

Non-muscle myosins and the podocyte

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Abstract

Proteinuria is often accompanied by a pathological change in the glomerulus that is referred as effacement of the podocyte foot processes. The highly dynamic podocyte foot processes contain an actin-based contractile apparatus comparable to that of pericytes, which needs to be precisely and temporally controlled to withstand high pressure in the capillaries and to maintain intact glomerular filtration properties. This review outlines the most recent concepts on the function of the podocyte contractile apparatus with a focus on the role of non-muscle myosins as they have been highlighted by studies in monogenic hereditary proteinuric diseases.

Keywords: actin cytoskeleton; focal segmental glomerulosclerosis; non-muscle myosin; podocyte foot processes; proteinuria

Introduction

The glomerulus is a highly specialized tuft of capillaries with a fairly high pressure (60 mmHg) and the ability to filter huge amounts of water and small solutes into the urinary space, while retaining at least 99.9% of albumin and large proteins [1, 2]. The importance of the glomerular filtration barrier (GFB) is emphasized by the fact that abnormalities of glomerular ultrafiltration properties reflected by urinary loss of proteins, lead to progressive renal insufficiency. The strong predictive value of proteinuria in chronic nephropathies is firmly established, and experimental evidence indicates that protein overload exerts toxic effects on tubular cells by induction of chemokine expression and complement activation that lead to interstitial inflammation and sustained fibrogenesis [3]. However, the direct cause and effect role of proteinuria in kidney disease progression is still a matter of debate [4, 5].

The GFB consists of three interacting layers: the glomerular fenestrated endothelium, the glomerular basement membrane (GBM) and the podocytes [1, 2], highly specialized kidney epithelial cells that consist of a cell body, major processes and foot processes. Podocyte foot processes form a characteristic interdigitating pattern with foot processes of neighbouring podocytes, leaving in between the filtration slits that are bridged by the slit diaphragm protein complex [1] (Figure 1). The key role of the podocyte in the GFB is exemplified by finding that patients with focal segmental glomerulosclerosis (FSGS), a kidney lesion characterized by alteration in podocytes, resulting in foot process effacement and disruption of the slit diaphragm, suffer from massive loss of proteins in the urines [6–8].

The complex structure of the podocyte is dependant upon a highly structured actin cytoskeleton. The arrangement of the podocyte cytoskeleton needs to be precisely and tempo-

rally controlled in order to withstand high pressure in the capillaries and to maintain intact filtration properties [9].

This review outlines the most recent concepts on the function of the podocyte cytoskeletal apparatus. The role of non-muscle myosins in regulating podocyte morphology and function will be discussed.

The podocyte is a contractile cell

In the past, the mesangial cell was thought to have the main contractile capability within the glomerulus. As we have learnt more about the anatomy and functionality of the podocyte, it has become apparent that the podocyte itself is a contractile cell.

Differentiated podocytes contract both spontaneously and in response to angiotensin II, analogous to smooth muscle cells [10]. Like smooth muscle cells, podocytes uptake glucose in response to insulin, a feature found in a very limited subset of cells in the body [11]. Due to these characteristics, podocytes have been recently compared to capillary pericytes, elongated contractile cells that wrap around precapillary arterioles outside the basement membrane [12] (Figure 1).

Podocyte foot processes contain long actin fibre bundles that run cortically and contiguously to link adjacent processes. Foot process effacement, the hallmark of podocyte injury and proteinuric kidney diseases, is accompanied by changes of these parallel contractile bundles into a dense network of disorganized filaments [13, 14].

In the healthy podocyte, such fibre bundles are thought to generate the contractile forces that help to actively modulate the permeability of the filtration barrier through changes in the foot processes morphology [12]. Exposure of mouse podocytes to fluid shear stress in a flow chamber, to mimic

