





Genetic Identification of Two Novel Loci Associated with Steroid-Sensitive Nephrotic Syndrome

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ABSTRACT

Background Steroid-sensitive nephrotic syndrome (SSNS), the most common form of nephrotic syndrome in childhood, is considered an autoimmune disease with an established classic HLA association. However, the precise etiology of the disease is unclear. In other autoimmune diseases, the identification of loci outside the classic HLA region by genome-wide association studies (GWAS) has provided critical insights into disease pathogenesis. Previously conducted GWAS of SSNS have not identified non-HLA loci achieving genome-wide significance.

Methods In an attempt to identify additional loci associated with SSNS, we conducted a GWAS of a large cohort of European ancestry comprising 422 ethnically homogeneous pediatric patients and 5642 ethnically matched controls.

Results The GWAS found three loci that achieved genome-wide significance, which explain approximately 14% of the genetic risk for SSNS. It confirmed the previously reported association with the HLA-DR/DQ region (lead single-nucleotide polymorphism [SNP] rs9273542, $P=1.59 \times 10^{-43}$; odds ratio [OR], 3.39; 95% confidence interval [95% CI], 2.86 to 4.03) and identified two additional loci outside the HLA region on chromosomes 4q13.3 and 6q22.1. The latter contains the calcium homeostasis modulator family member 6 gene *CALHM6* (previously called *FAM26F*). *CALHM6* is implicated in immune response modulation; the lead SNP (rs2637678, $P=1.27 \times 10^{-17}$; OR, 0.51; 95% CI, 0.44 to 0.60) exhibits strong expression quantitative trait loci effects, the risk allele being associated with lower lymphocytic expression of *CALHM6*.

Conclusions Because *CALHM6* is implicated in regulating the immune response to infection, this may provide an explanation for the typical triggering of SSNS onset by infections. Our results suggest that a genetically conferred risk of immune dysregulation may be a key component in the pathogenesis of SSNS.

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Steroid-sensitive nephrotic syndrome (SNSS) is the most common form of nephrotic syndrome in children, with an incidence of approximately 1–10 per 100,000.¹ The majority of affected children experience a chronic relapsing course. The onset of disease manifestations is commonly associated with a preceding activation of the immune system, typically by an upper respiratory tract infection.² As the name implies, SSNS is characterized by a therapeutic response to glucocorticoids as well as to

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other immunosuppressants. The apparent triggering of the disease by infection and the therapeutic effect of immunosuppressive treatment have suggested that SSNS is an autoimmune disorder.³

Investigating the genetic architecture of other autoimmune diseases through genome-wide association studies (GWAS) has proven to be successful in providing insight into their etiology and pathogenesis. Unsurprisingly, a common finding in these studies is the identification of association with the HLA region that contains numerous genes critical for the immune system, in particular enabling the distinction between self and foreign.⁴

Arguably, however, it is the identification of risk loci outside the HLA locus that can provide the most informative mechanistic insights into the pathogenesis of such diseases. Prominent examples from nephrology include membranous nephropathy and IgA nephropathy. In membranous nephropathy, GWAS identified association with *PLA2R1*, suggesting that the antibody formation against the PLA2R1 receptor observed in membranous nephropathy is a causal disease mechanism.^{5,6} Similarly, in IgA nephropathy, GWAS have highlighted the important role of the intestinal immune response as well as IgA1 antibody glycosylation in the pathogenesis of the disease.^{7–9}

Three GWAS of SSNS have recently been reported, but in all, association at genome-wide significance was identified within the HLA region only.^{10–12} We set out to perform a GWAS using the largest number of ethnically homogenous patients and controls studied to date in an attempt to identify additional loci associated with SSNS.

METHODS

Full details of all methods can be found in Supplemental Material.

Cohorts

DNA from pediatric patients diagnosed with SSNS and of reported European ethnicity were used in this study. SSNS was defined according to standard clinical criteria.¹³ DNA was acquired from the Prednisolone in Nephrotic Syndrome (PREDNOS) (EudraCT 2010–022489–29) and PREDNOS2 (EudraCT 2012–003476–39) trials¹⁴ as well as from collaborating clinicians at their affiliated institutions. Informed consent was obtained from all participants, and ethical approval was granted by the host institutions. Ethnically matched controls were drawn from publicly available datasets (Supplemental Figure 1). For the replication of our findings, we examined the results of previously published GWAS of SSNS.^{10–12}

Genotyping, Quality Control, and Imputation

Isolation of DNA, genotyping, quality control (QC), and imputation were performed using standard procedures. Patients were genotyped by University College London Genomics (Institute of Child Health, University College London, London, United Kingdom) on the Infinium Multi-Ethnic Global BeadChip v.A1 (Illumina). Controls had been genotyped on a variety of platforms (additional details are in Supplemental Material).^{15–17}

Significance Statement

Although steroid-sensitive nephrotic syndrome (SSNS) is considered an autoimmune disease, its etiology is poorly understood. Genome-wide association studies (GWAS) have provided important insights into other autoimmune diseases, but so far, such studies have reported associations only in the classical HLA region for SSNS. In a GWAS of a large cohort of European ancestry comprising 422 ethnically homogeneous pediatric patients and 5642 ethnically matched controls, the authors found two loci outside the HLA region associated with SSNS at genome-wide significance. The locus with strongest association contains the calcium homeostasis modulator family member 6 gene *CALHM6*, which has been implicated in the regulation of the immune system. These findings suggest that impaired downregulation of the immune system may be a key mechanism in the pathogenesis of SSNS.

