Journal of Population Therapeutics & Clinical Pharmacology

REVIEW ARTICLE
DOI: 10.47750/jptcp.2023.30.16.045

A Review on the Role of Actin Cytoskeleton genes of Podocytes in Childhood Nephrotic Syndrome

Pricilla Charmine[1], Vettriselvi Venkatesan[2], Sangeetha Geminiganesan[3], Bollam Rangaswamy Nammalwar[4], Mohanapriya Chinambedu Dandapani[5]*

[1]Research Scholar , Faculty of Clinical Research, Sriher

[2]Professor, Department of Human Genetics, Sriher

[3]Assistant Professor, Department of Pediatric Medicine, Sriher

[4]Senior Pediatric Nephrologist, Dr.Mehta's Children Hospital

[5]Assistant Professor, Department of Human Genetics, Sriher

***Corresponding author:** C.D. Mohana Priya, Assistant Professor, Department of Human Genetics, Sriher, Email: mohanapriya@sriramachandra.edu.in

Submitted: 24 April 2023; Accepted: 10 May 2023; Published: 02 June 2023

ABSTRACT

Podocytes play an essential role in establishing the glomerular filtration barrier. Podocyte dysfunction is central to the underlying pathophysiology of many common glomerular diseases, including nephrotic syndrome (NS), which often incites a progression to chronic kidney disease, affecting millions of patients worldwide. The role of podocytes in the pathogenesis of NS is best characterized by the discovery of genetic mutations, many of which regulate the actin cytoskeleton. Podocytes rely on an intact actin cytoskeleton to stabilise their unique cellular architecture and functions such as motility, cell division, intracellular transport, cellular trafficking of cargo and organelles and cell junction formation for the sustained function of glomerular filtration. This review briefly highlights the recent findings on role of actin cytoskeleton and the actin-associated proteins that take part in the assembly, maintenance and disassembly of actin cytoskeleton.

Keywords: *Nephrotic syndrome; Podocyte; Actin cytoskeleton; Actin-associated proteins*

INTRODUCTION

Nephrotic syndrome (NS) is considered to be a primary podocytopathy, indicating that podocyte loss or dysfunction is the central event in the development of NS[1]. Podocytes are highly differentiated epithelial cells with a unique cellular architecture consisting of a large cell body and interdigitating foot processes that not only enwrap glomerular capillaries but also interlink neighboring podocytes via slit diaphragms.[2,3] Together with a fenestrated endothelium and glomerular basement membrane (GBM), podocytes form part of the glomerular filtration barrier (GFB).[4] All the three components of GFB play an active role in preventing plasma proteins from entering the urinary ultrafiltrate, thus preventing proteinuria. Foot processes are not only involved in the formation of the filtration barrier, but are also essential for increasing the surface, thereby mediating efficient attachment to the GBM.[5] Podocyte has many unique properties that are critical to its participation in ultrafiltration. Since podocyte form an integral component of GFB, any injury to podocyte cause GFB to be disrupted, resulting in proteinuria and glomerular disease.

J Popul Ther Clin Pharmacol Vol 30(16):e330–e341; 19 June 2023.

Retraction and simplification of the podocyte foot process network, termed as foot process effacement in which they lose their structure and become diffuse, spreading out and leading to a reduction in filtration barrier function is a common feature in all glomerular diseases.[6]

Podocytes can be injured in many forms of human and experimental glomerular disease, including minimal change disease, focal segmental glomerulosclerosis (FSGS), membranous glomerulopathy, diabetic nephropathy, and lupus nephritis.[1,7] Podocyte injury is initially reversible. As long as the podocytes are just effaced and not lost, they display a remarkable capacity to regenerate foot processes within a short period of time, as occurs in minimal change disease, the classical example of the potential of podocytes to recover.[8,9] A decrease of podocyte number represents another form of injury that contributes to the loss of the glomerular function and leads to progressive renal failure. This can be caused by podocyte apoptosis, detachment from GBM and/or the inability to proliferate.[9,10]. Podocytes are terminally differentiated cells with no regenerative capacity. Therefore, a decline in podocyte number beyond a critical threshold leads to glomerular disease progression in human beings as well as experimental rodent models.[9,10]

Based on the molecular pathology of podocytes, at least four major causes of foot process effacement and proteinuria can be identified: (i) changes in the slit diaphragm complex and its spatiotemporal organization by lipid rafts,[2,3] (ii) interference with the GBM or the podocyte-GBM interaction,[11] (iii) reorganization of the actin cytoskeleton and its associated proteins[12] and (iv) modulations of the negatively charged apical membrane domain of podocytes.[13]

Podocyte cytoskeleton

Actin dynamics has emerged at the forefront of podocyte biology as actin cytoskeleton not only provide the structural framework for podocytes but are also essential for a variety of cellular functions such as motility, cell division, intracellular transport, cellular trafficking of cargo and organelles and cell junction formation. In order to form an actin filament, cells not only initiate actin polymerization (nucleation) but also maintain the filament by controlling depolymerization.[12,14] Beyond nucleation and maintenance of the filament, controlled branching and cross-linking are also essential to generate a complex structure such as lamellipodia.[15,16] at the leading edge which pulls the cell forward or propels a membrane protrusion. Intermediate filaments (IF), microtubules (MT), and microfilaments or actin fibres are the three major components of the eukaryotic cytoskeleton found in podocytes. Actin fibres are mostly found in foot processes, while intermediate filaments and microtubules are localized mainly in the cell body, and the primary processes.[12,17] All the three cytoskeletal components are in functional and physical contact with each other. Early pathological alterations in podocytes involve an active rearrangement of the FP actin cytoskeleton and the reorganization of the slit diaphragm, leading to FP effacement.[18] Effacement is thought to be due to a breakdown in the actin cytoskeleton of the foot processes, which is a complex contractile apparatus that allows podocytes to be dynamic in nature and reorganize themselves rapidly.

