Mechanisms of Glucocorticoid Resistance in Idiopathic Nephrotic Syndrome

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Nephrotic syndrome, characterized by massive proteinuria, hypoalbuminemia and edema, is one of the most common kidney diseases in children. Although glucocorticoids (GCs), the mainstay of therapy for over 50 years, are effective in most children, more than 20% develop GC resistant nephrotic syndrome (SRNS), among whom focal segmental glomerular sclerosis (FSGS) is a frequent pathological outcome and the cause of end-stage disease with a prevalence of 4% in the USA. Despite its clinical importance, the molecular basis of SRNS is unknown. In recent years, researchers have not only gained a new understanding of the roles of structural and functional abnormalities in GC receptors (GRs) in GC resistance, but have also gradually discovered close relationships between GC resistance in idiopathic nephrotic syndrome and podocyte-related molecules, like slit diaphragm (SD) molecules and so on. Here we mainly discussed these molecules and their physiological as well as pathological effects, including nephrin, podocin, CD2-associated protein (CD2AP), α-actinin-4, transient receptor potential cation channel 6 (TRPC6), phospholipase C epsilon-1 (PLCe1), Wilms' tumor suppressor gene 1 (WT1), Lmx1b, LAMB2, myosin 1e (MOY1E) and inverted formin 2 (IFN2). Mitochondrial cytopathies are also involved in GC resistance and well-reviewed [1, 2], which will not be discussed in detail in this review. To those SRNS without any genetic defects, immunological disturbances are always involved and should be stressed. In this article, recent progress in research on the mechanisms of GC resistance in idiopathic nephrotic syndrome is reviewed.

Role of glucocorticoid receptors’ structure and function

GRs are expressed in glomerular cells, such as podocytes [3], and translocate GCs to the nucleus [4]. The gene encoding the GR resides on chromosome 5q31-32 and includes
10 exons [5], among which exons 2–9 encode protein; exon 1, the 5'-untranslated region, plays an important role in cell type specific GR gene expression [5, 6]. It is well known that different splicing, modification and transcription of genes generate various polypeptides. Following promoters A, B and C, exon 1 of the GR gene has three isoforms (A, B and C), and different pre-RNA splicing of exon 1A generates three different transcripts (1A1, 1A2 and 1A3). All five of these exon 1 isoforms connect to the same area of exon 2. Similarly, alternatively spliced pieces of other exons produce different isoforms, such as GRα, GRβ, GRγ, GRA and GRP, of which the latter two are believed to be associated with the GC resistance phenotype. In addition, eight translation initiation sites on the GRα mRNA result in eight GRα isoforms (GRαA, B, C1–3 and D1–3). These GR isoforms have diverse cellular signal transduction potential [7, 8].

GRα is the only known receptor that binds GCs and has a positive transactivation potential [9]. When ligand-free, GRα forms a multi-protein complex with, for example, hsp90, hsp70, immunophilins, FKBP, Cyp40 and p23 in the cytoplasm [10, 11]. The configuration of these complexes helps the receptor maintain a high affinity for GCs. On GC binding, GRα detaches from its chaperones and translocates to the nucleus, where GR homodimers bind to a cis-acting sequence including GC response elements (GREs) and negative GREs in the promoter region of target genes, leading to the activation or repression of transcription, respectively [8]. Furthermore, GRα modulates the signal pathway through mutual interaction with coactivator and corepressor transcription factors [7, 8]. Coactivator molecules, such as CREB-binding protein and GR coactivator-2, activate the transcription of anti-inflammatory proteins. Corepressors, such as activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB), undergo cross-talk with GR homodimers, which can attenuate the proinflammatory response mediated by these proteins.

GRβ has the same first 727 amino acids as GRα, while GRα has an additional 50 amino acids [12]; thus, GRβ lacks the special GC-binding pocket possessed by GRα and fails to bind GCs [13, 14]. It is known that the predominant role of GRβ is to exert a negative effect on GRα-induced transcriptional activity. Research [7, 15, 16] has indicated that overexpression of GRβ leads to imbalance of the GRα/GRβ ratio and may be an important cause of GC resistance in some patients with nephrotic syndrome. The mechanism of this can be summarized as follows. 1) GRβ competes for GRE-binding with GRα [17]. 2) GRβ forms a heterodimer with GRα. Its unique carboxyl-terminal 15 amino acids form two critical residues, L733 and N734 [18], that stabilize the dimer, which represses the transcriptional activity of GRα. 3) Recent reports [19] have shown that GRβ has its own transcriptional activity independent of GRα. Through its AF-1 domain, GRβ can lodge into the transcriptional complex formed by GRα and other cofactors and then repress transcription [19, 20]. 4) It is hypothesized that GRβ directly modulates target gene transcription through "GRβ-specific response elements" [19].