Stringent QC steps for both single-nucleotide polymorphisms (SNPs) and individuals (including missingness, heterozygosity, and deviation from Hardy–Weinberg equilibrium) were carried out on patients and controls separately as well as on the combined cohort (Supplemental Figures 1–3). Principal component analysis was used to identify the subset of patients and controls of European ancestry (Supplemental Figure 4). The genomic inflation factor (λ) was calculated to estimate population stratification. Imputation was performed on the combined patient-control dataset with Beagle 5.0 (<https://faculty.washington.edu/browning/beagle/beagle.html>) using only markers passing stringent QC and present in all datasets.¹⁸ Only SNPs with a dosage R^2 of >0.8 were included. Data from the 1000 Genomes Project Phase 3 were used as a reference panel. Golden Helix SNP & Variation Suite version 8.8.1 (SVS; http://goldenhelix.com/products/SNP_Variation/index.html) and PLINK version 1.90 β (<https://www.cog-genomics.org/plink/1.9>) were used for analysis.¹⁹

GWAS and Conditional Analyses

The primary association analysis was performed using logistic regression under an additive model with adjustment for the first ten principal components of ancestry.

Conditional analysis of the lead SNPs was performed using a logistic regression model. A genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ was used. SVS was used for association testing and conditional analysis. A power calculation is detailed in Supplemental Material.

HLA Type Imputing

HLA imputation was performed using SNP2HLA v1.0.3 (<http://software.broadinstitute.org/mpg/snp2hla/>) with default parameters.²⁰ As input, a subset of 1189 SNPs from those selected for GWAS (post-QC) and overlapping with the SNP2HLA imputation HapMap European reference dataset was used. Logistic regression under an additive model with adjustment for the first ten principal components of ancestry was used to test for association of each HLA allele with SSNS. Conditional analysis of the lead HLA alleles was performed using a logistic regression model as well.

Expression Quantitative Trait Loci Analyses

Publicly available expression quantitative trait loci (eQTLs) databases were queried to ascertain whether variants significantly associated with SSNS were known to influence the expression levels of corresponding gene products in multiple tissues.

RESULTS

Study Cohort

DNA from a total of 712 anonymous patients with SSNS of reported European ethnicity were available to our project. The combined control dataset consisted of 6126 individuals of reported European ethnicity. After stringent QC and selection for European ancestry by principal component analysis, 422 patients and 5642 controls remained (Supplemental Figure 1), with a total of 158,217 overlapping SNPs. These were imputed to 5,216,266 high-quality genome-wide SNPs. The summary statistics are available in Supplemental Material.

GWAS Results

Our GWAS revealed three loci achieving genome-wide significance (Figure 1). Together, these loci explain approximately 14% of the genetic risk for SSNS. The strongest signal corresponded to a broad peak in the classic HLA region on chromosome 6p21.32. The three leading SNPs, in strong linkage disequilibrium with each other, are rs9273542 ($P=1.59\times 10^{-43}$; odds ratio [OR], 3.39; 95% confidence interval [95% CI], 2.86 to 4.03) and rs9273529 ($P=2.87\times 10^{-43}$; OR, 3.39; 95% CI, 2.85 to 4.03) in the intronic region of the gene *HLA-DQB1* and rs9273371 ($P=1.64\times 10^{-43}$; OR, 3.29; 95% CI, 2.78 to 3.89) intergenic between *HLA-DQA1* and *HLA-DQB1* (Figure 2A).

Conditional analysis on rs9273542 decreased the strength of the association such that the minimum P value achieved at this locus changed to $P=4.29\times 10^{-31}$ for rs2858317 centromeric of *HLA-DQB1* (Figure 2B). Joint conditioning on rs9273542 and rs2858317 significantly reduced the strength of the association to a minimum P value of $P=2.40\times 10^{-8}$ at rs3828799 (Figure 2C). These results indicate that the association at this locus is driven by at least two independent signals.

