STEROID RESISTANT NEPHROTIC SYNDROME-GENETIC CONSIDERATION

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Abstract

Nephrotic syndrome is defined as the association of massive proteinuria, hypoalbuminaemia, edema, and hyperlipidemia. It is separated to steroid-sensitive or steroid-resistant (SRNS) forms in respect to the response to intensive steroid therapy. SRNS usually progresses to end-stage renal failure. According to the North American Pediatric Renal Trials and Collaborative Studies SRNS constitutes the second most frequent cause of ESRD in the first two decades of life. Unfortunately, there is no curative treatment for majority of patients. Majority of the SRNS patients have the histologic picture of focal segmental glomerulosclerosis. Interestingly, the risk of recurrence in the kidney graft in patients with hereditary SRNS is lower than in those who do not have genetic background. The etiology and pathogenesis of SRNS has remained enigma for decades. The discovery of 39 dominant or recessive SRNS genes enabled better understanding of the function of the glomerular podocytes and slit membrane. Hildebrandt's group has shown that 85% of the SRNS cases with onset by 3 months of age and 66% with onset by 1 year of age can be explained by recessive mutations in one of four genes only (*NPHS1*, *NPHS2*, *LAMB2*, or *WT1*). The same group used modern diagnostic techniques such as the next generation sequencing and tested a large international cohort of SRNS patients ($n = 1783$ families). The diagnostic panel included 21 genes with a recessive mode of inheritance and 6 genes with a dominant mode of inheritance. Single-gene cause was detected in 29.5% (526 of 1783) of the families with SRNS that manifested before 25 years of age. The identification of causative single-gene mutations may have important therapeutic consequences in some cases. This is very important for patients who carry mutations in a gene of coenzyme Q10 biosynthesis (*COQ2*, *COQ6*, *ADCK4*, or *PDSS2*). In these patients the treatment with coenzyme Q10 may be indicated. Also, patients with recessive mutations in *PLCE1* may respond fully to the treatment with steroids or cyclosporine A. The patients with *CUBN* may benefit the treatment with vitamin B12. The detection of causative mutations may also be very important for familial genetic counseling and for prenatal diagnosis.

Key words: nephrotic syndrome, steroid-resistant, genetics, children

Nephrotic syndrome is a rare disease characterized clinically with the presence of edema and biochemically with massive proteinuria ($> 40 \text{ mg/m}^2/\text{h}$, or $> 50 \text{ mg/kg/d}$), hypoalbuminaemia ($< 25 \text{ g/l}$) and hyperlipidemia. In respect to the response to intensive steroid therapy it is classified as steroid-sensitive or steroid-resistant nephrotic syndrome (SRNS). SRNS usually progresses to end-stage renal disease (ESRD).

The prevalence of steroid resistant form is 15% in children while in adults it is higher – 40%. According to the North American Pediatric Renal Trials and Collaborative Studies SRNS constitutes the second most frequent cause of the ESRD in the first two decades of life [1]. Majority of the SRNS patients have the histologic picture of focal segmental glomerulosclerosis, but also minimal change disease and me-

sangioproliferative glomerulonephritis may be seen. Recurrence of the disease in the kidney graft is significantly lower in patients who have genetic background than in those who do not have it. The treatment of SRNS is a challenging task for the nephrologists due to its poor response to immunosuppressive drugs. High dose steroids, cyclosporamide, calcineurin inhibitors (cyclosporine and tacrolimus), mophetil mycophenolate and rituximab have been used with variable success rates in children and adults [2].

Pathogenesis

Circulating factor

It has been speculated that a circulating permeability factor is responsible for the pathogenesis of the disease due to the fact that many patients with FSGS develop proteinuria and recurrence of the disease shortly after transplantation. Cardiotrophin-like cytokine 1 (CLC-1) and anti-CD40 antibody have been incriminated as potential circulating permeability factors [3]. Recently Reiser's group suggested that soluble urokinase-type plasminogen activator receptor (suPAR) might be involved in the pathogenesis of primary FSGS and might be used as a useful diagnostic biomarker [4]. There is a strong evidence that serum suPAR levels are negatively associated with the renal function and can scarcely differentiate FSGS from the other glomerular/renal diseases. The researchers could not prove in vivo studies that suPAR upregulation could induce proteinuria or podocyte injury. One may hypothesise that a different form of suPAR, which cannot be measured by current enzyme-linked immunosorbent assay, might be the potential permeability factor and biomarker, but a lot of work has to be done to prove it.