The signal transduction mediated by GRα can also be interrupted by GR modification by phosphorylation, nitrosylation or ubiquitination [7, 8]. Phosphorylation of GRs can be achieved by several kinases, including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) [21] and P38 mitogen-activated protein kinase (MAPK) [22], which are activated by microbial superantigens, inflammatory cytokines or macrophage migration inhibitory factor. Inflammatory cytokines also generate increased nitric oxide, leading to the nitrosylation of GRs [23]. JNK can directly activate the transcription factor AP-1, preventing GRs from binding to GRE or NF-κB [21], as discussed in detail below.

GCs are conventionally considered to have immunosuppressive and anti-inflammatory effects. Actually, recent evidence [24] has shown that GCs may have a direct effect on podocytes in glomerular diseases through upregulating the expression of GRs. GCs are translocated to the nucleus by GRs, then modify gene expression to promote cell maturation and survival, stabilize the actin cytoskeleton and exert a positive effect on the key proteins nephrin, CD2AP, TRPC6, VEGF and IL-6. Not only do GCs prevent podocyte apoptosis [25], they also promote cell recovery from injury [26]. Additionally, Zhang et al discovered that GCs increase regeneration by augmenting the number of podocyte progenitors [27]. To FSGS, which is frequently resistant to GCs, studies have shown that circulating permeability
factor is the main culprit that induces podocyte foot effacement and proteinuria. Serum soluble urokinase receptor (suPAR) is one of the critical permeability factor, contributing to actin cytoskeleton reorganization and SRNS [28, 29]. Then calcineruin inhibitors, like cyclosporine A, emerge to be against proteinuria of FSGS patients and help to taper down the dose of GCs. It exerts the anti-proteinuric effect not only by immune-suppression, but also by inhibiting of uPAR signaling pathway and blocking dephosphorylation of synaptopodin, then, consequently, protecting podocyte integrity [29, 30]. Therefore, it would be interesting to further study the mechanism of GCs sensitivity and resistance in nephrotic syndrome, aiming at developing more effective therapies.

Role of podocyte-related molecules

In podocytes and podocytic processes, podocyte-related molecules, like SD molecules, are important components of the glomerular filtration barrier. Numerous SD molecules expressed by podocytes have recently been discovered and have been shown to be associated with GC resistance in idiopathic nephrotic syndrome, including NPHS1, NPHS2, ACTN4, CD2AP, TRPC6, PLCε1, MOY1E and IFN2. Molecules related with transcriptional activity in podocytes, such as WT1 and LMX1B are also critical to podocyte integrity. Additionally, LAMB1, secreted by podocytes, contributes to the structure of GBM, should also be stressed.

In SRNS, the genes encoding these molecules undergo various mutations, including homozygous and heterozygous missense, nonsense, frameshift, insertion, deletion and splice-site mutations, which result in structural and functional anomalies in the encoded proteins, and thereby to podocyte injury. Researchers have detected immature renal glomeruli in some children with SRNS, which might be a consequence of the mutation of cytoskeleton specific proteins. Thus, when mutation related with podocytes occurs, GCs may be unable to exert direct effects on disrupted podocytes, leading to GC resistance.

NPHS1 mutations

NPHS1 is located on human chromosome 19q13.1 and includes 29 exons. It encodes the podocyte-expressed protein nephrin, a member of the immunoglobulin superfamily and a single-pass transmembrane protein comprising eight extracellular C2-type Ig-like domains, a fibronectin type III-like motif, a single transmembrane domain and a cytosolic C-terminal tail [31]. In vitro experiments suggest that nephrin is highly flexible and changes its conformation easily [32]. Nephrin constitutes the structural basis of the SD with other proteins [33, 34]. It also plays an important role in signaling between podocytes [35].

Mutations of NPHS1 lead to congenital nephrotic syndrome (CNS) of the Finnish type in an autosomal recessive manner. Children with CNS have massive proteinuria and severe edema, are usually GC resistant and die within 2 years after birth [36]. Recent studies have confirmed that mutations of NPHS1 also occur in people of non-Finnish origin and in all three types of SRNS (CNS and childhood- and adult-onset SRNS) [37, 38]. The most prevalent mutations of CNS in the Finnish population include Finmajor (nt-121delCT, L41fsX91) and Finminor (c.3325C>T, R1109X), which were the first such mutations to be discovered [31]. To date, over 176 different mutations have been described [39]. Although the classical “Finnish type” mutation leads to GC resistance and progresses rapidly to end stage kidney disease, in several cases other NPHS1 mutations have been reported to be sensitive to GCs and the condition has not been so severe. In a worldwide cohort study of 42 non-Finnish cases of CNS [40], two patients exhibited a milder phenotype; one of them had a compound heterozygous truncated mutation in exon 10 and a missense mutation in exon 24. He achieved partial remission with GC treatment and maintained stable renal function. Kitamura [41] reported two cases of compound heterozygous missense mutations (C256R and V822M) in which remission of proteinuria was achieved without immunosuppressive treatment. Recently,
two siblings with a homozygous missense mutation (c.1760T>G, p. L587R) showed partial remission with GC treatment [36]. Researchers [40] have speculated that the severity of the disease phenotype may be due to different gene mutations. Fin major and Fin minor lead to the absence of nephrin from the SD, causing severe SRNS. About 75% of missense mutations lead to misfolding of nephrin, which becomes trapped in the endoplasmic reticulum and fails to express at the plasma membrane [42]. A point mutation may disturb the location of ligand binding to the extracellular nephrin domains. R160X, a nonsense mutation of exon 27, results in a milder CNS phenotype [43]. However, it remains difficult to clarify the correlations between genotype and phenotype, which is also influenced by, for example, gender and ethnicity. More work is required.

