



Renal Slit Diaphragm – the Open Zipper and the Failing Heart

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Abstract

Inherited forms of proteinuria constitute a rare and heterogeneous group of diseases, the most prominent of which is glomerular dysfunction, which leads to proteinuria. Investigation of the genetic background underlying these diseases has provided significant data on the normal operation of the glomerular filter. Among the different components of the glomerulus, the podocyte slit diaphragm is considered the main source for genetically derived protein alteration, which leads in turn to proteinuria. Investigation of the different proteins revealed that the lack of nephrin and podocin is the leading cause of several inherited forms of proteinuria. It was also proposed that the lack of podocin is linked to cardiac anomalies. This review suggests that the absence of slit diaphragm proteins and the open zipper phenomenon are associated with cardiac anomalies.

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Proteinuria was discovered some 40 years ago when Keen et al. [1] reported elevated urinary albumin excretion in newly identified hyperglycemic patients. The term microalbuminuria, however, first appeared in the medical literature in 1981 when Viberti [2] and Svendsen [3] and their teams reported a urine albumin excretion below the detection limit of a standard dipstick, but at a level that, as reported by Mogensen et al. in 1986 [4], was highly predictive of future overt nephropathy in patients with type 1 diabetes. Almost immediately it became evident that microalbuminuria can predict mortality, mostly from cardiovascular events, especially in patients with type 2 diabetes [5]. Microalbuminuria, defined as urinary albumin excretion of 20–200 µg/min or 30–300 mg in overnight and 24 hour urine collections respectively, has been identified as a cardiovascular and renal risk factor in both diabetic and non-diabetic subjects [6,7]. Similar to the connection between blood pressure and risk of cardiovascular events, mounting evidence indicates a continuous relationship between urinary albumin excretion and risk of cardiovascular mortality and morbidity. The Heart Outcome Prevention Evaluation study [8] also showed that the relationship between albuminuria and a cardiovascular event (i.e., the albumin/creatinine ratio) extends to as low as 0.5 mg/mmol. In concert with these findings, the Framingham Heart Study found that the 6 year risk of cardiovascular disease was threefold higher in non-hypertensive

non-diabetic subjects with urinary A/C above the gender-specific median (3.9 mg/min for men and 7.5 for women) than in those with urinary A/C below the median [9]. The mechanism that stands behind this connection is yet to be clarified. According to the Steno hypothesis [10], albuminuria might reflect a general vascular dysfunction, and leakage of albumin and other plasma macromolecules such as low density lipoproteins into the vessel wall may lead to inflammatory responses that, in turn, may start the atherosclerotic process. Moreover, the increased microvascular pressures and flows observed in diabetes and hypertension may act as injurious stimuli to the endothelium, leading to impaired vasodilatory function, excess matrix protein production, capillary basement membrane thickening, and sclerosis. In the heart, this may contribute to the impaired coronary hemodynamics associated with adaptive left ventricular hypertrophy and the consequent diminution of coronary reserve, increased diffusion distances, failure of angiogenesis to compensate, and secondary ischemic myocyte injury. An attractive hypothesis to tie both ends would be the presence of a common structural protein, such as podocin, which – in both kidney and heart – could provide the missing link to explain the albuminuria-cardiac event association.

Nephrotic syndrome

Ultrafiltration, the process of filtering the plasma across the glomerulus to permit the separation of minute particles and the formation of the ultrafiltrate, is the initial step, and one of the most important, in the excretory function of the kidney. The ultrafiltrate passes through the tubules during normal kidney function. The primary barrier for ultrafiltration of plasma in renal glomeruli consists of three layers: a fenestrated endothelium, a 300–350 nm thick, glomerular basement membrane, and slit pores, i.e., diaphragms located between the foot processes of the epithelial podocytes. This barrier is a highly sophisticated size-selective molecular sieve [Figure 1].

