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Common risk variants in *NPHS1* and *TNFSF15* are associated with childhood steroid-sensitive nephrotic syndrome

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To understand the genetics of steroid-sensitive nephrotic syndrome (SSNS), we conducted a genome-wide association study in 987 childhood SSNS patients and 3,206 healthy controls with Japanese ancestry. Beyond known associations in the *HLA-DR/DQ* region, common variants in *NPHS1-KIRREL2* (rs56117924, P = 4.94E-20, odds ratio (OR) = 1.90)

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and *TNFSF15* (rs6478109, P = 2.54E-8, OR = 0.72) regions achieved genome-wide significance and were replicated in Korean, South Asian and African populations. Trans-ethnic meta-analyses including Japanese, Korean, South Asian, African, European, Hispanic and Maghrebian populations confirmed the significant associations of variants in *NPHS1-KIRREL2* (P_{meta} = 6.71E-28, OR = 1.88) and *TNFSF15* (P_{meta} = 5.40E-11, OR = 1.33) loci. Analysis of the *NPHS1* risk alleles with glomerular *NPHS1* mRNA expression from the same person revealed allele specific expression with significantly lower expression of the transcript derived from the risk haplotype (Wilcox test p = 9.3E-4). Because rare pathogenic variants in *NPHS1* cause congenital nephrotic syndrome of the Finnish type (CNSF), the present study provides further evidence that variation along the allele frequency spectrum in the same gene can cause or contribute to both a rare monogenic disease (CNSF) and a more complex, polygenic disease (SSNS).

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Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in children. The prevalence of INS has been reported to be nearly 16 cases per 100,000.[1] There are substantial ethnic differences in the incidence of INS, creating a range of 2–7 cases per 100,000 children. The incidence of INS is higher among those with Asian ancestry compared with European ancestry.[2] In Japan, the estimated incidence of INS is 6.49 cases per 100,000 children.[3] Approximately 3% of children with steroid-sensitive nephrotic syndrome (SSNS) have a family history of SSNS.[4] The pathogenesis of INS is still unclear, although more than 50 causative single genes for steroid-resistant nephrotic syndrome have been found, and causative mutations in these genes were identified in approximately 30% of childhood and young adult patients with steroid-resistant nephrotic syndrome.[5,6] Recently, 6 genes associated with Rho-like small guanosine triphosphate–binding enzyme (GTPase) activity were identified as causes of nephrotic syndrome that can be partially treated with steroids.[7] *EMP2* mutations were identified in SSNS by a combination of linkage analysis and exome sequencing.[8] However, few patients with SSNS have a mutation in the above genes, indicating that alternative genetic architecture might drive the pathogenesis for most patients with SSNS.[9] Acute infections and insect stings are well known triggers of the onset and relapse of nephrotic syndrome.[10–13] These findings suggest that genetic and environmental factors are important in susceptibility to development of SSNS. To elucidate the genetic susceptibility factors of SSNS in children, 4 genome-wide association studies (GWASs) in well characterized case–control series have been performed. The *HLA-DR/DQ* region exhibited the most significant association with disease in European and Japanese populations,[14–17] with 2 non-*HLA* loci on 4q13.3 (*PARM1*) and 6q22.1 (*CALHM6*) also achieving genome-wide significance in only European children.[15,17] Given the limited sample sizes in these studies, larger cohorts and international collaborative studies are necessary to identify additional susceptibility factors. In a Japanese population, we performed a discovery GWAS with the largest sample size to date (987 childhood SSNS patients vs. 3206 controls), followed by an international replication and transethnic meta-analysis (Figure 1).

RESULTS

Subjects

Definitions of INS are shown in Supplementary Table S1. In the discovery stage, 1018 Japanese patients with childhood-onset SSNS were recruited, and 987 cases were included in the association analysis after quality control was conducted. Characteristics of the 987 patients are shown in Supplementary Table S2. The male-to-female ratio was 2.6:1. The median age at onset was 4.0 years (0.4–17.9 years). Renal biopsy was performed in 501 of 987 patients (51%) (for minimal change disease [N = 470; 93.8%], focal segmental glomerulosclerosis [N = 16; 3.2%], diffuse mesangial proliferation [N = 14; 2.8%], and focal mesangial proliferation [N = 1; 0.2%]). In total, 3331 healthy Japanese adults were recruited as controls.

Genome-wide single nucleotide polymorphism (SNP)–based association analysis

In the discovery stage, 1018 cases and 3331 healthy controls were genotyped by Japonica array. Whole-genome imputation was performed subsequently using the phased reference panel of 2036 healthy Japanese individuals. After data cleaning (Supplementary Figure S1A–E), 987 cases and 3206 controls with 6,834,340 autosomal single-nucleotide variants and short insertions and deletions were retained for association analyses. The power of discovery GWAS exceeded 80% to detect low-frequency variants (minor allele frequency [MAF] >0.5%) with genotypic relative risk >6.20, or common alleles (MAF ≥5%) with relative risk >2.05, or variants with an allele frequency = 50% conferring relative risk >1.48 at a significant *P* value threshold of 5E–08 under the additive model (Supplementary Figure S2). The inflation factor, λ, was 1.151 for all tested variants. After adjusting for sex and the first 4 principal components (PC1–4), the λ was 1.048, which decreased to 1.043 when variants in the *HLA* region (Hg19: chr6: 29,691,116–33,054,976) were excluded (Supplementary Figure S3A and B).

The most significant association was detected in the *HLA-DR/DQ* region (rs6901541, *P* = 2.80E–33, odds ratio [OR] = 2.49, 95% confidence interval: 2.15–2.89; Figures 2 and 3a). Signals in the *NPHS1-KIRREL2* region on 19q13.12 (rs56117924; *P* = 4.94E–20, OR = 1.90, 95% confidence interaval: 1.66–2.18; Figure 3b) and in the *TNFSF15* region on