**NPHS2 mutations**

* NPHS2 maps to chromosome lq25-q31 and includes eight exons encoding an integral membrane protein named podocin. Podocin is a newly identified member of the stomatin family of lipid raft-associated proteins and comprises 383 amino acids with a single ‘hairpin-like’ transmembrane domain. Its cytosolic C- and N-terminal domains are located in the cytoplasm. Podocin forms a homo-oligomeric complex that localizes to lipid rafts in the plasma membrane in the foot process of podocytes [44, 45]. These lipid rafts contain many signal transduction molecules and recruit nephrin and CD2AP with podocin [46, 47]. When the gene encoding podocin mutates, the distribution of nephrin and other key proteins in podocytes changes as a consequence. *NPHS2* mutations were initially reported in 2000, in children with autosomal recessive familial CNS. About 10–28% of all non-familial childhood cases of SRNS are caused by podocin mutations. Almost all patients with two recessive mutations of *NPHS2* are resistant to GC treatment. As mentioned above, different genotypes may influence the severity of disease. Kitamura et al. [48] reported a patient who was a compound heterozygote for the *NPHS2* mutations R168C and P271L. An expression study showed that the R168C mutant tended to become trapped within the ER, whereas the P271L mutant reached the plasma membrane and retained partial function in anchoring the SD; as a consequence, the disease had a milder clinical course. In 2006, Franceschini et al. [49] identified four relatively common non-synonymous gene variants: R229Q, G34E, A61V and A242V. R229Q was once believed to be harmless because it occurs in 2–4% of healthy individuals [49]. However, further studies have shown that R229Q decreases podocin binding to nephrin [50]. Although it may have only a weak biological effect, it is now known that compound heterozygosity for R229Q and p.A284V maybe characteristic of late childhood- or adult-onset SRNS [50-52]. Santin et al. [53] also found that the phenotypes of late childhood- and adult-onset SRNS are more similar to each other than to early childhood-onset SRNS. Researchers have speculated that specific podocin mutations might determine the age of onset of SRNS and that R229Q might be an ancient mutation that has expanded by population migration. Experts [54-56] recommend that adults with FSGS, especially in European countries, should be screened for R229Q first; those who are carriers should be tested for a second mutation. Unnecessary GC treatment should never be administered to compound heterozygous patients.

**ACTN4 mutations**

* ACTN4 locates on chromosome 19q13, *ACTN4* encodes α-actinin-4, the only member of the actinin family expressed in human podocytes [57]. Alpha-actinin-4 is an actin filament crosslinked protein with an actin-binding domain in the N terminus, four spectrin-like repeats in its center and two EF-hand motifs in the C terminus [58]. Alpha-actinin-4 is localized in contractile microfilaments within podocyte foot processes and contributes to the
unique morphological features of the podocyte foot process by regulating molecule adhesion and cytoskeletal dynamics [59, 60]. Mutations in ACTN4 are related to familial FSGS in an autosomal dominant manner [57, 61]. There are two main etiological mechanisms associated with the disorders caused by the mutants: "gain of function" and "loss of function" [62]. Gain-of-function mutations promote actin binding and abnormal aggregation of α-actinin-4 leading to podocyte damage. Such mutations often occur in the actin-binding domain (e.g., K225E, K256E). K256E is the mouse mutant corresponding to human K255E [60]. The K256E mutation increases the affinity of α-actinin-4 for F-actin, which reduces the ability of α-actinin-4 to interact with other binding proteins, disrupting the normal cytoskeleton [63]. Furthermore, the mutant may induce proteotoxicity in podocytes, impair the proteins' function by misfolding them and eventually cause apoptosis [60]. Loss-of-function mutation was not fully recognized as a pathogenic factor in human nephropathy until 2011 [62]. Before this, animal experiments [64, 65] had shown that ACTN4-absent mice exhibited abundant proteinuria with sclerosis of glomerular capillaries and podocyte foot process effacement, but whether humans were affected in the same way was unknown at that time. In 2011, Liu et al. [62] demonstrated that mutations in the non-actin-binding region (e.g., R310Q, Q348R) result in deficiencies of α-actinin-4 and its binding protein CLP36. This disruption of the α-actinin-4–CLP36 complex hinders RhoA signaling and the generation of traction force in podocytes. Clinically, ACTN4 mutations usually cause late childhood- and adult-onset nephrotic syndrome [57]. Choi et al. [66] reported a heterozygous mutation (p.S262F) in 3- and 4-year-old siblings with FSGS. However, because the siblings also had an NPHS1 substitution, which mutation caused the disease could not be determined.

