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In Focus

Fifteen years of research on nephrin: what we still need to know

Min Li[1], Silvia Armelloni[1], Alberto Edefonti[2], Piergiorgio Messa[3] and Maria Pia Rastaldi[1]

[1]Renal Research Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico & Fondazione D'Amico per la Ricerca sulle Malattie Renali, Milano, Italy, [2]Division of Pediatric Nephrology and Dialysis, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy and [3]Division of Nephrology, Dialysis, and Renal Transplant, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy

Correspondence and offprint requests to: Maria Pia Rastaldi; E-mail: mariapia.rastaldi@policlinico.mi.it

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In March 1998, the seminal work of Tryggvason's group was published [1], in which the protein mutated in congenital nephrotic syndrome of the Finnish type was discovered and termed 'nephrin'.

In the manuscript, besides identifying the mutations causative of the disease, the authors described for the first time the predicted molecular structure of nephrin, a transmembrane protein of the immunoglobulin superfamily. The protein was formed by an N-terminal signal peptide, followed by an extracellular domain containing eight Ig-like modules and one fibronectin type III-like module, and had a single transmembrane domain and an intracellular C-terminal domain. The extracellular part could be potentially heavily glycosylated and presented binding sites for heparan sulphate.

The authors concluded that the protein was 'likely to be an adhesion receptor and a signalling protein. The cytosolic domain contains nine tyrosines, some of which could become phosphorylated during ligand binding of nephrin' [1].

These initial data were confirmed by subsequent analyses which demonstrated that nephrin behaves as a signalling hub at the slit diaphragm, by binding to other slit diaphragm proteins and scaffolding molecules that transduce signals from phosphorylated nephrin to activate different intracellular pathways [2]. Fifteen years of research efforts have unequivocally established that nephrin is essential to glomerular filtration and to the health of podocyte foot processes.

The extracellular domain of nephrin contains free cysteines that allow formation of disulphide bonds with adjacent molecules. *Cis* and *trans* homophilic and heterophilic interactions of nephrin with itself and with Neph family proteins (Neph1, Neph2 and Neph3) are required to provide stability and maintain the health of the slit diaphragm [3–5]. Among the Ig-like molecules that form the slit diaphragm, the prominent importance of nephrin is not only confirmed by the fact that nephrin mutations are associated with the most severe forms of nephrotic syndrome, but also by the reduction in nephrin observed in numerous experimental and human glomerular diseases. In our experience, nephrin appears to be altered or down-regulated at the very first stages of almost all types of proteinuric diseases (Figure 1), when no changes \of other podocyte proteins, such as podocin, can be detected.

Likely, one of the best indirect proofs of the importance of nephrin in mammals is its absence in birds. Compared with mammalian glomeruli, the avian ones have larger slit diaphragms [6], and the genome of birds does not contain a coding sequence for nephrin, while expressing the other Neph family members [7]. Interestingly, birds excrete nitrogen mainly in the form of uric acid, which is not completely soluble in water and therefore requires a significant amount of proteins to be maintained in a colloidal suspension in the urine, forming the so-called urine spheres. These proteins need to pass the glomerular filtration barrier and nephrin absence seems to guarantee the necessary glomerular leakage. Ultimately, proteins are not lost thanks to a process of reabsorption in the lower colon.

Nephrin is an expression-restricted protein and a part from glomerular podocytes can be found in a few other mammalian cell types, such as neuronal cells, lymphocytes, testis cells and pancreatic β cells [8–11]. Recently, a role for nephrin has been proposed in the development of cardiac vessels [12].

