

Hereditary Podocytopathies in Adults: The Next Generation

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Abstract

Idiopathic nephrotic syndrome may have two underlying mechanisms: either (1) an alteration of the immune system resulting in the production of a putative circulating factor of glomerular permeability; or (2) mutations in the structural genes of the glomerular filtration barrier in which case patients are typically multidrug resistant and do not recur after transplantation. The latter forms have been recently recognized as “hereditary podocytopathies.” In the past few years, positional cloning approaches that allow the identification of gene mutations underlying diseases whose pathophysiology is unknown and animal models have helped decipher the pathophysiological mechanisms of the glomerular filtration process. Recently, the advent of next-generation sequencing (NGS) techniques has greatly facilitated the identification of numerous novel causative genes in hereditary podocytopathies. Moreover, it has revealed mutations in unexpected genes and has widened the phenotypes associated with podocyte gene mutations. The list of genes mutated in hereditary podocytopathies is constantly evolving and consists to date of more than 40 genes. However, the most recently identified genes are extremely rarely mutated and

may concern only a couple of families worldwide. These discoveries provided crucial insight into the pathophysiological mechanisms linking podocyte proteins to kidney function. This review will focus on monogenic podocytopathies affecting adult patients.

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Introduction

Idiopathic nephrotic syndrome may have two underlying mechanisms [1]: either (1) an alteration of the immune system resulting in the production of a circulating factor of glomerular permeability, yet to be identified – in which case patients are usually sensitive to immunosuppressive drugs, but may also be multidrug resistant with a high recurrence rate after renal transplantation; or (2) mutations in the structural genes of the glomerular filtration barrier. Patients are typically multidrug resistant, although partial remission may be observed with the calcineurin inhibitors ACEi and ARBs (i.e., reduced but persistent proteinuria and normal albuminemia), and do not recur after transplantation. The latter forms have been

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This review will focus on monogenic podocytopathies affecting adult patients. Late-onset podocytopathies are less frequent than those presenting during childhood, and are characterized by a slower progression to end-stage kidney disease (ESKD), incomplete penetrance, and variable expressivity, and mostly have an autosomal dominant (AD) inheritance.

Input from Positional Cloning

Positional cloning approaches (linkage analyses, homozygosity mapping) analyze the cotransmission of a morbid trait (i.e., proteinuria or nephrotic syndrome) with microsatellite markers spread along the whole genome. Once a putative locus has been defined, candidate genes within the candidate region are sequenced. These approaches allow the direct identification of genes involved in hereditary diseases, the pathophysiology of which is unknown. They require several informative families or large consanguineous families and may be hampered by incomplete penetrance. Despite these limitations, they led to the identification of several genes whose mutations underlie hereditary podocytopathies in adults.

NPHS2 and the Slit Diaphragm Signaling Platform

The *NPHS2* gene was identified through positional cloning by Boute et al. [3] in 2002. *NPHS2* mutations are the most frequent cause of autosomal recessive steroid-resistant nephrotic syndrome starting around 4 years of age with minimal changes or FSGS lesions on biopsy, and eventually leading to ESKD by 10 years of age. Thereafter, *NPHS2* mutations were identified in 42% of autosomal recessive cases but also 10–30% of sporadic cases [7–9]. *NPHS2* encodes podocin, an integral membrane protein with a hairpin-like structure located at the podocyte slit diaphragm [10]. It homodimerizes at the C-terminal domain and interacts with other key players of the slit diaphragm such as nephrin [11] or TRPC6 [12]. Its precise function remains uncertain, but podocin could play a key role in assembling slit diaphragm proteins on a signalling platform transducing extracellular signals to the podocyte cytoskeleton, thereby conferring plasticity to the podocyte.

Most mutations provoke a retention of the mutant protein in the endoplasmic reticulum [13, 14]. This has two major clinical consequences: endoplasmic reticulum-retained variants are associated with an earlier onset of nephrotic syndrome (starting around 2 years) than mutants correctly addressed at the plasma membrane (presenting around 10 years of age) [14, 15]. Therefore, the search for pharmacological chaperones able to properly localize podocin mutants at the slit diaphragm is ongoing and seems very promising to slow the progression of the disease.

Subsequent studies revealed that *NPHS2* mutations are the second cause of congenital nephrotic syndrome after nephrin gene mutations, and a leading cause of steroid-resistant nephrotic syndrome and primary FSGS in adults. Almost all adult-onset cases with *NPHS2* mutations carry the p.R229Q nonneutral polymorphism associated with a pathogenic mutation on the second allele [16–18]. This variant decreases the nephrin-binding capacity of podocin [16]. Our group demonstrated that the effect of the p.R229Q polymorphism might be far more complex than expected in a Mendelian disorder [19]. Indeed, the p.R229Q variant is only pathogenic when trans-associated to specific C-terminal mutations, which exert a dominant negative effect through an altered dimerization and mislocalization. On the contrary, patients bearing the p.R229Q variant at the homozygous state are asymptomatic. This has direct implications in genetic counseling since the risk of transmission of the disease may be definitely different from what is expected in autosomal recessive disorders, and also to evaluate the recurrence risk following renal transplantation [1, 19].

