Title: Novel insights in the genetics of steroid sensitive nephrotic syndrome in childhood

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Abstract

Steroid sensitive nephrotic syndrome (SSNS) is the most common form of nephrotic syndrome in childhood and there is growing evidence that genetics play a role in the susceptibility for the disease. Familial clustering has been observed and led to several studies on familiar SSNS trying to identify a monogenic cause of the disease. Until now, however, none of these have provided convincing evidence for Mendelian inheritance. This and the phenotypic variability within SSNS suggest a complex inheritance pattern, where multiple variants, interactions between those and the environment play a role in disease development. Genome wide association studies (GWAS) have been used to investigate this complex disease. We herein highlight new insights in the genetics of the disease provided by GWAS and how these fit into our understanding of the pathogenesis of SSNS.

Introduction

Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in children worldwide and is characterized by the leakage of protein from the blood into the urine through damaged glomeruli. INS affects approximately 5 in 100,000 children worldwide aged below 16 years, and has a 2:1 male predominance. Incidence varies by geographical region and ethnicity, with children of South Asian, African American, and Arabic ethnicity having the highest incidence of disease. [1]

Children with INS clinically present with heavy proteinuria, oedema, and low albumin, and can be classified according to their response to first-line treatment of corticosteroids. The majority of children (80-90%) respond to a course of corticosteroids within 4 weeks and are labelled as having steroid sensitive nephrotic syndrome (SSNS). The remaining 10-20% are non-responsive and are classified as having steroid resistant nephrotic syndrome (SRNS), leading to less favourable prognosis and often progression to end-stage kidney disease. In children with SSNS, the majority will experience at least one episode of relapse, and up to 50% of children will develop a frequently relapsing or steroid dependent course. [1]

Though disease prognosis is guided by response to corticosteroids, the underlying pathophysiology of INS remains unclear. Renal biopsies in children with INS are not performed routinely but typically show foot process effacement on electron microscopy consistent with minimal change disease (MCD), which is commonly associated with a steroid-responsive phenotype [2]. A smaller proportion of biopsies show segmental destruction of the glomerular capillaries in addition to foot process effacement, consistent with focal and segmental glomerulosclerosis (FSGS), which is

often associated with resistance to steroids [3]. Though these histological findings provide insight into the structural changes occurring in INS, we are still left with questions of why and how these changes occur. While effective treatment in form of corticosteroids and other immunosuppressants exists, this can be associated with severe side effects, including stunted growth, obesity and cataracts. In order to develop more specific treatments, a detailed knowledge of the molecular mechanisms is necessary.

In contrast to SSNS, our understanding of steroid resistant nephrotic syndrome (SRNS) has been influenced by the discovery of pathogenic variants in more than 60 genes that account for 10-30% of SRNS cases [4]. The majority of these genes unsurprisingly encode for proteins essential for the integrity and function of the glomerular podocyte. In SSNS, however, the genetic contributions to disease have been more elusive. So far, no confirmed Mendelian form of the disease has been identified, even though familial clustering is recognised [5]. This pattern is suggestive of a complex genetic background, where variations in the genetic code provide susceptibility to the disease but are not sufficient to cause the disease. One tool to investigate such complex genetic backgrounds is the genome-wide association study (GWAS) [6]. Recently, GWAS of SSNS have started to dissect the polygenic nature of this disease, and indeed have confirmed the long-standing observational findings that the immune system plays a critical role in SSNS development [7-10].

In this review, we will discuss what is currently known about the genetic architecture of SSNS in children, as well as our current understanding as to how these genetic factors could contribute to disease pathophysiology and prognosis.

Why do we believe genetics play a role in the development of SSNS?

Unlike in SRNS, there is no single gene that has been identified as causal for SSNS; however, there is epidemiologic evidence demonstrating that genetic risk plays an important role in the pathogenesis of SSNS. Firstly, there is familial aggregation of SSNS, suggesting shared genetic risk alleles leading to higher risk of developing disease [11-13]. It is estimated that 3% of children with SSNS have a first-degree relative with the same disease. Secondly, ethnic background influences both incidence and severity of disease phenotype [14,15]. Children of South Asian and African American descent have both been shown to have a higher incidence of INS than children of European descent. African American children are also more likely to show FSGS on renal biopsy and to develop steroid-resistance, compared with European children [15,16]. Genetic factors may not exclusively explain these trends in ethnicity influences and familial aggregation, as environmental factors may also play a role. It can therefore be hypothesized that susceptibility to developing SSNS is based on genetic risk variants in combination with environmental triggers.

