–4.25, and $p = 0.92$).

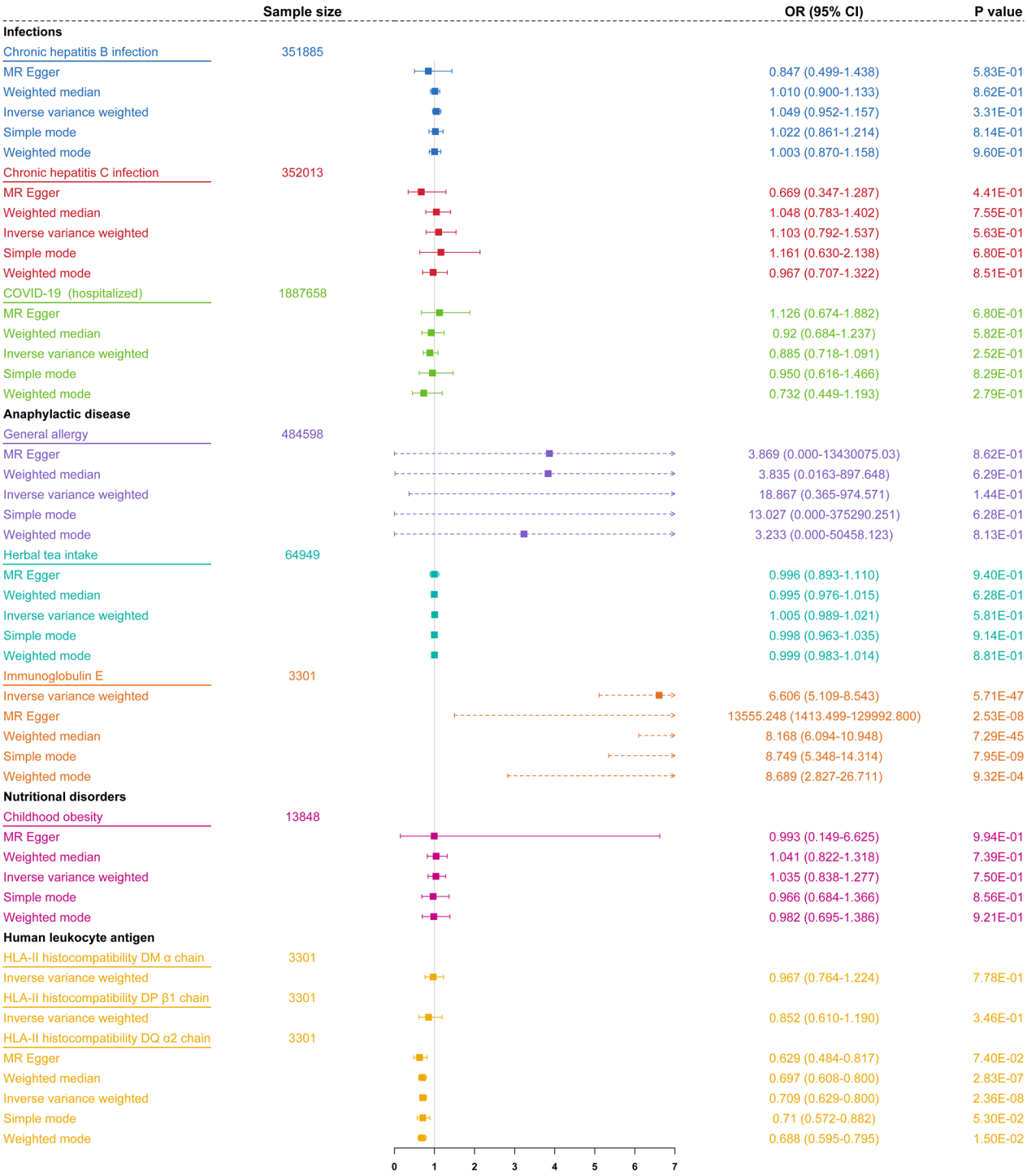


**Figure 4.** Univariate MR results for the associations between exposure traits and the risk of developing NS. The blue dots represent the estimates obtained *via* MR analysis, and the gray bars represent the 95% confidence intervals of the estimates. An or > 1 indicates increased risk, whereas <1 indicates decreased risk; or, odds ratio; CI: confidence interval.