TRPC6 and the Calcium Signaling Pathway in Podocytopathies

TRPC6 gene mutations are responsible for 5% of AD podocytopathies [12, 20, 21]. Affected patients present with nephrotic range proteinuria in their third or fourth decade of life and progress to ESKD within 10 years after onset. TRPC6 (Transient Receptor Potential) is a cationic channel that mediates calcium entry into cells and is involved in mechanosensation [22, 23]. *TRPC6* is expressed at the podocyte slit diaphragm, where it interacts with podocin and nephrin [12]. Several gain-of-function missense mutations cause an increase in intracellular calcium influx [5, 12], whereas others result in a loss-of-function phenotype [24]. This discovery unexpectedly suggested the implication of calcium signalling in the pathogenesis of FSGS.

ACTN4 and the Implication of Cytoskeleton Components in Podocytopathies

A genome-wide scan performed in a 100-member kindred allowed Pollak's group to map the first locus of AD podocytopathies on chromosome 19q13 [25, 26]. Linkage analysis including additional families helped to reduce the size of the region and led to the identification of three missense mutations in the *ACTN4* gene [27]. Subsequently, *ACTN4* mutations were evaluated to be responsible for approximately 4% of AD podocytopathies [28]. Patients typically present proteinuria during their teenage years or later and reach ESKD by the age of 50 years [26–30]. *ACTN4* encodes the actin-binding protein α -actinin-4, highly expressed in podocytes. Some mutations exert a gain-of-function effect by increasing the affinity of α -actinin-4 to actin, and/or inducing the formation of actin aggregates around the nucleus, with subsequent impairment of podocyte motility [27, 28, 31–33]. These results underlie the crucial importance of podocyte actin cytoskeleton integrity in kidney physiology.

INF2 and the Role of Formin Proteins in Podocytopathies

In 2010, heterozygous mutations in the *INF2* gene encoding the inverted formin 2 were identified in 12% of families with AD FSGS [4], and subsequently in 17% of pedigrees in a European cohort [34], thereby confirming that *INF2* mutations are the first cause of AD podocytopathies. Age at proteinuria onset ranges from 5 to 72 years and at ESKD onset from 13 to 70 years. As most AD disorders, these mutations have an incomplete penetrance and a variable expressivity. Conversely, less than 1% of sporadic cases with FSGS are mutated [34]. Formins are

large multidomain ubiquitous proteins that have essential roles in remodeling the actin and microtubule cytoskeletons [35]. *INF2* belongs to the diaphanous-related formin family, members of which are direct effectors of Rho-family GTPases [36]. The majority of known *INF2* mutations involve highly conserved residues of the diaphanous-inhibitory domain of the protein product, suggesting its crucial role. These data emphasize the role of a dynamic regulation of the cytoskeleton in podocyte physiology.

Input from Candidate-Gene Approaches

This approach aims to identify causative mutations in genes encoding proteins that could have a direct role in the pathophysiology of a particular disorder. It is based on scientific knowledge of the putative protein functions and interactions.

INF2 Mutations in Podocytopathies with Charcot-Marie-Tooth Disease

Since the 1960s, an increased prevalence of nephropathies, particularly FSGS, has been documented in patients with Charcot-Marie-Tooth disease, a frequent inherited chronic peripheral motor and sensory neuropathy [37]. The pathophysiological mechanism linking these two clinical entities was, however, unknown. *INF2* was known to interact with the Rho-GTPase *Cdc42* and the myelin component MAL (Myelin and Lymphocyte protein), both implicated in essential steps of myelination and myelin maintenance. Therefore, *INF2* was a good candidate to underlie this neuro-renal phenotype. Our group demonstrated that *INF2* mutations are the main cause of Charcot-Marie-Tooth disease with FSGS by identifying 75% of *INF2* mutations in a series of 16 affected families [34]. Conversely, no mutation of *INF2* was detected in patients with Charcot-Marie-Tooth disease, and no renal involvement either. Since the original publication, several other families have been described in the literature. Affected patients typically develop proteinuria between 7 and 30 years of age with FSGS and Charcot-Marie-Tooth disease of the intermediate type (i.e., with both demyelinating and axonal lesions, and intermediate median-nerve conduction velocities) during the second decade of life. *INF2* is expressed in Schwann cells, where it colocalizes and interacts with MAL, and *INF2* mutations alter both the polymerization and depolymerization properties of *INF2*, thereby perturbing myelination. These discoveries represent a major breakthrough. Indeed, although podocytes and peripheral nervous cells are highly specialized

cells with distinct functions, they share similar cellular machineries that, once disrupted, lead to renal and neurological diseases. They prompted physicians to search for proteinuria in all patients with Charcot-Marie-Tooth disease and, similarly, to consider a careful clinical neurological evaluation for patients with FSGS.