Vimentin, desmin, and nestin are the intermediate filament proteins expressed in mature podocytes.[17] Intermediate filament proteins that are expressed in mature podocytes include vimentin, desmin and nestin.[17] The presence of vimentin in differentiated podocytes underlines their mesenchymal features. IF fibers confer stability to the cell body that is constantly floating in the filtrate and exposed to enormous mechanical stress.[17] Podocyte foot processes consist of cortical actin filaments and actin-associated proteins, such as myosin, α-actinin and synaptopodin, which ensure the dynamic maintenance and reorganization of the cytoskeleton.

Foot processes can be divided into three domains: the apical membrane domain, the slit diaphragm and the basal membrane domain which is in contact with the GBM.[17] All three domains are physically linked to the FP actin cytoskeleton. The cortical actin network binds with specialised proteins of the slit diaphragm (especially nephrin, podocin, and NEPH1) at specific tethering points, beneath the plasma membrane of the foot process. In addition, FPs also express large macromolecular complexes called focal adhesions which bind the podocyte cytoskeleton to the extracellular matrix of glomerular structure and prevent their detachment.[19]

The slit diaphragm membrane, which links the foot processes, controls the ultrafiltration of

molecules by signaling to the actin cytoskeleton within the foot processes. Along with actin cytoskeleton, other structural components of the podocyte such as vimentin-rich intermediate filaments and microtubules, all contribute towards cell shape and rigidity. Microtubules regulate cell motility, vesicular transport, cell polarity, and organization and positioning of the membrane organelles. Actin and microtubules crosstalk enables changes at the slit diaphragm to be conveyed to the nuclei via microtubule-associated proteins, and enables the podocyte to respond to signals from the foot processes.[20] Although all the three components are essential for podocytes, actin cytoskeleton clearly dominates the research field in podocyte biology.

By signalling to the actin cytoskeleton within the foot processes, the slit diaphragm membrane, which connects the foot processes, controls the ultrafiltration of molecules. Other key structural components of the podocyte, such as vimentin-rich intermediate filaments and microtubules, all contribute to cell shape and rigidity in addition to the actin cytoskeleton. Microtubules control cell motility, vesicular transport, cell polarity, and membrane organelle arrangement and placement.

Role of actin cytoskeleton

Actin cytoskeleton that constantly undergoes polymerization and severing, shows a rather exclusive localization within secondary processes[15] (Schell and Huber, 2017) [15]. Actin filaments are made up of globular actin monomers and ATP is required for the efficient polymerization.[21] The actin cytoskeleton is known to be modulated by a number of polymerizing and severing proteins. The synthesis of actin dimers and trimers, which is the rate-limiting step for filament extension.[16] As soon as actin trimers are formed, they are rapidly added to the growing actin filament.

At the front of the cell, actin filaments are organized into branching and cross-linked structures known as lamellipodia. Filopodia are protrusions of aligned actin bundles that extend from the front of the cell allowing for directional movement. Actin-generated forces are necessary for maintaining cell shape and for cell motility. Actin polymerization produces pushing forces, whereas the sliding of actin filaments along myosin II filaments produces pulling forces.[16] Actin polymerization into filaments is tightly controlled by actin-binding proteins.[22]

Changes in actin dynamics appear to be a major driver in modifying podocyte morphology and glomerular permeability, since actin-mediated cell plasticity is a key feature of normal podocyte function. The function of the podocyte is based on the maintenance of highly ordered, parallel contractile actin filament bundles in foot processes. Podocyte injury can be caused by the reorganisation of the foot process actin cytoskeleton leading to podocyte foot process effacement and slit diaphragm disruption.

The podocyte foot processes are structurally shaped by an extensive actin cytoskeleton.[23] The foot processes are endowed with a microfilament-based contractile apparatus composed of actin, myosin-II, α-actinin, talin, paxillin, and vinculin, and are anchored to the GFB via an α3β1-integrin complex.[24] Disruption of the cytoskeleton is known to cause podocyte foot process effacement and fusion of filtration slits leading to slit diaphragm dysfunction, followed by proteinuria. Therefore, maintenance of the actin cytoskeleton and actin cytoskeletal dynamics are thus critical for normal podocyte function and preservation of the integrity of the GFB.

Genes involved in the actin cytoskeleton

Several genetic loss or gain-of-function models affecting the cytoskeleton and recapitulating hereditary human disorders highlight the importance of the podocyte actin cytoskeleton and podocyte–GBM interactions in the development of foot process effacement.[25] (Table :1).

TABLE 1: Actin-associated genes /proteins in podocytes

Genes	Actin-associated protein	Actin-related functions
ACTN4	α-Actinin-4	Bundling of actin fibers; crosslink actin filaments through its N-terminal actin-binding domain comprised of two calponin homology domains; link actin filaments to Z-lines in striated muscles; link focal adhesions to actin cytoskeleton; crosslink stress fibers; interact with