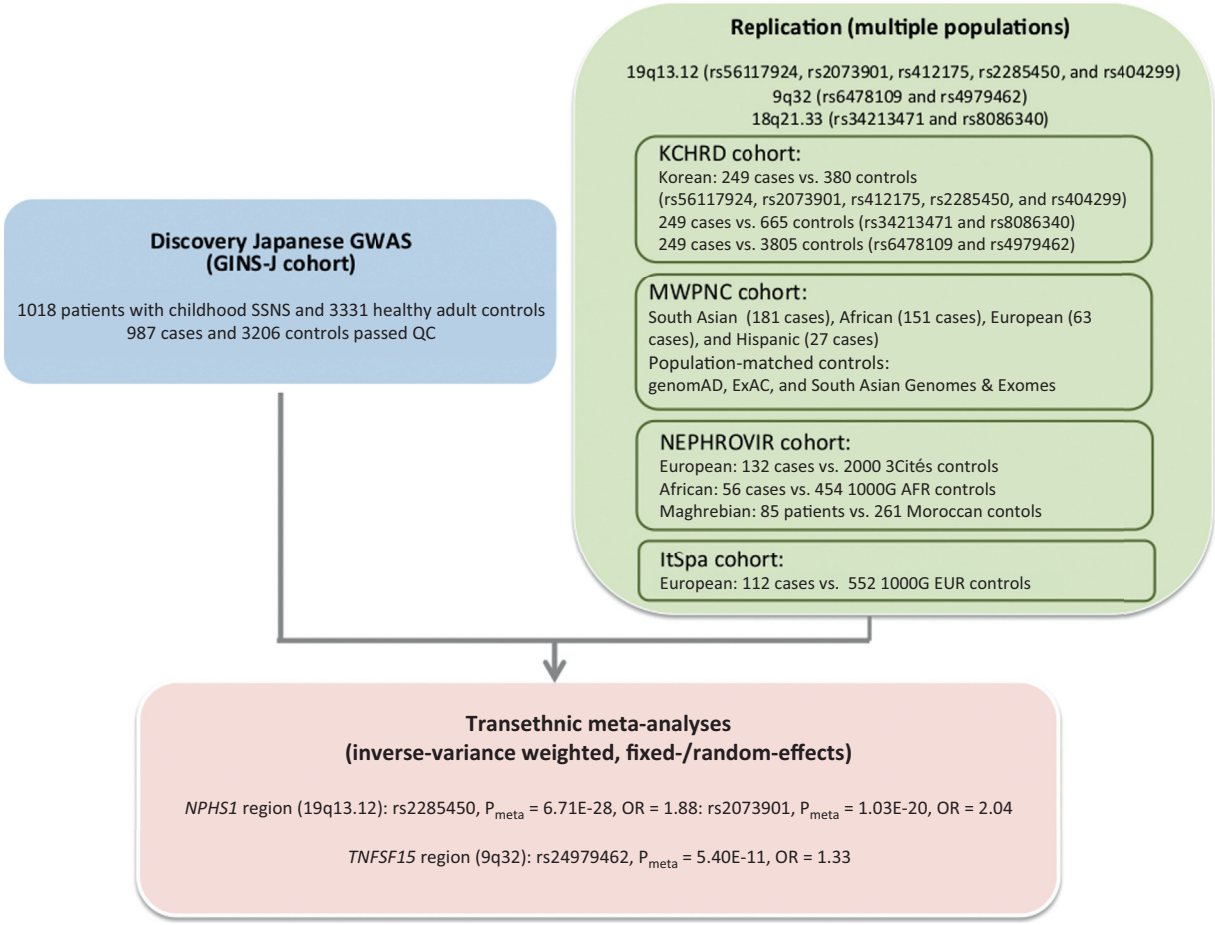

Figure 1 | Flowchart. Main outline of the discovery genome-wide association study (GWAS). Replication of candidate single-nucleotide polymorphisms outside *HLA* with multiple populations and transethnic meta-analysis. 3Cités, French 3-city (Bordeaux, Dijon, and Montpellier) cohort; AFR, African; EUR, European; ExAC, genomAD; GINS-J, The Research Consortium on Genetics of Childhood Nephrotic Syndrome in Japan; ItSpa, Italian and Spanish cohort; KCHRD, Korean Consortium of Hereditary Renal Diseases in Children; MWPNC, Midwest Pediatric Nephrology Consortium; NEPHROVIR, Children Cohort Nephrosis and Virus; OR, odds ratio; P_{meta}, *P* value in meta-analysis; QC, quality control; SSNS, steroid-sensitive nephrotic syndrome.

9q32 (rs6478109, $P = 2.54E{-}08$, OR = 0.72, 95% confidence interval: 0.64–0.81; Figure 3c) also achieved genome-wide significance ($P < 5E{-}08$). A signal in the *TNFRSF11A* region on 18q21.33 achieved marginal genome-wide significance (rs34213471, $P = 7.68E{-}08$, OR = 1.38, 95% confidence interval: 1.23–1.56; Figure 3d; Supplementary Table S3). Conditional analyses were performed in the novel non-*HLA* regions. No SNPs showed independent significance after conditioning on the lead SNP in each locus (Supplementary Figures S4–S6). Nine SNPs were chosen for targeted replication and meta-analysis: in the *NPHS1-KIRREL2* region, the lead SNP (rs56117924), 2 potential functional SNPs in linkage disequilibrium with the lead SNP (rs2073901 and rs2285450), and 2 SNPs in linkage disequilibrium with our lead SNP with sufficient frequency in European populations (rs412175 and rs404299) were selected (Supplementary Figure S7). In the *TNFSF15* region, in addition to the lead SNP (rs6478109), rs4979462 ($r^2 = 0.55$ with rs6478109 in the discovery Japanese set) was selected because it has been associated with autoimmune diseases in previous reports.[18,19] In the *TNFRSF11A* region, the lead SNP (rs34213471) and SNP in linkage disequilibrium with the lead SNP (rs8086340, $r^2 = 0.42$ with rs34213471 in the discovery Japanese set) were selected. All candidate SNPs were common variants (MAF > 5%) in the Japanese population (Table 1).

Replication and transethnic meta-analyses of candidate SNPs in non-*HLA* regions

Replication of 9 candidate SNPs was conducted in multiple populations including Korean, South Asian, African, European, Hispanic, and Maghrebian (Table 1). A value of $P < 0.05/9 = 5.55E{-}03$ was considered significant. The genome-wide significant signals in the *NPHS1–KIRREL2* region were replicated in Korean (rs2285450, $P = 1.32E{-}05$, OR = 1.98; rs412175, $P = 3.55E{-}05$, OR = 1.91; rs2073901, $P = 1.10E{-}04$, OR = 2.09; rs56117924, $P = 4.19E{-}04$, OR = 1.70; and rs404299, $P = 4.99E{-}04$, OR = 1.88), South Asian (Midwest Pediatric Nephrology Consortium [MWPNC]; rs404299, $P = 1.56E{-}12$, OR = 2.95; rs2285450, $P = 1.27E{-}04$, OR = 3.31; rs2073901, $P = 1.49E{-}04$, OR = 3.62;

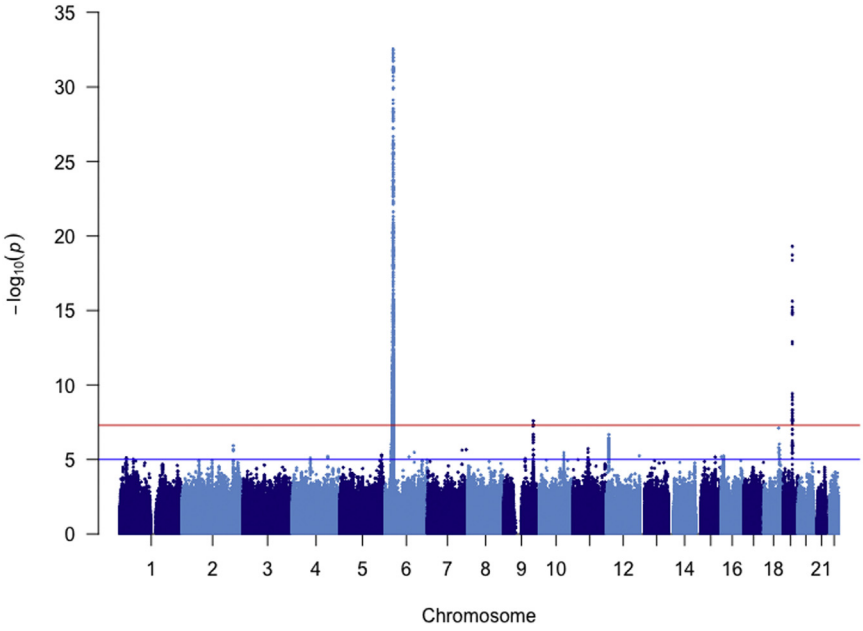

Figure 2 | Manhattan plot in the discovery genome-wide association study. In the discovery stage, 987 patients with childhood steroid-sensitive nephrotic syndrome and 3206 healthy adult controls with 6,834,340 autosomal single-nucleotide variants and insertions and deletions after whole-genome imputation were included. *P* values were calculated using logistic regression and adjusting for sex and principal components 1–4.

rs412175, $P = 2.77E{-}04$, OR = 2.39), and African (MWPNC) datasets (rs56117924, $P = 3.20E{-}05$, OR = 1.76). Candidate SNPs in the *TNFSF15* region were replicated in the Korean (rs6478109, $P = 1.09E{-}05$, OR = 0.65) and African (MWPNC) datasets (rs4979462, $P = 1.36E{-}03$, OR = 1.47). The association in the *TNFRSF11A* region was replicated in South Asians (MWPNC) (rs8086340, $P = 4.66E{-}05$, OR = 0.53). None of the 9 candidate SNPs achieved statistical significance in the European, Hispanic (MWPNC), or the African or Maghrebian (both NEPHROVIR) cohorts after multiple corrections. The limited power for detecting moderate associations in the replication stage should be taken into consideration (Supplementary Table S4).

