Trends in Pediatric Nephrotic Syndrome

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Abstract

Nephrotic syndrome (NS) is relatively common in children, with most of its histological types being minimal changed disease. Its etiology has long been attributed to lymphocyte (especially T-cell) dysfunction, while T-cell-mediated vascular hyperpermeability increases protein permeability in glomerular capillaries, leading to proteinuria and hypoproteinemia. Based on this etiology, steroids and immunosuppressive drugs that are effective against this disease have also been considered to correct T-cell dysfunction.

However, in recent years, this has been questioned. The primary cause of NS has been considered damage to glomerular epithelial cells and podocyte-related proteins. Therefore, we first describe the changes in expression of molecules involved in NS etiology, and then describe the mechanism by which abnormal expression of these molecules induces proteinuria. Finally, we consider the mechanism by which infection causes the recurrence of NS.

Core tips

There is no doubt that some vascular hyperpermeability factor is involved in the incidence of proteinuria in INS. However, no etiological molecule has been identified in INS as a factor for increasing the permeability of renal glomerular capillaries with

reproducibility and clinical consistency.

In addition, since the onset is sometimes observed in the family, there is high incidence of INS in East Asian children and there is the association of SSNS in childhood in Japan with the HLA-DR/DQ region, it is highly possible that some genetic factors are involved in the onset of NS.

In our opinion, INS is a multifactorial disease in which immunological stimuli, trigger the production of substances that impair podocytes, resulting in dysfunction of the slit membrane and cause proteinuria.

Introduction

Nephrotic syndrome (NS) is a chronic kidney disease that is relatively common in children, with an annual incidence of 2 to 7 per 100,000 in the pediatric population [1]. An epidemiological study of pediatric idiopathic NS (JP-SHINE study) was conducted in Japan, and found an incidence of 6.49 per 100,000, which is 3 to 4 times that reported for Caucasians [2]. The male-female ratio was 1.9, and 32.7% of patients had frequent recurrences during the 1- to 4-year observation period, which was similar to previous reports [2].

NS is classified into idiopathic (INS), secondary, and congenital depending on the cause and timing of proteinuria. INS accounts for 90% of NS in children. Furthermore, since more than 80% of INS in children is minimal change nephrotic syndrome (MCNS), more than 70% of NS in childhood is MCNS. This epidemiology differs strongly from that in adults [1].

Focal segmental glomerulosclerosis (FSGS) is the second most common disease in pediatric INS after MCNS. However, the difference between MCNS and FSGS has been debated for many years, with no conclusions being reached [3, 4]. It remains unclear whether they are distinct due to differing etiologies or stages/severity (early/mild for MCNS and advanced/severe for FSGS). The etiology of MCNS and FSGS has not yet been concluded.

Relationship between INS and T-cell function

Regarding INS etiology, the involvement of T-cell dysfunction proposed by Shalhoub in 1974 has long been supported [5]. In this study, steroid therapy showed a rapid and significant effect in INS patients, whose lymphocytes released vascular hyperpermeability factors into the culture supernatant. Additionally, INS patients were in remission when they suffered from measles, and malignant lymphoma patients often

had INS. Finally, the recurrence of INS patients was significantly higher during upper respiratory tract inflammation.

From these observations, it was concluded that lymphocytes (mainly T cells) in INS patients are dysfunctional and overproduce vascular hyperpermeability factors. These factors have been thought to increase vascular protein permeability in renal glomerular capillaries and lead to proteinuria [6, 7].

In fact, when the supernatant from immortalized T cells from NS patients is administered to rats, it effaces foot processes and causes proteinuria, but the normal control T-cell supernatant does not show such changes [8].

T cells include helper T cells (CD4 antigen-positive) that are presented with antigens from monocytes and macrophages and regulate immune responses, and killer T cells (CD8 antigen-positive) that damage virus-infected cells. Furthermore, helper T cells include Th1 and Th2 cells, which differ in cytokine secretion and effector functions. Th1 cells produce IL-2, IFN-γ and TNF-α, and Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. So, far, many groups have investigated the dynamics of blood cytokine levels in MCNS patients [9].

It has been reported that there is no significant difference between cytokine levels in remission in MCNS patients and controls, but IL-4 and IL-13 levels are elevated at the

onset of NS, that is, Th2-dominant fluctuations are observed. On the other hand, there have been some reports denying these fluctuations, and no consensus has been reached [10, 11].