Inherited forms of proteinuria comprise a rare and heterogeneous group of diseases, the most prominent being glomerular

A/C = albumin/creatinine ratio

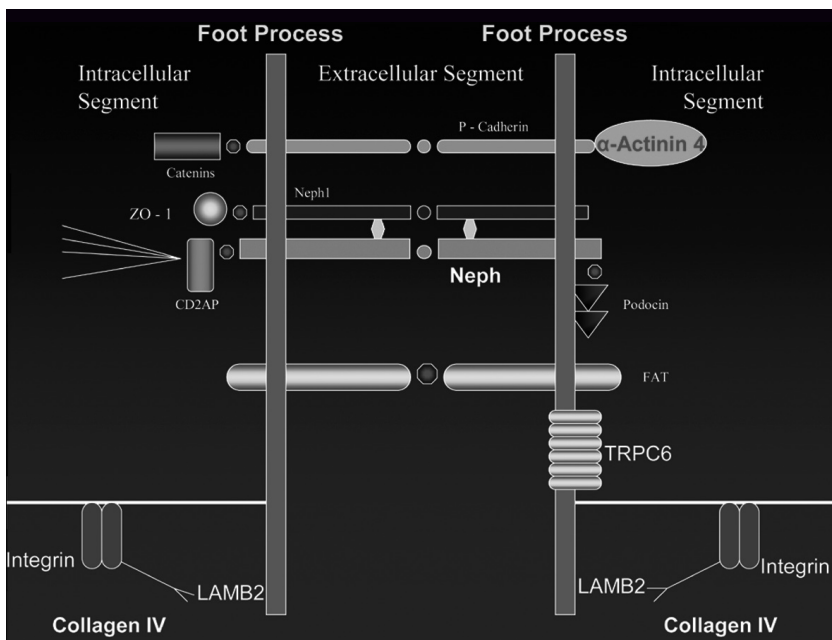


Figure 1. Slit diaphragm schematic presentation. The zipper-like structure of the slit diaphragm comprises nephrin, podocin and other proteins, and lies between two foot processes.

Neph = nephrin, Neph1 = nephrin1, ZO-1 = protein ZO-1, LAMB2 = laminin beta2, TRPC6 = transient receptor potential cation channel 6.

Slit diaphragm proteins and inherited forms of proteinuria

Podocytes are specialized epithelia with a cell body and several polypoid cellular extra-flections that cover the outer surface of glomerular basement membrane. The inter-podocyte connection is an electron-dense zipper-like structure composed of the extracellular components nephrin, nephrin homolog-1, Pcadherin and FAT, connected by other specialized structures such as podocin and CD2AP to the main cell body [Figure 1] [12,13]. The exact role of the slit diaphragm in the ultrafiltration process remained surprisingly obscure until recent years. The slit diaphragm proteins create a complex that contributes to the structure of the slit diaphragm, connects it to the cytoskeleton, and participates in signaling for the turnover of the glomerulus [Figure 2]. The congenital forms of nephrotic syndrome have been known to the nephrology community since the 1960s.

The role of nephrin in podocyte function and the nephrotic syndrome

The Finnish type, an autosomal recessive disease that starts in proteinuria *in utero* and leads to massive proteinuria, and nephrotic syndrome that develops shortly after birth, have drawn special attention. Though most common in Finland, many patients have been diagnosed around the world. Reporting on an Italian family with congenital nephrotic syndrome of the Finnish type, Gigante and co-authors [14] were the first to reach a molecular prenatal diagnosis. Investigating this disease's genetic background and mechanism led Kestila et al. [15] to sequence the critical 150 kb region of the *NPHS1* gene and term the gene product nephrin; this was shown to be a 1241-residue putative transmembrane protein of the immunoglobulin family of cell adhesion molecules, which was demonstrated by Northern blot and *in situ* hybridization to be specifically expressed in renal glomeruli. Nephrin was found only in the visceral epithelial cells of the glomeruli, as determined by *in situ* hybridization, indicat-