		other proteins such as β1-integrin, synaptodpodin, phosphatidylinositol 3-kinase and vinculin.
ANLN	Anillin	Scaffold protein linking RhoA with actin
ARHGAP 24	Rho GTPase-activating protein 24	Rho-activated GAP for Rac
ARHGDI A	Rho GDP-dissociation inhibitor α (RhoGDIα)	Inhibition of RhoGTPase activation
AVIL	Advillin	Ca^{2+} regulated actin-binding protein
CTNNA1	α-Catenin D	Adaptor for actin filaments and other actin-associated proteins; regulation of actin polymerization and suppression of actin branching.
	β/γ-Catenin	Anchorage of cell-cell contacts to actin filaments; communication between cell junctions and gene expression in the nucleus
CD2AP	CD2 associated protein	Modulating podocyte TGFβ response to prevent a proteolytic program that would culminate in decreased levels of essential cytoskeletal components such as dynamin and synaptopodin; Organization of cell-cell adhesions; anchorage of cell-cell adhesions to actin filaments.
CDC42	Cdc42	RhoGTPase; induction of filopodia formation.
CFL1	Cofilin-1	Severing of actin filaments; induction of actin branching
CTTN	Cortactin	Formation of branched cortical actin networks.
EZR	Ezrin	Linkage of actin to the cell surface; organization of cell-cell adhesions.
FAT1	Fat cadherin 1	Connects slit diaphragm and actin cytoskeleton.
FAK	Focal adhesion kinase	Signal mediator at focal contacts that regulates cell adhesion and actin organization.
INF2	Inverted formin 2	Elongate actin filaments at a rapid rate; actin-related protein 2/3 (Arp2/3) complex form branched actin networks; Acceleration of actin polymerization and depolymerization.
KANK1 KANK2 KANK	Kidney ankyrin repeat-containing protein	Regulates actin polymerization
RAPH1	Lamellipodin	Promotion of lamellipodia formation; regulation of focal adhesion dynamics.
MYO1E	Nonmuscle membrane-associated class I myosin	Non-muscle myosin generates tension by binding F-actin, which in turn generates the contractile forces that help the glomerular capillaries to resist the high intraluminal hydrostatic pressure.
NCK1/2	Nck1/2	Adaptor protein at cell-cell and cell-matrix contacts that regulates cell signaling and actin organization; stabilization of RhoA and actin stress fibers.
NPHS1	Nephrin	Phosphorylation of tyrosine residues of nephrin by Fyn allows binding of Nck adapter proteins, leading to the nucleation and polymerization of actin filaments.
PAK1/2	p21-activated kinase	Lamellipodia and protrusion formation; downstream target of Rac1 and Cdc42.
PXN	Paxillin	Cell-matrix adhesion; anchorage of focal contacts to actin filaments.
PLCG1	PLCγ1	Regulation of RhoGTPases and actin dynamics.
PLCε1	Phospholipase Cε1	PLC-gamma 1 binds to the actin-cytoskeleton (phosphotyrosine and F-actin) directly via its C-terminal SH2 domain.
PODXL	Podocalyxin	Modulates actin cytoskeleton
NPHS2	Podocin	Mechanosensing protein linking plasma membrane to actin cytoskeleton.
RAC1	Rac1	RhoGTPase; induction of lamellipodia formation.
RHO	RhoA	RhoGTPase; induction of stress fiber formation

SYNPO	Synaptopodin	Actin bundling; regulates RhoA activity; promoting the production of actin stress fibres; inhibition of lamellipodia and filopodia formation; Actin-associated protein for foot process motility.
TLN1	Talin	Cell-matrix adhesion; anchorage of focal contacts to actin filaments.
TNS2	Tensin2	Linkage of actin to integrins; organization of the cortical actin cytoskeleton; regulation of cell migration.
TPM1	Tropomyosin	Regulation of actin-myosin interactions in sarcomeric contraction; regulation of actin dynamics and cell migration.
TPRC6	Transient receptor potential channel6	Regulates Ca^{2+} signaling for mechanosensation Activates RhoA and Rac1.
WASL	N-WASP (Neural Wiskott-Aldrich syndrome protein)	Promotion of actin nucleation.

Nephrin

Nephrin, encoded by NPHS1, is perhaps the most important of the slit diaphragm proteins. Nephrin is a transmembrane protein with eight extracellular immunoglobulin domains, a fibronectin III domain, and an intracellular domain with several tyrosine residues.[26] Phosphorylation of these tyrosine residues by Fyn allows binding of Nck adapter proteins, leading to the nucleation and polymerization of actin filaments. Once the mature intercellular junctions have been formed, phosphorylation ceases and nephrin signaling stops, confirming a functional relationship between podocyte intercellular junction-associated proteins and actin cytoskeleton dynamics.[27] Nck adapter proteins also interact with N-WASP or p21-activated kinases (PAKs) to regulate the actin cytoskeleton.[28,29] PAKs, along with other molecules downstream of nephrin-like phospholipase cγ1 and phosphoinositide 3-OH kinase (PI3K), are thought to interact with Rac1 (and Cdc42 in the case of PAK) to mediate actin reorganization.[30]

Nephrin phosphorylation is thought to have a further role in actin dynamics through the regulation of lamellipodia formation, utilizing its ability to assemble protein complexes that can bind actin together into broad three dimensional structures that resemble lamellipodia.[3,32] Phosphorylation of nephrin via the Src family may contribute to the induction of rapid changes in the organization of the actin during the development of FP effacement.[27] It has also been shown to reverse the impact by depolymerizing actin with cofilin, an actin-depolymerizing protein having a natural role in the recycling of actin within podocytes effect by depolymerizing actin using cofilin, an actin-depolymerizing protein that has a natural role within podocytes in the recycling of actin.[32]

ACTN4

One of the first discovered cytoskeletal linked proteins causing specific podocyte dysfunction was actinin-4 (ACTN4).[33] α-Actinin-4 expressed in podocytes plays a key role in the maintenance of podocyte architecture. α-Actinin-4 is one of the actin crosslinking proteins in podocytes, and gain of function mutations have been associated with formation of aggregated and rapidly degraded cytoskeletal proteins. The putative function of α-actinin-4, is to crosslink actin filaments through its N-terminal actin-binding domain comprised of two calponin homology domains. α-Actinin is also required to link actin filaments to Z-lines in striated muscles.[34] where they crosslink stress fibers. Besides, α-actinin-4 interacts with a number of other proteins such as β1-integrin,[35] synaptodpodin,[36] vinculin[37] and phosphatidylinositol 3-kinase.[38]

Phosphorylation by focal adhesion kinase (FAK),[39] phosphoinositides binding,[38] and to intracellular calcium sensitivity,[40] may change the actin-binding properties and localization of α-actinin following various environmental stimuli. Interestingly, mutations in ACTN4 cause a wide range of functional impairments ranging from decreased protein stability,[41] gain-of-function, subcellular mislocalization, and increased filamentous actin (F-actin) affinity and binding capacity.[42] Aberrant sequestering of K256E α-actinin-4 reduced the mean number of actin-rich peripheral projections, impairs podocyte spreading, motility, and reduces the number of peripheral projections.[24] These alterations translate into impaired cytoskeletal dynamics characterized by decreased cellular spreading and

attenuation of podocyte migration.[24] Such intrinsic cytoskeletal derangements may underlie initial podocyte damage and foot process effacement encountered in ACTN4-associated FSGS.

