World Journal of Pediatrics (2018) 14:105–109
https://doi.org/10.1007/s12519-018-0156-4

EDITORIAL

The status quo and challenges of genetic diagnosis in children with steroid-resistant nephrotic syndrome

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Received: 27 February 2018 / Accepted: 28 March 2018 / Published online: 11 April 2018

Introduction

Idiopathic nephrotic syndrome (INS) is one of the most common renal diseases in the pediatric population, which is characterized by massive proteinuria, hypoalbuminemia, edema, and hyperlipidemia [1]. About 10–20% of affected children have steroid-resistant nephrotic syndrome (SRNS) and do not respond well to steroid therapy [2, 3]. Treatment of SRNS is very challenging due to its poor response to therapy and poor prognosis [4, 5]. Among the patients with SRNS, 20–40% will gradually progress to end-stage renal disease (ESRD) [6].

The etiology of SRNS can be genetic and non-genetic, such as infection- and immune-related factors [2]. The usage of genetic testing in the diagnosis of pediatric SRNS has significantly impacted its clinical management. Recent discoveries of genes encoding proteins crucial for the establishment and maintenance of glomerular filtration barrier have revealed the importance of glomerular epithelial cells (podocytes) in the pathogenesis of SRNS [7]. The most common histology of SRNS is focal segmental glomerular sclerosis (FSGS) [8]. About 11–50% of patients with FSGS are at risk of recurrence after kidney transplantation and eventually progress to terminal renal failure [8, 9], but the risk for recurrence is lower in FSGS patients with monogenic (single gene) forms of SRNS than those with nonhereditary forms [10]. One of the new factors that may predict response to therapy and renal outcomes is genetic variants of nephrotic syndrome [4]. Patients with genetic mutations are less likely to respond to immunosuppressant therapy and more likely to develop ESRD [11].

Genes known to cause SRNS

The first gene identified to cause SRNS was *NPHS1* [12]; its pathogenic variants cause Finnish-type congenital nephrotic syndrome (CNS). Later on, a number of disease-causing genes have been discovered. To date, about 53 genes are known to cause SRNS and/or FSGS with all types of modes of inheritance [8]. According to its symptoms, SRNS can be isolated or syndromic, and the following 28 genes are known to cause isolated nephrotic syndrome [3, 8, 12, 13]: *ADCK4*, *ARHGDIA*, *CD2AP*, *CFH*, *COQ2*, *COQ6*, *CUBN*, *DGKE*, *ITGA3*, *ITGB4*, *LAMB2*, *MEFV*, *MYO1E*, *NPHS1*, *NPHS2*, *PDSS2*, *PLCE1*, *PTPRO*, *SCARB2*, *SMARCAL1*, *TTC21B*, *ACTN4*, *ARHGAP24*, *INF2*, *LMX1B*, *PAX2*, *TRPC6*, and *WT1*.

Detection rate of genetic cause in children with SRNS

The variant detection rate of SRNS in children and young adults varies with different cohorts. Lipska et al. demonstrated that the overall mutation detection rate was 11% in an unselected adolescent cohort of the international PodoNet registry based on 227 patients aged 10–20 years [14]. Tan et al. identified underlying monogenic causes of SRNS in 11.1% of patients with SRNS in a single center of USA [9]. Trautmann et al. reported 23.6% SRNS cases of monogenic cause in a study of 1174 patients [15]. Disease-causing variants were detected in 26.2% of patients in the United Kingdom [16]. A different study identified pathogenic variants in 28.3% of Chinese SRNS patients. An international study detected a single-gene cause in 29.5% (526 of 1783) of families with SRNS that manifested before 25 years of age and 31.2% before the age of 18 years [10]. Santín et al.

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reported a genetic cause rate of 34% in patients with SRNS [17]. The detection rate is much higher and close to 50% in consanguineous families [7].

The detection rate of pathogenic variants is inversely correlated with the age of onset in the SRNS patients. The highest detection rate of 69.4% is in the youngest group of patients of 0–3 months, 49.7% in the group of patients of 4–12 months, and 10.8% in the group of patients of 13–18 years [7, 10]. Santín et al. showed that mutations were detected in 100% of congenital-onset, 57% of infantile-onset, 24 and 36% of early and late childhood-onset, 25% of adolescent-onset, and 14% of adult-onset SRNS patients [17].