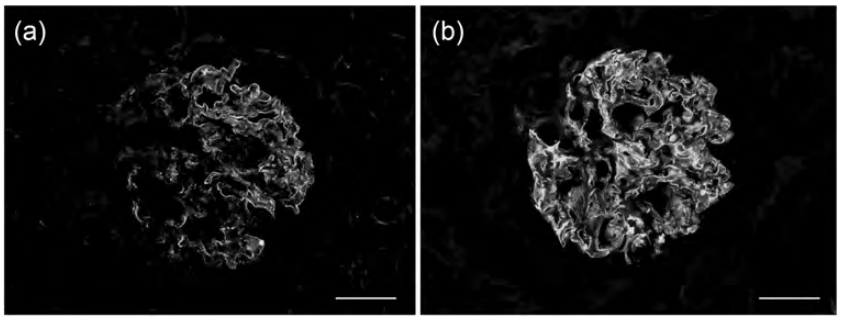

FIGURE 1: The same glomerulus from a case of minimal-change glomerular disease displays segmental loss of nephrin (**a**), but intact podocin staining along the tuft (**b**). Indirect immunofluorescence—magnification ×400, scale bar 50 μm.

The expression in neuronal cells is of particular interest, because the nephrin orthologues in *Caenorhabditis elegans* (Syg-2) and *Drosophila melanogaster* (Hibris) are crucial players in synapse targeting and positioning [13, 14], suggesting that, evolutionarily speaking, the original function of nephrin is that of a synaptic adhesion molecule.

Since its discovery, the neuronal expression of nephrin has been repetitively acknowledged [8, 15, 16]. Compared with the expression observed during development and at birth, in the adult rodent central nervous system [17], nephrin extends to the pons, but is reduced in the hippocampus. Adult rodents also display a diffuse presence of nephrin in basal ganglia and motor cortex, but complete negativity of the sensory cortex, suggesting the involvement of nephrin in distinct brain networks related to movement. The association of nephrin with movement activities is further confirmed by its presence in the Purkinjie cells of the cerebellum, and helps to explain the ataxic symptoms of nephrin-deficient mice, when their survival is prolonged by re-expressing nephrin only in the kidney [18].

The presence of nephrin in the central nervous system strongly supports a series of recognized similarities between podocytes and neuronal cells, which have been recently confirmed by an expression analysis conducted on both maturing and adult podocytes [19]. Podocytes and neurons are highly ramified post-mitotic cells characterized by specialized adhesion structures, the slit diaphragm in podocytes and the synapse in neuronal cells. Of note, the cytoplasmic insertion site of the slit diaphragm and the postsynaptic density of neurons are both lipid rafts, that is membrane regions of TritonX-100-resistant electron-dense material enriched in sphyngolipids, cholesterol and signalling proteins, such as nephrin [17].

In both podocytes and neuronal cells, the nephrin cytoplasmic domain can be phosphorylated by the Src family kinase Fyn [17, 20]. Interestingly, Fyn knockout mice not only show proteinuria, but also display alteration of long-term potentiation and spatial learning [21].

Phosphorylation is important for raft-mediated nephrin internalization and is an event needed for podocyte foot process development and maintenance, as demonstrated by the finding that phosphorylated nephrin recruits adaptor proteins such as Nck1/2, Grb2 and Crk1/2, resulting in the assembly of protein complexes that regulate actin polymerization [22].

Podocyte foot processes, as well as dendritic spines in neuronal cells, highly depend for their function on a dynamic actin cytoskeleton, and actin dynamics are influenced by nephrin in various manners. In fact, nephrin can recruit other actin-associated proteins like nWASp, Arp2/3 and, importantly, the regulatory p85 subunit of PI3 kinase [22]. Activated PI3K converts the plasma membrane lipid phosphatidylinositol-4,5-bisphosphate [PI(4,5)P2] to phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P3], which can regulate the activity of the actin filament-severing protein cofilin, inducing actin polymerization and maintaining a branched actin network.

It is well known that actin polymerization is a dynamic process that needs to be kept in a tight balance. Very recent data started shedding some light on this process by showing the involvement of Slit2-Robo2 activity in inhibiting nephrin-induced actin polymerization [23].

Phosphorylation of nephrin can also lead to the recruitment, phosphorylation and activation of phospholipase C γ1 (PLCγ1), which can trigger calcium signalling [22].

