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## The importance of genetic study in steroid-resistant nephrotic syndrome

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### ABSTRACT

Steroid-resistant nephrotic syndrome (SRNS) is a challenging clinical task. It has heterogeneous etiology and extremely variable clinical outcomes and generally progresses to end-stage renal disease (ESRD). Different gene mutations in podocyte's slit diaphragm, mitochondria, and cytoskeleton proteins, as well as glomerular basement membrane (GBM) have been associated with SRNS. These proteins regulate the function of the glomerular filtration barrier. Advances in genetic approaches and podocytology have led to discover the SRNS-causing genes that lead to a better understanding of the drug resistance. More than 45 genetic mutations have been recognized in the hereditary form of SRNS. This review offers an update on the current knowledge of steroid resistance-causing gene mutations in podocytes. Understanding the specific genes involved in SRNS would guarantee an optimum therapeutic benefit of steroid treatment.

#### Implication for health policy/practice/research/medical education:

The clinic needs novel therapeutic for patients with inherited glomerular disease that up to now have been suffering from lack of any specific and effective treatment. This review offers an update on the current knowledge of steroid resistance-causing gene mutations in podocytes.

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### Introduction

Steroid-resistant nephrotic syndrome (SRNS) is a challenging clinical task since it has heterogeneous etiology and extremely variable clinical outcomes (1). The prevalence of SRNS is 15% in children and 40% in adults (2). Renal histology in most of SRNS cases displays the presence of focal segmental glomerulosclerosis (FSGS), proliferative glomerulonephritis, and minimal change disease (MCD) (2,3). In children and adults, administration of high dose steroids, calcineurin inhibitors (CNI), cyclophosphamide (CP), rituximab, and mycophenolate mofetil have been exerted variable success rates (4). Even though in some cases persistent or temporary remission

can be obtained by immunosuppressive agents, some others display a multidrug-resistant phenotype (5). Most of the SRNS patients generally progress to end-stage renal disease (ESRD) and post-transplant recurrence (6,7).

The exact molecular pathophysiology of SRNS needs to be understood, however, genetic abnormalities or immune system dysfunction have been reported (8). A third of SRNS patients have one mutation in their podocyte genes, while the disease in others is triggered by an unclear circulating factor (3). Recently, abnormalities in podocyte genes have been recognized in congenital NS and SRNS cases (5,9).

Over the past 20 years, progresses in genomic tools such

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as next-generation sequencing (NGS) and chromosomal microarray (CMA) have discovered that more than 50 genes are connected with SRNS (3, 10). Most of these genes encode proteins that are located in the podocyte, slit diaphragm (SD), and glomerular filtration barrier (GFB), therefore, SRNS is considered as a podocytopathy (3, 11). Alterations in the architecture of podocyte lead to podocyte dysfunction or loss that increase its permeability and result in proteinuria. When any of the podocyte-related mutations exists, the effects of GCs on the podocytes are blunted and steroid-resistance happens. In this review, an update on recent findings of SRNS-related gene mutations will be put forward.

### Glomerular filtration barrier

Malfunction of the GFB is the common feature found in NS patients. GFB consists of three layers, podocytes, glomerular basement membrane (GBM), and fenestrated endothelium (Figure 1). Podocytes are highly differentiated epithelial cells that are connected by intercellular junctions called SD and create a compact layer on the urinary side of the GFB. GFB acts as a primary ultra-filter (12). The overall negative charge of GFB is related to negatively charged glycocalyx on the surface of podocytes and endothelial cells and also to sialoproteins and heparan sulfate located in GBM (13). Damages to any of the three layers and loss of GFB properties in sieving macromolecules lead to proteinuria. More importantly, podocytes destruction damages GFB integrity. SD interacts with the actin cytoskeleton and triggers signaling pathways involved in podocyte activity such as cell polarity, mechanosensation, endocytosis, and calcium flux (13). Therefore, it is expected that mutations in coding genes of SD components such as *WT1*, *PAX2*, and *nephrin* affect cellular functions and create morphological alterations such as apoptosis, and detachment from the GBM. Moreover, clinical evidence indicates that circulating agents, cytokine imbalance, and immune complex deposition are involved in podocytes injury in NS, since susceptibility to immune factors can be influenced by genomic variants found in these agents (13).