**CD2AP mutations**

The 80 kDa CD2AP encoded by chromosome 6p12 belongs to the immunoglobulin superfamily and serves as an intracellular ligand of T cell and natural killer cell CD2 receptors. It is widely expressed in almost all tissues except the brain and maintains stable connections between T cells and antigen-presenting cells. CD2AP has three Src homology 3 (SH3) domains at the NH₂ terminus followed by a proline-rich region containing SH3-binding sites; its coiled-coil domain is located at the COOH terminus. The SH3 domains anchor CD2 by identifying the proline-rich sequence in the intracellular C terminus of CD2. The COOH terminus mediates the protein–protein binding and participates in cytoskeleton remodeling [67]. Expressed in podocytes, CD2AP interacts with nephrin and podocin, maintaining the integrity of podocytes and SDS. These three proteins are interdependent on each other to maintain their structure and location [46]. CD2AP also participates in cell signaling pathways through stimulating the p85 regulatory subunit of phosphoinositide 3-OH kinase-dependent activation of the serine-threonine kinase AKT [68]. TGF-β induces these anti-apoptotic pathways via CD2AP adaptor protein. In the absence of CD2AP, the proapoptotic p38 MAPK pathway mediated by TGF-β is over-activated and accelerates podocyte apoptosis [69, 70]. Fully knocked-out mice (CD2AP⁻⁻) exhibited extensive foot process effacement and died of a disease resembling human nephrotic syndrome at approximately 6 weeks after birth [34]. Heterozygous CD2AP+/⁻ mice had abnormal glomeruli similar to the pathological changes seen in human FSGS [71]. Researchers [72] have also reported that CD2AP can bind dendrin, a transcriptional factor. Loss of CD2AP leads to the release of dendrin and increased expression of TGF-β1, which drives translocation of dendrin from the SD to the nucleus. Dendrin promotes expression of cytosolic CatL, which can reorganize the actin cytoskeleton to make podocytes sensitive to proapoptotic signals. Thus, CD2AP, deletion of which may mediate a proteolytic process, plays an important role in cell survival. Kim et al. [73] reported a heterogeneous mutation of exon 7 in two patients with primary FSGS; the mutation replaced cytosine with thymidine, leading to a lack of splicing at that site and loss of 80% of CD2AP protein. A homozygous mutation of CD2AP leading to a premature stop codon has also been described, resulting in
a slightly truncated protein. This mutation downregulated the expression of CD2AP and was discovered in a case of early-onset nephrotic syndrome [74]. Gigante et al. [75] screened for mutant CD2AP genes in 80 Italian sporadic FSGS patients and 200 healthy controls. Three mutations (c.904A>T, c.1120A>G and c.1573delAGA) were discovered in three unrelated patients who exhibited proteinuria and a high degree of GC resistance.

**TRPC6 mutations**

TRPC channels are a superfamily comprising seven different channels divided into four subfamilies (TRPC1, TRPC3,6,7, TRPC4,5 and TRPC2) [76]. Localized on chromosome 11q21-22, TRPC6 encodes an important Ca\(^{2+}\) channel in podocytes [77]. This 100 kDa protein has both its N and C termini located intracellularly and contains six transmembrane domains, the fifth and sixth of which form tetramers [76]. Most TRPCs are nonselective cation channels, but TRPC6 is a selective ion channel [78]. Independent of intracellular calcium concentration and membrane depolarization, TRPC6 can be activated by PLCε1 after the stimulation of a G(q)-protein-coupled or tyrosine kinase receptor; this is followed by the release of intracellular calcium from the endoplasmic reticulum [76]. The entrance of Ca\(^{2+}\) promotes the assembly and reorganization of actin, responsible for cell migration and endothelial permeability [79, 80]. TRPC6 is expressed throughout the kidney and specifically in the major and minor processes of podocytes. It also interacts with nephrin, podocin and CD2AP [81, 82]. Overexpression of TRPC6 results in certain glomerular diseases via podocyte dysfunction caused by abnormal Ca\(^{2+}\) reflux [83]. Recently, Yu et al. [78] showed that dexamethasone stabilizes the expression of TRPC6 by binding its receptor, which protects podocytes from injury and plays a role in preventing proteinuria. However, the mutant TRPC6 seems to reduce the effect of GCs. Winn et al. [77] showed that a missense mutation (P112Q) of TRPC6 is related to hereditary FSGS. Gigante M et al. [84] analyzed TRPC6 in 33 Italian children with sporadic early-onset SRNS. Three heterozygous missense mutations (c.374A>G, c.653A>T and c.2684G>T) were recognized. In addition, Santin et al. [85] identified three missense substitutions in non-familial cases. Mir et al. [86] detected a L395A missense variant in a sporadic FSGS patient. However, the precise genotype-phenotype relationship remains unknown. One hypothesis is that calcium overload activates calcium-dependent phosphatase calcineurin [83, 87]. FK506 and CsA, which are required by GC-resistant patients, exert their effect by inhibiting calcineurin [88, 89]. Therefore, mutations in TRPC6 might be correlated with GC resistance and the inhibition of calcineurin.