Structure of the glomerular barrier

In the past, the attention of the researches was addressed to the mesangial cell as the crucial target in the pathogenesis of SRNS. With the discovery of the structural podocyte genes this attention was redirected to the podocyte which became the principal cell and therefore the resulting pathology was termed podocytopathy. It was demonstrated in the rodent model that podocytes are essential for normal glomerular function and their structural and functional damage is sufficient to cause FSGS.

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The podocyte is very similar to the neuronal cell since it branches off cellular processes to cover the outside of the glomerular capillary, with primary, secondary and tertiary cellular processes, the latter called 'foot processes'. They interdigitate with foot processes from neighboring podocytes and create the glomerular slit membrane, which is very important for the filtering process and retention of protein in the blood stream. The structural and functional changes in the slit membrane result in the loss of its integrity, proteinuria and nephrotic syndrome. So far, 39 dominant and recessive genes have been discovered in patients with SRNS [5]. The identification of these genes was very important for our understanding of the physiology of glomerular filtration barrier and of the pathogenic mechanisms which operate in SRNS.

Identification of the first structural genes

Nephrin (NPHS1)

Ahvenainen et al in 1956 described a series of 8 infants from Finnish ancestry who presented with severe congenital nephrotic syndrome (CNS) and died within the first year of life [6]. In 1998 Kestilä and co-workers identified the gene responsible for the congenital nephrotic syndrome of Finnish type and entitled it nephrin (*NPHS1*) [7]. They characterized the nephrin as a transmembrane adhesion protein which belongs to the immunoglobulin family and which contains 1,241 amino acids. Nephrin takes part in cell signaling and is important in connecting the slit diaphragm to the actin cytoskeleton of the podocyte foot process. So far more than 200 mutations in *NPHS1* have been reported (www.biobase-international.com), which usually leads to an early and severe clinical form of CNS. Two founder mutations are typical for the Finnish patients: Fin-major (nt121(del2)) and Fin-minor (R1109X). The prevalence of the disease is 1/8000 in Finland. These babies are born with low birth weight ranging from 1500–3500 grams but the placenta is large (> 25% of the birth weight). The extrarenal abnormalities are usually absent. Proteinuria begins in utero and is detected in the first neonatal urine. As already mentioned,

these patients in the past died, but nowadays with the improvement of the nephrological care, they survive and have much better outcome [8]. This is achieved with daily albumin infusions, antiproteinuric therapy with indomethacin and captopril and in some cases with unilateral nephrectomy. In the most severe cases the bilateral nephrectomy is performed, the babies are treated with peritoneal dialysis and aggressive nutritional support until they achieve the body weight of 10 kg to undergo a kidney transplantation.

Podocin (NPHS2)

In 2000, Antignac's group from Paris described another gene, *NPHS2*, which encodes another structural podocyte protein named podocin [9]. Mutations in *NPHS2* can manifest early as an infantile nephrotic syndrome, but may also manifest later in life. Podocin interacts with nephrin and serves as a scaffolding function for the slit diaphragm.

Mutations in podocin (*NPHS2*) are the most common cause of childhood onset autosomal recessive steroid-resistant nephrotic syndrome (SRNS). The patients are resistant to the immunosuppressive therapy and rapidly progress to end-stage renal disease. The podocin variant R229Q combined with another pathogenic mutation has been associated with a mild phenotype with disease onset often in adulthood.

Jain et al reported a non-consanguineous family with three generations of individuals who were either compound heterozygotes for mutations in *NPHS2* (R138Q and Q215X) or who have inherited a mutation and a non-neutral polymorphism (R229Q) [10]. The father and the daughter had a childhood onset SRNS. Although the initial assessment pointed to the dominant inheritance of SRNS in this family a careful clinical and genetic testing of the relatives clarified the complex pattern of inheritance. It is of note that variant R229Q in combination with the pathogenic *NPHS2* mutation in *trans* position may cause SRNS of adult onset. Further, what makes the situation complicated, the grandfather in the family reported by Jain et al carried non neutral polymorphism R229Q and known pathogenic mutation R138Q but was healthy at the age of 69. This may be further explained by the recent report

by Tory et al. who showed that R229Q is only pathogenic when in *trans* association with specific *NPHS2* mutations [11]. Mutations in exons 1–6 of the *NPHS2* gene (which includes R138Q) are unlikely to be pathogenic in association with R229Q, as there was a healthy grandfather.