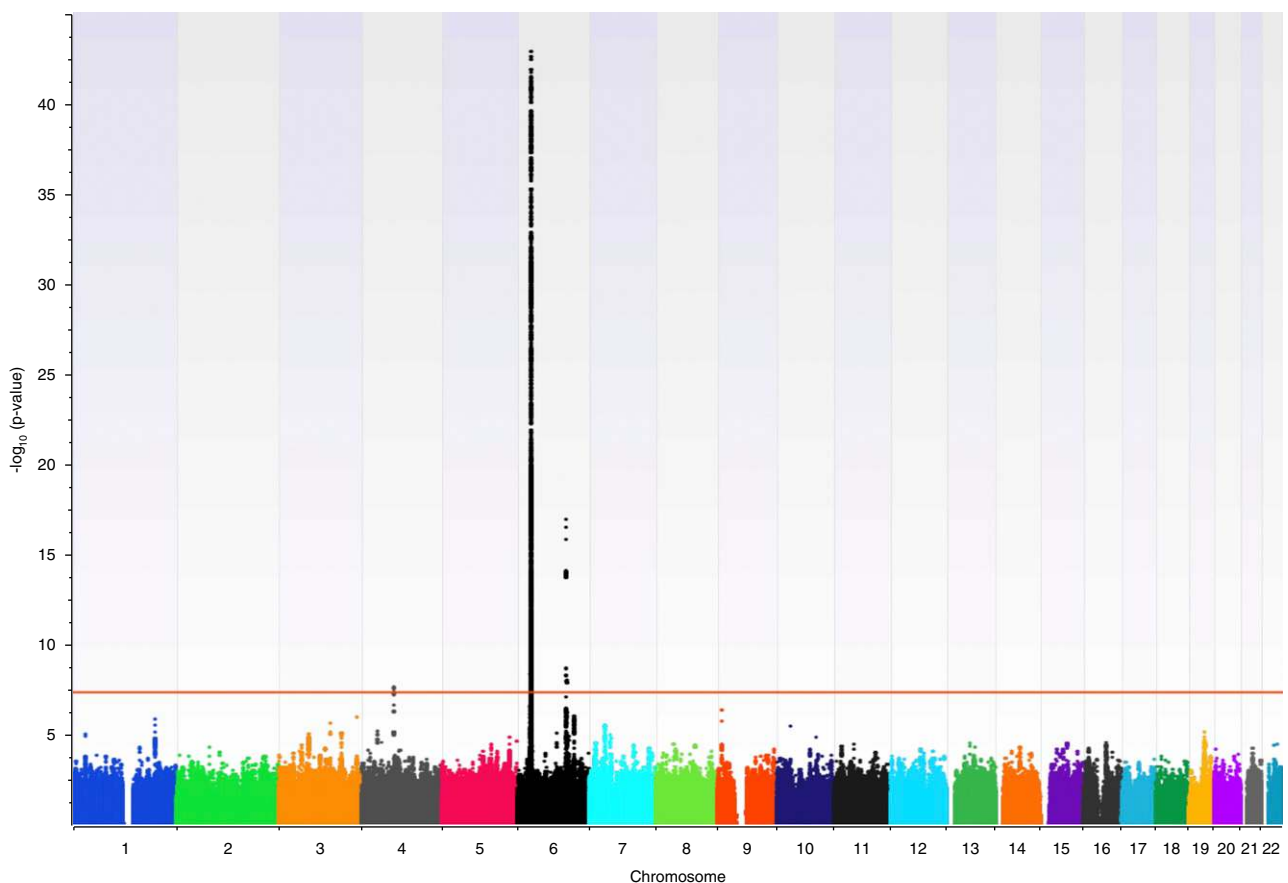


Figure 1. Manhattan plot. Genome-wide association studies (GWAS) for steroid-sensitive nephrotic syndrome (SSNS) comparing 422 European patients with 5642 ethnically matched controls. The x axis shows the chromosomal position. The y axis shows the log-transformed P value; the horizontal red line indicates the genome-wide significance threshold ($P=5\times 10^{-8}$). Three loci surpass this threshold on chromosomes 4 and 6.