Transethnic meta-analyses of the 9 SNPs, including discovery and replication sample sets, confirmed significant associations of the *NPHS1-KIRREL2* (rs2285450, *P* value in meta-analysis [P_{meta}] = 6.71E–28, OR = 1.88; rs2073901, $P_{meta} = 1.03E{-}20$, OR = 2.04) and *TNFSF15* regions (rs4979462, $P_{meta} = 5.40E{-}11$, OR = 1.33), but not of the *TNFRSF11A* locus (Table 2).

Gene-based analysis in the discovery stage

In a gene-based test, 6,834,340 autosomal variants were mapped to 18,644 protein-coding genes. Seventy-one genes achieved genome-wide significance ($P < 0.05/18{,}644 = 2.68E{-}06$) (Supplementary Figure S8; Supplementary Table S5). *NPHS1* (*P* value in gene-based analysis [P_{gene}] = 6.29E–18) and *KIRREL2* ($P_{gene} = 7.79E{-}14$) exhibited the most significant associations with disease outside the *HLA* region. In gene-set analysis, the major histocompatibility complex class II protein complex (*P* value modified by Bonferroni's method [*P*-Bonferroni] = 1.22E–03, Beta = 1.50), major histocompatibility complex class II receptor activity (*P*-Bonferroni = 6.17E–03, Beta = 1.61), luminal side of membrane (*P*-Bonferroni = 1.97E–02, Beta = 0.75), and innate immune response (*P*-Bonferroni = 1.97E–02, Beta = 0.17) were significantly associated with the disease (Supplementary Tables S6 and S7).

Post-GWAS analysis of the *NPHS1-KIRREL2* locus

Given that *NPHS1* was the most significant peak after the *HLA* region, that *NPHS1* and *KIRREL2* have key roles in podocyte biology, and that *NPHS1* is one of the most common Mendelian nephrotic syndrome genes, we focused our post-GWAS analysis on risk variants at this locus (19q13.12; 36.2–36.6 Mb). Given the biologic proximity of DNA to mRNA, and the availability of existing paired genomic and biopsy-derived kidney transcriptomic data from participants in the Nephrotic Syndrome Study Network (NEPTUNE) study,[20,21] we chose to investigate the relationship between the risk alleles at this locus and local mRNA expression.

We first tested the hypothesis that these risk SNPs altered expression of nearby genes as a cis expression quantitative trait locus (eQTL). However, there was no strong evidence that there were significant eQTLs for *NPHS1* or *KIRREL2* in kidney or other tissues (Supplementary Table S8). We also found it to be unlikely that the 2 synonymous variants (rs2285450, c.294 C>T; rs2073901, c.2223C>T) in *NPHS1* affected the secondary structure, using RNAsnp Web Server.[22]

We then turned our attention to studying the impact of the chromosome 19, five-SNP risk haplotype on *NPHS1* transcriptional regulation. To do this, we compared *NPHS1*

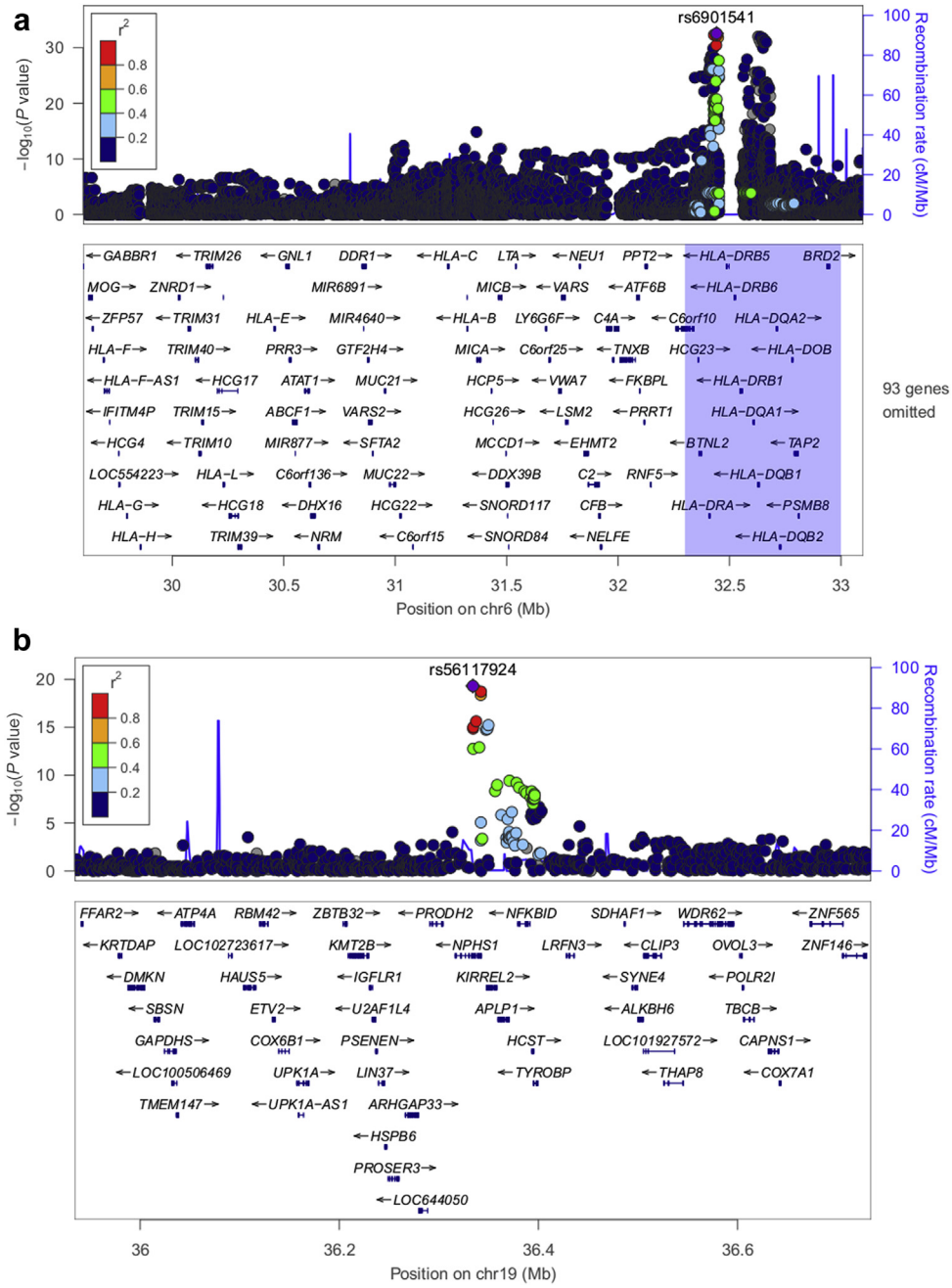

Figure 3 | Regional plots of loci with genome-wide significance ($P < 5E–08$) or marginal genome-wide significance in the discovery stage. (**a**) Classical *HLA* region on chromosome (chr) 6 exhibited the most significant association ($P = 2.80E–33$). (**b**) Candidate locus with genome-wide significance on chr 19 ($P = 4.94E–20$). (Continued)

expression of 4 NEPTUNE participants harboring the full 5 *NPHS1* risk haplotype with 183 participants without the full risk haplotype. The 4 participants with the risk haplotype did not have a significant difference in *NPHS1* expression (Wilcoxon test $P = 0.39$; Figure 4a). With no evidence that this risk haplotype harbors an eQTL for glomerular *NPHS1*, we hypothesized that the *NPHS1* transcript derived from the risk haplotype would be differentially expressed compared to that from the reference haplotype (allele-specific expression [ASE]), as ASE has been reported to be associated with susceptibility to diseases, including inflammatory bowel disease, autism spectrum disorder, and alcohol use disorders.[23–25] We used phasing and allele specific expression from RNA-seq (phASER) to quantify haplotype abundance, then quantified the "magnitude of ASE" as the degree of deviation from the expected 1:1 ratio of expression from each chromosome |0.5 – (haplotype A/total count)|. In patients with the risk haplotype, haplotype A harbors all 5 *NPHS1* risk variants; in patients without the risk haplotype, haplotype A was randomly selected from one of their 2 haplotypes. We found significantly lower expression of