Overlap between SSNS and SRNS

Though SSNS and SRNS have traditionally been considered to be separate disease entities with different prognoses and different treatment requirements, recent evidence suggests that perhaps these conditions represent a spectrum of a single disease [17]. Although genetic studies have revealed a plethora of genes, variants in which can cause SRNS, they only contribute to a minority (10-30%) of individuals with SRNS. For the remaining subset (>70%) current guidelines suggest trials with

immunosuppressive treatment assuming an underlying immunological process [18]. Some of these patients respond to further immunosuppression, despite their initial steroid resistance [Mason, 2020 #1371]. Interestingly, even in those patients with genes known to be causal for SRNS, such as *PLCE1* and *WT1*, responsiveness to immunosuppressives has also been documented [19,20]. Furthermore, it is observed that some individuals who are initially responsive to steroids, progress to developing resistance in later stages of the disease. Indeed, these patients even show evolutionary histopathologic changes on biopsy, transforming from MCD to FSGS as steroid-resistance develops [17]. These clinical observations highlight the difficulties in establishing a clear and unequivocal distinction between SRNS and SSNS. Nevertheless, every paediatric nephrologist will recognise the typical clinical picture of childhood SSNS, characterised by response to corticosteroid treatment within 4 weeks, age of onset between 2 and 6 years, and a 2:1 male predominance [6]. This is by far the most common form of childhood nephrotic syndrome and the phenotype of SSNS that this review will focus on.

Mendelian Inheritance of SSNS

Despite multiple studies on familial SSNS affecting individuals from more than one generation suggestive of a single-gene defect [11-13], no single gene has been confirmed to cause the disease in any of these families: Even when familial SSNS has been mapped to a genomic region through large family cohorts, researchers have been unable to find a causal gene. For example, using linkage studies in a consanguineous kindred with three affected children, Ruf *et al.* [5] were able to identify a locus of genome-wide significance on chromosome 2p linked with SSNS, labelling

a region that harboured more than 50 candidate genes, but further fine mapping and subsequent candidate gene analysis failed to identify any candidate causal variant and the locus has not been replicated in any other study so far.

To date, all of the genetic variants proposed to cause Mendelian SSNS can be classified as variants of uncertain significance. Most of the variants reported in the literature are from small, single-family studies and have not been validated in independent larger cohorts. When these variants are examined in the gnomAD database [21], many of them are shown to occur at high frequency in the general population. Recently, there has been consensus that although genetic sequencing advances have contributed greatly to our understanding of renal pathophysiology, they have also led to publication of numerous variants, especially in recessive diseases like nephrotic syndrome, where evidence of true variant pathogenicity remains insufficient [22]. Critical assessment of these variants is therefore essential, even when an identified gene matches with disease phenotype, with classification criteria available from the American College of Medical Genetics (ACMG) [23].

A recent example includes a large familial study focussed on partially-responsive nephrotic syndrome, where six genes were found to be associated with disease (*MAGI2*, *TNS2*, *DLC1*, *CDK20*, *ITSN1*, and *ITSN2*) [24]. These six genes were shown to interact with one another and converge on a single biochemical pathway: Rho GTPase regulation of the podocyte cytoskeleton. Rho-GTPases are important in forming and maintaining the foot processes of the glomerular filtration barrier, and therefore it is logical to suspect that defects in their function can lead to nephrotic syndrome [25]. On further examination, however, some of the identified genes were associated with only 1 or 2 families, and inheritance was often not statistically inferred between multiple families through linkage studies. In addition, the

study phenotypes show multiple individuals with SRNS or adult-onset disease, which does not fit with the classical phenotype of SSNS. Thus, although further families are needed for confirmation, genetic variation affecting the Rho GTPase pathway may well be implicated in familial clustering of proteinuric renal disease, but not with the typical childhood form of SSNS.