**PLCε1 mutations**

The PLCε1 gene is on chromosome 10q23 and encodes PLCε1, a member of the phospholipase enzyme family [90, 91]. PLC enzymes are divided into four classes: PLCβ, PLCγ, PLCδ and PLCε [92]. PLC works as a catalyst that promotes the hydrolysis of polyphosphoinositides to generate the second messengers, inositol-1,4,5 triphosphate (IP3) and diacylglycerol (DAG). IP3 releases Ca\(^{2+}\) from the endoplasmic reticulum while DAG is responsible for protein kinase C stimulation, both of which initiate cell growth and differentiation [91]. PLCε1 is highly expressed in podocytes and contains not only the conserved PLC part, but also a domain of guanine nucleotide exchange factor for Ras-like small GTPases and two C-terminal Ras-binding domains, through which it can be regulated by H-Ras. It can also interact with human BARF (γ-raf murine sarcoma viral oncogene homolog B1) and GTPase-activating protein 1, which is located in the basal part of developing podocytes [90, 91, 93]. Scientists [94] have found that PLCε1 mutation is a novel cause of DMS, which is characterized by the onset of nephrotic syndrome and rapid progression to end stage renal failure. Some patients presenting with nephrotic syndrome during the first
year of life exhibit GC resistance [95]. Recently, a large cohort study of patients with isolated DMS showed that 28.6% of families with DMS have loss-of-function mutations in \( PLCE1 \). Eight of the ten identified families are consanguineous [96]. Rasheed et al. [97] reported eight different mutants with a late onset of FSGS, two of which were novel variants (R561Q and K2173R).

\textbf{Myo1e mutations}

\( Myo1e \) locates in chromosome 15q21-q22, encoding a member of the nonmuscle class I myosins that belong to a subgroup of the unconventional myosin protein family. \( Myo1e \) is an actin-based molecular motor that contributes to junctional integrity in kidney podocytes. \( Myo1e \) is composed by an N-terminal motor domain, a neck domain and a tail domain. The N-terminal domain is responsible for actin binding and ATPase activity. The tail domain contains three tail homology (TH) regions that bind acidic phospholipids and prolin-rich proteins[98]. \( Myo1e \)-null mice exhibit glomerular filtration defects and, consequently, extensive proteinuria, of which the mechanism may involve changes of podocyte adhesion and cytoskeletal organization rather than loss of other podocyte-related molecules [99, 100]. Bi et al demonstrated that \( myo1e \) localizes in cell-cell junctions and its TH3 domain interacts with a component of SD and tight junctions, ZO-1 [98], contributing to the structure and function of SD. Simone et al. utilized homozygosity mapping and exome sequencing to identify an A159P substitution in \( MYO1E \) in three siblings with steroid-unresponsive FSGS. A159P mutation leads to molecular mislocation to the cytoplasm, impairing ligand binding and actin interaction [101]. Almost the same time, Mele et al. reported two mutations (A159P and Y695X) in \( MYO1E \) resulted in autosomal recessive GC resistant FSGS. Y695X mutation truncated the protein, lacking the domain for regulation of actin binding and ATP hydrolysis, which mimics the animal model of \( Myo1e \) deficiency. Cyclosporine A was partially effective in spite of high relapse rate [102]. To date, \( MYO1E \) mutations were homozygous and only reported in familial FSGS with some degree of consanguinity. So \( MYO1E \) mutations should be screened out in patients with familial FSGS.