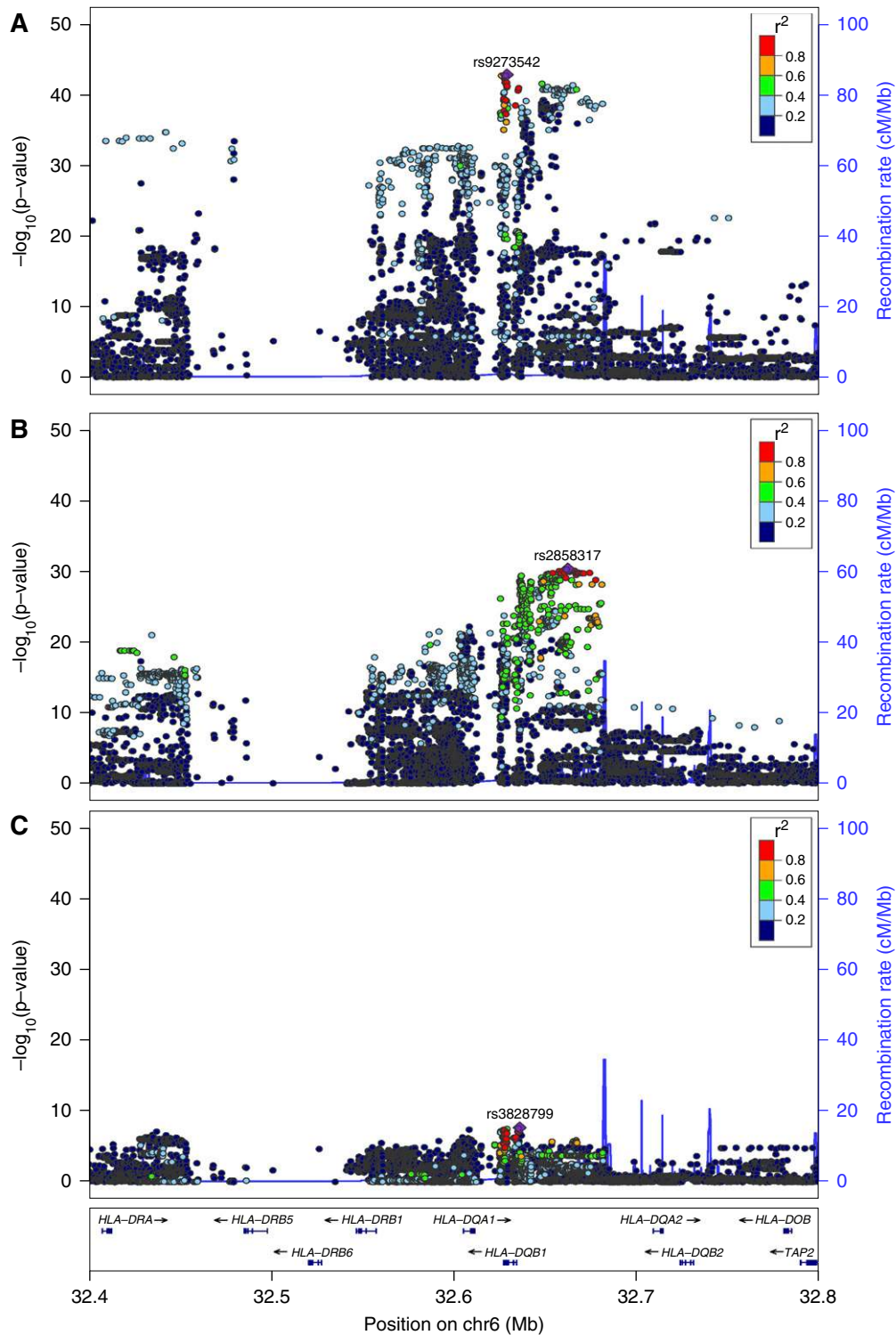


Figure 2. Locus zoom plot of the HLA-DR/DQ (6p21.32) association. The x axis indicates chromosomal position, the left y axis indicates the log-transformed P value, and the right y axis indicates the recombination rate. The purple diamond indicates the single-nucleotide polymorphism (SNP) with the smallest P value within each region. SNPs are colored on the basis of their pairwise linkage disequilibrium to the lead SNP as per 1000 Genomes European reference data according to the key. Recombination hotspots are indicated by blue vertical lines. Data are shown for 200 kb on either side of the lead SNP. (A) Unconditioned analysis. The lead SNP is rs9273542 in the gene *HLA-DQB1*, with a P value of 1.59×10^{-43} . (B) Postconditioning on rs9273542. The lead SNP is rs2858317 centromeric of *HLA-DQB1*, with a P value of 4.29×10^{-31} . (C) Postconditioning on rs9273542 and rs2858317. The lead SNP from this analysis is rs3828799 centromeric of *HLA-DQB1*, with a P value of 2.40×10^{-8} . Note that these results indicate that the association in the *HLA-DRB1* region is driven mainly by two independent HLA alleles (indicated by lead SNPs in A and B).

Our two lead SNPs (rs9273542 and rs2858317) are in strong linkage disequilibrium with rs4642516 (identified by Jia *et al.*¹²) and two markers rs1063348 and rs28366266 (identified by Debiec *et al.*¹¹).

The strongest association outside the HLA region was with a locus on chromosome 6q22.1. The three lead SNPs (rs2637678: $P=1.27\times 10^{-17}$; OR, 0.51; 95% CI, 0.44 to 0.60; rs2637681: $P=3.53\times 10^{-17}$; OR, 0.52; 95% CI, 0.44 to 0.61;

and rs2858829: $P=1.72\times 10^{-16}$; OR, 0.53; 95% CI, 0.45 to 0.62) are all in close linkage disequilibrium and localize around the gene *CALHM6* (previously called *FAM26F*) (Figure 3A). Genome-wide significance was lost by conditioning on rs2637678 (Figure 3B), indicating that a single signal is responsible for driving this association.

The third locus to reach genome-wide significance was on chromosome 4q13.3. The lead SNP, rs10518133, is in the