### *Heritability and genetic correlation*

The LDSC results (Table 2) revealed moderate total heritability estimates for both the HLA-II histocompatibility DQ α2 chain ($h^2$ = 0.12, SE = 0.18) and NS ($h^2$ = 0.002, SE = 0.0007). Additionally, the genetic correlation analysis indicated no significant genetic correlation ($p$ value = 0.78) between the HLA-II histocompatibility DQ α2 chain and NS, suggesting that the fraction of SNPs with an effect on both traits is small. This further implied that the IVs used in the MR analysis were less likely to have a direct effect on the outcome.

## Discussion

This is the first study investigating the causal correlation between potential exposure traits and the risk of developing NS. In this study, we conducted a systematic statistical

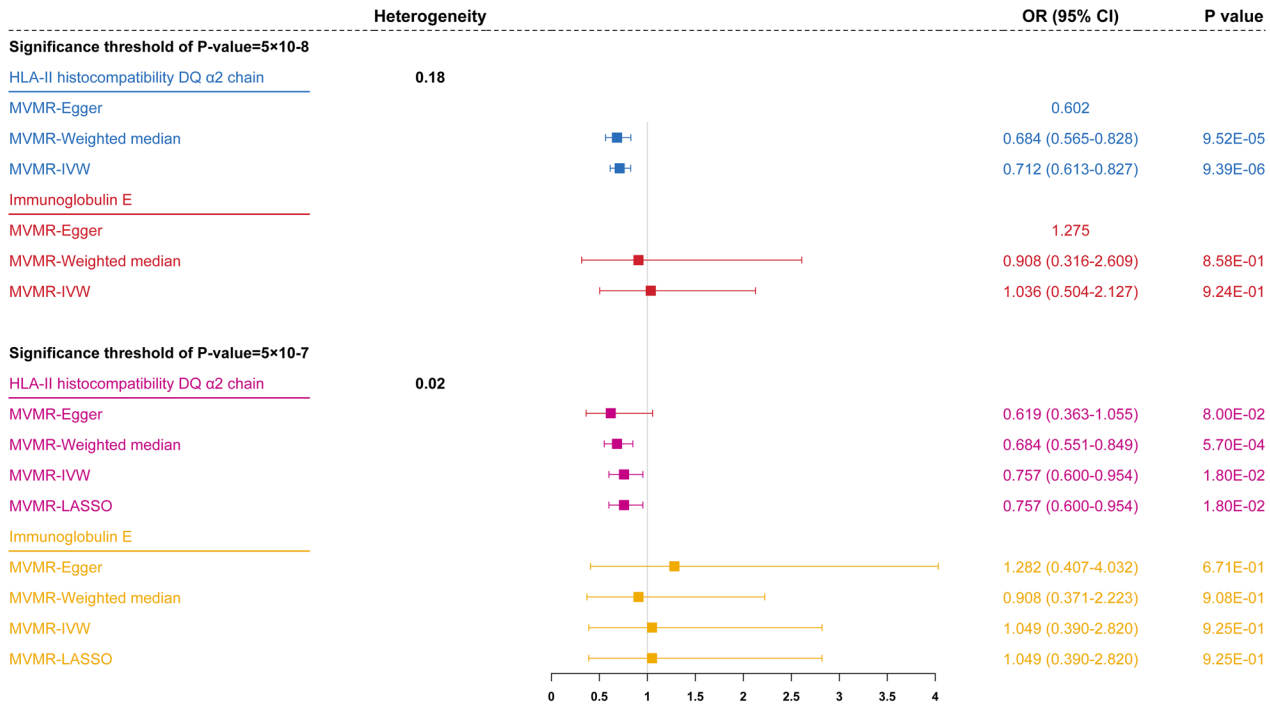


**Figure 5.** Multivariate MR results for the associations between exposure traits and the risk of developing NS. The blue dots represent the estimates obtained *via* MR analysis, and the gray bars represent the 95% confidence intervals of the estimates. An or > 1 indicates increased risk, whereas an or < 1 indicates decreased risk; or, odds ratio; CI: confidence interval; IVW: inverse variance weighting.