Reasons for the different observations may be differences in patient backgrounds, lack of standardization of analysis methods (such as sampling and timing), and there are no suitable *in vitro* cultured cells or *in vivo* animal models. At present, there is no established evidence that Th1 or Th2 dominance causes NS. Yap *et al.* found the elevated mRNA expression of IL-13 in the T cells of NS patients [12]. After that, an increase IL-13 concentration in blood and T-cell were confirmed by other groups [13, 14].

IL-13 receptors are expressed in glomerular epithelial cells, and the addition of IL-13 to cultured glomerular epithelial cells reduces barrier function [15]. Furthermore, since strong expression of IL-13 in rats causes MCNS-like nephropathy [16], it is possible that an increase in IL-13 in MCNS patients has an effect on the pathology. However, there is a report that the blood concentration of IL-13 is not necessarily high in MCNS patients [17], and future examinations of cytokine concentration in the renal region are necessary.

It has been reported that the expression of a molecule called c-mip (c-maf inducing protein) is increased in MCNS T cells [18]. Subsequent analysis revealed that c-mip expression was increased not only in T cells but also in glomerular epithelial cells when NS recurred [19]. Mice in which c-mip is overexpressed in glomerular epithelial cells show proteinuria, with c-mip modifying the tyrosine kinase signal by the slit membrane. C-mip has been suggested as a mediator causing glomerular epithelial cell damage in MCNS [19].

There have also been reports of the effectiveness of TNFα inhibitors in nephrotic patients [20] and of NF-kB pathway activation in the blood cells of MCNS patients [21], but the number of cases was small, and then no further examinations have been reported.

The CD25- and CD4-positive regulatory T-cell population has an inhibitory effect on the immune response and specifically expresses the transcription factor Foxp3. The forkhead box P3 (*FOXP3*) gene is thought to be the master gene in regulatory T-cell development and function. Examination of recurrence of MCNS revealed that the number of suppressive T cells was the same as normal, but the regulatory T cells of ability to suppress T-cell proliferation was reduced at the time of MCNS recurrence [22].

In addition, immune dysregulation, polyendocrinopathy, enteropathy, X-linked

syndrome, multiple endocrine disorders and digestive diseases caused by mutations in the FOXP3 gene are complicated by NS. A relationship between MCNS and regulatory T cells has been strongly suggested, while epigenomic changes in the lymphocytes of MCNS patients are also being investigated [23]. Changes in histone methylation [24] and DNA methylation [25] in MCNS have been reported, but there is currently no data on whether these are related to changes in lymphocyte function leading to MCNS. Since steroids induce epigenetic changes, this field is expected to gain interest, specifically in understanding the mechanism of steroid sensitivity in MCNS.

Relationship between INS and B cell function

Although the function of B cells in MCNS is extremely poorly understood compared to that of T cells, rituximab (a human monoclonal antibody against the B cell antigen CD20) is clinically effective against frequently relapsing NS. That is, it became clear that depletion of B cells is a treatment for MCNS [26]. However, it is unclear whether this arises from an effect of rituximab on B cells or a change in T-cell function mediated by B cells.

On the other hand, rituximab binds to acid sphingomyelinase-like phosphodiesterase 3b (SMPDL-3b), a protein expressed in glomerular epithelial cells. Serum from NS patients reduces SMPDL-3b expression levels in cultured glomerular epithelial cells,

induces cytoskeletal changes, and reduces the filtration barrier function, whereas rituximab increases SMPDL-3b expression level and suppresses the changes obtained with NS patient serum [27].

This suggests that rituximab may exert a proteinuria-suppressing effect directly on glomerular epithelial cells without the intervention of immune cells. However, the extent of involvement of this mechanism in the clinical effects of rituximab is unknown at this time.

Other factors

Hemopexin

Hemopexin is a blood factor potentially associated with MCNS. It is an enzyme involved in heme metabolism, and its administration to rats induces reversible proteinuria [28]. Hemopexin activity is increased in MCNS patients [29], and since hemopexin acts on the cytoskeleton of glomerular epithelial cells via nephrin *in vitro* [30], it may be involved in MCNS. However, this report included a small number of cases, and it is unclear whether its observations can be generalized.