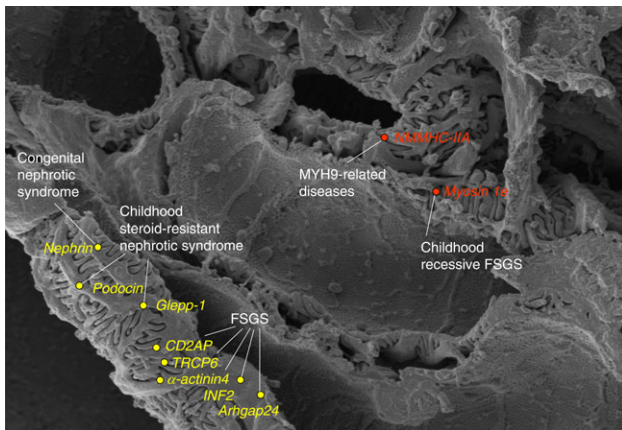


Fig. 1. Scanning transmission electron microscopy image of glomerular capillaries showing podocytes and their interdigitating foot processes surrounding the capillaries. Through continuous dynamic foot process reorganization regulated by the function of their contractile apparatus, the podocytes adapt their own morphology to resist the high intraluminal pressure, to regulate the permeability properties of the slit diaphragm and possibly also to modify the capillary diameter and flow, like the pericytes in the central nervous system. The latest advances in our understanding of the podocyte cytoskeletal apparatus have come from studies of monogenic forms of proteinuric diseases. The white writings show the proteins interacting or regulating the podocyte contractile apparatus along with the name of the syndromes that are caused by mutations in the corresponding genes.

the flow of the glomerular ultrafiltrate, activated intracellular signalling via tyrosine kinases and Rho kinases and induced cytoskeletal reorganization with enhanced recruitment of F-actin and α -actinin-4 at cell-cell contacts [15, 16]. Notably, prevention of shear stress-induced cytoskeleton reorganization by tyrosine kinase inhibitors caused podocyte loss [15, 16]. This finding provided the evidence that podocytes are mechanosensitive cells and that a fine regulation of the cytoskeleton is necessary to the podocyte in order to adapt to shear stress.

The function and regulation of the podocyte actin cytoskeleton

Upon polymerization, the soluble monomeric actin forms insoluble filaments with significant mechanical strength [17]. The processes involved in initiating a new filament from monomers or adding a branch to an existing filament is highly regulated. The actin-related protein (Arp) 2/3 complex is critical for the formation of branched filaments, while the formin family of proteins regulates the assembly of linear actin filaments [18]. Member 2 of the formin family, inverted formin 2 (INF2), inhibits actin polymerization in the podocyte by interacting with mammalian diaphanous-related formins (mDias), a Rho family of effectors that regulates actin dynamics [19].

In the apical membrane of foot processes, the actin cytoskeleton appears to be indirectly associated with the membrane glycoprotein podocalyxin through the adaptor proteins NHERF-1 and -2 and ezrin [9, 13]. Another transmembrane protein, glomerular epithelial protein-1 (GLEPP-1), a receptor tyrosine phosphatase, may regulate such interaction via phosphorylation events [20] (Figure 2).

At the slit diaphragm, multiple membrane proteins are connected to actin through a variety of adaptor and effector proteins. Nephlin, a major component of the slit diaphragm, binds the CD2-associated protein CD2AP, a cytoplasmic

adaptor protein which interacts with actin and the actin-binding proteins CapZ, cortactin and the actin polymerization complex Arp2/3 (Figure 2). This provides a signalling cascade from the slit diaphragm to the cytoskeleton (reviewed in [9] and [12]). The integral membrane protein podocin localizes to lipid microdomains in the slit diaphragm where it recruits nephrin and enhances nephrin signalling activity [12]. In lipid microdomains, podocin clusters and regulates the transient receptor calcium channel (TRPC)-6 [21] (Figure 2) and it has been suggested that this regulation gives the slit diaphragm the ability to act as a mechanosensor that enables the podocyte to remodel its cytoskeleton and contract its foot processes in response to mechanical stimuli. TRPC6 activation increases cytoplasmic Ca^{2+} levels, and TRPC6 overexpression leads to loss of actin stress fibres in cultured podocytes and to proteinuria in mice [22].

