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Commentary

The first identified heterozygous nonsense mutations in podocalyxin offer new perspectives on the biology of podocytopathies

Ido Refaeli, Michael R. Hughes and Kelly M. McNagny

The Biomedical Research Centre, University of British Columbia, Vancouver, Canada

Correspondence: Kelly M. McNagny (kelly@brc.ubc.ca)

In the last two decades, our understanding of the genetic underpinnings of inherited podocytopathies has advanced immensely. By sequencing the genomes of a large pool of families affected by focal segmental glomerulosclerosis (FSGS), researchers have identified a common theme: familial podocytopathies are frequently caused by genes selectively expressed in podocytes. Podocalyxin is a podocyte-specific surface sialomucin that has long been known to play important roles in podocyte morphogenesis and function. Few studies, however, have shown a conclusive link between mutations in the gene and FSGS complemented by functional evidence. In a fascinating new paper published in *Clinical Science*, Lin et al. identify two unrelated pedigrees in which dominant loss-of-function mutations in *PODXL* lead to adult-onset FSGS. Nonsense-mediated decay of the mutated *PODXL* transcripts leads to protein insufficiency, which in turn cause podocyte dysfunction through defects in motility and cytoskeletal organization. This is the first study to date that demonstrates, mechanistically, how autosomal dominant mutations in podocalyxin can lead to FSGS and renal insufficiency. Here, we summarize the experimental findings of this manuscript and propose, perhaps, a more controversial hypothesis: down-regulation of podocalyxin protein expression from podocytes is a critical turning point in the progression of most podocytopathies and may be mechanistically relevant to glomerulopathies in which podocyte damage is not necessarily induced by genetic lesions.

Our understanding of the genetic contributions to the incidence of podocytopathy has increased rapidly in the last two decades, largely due to the advent of next generation sequencing technologies. In a fascinating new paper published in *Clinical Science*, Lin F-J et al. used whole exome sequencing to identify two heterozygous nonsense mutations in the podocalyxin gene (human: *PODXL*; mouse: *Podxl)* linked to autosomal dominant (AD) focal segmental glomerulosclerosis (FSGS) [1]. FSGS, a podocyte-driven disease, is the most common diagnosis (39%) in patients biopsied for investigation of kidney abnormalities [2] and is a frequent cause of end-stage renal disease. Primary FSGS can be familial or sporadic, and typically presents with severe clinical features and resistance to steroid therapy [2–4]. A common theme to all variants of primary FSGS is lesions in genes that regulate podocyte structure and function [1,4]. Generally, autosomal recessive inheritance patterns of FSGS with homozygous or compound heterozygous mutations develop disease in early childhood [3,4], while AD inheritance patterns exhibit later onset, with some carriers never developing disease [3,5].

Podocalyxin, a member of the CD34 family of sialomucins, has long been understood to regulate podocyte morphogenesis and function [6]. Antibody studies first identified it as the single most highly glycosylated and negatively charged cell surface antigen expressed by podocytes in rodents and human [7]. Functionally, it was shown in animal models that neutralization of podocyte surface charge with polycation infusion or de-sialylation via neuraminidase treatment was sufficient to induce podocyte foot process effacement and proteinuria [7–9]. With the subsequent cloning of the encoding gene [10],

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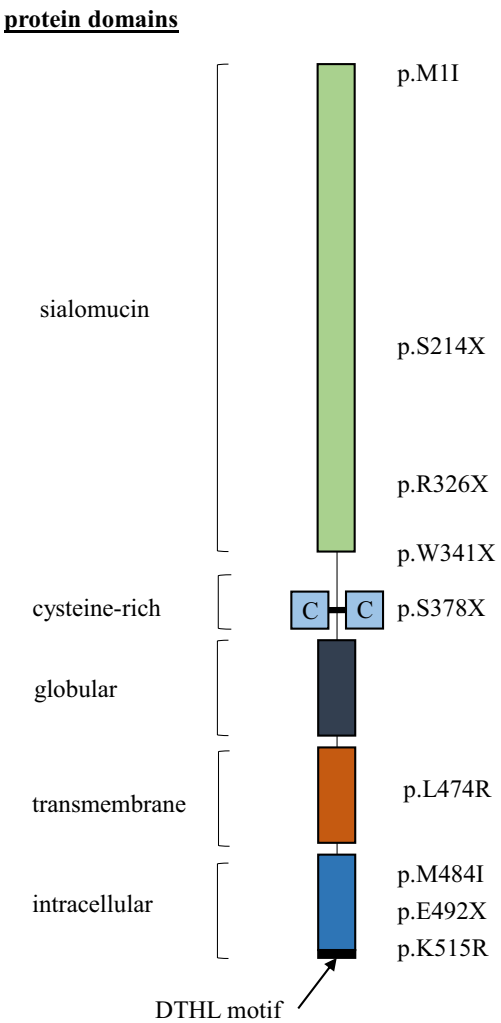