Individual gene in children with SRNS

NPHS1 and *NPHS2*, which encode nephrin and podocin, respectively, are the two most common genes implicated in the pathogenesis of SRNS [17]. Pathogenic variants in *NPHS1* are responsible for most CNS and some infantile forms of SRNS. Pathogenic variants in *NPHS2* are the most frequent causes of early onset autosomal recessive SRNS and account for 37.5% cases presenting in the first year of life. Hinkes and colleagues reported that as high as 85% of SRNS cases with age onset before 3 months and 66% of SRNS with age onset before 1 year can be explained by defects in one of the four genes (*NPHS1*, *NPHS2*, *LAMB2*, or *WT1*) [18]. Overall, pathogenic variants in autosomal recessive genes are more common in children with SRNS, while autosomal dominant gene variants are more prevalent in adults.

Inheritance mode

For the autosomal recessive SRNS, attention must also be taken to ethnic groups within different cohort studies, as causative genes differ significantly by ethnicity [19]. AR cases accounted for about 25% of Polish, Turkish, and Syrian patients in adolescents [14]. Sadowski et al. showed that four genes were major SRNS genes: *NPHS2* (9.93%), *NPHS1* (7.34%), *WT1* (4.77%), and *PLCE1* (2.17%), whereas *ADCK4* was the least common causative gene (0.17%) in an international cohort. Data from the PodoNet registry cohort also showed that *NPHS2*, *WT1*, and *NPHS1* were most commonly identified [15]. Similarly, the most frequent pathogenic genes were *NPHS1*, *NPHS2*, and *WT1* in the United Kingdom [16]. However, Wang et al. showed that the most common causative genes were *ADCK4* (6.67%), *NPHS1* (5.83%), *WT1* (5.83%), and *NPHS2* (3.33%) in Chinese children between 3 and 18 years old [6]. *ADCK4* is the most common causative gene, whereas there is a low prevalence of *NPHS2* variant [6].

For the autosomal dominant SRNS, the most prevalent genes are *INF2*, *WT1*, *TRPC6*, and *ACTN4* [19]. Mutations in *INF2* have recently been considered as the most common cause of AD nephrotic syndrome. Among a total of 325 AD FSGS families studied to date, 38 (12%) families with pathogenic sequence variants in *INF2* have been identified [14, 20–22]. Lipska et al. identified two *INF2* patients among 10 patients with AD FSGS [14]. Some studies suggested a very low (< 5%) incidence of *TRPC6* and *ACTN4* in sporadic SRNS cohorts [16, 23, 24]. Lipska et al. showed that none of the AD patients had a mutation in *TRCP6* or *ACTN4* in adolescents [14]. *WT1* mutations have a greater effect on sex determination and genital development in males than females, resulting in a predominance of the female phenotype among mutation carriers [25, 26].

Prognosis in genetic SRNS

Detection of a hereditary podocytopathy is a prognostic indicator of favorable and poor long-term outcomes [4]. Genetic SRNS is associated with a high rate of ESRD in 66% of patients. Patients with non-genetic disease developed ESRD less frequently than patients with genetic disease (27 versus 74%). Trautmann et al. showed that 10- and 15-year ESRD-free survival rates in patients with a genetic diagnosis (27 and 17%) were much lower than those in patients without a genetic diagnosis (53 and 48%) [4]. Further breakdown by genetic diagnosis showed largely uniform renal survival times of the major genetic entities, with estimated 10-year ESRD-free survival rates of 28% for *NPHS2*-associated nephropathy, 23% for *WT1*-associated disease, and 29% for the less common podocytopathies [4].

Modality of gene testing

In the past decades, the Sanger sequencing was used for the diagnosis of the monogenic causes of SRNS, which is a laborious and costly technique. Recently, the next-generation sequencing (NGS) is identified as a powerful technology and has made gene sequencing suitable for routine clinical diagnostics [27]. NGS can evaluate many genes simultaneously, thus made screening of variants in podocyte-specific genes possible. Typically, pathogenic variants in disease-causing genes identified by NGS are confirmed by Sanger sequencing. Three approaches for NGS analysis are used in clinical settings: gene panel, whole exome sequencing (WES), and whole genome sequencing (WGS) [7, 27].

Assignment of variants as disease causing in patients with SRNS

To identify if a variant is disease causing or not is quite a difficult issue in patients with SRNS, especially in patients without a familial history [27]. Therefore, assignment of variants as disease causing identified by NGS and Sanger sequencing should be validated by the following approaches [28]:

Baseline assumptions [27]: 1) full penetrance (age related); 2) defined clinical phenotype; 3) "mutation" implies that an allele changes the phenotype; and 4) known genes with similar phenotype have been excluded.

First principle: a validation of genetic variants requires familial/parental testing [27]. Targeted testing of the affected and unaffected family members across multiple generations significantly helps with the establishment of pathogenicity of genetic alterations in a family. A three-generation pedigree has been commonly used for the diagnosis and risk assessment of rare single-gene or chromosomal disorders.