At the basal membrane of foot processes, the integrin transmembrane proteins link the GBM to the intracellular actin cytoskeleton through a set of integrin- and actin-associated proteins, which include paxillin, talin, vinculin, α -actinin-4 and filamin [23, 24]. Integrins also regulate actin dynamics in response to extracellular stimuli by signalling through integrin-linked kinase (ILK) and the Arp2/3 complex to modulate actin polymerization, cell shape and motility [24]. In addition to integrins, the GBM is connected with the podocyte actin cytoskeleton through α - and β -dystroglycan complex, originally defined as an adhesion system of skeletal muscle cells (Figure 2). Decreased dystroglycan expression has been observed in proteinuric experimental and human renal diseases [13], showing that podocyte-matrix contacts are important to maintain foot process structure and function.

Most of the available information on the role of actin cytoskeleton in podocyte biology comes from the identification of the causes of monogenic forms of the nephrotic syndrome. The large majority of disease-associated mutations affect the slit diaphragm protein nephrin [25], the transmembrane receptors and channels GLEPP-1 and TRPC6 [26, 27] and the intracellular proteins podocin, CD2AP, α -actinin-4, INF2 and Arhgap24 [28–33], which either interact with the actin cytoskeleton or are involved in regulating cytoskeletal dynamics (Figures 1 and 2).

The podocyte is a motile cell

Evidence is accumulating that the podocyte is a motile cell and its motility must be finely regulated in order to maintain the function of the GFB [12]. A constant movement of the foot processes along the GBM has been hypothesized in order to enable proteins entrapped in the GBM to be flushed back [12].

Studies in rodents documented the recruitment of podocytes differentiating from glomerular parietal epithelium towards the capillary tuft, which sustains the physiological low-degree podocyte renewal [34]. However, in progressive renal diseases, podocyte injury and loss exacerbates the migration and proliferation of podocyte precursors, which may pave the way to crescent formation and subsequent sclerosis [35].

Alterations in podocyte motility *in vitro* correlate with proteinuria *in vivo*. Podocyte-specific expression of the FSGS-associated α -actinin-4 mutant K256E reduced podocyte migration *in vitro* and caused proteinuria in mice [36]. On the other hand, a hypermotile podocyte phenotype, as induced by hyperactivation of $\alpha v \beta 3$ integrin through activation of the urokinase receptor uPAR or by knockdown of the