**Table 2.** Heritability and genetic correlation of the HLA-II histocompatibility DQ α2 chain and NS.

| | Heritability | | | Genetic correlation | |
|---|---|---|---|---|---|
| Traits | $h^2$ (SE) | Intercept *p* value | Ratio of stratification | Correlation (SE) | *p* value |
| HLA-II histocompatibility DQ α2 chain | 0.12 (0.18) | 0.51 | −2.13 | 0.52 | 0.78 |
| NS | 0.002 (0.0007) | 0.05 | −0.45 | | |

NS: Nephrotic syndrome.

analysis of potential exposures and ultimately explored the causal relationships of 10 exposure traits with the risk of developing NS by conducting a GWAS in approximately 3,158,252 participants and applying genetic IVs to univariable and MVMR using data from the UKB, including over 342,531 cases of NS. Several studies have suggested that hyperuricemia is an independent risk factor for progression to end-stage renal disease in patients with NS, and it may even be correlated with an increased risk of venous thromboembolism and the occurrence of hypertension in childhood NS [26–28]. In the early phase of our study, we selected strongly correlated IVs [29] associated with hyperuricemia and univariable MR analysis revealed no significant causal relationships between hyperuricemia and NS (Table S8). Therefore, hyperuricemia was not included as a potential exposure factor in our study.

Our findings suggest that immunoglobulin E and the HLA-II histocompatibility DQ α2 chain have a causal connection with the risk of developing NS. After correction for immunoglobulin E, the HLA-II histocompatibility DQ α2 chain was still the predominant trait that accounts for the etiological risk of developing NS. Although univariable MR suggested a potential association between immunoglobulin E and NS, MVMR analyses refuted this association after correcting for the influence of the HLA-II histocompatibility DQ α2 chain. Nevertheless, our univariable MR analysis revealed possible evidence supporting a causal association between immunoglobulin E and the risk of developing NS for the first time from a genetic perspective. Numerous clinical studies have shown that immunoglobulin E is associated with NS. The chronological levels of serum immunoglobulin E revealed that extremely high serum immunoglobulin E levels preceded the onset or relapse of minimal-change NS [30–31]. A model for predicting minimal-change disease in 142 patients with NS showed that immunoglobulin E and G levels provide physicians with simple and valuable clinical markers for diagnosing minimal-change disease before renal biopsy [32]. NS has been reported to be associated with allergies in many studies. In children with SSNS, immunoglobulin E levels were found to be greater in patients who experienced relapse than in those who experienced remission, but no significant difference was found between those with and without a history of atopy in terms of their immunoglobulin E levels. Dilek Yılmaz et al. further suggested that increased IgE may reflect

the activation of immune mechanisms following various stimuli rather than a direct association with atopy [33]. These findings indicate that immunoglobulin E is necessary for immunoglobulin-mediated NS to occur.

Our principal findings are that after accounting for the effects of the HLA-II histocompatibility DQ α2 chain, the relationships of other exposure traits with the risk of developing NS attenuated markedly to null—in other words, the HLA-II histocompatibility DQ α2 chain is a critical entity that underlies the relationship between immune traits and the risk of developing NS. In recent years, genetic studies have provided evidence that human HLA histocompatibility plays an important role in the process of NS. However, the extent of their impact on determining NS remains uncertain. HLA histocompatibility is a crucial component of the human immune system and is encoded by a complex of genes located on the short arm of chromosome 6. These antigens are expressed by the major histocompatibility complex, which plays a pivotal role in determining tissue compatibility and rejection reactions during transplantation. HLAs are categorized into three classes on the basis of their distribution and function: Class I, Class II, and Class III. The classical class I antigens include HLA-A, HLA-B, and HLA-C, whereas class II antigens include HLA-DQ (HLA-DQA/DQB). In 2014, Gbadegesin et al. [34] conducted an exome array study and identified rs1129740 and rs1071630, which are located within HLA-DQA1, as candidate loci for SSNS in South Asian and White European patients. In 2018, Jia et al. [35] performed a GWAS with a replication study in a Japanese population and reported that the HLA-DR/DQ region is associated with childhood SSNS. A genomic association study dissecting the genetic components that contribute to the two main subphenotypes of NS *via* a GWAS strategy suggested that the HLA-DQA/DQB region is likely associated with disease relapse [36]. Unlike previous studies, our research further confirmed the causal relationship between the HLA-II histocompatibility DQ α2 chain and NS. One possible mechanism is that gene sets of the HLA-II histocompatibility DQ α2 chain and HLA-II receptor are strongly associated with the innate immune system, including the activation of antigen-presenting cells (APCs), as well as the adaptive immune system [37–38]. As the HLA-DQA/DQB heterodimeric surface molecule is expressed on professional APCs, including macrophages, B lymphocytes and monocyte-derived dendritic cells (MoDCs), specific peptides are presented by APCs through HLA-DQA/DQB, enabling direct interactions with the T-cell receptor of CD4+ T lymphocytes and eliciting a proinflammatory cytokine-rich microenvironment [39], ultimately leading to the development of NS. Single amino acids in one HLA-DQA/DQB protein may play a key role in HLA functions by altering the local 3-dimensional conformation of certain HLA-II histocompatibility molecules [40]. Interestingly, different patients with NS carry different HLA-II histocompatibility DQ molecules, resulting in varying disease manifestations, such as disease recurrence. Therefore, the results of our study are highly important for the future diagnosis of NS, such as peptide docking-based binding assays, which may help to confirm these special types of NS.