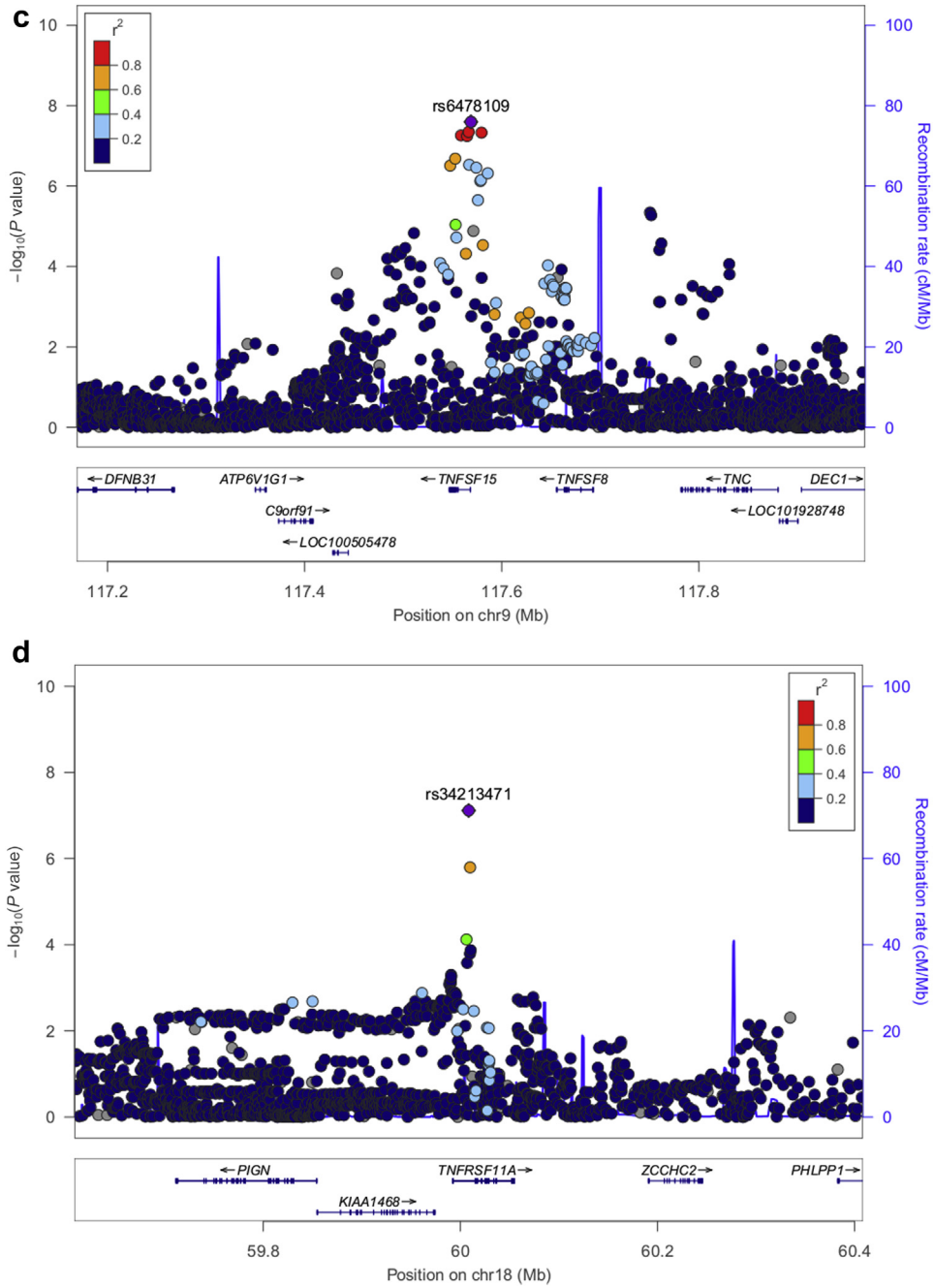

Figure 3 | (Continued) (**c**) Candidate locus with genome-wide significance on chr 9 (P = 2.54E–08). (**d**) Candidate locus with marginal genome-wide significance on chr 18 (P = 7.68E–08).

the *NPHS1* transcript from the risk haplotype (Table 3). The magnitudes of ASE in the 4 risk haplotype samples were 0.14, 0.21, 0.22, and 0.22, whereas the median of the other 183 patients was 0.03 (interquartile range: 0.01–0.05, Wilcoxon test for difference in ASE, P = 9.3E–4; Figure 4b).

HLA fine-mapping

HLA association analysis in the present study confirmed our previous findings with more significant P values.[16] Details of *HLA* fine-mapping are shown in Supplementary Tables S9–S20 and Supplementary Figure S9A–G). *HLA-DRB1*08:02–DQB1*03:02* was the most significant susceptibility haplotype (corrected P value [Pc] = 1.16E–22, OR = 3.38), with a more significant and stronger association than *HLA-DRB1*08:02* (Pc = 2.60E–22, OR = 2.66) or *HLA-DQB1*03:02* (Pc = 2.59E–10, OR = 1.71) alone. *HLA-DRB1*13:02–DQB1*06:04* was the most significant protective haplotype (Pc = 1.63E–16, OR = 0.18). One individual in the case group (1 of 870 = 0.11%) and

Table 1 | SNPs selected from candidate loci in the discovery GWAS and replication of candidate SNPs in multiple populations

							Discovery GWAS and replication							
CHR	SNP	BP	A1	A2	Related gene(s)		Consortium	Population	N of cases	N of controls	A1_cases	A1_controls	OR (95% CI)	*P* value
19	rs56117924	36,334,182	A	G	*NPHS1*	Intron variant	GINS-J	Discovery Japanese GWAS	987	3206	0.27	0.17	1.90 (1.66–2.18)	4.94E-20
							KCHRD	Korean	247	378	0.25	0.17	1.70 (1.26–2.27)	4.19E-04
							MWPNC	South Asian	181	176	0.04	0.03	1.34 (0.57–3.27)	4.68E-01 (NS)
								African	158	4345	0.23	0.14	1.76 (1.32–2.31)	3.20E-05
								European	63	7711	0.02	0.00	19.10 (2.07–85.83)	6.39E-03 (NS)
								Hispanic	27	424	0.02	0.02	1.21 (0.03–8.36)	5.81E-01 (NS)
							NEPHROVIR	African	56	454	0.20	0.17	1.19 (0.66–2.16)	5.62E-01 (NS)
								European	/	/	/	/	/	/
								Maghrebian	85	261	0.05	0.03	1.53 (0.50–4.63)	4.55E-01 (NS)
							ItSpa	European	/	/	/	/	/	/
19	rs2073901	36,334,485	A	G	*NPHS1*	Synonymous codon	GINS-J	Discovery Japanese GWAS	962	3171	0.15	0.08	1.93 (1.62–2.29)	1.77E-13
							KCHRD	Korean	249	370	0.16	0.08	2.09 (1.44–3.04)	1.10E-04
							MWPNC	South Asian	181	8255	0.04	0.01	3.62 (1.87–6.45)	1.49E-04
								African	158	5202	0.01	0.01	1.36 (0.27–4.16)	4.91E-01 (NS)
								European	62	33,361	0.00	0.00	4.15 (0.26–67.45)	1 (NS)
								Hispanic	27	5789	0.00	0.00	8.49 (0.50–145.19)	1 (NS)
							NEPHROVIR	African	56	454	0.02	0.01	2.98 (0.45–19.88)	2.60E-01 (NS)
								European	/	/	/	/	/	/
								Maghrebian	/	/	/	/	/	/
							ItSpa	European	/	/	/	/	/	/
19	rs412175	36,342,103	C	T	*NPHS1, KIRREL2*	Intron variants upstream variant 2KB	GINS-J	Discovery Japanese GWAS	961	3157	0.25	0.15	1.93 (1.67–2.23)	4.25E–19
							KCHRD	Korean	247	374	0.25	0.15	1.91 (1.41–2.60)	3.55E-05
							MWPNC	South Asian	181	176	0.17	0.08	2.39 (1.45–4.02)	2.77E-04
								African	158	4330	0.50	0.44	1.26 (1.00–1.59)	4.38E–02 (NS)
								European	63	7692	0.04	0.03	1.24 (0.39–2.99)	6.07E–01 (NS)
								Hispanic	27	423	0.06	0.06	0.94 (0.18–3.06)	1 (NS)
							NEPHROVIR	African	56	454	0.56	0.46	1.49 (0.91–2.44)	1.12E–01 (NS)
								European	132	2000	0.04	0.04	0.97 (0.46–2.06)	9.37E–01 (NS)
								Maghrebian	85	261	0.18	0.15	0.93 (0.49–1.76)	8.14E–01 (NS)
							ItSpa	European	112	552	0.05	0.03	1.13 (0.49–2.60)	7.68E–01 (NS)
19	rs2285450	36,342,267	A	G	*NPHS1, KIRREL2*	Synonymous codon upstream variant 2KB	GINS-J	Discovery Japanese GWAS	963	3163	0.25	0.15	1.93 (1.68–2.23)	1.91E-19
							KCHRD	Korean	246	379	0.25	0.15	1.98 (1.46–2.70)	1.32E-05
							MWPNC	South Asian	181	7983	0.04	0.01	3.31 (1.80–5.66)	1.27E-04
								African	158	4750	0.18	0.13	1.45 (1.06–1.96)	1.18E–02 (NS)
								European	63	30,946	0.01	0.00	4.89 (0.12–28.41)	1.88E–01 (NS)
								Hispanic	27	5377	0.00	0.01	1.25 (0.08–20.38)	1 (NS)
							NEPHROVIR	African	56	454	0.18	0.16	1.20 (0.64–2.25)	5.79E–01 (NS)
								European	/	/	/	/	/	/
								Maghrebian	85	261	0.06	0.02	2.13 (0.72–6.25)	1.71E–01 (NS)
							ItSpa	European	/	/	/	/	/	/