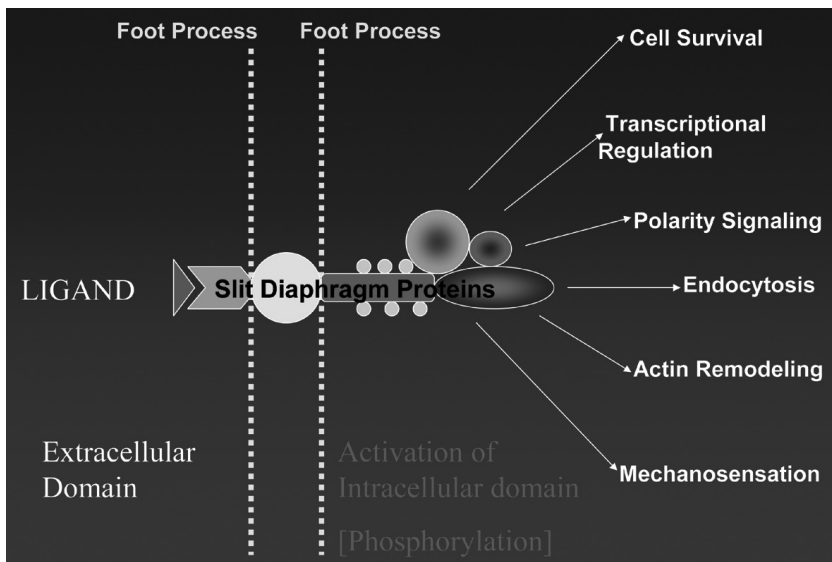


Figure 2. Signal transduction by slit diaphragm, which is critical for podocyte integrity and function

dysfunction, which leads to proteinuria. Investigation of the genetic background underlying these diseases has provided significant data on the normal operation of the glomerular filter. These diseases regardless of their cause can often progress to end-stage renal failure. Among the different components of the glomerulus, the podocyte slit diaphragm is considered the main source for genetically derived protein alteration, which leads in turn to proteinuria [11].

ing its importance for the development or maintenance of the glomerular filtration barrier. Investigation of the Finnish type nephrotic syndrome enhanced our understanding of deficiencies underlying nephrotic syndrome and other primary and secondary kidney diseases, thereby revealing the mechanisms of normal slit diaphragm function.

There are two main Finnish mutations: Fin-major and minor, both causing lack of nephrin and absence of the slit

diaphragm between the podocytes. Laakkonen et al. [16] showed that up to 8% of the patients with the Finnish-type nephrotic syndrome had also developed neurologic deficits, which were predominantly of a motor nature. The nephrin-slit diaphragm protein complex contains a group of scaffolding proteins that function to connect junctional membrane proteins to the actin cytoskeleton and signaling cascades. Using mass spectrometry, six nephrin-binding proteins were identified: MAGI-2/S-SCAM (membrane-associated guanylate kinase inverted 2/synaptic scaffolding molecule), IQGAP1 (IQ motif-containing GTPase-activating protein 1), CASK (calcium/calmodulin-dependent serine protein kinase), alpha-actinin, alpha1 spectrin, and beta1 spectrin. All of these scaffolding proteins are often associated with cell junctions. Immunofluorescence demonstrated that these proteins were expressed in glomerular epithelial cells, where they co-localize with nephrin in the foot processes. MAGI-2/S-SCAM is first detected in junctional complexes in podocytes after their migration to the base of cells. During glomerular development, IQGAP1 is expressed in the junctional complexes between the earliest identifiable podocytes [17].

Integrin-linked kinase has been implicated in the pathogenesis of proteinuria and congenital nephrotic syndrome. However, the function of ILK in glomerular podocytes in a physiologic setting remains unknown. Dai and colleagues [18] generated a mouse model in which the *ILK* gene was selectively disrupted in podocytes by using the Cre-LoxP system. Podocyte-specific ablation of ILK resulted in severe albuminuria, glomerulosclerosis and kidney failure, which led to animal death beginning at age 10 weeks. Podocyte detachment and apoptosis were not observed at 4 weeks of age when albuminuria became prominent, indicating that they are not the initial cause of proteinuria.

Electron microscopy revealed an early foot process effacement as well as morphologic abnormality in ILK-deficient podocytes. ILK deficiency caused an aberrant distribution of nephrin and alpha-actinin-4 in podocytes, whereas the localization of podocin and synaptopodin remained relatively intact. Co-immunoprecipitation demonstrated that ILK physically interacted with nephrin to form a ternary complex, and alpha-actinin-4 participated in ILK/nephrin complex formation. Therefore, ILK plays an essential role in specifying nephrin and alpha-actinin-4 distribution and in maintaining the slit diaphragm integrity and podocyte architecture. These results also illustrate that the integrin and slit diaphragm signals in podocytes are intrinsically coupled through an ILK-dependent mechanism.