\textbf{INF2 mutations}

Locating on chromosome 14q32.33, \( INF2 \) encodes a member of the forming family of proteins. Formins remodel actin cytoskeleton and govern dynamic events like cell morphogenesis and cytokinesis [103]. \( INF2 \) regulates lamellipodial actin dynamics and SD trafficking by inhibiting actin polymerization mediated by Rho/diaphanous-related formins and interacting with lipid raft components [104]. Diaphanous-related formins contain the forming homology domains FH1/FH2 and the diaphanous autoregulatory domain (DAD) in C terminal whereas the diaphanous inhibitory domain (DID) is located in the N-terminal. FH1 and FH2 are responsible for acceleration of filament elongation and actin assembly, respectively, of which the latter can be inhibited by DID and DAD interaction. Rho GTPases can relieve the inhibition. However, DID-DAD interaction does not influence \( INF2 \) polymerization. In contrast, \( INF2 \) depolymerization is inhibited by the competition for actin monomer binding of DID-DAD [105]. Mutations in \( INF2 \) have been reported to be a major cause of autosomal dominant FSGS while sporadic cases are rarely seen. Barua et al concluded \( INF2 \) mutations reported in the literature [106]. Additionally, \( INF2 \) mutations cause many cases of Charcot-Marie-Tooth syndrome (CMT) associated with FSGS. \( INF2 \) strongly expressed in podocytes and Schwann cell cytoplasm. All mutations detected are located in the DID domain and mutations reported in the cases of FSGS with CMT are concentrated in the inner face of the central core of DID. The mutations severely disrupt DID function, affecting its depolymerization effect and the interaction of \( INF2 \) with myelin-specific proteins [107, 108],
which might result in abnormal actin accumulation and INF2 dislocation [105, 109]. Further studies about the mechanisms about renal and neural defects induced by INF2 mutations are expected.

WT1 mutations

Wilms’ tumor was first described by Max Wilms in 1899. It is known as a highly malignant nephroblastoma [110]. Investigators first identified constitutional deletions of the 11p13 chromosome in children with Wilms’ tumor in 1978 [111]. In 1990, Haber et al. [112] isolated the Wilms’ tumor suppressor gene WT1 by deletion analysis. WT1 has been recognized as a tumor-suppressor gene, but the wild type is often overexpressed in various cancers. The WT1 gene has 10 exons encoding a protein with four zinc fingers and a proline- and glutamine-rich transactivation domain, the structure of which is similar to that of transcription factors. The first exon encodes the transactivation domain alone, while exons 7–10 encode the four zinc fingers responsible for DNA and RNA binding [113]. During nephrogenesis, the WT1 protein plays an important role in the induction of the mesenchymal–epithelial transition and the formation of nephrons [114]. After kidney maturation, WT1 is expressed within podocytes [115]. The WT1 gene is spliced at the 17AA and KTS sites, yielding four basic isoforms (17AA(+))KTS(+), 17AA(+)KTS(-), 17AA(-)KTS(+) and 17AA(-)KTS(-)), each of which has different functions [116]. The 17AA(-)KTS(-)WT1 isoform inhibits G1/S progression during the cell cycle, suggesting a tumor-suppressor role, while Ito et al. [116] have shown that the 17AA(+))WT1 isoforms (both KTS(+) and KTS(-)) disturb apoptosis through the intrinsic apoptosis pathway. Thus, imbalanced expression of different WT1 isoforms results in abnormal podocyte hyperplasia and abnormal differentiation, inducing renal inflammation or tumor [117]. WT1 can also modify the cytoskeleton of podocytes through downregulating target genes encoding key podocyte proteins [118, 119].

There are three main nephropathies associated with WT1 mutation. The first is Denys–Drash syndrome (DDS), characterized by SRNS with diffuse messangial sclerosis (DMS), XY pseudohermaphrodism and Wilms’ tumor [115]. Over 80 mutations have been reported in patients with DDS, most of which are missense in exon 8 or 9 encoding zinc fingers 2 and 3 [120]. The most common mutation is R394W (1180C>T, exon 9). It has been shown that, in the podocytes of DDS patients, WT1 mutants result in overexpression of Pax-2, which should be repressed after early nephrogenesis [110]. Second, mutations in the donor splice site at intron 9 lead to Frasier syndrome (FS), which is described as a combination of complete XY gonadal dysgenesis, FSGS and gonadoblastoma in 46, XY patients and as nephropathy alone in 46, XX patients [121]. The third nephropathy is isolated SRNS. Carrying the same mutation as that in DDS patients, some affected individuals exhibit only the isolated clinical manifestations of SRNS. This phenotype is usually found in females or males with genitourinary malformations [120, 122].

LMX1B mutations

Encoding by Lmx1b, which is located on chromosome 9q34, LMX1B belongs to the LIM-homeodomain family of more than nine transcriptional factors. It contains two cysteine-rich N-terminal zinc-binding LIM domains, one homeodomain and a C-terminal glutamine-rich domain. The LIM domains interact with other transcription factors or modifiers, whereas the homeodomain is responsible for the binding of promoters [123]. LMX1B is one of transcription factors necessary for normal podocyte function and development [1]. Mutations of Lmx1b cause nail–patella syndrome (NPS), which is an autosomal dominant disease causing nail dysplasia, patellar abnormalities, elbow dysplasia, iliac horns, nephropathy and glaucoma [124, 125]. The first mutation of Lmx1b was discovered in 1998 [124]. Following
this finding, absence or inactivation of the entire homeodomain was held responsible for the related pathologies, which did not agree with the fact that only a few peptides are disturbed by this mutation [103, 104].