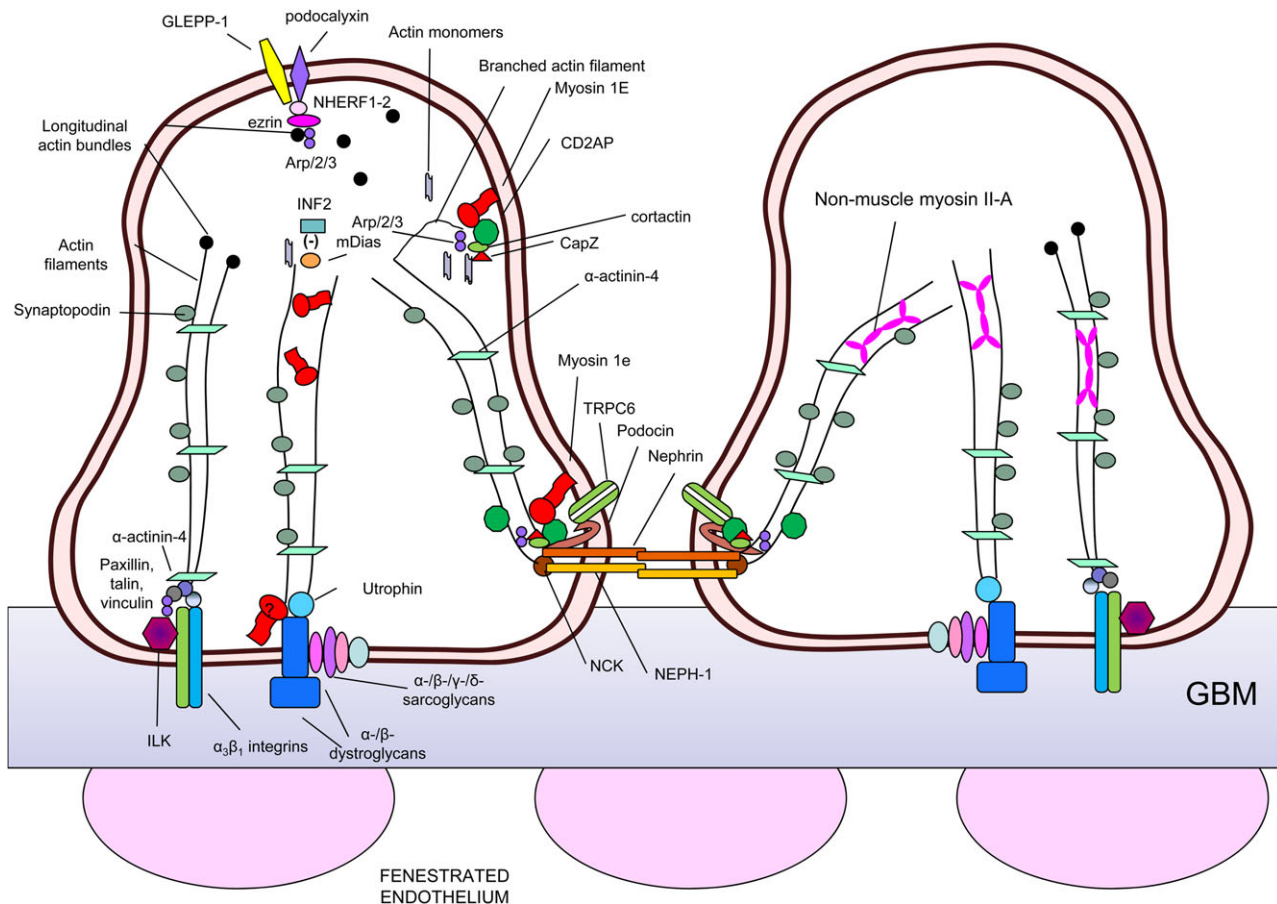


Fig. 2. Schematic representation of the podocyte foot process cytoskeleton organization with the proposed functions of myosin 1e (left) and non-muscle myosin II-A (NMII-A) (right). Only a subset of interactions is shown. In the apical membrane of foot processes, the membrane glycoprotein podocalyxin interacts with the actin cytoskeleton through the adaptor proteins NHERF-1 and -2 and ezrin. The receptor tyrosine phosphatase GLEPP-1 regulates such interaction. At the slit diaphragm, nephrin binds CD2AP and Nck, which interact with actin and the actin-binding proteins CapZ, cortactin and the actin polymerization complex Arp2/3. Podocin recruits nephrin and enhances nephrin signalling activity. Podocin also clusters and regulates the TRPC6. Synaptopodin binds to α -actinin-4 regulating its actin bundling activity. Inverted formin 2 (INF2) modulates actin polymerization by interacting with mammalian diaphanous-related formins (mDias). At the basal membrane of the foot processes, the integrin transmembrane proteins link the GBM to the intracellular cytoskeleton through paxillin, talin, vinculin and α -actinin-4. Integrins also regulate actin by signalling through ILK and Arp2/3. In addition to integrin, the GBM is connected with the podocyte actin cytoskeleton through utrophin and the α - and β -dystroglycan complex, which in turn is laterally associated with members of the sarcoglycan family. Myosin 1e (left), thanks to its strong affinity for membrane phospholipids, contributes to recruitment of CD2AP to the podocin–nephrin-rich lipid microdomains in the slit diaphragm. Myosin 1e cross-links actin filaments via its tail and by dynamic ATP-dependant dissociation-reassociation with actin, allows filopodia and lamellipodia formation and cell movement. Via indirect interaction with the CD2AP–CapZ–cortactin–Arp2/3 complex, myosin 1e could be involved in the polymerization of branched actin filaments. Non-muscle myosin II-A (NMII-A, right) interacts dynamically with F-actin to contract the cytoskeleton through the formation of bipolar filaments to maintain membrane tension and cell shape. At variance with Myo1e, NMII-A negatively regulates membrane ruffling and cell migration.

GTPase-activating protein Arhgap24 [33], has been associated with proteinuria in mice and with FSGS in humans [37, 38]. The above studies underline the importance of a fine-tuning of podocyte motility for glomerular permselectivity properties.