The findings of the present study have been enabled by 2 recent scientific advances. First, our study fully utilized publicly available genetic repositories. This is the first study in which an MVMR design was utilized to investigate the causal effect of epidemiological exposure traits on the risk of developing NS. The availability of large-scale epidemiological exposure trait data and GWAS genotyping in public databases, such as the UK Biobank, GWAS catalog, Integrative Epidemiology Unit (IEU) GWAS database and FinnGen database, provides sufficiently large numbers to permit the identification of robust genetic variants (and therefore suitable genetic IVs) to conduct MR analyses. Determining the independent causal effect of each parameter on the risk of developing NS is an excellent solution to the vertical pleiotropy caused by the interaction effects of immune factors. Second, methodological developments in MR to include more than one trait allow for direct effects of multiple exposures to be assessed simultaneously and without the risk of introducing certain forms of bias, such as collider bias. The above technologies have significantly improved the possibility of deriving epidemiological conclusions related to the risk of developing NS: As an immune factor, the HLA-II histocompatibility DQ α2 chain. plays a critical role in the causal risk of developing NS but not in ulcerative colitis. Immune-related disorders and abnormal immune responses may lead to the different subtypes of NS and its pathological changes, resulting in immune-mediated glomerulonephritis and damage to the kidneys.

This study also has certain limitations. According to the principles of MR, the exposure and outcome data should be derived from the same ethnic group. However, owing to the limitations of existing public HLA histocompatibility resources, only five HLA class II histocompatibility traits from European individuals were assessed in this study. HLA class II histocompatibility data from other ethnic groups were not collected or analyzed. Owing to the limitations of public GWAS resources and the small sample size for specific pathological types of NS, such as minimal change disease (MCD) and FSGS, these subtype analyses of NS were not performed. However, childhood SSNS was subjected to MR analysis in our study and it revealed no significant causal relationships between the HLA-II histocompatibility DQ α2 chain and the risk of developing childhood SSNS. This result contradicts existing studies [41], including those of our study, and the possible reasons are as follows. First, the small number of children with SSNS ($n=132$) led to a lack of effective IVs, resulting in a false-negative outcome. Second, different types of NS may exhibit varying HLA histocompatibility dependencies, but owing to the lack of publicly available HLA histocompatibility genetic data or GWAS data on different subtypes of NS for analysis, this hypothesis cannot be confirmed at present. We look forward to the publication of large sample multiethnic and multisubtype GWAS data for NS in the future.

## Conclusion

In conclusion, through extensive research on the epidemiological risk factors for NS, our findings demonstrate that the HLA-II

histocompatibility DQ α2 chain is causally associated with the risk of developing NS. These findings could help prioritize the epidemiology and prevention of NS and motivate the development of future studies to provide further insight into the mechanisms linking subtypes of NS and kidney disease.

## Acknowledgments

All of the authors accept responsibility for the full content of this manuscript and approve its submission. We are grateful to Dr. Daoqi Wu for his technical assistance.

## Ethics approval

Only publicly available data were used for this study. Ethical approval for all of the data used can be found in the original publications.

## Authors' contributions

HC conducted the bioinformatics analysis and drafted the article. HC and QL proposed and conducted the study. HY1, XY and FG were responsible for data collection and data extraction. HY2 and MW were responsible for the data analysis.

## Consent for publication

All of the authors agreed to publish this article.

## Disclosure statement

All of the authors declare that they have no competing interests.

## Funding

This study was funded by the China National Natural Science Foundation [82200788, 81970618, and 82170720], National Key R&D Program of China [2022YFC2705101], and Youth Program of National Clinical Research Center for Child Health and Disorders in Children's Hospital of Chongqing Medical University, Chongqing, China [NCRCCHD-2022-YP-02]. The authors declare that they have no relevant financial interests.

## Data availability statement

The datasets analyzed during the current study are available from the UK Biobank (https://www.ukbiobank.ac.uk/), GWAS catalog (https://www.ebi.ac.uk/gwas/home), Integrative Epidemiology Unit (IEU) GWAS database (https://gwas.mrcieu.ac.uk/) and FinnGen database (https://www.finngen.fi/en/access_results).

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