Figure 1. Mapping of AD and recessive mutations in human podocalyxin associated with familial podocytopathies

P.M1I and p.W341X exhibited an autosomal recessive inheritance and were identified as compound heterozygous in a Korean child born with congenital nephrotic syndrome, omphalocele, and microcoria [12]. p.326X and p.S378X are dominant mutations associated with familial FSGS in the Chinese and Indian pedigrees described by Lin et al., respectively [1]. p.S214R, p.L474R, p.M484I, PE492X, and p.K515R were identified by Barua et al. FSGS pedigrees bearing AD inheritance patterns [5].

podocalyxin became a prime candidate for genetically linked podocytopathies. In a surprising twist, however, deletion of the *Podxl* gene in mice led to perinatal lethality due to hypertension and a complete failure to produce urine, in contrast with the proteinuria observed in FSGS or other glomerular disorders [11]. The mouse phenotype was attributed to an inability of the immature $Podxl^{-/-}$ podocytes to disassemble adherens and tight junction between neighboring immature podocytes and a corresponding failure to form foot processes. In humans, a similar phenotype was also recently observed in a Korean child carrying compound heterozygous nonsense mutations in *PODXL* [12]. Finally, germline deletion of *PODXL* in human pluripotent stem cells prior to kidney organoid differentiation leads to podocyte structural defects that phenocopy those observed in mouse *in vivo* [13]. In aggregate, these studies suggest that podocalyxin plays a critical role in dissolution of junctional complexes in immature podocytes and that excessive podocyte adhesion, anuria and hypertension, manifests in its absence.

Strikingly, in their study, Lin et al. present compelling clinical and mechanistic evidence to suggest that heterozygous nonsense mutations in *PODXL* can elicit familial FSGS and proteinuria in adults. They identify two unrelated pedigrees of Chinese (p.Arg326X) and Indian (p.Ser378X) descent with renal insufficiency and biopsy-proven FSGS for which heterozygous nonsense mutations in *PODXL* correlated with disease (Figure 1). From the Chinese pedigree (p.Arg326X), they obtained peripheral blood mononuclear cells and performed real-time PCR and Western blotting

Table 1 Urinary podocalyxin concentration in glomerulonephritis

Indication	Study	uPODXL Concentration
Diabetic nephropathy (DN)	Hara et al. [19]	27.3 ± 3.3 ng/μmol Cr (cf. 7.1± 0.5 in healthy controls)
	Shoji et al. [21]	58.9 mg/g Cr in diabetic patients with macroalbuminuria (2-fold more than patients with normoalbuminuria)
Focal segmental glomerulosclerosis (FSGS)	Hara et al. [19]	37.1 ± 11.7 ng/μmol Cr (cf. 7.1± 0.5 in healthy controls)
	Zhu et al. [20]	10.6-fold increase in uPODXL/uAQP2 concentration relative to healthy controls
Membranous nephropathy (MN)	Hara et al. [19]	71.4 ± 3.8 ng /μmol Cr (cf. 7.1± 0.5 in healthy controls)
	Zhu et al. [20]	4.4-fold increase in uPODXL/uAQP2 concentration relative to healthy controls
Lupus nephritis (LN)	Hara et al. [19]	44.3 ± 10.8 ng/μmol Cr (cf. 7.1± 0.5 in healthy controls)
	Ikuma et al. [18]	311.0 (155.8-633.5) μg/g Cr in LN patient group (cf. 127.0 (69.3–177.0) in lupus patients without nephritis)
	Zhu et al. [20]	5.9-fold increase in uPODXL/uAQP2 concentration relative to healthy controls
IgA nephropathy (IgAN)	Hara et al. [19]	14.4 ± 10 ng/μmol Cr (cf. 7.1± 0.5 in healthy controls)
	Asao et al. [22]	82 μg/g Cr in patients with IgAN and FSGS with poor prognosis (cf. 50 for patients with good prognosis)
IgM nephropathy (IgMN)	Zhu et al. [20]	9.8-fold increase in uPODXL/uAQP2 concentration relative to healthy controls
Obesity	Suwanpen et al. [23]	A higher number of PODXL-positive cells in urine correlates with higher BMI ($r = 0.343$, $p = 0.008$)