Complex inheritance of SSNS

In contrast to Mendelian inheritance, complex inheritance arises from common variation in multiple genes. The relevant variants are not necessarily in coding regions, indeed, the vast majority of variants identified in complex disease lie outside coding regions [26]. Many of those are found to be in areas relevant for gene regulation, such as promoters, enhancers *etc.*, where their presence alters the expression of genes. Additionally, complex interactions between these variants are assumed to have an influence on the risk of disease development [27]. This makes the study of complex disease challenging and single-family studies are not sufficient to identify relevant variants.

To identify these variants, a hypothesis free approach is necessary, where variants across the whole genome and not limited to coding regions are examined. One powerful approach to achieve this is the genome-wide association study (GWAS). GWAS investigates in a hypothesis-free fashion by using variants distributed over the whole genome as markers to tag risk loci in the genome. In a case-control design every single marker is tested if the frequency of an allele is significantly different between affected and unaffected individuals, this implies a risk modifying effect of the allele itself or nearby (ungenotyped) allele(s) that tend to be inherited alongside the

tested allele, a situation termed linkage disequilibrium (LD). Since the frequency of some alleles is different in different ethnic groups or geographical regions it is important to ensure that the ancestry of cases and controls are as well-matched as possible and various statistical tools exist to quantify and, to an extent, correct for any known or hidden population stratification. Because a GWAS typically tests many thousands of independent markers, a high degree of statistical certainty is required to avoid observing apparent association purely as a consequence of observing the tail of a null distribution and conventionally a p-value of $5x10^{-8}$ is accepted as statistically significant in this type of experiment. Because statistical power is a function of both the strength of the genetic effect (*i.e.* the relative risk with each copy of the allele) and the frequency of the allele in the population, GWAS are poorly suited to detecting the effects of rare alleles unless the magnitude of their effect on disease risk is large. In addition, by convention, novel associations should be replicated in an independent set of cases and controls before they are regarded as providing strong evidence implicating a gene or locus. It is important to keep in mind, as stated above, that the markers tested rarely include all of the potentially causative variants and rather are linked to (*i.e*. inherited with) the responsible allele.

The role of HLA in SSNS

A handful of studies investigating complex inheritance patterns of SSNS have been published (Table 1). The majority of these studies used candidate gene approaches, where an *a priori* selection of candidate genes is required (summarized in Table 1 and reviewed in [28]). By far the strongest association found in all these

studies is located within the HLA-DR/DQ region, specifically in and around *HLA-DQA1* and *HLA-DQB1*.

The first exome wide association study, which is not limited to a selection of genes, but still to coding regions, was published by Gbadegesin *et al.* [29], who identified four variants (rs1129740, rs9273349, rs1071630, and rs1140343) in the HLA-DQA1/HLA-DQB1 locus associated with SSNS in a South Asian cohort. The two variants in *HLA-DQA1*, rs1129740 and rs1071630, were replicated in an independent European cohort and the association with *HLA-DQA1* has since been confirmed by other independent studies [29,7,9].

To date, there are 4 GWAS published on SSNS revealing new insights in the genetics of SSNS [7-10].

Debiec *et al.* [7] performed four separate GWAS in children with SSNS (discovery cohorts: European with 132 cases versus 2000 controls, African with 56 cases versus 454 controls, and Maghrebian with 85 cases versus 261 controls; replication cohort: European with 133 cases versus 552 controls) followed by a transethnic meta-analysis across all four cohorts. The group identified one SNP (rs1063348) reaching genome-wide significance across ethnicities located around *HLA-DQB1*. Subsequent conditional analysis revealed a further independent association (rs28366266) around *HLA-DRB1* and after conditioning on both, another SNP at this locus, rs9348883, which lies within introns of *HCG23* and *LOC101920163* (a minimally characterized long noncoding RNA) and in close proximity to *BTNL2,* remained significant across ethnicities [7].

A subsequent burden analysis of the two risk alleles in the HLA-DR/DQ region demonstrated that an increased number of risk alleles was associated with an increased SSNS risk as well as decreased age at onset of disease. Additionally, the

lead variant (rs1063348) around *HLA-DQB1* was associated with significantly decreased expression of *HLA-DRB1, HLA-DRB5*, and *HLA-DQB1* in the glomerulus in children with SSNS (North American Nephrotic Syndrome Study Network-NEPTUNE cohort). Both lead variants, rs1063348 and rs28366266 were expression quantitative trait loci (eQTLs- a locus known to affect the expression of usually surrounding genes in different cell lines) for several HLA genes, including blood and EBV-transformed lymphocytes. These findings underline the central role of the immune system in SSNS, but also demonstrate the complexity of the mechanisms underlying disease development and the importance of understanding the cell type specificity of these eQTLs.