### Podocyte single genes and pathogenic pathways of SRNS

Investigations on the disease mechanism have indicated the participation of more than 50 single genes in the induction of SRNS. Corresponding proteins of these genes are mostly located in glomerular podocytes and SD and their mutation is associated with the pathogenesis of SRNS (6,8). Sadowski et al measured the frequency of *NPHS2* and *WT1* mutations in unrelated families with SRNS and detected 29.5% single gene cause in families with SRNS (14). Previous data show that most of SRNS patients (85%) with early-onset of three months and 66% of patients with the onset of one year of age carry mutations in one of podocyte *NPHS1*, *WT1*, *LAMB2*, or *NPHS2*

genes (15). However, the frequency of genes mutations varies in different studies and populations. For instance, *ADCK4* (0.17%) is the least common mutation in a study performed on international unrelated families; however, the same gene is the most common mutation (6.67%) in Chinese children with SRNS (16). Mitochondrial-related gene mutations are also involved in steroid resistance. Beyond diagnostic value, the identification of causative mutations may present therapeutic significances in some patients (2). Table 1 lists an updated number of podocyte-SRNS-related genes and their abrogated mechanism after mutation. In the following section, we provide the most reported gene abnormalities associated with SRNS (Figure 1).

### Nephrin

Nephrin protein encodes by *NPHS1* gene (38). Different *NPHS1* mutations are associated with congenital NS that influence infants before three months of age (14,39). Congenital NS is associated with in-utero proteinuria, post-natal SRNS, and ESRD. Two recognized common mutation defects in *NPHS1* are ‘Fin major’ (c.121delCT) and ‘Fin minor’ (p.R1109X) in congenital NS patients. Both mutations create premature stop codons at N- and C-terminal regions of nephrin, respectively, and produce a truncated protein, with impaired SDs function (40).

Due to the absence of native nephrin protein in mutant kidney cells, slits in the SDs are narrowed. Another mutation associated with congenital NS is homozygous p. R1160X mutation that leads to mild complications and spontaneous disease remission in children (41). In addition to congenital NS, compound heterozygosity in *NPHS1* gene is detected in early childhood onset NS with late childhood remission in some cases (42). Homozygous or compound heterozygous polymorphisms in *NPHS1* are detected in cases suffering from FSGS and SRNS (43), most of these cases were children (in the range of 8 months to seven years). *NPHS1* mutations are also reported in a 27-year FSGS case which partially responded to immunosuppression. Hence, *NPHS1* mutations are identified in patients with congenital NS and early-onset NS. Moreover, rare mild mutations have been reported in patients with late-onset SRNS.

### Podocin

*NPHS2* mutations first found in early-onset autosomal recessive familial and sporadic SRNS (44). *NPHS2* gene harbors 8 exons and encodes podocin, an interrelated protein with nephrin. Some mutations in the coding sequence of gene may alter its structure and stability and thus, disrupt its functional association and cause proteinuria (45). *NPHS2* mutations are associated with infantile-onset, SRNS accompanied by FSGS, and progression to renal failure. Mutations (about 126) have been reported that mostly correspond for missense

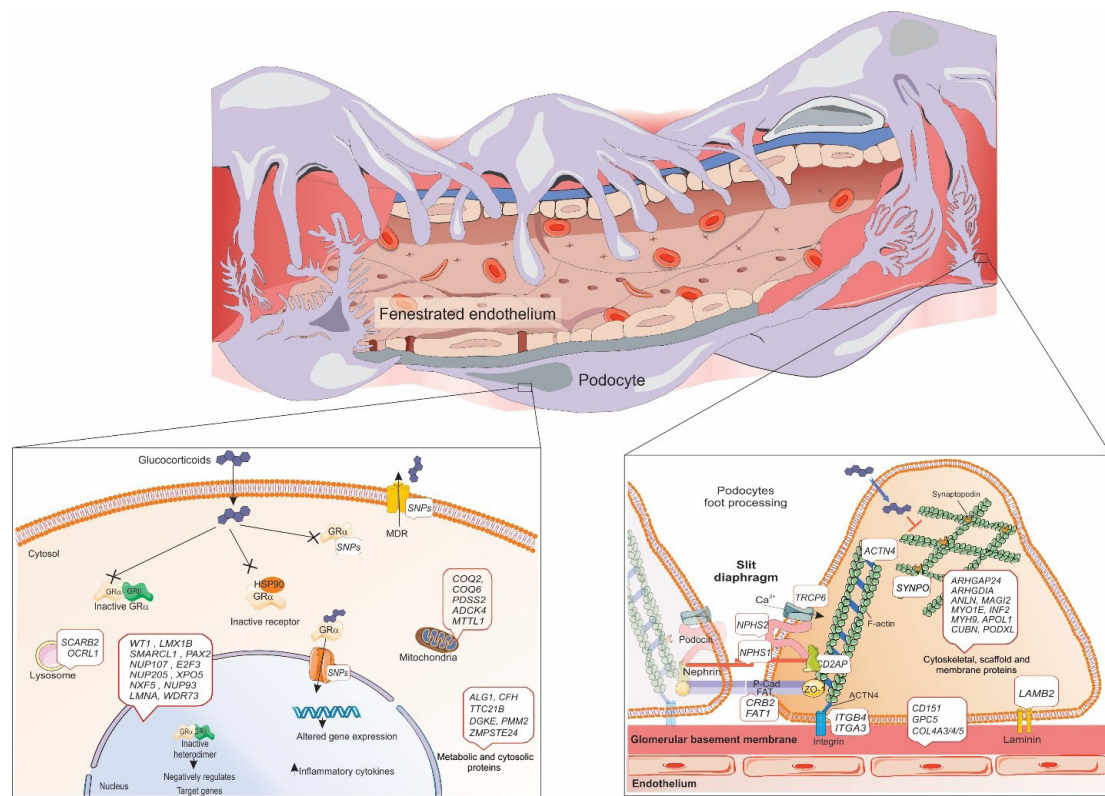
**Table 1.** Identified mutations and their role in SRNS-related podocyte genes