Second principle: the validation of genetic variants requires functional study [29–31]. The following methods can be used to validate variants of genes known to cause disease: 1) in silico prediction of the gene encoding protein structure and function [30] (e.g., SIFT, PolyPhen-2, and VariantsTaster), as well as its homology and conservation across species (e.g., BLASTn and BLASTx); 2) impact of mutant gene function in cultured cells or animal models [32, 33] [e.g., exogenous expression, transgenic animal model (Zebrafish, *Drosophila*, *C. elegans*, *Xenopus*, and mouse), and gene knock-in/knock-out].

Patient treatment of genetic SRNS

Although our ability to identify individuals at risk for genetic diseases often exceeds our ability to prevent or treat the diseases, existing studies show that the exact genetic test results will help guide the treatment [3]. For example, variants in the *NPHS2* gene should avoid steroid and immunosuppressive agents, and renal transplantation is the suitable choice for these patients; patients with *ADCK4* or *CUBN* variants may be amenable to supplementation with coenzyme Q10 or vitamin B12 [10]; patients with recessive variants in *PLCE1* may respond fully to the treatment with steroids or cyclosporine A (CsA) [1]; and patients with a *WT1* mutation may respond to CsA and methylprednisolone pulses [4]. However, Buscher et al. showed that only 3% of patients with genetic SRNS experienced a complete remission and 16% of patients with genetic SRNS experienced a partial remission after CsA therapy [34].

The including criteria of genetic testing in patients with nephrotic syndrome

Then, when should the patients undergo genetic test? According to the literature [7], 1) positive family history of SRNS or kidney diseases; 2) congenital (< 3 months) or early age of onset of SRNS (< 1 year), and some experts suggest that genetic analysis should be offered to all individuals who manifest with SRNS before the age of 25 years; 3) lack of response to immunosuppression; 4) FSGS or diffuse mesangial sclerosis in renal pathology; 5) presence of extrarenal manifestations (syndromes); and 6) reduced glomerular filtration rate or renal failure.

Challenges in genetic testing

First, different companies have developed competing platforms for panel testing and whole exome sequencing [7] as well as different methodologies and reagents for enrichment of targets of interest. These platforms and methods differ in various aspects, such as the length of reads, coverage, and read depth. Similarly, NGS analysis pipelines also bring in variations during data processing. Different variant filtering parameters such as allele frequency, population frequency, computational prediction, and pathogenicity from databases can be applied and the stringency of which may significantly affect the sensitivity of analysis.

Another huge challenge is variant interpretation and analysis [7, 27, 35, 36]. Because WGS and WES generate a huge amount of variants, it is very important that the interpreting follows strict standard criteria to reduce false-positive and false-negative rates, both of which can lead to serious consequence during clinical management. In addition, pseudogenes and other build-in errors in reference sequences increase false-positive rate. Moreover, the normal variation in the human population can be huge among different ethnic backgrounds, making variant classification even more challenging. These challenges are more prominent in genes lacking an easy and high throughput functional assay to establish pathogenicity. These variants include deletion, insertion, nonsense variants, missense variants, and splicing variants. Some variants lead to structural changes in proteins that ultimately affect biological functions, while others do not. Therefore, it is crucial to classify the pathogenicity of a variant. To add more complexity of interpretation, there is no well-defined genotype–phenotype correlation [37]. Moreover, genes with reduced penetrance and diseases with genetic heterogeneity make it even more difficult to reach a diagnosis.

To prevent the recurrence of genetic diseases, clinical genetic counseling is particularly important and necessary

[38]. Genetic counseling should be offered to any patient and his/her family affected with an inherited disorder. The only way to offer accurate services is to have experienced clinical geneticists and syndromologists, and to know how to better use specialized databases, which help making more clinical diagnoses.

Limitation and expectation

Genetic tests have some limits, including the possibility of uninformative results, the inability to predict the exact age of onset, or the severity of symptoms and, in the case of multifactorial diseases, the inability to predict if the individuals will develop the disease in question [39]. Further research is needed to yield useful information,including connecting podocyte physiology and the immune system, and therapeutic innovations. With the rapid development of genetic testing techniques, researches in INS improve quickly as well; some current issues will be solved in the near future, and we will discover other more variants as the underlying causes of childhood SRNS.

Author contributions YYJ and BYF wrote the initial draft. JHM reviewed and revised the manuscript. All the authors approved the final version.

Funding This study was supported by the National Natural Foundation of China (81470939 and 81770710) and Natural Science Foundation of Zhejiang Province (LH14H050002, LY15H050001, Q18H050002).

Compliance with ethical standards

Ethical approval Not needed.

Conflict of interest All the authors disclosed no conflict of interest.

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