(Continued on following page)

19	rs404299	36,349,752	A	G	*KIRREL2*	Missense variant (Ala to Thr)	GINS-J	Discovery Japanese GWAS	987	3206	0.16	0.09	2.00 (1.69–2.36)	6.01E-16
							KCHRD	Korean	248	374	0.16	0.09	1.88 (1.32–2.69)	4.99E-04
							MWPNC	South Asian	181	8245	0.13	0.05	2.95 (2.11–4.06)	1.56E-12
								African	158	5203	0.43	0.38	1.20 (0.95–1.52)	1.10E–01 (NS)
								European	63	33,348	0.03	0.04	0.87 (0.23–2.28)	1 (NS)
								Hispanic	27	5789	0.06	0.03	2.03 (0.40–6.33)	1.96E–01 (NS)
							NEPHROVIR	African	56	454	0.50	0.39	1.53 (0.95–2.47)	7.85E–02 (NS)
								European	132	2000	0.04	0.04	0.98 (0.46–2.09)	9.60E–01 (NS)
								Maghrebian	85	261	0.14	0.12	0.78 (0.38–1.64)	5.15E–01 (NS)
							ItSpa	European	112	552	0.05	0.03	1.34 (0.57–3.14)	5.07E–01 (NS)
9	rs4979462	117,567,013	T	C	*TNFSF15*	Intron variant	GINS-J	Discovery Japanese GWAS	987	3206	0.53	0.47	1.34 (1.20–1.49)	2.96E-07
							KCHRD	Korean	248	3805	0.38	0.35	1.18 (0.98–1.43)	8.78E–02 (NS)
							MWPNC	South Asian	180	176	0.09	0.06	1.56 (0.84–2.96)	1.30E–01 (NS)
								African	147	4343	0.41	0.32	1.47 (1.15–1.87)	1.36E-03
								European	62	7716	0.03	0.01	2.45 (0.65–6.54)	8.84E–02 (NS)
								Hispanic	27	422	0.24	0.23	1.06 (0.51–2.06)	8.70E–01 (NS)
							NEPHROVIR	African	56	454	0.46	0.36	1.65 (1.03–2.65)	3.87E–02 (NS)
								European	132	2000	0.01	0.01	0.80 (0.11–6.10)	8.33E–01 (NS)
								Maghrebian	85	261	0.06	0.07	0.56 (0.18–1.72)	3.08E–01 (NS)
							ItSpa	European	112	552	0.02	0.02	2.20 (0.52–9.32)	2.84E–01 (NS)
9	rs6478109	117,568,766	A	G	*TNFSF15*	Upstream variant 2KB	GINS-J	Discovery Japanese GWAS	987	3206	0.32	0.39	0.72 (0.64–0.81)	2.54E-08
							KCHRD	Korean	248	3805	0.38	0.48	0.65 (0.54–0.79)	1.09E-05
							MWPNC	South Asian	176	176	0.21	0.24	0.84 (0.58–1.21)	3.24E–01 (NS)
								African	154	4349	0.07	0.11	0.60 (0.37–0.94)	2.24E–02 (NS)
								European	59	7694	0.28	0.33	0.79 (0.51–1.20)	2.62E–01 (NS)
								Hispanic	26	423	0.27	0.22	1.29 (0.63–2.50)	4.31E–01 (NS)
							NEPHROVIR	African	56	454	0.07	0.10	0.69 (0.29–1.68)	4.17E–01 (NS)
								European	132	2000	0.29	0.33	0.97 (0.71–1.33)	8.44E–01 (NS)
								Maghrebian	85	261	0.16	0.18	0.58 (0.31–1.10)	9.43E–02 (NS)
							ItSpa	European	112	552	0.31	0.32	1.26 (0.83–1.93)	2.69E–01 (NS)
18	rs8086340	60,006,978	C	G	*TNFRSF11A*	Intron variant	GINS-J	Discovery Japanese GWAS	986	3199	0.43	0.46	0.81 (0.73–0.91)	2.66E-04
							KCHRD	Korean	247	648	0.37	0.41	0.87 (0.70–1.08)	1.92E–01 (NS)
							MWPNC	South Asian	181	176	0.31	0.43	0.53 (0.39–0.73)	4.66E-05
								African	158	4342	0.43	0.45	0.94 (0.74–1.19)	5.93E–01 (NS)
								European	63	7695	0.45	0.44	1.04 (0.72–1.49)	8.46E–01 (NS)
								Hispanic	27	422	0.37	0.36	1.03 (0.55–1.88)	9.22E–01 (NS)
							NEPHROVIR	African	56	454	0.45	0.43	1.07 (0.66–1.73)	7.99E–01 (NS)
								European	132	2000	0.44	0.46	0.76 (0.57–1.03)	8.07E–02 (NS)
								Maghrebian	85	261	0.50	0.52	1.09 (0.69–1.73)	7.01E–01 (NS)
							ItSpa	European	112	552	0.48	0.45	1.23 (0.83–1.82)	2.87E–01 (NS)

(*Continued on following page*)

Table 1 | (Continued) **SNPs selected from candidate loci in the discovery GWAS and replication of candidate SNPs in multiple populations**