Additional support for the involvement of the HLA-DR/DQ region in SSNS came from a GWAS performed by our group investigating a large European cohort (422 cases versus 5642 controls) [8].

The strongest signal (rs9273542) was located within the HLA-DR/DQ region, in the intronic region of the gene *HLA-DQB1.* Conditional analysis on the lead SNP revealed another independently associated SNP (rs2858317) centromeric of *HLA-DQB1*, demonstrating that the association in this locus was driven by at least 2 independent signals. Further, the lead SNPs identified were in strong LD with the two lead markers (rs1063348 and rs28366266) identified by Debiec *et al*..

There are also two GWAS in Japanese SSNS patients (224 vs 419 controls and 987 cases vs 3206 controls, respectively) confirming the association with HLA-DR/DQ is important in a different ethnicity [9,10].

The HLA region is known as the most polymorphic genetic region in humans and SNPs in the HLA region are often tightly linked to each other. Therefore, even

though the HLA-DR/DQ region is clearly significantly associated with SSNS susceptibility, further analysis (termed HLA fine-mapping) is required to pinpoint the exact HLA alleles associated with a disease. This can be achieved by HLA imputation, where four-digit HLA alleles are defined, which correspond to specific HLA molecules. A summary of HLA alleles significantly associated with SSNS is provided in Table .

Imputation of HLA alleles identified the composite haplotype *HLA-DQA1*02:01; HLA-DRB1*07:01; HLA-DQB1*02* associated with the strongest risk of disease [7,8]. The same risk haplotype was identified in a group of South Asian children with SSNS [30]. Conversely, *HLA-DQA1*01, HLA-DQA1*01:03* and *HLA-DRB1*13* appear to be protective in the European cohort and also in the South Asian cohort [30]. The fact that these haplotypes are preserved over different ethnicities supports their relevance in disease pathogenesis.

Remarkably, in both Japanese cohorts studied [9,10], the common risk haplotype of the European and South Asian population (*HLA-DQA1*02:01; HLA-DRB1*07:01; HLA-DQB1*02)* was not replicated. The haplotype associated with the strongest risk for disease was *HLA-DRB1*08:02, DQB1*03:02*, and the haplotype with the strongest protective association was *HLA-DRB1*13:02, DQB1*06:04* [9]. Based on the Allele Frequency Net Database [31], these specific haplotypes seem to be extremely rare in Europeans, where HLA-DRB1*08:02 has an allele frequency of only 0-0.03%, in contrast to 4.2% in the Japanese population [32]. This low frequency means that a very much larger study than seems feasible at the moment would be needed to have sufficient power to reliably confirm or refute an association of this alleles with disease in Europeans.

The involvement of the HLA system suggests that SSNS may be an unintended consequence of the immune system responding to an infection and this fits with the

clinical observation that manifestations of the disease are typically preceded by an upper respiratory infection. The fact that different HLA alleles appear to be involved in different ethnicities may indicate that the infectious trigger may vary by geographic region.

Non-HLA candidate genes for SSNS

While the association of SSNS with the HLA class II locus is confirmed by several studies, it is not clearly understood how the different HLA alleles impact susceptibility to SSNS or how they modify the risk for immune disease. It is possible that different HLA alleles facilitate altered antigen presentation via HLA class II variants to the immune system, and that this is enhanced by impaired regulatory mechanisms of the immune system; however, these hypotheses have yet to be experimentally tested. Thus, a crucial first step in understanding the pathogenesis of SSNS is through the detection of associations outside the HLA region. Prominent examples from nephrology include membranous nephropathy (MN): in MN, a GWAS identified a locus over *PLA2R1*, acting as an antigen in the kidney, thus suggesting a genetic predisposition to antibody formation against the PLA2R1 receptor as a crucial disease mechanism [33,34]. Arguably, it would be the detection of non-HLA genes that substantially increase our understanding of SSNS pathogenesis.