The obvious dependence of podocyte motility on the cytoskeleton begs the question of a possible role of non-muscle myosins in this podocyte function.

Non-muscle myosins

Myosins are molecular motors that power an assortment of movements in the cells by translocating cargo along actin filaments in an ATP-dependant manner. Members of the myosin superfamily, which includes at least 24 classes, are defined by the presence of a heavy chain with a conserved catalytic domain that in most myosins is followed by an α -helical light chain-binding region [39].

Class II myosins include the conventional myosins involved in muscle contraction, skeletal muscle myosins, cardiac myosin and the smooth muscle myosin, but also three ubiquitously expressed non-muscle myosins, NMII-A, NMII-B and NMII-C, whose heavy chains are encoded by MYH9, MYH10 and MYH14 genes, respectively [39, 40]. Each NMII molecule is composed of a pair of heavy chains and two pairs of light chains. The NMII heavy chain has a globular-shaped motor head domain at one end, containing the enzymatic activity that hydrolyses MgATP, followed by a neck participating in light chain binding [41] and by an α -helical tail responsible for both dimerization of two heavy chains and association of the myosin molecule into functional filaments (Figure 2, right). Such bipolar filaments can cross-link and exert force on actin filaments and are involved in a variety of cellular processes, including cell adhesion, cytokinesis and shape maintenance.

The unconventional non-muscle myosins make up all other myosin classes [39] and appear to be essential players

in the establishment and maintenance of cortical tension and related functions, such as motility, endocytosis and exocytosis [42]. Class I myosins have also been involved in actin polymerization and assembly through interactions with the Arp2/3 complex [43]. All Class I myosins contain a head motor domain that binds actin and ATP, a light chain-binding neck domain that binds one or more calmodulin light chains and a cargo-binding tail [42].

The non-muscle myosin network and its function in the podocyte

In 1988, by immunoelectron microscopy, Drenckhahn and Franke [44] provided the first evidence of the presence of myosin molecules in podocyte foot processes of rat and human kidneys. In a more recent study, exposure of a mouse podocyte line to biaxial cyclic stress revealed the formation of radial stress fibres showing an intense staining for non-muscle myosin [16]. Such an event was associated with reduction of the podocyte body size, while the processes became thin and elongated. Inhibition of Rho kinase disassembled stretch-induced radial stress fibres and altered cell morphology [16]. Myosins generate tension, and this study suggests that interaction between myosins and the actin cytoskeleton may be important to allow the podocytes to generate the contractile forces to resist the high intraluminal hydrostatic pressure and to actively change their own morphology.

More fine molecular characterization disclosed several members of the myosin superfamily that are expressed in podocytes. Nabet *et al.* [45] identified a subset of messenger RNAs that were enriched for transcripts encoding actin-binding proteins, which included those of several Class I myosins and of the Class II myosins, NMII-A, NMII-B. At the protein level, three Class I myosins, Myo1b, Myo1c and Myo1e, and NMII-A have been found in human podocytes [46], while NMII-B was localized in mesangial cells [47]. Notably, a recent report identified Myo1b, Myo1c, Myo1d and Myo1e in the podocyte slit diaphragm proteome [48].

Myo1c

Myo1c is a widely expressed Class I myosin that localizes at the cell periphery and plasma membrane, particularly at the leading edge of motile cells [49].

In kidney rat sections, Myo1c was localized in the podocyte body and in foot processes close to the slit diaphragm [50]. By immunoprecipitation of human podocyte cell lysates, an interaction between Myo1c and nephrin and the nephrin-binding protein Neph1 was documented [50]. Myo1c and Neph1 colocalized in rat glomeruli and cultured human podocytes, mainly enriched at the plasma membrane and cell junctions. Notably, membrane localization of both nephrin and Neph1 was significantly reduced in podocytes in which Myo1c was knocked down. Myo1c knockdown podocytes exhibited decreased cell migration and increased permeability to bovine serum albumin [50], which suggested a role of Myo1c in podocyte function. However, mutations in Myo1c have been found in patients with deafness without any renal defects [51], indicating that other non-muscle myosins may offset loss of Myo1c function in podocytes *in vivo*.