CD2-AP

The slit-diaphragm is anchored to the podocyte by adaptor proteins (CD2-AP, podocin, α-actinin-4). Recombinant mice deficient in CD2-AP,[43] podocin,[44] or α-actinin-4,[45] all develop significant proteinuria, although typically less severe than in nephrin-deficient mice. Thus, defects in podocyte-slit-diaphragm proteins lead to massive proteinuria, suggesting that the podocyte-slit diaphragm complex is critical to maintaining the GFB.

CD2AP is recruited by Rac1 to regulate cell–cell contacts in the podocyte.[46] RhoA has been found to be involved in a signalling pathway mediated by CD2AP, despite the fact that the other Rho GTPases have not been shown to have direct interactions with CD2AP.[47] An elegant study demonstrated that lack of CD2AP promoted upregulation of cytosolic cathepsin L (CatL) via translocation of dendrin to the nucleus, resulting in the proteolysis of synaptopodin, dynamin, and RhoA, which regulate the actin cytoskeleton.[47] Furthermore, CD2AP has been shown to play a role in modulating podocyte TGFβ response in order to prevent a proteolytic program that would culminate in decreased levels of essential cytoskeletal components such as dynamin and synaptopodin.[15] A similar cellular phenotype was reported in the case of missense mutations of ARHGAP24, a small GTPase modulator which is specifically expressed in podocytes resulted in increased membrane ruffling dynamics, a phenotype which is thought to partially reflect podocyte foot process effacement in vitro.[48]

Transient receptor potential-6 channel (TRPC6)

Transient receptor potential-6 channel (TRPC6) is a Ca^{2+}-permeable nonselective cation channel at the slit diaphragm that interacts with nephrin and podocin. Mutations in TRPC6 result in late-onset autosomal dominant FSGS, which, interestingly, mostly results in a pathological increase in calcium influx.[49] TRPC-mediated calcium influx has been shown to promote RhoA activity and inhibits podocyte migration. Excessive calcium influx associated caused by TRPC6 mutations may render the podocyte too "stiff" to respond to cues in the environment, resulting in stress fiber and cytoskeleton disorganization and cell death.[50] TRPC6 also allow the slit diaphragm to detect membrane stretch and respond to this by remodeling the actin cytoskeleton to a contractile state.[51]

Synaptopodin

Synaptopodin, an actin-binding protein is highly expressed in differentiated podocytes.[52] Synaptopodin interacts with α-actinin and regulates the actin-bundling activity of α-actinin.[36] Synaptopodin also interacts directly with CD2AP and MAGI-1 (membrane-associated guanylate kinase), two adaptor proteins that link cell surface receptors to the actin cytoskeleton at the slit-diaphragm and the basement membrane, respectively.[53] Besides providing a physical linkage to the actin cytoskeleton, synaptopodin promotes the production of actin stress fibres which is essential for the stabilization of the cytoskeleton by positively regulating RhoA activity, via Smurf1-mediated ubiquitination of RhoA, thereby preventing the targeting of RhoA for proteasomal degradation.[36,54] **Kemeny et al. (1997)**[55] reported a loss of synaptopodin expression in a renal biopsy specimen from patients with FSGS. This observation was confirmed by a study of 13 children with FSGS, in whom synaptopodin expression was absent from areas of sclerosis and weak in nonsclerotic glomeruli.[56]

Formins

Inverted formin 2 (INF2), a member of the formin protein family, is another cytoskeletal gene promote actin filament assembly by accelerating filament nucleation and elongation and by blocking filament capping.[57] INF2 can also accelerate F-actin depolymerization and filament severing.[58] Mutations in the INF2 gene are a common cause of familial FSGS. In general, INF2 promotes actin polymerization by staying associated with the growing barbed end of an actin filament and by directly binding mammalian diaphanous-related formin (mDia).[57] Majority of INF2 mutations are found in the autoinhibitory region (diaphanous inhibitory domain), implying increased activity of this protein promotes glomerular dysfunction.[59] INF2 interacts with the actin-modifying proteins

profilin and CAPZ, whereas INF2 mutations disrupt these interactions.[60]

Rho GTPases

The small GTPases of the Rho family (RhoA, Rac1, Cdc42) are the most important proteins in regulating actin dynamics. For example, Rac1 can stimulate Arp2/3 and enhance the production of branching actin, as well as induce actin polymerization at cell–cell junctions to reinforce cell–cell contacts.[61] Rac1 activates p21-activated kinase (PAK), which phosphorylates and activates Lim kinase (LIMK). Activated LIMK mediates cofilin phosphorylation and inhibits actin filament depolymerization, thus limiting the amount of actin turnover and increasing stress fiber formation.[62] The Rho GTPases cycle between two distinct conformational states: they are active when bound to GTP and inactive when bound to GDP.[63] The complex interplay between these small GTPases and their tightly controlled spatial and temporal distribution play a significant role in modifying the podocyte cytoskeleton and cell–cell adhesion. RhoA has a role in the initial events of protrusion and is activated at the cell edge synchronous with edge advancement, whereas Cdc42 and Rac1 are activated just behind the cell edge 40 seconds later than RhoA, and activate pathways implicated in the reinforcement and stabilization of newly expanded protrusions.[64]

Rho GTPases activate two different kinds of molecules that directly stimulate actin polymerization, WASP/ WAVE proteins and Diaphanous-related formins (DRFs). WASP/WAVE proteins induce actin polymerization via the Arp2/3 complex, which stimulates the formation of a new actin filament branching off an existing filament.[65] Cdc42 binds directly to WASP and N-WASP and stimulates their activation of the Arp2/3 complex.[66] The ability of WASP proteins to stimulate actin polymerization is also regulated by phosphorylation and by protein–protein interactions, for example they bind via their proline-rich region to the Src homology 3 (SH3) domain of cortactin, a well known regulator of both actin dynamics and endocytosis.[66,67]

The DRFs (Dia1, Dia2 and Dia3) stimulate the nucleation and extension of non-branching actin filaments. They bind as dimers at the barbed (or plus) end of actin filaments, preventing the binding of capping proteins, which normally terminate actin polymerization.[68] Each DRF is activated by a distinct subset of Rho GTPases.