ARHGAP24 and the Role of GTPases in Podocytopathies

Using a candidate-gene approach, Akilesh et al. [38] sequenced *ARHGAP24* encoding the actin-regulating protein Rho-GAP 24, highly expressed in podocytes in 310 patients, and identified a loss-of-function heterozygous mutation in one family with AD podocytopathy. The index case had died of ESKD at 29 years of age, and two offsprings had reached ESKD at 12 and 20 years of age, respectively. Rho-GTPases belong to a complex pathway of actin-regulating proteins that provides numerous other interesting candidate genes potentially involved in hereditary podocytopathies.

Input from NGS

Assuming that coding sequences (or exons) represent less than 2% of the genome but contain 85% of known disease-causing variants, techniques have been developed that capture all exons (“Whole Exome”) and massively sequence them in parallel (“Whole Exome Sequencing”) [39]. This cost-effective strategy is useful to search for new causative genes and also in routine genetic testing. However, it raises many challenges both from technical and diagnostic standpoints, and generates large variant data sets that should be filtered in silico to identify potential candidate genes, whose pathogenicity will have to be demonstrated by segregation and functional studies. It has yielded surprising results on podocytopathies, not only by identifying new genes, but also by redefining phenotypes related to known genes.

ANLN and Cell-Cycle Regulating Proteins in Podocytopathies

Mutations in the *ANLN* gene were identified by exome sequencing combined to linkage analyses in two families with autosomal FSGS diagnosed from 9 to 69 years, with ESKD occurring between 35 and 75 years [6]. The encoded protein anillin is an actin-binding protein that plays a key role in cytokinesis, and interacts with the slit diaphragm protein CD2AP and the formin mDia2 during cell division. Anillin is overexpressed on kidney biopsies of patients with

idiopathic FSGS. In vitro, anillin mutants have an impaired binding to CD2AP and cause an abnormal podocyte motility. These results add to the expanding group of podocyte cytoskeleton genes involved in podocytopathies.

ADCK4 and Mitochondrial Components in Podocytopathies

Using exome sequencing and homozygosity mapping, Ashraf et al. [40] identified mutations of the *ADCK4* gene implicated in coenzyme Q₁₀ (CoQ₁₀, ubiquinone) biosynthesis in seven families with isolated podocytopathies. CoQ₁₀ is a key component of the mitochondrial respiratory chain and a potent antioxidant [41]. Subsequently, *ADCK4* mutations were described in a large cohort with a mostly renal limited phenotype and a teenage onset, ESKD during the second decade of life, and mild neurological features or retinitis pigmentosa in only 3 of the 26 patients [42]. A dozen enzymes are required for CoQ₁₀ synthesis, the mutation of which leads to heterogeneous multisystemic disorders associating myopathy, seizures, ataxia, deafness, optic atrophy, and cardiomyopathy, among others. *COQ2*, *COQ6*, *PDSS2*, or *ADCK4* gene mutations are a cause of podocytopathy and altogether, mutations in these genes are identified in about 1% of SRNS cases [43]. This is a crucial diagnosis to make since early CoQ₁₀ supplementation may improve the symptoms and, in particular, reduce proteinuria.

NGS Findings and Redefinition of Known Gene-Associated Phenotypes

LMX1B and Podocytopathies without Nail Patella Syndrome

By linkage analysis and exome sequencing in a large pedigree with adult-onset AD-FSGS and no extrarenal features, a novel *LMX1B* mutation was identified. This was unexpected, since *LMX1B* mutations cause Nail-Patella syndrome associating dysplasia of the patellae, nails and elbows, iliac horns, glaucoma, and in some cases FSGS with specific lesions characterized by the presence of type III collagen fibrils in the glomerular basement membrane by electron microscopy [44]. Mutations in three other families were subsequently identified that involve the same domain of the encoded transcription factor [45, 46] and are expected to diminish the interaction between the *LMX1B* homeodomain and DNA molecule. These data first demonstrated that mutations in genes involved in syndromic forms of podocytopathies may also be responsible for isolated FSGS and prompted to include these genes in NGS diagnosis strategies.