Angptl4

In 2011, Chugh *et al.* found an increase in Angiopoetin-like 4 (Angptl 4) levels in the blood of MCNS patients [31]. Angptl4 expression is also enhanced in epithelial cells in the glomeruli of MCNS patients, and proteinuria occurs when Angptl4 is strongly

expressed specifically in glomerular epithelial cells in mice [31]. Therefore, it was suggested that an increase in Angptl4 leads to MCNS, but this possibility has now been refuted. Subsequent analysis revealed that mice expressing Angptl4 in the liver did not exhibit proteinuria, and that Angptl4 in the blood acted on glomerular endothelial cells and had a proteinuria-lowering effect [32]. Interestingly, Angptl4 levels are elevated by lowering blood albumin, but Angptl4 suppresses lipoprotein lipase (LPL) activity, which suppresses the conversion of triglycerides to free fatty acids and causes hyperlipidemia [32]. Therefore, Angptl4 may play a role in NS hyperlipidemia.

CD80

CD80 (B7-1) is a membrane protein that is expressed on activated B cells and antigen-presenting cells. It binds to CD28 on CD4 + T cells in response to T-cell receptor activation and promotes T-cell proliferation. Thus, interaction co-stimulation signaling between CD80 and CD28 mediates the interaction between T cells and B cells or antigen-presenting cells and regulates the adaptive immune response. On the other hand, cytotoxic lymphocytes-associated antigen-4 (CTLA-4), which is a negative co-stimulatory receptor, also binds to CD80 as a ligand, but its affinity is ten times higher than that of CD28 and CD80, and therefore strongly inhibits the binding of CD28 and CD80.

Animal experiments have shown that when glomerular epithelial cells are stimulated and injured, they express CD80 [33]. Urinary CD80 levels increase during recurrence of MCNS, which is not seen in FSGS patients or those in remission, suggesting that changes in CD80 expression may be specific to MCNS. [34]. The addition of serum from MCNS patients to cultured podocytes has been shown to increase CD80 expression *in vitro* [35], suggesting that there is a close relationship between MCNS and CD80 expression. It is ~~supposed~~ believed that these are not only involved in the onset and recurrence of MCNS, but are also ~~can be expected as~~ potential biomarkers for differentiating MCNS from FSGS.

A two-hit hypothesis has been proposed, whereby the induction of CD80 expression by a serum stimulus is the first hit, and the subsequent decrease in CTLA4 expression that suppresses the CD80 signal is the second *hit* [36].

Abatacept is a chimera of CTLA4 and IgG that binds to CD80 and suppresses the CD80-CD28 signal, attenuating the immune response. Therefore, several groups have recently investigated whether suppressing CD80 on glomerular epithelial cells by abatacept leads to an attenuation of proteinuria. Yu *et al.* reported the administration of abatacept to 5 FSGS patients (4 rituximab-resistant and 1 steroid-resistant NS) and the improvement of nephrotic-level proteinuria in all of them [37].

On the other hand, Garin *et al.* reported that abatacept had a temporary inhibitory effect on proteinuria in MCNS patients, whereas there was no change in proteinuria in FSGS patients despite a decrease in urinary CD80 antigen [38]. Another group has reported that abatacept has a poor effect on proteinuria in FSGS patients [39]. Future cases need to be collected to analyze the involvement of CD80 and abatacept on NS.

Genetic factors

More than 50 genes mutated in hereditary podocytopathies have been identified (Table 1). The causative gene of congenital and steroid-resistant NS is being elucidated. Depending on the gene mutated, NS can be roughly classified into three types for convenience: congenital NS developing symptoms early in life (*NPHS1, NPHS2, NPHS3, CD2AP, MYO1E, PTPRO* etc.), NS with an adult onset in the form of autosomal dominant inheritance (*TRPC6, ACTN4, INF2* etc.), and NS with symptoms in other organs (*WT1, LAMB2, LMX1B, MYH9* etc.). Many of these genes encode proteins that are strongly expressed in glomerular epithelial cells, so these genetic diseases are considered podocyte diseases. In Western studies, two-thirds of infant NS cases developing within the first year of life are explained by four gene mutations (NPHS1, 24%; NPHS2, 38%; LAMB2, 5%; and WT1, 3%). It has also been reported that in steroid-resistant congenital NS that develops under 2 years of age, mutations in 24 of

the currently known genes are found in nearly 90% of cases [40]. The analysis of more than 2000 cases of steroid-resistant NS (SRNS) found that 30% of cases were explained by 27 known genes [41].