Gene	Chromosome	Mutation	Mechanism of mutation	Ref.
<i>LAMB2</i>	3p21.3	c.737G>A/ R246Q c.253delG /p.Glu85Lysfs*27 3 (hom, p, m)	Perturbs polymerization of laminin heterotrimer and secretion of the mutant laminin trimer into the GBM, leading to altered GBM laminin composition.	(17)
<i>NUP160</i>	11p	R11733 and E803K	Both mutations are pathogenic in the patient with SRNS and FSGS. The missense E803K severely impairs nucleocytoplasmic transport and other NPC-associated functions required for normal nephrocyte physiology.	(18)
<i>NUP93</i>		c.2137-18G>A	The intronic variant caused exon skipping.	(19)
<i>SMARCAL1</i>	2q35	c.2542 G>T/p.E848x	SWI/SF2-related protein involves in chromatin remodeling, SMARCAL1 possibly regulates expression of podocyte proteins.	(20)
<i>TP53RK</i>	20q13	c.194A > T, p.Lys65Met	It is a subunit of KEOPS. Mutation leads to impaired cell proliferation, translational attenuation, ER stress, activation of DDR signaling, increased apoptosis, and defects in actin regulation,	(21)
<i>CFH</i>	1q31	c.2195C>T, p.Thr732Met	Dysregulation of alternative pathway of complement at the microvascular level, and capillary wall injury	(22)
<i>COQ2</i>	4q21	c.890A>G/ p.Y297C	Deficiency in CoQ10 biosynthesis	(23)
<i>CoQ6</i>	14q24	c.1058 C>A /p.A353D	Deficiency in CoQ10 biosynthesis	(24)
<i>PSS2</i>	6q21	c. 964 C→T, p.322 glutamine to a stop codon/ c. 1145 C→T, p. 382 Serine to Leucine	Deficiency in CoQ10 biosynthesis	(25)
<i>ACDK4</i>	19 q13	c.532C>T;p.R178W	Decreases CoQ10 levels and mitochondrial respiratory enzyme activity and reduces migration.	(26)
<i>PTPRO</i>	12p12	c.2627+1G>T (p.Glu854_Trp876del) c.2745p1G>A (p.Asn888Lysfs*3)	Mutant protein lacks the tyrosine protein phosphatase domain and the substrate-binding regions and prohibits tyrosine phosphorylation of tight junction proteins which plays a major role in controlling paracellular permeability, cell signaling, and actin cytoskeleton remodeling.	(27)
<i>GAPVD1</i>	9q33	c.1240 C .G /p.Leu414Val c.2810 G .A /p.Arg937Gln	Affects binding affinity to nephrin and RAB5, regulator of endocytosis, and podocyte migration rate.	(28)
<i>ANKFY1</i>	17p13	c.284 G .T/ p.Arg95Leu	Affects binding affinity to RAB5 and podocyte migration rate	(28)
<i>KANK1</i>	9p24	C1360G>A/ p.E454K	Podocyte dysfunction, probably by dysregulating RHO GTPase signaling	(29)
<i>ARHGDI1</i>	17q25	c.358C>T R120X and c.518 G>T/G173V)	Mutations abrogate interaction with RHO GTPases such as RAC1 and CDC42 and result in a migratory phenotypic change in podocytes	(30)
<i>ARHGAP24</i>	4q21	c.473A>G /Q158R	Dysregulates Rac1-GAP activity which controls podocyte migration	(31)
<i>MAGI2</i>	7q21	Familial: c.3998delG:p.(Gly1333Alafs*141) Sporadic: c.64_71delAGGAACCC:p.(Arg22Glyfs*7) together with c.3526_3533dupCTGGCAGA:p. (Glu1178Aspfs*9)	Alter actin cytoskeletal regulation through RhoA signaling and possibly Destroy the SD formation and podocyte foot process effacement, and severe glomerular pathology	(32)