BTNL2

The lead SNP in the African cohort identified by Debiec *et al.* [7] was a missense variant in the gene *butyrophilin like-2 (BTNL2*) and subsequent metanalysis

of all four cohorts identified an independently associated SNP in close proximity of *BTNL2*.

BTNL2 (Butyrophilin-like 2) has not been associated with SSNS elsewhere, but with multiple other immune mediated diseases [35,36]. It is thought to be a negative regulator of T cell activity by suppressing T cell proliferation and activation (Figure 1) [37,38]. *BTNL2* induced FoxP3 expression and regulatory T cell (Treg) differentiation [37]. However, the precise mechanism between variants in *BTNL2* and the development of proteinuria remains unclear and this association was not replicated in an independent group.

CALHM6/DSE and PARM1

Our group identified two signals outside the HLA region, the strongest on chromosome 6 between the genes *CALHM6* (*Calcium Homeostasis Modulator Family Member 6)* and *DSE (Dermatan sulfate epimerase-1)* [8]. And the other on chromosome 4 within the intronic region of the gene *PARM1 (Prostate androgen-regulated mucin-like protein 1)*. Although we did not replicate these findings in an independent cohort, Debiec *et al.* identified two SNPs outside the HLA region reaching suggestive levels of significance, one of which (rs2858829) is identical to one of the lead SNPs identified by us between the genes *CALHM6* and *DSE* [8,7]. This could be considered as an independent replication for this locus, however, as the direction of effect (risk or protection) is not reported by Debiec *et al.* results still have to interpreted cautiously.

CALHM6 (*Calcium Homeostasis Modulator Family Member 6)*, previously also annotated as FAM26F or INAM (IRF-3-dependent NK-activating molecule), is thought

to be involved in infection, stress and immune response [39]. *CALHM6* expression is high in lymphocytes, especially in naïve and memory B cells as well as CD4+ T cells, and is regulated by various immune stimuli, with INF-γ known as the strongest stimulator for expression [40,41]. The function of the protein encoded by *CALHM6* remains unknown, but it is predicted to be a cation channel with an immunoglobulin-like fold [39]. Another member of the CALHM superfamily has been shown to be an ATP release channel and ATP is a reported trigger of apoptosis also in immune cells [42-44]. The lead variants associated with SSNS in our study are strong expression quantitative trait loci (eQTL) for *CALHM6*, with the risk allele decreasing expression of *CALHM6*. Downregulation of *CALHM6* could affect its immune regulatory role. The presence of the variant could lead to an imbalance in the CD4+ T helper (Th) cell subgroups, Th17 and Tregs cells, which has been associated with SSNS pathogenesis [45,46]. The beneficial role of glucocorticoids in SSNS could be partially explained by an upregulation of *CALHM6*, which was shown in rheumatic arthritis patients where *CALHM6* was significantly upregulated in CD4+ T cells of steroid responders in comparison to non-responders [47]. (Figure 1)

CALHM6 has further been shown to be expressed on memory B cells. Recovery of memory B cells after Rituximab treatment has been related to SSNS relapse, hence decreased expression of *CALHM6* in memory B cells of SSNS patients could disturb regulatory processes in this cell line [48]. Thus, altered immune regulatory mechanism because of reduced *CALHM6* expression could affect B cells or T cells or perhaps both lymphocyte types in parallel. (Figure 1)

DSE (Dermatan sulfate epimerase-1), is the neighbouring gene to *CALHM6* and encodes the enzyme dermatan sulfate epimerase-1, that converts chondroitin D-glucuronic acid to dermatan L-iduronic acid (IdoA) during the biosynthesis of dermatan

sulfate (DS). Together with chondroitin sulfate (CS), DS is a member of the large family of polysaccharides called glycosaminoglycans (GAGs), which are an essential part of the glycocalyx surrounding the endothelial cells of the glomerular filtration barrier [49]. Changes in the CS/DS content and modifications have been found in different animal models for renal disease [50]. A study in humans examining the expression of different DS domains in the glomerulus, found that in healthy kidneys, neither DS domain is expressed in the glomerulus, whereas in patients with glomerular disease (FSGS, MN and SLE) DS domains were highly expressed [51]. The variants detected by our group are eQTL for *DSE* possibly leading to an increased expression of *DSE* in SSNS patients. This could lead to an increased production of DS in the kidneys and successive alteration in the CS/DS content in the glomerulus. However, it remains to be elucidated what role DS plays in the pathophysiology of SSNS and how it possibly leads to structural changes in the podocyte's cytoskeleton. (Figure 1)

PARM1 (Prostate androgen-regulated mucin-like protein 1) encodes a protein that has mainly been linked and investigated in relation to prostate cancer [52]. Expression is the highest in androgen dependent cell lines, and there is currently no obvious association of *PARM1* with renal or autoimmune disease [52]. Furthermore, the association with *PARM1* was not replicated elsewhere.