WT1 gene

WT1 mutations can clinically present with three different phenotypes associated with steroid resistant nephrotic syndrome: (i) Denys-Drash syndrome (DDS) (ii) Frasier syndrome (FS) and (iii) isolated nephrotic syndrome. DDS patients besides early nephrotic syndrome have ambiguous genitals and tend to develop Wilms tumor. The typical renal histology picture is of diffuse mesangial sclerosis. Patients with Frasier syndrome present with SRNS later than DDS patients and have male pseudohermaphroditism. Focal segmental glomerulosclerosis is the dominant histological picture. Patients with "isolated nephrotic syndrome" do not tend to develop Wilms tumor and there are no major urogenital abnormalities. In majority of the patients (> 95%) with nephrotic syndrome and *WT1* mutations the location of the mutations is in exon 8 or 9 of the *WT1* gene. Typically Frasier patients have mutations in the donor splice-site of intron 9. The diagnosis of the *WT1* mutation in patients with SRNS is of utmost clinical importance in respect of the further careful clinical surveillance for prevention of the development of Wilms tumor or gonadoblastoma.

Genetics of SRNS in the next generation sequencing era

In the past, the diagnosis of the monogenic causes of SRNS was performed using the Sanger sequencing, which is a laborious and costly technique. With the advance in the genetic technologies, it is now possible to test > 30 genes simultaneously by using high throughput PCR amplification and sequencing approaches. Gene panels include known genes and enable faster and cheaper diagnosis compared with the classic Sanger sequencing [12]. The whole exome sequencing enables sequencing of the whole human exome (300,000 exons) and

detection of new SRNS genes, particularly in patients who had negative panel results. Owing to whole exome sequencing many new SRNS genes have been detected. Very interesting and valuable information was gathered from two big international studies (Hildebrand's group and PODONET study).

Hildebrandt's study

The Hildebrandt's group used modern diagnostic techniques such as next generation sequencing and tested a large international cohort of SRNS patients (n = 1783 families) [13]. The diagnostic panel included 21 genes with a recessive mode of inheritance (*NPHS2*, *NPHS1*, *PLCE1*, *LAMB2*, *SMARCAL1*, *COQ6*, *ITGA3*, *MYO1E*, *COQ2*, *CUBN*, *ADCK4*, *DGKE*, *PDSS2*, *ARHGDI*, *CD2AP*, *CFH*, *ITGB4*, *NEIL1*, *PTPRO/GLEPPI*, *SCARB2*, and *MEFV*) and 6 genes with a dominant mode of inheritance (*WT1*, *INF2*, *TRPC6*, *ARHGAP24*, *ACTN4* and *LMX1B*). Causative mutations were detected in 21 of 27 tested genes. The genetic diagnosis was established in 526 of 183 families (29.5%). This study showed that four genes were major SRNS genes: *NPHS2* (9.93%), *NPHS1* (7.34%), *WT1* (4.77%) and *PLCE1* (2.17%). The highest rate of mutation detection of 69.4% was in the youngest group of patients (0–3 months) and this percentage decreased with the older age. The second important finding from this study was that mutations in recessive genes were more common in children while dominant genes were prevalent in adults. Dominant genes are less penetrant and cause SRNS in adults with phenotypic variability. The most prevalent dominant genes are *INF2*, *TRPC6* and *ACTN4*.

The identification of causative single-gene mutations may have important therapeutic consequences in some cases. This is very important for patients who carry mutations in a gene of coenzyme Q10 biosynthesis (*COQ2*, *COQ6*, *ADCK4*, or *PDSS2*). In these patients the treatment with coenzyme Q10 may be indicated. An illustrative example is presented by this group; a 5-yearold girl presented with SRNS and a causative homozygous mutation was detected in the *COQ6* gene [14]. She had previously responded partially to the treatment with cyclosporine A. The treatment with coen-

zyme Q10 was commenced during the remission and her proteinuria was minimal. Following the inadvertent interruption of coenzyme Q10 administration, the proteinuria rose into the nephrotic range, but after the reinstitution of therapy, the proteinuria normalized.

