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An update: the role of Nephrin inside and outside the kidney

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Nephrin is a key molecule in podocytes to maintain normal slit diaphragm structure. Nephin interacts with many other podocyte and slit diaphragm protein and also mediates important cell signaling pathways in podocytes. Loss of nephrin during the development leads to the congenital nephrotic syndrome in children. Reduction of nephrin expression is often observed in adult kidney diseases including diabetic nephropathy and HIV-associated nephropathy. The critical role of nephrin has been confirmed by different animal models with nephrin knockout and knockdown. Recent studies demonstrate that knockdown of nephrin expression in adult mice aggravates the progression of unilateral nephrectomy and Adriamycin-induced kidney disease. In addition to its critical role in maintaining normal glomerular filtration unit in the kidney, nephrin is also expressed in other organs. However, the exact role of nephrin in kidney and extra-renal organs has not been well characterized. Future studies are required to determine whether nephrin could be developed as a drug target to treat patients with kidney disease.

Nephrin, podocytes, kidney, slit diaphragm, proteinuria, cell signaling pathway

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The filtration property of the kidney localizes to the glomerular capillary wall, which forms a specialized filter that allows the passage of small plasma molecules, including water and waste products, from the circulating blood into the primary urine for excretion. This filtration barrier consists of three layers: the fenestrated endothelium, glomerular basement membrane (GBM) and podocytes. Podocytes enclose the outer aspect of the glomerular capillary wall and is the final barrier to filtration. Cytoplasmic extensions of the podocytes, called foot processes (FP), interdigitate each other from adjacent podocytes. Specialized cell-cell junctions between the FP, called slit diaphragms (SD), form a zipper-like structure that functions as a sieve to restrict the passage of large molecules.

Nephrin, an integral transmembrane protein of the immunoglobulin superfamily, is the first protein identified in the SD in 1998 [1]. Nephrin has crucial functions both in controlling the podocyte SD structure and as a hub of signaling pathways. Although many years' research efforts have been made, we are still far from having a complete understanding of nephrin's role in podocyte as well as in other cell types. In this paper, we will review research progress related to the role of nephrin both inside and outside of the kidney.

1 Molecular structure of nephrin

NPHS1, gene that code Nephrin, was first cloned in 1998 by Kestilä and colleagues [1]. *NPHS1* is assigned to the long arm of chromosome 19, 19q13.1, with a telomere-to-centromere orientation [1,2]. The size of *NPHS1* gene is 26 kb and it contains totally 29 exons. Northern hybridization and *in situ* hybridization suggest that *NPHS1* is mostly localized in the glomerulus but also found in the brain, pancreas, testis, heat, spleen and lymphoid tissues.

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Nephrin is a transmembrane protein with 1,241 residues and a calculated molecular weight of 135 kD without post-translational modifications [1]. Nephrin composes of an extracellular domain containing eight IgG-like modules and one fibronectin type III-like motif, a single transmembrane domain and a short intracellular domain containing nine tyrosines. The IgG-like domains are of type C2 which is typically found in proteins participating in cell-cell [3–5] and cell-matrix interactions [6]. Nephrin has two cysteine residues in each IgG-like domain, which can form disulfide bridges between Nephrin molecules or with other proteins [1,7], leading to either homophilic interaction of Nephrin molecules or heterophilic interactions of Nephrin with other SD proteins.

In sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, Nephrin runs as a 185–200 kD protein doublet suggesting posttranslational modifications [8,9]. In the extracellular domain of Nephrin there are 10 potential sites for N-glycosylation [1] and glycosylation of Nephrin is critical for its proper folding and localization in the plasma membrane [10].

Several spliced variants of Nephrin have been identified in both rats and humans. Nephrin-α, MW 166 kD, lacks the nucleotides 3167–3286 of exon 24, which codes the transmembrane spanning domain, produces a truncated soluble form of Nephrin. The function of Nephrin-α is still unknown [11,12].

2 Location of Nephrin in the glomerulus

In kidney, Nephrin exists exclusively at the filtration areas of the podocyte foot processes in glomerulus [13]. Nephrin molecules were proposed to interact in the slit through homophilic and heterophilic interactions of Nephrin with itself and with Neph family proteins forming a porous substructure in the SD. Nephrin mRNA was first detected in the late S-shaped bodies during the kidney development. Nephrin mRNA also shows a typical splicing pattern apparently regulated differently in various disease entities [11]. Nephrin mRNA has a very unique regulation by natural antisense mRNA [14] and bidirectional regulation with a closely related molecule, filtrin [15].

Nephrin is also expressed in pronephric glomerulus of zebrafish and medaka in which Nephrin needs to be integrated to the membrane before the formation of the SD and moving to the proper site to form the SD [16]. Nephrin is absent in the slit diaphragms of birds. Birds have larger SD as compared with mammalian glomeruli [17], and the genome of birds does not contain a coding sequence for Nephrin [18]. Birds excrete nitrogen mainly in the form of uric acid, which is not completely soluble in water and requires a certain amount of proteins to be maintained in a colloidal suspension in the urine. Due to the absence of Nephrin, proteins could pass the glomerular filtration barrier. This is an indirect evidence to support the importance of Nephrin in mammals.