Myosin 1e

Myo1e is a long tail Class I myosin. In mammalian cells, Myo1e localizes to phagocytic cups [52], adherens-type in-

tercellular junctions [53] and clathrin-containing puncta at the plasma membrane [54]. The Myo1e tail binds to membranes via two sites, a primary site that binds negatively charged phospholipids via electrostatic interactions and a second low-affinity phosphoinositide-specific site [55]. The Myo1e tail also binds the endocytic proteins dynamin and synaptojanin involved in actin dynamics and expression of a dominant-negative Myo1e mutant in human fibroblasts led to defects in endocytosis [54]. Myo1e anchored to membrane phospholipids may help concentrate Myo1e-binding proteins at actin-rich regions of the membrane (Figure 2, left) [55].

Myo1e is expressed in a wide variety of tissues, including the spleen, small intestine, pancreas, brain, kidney and the immune system [56]. In human and mouse kidneys, Myo1e is predominantly expressed in the glomerulus, where it colocalizes with the podocyte-specific marker synaptopodin in podocytes, but not with the endothelial marker VE-cadherin [57, 58]. Myo1e labelling in mouse and human podocytes was found both in foot processes and cell bodies [58] and predominantly localized on the bottom surface of the plasma membrane, colocalizing with F-actin at lamellipodia tips [57, 58], which implicates Myo1e as a key component of the foot process cytoskeleton (Figure 2).

Interestingly, studies from our group showed that Myo1e partially colocalized with CD2AP at the cell membrane in cultured human podocytes [57]. CD2AP regulates actin dynamics by transferring the signalling cascade from the slit diaphragm to the cytoskeleton [9]. CD2AP knockdown in mouse podocytes disrupted actin cytoskeleton, reduced cell adhesion and spreading and disturbed the cell cycle [59]. We hypothesize that Myo1e, thanks to its strong affinity for membrane phospholipids, may have a critical role in recruiting CD2AP to the podocin–nephrin-rich lipid microdomains in the slit diaphragm (Figure 2). Whether Myo1e and CD2AP directly bind to each other or whether they indirectly interact through actin or actin-binding molecules remains to be established.

Myo1e is a motor protein that cross-links actin filaments via an ATP-insensitive binding site in its tail [60]. This cross-linking results in triphasic actin activated ATPase activity—binding of ATP to actomyosin complex, dissociation of myosin from actin and ATP cleavage, myosin re-association to the next binding site on actin—that allows filopodia and lamellipodia formation and cell movement [60]. In a scrape-wound assay, we found that over-expression of wild-type Myo1e increased the motility of human podocytes [57]. Conversely, Myo1e knockdown in human podocytes and Myo1E knockout in mouse podocytes impaired cell migration [57].

The critical role of Myo1e in renal physiology and pathophysiology is supported by findings that Myo1e genetic deficiency both in humans and in mice severely and selectively alters kidney morphology and function [57, 58]. By whole-genome linkage analysis and high-throughput sequencing of the area of positive linkage in a family with childhood-onset recessive nephrotic syndrome, followed by direct sequencing of an additional 52 unrelated patients, we identified two homozygous mutations in MYO1E gene segregating with the disease in two families [57]. One mutation causes replacement of alanine-159 with proline in the ATP-binding pocket close to the actin-binding domain of Myo1E. The other mutation causes protein truncation at tyrosine-695, at the start of the calmodulin-binding domain.

Both children with homozygous MYO1E mutations [57] and Myo1e knockout mice [58] developed high-level proteinuria early in life, followed by kidney inflammation and chronic renal disease. Like MYO1E-mutated patients, Myo1e-deficient mice exhibited signs of FSGS in the kidney with

thickened and disorganized GBM and effaced podocyte foot processes [57, 58].

Expression studies in cultured human podocytes documented that while wild-type recombinant Myo1e properly colocalized with F-actin and CD2AP at the plasma membrane, the FSGS-associated A159P mutant showed a cytoplasmic localization, associated with the shift of CD2AP–Myo1e complexes in the cytoplasm [57]. At variance with wild-type recombinant Myo1e, the A159P mutant failed to promote podocyte motility.