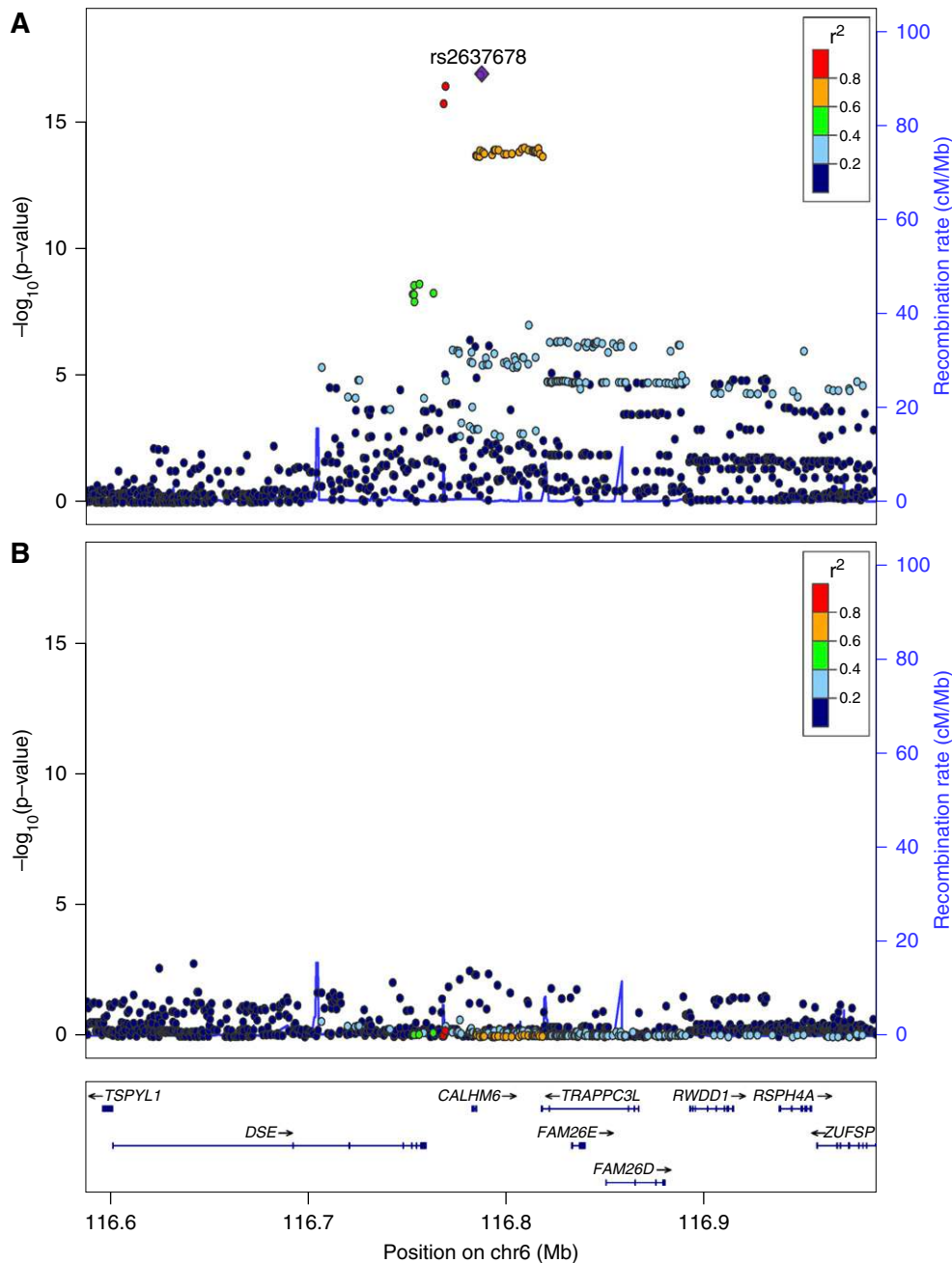


Figure 3. Locus zoom plot of the *CALHM6* (6q22.1) association. The composition of this figure is as per Figure 2. (A) Unconditioned analysis. The lead single-nucleotide polymorphism (SNP) is rs2637678 with a P value of 1.27×10^{-17} . (B) Postconditioning on rs2637678. No additional SNPs reached genome-wide significance level, indicating that the association with *CALHM6* is driven by a single haplotype.

intronic region of the gene *PARM1*, and it was associated at $P=2.50 \times 10^{-8}$ with OR of 1.96 (95% CI, 1.57 to 2.45) (Figure 4A). Genome-wide significance was also lost at this locus by conditioning on the lead SNP, indicating that a single signal is driving the association (Figure 4B).

A Manhattan plot as well as locus zoom plots of the identical regions using genotyped markers only are provided in Supplemental Figures 5–8, and details for the lead genotyped SNPs at the respective loci are listed in Supplemental Table 1.

Classic HLA Type Analyses

HLA allele imputation was performed for HLA class 1 (*HLA-A*, *-C*, and *-B*) and class 2 (*HLA-DRB1* and *-DQB1*) genes. Genome-wide significance for SSNS was achieved for nine HLA alleles, including high-resolution subtypes at *DQA1*01*, *DQA1*02*, *DQA1*07*, *DQA1*13*, and *B*8* (Supplemental Figure 2, Table 1). The strongest association was observed with *HLA-DQA1*02:01*, which resides on a haplotype with *HLA-DRB1*07:01* (indicated by equal allele frequencies in