BMI, body mass index; cf., compare to; Cr, creatinine; uAQP2, urinary aquaporin-2; uPODXL, urinary podocalyxin.

experiments to assess podocalyxin expression. They found a statistically significant decrease in both relative mRNA (∼50%) and protein (∼50%) levels compared with normal controls. Together, these experiments suggested that the heterozygous nonsense mutations identified in the Chinese and Indian pedigrees may cause instability and degradation of podocalyxin mRNA and the down-regulation of podocalyxin protein expression in human tissue. To test this hypothesis, the authors transfected HEK 293T cells with plasmids expressing mutant (p.Arg326X and p.Ser378X) pEGFP-PODXL constructs. When compared with control mRNA, the mutant pEGFP-PODXL transcripts were susceptible to nonsense-mediated decay (NMD), as the authors showed that mutant mRNA levels were rescued in cells incubated with the NMD inhibitor, cycloheximide. These important experimental findings provide the first mechanistic evidence to suggest that dominant heterozygous loss-of-function mutations in podocalyxin can lead to protein insufficiency. Indeed, previous studies addressing AD *PODXL* mutations showed no differences in protein expression, localization, and binding partner interactions, making it challenging to infer disease mechanisms [5].

Because of these findings, the authors continued to investigate the consequences of podocalyxin down-regulation on podocyte biology *in vitro*. They performed *Podxl* knockdown experiments in cultured mouse podocytes and assayed the activities of RhoA and ezrin, both of which regulate podocyte maintenance and motility/adhesion [9,14]. Knockdown by siRNA led to decreased levels of phospho-ezrin (i.e., its active form) and active RhoA (i.e., GTP-bound). Using both transwell migration assays and wound-healing assays, the authors showed that podocalyxin knockdown impairs podocyte motility, measured by the number of migrating cells and percent wound closure, respectively.

These new data pose an important question: Do heterozygous nonsense mutations in *PODXL* spontaneously cause disease or do they confer disease susceptibility? The hypothesis that they are disease-causing is supported by their tight segregation with disease in affected families and the functional *in vitro* data implicating reduced podocalyxin levels in podocyte dysfunction [1]. It is also possible, however, that heterozygous nonsense mutations increase an individual's susceptibility to podocytopathy without spontaneously causing disease. Notably, $Podxl^{+/-}$ mice have a normal lifespan at steady state with functional kidney architecture capable of normal urine filtration [11]. One might predict, however, that when challenged with the appropriate environmental stress (diet, nephrotoxins, etc.) these mice may show an enhanced susceptibility to renal disease. Alternatively, it is possible that variation in modifier genes plays a role in hastening the onset or progression of FSGS in patients with heterozygous nonsense mutations in *PODXL*. This speculation is supported by the intrafamilial clinical heterogeneity in the AD-FSGS pedigrees described to date [1,5,12]. Because extensive clinical chart history from the pedigrees in the Lin et al. study was not available, it is difficult to ascertain whether such variables could have influenced the severity or onset of the FSGS lesions. Nevertheless, the linkage between allele loss and disease is remarkable.

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Perhaps of more general interest is the notion that loss of podocalyxin expression or post-translational modifications could play a role in a wider slate of renal diseases than FSGS. As stated above, experimentally induced alterations in podocalyxin glycosylation have been shown to cause renal disease in animal models. Likewise, mutations in glycosyltransferase *C1galt1* in mice have shown remarkably selective defects in kidney function and these studies have implicated alterations in podocalyxin glycosylation as the cause of proteinuria [15,16]. Finally, down-regulation of podocalyxin from the surface of podocytes is consistently associated with foot process effacement and sclerosis in patients [17] and, intriguingly, shedding of podocalyxin into the urine has been robustly documented to indicate podocyte injury and disease progression in patients with lupus nephritis [18–20], diabetic nephropathy [19,21], IgA nephropathy [19,22], IgM nephropathy [20], membranous nephropathy [19,20], and obesity-related nephrosis [23] (Table 1). It is therefore worth considering that although this exciting paper now firmly establishes that variations/deletions in podocalyxin's coding sequence are linked to disease, it may also herald future studies that implicate alterations in protein expression or post-translational modifications as more common mechanistic focal points in podocyte deterioration.

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I.R., M.R.H., and K.M.M. wrote the manuscript.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AD, autosomal dominant; BMI, body mass index; cf., compare to; Cr, creatinine; DN, Diabetic nephropathy; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; IgMN, IgM nephropathy; LN, Lupus nephritis; MN, Membranous nephropathy; NMD, nonsense-mediated decay; uAQP2, urinary aquaporin-2; uPODXL, urinary podocalyxin.

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