It is ~~key~~ important to understand to what extent genetic background is involved in the onset of steroid-sensitive NS (SSNS) and MCNS. Familial onset of SSNS is rare, in fact, it was reported that the onset of SSNS in the sibs is 3% [42]. Certainly, the frequency of known genetic abnormalities in SSNS is extremely lower than that in SRNS. For example, the analysis of 38 SSNS patients did not find any genetic abnormalities [43]. Minor nephrin abnormalities have been reported in siblings with proteinuria [44]. In addition, a mutation in *LMX1B*, the causative gene of Nail-Patella syndrome, has been found in patients with proteinuria without extrarenal symptoms [45]. Furthermore, a gene mutation in *EMP2* was found by analysis of familial SSNS that developed in early childhood [46]. *EMP2* is expressed in glomerular epithelial and endothelial cells, regulates the expression of the membrane protein caveolin, and its mutation is thought to cause morphological changes to epithelial cells. Additionally, mutations of the kidney ankyrin repeat-containing proteins (KANKs) 1, 2 and 4 known as the cause of SRNS have also been found in SSNS ~~/ MCNS~~ patients [47].

Ashraf *et al.* focused on a family with SSNS and performed a whole exome analysis of its members. A novel causative gene, called *ITSN2*, was identified in this family. By combining this result with those from the genomic analysis of NS families with a blood relative, six novel causative genes were identified. The 17 families with mutations in this gene had an NS which was partially sensitive to steroid treatment. Interestingly, all identified genes were involved in the same pathway (Rho signaling) and were found to interact with each other. This pathway also includes genes involved in SRNS, which is indicative of a common mechanism in SSNS and SRNS. In addition, this study suggested that steroids also act on this signaling pathway [48].

These facts suggest that gene mutations affect glomerular epithelial cell function.

Large-scale studies have begun on not only causative genes whose mutations determine the onset of disease, but also polymorphisms in susceptibility genes that increase the risk of onset. In the case of diseases affected by multiple susceptibility genes, the magnitude of the risk of developing the disease is expressed by the “odds ratio.” Specifically, it is expressed as a numerical value indicating how many times the risk of developing the disease is higher in a person who has a susceptibility gene than that of a person who does not have the susceptibility gene.

Genome-wide association studies (GWAS) are comprehensive analyses of the single nucleotide polymorphisms (SNPs) an individual has in their genome. A GWAS was performed in less than 200 cases of acquired NS in Japan, and an SNP in the intron of *GPC5*, which encodes Glypican-5, was found to correlate with NS onset. Glypican-5 is expressed in glomerular epithelial cells and its specific knockdown in these cells turns mice resistant to the development of experimental proteinuria. It is believed that the expression levels of this gene define susceptibility to glomerular epithelial cell damage [49].

In a GWAS of about 200 childhood-onset SSNS cases, the proportion of *HLA-DQA1* polymorphisms on chromosome 6 was significantly increased in SSNS (odds ratio 2.1) [50]. Xiaoyuan *et al.* performed a GWAS using an SNP array optimized for Japanese patients, including 224 pediatric SSNS patient and 419 healthy subject control specimens. As a result, SNPs showed a significant genome-wide association in the *HLA-DR, DQ* region of the short arm of chromosome 6. This result was also confirmed in another cohort consisting of 213 pediatric SSNS patients and 710 healthy controls [51].

A GWAS using an SNP array optimized for Japanese patients was performed on 987 pediatric SSNS patients and 3,206 healthy controls. As a result, in addition to the

HLA-DR, DQ region, variants (polymorphisms) showing a significant genome-wide association with the *NPHS1-KIRREL2* region of chromosome 19 19q13.12 were identified. Furthermore, the relationship between multiple *NPHS1* variants and glomerular *NPHS1* mRNA expression was investigated. The expression of *NPHS1* mRNA from chromosomes having haplotypes with these risk variants was reduced. It has been clarified that *NPHS1* is involved in expression regulation [52].

Although polymorphisms in the multiple susceptibility genes do not cause the disease, they can have a significant impact on the risk of developing NS. These macroscopic genome analyses, which are expected to gain popularity in the future, are effective not only for clarifying the dynamics of susceptibility genes but also for establishing the genetic differences found in populations such as specific ethnic groups and races.

Mechanism of glomerular epithelial cell damage in NS

As mentioned above, various genetic abnormalities can cause NS. It has also been suggested that changes in circulatory factors and local tissues may be involved in the onset of non-genetic NS. Despite these various causes, changes in glomerular epithelial cells are common throughout NS. In particular, fusion of the foot process is observed in most cases, and basement membrane detachment, vacuolar degeneration, and inclusion body formation are strongly associated with barrier rupture.

Glomerular epithelial cells receive chemical or mechanical stimuli from the glomerular blood vessels and Bowman's cavity to transmit intracellular signals [53]. These signals control the development, morphogenesis, and maintenance of morphology of glomerular epithelial cells, and are closely related to proteinuria [54].