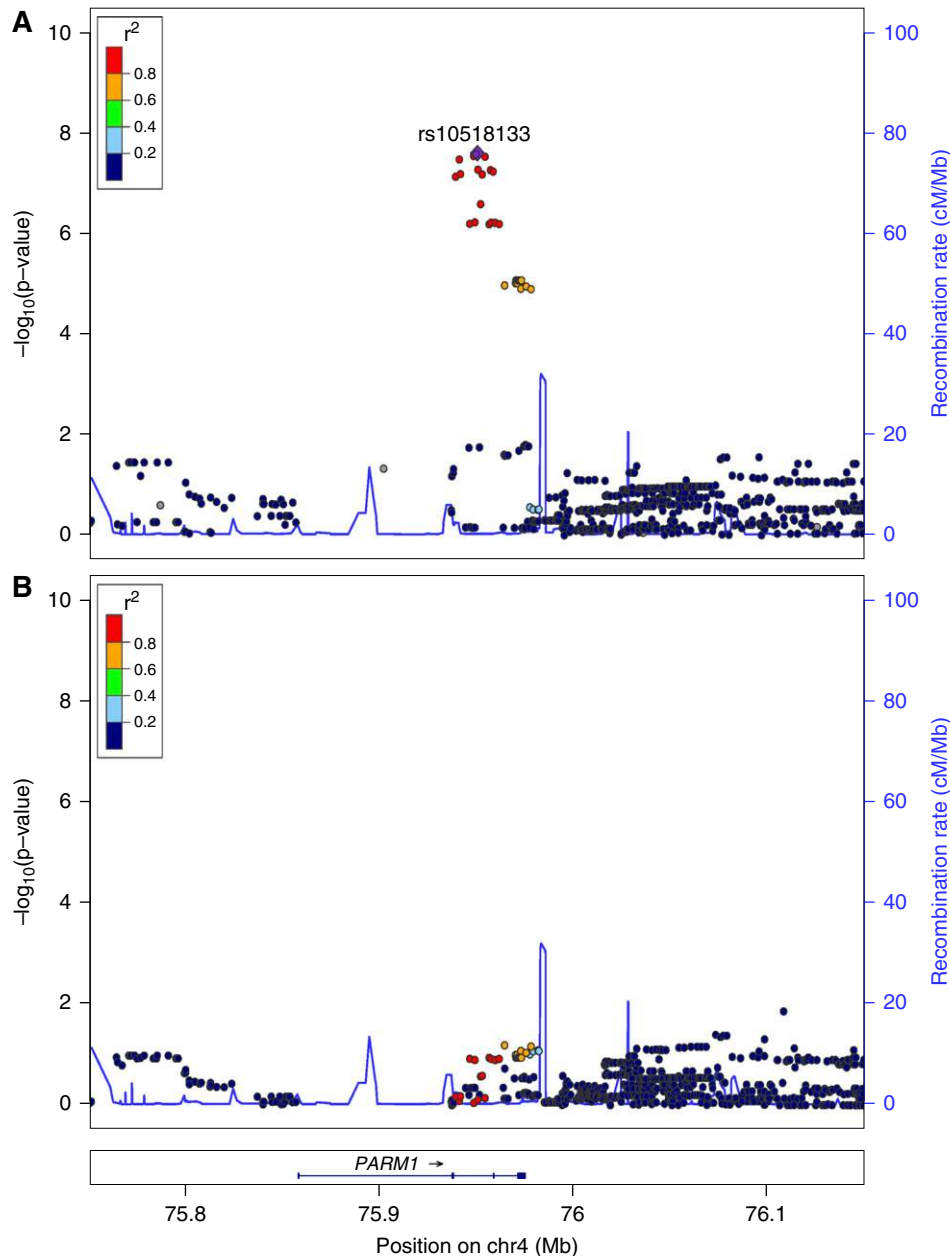


Figure 4. Locus zoom plot of the *PARM1* (4q13.3) association. The composition of this figure is as per Figure 2. (A) Unconditioned analysis. The lead single-nucleotide polymorphism (SNP) is rs10518133 in the gene *PARM1*, with a P value of 2.50×10^{-8} . (B) Post-conditioning on rs10518133. No additional SNPs reached genome wide significance level, indicating that the association with *PARM1* is driven by a single haplotype.

Table 1. Association of imputed classic HLA alleles with steroid-sensitive nephrotic syndrome

HLA Allele	MAF Patients	MAF Controls	OR	95% CI	P Value
HLA_DQA1*02:01	0.35	0.15	3.42	2.80 to 4.16	1.06×10^{-32}
HLA_DQA1*01	0.13	0.38	0.36	0.30 to 0.43	1.90×10^{-31}
HLA_DRB1*07:01	0.35	0.15	3.26	2.68 to 3.97	5.62×10^{-31}
HLA_DQB1*02	0.40	0.21	2.43	2.04 to 2.91	9.77×10^{-22}
HLA_DQA1*01:03	0.02	0.09	0.24	0.15 to 0.38	1.79×10^{-14}
HLA_DRB1*13	0.04	0.11	0.31	0.22 to 0.44	2.41×10^{-14}
HLA_DRB1*13:01	0.02	0.08	0.23	0.15 to 0.37	3.18×10^{-14}
HLA_DQA1*01:01	0.08	0.15	0.46	0.35 to 0.59	1.53×10^{-10}
HLA_B*08:01	0.20	0.13	2.95	2.05 to 4.23	9.17×10^{-09}

Minor allele frequencies (MAFs) for patients and controls and odds ratios (ORs) with 95% confidence intervals (95% CIs) for each of the HLA alleles achieving genome-wide significance are shown.

patients and controls). The *HLA-DQA1*01* allele was protective and remained independently associated at genome-wide significance after conditioning on *HLA-DQA1*02:01* ($P=1.24 \times 10^{-31}$; OR, 0.31; 95% CI, 0.25 to 0.38).

eQTL Analyses

The three leading SNPs at the *CALHM6* locus (rs2637678, rs2637681, and rs2858829) exhibit strong cis-eQTL effects in the GTEx database.²¹ For all three SNPs, the highest normalized effect size (NES) is seen in EBV-transformed lymphocytes (rs2637678: NES=0.66; $P=7.2 \times 10^{-10}$; rs2637681: NES=0.67; $P=1.9 \times 10^{-9}$; and rs2858829: NES=0.66; $P=3.2 \times 10^{-9}$). A strong cis-eQTL effect is also noted in the eQTLgen database from blood (Z score=43.18; $P=3.27 \times 10^{-310}$), which also involves the neighboring genes *DSE*, *RWDD1*, and *NT5DC1*.²² In both databases, the minor allele is associated with increased expression of *CALHM6*. Of note, no expression of *CALHM6* is noted in whole kidney in the GTEx, NephQTL, and Human Kidney Atlas Expression (HKAE) databases.^{23,24} The HKAE database suggests specific expression in glomerulus, but this could not be confirmed in the other databases.