**Table 1.** Continued

Gene	Chromosome	Mutation	Mechanism of mutation	Ref.
<i>CRB2</i>	9q33	c.1859G>C (p.Cys620Ser) c.1886G>C p.Cys629Ser	It is identified in extracellular tenth EGF-like domain of CRB2 and probably has an important role in CRB2 function in podocytes.	(33)
<i>MYO1E</i>	15q22	p.A159P/ c. 475 G>C	It is a podocyte cytoskeletal protein; mutation impairs ligand binding and actin interaction in the MYO1E motor domain.	(34)
<i>NEIL1</i>	15q24	E181K/ c. 541G>A	Impairs encoding base excision DNA repair glycosylase	(34)
<i>NUP93</i>	16q13	Deletion of exon 13, p.Arg388Trp and p.Lys442Asn fs*14) p.Lys442Asnfs*14, p.Gly591Val and p.Tyr629Cys	Impairs pore formation Disrupt NUP93-SMAD4 or NUP93- importin7 interactions	(35)
<i>NUP205</i>	7q33	Homozygous mutation (c.5984T>C) (p.Phe1995Ser).	Abrogates interaction with NUP93 and impairs NPC assembly	(35)
<i>XPO5</i>	6p21.1	(c.1654G>A)( p.Val552Ile)	Abrogates SMAD4 interaction and nuclear export	(35)
<i>HP</i>		p.K320N and p.R227P		(36)
<i>NUP107</i>	12q15	(c.2492A>C [p.Asp831Ala])	Impairs NUP107 and NUP133 interaction and NUP incorporation to NPC	(37)

LAMB2: Laminin subunit b2; KIRREL2 Neph3 or filtrin encodes a protein with five Ig domains; HP: Haptoglobin; NPC: Nuclear pore complex; SMARCAL1: SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1; TP53RK: TP53 regulating kinase; CFH: Complement factor H; COQ2: coenzyme Q2; PDSS2: Decaprenyl-diphosphate synthase subunit 2; PTPRO: Protein tyrosine phosphatase receptor type O; GAPVD1: GTPase Activating Protein And VPS9 Domains 1; ANKFY1: Ankyrin Repeat And FYVE Domain Containing 1; KANK1: Kidney ankyrin repeat-containing protein 1; ARHGDI1: Rho GDP-dissociation inhibitor 1; ARHGAP24: Rho GTPase-activating protein 24; MAGI2: Membrane-associated guanylate kinase, WW, and PDZ domain-containing 2; CRB2: Crumbs homolog 2; MYO1E: Myosin 1E; NEIL1: NEI endonuclease VIII-like 1; NUP: Nucleoprotein. SD: slit diaphragm; KEOPS: Kinase, Endopeptidase and Other Proteins of small Size, ER: endoplasmic reticulum, DDR: DNA damage response.



**Figure 1. Podocyte genetic mutations associated with steroid-resistant nephrotic syndrome (SRNS).** Glomerular filtration barrier (GFB) consists of 3 layers, podocytes, glomerular basement membrane (GBM), and fenestrated endothelium. Podocytes are linked by intercellular slit diaphragm (SD) and made a condensed layer on the urinary side of the GFB. Gene mutations are grouped based on their function and location within the GFB and the full names of their-encoded proteins are as follows:

COQ: coenzyme Q, *PDS2*: prenyl-diphosphate synthase subunit 2, *ADCK4*: AarF domain-containing kinase 4, *MTTL1*: mitochondrial lyencoded tRNA leucine1, *SCARB2*: scavenger receptor class B, *PMM2*: phosphomannomutase2, *ALG1*: chitobiosyldiphosphodolichol  $\beta$ -mannosyltransferase, *WT1*: Wilm's tumor protein 1, *SMARCAL1*: SMARCA-like protein, *NUP*: nuclear pore complex protein, *XPO5*: exportin 5, *LMX1B*: LIM homeo box transcription factor1b, *NXF5*: nuclear RNA export factor 5, *PAX2*: paired box protein, *E2F3*: E2F3 transcription factor, *LMNA*: lamina A and C, *ZMPSTE24*: zinc metallopeptidase STE 24, *NPHS1*: nephrin, *NPHS2*: podocin, *CD2AP*: CD2-associatedprotein, *PLCE1*: phospholipase C epsilon 1, *TRPC6*: transient receptor potential channel 6, *FAT1*: FAT atypical cadherin 1, *PTPRO*: protein tyrosine phosphatase receptor type O, *CRB2*: Crumbs family member 2, *ACTN4*:  $\alpha$ -actinin-4, *SYNPO*: synaptopodin, *PODXL*: podocalyxin, *INF2*: inverted formin 2, *MYO1E*: myosin 1E, *MAGI2*: membrane associated guanylate kinase, *PTPRO*: protein-tyrosine phosphatase-R O, *ARHGAP24*: Rho-GDP-dissociation inhibitor alpha, *ARHGAP24*: Rho-GTPase-activating protein24, *ANLN*: anillin actin-binding protein, *CUBN*: cubilin, *APOL1*: apolipoprotein L1, *EMP2*: epithelial membrane protein 2, *IFT139*, *TTC21B*: tetratricopeptide repeat protein21B.

mutations. The type and frequency of these identified mutations vary among different ethnics (46). In a worldwide cohort study, investigations indicated one or more loss of function mutations in *NPHS2* like p.R138Q (39.1%) in patients with congenital NS (47). p.R138Q is mainly associated with earlier-NS onset compared to other podocin mutations and leads to severe phenotype. p.R138Q mutation produces a truncated-podocin that is entrapped in the endoplasmic reticulum and disturbs nephrin transport to its target site in the cell membrane (48) and leads to early-onset SRNS. In children before six years of age, homozygous p.R138Q mutations lead to NS. In a study, children from 93.3% of families with NS exhibited the disease in the first year of life. These patients carried p.R138Q mutation or more than one missense *NPHS2* mutation in their genome (49). p.R138X is the other *NPHS2* mutation in which truncated podocin cannot interact with membrane lipid rafts and nephrin

signaling is prohibited (48). The phenotype of this mutation is observed as early and late-onset SRNS, FSGS, and also in diffuse mesangial proliferation. p.A284V and p.R229Q have been detected as the most common variants in Chilean SRNS children (50). Combination of p.R229Q and p.A284V leads to resistance to steroids and other types of immunosuppression. In these patients, FSGS progressed to ESRD in an average time of 8 years (51). The binding affinity of p.R229Q-mutated-podocin to nephrin is reduced that explains the mild phenotype of the mutation and late-onset of disease (52). Adult-onset SRNS is associated with various *NPHS2* mutations, mostly with R229Q (51,53). A compound heterozygous variant of *NPHS2* R229Q and a pathogenic variant in exon 7 or 8 predispose carriers to develop NS in adulthood and cause resistance to corticoids or immunosuppressors. However, if the pathogenic variant is on exons 1 to 6, the risk decreases (54). Most identified *NPHS2* mutations are

associated with SRNS. This necessitates investigation to find *NPHS2* mutations in patients with SRNS to discover if patients are sensitive to steroids (39).