Slit membrane complexes such as Nephrin, Neph1, and Podocin play a major role in controlling the cytoskeletal structure of glomerular epithelial cells, and various adapter proteins are used in the intracellular region of slit membrane proteins, due to stimulation-dependent phosphorylation [55, 56]. The slit membrane functions as a conversion point for receiving extracellular signals such as humoral factors [19, 57]. This signaling system is extremely important for executing reversible morphological changes in epithelial cells and as the point of action of NS drugs.

Significance of viral infection in the onset and recurrence of INS in children

There are many reports on the immunological background of INS patients and abnormalities in renal glomeruli. In recent years, there have been an increasing number of research papers on relationship between upper respiratory tract infection (URI) and the onset and recurrence of INS.

In children, it has been known for over 30 years that the onset and recurrence of INS are observed in URI. Specifically, about 70% of INS recurrences are triggered by URI [58].

Despite interesting findings reported in recent years, the molecular mechanism that links URI to the onset and recurrence of INS has not been elucidated.

Involvement of Toll-like receptors in INS pathology

Innate immunity plays an important role in the initial recognition of pathogens (e.g., bacteria, viruses, and parasites), phagocytosis or digestion, and the subsequent induction of an inflammatory response and the induction of acquired immunity. Macrophages, neutrophils, and phagocytes such as dendritic cells play a central role in this process. These cells express pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) and transmit activation signals through PRRs. The Toll-like receptor (TLR) family of PRRs, consist of 13 types reported in humans, each of which recognizes different PAMPs such as proteins lipids, and nucleic acids of bacteria, viruses, and parasites. TLRs have specific signaling pathways depending on the adapter molecule which lead to the induction of differential gene expression patterns. The main signal transduction pathways are the MyD88-dependent and TRIF-dependent pathways. The former is involved in the induction of the inflammatory response through nuclear factor-κB (NF-κB) activation, and the latter activates the interferon regulatory factor (IRF, a transcription factor) which finally induces type I interferon and is involved in the antiviral response.

There are some reports that the expression of TLR-3 and TLR-4 in peripheral blood mononuclear cells is enhanced at the time of INS onset or recurrence [59, 60]. Mishra *et al.* compared the mRNA expression levels of TLR-3, TLR-4, and CD80 using peripheral blood mononuclear cells (PBMC) of 40 SSNS cases (25 of whom were initial or recurrent and 15 were in remission; histological type was mainly MCNS), 30 cases of SRNS (tissue type was mainly FSGS) and 23 control children. The mRNA expression levels of these molecules were increased in patients with initial and recurrent SSNS. On the other hand, patients with SRNS displayed a decreased expression compared to those of normal controls [60].

TLR-3 is localized in the cell and recognizes viral double-stranded RNA (dsRNA), while TLR-4 is present on the cell surface and recognizes sugars, lipids, and proteins derived from the virus [61]. Therefore, the fact that the expression of these TLRs is enhanced is consistent with the fact that many INS recurrences are triggered by URI.

Involvement of alveolar surfactant protein in recurrence of INS

When MCNS patients relapse with URI, their levels of pulmonary surfactant proteins SP-A (surfactant protein A) and SP-D (surfactant protein D) in the serum increase. As a result of activating signal-regulatory protein-α (SIRPα), structural changes (such as

disappearance of podocyte foot protrusions) occur, resulting in the appearance of proteinuria [62].

This inference is based on the elevation of SP-A and SP-D levels in the serum collected at the time of recurrence of MCNS patients. SIRPα is stimulated by adding the MCNS patient's serum at the time of recurrence to cultured podocytes, and protein phosphatase non-receptor type 1(PTPN1) is released, which dephosphorylates nephrin, activates podocyte NF-κB, promotes CD80 and pro-inflammatory cytokine production, and causes structural podocyte changes. SIRPα is a transmembrane protein that contains a tyrosine phosphorylation site in the cytoplasmic region and is expressed in dendritic cells, macrophages, nerve cells, and microglia. SIRPα is also expressed in podocytes, and it was clarified that it is involved in the regulation of podocyte structure and function as one of the major tyrosine phosphorylated proteins in renal glomeruli [63-65].

In addition, SP-A and SP-D, which are mainly produced by alveolar type II epithelial and Clara cells, are known as useful biomarkers of interstitial pneumonia, but they are also SIRPα agonists [66]. Therefore, a hypothesis that SP-A and SP-D serum levels increase during URI causing abnormalities SIRPα in podocytes and leads to recurrence of INS can be formulated.