No significant eQTLs were found for rs10518133 (tagging the *PARM1* locus) in any tissue in the GTEx database, but in eQTLgen, the risk allele is associated with significant downregulation of *PARM1*.

DISCUSSION

This study has confirmed the association of SSNS with the HLA locus and identified two additional loci on chromosome 6 and chromosome 4 achieving genome-wide significance.

HLA Locus

By far, the most associations identified by GWAS of human diseases are located in the HLA region.²⁵ The vast majority of these phenotypes are autoimmune or infectious diseases. The necessity for an appropriate and ever-evolving response to infection drives variation in HLA peptide binding grooves and some of these variants can lead to inappropriate responses against antigens of the host.²⁶ Thus, unsurprisingly and consistent with previous studies, the strongest signal for association with SSNS that we identified was in the HLA region.^{10–12} The lead SNP, rs9273542 (Table 2), is located within the *HLA-DR/DQ* region, specifically *HLA-DQB1*.

Imputation of HLA alleles identified that *HLA-DQA1*02:01* and *HLA-DRB1*07:01* are associated with the strongest risk, whereas *HLA-DQA1*01* seems to be independently protective. *HLA-DRB1*13* ($P=2.41 \times 10^{-14}$) is also protective, which is similar to the results of the study by Jia *et al.*,¹² which showed in an Asian population that *HLA-DRB1*13:02* and *HLA-DQB1*06:04* have protective effects. In the study by Debiec *et al.*,¹¹ serial conditional analysis of the HLA association revealed an independently associated SNP located within *BTNL2* (rs9348883), although this did not reach genome-wide significance. We were unable to confirm an independent association with this gene, which may reflect the different ethnicities analyzed, because the *BTNL2* signal was primarily driven by the African cohort in that study.

Associations Outside the HLA Region

The adaptive immune response is triggered by HLA peptide-epitope binding, but it is modulated by regulatory mechanisms, elements of which are encoded in non-HLA genes.⁴ Arguably, it is the identification of these genes that can provide

Table 2. Effect estimates for lead single-nucleotide polymorphism

Locus	Gene	SNP	DR2	Minor Allele	MAF Patients	MAF Controls	OR	95% CI	P Value
6p21.3	<i>HLA-DQB1</i>	rs9273542	0.89	T	0.51	0.24	3.39	2.86 to 4.03	1.59×10^{-43}
6q22.1	<i>CALHM6/FAM26F</i>	rs2637678	0.96	C	0.26	0.40	0.51	0.44 to 0.60	1.27×10^{-17}
4q13.3	<i>PARM1</i>	rs10518133	0.93	A	0.12	0.06	1.96	1.57 to 2.45	2.50×10^{-8}

Minor allele frequencies (MAFs) and odds ratios (ORs) with 95% confidence intervals (95% CIs) for each of the minor alleles of the lead single-nucleotide polymorphisms (SNPs) from the three loci achieving genome-wide significance. Dosage R^2 (DR2) indicates the Beagle imputation quality score.

the most informative insights into the complex architecture of the dysregulated immune response in specific diseases. We here describe the discovery of two loci outside the HLA region achieving genome-wide significant associations with SSNS.

CALHM6 Association (6q22.1)

The strongest signal outside the HLA region is on chromosome 6q22.1, and the lead SNP, rs2637678 (Table 2), is located very near the gene *CALHM6* (calcium homeostasis modulator family member 6), previously also annotated as *FAM26F* or *INAM* (IRF-3–dependent NK-activating molecule).²⁷ Of note, this locus had been identified as a potential signal in SSNS by Debiec *et al.*,¹¹ but it did not reach genome-wide significance. The 6q22.1 SNP reported by Debiec *et al.*¹¹ is identical to one of the lead SNPs identified in this study (rs2858829), and it was associated with a *P* value of 6.8×10^{-8} . Importantly, the existence of this published suggestive association at rs2858829 provides independent confirmatory evidence for our genome-wide significant finding.

The apparent absence of expression of *CALHM6* in the kidney is in contrast to the high expression in lymphocytes and consistent with the notion that impaired immune regulation is a key risk factor for the development of SSNS. Indeed, *CALHM6* is thought to have an important role in the regulation of the immune system by facilitating interactions and potential synapses between immune cells.²⁸ It is differentially regulated in response to various immune stimuli, especially IFN- γ , with predicted IFN-stimulated response element and signal transducer and initiator of transcription binding sites in its promoter.²⁹ Members of the *CALHM* family are thought to belong to the ATP release channel superfamily.³⁰ *CALHM6* is highly expressed in lymphocytes, and ATP is a recognized trigger for apoptosis, including of immune cells.^{31,32} A key effect of glucocorticoids on the immune system is the induction of lymphocyte apoptosis.³³ Thus, *CALHM6* may play a role in mediating this effect, and impaired *CALHM6* function may exacerbate an exaggerated immune response, leading to SSNS, which can be suppressed by pharmacologic doses of glucocorticoids. Of note, the same locus with the same risk allele has been previously associated with another autoimmune disease highly sensitive to steroid treatment, ulcerative colitis, providing additional evidence for the importance of *CALHM6* in immune response regulation.³⁴

CALHM6 eQTL Analyses

We performed an eQTL analysis to further assess potential associations between our identified variants and gene expression.³⁵ Of note, for the lead variants in *CALHM6*, the presence of the minor allele is protective against SSNS so that the major allele is the risk allele and enriched in patients. Interestingly, the lead variant (rs2637678) at the *CALHM6* locus is a known eQTL, and the risk allele is associated with decreased *CALHM6* expression. This is consistent with the hypothesis that the presence of the risk allele may impair lymphocyte apoptosis and thus, lead to less effective downregulation of an immune response.