CHR	SNP	BP	A1	A2	Related gene(s)	Discovery GWAS and replication						
						Consortium	Population	N of cases	N of controls	A1_cases	A1_controls	OR (95% CI)	*P* value
18	rs34213471	60,008,436	A	C	*TNFRSF11A* Intron variant	GINS-J	Discovery Japanese GWAS	987	3206	0.36	0.31	1.38 (1.23–1.56)	7.68E-08
						KCHRD	Korean	247	658	0.31	0.28	1.16 (0.92–1.47)	2.08E–01 (NS)
						MWPNC	South Asian	172	176	0.23	0.16	1.54 (1.04–2.28)	2.49E–02 (NS)
							African	146	4357	0.01	0.01	0.74 (0.09–2.80)	1 (NS)
							European	60	7717	0.03	0.07	0.48 (0.13–1.27)	1.41E–01 (NS)
							Hispanic	26	424	0.06	0.10	0.52 (0.10–1.67)	2.75E–01 (NS)
						NEPHROVIR	African	56	454	0.02	0.01	2.50 (0.33–19.04)	3.77E–01 (NS)
							European	132	2000	0.08	0.06	1.26 (0.69–2.28)	4.52E–01 (NS)
							Maghrebian	85	261	0.06	0.01	4.07 (0.99–16.68)	5.10E–02 (NS)
						ItSpa	European	112	552	0.04	0.05	0.65 (0.28–1.53)	3.25E–01 (NS)

A1, minor alleles in discovery Japanese sample set (effect alleles); A2, major alleles in discovery Japanese sample set (reference alleles); A1_Cases, allele frequency of A1 in cases; A1_Controls, allele frequency of A1 in controls; ala, alanine; BP, physical position according to Hg19; CHR, chromosome; CI, confidence interval; GINS-J, The Research Consortium on Genetics of Childhood Idiopathic Nephrotic Syndrome in Japan; GWAS, genome-wide association studies; ItSpa, Italian and Spanish cohort; KCHRD, Korean Consortium of Hereditary Renal Diseases in Children; MWPNC, Midwest Pediatric Nephrology Consortium; NEPHROVIR, Children Cohort Nephrosis and Virus; NS, nonsignificant; OR, odds ratio; SNP, single-nucleotide polymorphism.

15 healthy controls (15 of 2903 = 0.52%) were heterozygotes for the susceptible haplotype (*HLA-DRB1*08:02–DQB1*03:02*) and the protective haplotype (*HLA-DRB1*13:02–DQB1*06:04*), suggesting a dominant effect of the protective haplotype over the susceptible haplotype, although the difference was not statistically significant ($P = 0.14$; Supplementary Table S20).

Variance explained by all autosomal variants in the current study

The observed heritability of childhood SSNS explained by genome-wide variants was estimated to be 41.1% (±14.0%). The heritability at the liability scale was 14.8% (±5.0%), assuming a population prevalence of 0.016%. Considering the strong association of the *HLA* region, the observed heritability was estimated to be 31.6% (±13.6%), and it became 11.4% (±4.9%) after transformation when variants on chromosome 6 were excluded. Common variants (MAF > 5%) explained 88%–90% of the disease heritability (Supplementary Table S21; Supplementary Figure S10).

DISCUSSION

The present Japanese GWAS with the largest sample size to date and replication in multiple continental populations identified common variants in the *NPHS1* and *TNFSF15* regions as new susceptibility factors for childhood SSNS. Post-GWAS analysis of the chromosome 19 locus identified a potential transcriptional mechanism by which the *NPHS1* risk haplotype may contribute to disease. Finally, the larger sample size empowered additional fine-mapping of the previously implicated *HLA* loci.[16] Altogether, these findings markedly expand and improve our understanding of the genetic background of childhood SSNS. They identify nephrin, the proinflammatory cytokine tumor necrosis factor superfamily member 15 (TNFSF15), and their associated molecules as new targets for biologic inquiry to better understand SSNS and potentially for therapeutic development. Finally, the association with common variants in the *NPHS1* locus provides another example showing that Mendelian kidney disease genes can harbor susceptibility variants for a more common multifactorial disease (SSNS).

Rare mutations in *NPHS1* cause congenital nephrotic syndrome of the Finnish type, a rare monogenic nephrotic syndrome that is steroid resistant and has a poor renal prognosis.[26] Surprisingly, the present study revealed that variants in *NPHS1* are associated with susceptibility to SSNS.

Although *NPHS1* has never been implicated in genome-wide scans for SSNS, a candidate association study between this gene's variants and SSNS in East Asian patients supports our current findings. In our present study, one of the significant SNPs in the *NPHS1* locus was the synonymous variant rs2285450 (c.294 C>T) in exon 3. Previously, Sun *et al.* reported a higher frequency of the rs2285450 minor allele in Chinese sporadic nephrotic syndrome patients from Singapore than in controls (20% vs. 13%, $P = 0.025$) and showed that the risk allele resulted in a decreased ability to inhibit TRPC6 currents in HEK293-M1 cells via patch clamp,

which might account for susceptibility to proteinuria.[27,28] This study provides independent support for rs2285450 as a risk factor for SSNS and suggests an alternative mechanism for susceptibility to nephrotic syndrome requiring further inquiry.

Using paired human genetic and glomerular transcriptomic data, we did not observe differences in total *NPHS1* expression as a function of this risk haplotype. However, RNA-seq data allowed observation of significant ASE resulting in lower *NPHS1* expression from the haplotype harboring the risk alleles (Supplementary Figure S11). A number of the risk variants at this locus were synonymous exonic changes in *NPHS1*. We therefore focused more on *NPHS1* and did not pursue discussion of the role of *KIRREL2* in potential disease mechanisms. Future work should include: (i) validating this observation in additional patients with genotyping and kidney expression data; and (ii) gaining a mechanistic understanding of how this ASE of *NPHS1* is contributing to or causing the association with SSNS that we observed. Potential hypotheses for dysfunction include: (i) increased burden on the cell to produce the necessary amount of nephrin from the reference chromosome, leading to cellular stress and increased susceptibility to injury; and (ii) potentially dysfunctional nephrin protein produced from the risk haplotype (e.g., via differential posttranslational modifications) resulting in an abnormal glomerular filtration barrier.

Genetic polymorphisms at the 9q32 locus are linked with several autoimmune and inflammatory diseases.[23,29–31] The most likely disease-causing gene within 9q32 is *TNFSF15*, which encodes TNFSF15. In the present study, the significant association of rs4979462 was replicated independently and further strengthened by a transethnic meta-analysis. Previously, Hitomi *et al.*[32] identified rs4979462 as a functional variant by *in vitro* functional analysis using luciferase assay and electrophoretic mobility shift assay. Super-shift assay clarified that the risk allele (T) of rs4979462 generated a novel NF-1 binding site.[32] In addition, several reports have shown that the risk allele of rs4979432 affects the expression level of *TNFSF15* mRNA.[30,32] Further studies are required to confirm the association of *TNFSF15* with SSNS in children.

In the present study, the variants in *NPHS1* and *TNFSF15* loci were identified and replicated mainly in the East Asian (Japanese and Korean) and South Asian populations, in which a higher incidence of disease has been reported. In contrast, the frequencies of risk alleles of candidate SNPs in *NPHS1* were rare in people of European ancestry (Table 1). These findings may partially explain the epidemiologic difference among populations from the perspective of disease-associated polymorphisms. This difference is further illustrated by the absence of significant signals in our Japanese dataset in the *CALHM6/FAM26*F and *PARM1* regions, which were detected in a homogeneous cohort of European ancestry.[15,17]

With the predominant contribution of *HLA-DR/DQ* genes, gene sets of major histocompatibility complex class II protein complex and major histocompatibility complex class II receptor activity were strongly associated with the disease. Innate immune response was also identified as a significant gene set with a moderate effect. The innate immune system including the activation of professional cells (antigen-presenting cells and B cells/Toll-like receptors), as well as the adaptive immune system, in which *HLA* molecules play a crucial role, is involved in the disease process and response to treatment.[33,34]

Around 90% of the heritability of childhood SSNS was contributed by common variants, although variants with relatively rare allele frequencies (MAF: 0.5%–5%) were also taken into consideration. However, a large proportion of the disease heritability remains unaccounted for. Other disease-associated variants in non-*HLA* regions might be identified by future genome-wide studies with larger sample sizes, especially for patients of European and other non–East Asian ancestries, as disease-associated polymorphisms in non-*HLA* regions are still largely unknown.

