Genetics of Proteinuria: An Overview of Gene Mutations Associated with Nonsyndromic Proteinuric Glomerulopathies

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Heritable causes of proteinuria are rare and account for a relatively small proportion of all cases of proteinuria affecting children and adults. Yet, significant contributions to understanding the mechanistic basis for proteinuria have been made through genetic and molecular analyses of a small group of syndromic and nonsyndromic proteinuric disorders which are caused by mutations encoding structural components of the glomerular filtration barrier. Technological advances in genomic analyses and improved accessibility to mutational screening at clinically approved laboratories have facilitated diagnosis of proteinuria in the clinical setting. From a clinical standpoint, it may be argued that a genetic diagnosis mitigates exposure to potentially ineffective and harmful treatments in instances where a clear genotype-phenotype correlation exists between a specific gene mutation and treatment nonresponsiveness. However, cautious interpretation of risk may be necessitated in cases with phenotypic heterogeneity (eg, variability in clinical or histological presentation). This review summarizes gene mutations which are known to be associated with proteinuric glomerulopathies in children and adults.

Key Words: Proteinuria, Nephrotic syndrome, Glomerular filter, Podocytes, Slit diaphragm, Genetics

The glomerular filtration barrier is a physiologic module in the kidney, which functions to generate a plasma ultrafiltrate devoid of protein.[1] It comprises three principal layers: glomerular capillary endothelial cells, glomerular basement membrane (GBM), and podocytes. Defects in any of the three components of the glomerular filtration barrier can result in proteinuria.[1] Nephrotic syndrome defines a clinical scenario characterized by severe proteinuria (>40 mg/m^2/h), edema, hypoalbuminemia, and hyperlipidemia.[2] To date, 11 podocyte-specific genes (*NPHS1*, *NPHS2*, Wilms tumor-1 [*WT1*], phospholipase C epsilon 1 [*PLCE1*], LIM homeobox transcription factor 1 beta [*LMX1B*], SWI/SNF2-related matrix-associated, actin-dependent regulator of chromatin, subfamily a-like 1 [*SMARCAL1*], coenzyme Q2 homolog [COQ2], CD2-associated protein [*CD2AP*], alpha-actinin-4 [*ACTN4*], transient receptor potential channel, subfamily 6 [*TRPC6*], and inverted formin 2 [*INF2*]) and 4 genes encoding GBM components (type IV collagen alpha 3, 4, and 5 chains [*COL4A3*, *COL4A4*, *COL4A5*, respectively], and laminin beta-2 [*LAMB2*]) have been identified as the cause of familial and nonfamilial cases of hereditary forms of nephrotic syndrome which present across a broad age range and wide spectrum of histological variants.[3-5] Table 1 provides a comprehensive list of syndromic and nonsyndromic conditions associated with proteinuria for which a gene mutation has been identified. However, a complete description of all phenotypes associated with these gene mutations is beyond the scope of this review.

Mutations in *NPHS1* and *NPHS2*, encoding nephrin and podocin, respectively, account for more than 85% of cases of nephrotic syndrome in the first year of life (NSFL)[6] and more than 40% of cases of steroid-resistant nephrotic syndrome in children and adults.[7] Nephrin is a transmembrane protein of the immunoglobulin superfamily and is a major component of the podocyte slit diaphragm.[8,9] Slit diaphragms are multiprotein complexes forming a bridge between interdigitated foot processes of neighboring podocytes which have a direct role in glomerular filtration.[1] At the level of the slit diaphragm, nephrin physically interacts through its carboxy-terminal domain with podocin (Fig 1A), which is a hairpin-like protein member of the stomatin family.[10] A key function of podocin is to recruit and stabilize nephrin at the slit diaphragm within lipid-rich plasma membrane microdomains.[11] The nephrin-podocin complex also interacts physically with the CD2 adaptor protein (encoded by *CD2AP*; Fig 1A), which associates with actin and is critical for coupling the slit diaphragm complex to the podocyte actin cytoskeleton.[10] Heterozygous *CD2AP* mutations have been associated with focal segmental glomerulosclerosis (FSGS) in pediatric and adult cases and are associated with reduced expression of *CD2AP* as well as nephrin and podocin in renal biopsies.[12-14] *PLCE1* encodes a receptor-regulated phospholipase C family member (PLCε1), which is involved in hydrolyzing membrane phospholipids and regulating intracellular calcium.[6] PLCε1 also interacts with IQGAP1 (Fig 1A), a podocyte-specific cell junction-associated protein that binds nephrin and is implicated in cell adhesion.[17] *PLCE1* mutations are a cause of nephrotic syndrome in children aged <1 year and most frequently are associated