### **WT1 protein**

Wilm's tumor protein (WT1) gene mutations are reported in children (5%–9%) with early-onset steroid SRNS (16). A higher incidence is reported in infantile or congenital NS and in children with diffuse mesangial sclerosis (DMS) (14, 55). *WT1*, harbouring 10 exons, encodes for a transcriptional factor protein that is participated in a wide variety of physiological functions including induction of the mesenchymal-epithelial transition process and various developmental and transcriptional pathways of nephrogenesis (56). In mature developed kidneys, WT1 is expressed in epithelial cells of Bowman's capsule and podocytes (57). WT1 isoforms have different roles in the development and physiology of the kidney (58, 59). *WT1* gene mutations affect the production of SD proteins such as nephrin and podocalyxin (60). Denys-Drash syndrome (DDS), NS type 4 (NPHS4), and Frasier syndrome (FS) are three main nephropathies associated with WT1 mutation and are associated with SRNS. DDS patients are suffering from early-onset SRNS, male pseudo-hermaphroditism, and increased probability of developing Wilm's tumors. WT1 coding gene (exons 7-10) encodes four zinc fingers that specifically recognize DNA and RNA sequences and most dominant missense mutations in *WT1* occur in exons 8 and 9 (zinc fingers 2 and 3) (57). The most frequent *WT1* mutation, p.R394W (exon 9), leads to upregulation of Pax-2 transcription factor that is normally suppressed by WT1 (61). Pax-2 is involved in nephrogenesis and podocyte differentiation (62). There is a positive correlation between renal tubular damage and Pax-2 concentration. Frasier syndrome mostly occurs due to mutations in the donor splice site at intron 9 (IVS9 +4C>T). Alternative splicing in exon 9 inserts KTS amino acids between the WT1 zinc fingers (third and fourth ones). Presence or absence of KTS determines the location of protein isoforms. WT1 isoforms without KTS locate in the nucleus and in contrast to WT1 with KTS, actively participate in transcription. The mutation identified as IVS9 +4C>T in the intron 9 splice-site, decreases the production of WT1 with KTS and results in FS phenotype (63). FS is described by the presence of complications such as FSGS and progressive glomerulopathy, and gonadoblastoma. This mutation is also found in FSGS patients and normal females (64). Kidney clinical symptoms are detectable at early ages (2-6 years old) and are identified by progressive proteinuria, steroid resistance, FSGS, and finally progression to ESRD. Children with NPHS4 are recognized with isolated DMS, SRNS, and rapid progression to ESRD. In contrast to the concept that NS patients with *WT1* mutations are not sensitive to steroid therapy, there are reports, indicating successful treatment with steroids. Children with FSGS,

*WT1* mutations, or nephrotic proteinuria showed sensitivity to CsA therapy (57). This observation suggests the existence of an unknown subset of *WT1* mutations capable to reduce proteinuria upon CsA therapy.

### **Phospholipase C Epsilon-1**

Phospholipase C Epsilon-1 (*PLCE1*) coding gene is highly associated with SRNS (65). *PLCE1* hydrolysis phospholipids in membrane to create inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG), the second messenger molecules initiating cell growth intracellular pathways. This enzyme participates in signaling pathways and induces structural development in podocytes. In NS type 3 (NPHS3) patients, homozygous or compound heterozygous *PLCE1* mutations have been identified. These patients develop severe early-onset NS associated with SRNS, proteinuria, and rapid ESRD development (66). Moreover, the association of *PLCE1* mutations with congenital NS, DMS, and in the non-negligible proportion of patients with FSGS with no *NPHS2* mutation is reported (66-68). *PLCE1* mutations have been detected in hereditary and sporadic forms of FSGS (65). There are reports indicating that patients with *PLCE1* truncating mutations, p.R493X and p.Q1854X successfully responded to therapy (66). The authors suggested that children partially responding to therapy, probably another family member of PLC protein may take over and via an indefinite mechanism compensate for the loss of *PLCE1* function. Consequently, cases with *PLCE1* mutations may respond well to immunosuppressive treatment in the absence of other gene mutations (69).

### **CD2-associated protein**

CD2-associated protein (*CD2AP*) is associated with the C-terminal of cell adhesion molecule (CD2) on podocytes, natural killer cells, and T lymphocytes. *CD2AP* interacts with cytoskeletal content of podocytes, *fyn*, and *synaptopodin*, and maintain the architecture of the cells (70). Moreover, its interaction with nephrin and podocin has been indicated which emphasizes on the role of *CD2AP* in protein signaling (71). *CD2AP* heterozygous (p.T374A) and homozygous (p.R612X) gene mutations are found in steroid-resistant FSGS patients (72-74). Recessive *CD2AP* mutations are not prevalent in SRNS children (75). p.R612X homozygous mutation resulting in a premature stop codon gives rise to production of a truncated protein with lower ability to bind F-actin diagnosed in a FSGS patient. Absence of *CD2AP* in mice caused massive proteinuria and death shortly after birth. Heterozygote mutation of *CD2AP* in mice resulted in the occurrence of glomerular injury after nine months of birth (76).