Certain viruses that are prone to the onset and recurrence of INS in children

Approximately 85% of microorganisms that cause URI, the so-called cold syndrome, are viruses. The main causative viruses are rhinovirus and coronavirus, followed by RS virus, parainfluenza virus, and adenovirus. It is well known that pediatric INS patients are prone to recurrence when suffering from cold syndrome. There were various studies examining the link between recurrence and the causative virus such as RS virus, influenza virus A and B, parainfluenza virus., varicella herpes zoster virus, and adenovirus, but it was unclear whether a specific pathogen was involved in recurrence. In 2017, two facilities reported that infection with a specific virus was involved in recurrence. Ching *et al.* proposed the hypothesis that rhinovirus (HRV) infection leads to increased expression of CD80 in the renal podocytes of patients and causes recurrence [67]. Ching *et al.* examined 32 MCNS patients who relapsed during URI due to HRV, using PBMC and renal biopsy tissue, and compared the patients with CD80-positive T cells of PBMC to control children ~~of~~ with PBMC. The ratios of CD80-positive T cells to CTLA-4 positive T cells and the ratios of Th17 to Treg increased at the time of recurrence in MCNS when compared to those in control children, but they normalized during the remission period. Furthermore, in an immunostaining study using renal tissue of MCNS patients who underwent renal biopsy

at the time of recurrence, CD80 was strongly expressed renal glomeruli, but CTLA-4 was weakly expressed. It is speculated that HRV infection increases the CD80 CTLA-4 ratio of PBMC in MCNS patients, resulting in an increase in the Th17 Treg ratio. As a result, the expression of CD80 in podocytes is enhanced and structural podocyte changes occur, leading to recurrence [67].

The Epstein-Barr (EB) virus is a double-stranded DNA herpesvirus found in cultured cells of Burkitt lymphoma that frequently occurs in children in equatorial Africa. It is also called human herpesvirus type 4 (HHV4). A characteristic of herpesviruses, including EB virus, is that they cause latent infections centered on B lymphocytes [68]. Dossier *et al.* have proposed the etiologic significance of the EB virus in INS because of findings of infection and reactivation of the EB virus in pediatric patients with initial INS [69, 70]. According to them, about half of children with INS have amplification of EB virus DNA. This amplification occurs in a locus with a previously reported monobasic polymorphism in children with SSNS (6p21.32), associated with the ability to produce Epstein-Barr virus nuclear antigen 1 (EBNA1). Additionally, depletion of B cells with rituximab relieves INS, but the cells that are persistently infected with EB virus are B cells. These facts were cited as the basis for the EB virus etiology [70].

On the other hand, it is a well-known fact that pediatric INS resolved due to viral infections, such as influenza and measles [71, 72].

It has been reported that CD25, CD4, Foxp3, and regulatory T cells (Tregs) levels increase in the blood during measles, and that changes in the T-cell-producing cytokine balance during measles are involved in NS remission [73]. An increase in the number of Tregs was observed in response to intercurrent influenza B virus infection and prednisolone administration, along with a parallel decrease in the amount of proteinuria [74]. Moreover, both influenza virus infection and glucocorticoid administration, which is the key treatment for INS, increase the number of Tregs [75, 76]. Therefore, it may be hypothesized that Tregs play an important role in INS pathogenesis in patients with INS complicated by influenza B and measles infections.

New insights in the drugs of MCNS

1) ~~New insights in~~ Glucocorticoid (GC) ~~of MCNS~~

Approximately 80% of pediatric MCNS patients are in remission with GC, but ~~there are many unclear points about~~ how GC improves MCNS remains unclear. GC may act directly on podocyte receptors to suppress the appearance of proteinuria. In fact, dexamethasone has a significant effect on the structure and function of human

podocytes [77], and has been shown to suppress the intracellular signaling of podocyte NFκB [78].

2) ~~New insights in~~ Cyclosporine (CsA) ~~of MCNS~~

~~The mechanism of CsA in MCNS was thought to be~~ The suppression of intracellular signal transduction of activated T cells was thought to be a possible mechanism of CsA in MCNS. CsA ~~is also thought to~~ acts on the calcineurin-dependent dephosphorylation of synaptopodin in podocytes to stabilize the actin cytoskeleton and reduce proteinuria [79].

3) ~~New insights in~~ Rituximab ~~(RTX) of MCNS~~

RTX, a monoclonal antibody that acts against the B cell surface antigen CD20, is also highly effective in MCNS. ~~But~~, However, its mechanism of action is not well known.