Almost a decade ago, Rhor et al. [126] demonstrated that LMX1B bound to the promoter region of NPHS2 encoding podocin, and the absence of LMX1B, resulted in severely impaired GBM and podocytes. Harendza et al. [127] further indicated that podocin is specifically regulated by LMX1B. Additionally, Bongers et al. [128] found that patients with mutations in the homeodomain exhibited more severe proteinuria than those with mutations in the LIM domains. Kevin and colleagues [123] believe that two distinct mechanisms underlie the kidney problems seen in NPS. In those who lose filtration function with age but without progressive proteinuria, the possible mechanism is progressive thickening of the GBM in the absence of podocyte dysfunction. However, some patients suffer massive proteinuria and develop end stage renal failure. These authors speculate that, besides the heterozygous Lmx1b mutation, another mutation or polymorphism of a key gene in podocyte function regulated by Lmx1b may exist, such as NPHS2, CD2AP or ACTN4. The combination of mutations leads to podocyte dysfunction.

**LAMB2 mutations**

The human LAMB2 gene lies in chromosome band 3p21 and comprises 32 densely packed exons encoding a protein of 1798 amino acids – the β subunit of laminin [129]. Laminin is a family of heterotrimeric macromolecules comprising at least 15 different combinations of α, β and γ chains [130]. For example, the major laminin trimer in the mature glomerular basement membrane (GBM), which is secreted by podocytes and endothelial cells, comprises α5, β2 and γ1 chains, and is thus called LM-521 [131]. During glomerulogenesis, a transition occurs from LM-111 and LM-511 in the immature to LM-521 in the mature GBM [132, 133]. Most laminins have one “long arm” formed by α, β and γ chains through coiled-coil interactions and three “short arms” with NH2-terminal globular domains that mediate polymerization extracellularly [134, 135]. Laminin is responsible for the initiation of GBM formation [135]. Specific mutations of LAMB2, which encodes the β chain of LM-521, are associated with Pierson syndrome (microcoria–congenital nephrosis syndrome), which is characterized by CNS with diffuse mesangial sclerosis as well as neurodevelopmental deficits and ocular malformations [94]. Although these symptoms were first described in 1963 [136], this syndrome was not recognized as a distinct entity until 2004 [94]. Recently, Matejas et al. [129] reviewed all 49 of the mutations of LAMB2 discovered from 2004 to 2010, of which eight missense mutations and two frame deletions were identified as causative mutations. These mutations disrupt the highly conserved residues of the β2 chain either homozygously or heterozygously with another bona fide mutation on the second allele. The mutations create premature stop codons (e.g., c.5258dupA) and lead to failure of the truncated C terminus to assemble a trimeric complex. Missense and frame deletions (e.g., p.R246W) cluster in the LN domain of laminin β2 crucial for laminin polymerization and the structure of the GBM. R246W, a missense mutation of a highly conserved arginine in the NH₂-terminal LN domain, disturbs LM-521 synthesis, secretion and stability, causing severe but slightly delayed nephrosis [94]. Intriguingly, in contrast to R246W, R246Q seems to be a milder mutant in which the same arginine is exchanged for glutamine. Patients carrying R246Q tend to exhibit impaired secretion of laminin-521 with increased compensatory accumulation of ectopic laminin chains, which worsens the proteinuria. However, increased expression of the mutant protein can overcome this secretion defect and even improve GBM permselectivity [137]. Whether overexpression of the R246Q mutant can ameliorate GC resistance is unknown.
Clinically, the genetic screenings targeted these genes mentioned above are ongoing [138-140]. In the first year of life, two thirds of nephrotic syndrome can be explained by single-gene mutations in four genes: NPHS1, NPHS2, LAMB2 and WT1 [141]. Later in life other gene mutations are responsible for steroid-resistant FSGS. WT1, LAMB2 and LMX1B mutations should be suspected when coming across SRNS patients with other syndromes or multiorgan defects. Patients with autosomal dominant FSGS should have genetic tests of TRPC6, INFG2, MOY1E and ACTN4. Joshi et al. had a detailed review of genetic testing for SRNS[142]. To nephrologists, genetic screening not only helps to choose the best therapeutical strategies for the patients, but also renders opportunity to discover novel mutations and potential mechanisms of SRNS.