RhoGTPases are essential for the organization of the actin cytoskeleton and promote specialized actin structures such as stress fibers (RhoA), lamellipodia (Rac1) and filopodia (Cdc42).[69] Both integrins and dystroglycans are coupled via various adapter molecules to the podocyte cytoskeleton, allowing the transfer of mechanical stress from the extracellular matrix to the FP actin cytoskeleton.[18] (Figure ;1)

Fig.1 Rho Signaling Pathway

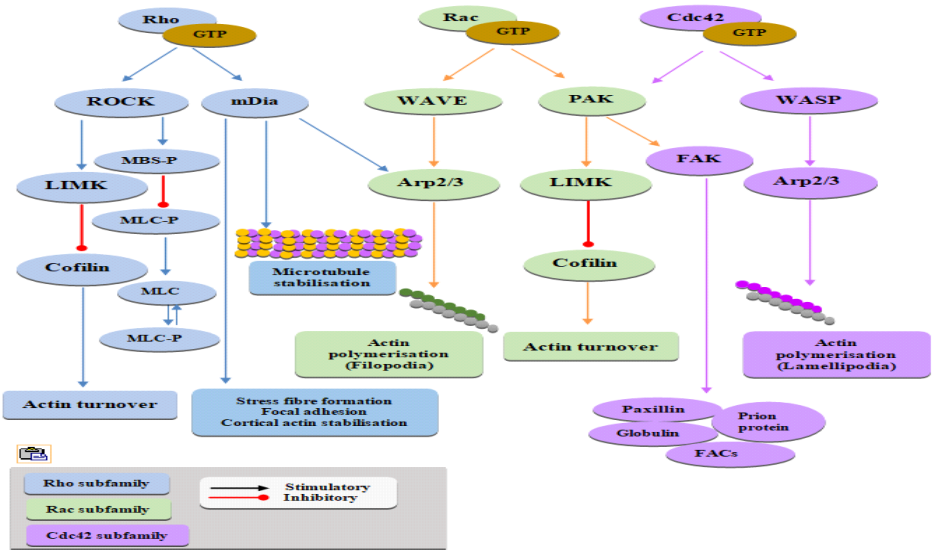

FIGURE 1: Rho Signalling Pathway

J Popul Ther Clin Pharmacol Vol 30(16):e330–e341; 19 June 2023.

Neph1

Nephrin-Neph1 complex transduces phosphorylation-mediated signals that assemble an actin polymerization complex at the podocyte intercellular junction. Upon engagement, Neph1 is phosphorylated on specific tyrosine residues by Fyn, which results in the recruitment of Grb2, an event that is necessary for Neph1-induced actin polymerization at the plasma membrane. Importantly, Neph1 and Nephrin directly interact and, by juxtaposing Grb2 and Nck1/2 at the membrane following complex activation, cooperate to augment the efficiency of actin polymerization.[70]

Podocalyxin

Podocalyxin's C-terminal binding motif (DTHL) interacts with Na^+/H^+ exchanger regulator proteins (NHERF1 and NHERF2) and actin-binding protein ezrin, and can modify the cytoskeleton through these interactions.[71] When podocalyxin is knocked down, podocytes develop abnormal cell morphology. Knockout of the PODXL gene in mice results in loss of foot processes and loss of the slit diaphragm entirely causing a phenotype of anuric renal failure, omphalocele, and perinatal death.[72] A reported loss-of-function mutation in PODXL has been reported clinically with a very similar phenotype to the one observed in PODXL knockout mice.[73]

Focal adhesions

In podocytes, focal adhesions link the GBM to the actin cytoskeleton of foot processes and are under significant tensile and shear stress forces. To withstand these forces, the actin cytoskeleton in focal adhesions flows in a retrograde direction in the vicinity of the podocyte membrane in contact with the GBM.[74] In addition, actin is arranged in linear filaments (stress fibers) in the vicinity of focal adhesions and actin filaments are also cross-linked by myosin II and α-actinin, allowing for increased podocyte adhesion to the GBM via actomyosin contractility.[74] Key components of focal adhesions that link the cell exterior to the FP actin array include integrins and GTPases.

MYO1E

One of the essential crosslinkers is Myo1e, a non-muscle class I myosin, whose gene mutations underlie FSGS.[75] Without appropriate actin binding, actin and myosin dynamics are impaired, leading to ineffective contractions. Actin by itself is inert and needs appropriate bundling and coupling with myosin to form the contractile apparatus that provides the mechanical force needed for movement. The primary role of non-muscle myosin is to generate tension by binding F-actin, which in turn generates the contractile forces that help the glomerular capillaries to resist the high intraluminal hydrostatic pressure.[75] Since actin needs myosin for movement, any mutations affecting myosin can cause diseases which alter the actin cytoskeleton. Most notable in regards to podocytes are mutations in the MYH9 gene, which encodes non-muscle myosin class II isoform A, and MYO1E, which is a membrane-associated class I myosin.[75]

Anillin and Advillin

Proteins anillin, coded on the ANLN gene, and advillin, coded on AVIL gene, are both F-actin-binding proteins needed for podocyte motility and both have mutations that are associated with kidney disease.[77,78] Besides, anillin and advillin also interact with CD2AP and PLCε1, respectively, of the slit diaphragm,[77] to regulate and coordinate motility while maintaining the filtration barrier.[78]

Dynamin

Dynamin-actin interactions promote GTP-dependent dynamin oligomerization, which releases a capping protein gelsolin from the barbed ends resulting in potent actin polymerization from the fast, growing barbed ends which in turn lead to focal adhesion maturation.[79]

CONCLUSIONS

The above review will not only improve our understanding of the molecular mechanisms regulating podocyte actin dynamics and the events underlying podocytopathies but also identify the potential areas for the discovery of novel therapeutic targets to treat glomerular diseases.