NPHS1 and TNFSF15

In the latest study by Jia *et al.,* common variants in two loci outside the HLA region were associated with SSNS in Japanese children. The first locus was on chromosome 19 in the *NPHS1-KIRREL2* region and the second locus on chromosome 9 in the *TNFSF15* region. They went on to replicate these risk variants in *NPHS1* in a

Korean, South Asian, and in one of two African (South Asian Midwest Pediatric Nephrology Consortium, MWPNC) cohorts. In contrast, none of the risk variants in *NPHS1* were replicated in a European, Hispanic, Maghrebian or in the second African (NEPHROVIR) cohort. These results, revealing differences between European and Asian (Japanese) GWAS cohorts, would suggest that different genetic risk factors are operating in European compared with Japanese SSNS patients. This is further supported by the absence of significant association in the Japanese groups with the *CALHM6/DSE* and *PARM1* region. However, these findings have to be replicated in large cohorts and thus should be interpreted with caution.

NPHS1 encodes the protein Nephrin, a structural component of the slit diaphragm and essential part of the glomerular filtration barrier. Rare variants in *NPHS1*, are a well-established monogenic cause of congenital nephrotic syndrome (Finnish type), which is steroid resistant. The association of common variants in and around *NPHS1* with SSNS is therefore surprising. The group could not find evidence that the identified risk variants are eQTLs for *NPHS1* or *KIRREL2* by interrogation of public databases and this conclusion was supported by analysis of the expression of *NPHS1* in four patients compared to 183 controls, where no difference in the overall expression of *NPHS1* was found. However, they subsequently analysed the allele specific expression, where the expression of the risk haplotype harbouring the risk variants is compared to the expression of the reference haplotype without risk variants. The expected ratio would be 1:1 if there was no effect on gene expression of the risk allele but the risk haplotype was found to be less abundant than the reference haplotype. However, the numbers used in this expression analysis are very small and therefore must be replicated in larger groups [10]. (Figure 1)

The second peak outside the HLA region in the Japanese study was in the region of the gene *TNFSF15* (TNF super-family member 15). Variants in the region of this gene have been associated with several autoimmune and inflammatory disease. The lead variant (rs4979462) identified in the study by Jia *et al.* was previously investigated in relation to primary biliary cirrhosis, where the presence of the variant led to a higher endogenous T*NFSF15* expression and generation of a novel NF-1 binding site [53]. However, further studies have to be performed to investigate the relevance of these findings in SSNS patients.

Conclusion

Genetic studies in patients with steroid sensitive nephrotic syndrome have taught us firstly that the disease is rarely, if ever, inherited as a monogenic trait and instead has a complex genetic basis; secondly, the strongest genetic risk factors, in multiple different populations, are HLA alleles, implying that the adaptive immune system plays a key role in the pathophysiology of the disease. The association of different HLA alleles, and different non-HLA genes, with the disease in different ethnic groups suggests that the disease, as encountered in different parts of the world, may be more varied (in terms of its underlying mechanism) than is apparent clinically.

These findings highlight the large gaps in our understanding of SSNS. Future work should be focussed on the following strategies: Firstly, on replicating and extending genetic analyses in large cohorts of patients with SSNS from different ethnic backgrounds, to further define differences, and commonalities, in the genetic architecture across different populations.

Secondly, on further investigating the role of the identified variants on SSNS, for instance by looking at tissue specific expression of the relevant genes and their

interactions in order to understand how genetic variation at these loci can lead to glomerular disease.

Thirdly, to determine the genetic risk variants associated with the variable phenotypes of SSNS, such as age or pattern and number of disease relapses. These "genetic signatures" could then be used to optimize immunosuppressive treatments and greater predict disease prognosis for patients with SSNS.