### **Inverted formin 2**

Inverted formin 2 (*INF2*), plays a role in the regulation of

actin polymerization of the cytoskeleton in different organs such as heart, placenta, kidney, and liver. Heterozygous *INF2* mutations are found in hereditary kidney disease, FSGS5 (OMIM #613237) and a subgroup of cases with Charcot-Marie-Tooth (CMT) disease-associated FSGS (77). *INF2*, a member of formin family, consists of a diaphanous inhibitory domain (DID), a C-terminal diaphanous autoregulatory domain (DAD), and the formin homology domains (FH1 and FH2). FH2 interaction with actin is necessary to regulate the polymerization of the cytoskeleton and DID-DAD interaction blocks this function (78). *INF2* mutations mostly are located on exon 2 (c.317G>C (p.Arg106Pro), c.451T>C (p.Cys151Arg)) and 4 (c.530G>A (p.Arg177His), c.550G>A (p.Glu184Lys)) of DID domain and are related to familial and rarely sporadic (p.R218Q) autosomal-dominant FSGS patients (79, 80). Other mutations related to other exons (e.g. p.L245P, exon 6) are also reported. Then, to find disease-related mutations, the entire *INF2* gene should be considered (81). *INF2* mutations are more frequent in late-onset SRNS and cause proteinuria in early adolescence or adulthood and progress to ESRD. The reduced expression level of *INF2* indicates podocyte damage and is associated with the severity of SRNS (82).

#### TRPC6

Transient receptor potential canonical 6 subfamily C, member 6 (TRPC6), is a Ca<sup>2+</sup> ion channel and regulates intracellular calcium influx into the podocytes. Mutations in *TRPC6* lead to late-onset FSGS2 (OMIM #603965) (83). Moreover, *TRPC6* mutations are involved in childhood-onset SRNS and FSGS in familial and sporadic SRNS cases (84-86). Mir et al investigated *TRPC6* gene variants in 25 children with SRNS and found 5 patients with hereditary and 11 patients with sporadic SRNS carrying (c.171+ 86 G>C and c.171 + 16 A>G) intronic nucleotide substitution, A404V missense, and N561N synonymous variants. Resistance to CsA and CP treatment was observed in these patients (84). Riehle et al identified 19 *TRPC6* mutations related to FSGS consisted of both gain- and loss-of-function mutations (83). Gain-of-function mutations (p.E897K, p.R895C, p.P112Q) augment ion flux into podocytes and induce Ca<sup>2+</sup> ion signaling. These mutations are mostly presented in patients with adolescence or early adulthood- onset of disease (86). On the contrary, loss of function mutations (N125S, L395A, G757D, L780P) is associated with early-onset of SRNS/FSGS in patients (83). It should be considered that there are differences in the phenotype of these mutants in different populations. For instance, promoter mutation (-254C>G) identified in SRNS patients is not related to therapy susceptibility in Indian population (87), however, a probable association is indicated in Chinese children (88). Resistance to therapy is indicated in SRNS patients, presenting various *TRPC6* mutations. Santin et al identified p.L780P variant, a

novel missense substitution, in a 7-year of age mesangial proliferative FSGS patient. They responded partially to CsA and MMF and no susceptibility to prednisolone was observed. No clinical symptoms was identified in her family members with the same substitution (89). Therefore, *TRPC6* gene screening in SRNS with FSGS patients could be considered after the exclusion of other possible associated genes.

#### Actinin alpha-4

Actinin alpha 4 (ACTN4), an actin-filament crosslinking protein, organizes cytoskeletal architecture. Dominant *ACTN4* mutations occur within the actin-binding domain of the protein and affect the binding ability of  $\alpha$ -actinin to adhesion molecules, perturb filament assembly in podocytes, and interfere with their function (90, 91). Furthermore, *ACTN4* interacts with the glucocorticoid receptor (GR) and activates target gene transcription (92). Multiple missense mutations in *ACTN4* coding gene and loss of protein expression have been associated with adult-onset autosomal dominant form of familial FSGS (OMIM #603278)(75).

*ACTN4* mutations (K255E, T259I, and S262P) found in three unrelated families and more abundantly happen in the binding domain and elevate its binding affinity to filamentous actin in comparison to wild-type protein (93-95). Identified mutations caused low-grade proteinuria in early adulthood and can progress to ESRD. W59R and I149del are two other identified mutations in *ACTN4* that are found in FSGS patients (91). These five mutations occur in low proportion, around 4 % of familial FSGS (96). Two mutations found in the same location (S262F and S262P) in FSGS patients with early childhood-onset (97). Patients with S262F mutation were two siblings and their father was also carrying the germline mosaicism for the mutation. They demonstrated variable clinical and pathological features. The siblings were found to have a substitution in *NPHS1*, Thr5Met. The function of this substitution is not clear (97). It is recommended to screen the *ACTN4* gene in kids with infantile- or childhood-onset FSGS in cases when screening for mutations in other candidate genes is negative.