In the present study, the sample sizes in replication cohorts with different ancestries have limited the power for the replication of signals with smaller effect sizes. Population stratification might exist in the replication stage due to lack of adjustment. Moreover, batch effects might have occurred between cases and controls when allele frequencies from public databases were utilized as population-matched controls.

In summary, the discoveries here provide new SSNS loci. The *NPHS1* locus, in particular, provides a provocative new concept for the disease's pathogenesis, linking and reinforcing an emerging paradigm in nephrology—in certain genes harboring Mendelian variants, more common alleles can increase the susceptibility to multifactorial, polygenic diseases, as previously demonstrated for *UMOD, COL4A3,* and *NPHS2*.[26,35-41] Finally, the current findings again emphasize the importance of performing genomic research in diverse populations. By doing so, we discovered specific SNPs that are likely to have more impact on East and South Asian populations, while identifying genes and loci that may be important in all populations, to which we would be statistically blind if using a European discovery cohort.

METHODS

See Supplementary Methods for additional details.

Samples

In the discovery stage, 1018 Japanese patients diagnosed with childhood SSNS (onset age <18 years) were recruited. Patients with a history of steroid resistance during follow-up were excluded. Overall, 3331 Japanese healthy adults were recruited as controls. All participants provided written informed consent.

Genotyping and whole-genome imputation in the discovery stage

In the discovery stage, 1018 cases and 3331 controls were genotyped using the Affymetrix Japonica array.[42] Nineteen samples were excluded by low call rate (<97%) during the genotype calling process. Nine controls with ambiguous sex were excluded. Then, whole-genome imputation was performed with IMPUTE4[43] (version 2.3.1) using a phased reference panel of 2036 healthy Japanese individuals. After whole-genome imputation, there were 22,049,786 autosomal single-nucleotide variants and short insertions and deletions with info

Table 2 | Transethnic meta-analysis of candidate SNPs

CHR	SNP	BP	Effect allele	*P*_value	OR (95% CI)	*P*_heterogeneity	I^2	Model
19	rs56117924	36,334,182	A	2.73E–07	1.76 (1.42–2.18)	7.51E–02	45.66	Random-effect
19	rs2073901	36,334,485	A	1.03E–20	2.04 (1.76–2.38)	4.09E–01	2.04	Fixed-effect
19	rs412175	36,342,103	C	6.21E–05	1.52 (1.24–1.87)	1.58E–02	55.80	Random-effect
19	rs2285450	36,342,267	A	6.71E–28	1.88 (1.68–2.11)	1.58E–01	33.85	Fixed-effect
19	rs404299	36,349,752	A	8.64E–04	1.56 (1.20–2.03)	1.21E–04	72.92	Random-effect
9	rs4979462	117,567,013	T	5.40E–11	1.33 (1.22–1.44)	5.16E–01	0.00	Fixed-effect
9	rs6478109	117,568,766	A	7.86E–04	0.78 (0.68–0.90)	7.01E–02	43.21	Random-effect
18	rs8086340	60,006,978	C	4.58E–02	0.88 (0.77–1.00)	3.28E–02	50.57	Random-effect
18	rs34213471	60,008,436	A	5.61E–02	1.22 (0.99–1.50)	9.63E–02	39.23	Random-effect

BP, physical position according to Hg19; CHR, chromosome; CI, confidence interval; I^2, percentage of total variation across studies due to heterogeneity; OR, odds ratio; *P* heterogeneity, *P* value of Cochran Q test for heterogeneity; Model, meta-analysis method used: fixed-effect = inverse-variance method based on a fixed-effects model; random-effect = inverse-variance method based on a random-effects model; SNP, single-nucleotide polymorphism.

score >0.5. Quality control was conducted using the following threshold: individual missing rate <3%, single-nucleotide variant / insertions and deletions call rate ≥97%, MAF ≥0.5%, and Hardy-Weinberg equilibrium $P \geq 0.0001$ in healthy controls. An identical-by-descent test was performed using a threshold of Pi-hat >0.1875. Principal component analysis was performed using genome-wide complex trait analysis (version 1.26.0)[44] for cases, controls, and HapMap Phase III data (113 CEU [a northwestern European population], 113 YRI [the Yoruba in

Ibadan, Nigeria], 84 CHB [the Han Chinese in Beijing, China], and 86 JPT [the Japanese population in Tokyo, Japan]); samples identified as outliers were excluded (Supplementary Figure S1A–E).

Genome-wide association analyses in the discovery stage

Genome-wide association analyses and single-nucleotide variant–based conditional analyses were conducted using logistic regression, adjusting for sex and the first 4 principal components (PC1 to PC4) by PLINK 1.9. R package "qqman" was utilized to generate Manhattan and QQ plots. Regional plots were generated using Locuszoom (1000 Genomes Nov 2014 ASN was used as linkage disequilibrium reference).[45]

Gene-based test and gene-set analysis

Gene-based test and gene-set analyses were performed by MAGMA v1.6[46] (implemented through FUMA[47]). The Genotype-Tissue Expression (GTEx) database[48] and the NephVS eQTL Browser (NephQTL)[21] were utilized to find eQTLs affecting the expression of various genes in various tissues and kidney-specific tissues.

Replication of candidate SNPs

Replication was carried out in multiple populations. Participants in the Korean dataset were recruited from South Korea. The MWPNC cohort included 181 patients of South Asian ancestry recruited from the US and Sri Lanka, 158 patients of African ancestry recruited from

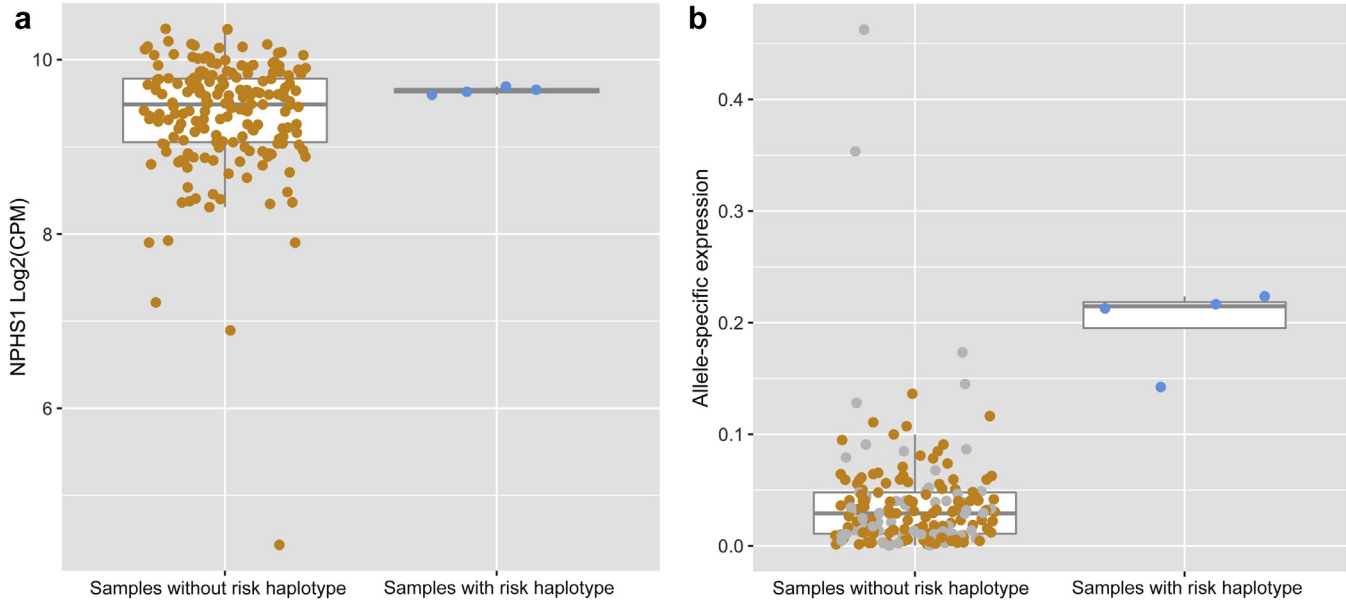

Figure 4 | Glomerular *NPHS1* mRNA expression in Nephrotic Syndrome Study Network (NEPTUNE) cohort. (**a**) *NPHS1* fragments per kilobase million expression comparing samples with versus without *NPHS1* risk haplotype. Samples with the *NPHS1* risk haplotype do not show significantly different expression levels (Wilcoxon test, $P = 0.39$). (**b**) Allele-specific expression (ASE) comparing samples with versus without *NPHS1* risk haplotype. ASE = |0.5 – (haplotype A / total reads)|. In patients with the risk alleles, haplotype A harbors all 5 *NPHS1* risk variants; in patients without the risk haplotype, haplotype A is randomly selected from 1 of their 2 haplotypes. Samples with less than 2 heterozygous single-nucleotide polymorphisms or in the bottom 10% of total counts are indicated in gray. Samples with the *NPHS1* risk haplotype show significant allele-specific expression with lower expression of the risk haplotype (Wilcoxon test, $P = 9.3E–4$). CPM, counts per million.