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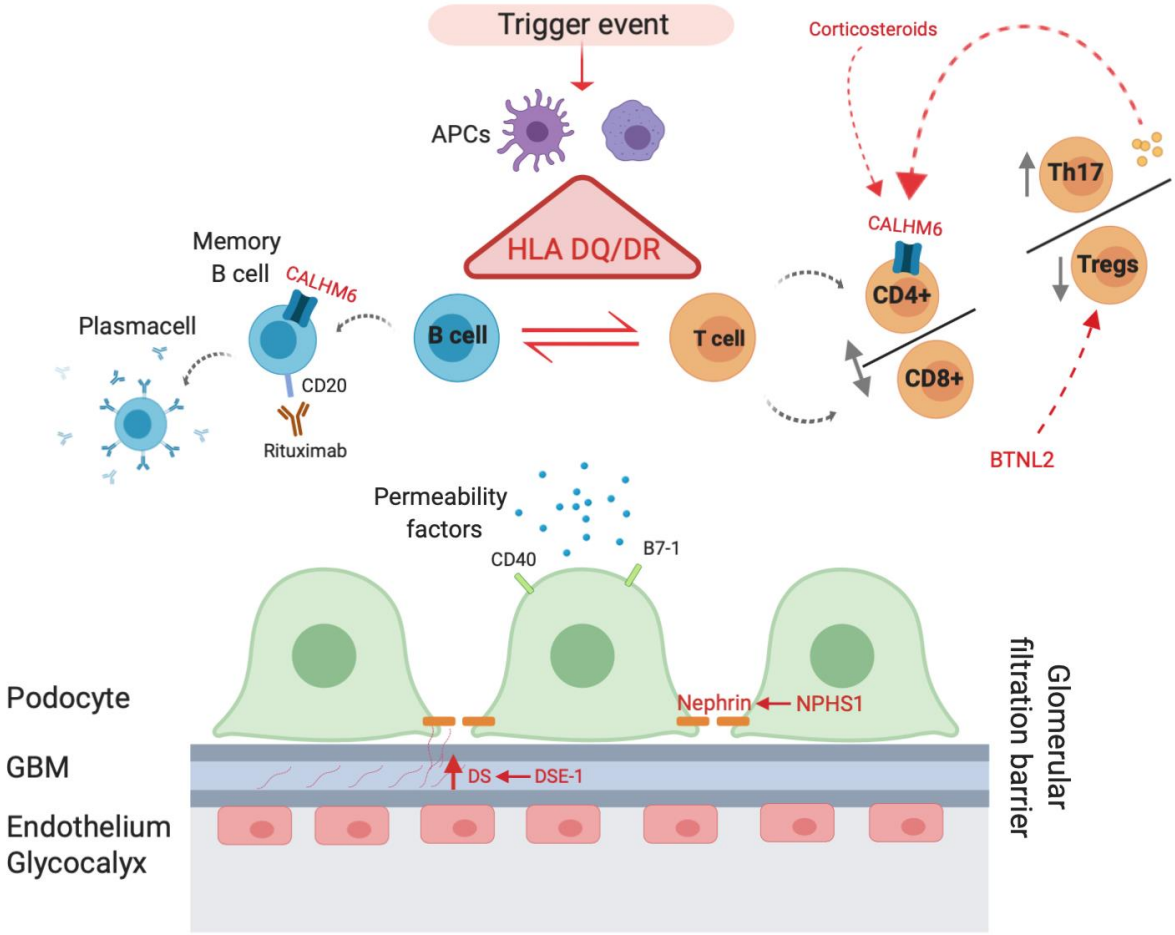
Figure 1 How candidate genes could influence the pathophysiology of SSNS



Legend Figure 1 Summary of proposed mechanisms leading to SSNS.
APCs: Antigen-presenting cells; GBM: glomerular basement membrane; *BTNL2: Butyrophilin-like 2; CALHM6: Calcium Homeostasis Modulator Family Member 6; DSE: Dermatan sulfate epimerase-1;* DS: dermatan sulfate;