Also, patients with recessive mutations in *PLCE1* may respond fully to the treatment with steroids or cyclosporine A [15, 16]. The patients with *CUBN* may benefit the treatment with vitamin B12 [17]. Individuals with *ARHGDI* may theoretically be responsive to the eplerenone treatment.

Podonet Study

The PodoNet Consortium has established an international registry for studying congenital nephrotic syndrome and childhood-onset steroid-resistant nephrotic syndrome. Since 2009 more than 1655 patients' data have been collected and mutation screening has been performed in 1184 patients [18]. In majority of the patients (64%) the SRNS manifested in the first five years of life and the congenital form accounted for 6% of all patients. The most common histological picture was FSGS. Genetic disease was identified in 23.6% patients. Causative mutations were detected in 14 SRNS genes and the most common mutated genes were *NPHS2*, *WT1* and *NPHS1*. This study showed similar trend as Hildebrand's study – the percentage of patients with genetic form decreased with the increased age; this percentage was 66% in patients with congenital nephrotic form, while in school children and adolescents it decreased to 15–16%. This study showed presence of extra-renal abnormalities in 17.3% patients. The most common abnormalities were: short stature 5.1%, mental retardation 3.9%, anomalies in the central nervous system 2.5, facial dysmorphism 2.2%, cardiac structural disorders 2.2%, urogenital anomalies 2.0%, visual impairment 1.9%, hearing disorder 1.5% etc. Concerning the treatment outcome the best effects were observed with the administration of calcineurin inhibitors and rituximab with 40%–45% of the patients achieving complete remission. This study revealed two additional important conclusions (i) that genetic diagnosis (not histology) was predictive for immunosuppressive treatment response and (ii) that post-transplant

disease recurrence was significantly lower in patients with genetic diagnosis (4.5% versus 25.8%).

Other studies

In a small Brazilian study 27 steroid-resistant nephrotic syndrome patients were tested for mutations in *NPHS1*, *NPHS2* and *WT1* gene [19]. Only mutations in *NPHS2* were detected confirming the hereditary character of the kidney disease in only 14.8% of patients.

A molecular genetic analysis of childhood nephrotic syndrome was performed in a cohort of 49 Saudi Arabian families (62 cases) [20]. The following genes were screened: *NPHS1*, *NPHS2*, *LAMB2*, *PLCE1*, *CD2AP*, *MYO1E*, *WT1*, *PTPRO* and Nei endonuclease VIII-like 1 (*NEIL1*). Causative mutations were identified in 25 families (51%). The most common mutated genes were *NPHS2* (22%), *NPHS1* (12%) and *PLCE1* (8%).

Giglio et al performed next-generation sequencing analysis of 19 target genes in a cohort of 31 children affected by sporadic steroid-resistant nephrotic syndrome and 38 patients who had steroid-sensitive clinical phenotype [21]. The genetic background was established in 32.3% of the children with steroid-resistant disease but zero of 38 children with steroid-sensitive disease. The response to immunosuppressive treatment in children with genetic and non-genetic steroid resistant nephrotic syndrome was 0 and 57.9% respectively.

Hildebrandt's group reported that mutations in *EMP2* can cause childhood-onset nephrotic syndrome. Interestingly mutations in this gene were detected in 4 individuals from three families who showed steroid resistant or steroid sensitive phenotype [22].

Bińczak-Kuleta et al analyzed *NPHS1*, *NPHS2*, *WT1* and *LAMB2* genes in 33 Polish children with SRNS due to the focal segmental glomerulosclerosis (FSGS), manifesting before the age of 13 years old [23]. No pathogenic mutations were found in *NPHS1* or *LAMB2* genes. Causative mutations of *NPHS2* and *WT1* were detected in 7 of 33 patients (21%), including those with nephrotic syndrome manifesting before one year old: five of seven patients.

Jaffer et al studied a cohort of 484 subjects from the southern Indian population for the presence of four missense mutations G92C,

P118L, R138Q and D160G within the *NPHS2* gene [24]. These mutations were found in 18.5% of steroid-resistant patients in their study group, but not in controls and steroid sensitive patients.