Despite their special morphology and function, there is considerable compositional similarity between the podocyte slit diaphragm and typical junctional complexes of other epithelial cells. These findings support the hypothesis of the cell signaling role of slit diaphragm proteins [Figure 2].

Another important member of the slit diaphragm is podocin, whose role and function were also determined using the same strategy. Doublrier and team [19] found that serum and plasma factors from patients with focal segmental glomerulosclerosis

rapidly induced redistribution and loss of nephrin in podocytes. This effect was associated with cytoskeleton redistribution and inhibition by cytochalasin B and sodium azide. In contrast, podocin expression was unchanged after incubation with serum and plasma factors from FSGS patients for short periods, but was markedly reduced at 24 hours. These results demonstrate that serum and plasma factors from FSGS patients may directly affect nephrin and podocin in human podocytes, thus providing new insights into the mechanisms causing proteinuria in FSGS [19].

CD2AP, another key slit diaphragm protein, interacts with nephrin and localizes to the cytoplasmic face of the slit diaphragm [20]. Mice completely lacking CD2AP die of massive proteinuria 6 weeks after birth, suggesting a critical role for CD2AP in slit diaphragm function. Moreover, CD2AP haploinsufficiency seems to be linked to glomerular disease susceptibility in both mice and humans, further supporting the critical role of CD2AP for the integrity of the glomerular filter.

Donoviel et al. [21] identified nephl1, another immunoglobulin superfamily protein [Figure 1] that localizes to the slit and causes congenital nephrotic syndrome in knockout mice. Nephl1 is a member of a family of immunoglobulin adhesion molecules that are expressed in podocytes and interact with podocin [20]. Recently, the adhesion protein and member of the protocadherin superfamily of protein FAT1 was shown to localize to the slit membrane of podocytes [20]. Targeted deletion of the *fat1* gene in mice leads to nephrotic syndrome and podocyte changes that resemble nephrin mutation [20].

More recent studies have demonstrated the novel role for a channel protein within the slit diaphragm. Winn and colleagues [22] suggested that abnormalities in calcium signaling may play a causal role in certain familial forms of podocyte disease. By performing haplotype analysis of a large New Zealand kindred with autosomal dominant FSGS, they discovered a P112Q missense mutation in transient receptor potential cation channel 6, which was specific to all 21 affected family members screened, resulting in increased calcium influx and exaggerated inward current following exposure to the G protein-coupled agonist angiotensin II. Studies by Reiser et al. [23] confirmed expression of TRPC6 in a predominant podocyte distribution, where it localized to the slit diaphragm as a binding partner of podocin and nephrin. Furthermore, upon screening other pedigrees of familial FSGS, they identified additional variants of this gene associated with a gain of function mutation resulting in aberrant calcium signaling.

Podocin – a slit diaphragm protein

Podocin is a membrane-associated protein of the band-7-stomatins family, which interacts with the cytosolic tail of nephrin. Mutations in the podocin gene (*NPHS2*), localized at chromosome 1q25, cause severe podocyte alterations and nephrotic syndrome [24]. It is an integral protein of 383 amino acids with a membrane domain forming a hairpin structure with two cytoplasmic ends at

ILK = integrin-linked kinase

FSGS = focal segmental glomerulosclerosis

TRPC6 = transient receptor potential cation channel 6

the C- and N-terminus. Oligomerization of podocin clusters and nephrin assembles the slit diaphragm where CD2AP serves as an adapter to the overall network [13,25]. In fact, the presence of an intact podocin is a prerequisite for the transport of nephrin to the membrane and for podocyte intracellular signaling.

Mutations in the *NPHS2* gene cause autosomal recessive nephrotic syndrome and has been linked to proteinuria in some populations. In a genome-wide scan of African-American siblings, evidence was found for a link between chromosome 1q25 in a region of the *NPHS2* gene and end-stage renal disease. Dusel and team [26] found that uncommon variants in the *NPHS2* gene may play a role in the development of non-diabetic end-stage renal disease.

Nakhoul et al. [27] demonstrated in adriamycin-induced nephrotic syndrome in the rat that treatment with enalapril alone or in combination with losartan resulted in significant preservation of podocin. The same treatment was also found to decrease proteinuria. Restoration of nephrin was shown to restore ultrafiltration and decrease daily and accumulative proteinuria; on the other hand, not all the drugs that restore podocin had the same favorable effect on proteinuria.