CONFLICT OF INTEREST

The authors report no conflicts of interest and they are responsible for the content and writing of this article.

ACKNOWLEDGEMENT

We would like to acknowledge the funding agency DBT (Biocare scheme),Ministry of Health New Delhi (Project:No.102/IFD/SAN/135/2019-2020) for supporting the research work.

REFERENCES

1. Kopp JB, Anders HJ, Susztak K, Podestà MA et al. Podocytopathies. Nat Rev Dis Primers. 2020;6(1):68.
2. Huber TB, Benzing T. The slit diaphragm: a signaling platform to regulatepodocyte function. Curr Opin Nephrol Hypertens 2005;14:211–216.
3. Garg P. A review of podocyte biology. Am J Nephrol. 2018;47:3–13.
4. Daehn IS, Duffield JS. The glomerular filtration barrier: a structural target for novel kidney therapies. Nat Rev Drug Disc. 2021;20(10):770–788.
5. Lennon R, Randles MJ, Humphries MJ. The importance of podocyte adhesion for a healthy glomerulus. Front Endocrinol. 2014;5:160–1177.
6. Kriz W, Lemley KV. A potential role for mechanical forces in the detachment of podocytes and the progression of CKD. J Am Soc Nephrol. 2015;26:258–269.
7. Somlo S, Mundel P. Getting a foothold in nephrotic syndrome. Nat Genet. 2000;24:333–335.
8. Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohno K, Holzman LB, Wiggins RC: Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. J Am Soc Nephrol 2005;16:2941–2952.
9. Trimarchi H. Podocyturia: What is in a name?. J Transl Intern Med 2015;3(2), 51–56.
10. Campbell KN, Tumlin JA. Protecting Podocytes: A key target for therapy of focal segmental glomerulosclerosis. Amer J Nephrol. 2018; 47 (1) 14–29.
11. Greka A, Mundel P. Cell biology and pathology of podocytes. Annu Rev Physiol. 2012; 74:299–323.
12. Blaine J, Dylewski J. Regulation of the actin cytoskeleton in podocytes. Cells 2020;9(7): 1700.
13. Orlando RA, Takeda T, Zak B, Schmieder S, Benoit VM, McQuistan T, Furthmayr H, Farquhar MG (2001) The glomerular epithelial cell anti-adhesin podocalyxin associates with the actin cytoskeleton through interactions with ezrin. J Am Soc Nephrol 12:1589–1598.
14. Pollard TD and Borisy GG. (2003). Cellular motility driven by assembly and disassembly of actin filaments. Cell;112(4), 453–465.
15. Schell and Huber, 2017) [20]. Schell C, Huber,TB. (2017). The evolving complexity of the podocyte cytoskeleton. J Amer Society Nephrol 28(11):3166–3174.
16. Svitkina T. The actin cytoskeleton and actin-based motility. Cold Spring Harb. Perspect. Biol. 2018;10(1):a018267.
17. Faul, 2014. The podocyte cytoskeleton: Key to a functioning glomerulus in health and disease. Liu Z-H, He JC (eds): Cell biology and pathology of podocyte. Contrib Nephrol. Basel, Karger, 2014, vol 183, pp 22–53.
18. Oh J, Reiser J, Mundel P. Dynamic (re)organization of the podocyte actin cytoskeleton in the nephrotic syndrome. Pediatr Nephrol (2004) 19:130–137.
19. Perico L, Conti S, Benigni A, Remuzzi G. Podocyte-actin dynamics in health and disease. Nat Rev Nephrol. 2016;12:692–710.
20. Kim JH, Konieczkowski M, Mukherjee A, Schechtman S, Khan S, Scheling JR, Ross MD, Bruggeman LA, Sedor JR. Podocyte injury induces nuclear translocation of WTIP via microtubule-dependent transport. J Biol Chem. 2010;285:9995–10004.
21. Campellone KG, Welch MD. A nucleator arms race. Cellular control of actin assembly. Nat Rev Mol Cell Biol. 2010;11:237–251.
22. Lee S.H., Dominguez R. Regulation of actin cytoskeleton dynamics in cells. Mol. Cells. 2010;29:311–325.
23. Ransom RF, Lam NG, Hallett MA et al. Glucocorticoids protect and enhance recovery of cultured murine podocytes via actin filament stabilization. Kidney Int 2005; 68: 2473–2483.
24. Michaud JLR, Chaisson KM, Parks RJ, Kennedy CRJ. FSGS-associated a-actinin-4 (K256E) impairs cytoskeletal dynamics in podocytes. Kid Intern. 2006;70:1054–1061.
25. Machuca E, Benoit G, Antignac C: Genetics of nephrotic syndrome: connecting molecular genetics to podocyte physiology. Hum Mol Genet 2009;18:R185–R194.
26. Patrakka J, Tryggvason K. New insights into the role of podocytes in proteinuria. Nat Rev Nephrol 2007;5:463-8.