Notably, podocytes expressing wild-type Myo1e had a normal polygonal shape, like untransfected podocytes. Conversely, several podocytes expressing mutant A159P–Myo1e showed an abnormal morphology with rounded-up cells that tended to detach from the culture plate (C. Mele, P. Iatropoulos, S. Buelli, M. Noris, personal communication). Altogether these findings indicate that Myo1e function is important for maintaining podocyte structure, for podocyte motility and in regulating the crosstalk between podocytes and their matrix.

Non-muscle myosin II-A

NMII-A is a conventional non-muscle myosin expressed in most cells and tissues [41,61]. In the kidney, it is expressed in the glomerulus but also in tubular cells and endothelial cells of interlobular arteries and arterioles and peritubular capillaries.

NMII-A interacts dynamically with F-actin to contract the cytoskeleton and interacts statically with F-actin to maintain membrane tension and cell shape [41]; consequently, it is plausible that NMII-A dysfunction may alter the structure and function of the cytoskeleton in podocytes. NMII-A disruption by gene targeting or siRNA knockdown in mammalian cell lines causes pronounced defects in cellular contractility, focal adhesions and actin stress fibres organization [62]. Ablation of NMII-A in embryonic stem cells disrupt cell–cell adhesions and the normal localization of major junction proteins, such as E-cadherin, and β -catenin, which is relevant because the podocyte intercellular junction is molecularly similar to adherent junctions and tight junctions. Despite its role in cell contractility, NMII-A has been shown to negatively regulate cell migration. Indeed, NMII-A knockdown human fibroblasts and NMII-A knockout mouse embryonic stem cells displayed increased migration and exaggerated membrane ruffling associated with stabilization of microtubules [63]. It has been proposed that NMII-A continuously restrains random cell migration under normal conditions by cross-talk with the microtubule system. If this would apply to podocytes, we would speculate that NMII-A and Myo1e could exert concerted opposite effects on podocyte motility thus contributing to finely regulating this critical podocyte function.

Knockdown of NMII-A in zebrafish embryos resulted in malformation of the glomerular capillary tuft characterized by few and dilated capillaries of the pronephros. Endothelial cells failed to develop fenestrations, mesangial cells were absent or reduced and the GBM appeared non-uniformly thickened [64]. This study suggested an important role of NMII-A for the proper formation and function of the glomerulus in the zebrafish that is unrestricted to the podocyte.

Point mutations in the gene MYH9 encoding the NMII-A heavy chain NMMHC-IIA underlie autosomal-dominant syndromes. These syndromes, now referred to as MYH9-related diseases (MYH9-RDs), were formerly called May-Hegglin, Fechtner, Sebastian and Epstein syndromes [65] and manifest as macrothrombocytopenia, granulocyte inclusions, cataracts and sensorineural deafness. Patients with MYH9

mutations often develop proteinuria and progressive renal disease [66]. Electron microscopy of renal biopsies showed features of FSGS with focal and segmental foot process effacement [67].

The presence of a mutation in a single allele is sufficient to impair the normal properties of NMMHC-IIA, which requires the formation of bipolar filaments that in the heterozygous mutation carrier are probably composed of a mixture of heterodimers and homodimers of both the mutant and wild-type heavy chains. The position of mutations predicts the clinical manifestations of MYH9-related diseases. Indeed, the large majority of patients with mutations (such as R702C or R702H) in the motor domain of NMMHC-IIA present with severe thrombocytopenia and develop nephritis and deafness before the age of 40 years, while those with mutations in the tail domain have mild thrombocytopenia and only 10% of them develop nephritis and hearing impairment [66]. The R702C NMMHC-IIA mutant showed a striking reduction of *in vitro* ATPase activity and severely impaired ability of moving actin [68]. It is likely that in heterozygous R702C mutation carriers, the mutant protein copolymerizes with wild-type NMMHC-IIA and severely affects the function of resulting filaments, with a dominant-negative effect. On the contrary, mutations in the tail domain have been all predicted to hamper the NMMHC-IIA assembly into filaments. As a consequence, the capacity of mutant NMMHC-IIA to copolymerize with wild-type molecules into filaments is reduced and therefore their ‘poisoning effect’ on the normal protein is less severe.