Idiopathic nephrotic syndrome is the most common glomerular disease in childhood. On the basis of the patients' responses to steroid therapy, it is divided into steroid-sensitive and steroid-resistant nephrotic syndrome. Between 10 and 20% of patients fail to respond to steroid treatment and may progress to end-stage renal failure.

Furthermore, mutations in *NPHS2* cause autosomal recessive SRNS [28]. Yu et al. [29] who studied 23 Chinese children with sporadic SRNS found mutations in *NPHS2*.

Podocin and the failing heart

While both nephrin and podocin were found to be expressed only in the kidney's glomeruli, it was also shown that podocin mRNA is expressed in the human fetal heart, and was suggested to contribute to normal cardiac development.

Frishberg and collaborators [30] examined 22 children with SRNS from six unrelated Arab families that were homozygous for the R138X mutation in *NPHS2*. Eighteen of the 22 patients underwent cardiac evaluation at the diagnosis of SRNS, although they had normal blood pressure and preserved renal function. Cardiac anomalies were detected in 16 children (89%) and consisted of left ventricular hypertrophy, pulmonary stenosis, discrete sub-aortic stenosis, Ebstein anomaly, and ventricular septal defect. The remaining four individuals were assessed for cardiac defects only once they had end-stage renal failure. They had severe left ventricular hypertrophy and experienced repeated episodes of heart failure. In addition, two control groups were evaluated in the same manner. One group consisted of 37 siblings without nephrotic syndrome; in this group only one carrier had a cardiac defect. The second group included 22 children with persistent nephrotic syndrome as a result of other causes; none of them had a cardiac anomaly. The authors concluded that cardiac

disorders in a homozygote for mutations in *NPHS2* cannot be attributed to an association by chance or to a state of persistent nephrotic syndrome. They recommend cardiac evaluation at the time of podocin mutation-related SRNS diagnosis. Unfortunately, there are no experimental data on the association of podocin mutations and heart failure such as in knockout mice to podocin. The mechanisms underlying the association between mutations of podocin and heart failure are unknown. However, as suggested by these authors, since human podocin mRNA is expressed in the fetal heart, it may have a role in normal cardiac development. Additionally, since some patients with familial focal segmental glomerulosclerosis display mutation in the TRPC6 cation channel [22], it is tempting to suggest that podocin abnormalities may interfere with calcium signaling and hence play a contributory role in certain familial forms of heart failure.

Conclusion

Podocin and nephrin have been shown to be major factors in the development and mechanism of the inherited forms of nephrotic syndrome. Podocin has also been linked to heart anomalies in patients with SRNS. The cardio-renal axis is well investigated both in mechanism and therapy in the non-exclusively renal diseases such as hypertension. The data presented here suggest the importance of evaluating and investigating the same axis in the inherited forms of nephrotic syndrome.

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SRNS = steroid-resistant nephrotic syndrome

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Take rest, a field that has rested gives a bountiful crop

Ovid (1st century BCE), Roman poet

Capsule

A loss of intestinal fortitude

The large-scale and rapid depletion of CD4+ T cells in the weeks after HIV infection occurs predominantly in the gastrointestinal tract. Accompanying this loss is a sustained whole-scale activation of the immune system, which corresponds directly with the eventual progression to AIDS. Brenchley et al. propose that the two processes are tightly coupled, with impaired intestinal integrity leading to the translocation of gut microbes, or some of their constituent components, which overstimulate the immune system. Circulating levels of bacterial lipopolysaccharide (LPS), which was used as a marker for microbial translocation, were markedly elevated in the sera of chronically infected HIV individuals and in macaques experimentally infected with the simian immunodeficiency virus (SIV).

This increase corresponded directly with footprints of immune activation, including circulating cytokines, antibodies to LPS, and immune-cell turnover. In HIV patients undergoing highly active antiretroviral therapy, LPS levels were decreased, with a corresponding rebound in CD4+ T cell numbers. Furthermore, in the absence of pathology – as typified in the infection of natural primate hosts for SIV – signs of substantial microbial translocation or immune activation were not apparent. The link between HIV infection, integrity of the mucosal immune system, and chronic peripheral immune activation may prove important to consider in future therapies for HIV infection.

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