~~how RTX is effective against MCNS.~~

It ~~has been considered~~ speculated that the depletion of B cells may reduce self-reactive T cells through cell-cell interactions [80]. Fornoni et al indicated that RTX not only recognizes CD20 on the surface of B cells, but also binds to and protects podocyte SMPDL-3b ~~to~~ preventing the destruction of the actin cytoskeleton and suppressing proteinuria [27].

Why don't we still understand the cause of MCNS?

Among the genetic abnormalities identified for congenital NS and SRNS, many have been found to be explained by glomerular epithelial cell abnormalities, however, many aspects of MCNS pathogenesis remain unknown. There are various possible reasons for this.

(1) Factors other than the currently analyzed blood factors.

(2) Involvement of not one but multiple factors (Genetic, immunological or circulatory factors etc.)

(3) Caused by a combination of such factors (eg, glomerular epithelial cell factor + immunological factor, T cell factor + B cell factor,1st hit + 2nd hit, etc.)

Considering these problems, carrying out comprehensive analysis, such as analysis of genome, epigenome, proteome, and transcriptome using a large cohort will be essential for future studies. Additionally, clarifying the genetic background of patients with a familial history may provide an opportunity to approach the more common cause of idiopathic INS.

Conclusions

There is no doubt that some vascular hyperpermeability factor is involved in the incidence of proteinuria in INS. However, no etiological molecule has been identified in

INS as a factor for increasing the permeability of renal glomerular capillaries with reproducibility and clinical consistency.

In addition, since the onset is sometimes observed in the family, there is high incidence of INS in East Asian children [2] and there is the association of SSNS in childhood in Japan with the *HLA-DR DQ* region [51], it is highly possible that some genetic factors are involved in the onset of NS.

In our opinion, INS is a multifactorial disease in which immunological stimuli, trigger the production of substances that impair podocytes, resulting in the dysfunction of the slit membrane and causing proteinuria.

Conflict of Interest Statement

The author declare that this manuscript has no conflict of interest.

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1 Table 1. Genetic forms of podocytopathies.

Gene	Inheritance	OMIM ID	Pathology	Function	Features
NPHS1	AR	602716	FSGS/MCD	slit membrane	Congenital. Finish type.
NPHS2	AR	604766	FSGS/MCD	slit membrane	Develop ESRD in the first or second decades
CD2AP	AR	607832	FSGS	slit membrane	Severe early-onset SRNS
CRB2	AR	609720	FSGS	slit membrane	Child onset SRNS
FAT1	AR	600976	FSGS	slit membrane	First or second decade onset SRNS Tubular ectasia, haematuria and facultative neurological involvement
TRPC6	AD	603652	FSGS	slit membrane	Both child and adult onset SRNS
MYO1E	AR	601479	FSGS	Actin binding	Child onset SRNS
PLCE1	AR	608414	FSGS/MCD	Actin binding	Infantile to child onset SRNS
INF2	AD	613237	FSGS	Actin binding	complicated by Charcot-Marie-Tooth dis- ease
ACTN4	AD	604638	FSGS	Actin binding	Adult onset SRNS
MYH9	AD	160775	FSGS/MCD	Actin binding	complicated by Epstein syndrome
ANLN	AD	616027	FSGS	Actin binding	Both child and adult onset SRNS
KANK1	AR	607704	MCD	Actin regulation	
KANK2	AR	614610	MCD	Actin regulation	Early-onset SSNS
KANK4	AR	614612	FSGS	Actin regulation	Early-onset SRNS
ARHGDIA	AR	601925	FSGS/DMS	Actin regulation	Onset age is younger

					than 3 years
ITSN1	AR	602442	FSGS/MCD	Actin regulation	SSNS
ITSN2	AR	604464	FSGS	Actin regulation	SSNS
MAGI2	AR	606382	MCD	Actin regulation	SSNS
TNS2	AR	607717	FSGS/MCD	Actin regulation	SSNS
DLC1	AR	604258	FSGS	Actin regulation	SSNS
ARHGAP24	AD	610586	FSGS	Actin regulation	
LAMB2	AR	609049	DMS/FSGS	Integrin and laminin	Pierson syndrome
ITGA3	AR	605025	FSGS	Integrin and laminin	Infantile onset SRNS Congenital interstitial lung disease and mild epidermolysis bullosa.
ITGB4	AR	147557	FSGS	Integrin and laminin	Congenital or infantile onset SRNS Epidermolysis bullosa and pyloric atresia
WT1	AD	256370	DMS/FSGS	Nucleus	Denys-Drash syndrome Frasier syndrome Wilms tumor
LMX1B	AD	161200	FSGS/MCD	Nucleus	Nail-patella syndrome
SMARCAL1	AR	606622	FSGS	Nucleus	Schimke immunoosseous dysplasia
NUP93	AR	614351	FSGS	Nucleoporins	Child onset SRNS
NUP107	AR	607617	FSGS	Nucleoporins	Child onset SRNS
NUP205	AR	614352	FSGS	Nucleoporins	Early onset SRNS