At variance with humans, the location of the MYH9 mutations does not seem to influence the disease manifestation in mice. Three knockin mutant Myh9 mouse lines, each carrying one of the three most frequent mutations, found in patients (R702C in the motor domain, D1424N and E1841K in the tail domain) developed very similar haematological, eye and kidney phenotypes [69]. The earliest pathological changes were FSGS and global glomerulosclerosis, with altered podocyte architecture, typically foot process effacement. All these changes closely resemble those of patients with MYH9-RD supporting the association between MYH9 deficiency and podocyte abnormalities. Consistently, mice that were ablated for Myh9 exclusively in podocytes developed spontaneous glomerulosclerosis with foot process effacement and loss of the filtration slits [69].

Whether NMII-A is perturbed in non-syndromic FSGS is unknown. Interestingly, a recent case report [70] described a paediatric patient with idiopathic FSGS in whom proteinuria recurred within hours of deceased donor transplantation but who responded to plasmapheresis. Exposure of cultured podocytes to plasmapheresis effluent from this patient caused a rapid dissociation of NMII-A from actin fibres and diffuse distribution of NMII-A throughout the cytoplasm that paralleled actin depolymerization and the disappearance of the stress fibres in the centre of cells. These data would suggest that a circulating factor in idiopathic FSGS disrupts normal NMII-A function in podocytes [70].

The MYH9-APOL1 locus in chronic kidney disease

MYH9 gene variants have been recently involved in the development of more common forms of nephropathy. Mapping by admixture linkage disequilibrium—a genome-wide method to detect disease-causing gene variants—identified an haplotype (E1), including MYH9 intronic variants that confer risk for non-diabetic causes of end-stage renal disease (ESRD), FSGS and HIV-associated nephropathy in African American

patients [71,72]. A replication study in three European populations displayed an association between MYH9 single-nucleotide polymorphisms that are not part of the E1 haplotype and serum creatinine levels [73]. Sequencing of MYH9 has, however, failed to identify non-synonymous coding variants and the role of MYH9 in common renal disease remains controversial [74].

Two subsequent studies [75, 76] found that two coding sequence variants (G1 and G2) within the last exon of the APOL1 gene, which is adjacent to MYH9, were associated with both FSGS and non-diabetic causes of ESRD in African-American patients. In independent samples of African-American and Hispanic individuals, the same APOL1 risk variants were more strongly associated with non-diabetic causes of ESRD than were MYH9 variants [75, 77]. Crucially, none of the MYH9 variants remained significantly associated with renal disease after adjusting for APOL1 variants [76, 77]. APOL1 encodes apolipoprotein L1, an HDL-associated protein that plays a main role in the human innate immune response against many *Trypanosoma* species [78] that cause sleeping sickness. Interestingly, only sera of individuals with at least one copy of the APOL1 G1 or G2 mutations were able to lyse the *Trypanosoma Brusei Rhodiense* that is endemic in West Africa. It has been hypothesized that APOL1 mutations spread in West Africa owing to selection pressure resulting from the adaptive advantages conferred by protection against trypanosomiasis [78].

Conclusions

The discovery of several non-muscle myosins in the podocytes with a predominant localization at the foot process and the slit diaphragm, and MYO1E and MYH9 mutations in FSGS patients (Figure 1) highlighted the role of myosins in regulating podocyte structure and function. Non-muscle myosins appear to be involved in transferring the signalling cascade from the slit diaphragm to the cytoskeleton, in podocyte-matrix interaction and in regulating podocyte motility (Figure 2). Further studies will be necessary to dissect the cross-talk among the different non-muscle myosins that converge on the podocyte actin cytoskeleton.

Interestingly, most patients with MYO1E mutations responded to the calcineurin inhibitor cyclosporine A (CsA) [57]. There is evidence that CsA may stabilize the actin cytoskeleton of the podocyte by blocking calcineurin-induced synaptopodin dephosphorylation [23, 79]. Thus, discovery of the effects of non-muscle myosin in podocyte physiology and pathophysiology underscores the potential of cytoskeletal-stabilizing agents for treatment of FSGS.

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Conflict of interest statement. None declared.

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