In a report from Turkey 295 children with SRNS were included in mutational analysis of the *NPHS2* gene [25]. Forty-one patients (13.8%) had familial nephrotic syndrome. The mutation detection rate was 24.7% for all patients and the most common mutated exon was exon 5 (52 allele). The patients with mutations in exon 4 had increased risk for progression to end-stage renal disease (ESRD). The mean time of progression to renal failure and ESRD in patients with mutations (1.8 ± 2.5 years) was significantly shorter than in patients without mutations (3.7 ± 4.0 years).

Future perspectives and clinical implications

There is evidence that angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) may have beneficial effect in patients with SRNS, but the mechanism of the action was obscure. Recently a calcium mediated pathway has been identified that connects the angiotensin type 1 receptor (AT1R) to podocyte cytoskeletal dynamics, which is important for a functioning glomerular filtration barrier [26]. The discovery of the two Ca^{2+} permeant channels TRPC5 and TRPC6 as mediators of this pathway not only bolstered the importance of podocyte cytoskeleton dynamics but also revealed promising drug targets for the treatment-resistant nephrotic syndrome.

From a clinical point of view the detection of causative mutations is important because:

- There is no benefit from immunosuppressants;
- No recurrence after renal transplantation;
- No need for renal biopsy;
- Living related transplant donors may be acceptable particularly in those with recessive mutations;
- Familial genetic counseling;
- Prenatal diagnosis;
- Carriers of *WT1* mutations should be monitored for Wilm's tumor and gonadoblastoma.

Therefore, pediatric and adult nephrologists should learn about the genetics of SRNS. Detection of new genes and pathophysiology

events which are important for a provision of a normal function and integrity of the glomerular barrier will enable appropriate pharmacological treatment [27, 28, 29, 30]. The achievement of SRNS genetics is an example of personalized medicine where modern research innovations are translated into the clinical practice.

The authors declare no conflict of interests.

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Резиме

СТЕРОИД РЕЗИСТЕНТЕН НЕФРОТСКИ СИНДРОМ – ГЕНЕТСКО РАЗГЛЕДУВАЊЕ

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Нефротски синдром се дефинира како асоцијација на масивна протеинурија, хипоалбуминемија, едеми и хиперлипидемија. Се разликува стероид сензитивна и стероид резистентна форма (СРНС) во однос на одговор на интензивна стероидна терапија. СРНС обично прогредира во терминална уремија. Според North-American Pediatric Renal Trials and Collaborative Studies, СРНС претставува втора најчеста причина на терминална уремија во првите две декади од животот. За жал, за повеќето пациенти не постои третман на излекување. Поголем број СРНС-пациенти имаат хистолошка слика на фокална сегментна гломерулосклероза. Интересно, ризикот од релапс на болеста во бубрежниот трансплантат кај пациенти со генетска форма е помал отколку кај оние што немаат генетска основа. Етиологијата и патогенезата на СРНС беше енигма со децении. Откритието на 39 доминантни и рецесивни СРНС-гени овозможи подобро познавање на функцијата на гломеруларните подоцити и slit membrane. Групата на Hildebrandt покажа дека 85% на СРНС-случаи со почеток до три месеци возраст и 66% со почеток до една година можат да се објаснат со мутации во еден од четирите гена (*NPHS1*, *NPHS2*, *LAMB2*, или *WT1*). Истата група ги користела модерните дијагностички техники како што е next generation sequencing и тестираше голема интернационална кохорта на СРНС пациенти (n = 1783 фамилии). Дијагностичкиот панел вклучувал 21 рецесивен и 6 доминантни гени. Моногенска причина на СРНС била детектирана кај 29.5% (526 од 1.783) на фамилии кај кои СРНС се манифестираше пред 25 година од животот. Идентификација на моногенските мутации може да има значајни терапевтски импликации во одредени случаи. Ова е важно за пациенти кои носат мутации во гените на коензим Q10 биосинтезата (*COQ2*, *COQ6*, *ADCK4*, or *PDSS2*). Кај овие пациенти третман со коензим Q10 може да биде индициран. Исто така, па-

циенти со рецесивни мутации во *PLCE1* можат комплетно да одговорат на третман со стероиди и циклоспорин А. Пациенти со *CUBN* мутации можат да имаат корист од третман со витамин Б12. Детекција на причинските мутации е исто

многу важна за фамилијарно генетско советување, како и за пренатална дијагноза.

Клучни зборови: нефротски синдром, стероид-резистентен, генетика, деца