Detailed understanding of calcium signalling in podocytes constitutes a rapidly growing field of investigation, particularly after the discovery that mutations of the transient receptor potential calcium channel TRPC6 cause a genetic form of focal segmental glomerulosclerosis. Increased calcium entrance in podocytes, due to the gain of function TRPC6 mutations, or to increased expression of the channel in acquired forms of nephrotic syndrome, leads to podocyte damage [24].

TRPC6 has been shown to interact with podocin [25], whereas another potent calcium channel, the ionotropic NMDA glutamate receptor (NMDAR), directly interacts with nephrin [17]. Imbalances of NMDAR activity, either the blockade or an excessive activation, are known to be harmful to neuronal cells, and the same is true for podocytes. Sustained activation of the NMDAR by its specific agonist results in oxidative stress leading to apoptotic cell death [26]. Similarly, blockade of NMDAR by the specific antagonists norketamine and MK-801 increases albumin loss in mice and humans, and causes profound remodelling of the actin–myosin podocyte cytoskeleton and disappearance of nephrin from podocyte cell processes [27].

First analyses by the group of Tryggvason provided information on the nephrin promoter, showing consensus sequences for the transcription factors GATA-1, GATA-2, NF-1 and AP-2 in the immediate region preceding the start of

human nephrin transcription [28] and identifying potential transcription factor recognition sites which are conserved between human and mouse, such as GATA-1, GATA-2, AP4, Ets-1, NFAT, deltaEF1 and MZF1 binding sites [16].

Subsequent studies have shown that the nephrin gene can be regulated by the transcription factors WT1, Sp1 and Snail [29–31], have implicated the transcription factor PTf1a in nephrin expression in the central nervous system [32] and have described response elements for retinoic receptors and vitamin D receptor in the rodent nephrin promoter [33].

In this issue of *NDT*, Ristola *et al.* identify the transcription factor GABP as a positive regulator of nephrin expression. Interestingly, GABP has been shown to cooperate with Sp1 to increase responsiveness to retinoic receptors in myeloid cells [34], and is known to regulate genes involved in the formation of the neuromuscular junction, such as utrophin [35].

Despite this evidence, we are still far from having a complete picture of the precise sequence of events which control nephrin transcription in development, maintain nephrin expression in healthy podocytes and intervene in nephrin changes during disease. Furthermore, other questions remain unanswered, such as the role played by two described variants of nephrin, one found in the kidney that lacks the transmembrane domain [36] and one in the brain that lacks the extracellular signalling domain [16].

Therefore, research on nephrin is far from concluded and additional information is certainly required to gain complete knowledge on nephrin properties and its role in podocytes as well as in other cell types.

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CONFLICT OF INTEREST STATEMENT

The manuscript has not been submitted to any other journal.

(See related article by Ristola *et al.* Regulation of nephrin gene by the Ets transcription factor, GA-binding protein. *Nephrol Dial Transplant* 2013; 28: 846–855.)

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Inflammation from dialysis, can it be removed?

Steven G. Achinger[1]
and Juan Carlos Ayus[2,3]

Correspondence and offprint requests to: Juan Carlos Ayus; E-mail: carlosayus@yahoo.com

[1]Department of Nephrology, Watson Clinic, LLP – Lakeland, FL, USA,
[2]Renal Consultants of Houston, Houston, TX, USA and
[3]Nephrology and Medicine, Hospital Italiano, Buenos Aires, Argentina

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ABSTRACT

Mortality among hemodialysis patients remains unacceptably high in the USA, especially among newly diagnosed end-stage renal disease patients. Chronic inflammation is a risk factor for cardiovascular disease among HD patients. It has been shown that complications of the arteriovenous (AV) access are not just limited to overt infectious complications but they may also pose a threat as a haven for occult infection and can aggravate the chronic inflammatory state. This inflammatory state is characterized by failure to thrive, erythropoietin-resistant anemia, hypoalbuminemia, elevated plasma C-reactive protein levels, which are well-known risk factors for increased morbidity and mortality on dialysis. In this issue, Wasse *et al.* presents a paper that demonstrates in a large cohort that failed AV grafts are associated with increased chronic inflammatory markers. They have provided a