27. Verma R, Kovari I, Soofi A. et al. Nephrin ectodomain engagement results in Src kinase activation, nephrin phosphorylation, Nck recruitment, and actin polymerization. J Clin Invest. 2006;116:1346–1359.
28. Jones N, Blasutig IM, Eremina V, Ruston JM, Bladt F, Li H, Huang H, Larose l, Li SS, Takano T, Quaggin SE, Pawson T. NCK adaptor proteins link nephrin to the actin cytoskeleton of kidney podocytes. Nature 2006;440:818–823.
29. Zhu J, Attias O, Aoudjit L, Jiang R, Kawachi H, Takano T: p21-activated kinases regulate actin remodeling in glomerular podocytes. Am J Physiol Renal Physiol. 2010;298:F951– F961.
30. Zhu J, Sun N, Aoudjit L, Li H, Kawachi H, Lemay S, Takano T. Nephrin mediates actin reorganization via phosphoinositide 3-kinase in podocytes. Kidney Int. 2008;73: 556-566.
31. Venkatareddy M, Cook L, Abuarquob K, Verma R, Garg P. Nephrin regulates lamellipodia formation by assembling a protein complex that includes Ship2, filamin and lamellipodin. PLoS One 2011;6:e28710.
32. Garg P, Verma R, Cook L, Soofi A, Venkatareddy M, George B, Mizuno K, Gurniak C, Witke W, Holzman LB: Actin-depolymerizing factor cofilin-1 is necessary in maintaining mature podocyte architecture. J Biol Chem. 2010;285:22676–22688.
33. Kaplan JM, Kim SH, North KN, Rennke H, Correia LA, Tong HQ, Mathis BJ, Rodrguez-Pe´rez JC, Allen PG, Beggs AH, Pollak MR. Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. Nat Genet. 2000;24:251–256.
34. Gautel M, Djinović-Carugo K. The sarcomeric cytoskeleton: from molecules to motion. J Exp Biol. 219(2):135–145.
35. Otey CA, Carpen O. Alpha-actinin revisited: a fresh look at an old player. Cell Motil Cytoskeleton 2004; 58: 104–111.
36. Asanuma K, Kim K, Oh J et al. Synaptopodin regulates the actin-bundling activity of alpha-actinin in an isoform-specific manner. J Clin Invest 2005;115: 1188–1198
37. Rajfur Z, Roy P, Otey C et al. Dissecting the link between stress fibres and focal adhesions by CALI with EGFP fusion proteins. Nat Cell Biol 2002; 4: 286–293.
38. Fraley TS, Tran TC, Corgan AM et al. Phosphoinositide binding inhibits alpha-actinin bundling activity. J Biol Chem. 2003;278: 24039–24045.
39. Izaguirre G, Aguirre L, Hu YP et al. The cytoskeletal/non-muscle isoform of alpha-actinin is phosphorylated on its actin-binding domain by the focal adhesion kinase. J Biol Chem. 2001; 276: 28676–28685
40. Furukawa R, Maselli A, Thomson SA et al. Calcium regulation of actin crosslinking is important for function of the actin cytoskeleton in Dictyostelium. J Cell Sci 2003; 116: 187–196.
41. Bartram MP, Habbig S, Pahmeyer C, et al. Three-layered proteomic characterization of a novel ACTN4 mutation unravels its pathogenic potential in FSGS. Hum Mol Genet. 2016;25:1152–1164.
42. Weins, A. Schlondorff JS, Nakamura F, Denker BM, Hartwig JH, Thomas P. Stossel TP, Martin R. Pollak MR. Disease-associated mutant α-actinin-4 reveals a mechanism for regulating its F-actin-binding affinity. Proc Natl Acad Sci USA. 2007;104:16080–16085.
43. Shih NY, Li J, Karpitskii V, Nguyen A, Dustin ML, Kanagawa O, Miner JH, Shaw AS Congenital nephrotic syndrome in mice lacking CD2-associated protein. Science. 1999;286:312–315.
44. Roselli S, Heidet L, Sich M et al. Early glomerular filtration defect and severe renal disease in podocin-deficient mice. Mol Cell Biol. 2004;24:550–560.
45. Kos CH, Le TC, Sinha S, et al. Mice deficient in alpha-actinin-4 have severe glomerular disease. J Clin Invest. 2003;111:1683–1690.
46. Van Duijn TJ, Anthony EC, Hensbergen PJ, Deelder AM, Hordijk PL: Rac1 recruits the adapter protein CMS/CD2AP to cell-cell contacts. J Biol Chem. 2010;285:20137–20146.
47. Yaddanapudi S, Altintas MM, Kistler AD, Fernandez I, Moller CC, Wei C, et al: CD2AP in mouse and human podocytes controls a proteolytic program that regulates cytoskeletal structure and cellular survival. J Clin Invest 2011;121:3965–39.
48. Akilesh S, Suleiman H, Yu H, Stander MC, Lavin P, Gbadegesin R, Antignac C, Pollak M, Kopp JB, Winn MP, Shaw AS: Arhgap24 inactivates Rac1 in mouse podocytes, and a mutant form is associated with familial focal segmental glomerulosclerosis. J Clin Invest. 2011;121:4127–4137.
49. Dryer SE, Reiser J. TRPC6 channels and their binding partners in podocytes: role in glomerular filtration and pathophysiology. Am J Physiol Renal Physiol. 2010;299:F689–F701.
50. Tian D, Jacobo SM, Billing D et al. Antagonistic regulation of actin dynamics and cell motility by TRPC5 and TRPC6 channels. Sci Signal 2010;3: ra77.
51. Huber TB, Schermer B, Benzing T. Podocin organizes ion channel-lipid super complexes: implications for mechanosensation at the slit diaphragm Nephron Exp Nephrol. 2007;106: e27-e31.
52. Mundel P, Heid HW, Mundel TM et al. Synaptopodin: an actin-associated protein in