Role of the immune system

Patients of SRNS without any genetic defects are kind of population that might have promising outcomes with alterations of therapies other than GCs. So it is critical to shed light on immune system of SRNS patients since it is universally accepted that GC resistance is closely related to circulating factors, including lymphocytes, cytokines and transcription factors. Normally, GRs inhibit inflammation via interference with the genes encoding proinflammatory factors, especially AP-1 and NF-κB [143]. Belonging to the basic region-leucine zipper (bZIP) family, AP-1 is a collective term for dimeric transcription factors comprising a Jun family member (c-Jun, v-Jun, Jun-B or Jun-D) homodimerized with another Jun protein or heterodimerized with a Fos protein (c-Fos, Fos-B, Fra-1 or Fra-2). The combination can also unite other bZIP family members, such as the activating transcription factor and Maf families. The composition of the subunits directly determines the transcriptional activation of AP-1, which is responsible for the overexpression of several proinflammatory cytokines and the tissue-destructive enzyme collagenase [144]. Proinflammatory cytokines usually activate AP-1 by phosphorylating c-Jun and Fos after the phosphorylation of JNK or ERK, respectively, caused by the MAPK cascade of reactions. GCs upregulate MAPK phosphatase-1 mRNA, which blocks the activation of MAPK pathways, including p38, JNK and ERK signal transduction [109, 110]. Furthermore, GRα represses AP-1 activity by direct protein–protein interference with c-Jun and c-Fos, inhibiting its binding to DNA. AP-1 can also inhibit GCs, because its components competitively bind to GRs and suppress their action [144]. Tsitoura et al. [145] found that CD28- or IL-2-mediated costimulation abrogated this suppressive effect of GCs on c-Fos expression and AP-1 function, which indicates that different quantities and the quality of stimulation might lead to different GC responses, varying from suppression to total resistance. Intriguingly, it is known that AP-1 regulates basal chromatin structure and enhances GR binding to the gene [146]. Thus, scientists believe that the interaction of GCs and AP-1 is not as straightforward as it appears [147].

NF-κB belongs to the Rel family and comprises two subunits: p50 and p65 [148]. It remains inactive as it binds to endogenous inhibitor IκB family proteins. After antigenic stimulation, IκB kinase α and β phosphorylate IκB and release NF-κB, which subsequently translocates to the nucleus to bind target genes [149]. It is known that patients with SRNS tend to have lower levels of the NF-κB p65 subunit than do total or partial GC responders [150]. Expression of p50 remains the same in SSNS and SRNS patients. An in vitro study showed that only cells transfected with p65/p50 heterodimers or p60 homodimer could respond to GCs, whereas cells with only p50 homodimer were resistant to GCs [151]. Therefore, the interference of GCs with NF-κB relies on the transactivation domain of p65, absence of which can result in GC resistance [152]. Aviles et al. [152] concluded that there are at least three mechanisms for this phenomenon: 1) lack of the necessary protein–protein interactions, mainly through via the p65 subunit and GRα; 2) impairment of cellular nuclear export function and 3) decreased affinity of NF-κB for the GC-induced leucine zipper, which normally binds to the p65 subunit to inhibit NF-κB nuclear translocation.
T helper subtype 1 (Th1) cells, which promote opsonization and the delayed-type hypersensitivity response, generate IL-2, IFN-γ and tumor necrosis factor-beta. T helper subtype 2 (Th2) cells produce IL-4, 5, 6, 10 and 13, promoting eosinophil differentiation and humoral responses [153]. It has been noted that an imbalance between Th1 and Th2 cells may induce SRNS [154, 155], though this view is controversial. Several studies have demonstrated that patients who are unresponsive to GCs or prone to relapse tend to exhibit immunologic switching from Th2 to Th1 cells [154]. However, in the study of Carlotti et al. [156], the levels of Th1- and Th2-related cytokines were similar in GC sensitive and GC resistant groups; differences may be the result of different lymphocyte stimuli in various studies. Studies have also revealed that Th2 cells play a predominant role in minimal-change nephrotic syndrome, membranous glomerulonephritis and IgA nephropathy [157, 158]. Meanwhile, an association between nephrotic syndrome and allergy arises in numerous studies, among which there is a hypothesis concerning the active role of IgE in the pathogenesis of nephrotic syndrome [159]. Synthesis of IgE is closely related to IL-4, 5 and 13, and serum levels of IgE are comparatively higher in the relapse phase of nephrotic syndrome than in the remission phase. Patients with high IgE have shorter remission times and are more susceptible to relapse. However, there are also reports of nephrotic children with normal serum IgE levels [160], so whether high IgE indicates a GC unresponsive phenotype remains unclear.

Conclusion

The mechanism of GC resistance in nephrotic syndrome remains unclear, but may possibly be the result of concurrent immunologic disorder and gene defects in either the GR or key molecules. There is room for further studies of the exact relationship between phenotype and genotype in GC resistance. Recent progress in research is paving the way for improved understanding of the mechanism of GC resistance in nephrotic syndrome.

Conflict of interest statements

All the authors declared no competing interests.

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References


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