#### Mitochondrial and other rare genes

Abnormal aggregated mitochondria in basement membrane, podocytes, and epithelial cells of Bowman's capsule has been detected as an underlying cause of FSGS. It seems mutations in mitochondrial DNA are also involved in the pathogenesis of FSGS (98). FSGS is predominantly associated with a subclass of mitochondrial disorder, lactic acidosis, mitochondrial encephalomyopathy, and stroke-like episodes (MELAS). However, mitochondrial DNA (mtDNA) 3243A>G mutation is also identified in patients with FSGS associated with chronic progressive external ophthalmoplegia (CPEO), hearing loss, (cardio)

myopathy, and diabetes mellitus (99). A3243G transition is reported to be associated with SRNS in an adult patient (100). In vitro investigations indicated A3243G mutation leads to deficiency in the mitochondrial respiratory chain and impaired protein synthesis process. Other tRNA mutations, m.5538G>A, m.A5728G, m.A5843G (tRNA<sup>Tyr</sup>) are found in FSGS-associated mitochondrial diseases (101-103). These mutations are related to a variety of symptoms. A4269G mutation (tRNA<sup>Leu</sup> gene) in a patient showed association with mitochondrial encephalomyopathy and hearing loss, epilepsy, FSGS, and myopathy (104). From these studies, it is recommended that, in patients with heterogeneous complications in muscle and nervous systems, diabetes, loss of hearing, and renal diseases, mitochondrial DNA mutations should be considered.

Coenzyme Q (CoQ10) is a lipophilic antioxidant and dehydrogenase cofactor located in the mitochondrial respiratory chain and involves in the biosynthesis of pyrimidines and production of ATP (105). Mutations in CoQ10 represent a wide range of symptoms from deafness, retinitis pigmentosa, ataxia, encephalomyopathy to multi-organ involvement and kidney diseases (105). These manifestations are both in adult- and adolescence-onset with the familial and sporadic origin. Association between SRNS and mutations in coding genes of *COQ2* (106), *COQ6* (107,108), and *PDSS2* (105), and *aarF* domain containing kinase 4 (*ADCK4*) (26) (enzymes in CoQ10 biosynthetic pathway) have been demonstrated. Mutations in *ADCK4*, located in foot processes and mitochondria of podocytes, are related to glomerular disease phenotype and CoQ10 deficiency (26).

#### Other genes

Among potential factors engaged in the development of steroid resistance, polymorphisms in cytokine genes should be mentioned, especially in IL-4, IL-6, TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ) genes (109). Jafar et al demonstrated correlations between NS and polymorphisms in IL-6 (G174C), TNF- $\alpha$  (G308A), and IL-4 (C590T) as well as their marked role in diagnosis of SRNS. In addition, the IL-4 polymorphism correlated with predisposition to the minimal change NS (109).

#### Concluding remarks

FSGS is a histologic pattern of glomerular injury that may happen in several circumstances. Most of the time, it is secondary to conditions with glomerular hyperfiltration. FSGS could be mediated by an unknown circulating factor(s) that affect the podocytes and glomerular filtration barriers. A small but significant subset of FSGS cases causes by mutations in single genes which affect podocyte development, structure, and function. Clinically, monogenic FSGS most often presents as SRNS (110). Recently, more podocyte-centric thinking has been developed by the discovery that different heritable forms

of NS are initiated by mutations of proteins involved in podocyte development, structure, and function.

We clearly and desperately need novel therapeutic methods for patients with inherited glomerular disease that up to now have been suffering from lack of any specific and effective treatment. While we are pursuing those efforts, it is imperative to know that genetic testing does not give absolute answers to all existing questions in this area (90, 110). The current movement is giving support to establish genetic, epigenetic, and signaling studies toward precision and personalized medicine in the field of nephrology.

Diagnostic genetic testing could be aimed to identify common gene mutations at the first stages of the disease and deliver more personalized treatment (13). Additionally, they have prognostic information and prevent exposure to unnecessary treatment that patient is unlikely to benefit from (4). Since the SRNS hereditary forms comprise a large number of genes, NGS technology can be used for simultaneous evaluation of all genes involved in podocytopathy (6). The PodoNet Registry has provided complete genetic and clinical information on SRNS cases and aims to make essential visions on genotype-phenotype associations, the efficacy of treatment, and long-term outcomes of the patients and kidney comprising recurrence of disease after transplant (1).

#### Authors' contribution

SZV and MRA designed the study. HMS, SMH, ZNAF and MH prepared the first draft. SZV, MRA and MAMS revised the manuscript. All authors read and signed the final paper.

#### Conflicts of interest

The authors declared no competing interests.

#### Ethical considerations

Ethical issues including plagiarism, double publication, and redundancy have been completely observed by the authors.

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