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with diffuse mesangial sclerosis on renal biopsy,[6,18] although FSGS has been reported.[17] In contrast, *TRPC6* and *ACTN4* mutations are the cause of adult-onset familial FSGS (Online Mendelian Inheritance in Man [OMIM] number 603278 and 603965, respectively).[20-23] *TRPC6* encodes a family member of the transient receptor potential cation channel subfamily C-6, which is involved in the regulation of intracellular calcium.[24] Within podocytes, TRPC6 protein is localized to podocyte foot processes in the vicinity of the slit diaphragm where it interacts with nephrin and podocin[23] (Fig 1A). *ACTN4* encodes alpha-actinin-4, which belongs to a family of actin-binding proteins that regulate cytoskeletal dynamics.[25] In addition to genes encoding podocyte-expressed structural proteins, *WT1* and *LMX1B* are two podocyte-expressed genes which encode transcription factors that are found to be mutated in two heritable syndromes associated with proteinuria. Heterozygous de novo mutations in *WT1*, encoding the *WT1* transcription factor, cause Denys–Drash syndrome (male pseudohermaphroditism, diffuse mesangial sclerosis, and Wilms tumor; OMIM number 194080)[26,27] as well as Frasier syndrome (male pseudohermaphroditism, FSGS, and gonadoblastoma; OMIM number 136680).[28,29] *WT1* mutations have also been detected in young females without gonadal defects who present with isolated FSGS.[30,31] *LMX1B* mutations are the cause of Nail-patella syndrome (OMIM number 161200), which is an autosomal dominant disorder featuring nail dysplasia, absence of malformed patellae, elbow and hip dysplasia, and FSGS.[32]

CLINICAL SUMMARY

- Homozygous or compound heterozygous mutations in either *NPHS1* or *NPHS2* account for 85% of the cause of NSFL.
- Recessive *NPHS2* mutations are responsible for approximately 40% of familial and 6% to 17% of sporadic cases of steroid-resistant nephrotic syndrome.
- Mutations in genes encoding regulators of the complement system (eg, *CFH, CFI, CFHR5*) have emerged as important genetic causes of proteinuric glomerulopathy.

Glomerular endothelial cells are characterized by the presence of fenestrations, which are 70 to 100-nm pores that constitute 20% to 50% of the entire endothelial surface of the glomerulus.[1,33] Podocyte-derived vascular endothelial growth factor (VEGF) plays a critical role in development and maturation of endothelial fenestrae.[34] This was revealed in newborn mice after podocyte-specific deletion of mouse *Vegf* (encoding VEGF-A), which resulted in severe glomerular defects and endothelial cells lacking fenestrae. A role for VEGF in regulating glomerular filtration barrier permselectivity in humans is suggested by several reports from different centers that describe the onset of proteinuria in more than 30 cancer patients following administration of bevacizumab, which is a humanized monoclonal antibody against VEGF.[35-37] Cell type-specific genetic deletion studies in mice have further revealed that the glomerular endothelial cell is the cellular target of VEGF activity in maintaining glomerular filtration barrier permselective function. This was demonstrated in mice after conditional removal of the gene *Kdr* encoding the VEGF receptor, VEGFR-2, which resulted in proteinuria exclusively when *Kdr* was deleted in endothelial cells but not in podocytes.[38] These studies in humans and mice draw attention to the importance of regulating local VEGF activity on maintenance of glomerular filtration barrier permselectivity. Notwithstanding the critical importance of VEGF protein in maturation of the endothelial layer of the glomerular filtration barrier, *VEGF* or *KDR* gene mutations have yet to be identified in humans with proteinuric glomerulopathies.

Mutations encoding major components of the GBM are the cause of Alport syndrome and Pierson syndrome, which are two hereditary conditions associated with microhematuria, proteinuria, and progressive glomerulopathy.[39,40] The main components of the GBM are type IV collagen, laminins, nidogen, and proteoglycans[41] (Fig 1B). Mutations encoding *COL4A5* cause X-linked Alport syndrome (OMIM number 301050),[42] whereas mutations in alpha 3 and alpha 4 type IV collagen chains (encoded by *COL4A3* and *COL4A4*, respectively) are the causes of autosomal dominant and recessive forms of Alport syndrome (OMIM number 104200 and 203780, respectively).[43,44] Pierson syndrome (OMIM number 609049), caused by mutations in *LAMB2* (encoding laminin beta-2 [β2] subchain of laminin-11), is an autosomal recessive condition with variable ocular and neurological defects in association with proteinuria in the first year of life.[40] Laminins constitute a family of heterotrimeric glycoproteins consisting of alpha, beta, and gamma subunits, which are crucial components of all basement membranes and are important for mediating matrix-cell interactions through binding to cell receptors.[45] The defect in Pierson syndrome is because of the loss of *LAMB2* expression in mature GBM,[46] which results in defective laminin-integrin interaction between the GBM and the podocyte actin cytoskeleton[47] (Fig 1B). Heterozygous mutations in *MYH9*, a podocyte-expressed gene encoding nonmuscle myosin IIA, are the cause of a rare group of platelet disorders (ie, Fechtner syndrome, OMIM number 153640; May-Hegglin Anomaly, OMIM number 155100; Sebastian syndrome, OMIM number 605249; Epstein syndrome, OMIM number 153650), which are associated with Alport-like nephropathy with variable penetrance.[48] Recently, *MYH9* and a neighboring gene, *APOL1*, have been implicated as susceptible genes for idiopathic