A) *BTNL2* is assumed to be involved in immune regulation, serving as a negative T-cell regulator [38]. Further, it is assumed to promote expression of Foxp3, a transcription factor necessary for regulatory T cell (Treg) development and function [37]. Variants in this gene, could alter its immune regulatory function with increased activation of T cells and downregulation of regulatory T cells.
B) *CALHM6* is known to have an important immune regulatory function, possibly via mediating apoptosis of CD4+ lymphocytes. IFN-y is a strong inducer of *CALHM6* expression. IFN-γ is also produced by Th17 cells, and hence could be the mediator of an autoregulatory mechanism of CD4+/Th17/Tregs cells. Regulatory variants could downregulate the expression of *CALHM6*, which could lead to an alteration of this immune regulatory mechanism. In contrast, corticosteroids increase the expression of *CALHM6*, hence the treatment with corticosteroids could restore the immune regulatory function of *CALHM6*. This then leads to remission of nephrotic syndrome.
C) *CALHM6* is also expressed on memory B cells a subpopulation of B cells which also carry CD20. The recovery of memory B cells after rituximab has been linked to relapse of nephrotic syndrome [48]. Hence, altered immune regulatory mechanism because of downregulated *CALHM6* in memory B cells could be involved in the pathomechanisms of SSNS. Treatment with Rituximab addresses those B cells, which also show altered *CALHM6* expression, and hence the removal of these cells can induce remission.
D) Another pathway could be that regulatory variants upregulate the expression of *DSE*, which consequently leads to an increased production of DS in the glomerulus [8]. Increased expression of DS has been associated previously with FSGS [Lensen, 2015 #390]. However, how this could play a role in SSNS pathophysiology remains to be elucidated.
E) Common variants in *NPHS1* could affect the expression of *NPHS1*, encoding the protein Nephrin, a structural component of the slit diaphragm and essential part of the glomerular filtration barrier. However, how this could play a role in the episodic manifestation of SSNS has to be further investigated.

Table 1 Overview of risk loci identified for SSNS

Study population	Number of cases	Gene	References
Candidate gene approaches			
UK Caucasian	40	*HLA-DR7* *HLA-DQW2*	Clark *et al.* 1990 [54]
US Caucasian	32	*HLA-DQW2*	Lagueruela *et al.* 1990 [55]
French and German	161	*HLA-DR7* *HLA-DQB* *HLA-DQA*	Konrad *et al.* 1995 [56]
Japanese	30	*HLA-DQB1*	Kobayashi *et al.* 1995 [57]
Taiwanese	59	*HLA-DQB1* *HLA-DR*	Huang *et al.* 2009 [58]
South Asia	76	*HLA-DRB1* *HLA-DQB1*	Ramanathan *et al.* 2015 [59]
African American	65	*HLA-DQA1*	Adeyemo *et al.* 2018 [30]
[1] South Asia USA white	214 100	*HLA-DQA1*	Gbadegesin *et al.* 2015 [29]
Genome wide association studies			
[2] European, African, Maghrebian	385	*HLA-DQA1* *HLA-DQB1* *HLA-DRB1* *BTNL2*	Debiec *et al.* 2018 [7]
Japanese Replication cohort	224 216	*HLA-DRB1* *HLA-DQB1*	Jia *et al* 2018 [9]
European	422	*HLA-DQA1* *HLA-DQB1 HLA-DRB1* *CALHM6/DSE*	Dufek *et al.* 2019 [8]
[2]Japanese	987	*HLA-DR/DQ* *NPHS1/KIRREL2* *TNFSF15*	Jia *et al.* 2020 [10]

[1]Exome wide association study
[2]Transethnic meta-analysis

Table 2 Overview of HLA alleles significantly associated with SSNS

HLA allele	Dufek *et al.* [8]	Debiec *et al.* [7]	Adeyemo *et al.* [30]	Jia *et al.* [9]	Jia *et al.* [10]
	European	European	South Asian	Japanese	Japanese
Deleterious					
*HLA-DQA1*02:01*	X	X	X		
*HLA-DRB1*07:01*	X	X	X		
*HLA-DQB1*02*	X	X	X		
*HLA-DRB1*08:02*				X	X
*HLA-DQB1*03:02*				X	X
Protective					
*HLA-DQA1*01*	X		X		
*HLA-DQA1*01:03*	X				
*HLA-DRB1*13*	X				
*HLA-DRB1*13:02*				X	X
*HLA-DQB1*06:04*				X	X

Legend Classical HLA alleles identified in SSNS cohorts to be associated with the disease.