telencephalic dendrites and renal podocytes. J Cell Biol. 1997; 139: 193–204.
53. Patrie KM, Drescher AJ, Welihinda A et al. Interaction of two actin-binding proteins, synaptopodin and alpha-actinin-4, with the tight junction protein MAGI-1. J Biol Chem. 2002; 277: 30183–30190.
54. Asanuma K, Yanagida-Asanuma E, Faul C, Tomino Y, Kim K, Mundel P. Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling. Nat Cell Biol. 2006;8:485–491.
55. Kemeny E, Durmuller U, Nickeleit V, Gudat F, Mihatsch MJ (1997) Distribution of podocyte protein (44 KD) in different types of glomerular diseases. Virchows Arch. 431:425–430.
56. Srivastava T, Garola RE, Whiting JM, Alon US. Synaptopodin expression in idiopathic nephrotic syndrome of childhood. Kidney Int. 2001;59:118–125.
57. Hegsted, A., Yingling, C. V., & Pruyne, D. (2017). Inverted formins: A subfamily of atypical formins. Cytoskeleton 74(11), 405–419.
58. Chhabra ES, Higgs HN. INF2 is a WASP homology 2 motif-containing formin that severs actin filaments and accelerates both polymerization and depolymerization. J Biol Chem. 2006;281(36):26754–67.
59. Sun H, Schlondorff J, Higgs HN, Pollak MR: Inverted formin 2 regulates actin dynamics by antagonizing Rho/diaphanous-related formin signaling. J Am Soc Nephrol. 2013; 24:917–929.
60. Rollason R, Wherlock M, Heath JA, Heesom KJ, Saleem MA, Welsh GI. Disease causing mutations in inverted formin 2 regulate its binding to G-actin, F-actin capping protein (CapZ alpha-1) and profilin 2. Biosci Rep. 2016;36:e00302.
61. Pollard TD. Regulation of actin filament assembly by Arp2/3 complex and formins. Annu Rev Biophys Biomol Struct. 2017;36:451–77.
62. Tang DD, Gerlach BD. The roles and regulation of the actin cytoskeleton, intermediate filaments and microtubules in smooth muscle cell migration. Respir Res. 2017;**18,** 54.
63. Welsh GI , Saleem MA. The podocyte cytoskeleton - key to a functioning glomerulus is health and disease. Nat Rev Nephrol. 2011;8:14-21.
64. Machacek M, Hodgson L, Welch C, Elliott H, Pertz O, Nalbant P, Abell A, Johnson GL, Hahn KM, Danuser G. (2009). Coordination of Rho GTPase activities during cell protrusion. Nature. 461(7260):99–103.
65. Forget MA, Desrosiers RR, Gingras D, Béliveau R. Phosphorylation states of Cdc42 and RhoA regulate their interactions with Rho GDP dissociation inhibitor and their extraction from biological membranes. Biochem J. 2002;361(2):243-54.
66. Millard TH, Sharp SJ. Machesky LM. Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. Biochem. J. 2004;380(1):1–17.
67. Martinez-Quiles N, Ho HY, Kirschner MW, Ramesh N, Geha RS. Erk/Src phosphorylation of cortactin acts as a switch on-switch off mechanism that controls its ability to activate N-WASP. Mol Cell Biol. 2004;24(12):5269-80.
68. Kovar DR. Molecular details of formin-mediated actin assembly. Curr Opin Cell Biol. 2006;18(1):11–17.
69. Etienne-Manneville S, Hall A (2002) Rho GTPases in cell biology. Nature 420:629–635.
70. Garg P, Verma R, Nihalani D, et al. Neph1 cooperates with nephrin to transduce a signal that induces actin polymerization. Mol Cell Biol. 2007;27:8698–8712.
71. Nielsen JS, McNagny KM. The role of podocalyxin in health and disease. J Am Soc. Nephrol. 2009;20(8):1669–1676.
72. Doyonnas R, Kershaw DB, Duhme C, Merkens H, Chelliah S, Graf T, McNagny KM. Anuria, omphalocele, and perinatal lethality in mice lacking the CD34-related protein podocalyxin. J Exp Med. 2001;194(1):13–27.
73. Kang HG, Lee M, Lee KB, Hughes M, Kwon BS, Lee S, McNagny KM., Ahn YH, Ko JM, Ha IS, et al. Loss of podocalyxin causes a novel syndromic type of congenital nephrotic syndrome. Exp. Mol. Med. 2017;49(12):e414.
74. Sever S, Schiffer M. Actin dynamics at focal adhesions: A common endpoint and putative therapeutic target for proteinuric kidney diseases. Kidney Int. 2018;93(6):1298–1307.
75. Mele C, Iatropoulos P, Donadelli R, Calabria A, Maranta R, Cassis P, Buelli S, Tomasoni S, Piras R, Krendel M, et al. MYO1E mutations and childhood familial focal segmental glomerulosclerosis. N. Engl. J. Med. 2011;365(4):295–306.
76. Pecci A, Ma X, Savoia A, Adelstein RS. MYH9: Structure, functions and role of non-muscle myosin IIA in human disease. Gene. 2018;664:152–167.
77. Gbadegesin RA, Hall G, Adeyemo A, Hanke N, Tossidou I, Burchette J, Wu G, Homstad A, Sparks MA, Gomez J. et al. Mutations in the gene that encodes the F-actin binding protein anillin cause FSGS. J. Am. Soc. Nephrol. 2014;25(9):1991–2002.
78. Hall G, Lane BM, Khan K, Pediaditakis I, Xiao J, Wu G, Wang L, Kovalik ME, Chryst-Stang M, Davis EE. et al. The human FSGS-causing ANLN R431C mutation induces dysregulated PI3K/AKT/mTOR/Rac1 signaling in

podocytes. J Am Soc Nephrol. 2018;29 (8):2110–2122.
79. Gu C, Yaddanapudi S, Weins A, Osborn T, Reiser J, Pollak M, Hartwig J, Sever, S. Direct dynamin-actin interactions regulate the actin cytoskeleton. EMBO J. 2010;29(21):3593–3606.