However, it is important to note that GWAS identify variants that represent a haploblock rather than a specific gene associated with the disease. This is best illustrated by the initial controversy over the association of *MYH9* versus *APOL1* with nondiabetic kidney disease in black individuals.³⁶ Thus, it is possible that another gene in the haploblock with *CALHM6* may actually be causally associated. Indeed, in the eQTLGen database, rs2637678 also has highly significant eQTL effects on neighboring genes. *CALHM6* was prioritized on the basis of its proximity to the GWAS signal and its known function, but additional studies will be required to establish with certainty if this is indeed the gene responsible for driving the observed association.

PARM1 Association (4q13.3)

In addition, we found a genome-wide significant association with a locus on chromosome 4q13.3 (lead SNP rs10518133; *P* = 2.50×10^{-8} ; OR, 1.96), which is located within the gene *PARM1* (prostate androgen-regulated mucin-like protein 1). Interestingly, this locus is near a locus reported by Debiec *et al.*¹¹ as achieving suggestive evidence of association with SSNS, with their lead SNP located within *BTC* (Betacellulin), the next gene upstream of *PARM1* on chromosome 4. The lead SNP at 4q13 reported by Debiec *et al.*¹¹ and the *PARM1* SNP identified in this study are approximately 250 kb apart, but they are separated by a strong recombination hotspot (>50 cM/Mb) (Supplemental Figure 3). This hot spot is at least equally strong in African populations (Supplemental Tables 2 and 3) so that the different ethnicities cannot explain the separation of this locus between the previous study and our study.

Limitations

Our study has several limitations. First, only limited clinical information on our patients is available. However, the majority of patients were obtained through two clinical trials (the PREDNOS and PREDNOS2 trials), which recruited from >100 pediatric units across the United Kingdom, and it is thus highly unlikely that our patient cohort is substantially different from other SSNS cohorts. Indeed, the available data, such as the 2:1 male:female ratio, match perfectly with published data.¹³

Second, we used publicly available genotype data for controls, and therefore, the data from different genotyping platforms needed to be combined. As detailed in Supplemental Figure 1, this led to a limited set of overlapping genotyped SNPs, and the majority of SNPs used in our final analysis were imputed. However, imputation has become an accepted tool in GWAS, and the fact that the analysis with genotyped markers only (Supplemental Figures 5–9) identifies the same loci, albeit with higher *P* values, provides strong evidence that these loci are genuinely associated with the phenotype and have not been identified due to imputation artifacts.³⁷

The most important limitation, however, is the lack of a replication cohort. The independent identification of loci at both 6q22.1 and 4q13.3 by Debiec *et al.*¹¹ provides strong confirmatory evidence for our results. Yet, as detailed above, the

lead SNP at 4q13.3 identified in that study is separated from our lead SNP by a recombination hotspot. It, therefore, remains to be determined whether this region is truly associated with SSNS or not and if so, if these loci are independent from each other. Moreover, Debiec *et al.*¹¹ did not provide detailed information on the risk allele at 6q22.1, and we are thus unable to assess whether the allelic effect is identical in both studies. Additional independent replication is thus needed to confirm the discovery of these loci. Our study identifies two loci outside the HLA region with genome-wide significant association with SSNS and thus, provides important insight into the pathogenesis of SSNS. Because *CALHM6* is implicated in regulating the immune response to infection, this may provide an explanation for the typical triggering of disease onset by infections. Additional studies are needed to provide independent replication of our findings and investigate the precise mechanisms and whether these could be amenable to specific treatments.

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SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at <http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2018101054/-/DCSupplemental>.

Supplemental Material. Supplementary results, materials and methods, acknowledgments, and summary statistics.

Supplemental Figure 1. Flowchart of quality control steps and GWAS.

Supplemental Figure 2. Results of classical HLA type association analysis.

Supplemental Figure 3. Locus Zoom plot of the association on chromosome 4.

Supplemental Figure 4. Principal component analysis of patients and controls.

Supplemental Figure 5. Manhattan plot with genotyped markers only.

Supplemental Figure 6. Locus zoom plot with genotyped markers only for the HLA locus.

Supplemental Figure 7. Locus zoom plot with genotyped markers only for the *CALHM6* locus.

Supplemental Figure 8. Locus zoom plot with genotyped markers only for the chromosome 4q13.3 locus.

Supplemental Figure 9. Linkage disequilibrium of SNP in the HLA region associated with SSNS.

Supplementary Table 1. Effect estimates for the three lead genotyped SNPs at the associated loci.

Supplementary Table 2. Linkage equilibrium at the chromosome 4q13.3 locus.

Supplementary Table 3. List of SNPs with a suggestive association.

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