XPO5	AR	607845	FSGS	Nucleoporins	Speech development delay
COQ2	AR	609825	FSGS/CG	CoQ10 biosynthesis	Early-onset NS
COQ6	AR	624647	FSGS	CoQ10 biosynthesis	Early-onset NS. Hearing loss
PDSS2	AR	610564	FSGS	CoQ10 biosynthesis	Leigh syndrome
MTTL1	AR	590050	FSGS	CoQ10 biosynthesis	
SGPL1	AR	603729	FSGS	S1P metabolism	Hyperpigmentation, increased ACTH, hypoglycemia, and hypocalcemia with seizures, ichthyosis, primary hypothyroidism and developmental delay
SCARB2	AR	602257	FSGS	Lysosome	Progressive myoclonic epilepsy

May, 15, 2021

Dear Dr. Li Zuo,, Editor-In-Chief, *World Journal of Nephrology*

We thank referees for careful reading our manuscript and for giving useful comments. We have revised the manuscript We have revised the manuscript NO 66103, entitled " Nephrotic Syndrome in children." on the basis of Referee's comments.

We have made major revisions according to the reviewer's pointed out and it is in English proofreading. Revisions in the text are shown using red highlight for additions and changes, and strikethrough font for deletions.

We look forward to a publication of our manuscript in *World Journal of Nephrology*.

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

1. Present title does not reflect the manuscript hypothesis completely. this should be the precised one. Abstracted the main subject very well. Key words: does not reflected the main/ focused subject of manuscript. Background was described well but significance and present status was not described properly. still to focus on the main hypothesis.

Response: It was fixed in accordance with you pointed out (title, key words).

2. Methods described in details. Results/ observation made by reviewing the subject will definitely helps in progress the research in the field. The manuscript interpret the findings/ reviews adequately and appropriately, focusing the key points clearly. the subject is discussed significance in relation to clinical practice. Tables illustrative of the paper contents appropriately. The manuscript well, concisely and coherently organized, but few of the grammatical mistakes are there.

Response: The manuscript has been carefully reviewed by an experienced editor whose first language is English.

3. The limitations of the study is not described. The future directions and the questions/issues that remain to be solved are not described. The publication impact the clinical practice in relation to therapeutic aspects of minimal change nephrotic syndrome. Research methods and reporting of the manuscript not completely followed the PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review.

Response: It was fixed in accordance with you pointed out (P24 L18–P25 L14)

4 LANGUAGE QUALITY

Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

Response: The manuscript has been carefully reviewed by an experienced editor whose first language is English.

5 EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) *Science editor:*

1 Scientific quality: The manuscript describes a review of the nephrotic syndrome in children. The topic is within the scope of the WJN. (1) Classification: Grade C; (2) Summary of the Peer-Review Report: The manuscript well, concisely and coherently organized. However, the limitation of the study is not described. The questions raised by the reviewer should be answered; and

(3) Format: There is 1 table.

(4) References: A total of 80 references are cited, including 1 reference published in the last 3 years;

(5) Self-cited references: There are no self-cited references; and

(6) References recommend: The authors have the right to refuse to cite improper references recommended by peer reviewer(s), especially the references published by the peer reviewer(s) themselves. If the authors found the peer reviewer(s) request the authors to cite improper references published by themselves, please send the peer reviewer's ID number to the editorialoffice@wjgnet.com. The Editorial Office will close and remove the peer reviewer from the F6Publishing system immediately. 2 Language evaluation: Classification: Grade B. A language editing certificate issued by enago was provided. 3 Academic norms and rules: No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. No financial support was obtained for the study. The topic has not previously been published in the WJN. 5 Issues raised:

(1) The "Author Contributions" section is missing. Please provide the author contributions;

Response: It was fixed in accordance with you pointed out (P1 L6)

(2) PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references.

Response: It was fixed in accordance with you pointed out (references)

Please revise throughout; and (3) The column should be minireviews. 6 Recommendation: Conditional acceptance.

(2) *Editorial office director:*

(3) *Company editor-in-chief:*

I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Nephrology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

Sincerely yours,

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