Table 3 | *NPHS1* allele-specific expression for samples harboring risk haplotype

Patient[a]	Risk haplotype counts	Total counts	Risk haplotype/ total counts	Magnitude of allele-specific expression
1	3006	10,466	0.287	0.213
2	4823	13,484	0.358	0.142
3	826	2914	0.283	0.217
4	2135	7723	0.276	0.224

[a]Order of samples follows Figure 4b from left to right.

the US and Nigeria, and 63 European and 27 Hispanic patients recruited from the US. The NEPHROVIR cohort included 132 European, 56 African, and 85 Maghrebian children with SSNS, recruited in the Paris area; 2000 European controls from 3 cities and 454 controls from the 1000G African cohort and 261 Moroccan controls were used as population-matched controls, respectively. The Italian and Spanish (ItSpa) cohort comprised 112 European patients from Italy and Spain and 552 controls from the 1000G European cohort. Detailed information on the datasets of each population is shown in the Supplementary Methods and Table 1.

In the replication stage, logistic regression was performed in the Korean dataset using individual genotype data. *P* values were calculated by Pearson's χ^2 test or Fisher's exact test in the MWPNC cohort and public databases. For cell = 0 in the χ^2 test, 0.5 was added to each of the 4 cells to calculate the ORs, standard error (SE), and 95% confidence interval. In the NEPHROVIR and ItSpa datasets, association analyses were carried out under the additive model.

Transethnic meta-analysis

Transethnic meta-analysis was conducted using the inverse-variance method based on fixed- or random-effects models by "META."[49] Heterogeneity was considered when the Cochran Q test had a *P* value < 0.10. $P_{meta} < 5E{-}08$ was considered the genome-wide significance threshold for the meta-analysis.

ASE analysis in the NEPTUNE cohort

Total RNA from glomerular biopsies and 30X whole-genome sequencing was performed on 269 and 625 samples from NEPTUNE[20] (see supplementary data[20] for more detail). Variants on chromosome 19 were filtered, removing MAF < 0.0001, insertions and deletions, genotype quality score < 20, and missing $> 10\%$. Variants were phased with Eagle v2.4.1[50] on the Michigan Imputation Server[51] using the 1000 Genomes Phase 3 reference panel.[52] Samples harboring all 5 chromosome 19 risk alleles (rs56117924, rs2073901, rs412175, rs2285450, and rs404299) were identified. Gene quantification (Log2CPM) was calculated with the edgeR trimmed mean of M values (TMM)-normalization method.[53] *NPHS1* and the surrounding 1KB intergenic region (chr19: 36,315,274–36,343,895) were specifically focused upon from the bam files. The *NPHS1* bam files and phased whole-genome sequencing were input into phASER[54] to perform haplotype phasing. Due to a high recombination hotspot within *NPHS1*, we found inconsistent phasing of rs2071347 (*NPHS1*, exon 26) when comparing different reference panels and methods, and therefore removed it from downstream analyses. We used phASER to calculate haplotype-specific expression by summing RNA-seq counts across all heterozygous SNPs. Samples with less than 20 total reads across *NPHS1* were removed. For the 187 remaining samples, we calculated ASE as $|0.5 - (\text{haplotype A / total reads})|$. In patients with the risk haplotype, haplotype A harbors all 5 *NPHS1* risk variants; in patients without the risk haplotype, haplotype A is randomly selected from one of their 2 haplotypes. We then compared ASE and gene expression for samples with and without the risk haplotype with a Wilcoxon rank-sum test in R. The total reads and number of supporting heterozygous SNPs varied across samples. Samples with lower power to detect ASE, less than 2 heterozygous SNPs, or in the bottom 10% of total reads were nonetheless included for completeness and are indicated as gray points in Figure 4a and b.

HLA fine-mapping

Methods for *HLA* imputation and *HLA* genotyping are shown in the Supplementary Methods.

Power calculation

The power of discovery GWAS (987 cases and 3206 controls) was calculated using the R package "CATS".[55] Assuming a disease prevalence of 0.016%, study power was calculated separately under the additive model for variants with an allele frequency of 0.5%, 5%, and 50%, with a significance threshold $\alpha = 5E{-}08$ (Supplementary Figure S2). The Bioinformatics Institute's Online Sample Size Estimator[56] was used to estimate the power of replication for each candidate SNP.

Heritability estimates

The disease heritabilities explained by genome-wide variants were estimated using genome-wide complex trait analysis[43,57] (genome-wide complex trait analysis—LDMS method), assuming a disease prevalence of 0.016% in the Japanese population. All variants that passed the quality control procedure after whole-genome imputation were included in the analysis. Variants were grouped as common variants (MAF $> 5\%$) or uncommon variants ($0.5\% <$ MAF $\leq 5\%$) when making genetic relationship matrixes during the calculation. Details are shown in the Supplementary Methods.

APPENDIX

The Research Consortium on Genetics of Childhood Idiopathic Nephrotic Syndrome in Japan

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Notes. The Research Consortium on Genetics of Childhood Idiopathic Nephrotic Syndrome in Japan, Korean Consortium of Hereditary Renal Diseases in Children, Midwest Pediatric Nephrology Consortium (Genetics of Nephrotic Syndrome study group), and NEPHROVIR.

Supplementary step-wise conditional analyses in *HLA* region and *HLA* fine-mapping.

Supplementary Methods.

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Table S2. Clinical information of patients in the discovery GWAS and international replication study.

Table S6. Significant gene sets with P values < 0.05 after Bonferroni correction in gene-set analysis by MAGMA.

Table S9. Step-wise conditional analyses in *HLA* region.

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Table S11. *HLA* alleles significantly associated with Japanese childhood SSNS in the discovery stage using HLA-imputation data.

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Table S21. Heritability estimation using autosomal variants in discovery Japanese sample set.

Figure S1. Principal component analysis in the discovery GWAS using HapMap Phase III samples as a reference (113 Utah residents with ancestry from northern and western Europe [CEU], 113 Yoruba in Ibadan [YRI], 84 Han Chinese in Beijing [CHB], and 86 Japanese in Tokyo [JPT]).

Figure S2. Power of the discovery GWAS.

Figure S3. Quantile–quantile (Q–Q) plot of P values for SNPs calculated using logistic regression with an adjustment for sex and PC1–4 (987 cases with childhood SSNS and 3206 healthy controls).

Figure S4. Conditional analysis in the candidate locus on chromosome 19.

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Figure S7. The location and annotation of 5 SNPs selected for replication in the *NPHS1-KIRREL2* region (A).

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Figure S11. Schematic diagram of allele-specific expression of *NPHS1*. Both risk and non-risk samples show the same total reads.

Supplementary File (Excel)

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Table S18. Association analysis of HLA-DRB1-DQB1-DPA1-DPB1 haplotypes with childhood SSNS in the discovery stage using HLA-imputation data.

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