In Focus

Genetic testing for podocyte genes in sporadic focal segmental glomerulosclerosis

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Focal segmental glomerulosclerosis (FSGS) is a clinicopathological entity that manifests with severe proteinuria and/or nephrotic syndrome. FSGS is considered to represent a pattern of glomerular scarring, rather than a specific disease entity. The histological hallmark of FSGS is characterized by sclerosis that involves some, but not all, glomeruli and the affected glomeruli typically show involvement of only a portion of the capillary tuft.

The aetiology of FSGS is diverse and can be divided into primary and secondary causes. Identifying the exact cause of FSGS has important therapeutic and prognostic consequences. Secondary FSGS is usually associated with a maladaptive response of the glomerulus to hyperfiltration or nephron loss, and can occur in conditions like hypertension, obesity, viral infection or drug toxicity. Secondary FSGS can also result from nonspecific scarring in the context of other glomerulopathies, including diabetic nephropathy, IgA nephropathy and lupus nephritis. Primary FSGS can be either ‘genetic’ or ‘idiopathic’. Genetic primary FSGS can be associated with mutations in several podocyte-associated genes, while idiopathic primary FSGS might be caused by either as yet unknown mutations, or by a circulating permeability factor [1].

In the current issue of Nephrology Dialysis Transplantation, Laurin et al. have examined the utility of genetic testing in sporadic FSGS. For this, pathogenic mutations in five podocyte-related genes (i.e. NPHS2, ACTN4, INF2 and PLCE1) and APOL1 risk alleles were tested in 28 children and 37 adults with sporadic FSGS. The authors identified biallelic pathogenic NPHS2 mutations in two Caucasian children. In addition, one novel nonsynonymous variant in INF2 was detected in an African American patient with adult-onset FSGS that could be potentially pathogenic.

Some clinical features might be helpful in differentiating primary and secondary FSGS. Patients with secondary FSGS commonly present with slowly increasing proteinuria in the non-nephrotic range, while patients with primary FSGS show acute onset of nephrotic range proteinuria, often associated with features of nephrotic syndrome.

The role of genetic factors in the development of primary FSGS has become increasingly obvious. Podocyte disorders lead to a spectrum of clinical presentations like congenital nephrotic syndrome, minimal change disease and FSGS. The heterogeneity of these disorders is reflected by the variability in disease phenotypes. Age of presentation, severity of proteinuria, response to steroids, time to dialysis and recurrence after transplantation may vary depending on the mechanism of injury. This complexity supports the impression that primary FSGS is probably a common downstream manifestation of a number of different mechanisms leading to glomerular damage.

As for histopathological characteristics of different forms of FSGS, the Columbia classification distinguishes five subtypes, i.e. tip lesion, cellular, perihilar, collapsing and not otherwise specified. These variants are defined by light microscopy features, some of which correlate with prognosis and response to therapy. Although the perihilar variant has more often been associated with secondary forms of FSGS [2, 3], the Columbia classification does not clearly differentiate between primary or secondary FSGS.

Podocyte foot process effacement is an ultrastructural feature of all glomerular proteinuric diseases and does not discriminate between different variants of primary and secondary FSGS. However, morphometric determination of the foot process width by electron microscopy can help to distinguish primary from secondary FSGS. There is only little overlap between foot process width in primary and secondary FSGS, and a cut-off of 1500 nm accurately differentiated primary from secondary FSGS [4]. However, there is no evidence that the degree of foot process effacement might also help to distinguish genetic from non-genetic primary FSGS.

The last decades have witnessed an increase of interest in the role of the podocyte in primary FSGS. Podocytes are
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highly differentiated postmitotic cells whose function is based on their specific architecture. Numerous proteins important for the normal function of podocytes and the development of proteinuria have been identified. Mutated genes in familial forms of FSGS encode podocyte proteins involved in the maintenance of glomerular slit diaphragm, which, in turn, are responsible for holding back macromolecular structures like proteins within the vascular bed. The affected proteins include nephrin (NPHS1), a cation channel (TRPC6), α-actinin 4 (ACTN4), podocin (NPHS2), formin (INF2), an adaptor protein (CD2AP), a non-muscle class I myosin (Myo1E), a RhoA-activated Rac1 (ARHGAP24) and a phospholipase C (PLCE1). Some of these genes identified for familial cases of non-syndromal FSGS may also be important in the more common, so-called sporadic versions of the disease. In addition, mutations in not-podocyte specific genes associated with syndromes featuring FSGS, including COQ2, LMXB1 and WT1, may also be relevant in sporadic FSGS.

Mutations in the NPHS2 gene are the most frequent cause of steroid-resistant nephrotic syndrome and also the most prevalent causative gene mutation in childhood-onset sporadic primary FSGS and related syndromes, as evidenced by a considerable number of public cohort. The frequencies observed in studies of >10 patients vary from 0 to 9%, which probably relates to differences in cohort age of disease onset, specific FSGS patient characteristics and ethnicity. Also, a mitochondrial pattern of FSGS inheritance has been reported, the most frequently encountered mutation being an A to G point mutation at position 3243 in the mitochondrial DNA gene MTTL1. Limited data are available for sporadic FSGS in African American children. In African American FSGS patients, two sequence variants (G1 and G2 risk alleles) in the APOL1 gene are associated with FSGS.

Selecting genes to be investigated in FSGS is often difficult because of the sheer number of candidate genes. It has been proposed that this may be limited by taking specific clinical and pathological features into account. Thus, several diagnostic flow charts for genetic analysis of FSGS patients have been published to aid geneticists and/or nephrologists with the selection of candidate genes for DNA testing [5]. However, the application of now widely available next generation sequencing (NGS) technologies, which allow rapid sequencing of a large number of genes, seems much more attractive for testing of genetic defects that may be involved in primary FSGS.

There are two approaches to the implementation of NGS technology in the diagnostic work-up of FSGS patients. One approach is to sequence a large number of candidate genes selected for clinical relevance, i.e. known disease causing genes (or risk alleles). An alternative approach is to sequence the entire exome and to filter the data focusing on clinical relevant genes, followed by a more broad data analysis of all other genes (if necessary). Costs and turn-around times of NGS tests decrease rapidly, and it can be envisioned that whole exome sequencing will take a central position in the diagnostic workflow in sporadic primary FSGS in the near future.

The performance of NGS technologies in genetic diagnostics of primary FSGS will keep on improving in the future. Bioinformatic data analysis of NGS sequencing data remains a challenge.

It involves the use of error-prone single nucleotide polymorphism (SNP) databases and software to make predictions about possible pathogenicity of mutations. Specific diagnostic tests (functional genomics) to confirm pathogenicity of observed genetic variants will often be required. The development of a collection of sophisticated and preferably non-invasive diagnostic tests for functional follow-up of NGS molecular genetic results is the next challenge in the examination of primary FSGS patients.

Considering the large discrepancies reported in literature in the percentage of podocyte specific gene mutations found in sporadic FSGS, NGS technologies might be preferred to sequence analysis of single or selected genes. Moreover, the role of multigenic inherited podocyte gene mutations may be substantial as well in FSGS [6, 7]. NGS enables examining different candidate genes for the presence of this potential bigenic heterozygosity in primary FSGS patients.

The developments in sequencing technology and bioinformatics are rapidly changing the landscape of primary FSGS diagnostics and will soon include broad genetic testing as a standard procedure. Hopefully, this will not only facilitate diagnostic classification and prognostication, but also contribute to development of more differentiated and novel treatment modalities.

CONFLICT OF INTEREST STATEMENT

None declared.


REFERENCES


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