Collagen IV Genes in Podocytopathies

Similarly, *COL4A3* variants were first identified by exome sequencing in children with steroid-resistant nephrotic syndrome, and subsequently *COL4A3* and *COL4A4* mutations were found in 10–12% of patients with AD FSGS, mostly diagnosed during adulthood [47]. Subsequent reports confirmed that *COL4A3* and *COL4A4* mutations are rather frequent causes of AD FSGS (~10–12%) [48, 49]. Mutations in these genes encode the $\alpha 3$ and 4 chains of collagen IV, respectively, and are responsible for Alport syndrome. Dominant forms of the disease have a high degree of phenotypic variability with inconstant gross hematuria and hearing loss [50]. None of the patients had characteristic lesions suggestive of Alport syndrome by electron microscopy. Nevertheless, all patients with available data had associated hematuria. These data emphasize the need to perform *COL4A3* and *COL4A4* gene screening in familial FSGS, especially in adult patients with or without microscopic hematuria or deafness.

Kidney Development Genes in Podocytopathies

WT1 encodes a transcription factor that plays an essential role in kidney and genital tract development [51, 52]. *WT1* mutations cause a wide spectrum of syndromes of AD inheritance [53]: Denys-Drash syndrome in case of exon 8 and 9 mutations (male pseudohermaphroditism, diffuse mesangial sclerosis, and predisposition to Wilms' tumors) and Frasier syndromes related to specific splice site mutations in exon 9 (FSGS and male pseudohermaphroditism with increased susceptibility to gonadoblastomas in 46,XY patients) [54, 55]. Surprisingly, *WT1* mutations were identified in pedigrees with isolated AD FSGS, including males without genital abnormalities or developmental tumors who transmitted the mutation, as observed through classical Sanger sequencing and more recently exome sequencing [11, 56–59]. These results suggest to perform *WT1* testing in females with sporadic FSGS but also in males with familial AD FSGS.

PAX2 mutations were also identified by exome sequencing in seven families with AD FSGS, mostly diagnosed in the second to fourth decades of life [60]. Heterozygous mutations of *PAX2* were thus far known to cause congenital anomalies of the kidney and urinary tract (CAKUT), as well as papillorenal syndrome (renal hypodysplasia and optic nerve coloboma). Mutated patients may have either normal kidney ultrasound or echoic or small kidneys, dilated renal pelvis or calices [60]. Most patients do not have any extrarenal symptom. *PAX2* is a transcription factor expressed during the development of

the kidney as well as the otic and optic vesicles and the hindbrain. *PAX2* mutations could result in FSGS secondary to nephron loss, but through dysregulation of target genes such as the transcription factor *WT1* [60].

Ciliary Genes in Podocytopathies

Mutations of *TTC21B* encoding the ciliary retrograde intraflagellar transport-A protein IFT139 were initially identified as a cause of nephronophthisis [61]. Unexpectedly, its most common mutation (p.P209L) was identified by exome sequencing combined to linkage analyses in families with FSGS, making *TTC21B* the first ciliary gene involved in a glomerular disorder. The related phenotype associates late-onset proteinuria in teenage years, high blood pressure, ESKD in adulthood, and both FSGS lesion and tubular basement membrane thickening on kidney biopsies [61].

These findings modify our understanding of hereditary kidney diseases, previously classified as either “primary glomerular” or “primary tubulo-interstitial” disorders, and opens a new chapter in nephrology textbooks on “primary tubulo-glomerular” diseases.

Conclusions

NGS techniques have greatly facilitated the screening of podocyte gene mutations [43, 62, 63] and have revealed that hereditary forms of podocytopathies are far more common than previously thought. However, causative gene mutations are identified in only a third of pediatric patients and even fewer adult patients [64]. Numerous further genes are therefore expected to be mutated in these disorders. Nevertheless, these diagnosis approaches face the difficulty of determining the causal mutation(s) amongst a great number of potential pathogenic variants. Targeted podocyte gene sequencing panels are now useful tools for fast and rather cheap genetic diagnosis, but also to overcome some ethical issues raised by exome sequencing. The constant advances in the pathophysiology of podocytopathies also open the way to therapeutic innovations. A promising therapy, still explored at a basic level, involves protein chaperones that could redirect the trafficking of missense mutant proteins to the plasma membrane when abnormally retained in the endoplasmic reticulum. Additional promising results have been obtained with drugs that stabilize the podocyte actin cytoskeleton. Podocytopathies are paradigmatic disorders to illustrate the paramount clinical relevance that genetic research can have for affected patients and families. Genet-

ic diagnosis is of utmost importance for genetic counseling, to avoid ineffective and potentially harmful therapies, and even to start early suitable treatment, such as ubiquinone in CoQ₁₀ deficiency, and hopefully in the near future, to offer specific mutation-based therapies [65–67].

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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