3 Regulation of Nephrin expression

3.1 Interaction with the other SD proteins

Loss of Nephrin does not affect podocyte viability, suggesting that Nephrin can be compensated by other podocyte molecules during glomerular development [19]. Nephrin can interact with the cytoskeleton through several proteins, but the relationship between Nephrin and the other podocyte molecules has not been fully demonstrated.

3.1.1 CD2 adaptor protein (CD2AP)

CD2AP is an intracellular protein initially found in T-lymphocyte molecule CD2. In glomerulus, CD2AP is also located at the SD and it can interact with Nephrin via its C-terminal domain [20,21]. CD2AP is an adaptor protein that is also important for the maintenance of the SD. The N-terminal domain of CD2AP can bind to p85 and facilitate the Nephrin-induced AKT signaling [22], which protects podocytes from apoptosis. CD2AP enhanced the small ubiquitin-related modification of CIN85, which increase the binding of CIN85 to Nephrin [23], induces Nephrin ubiquitination and endocytosis [24].CD2AP knockout ($CD2AP^{-/-}$) mice are born healthy but develop a rapid-onset nephrotic syndrome at three weeks of age and die of renal failure at six weeks [21,25]. Kidneys from $CD2AP^{-/-}$ mice initially exhibit normal Nephrin localization, but with aging the FP become effaced [26] and deficiency of CD2AP leads to a loss of expression of the SD protein Nephrin in podocytes.

3.1.2 Podocin

Podocin has been shown to be expressed only in the podocytes, and serve as a scaffolding protein in the SD complex. Podocin is an integral membrane protein with both N and C-terminal domains directed into cytosol. Podocin associates via its C-terminal domain with cytoplasmic part of Nephrin at the specialized lipid raft microdomains of the plasma membrane [27,28]. *NPHS2*, the gene that codes podocin, is mutated in autosomal recessive steroid-resistant nephrotic syndrome and has a similar phenotype of CNF. Podocin-deficient mice also develop proteinuria during ear-

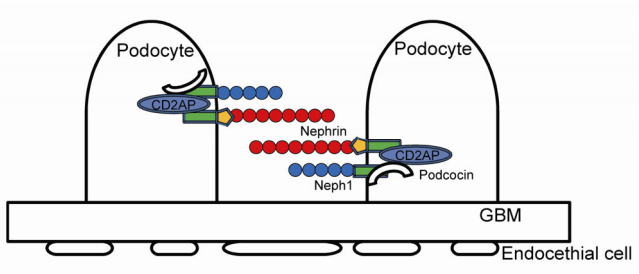

Figure 1 (color online) Location of Nephrin in podocyte.

ly antenatal period, and die in the five weeks after birth. Electron microscopy showed fusion of foot processes and massive mesangial sclerosis with vastly reduced Nephrin expression [29]. Podocin can recruit Nephrin into lipid rafts, connect via its C-terminal domain with CD2AP and then activate mitogen-activated protein kinase cascades [22,28]. And knocking down podocin expression in a cultured podocyte cell line by mRNA interference decreased Nephrin expression by 70% and altered Nephrin localization from the membrane surface to the nuclear area [30].

3.1.3 Neph proteins

The Neph protein family comprises three members: Neph1, Neph2 and Neph3. They belong to the immunoglobulin superfamily and are composed of a short intracellular domain and an extracellular domain with five Ig-like motifs [31,32]. Neph1 is a protein with weak homology and structural similarity to Nephrin.Neph1 can form a heterophilic interaction with Nephrin, via *cis*- or *trans*-interactions at the podocyte intercellular junction. [33,34]. The dissociation of Neph1 from Nephrin induced proteinuria in FSGS [35]. The Neph1-Nephrin complex cooperate to induce actin filament nucleation and elongation in a tyrosine phosphorylation dependent fashion at the plasma membrane by recruiting the cytoskeletal adaptor protein Nck1/2 and other proteins of the actin polymerization complex [36,37]. The lack of Neph1 leads to prenatal lethality with proteinuria and podocyte foot processes effacement in Neph1 knockout mice [38]. Neph1 also interacts with other SD proteins like podocin [39] and ZO-1 [40].

Neph2 can also form homodimers and interacts specifically with the extracellular domain of Nephrin both *in vitro* and *in vivo*. The extracellular domain of Neph2 is cleaved under physiological conditions [41]. Neph3 is a pleiotropic gene active during distinct stages of tissue differentiation and associates directly with the regulation of both glomerular and neural development [42].

3.1.4 Zonula occludens-1 (ZO-1)

ZO-1 is a tight junction protein in the cytoplasmic base of SD. In diabetes, ZO-1 expression decreases and redistributes from podocyte membrane to the cytoplasm [43]. The lack of Nephrin does not affect the expression of ZO-1 in mice. However, injection of antibodies against p51 antigen, an epitope of Nephrin molecule, induces nephrosis in rats and leads to progressive decline of ZO-1. It was recently shown that ZO-1 may serve as a cytoplasmic organizer by coupling the Nephrin-Neph1 complex to the actin cytoskeleton and recruiting appropriate signal transduction components to the SD area [9,44,45].

3.1.5 Alpha-actin 4 (ACTN4)

The alpha-actinin-4 is an actin-bundling protein [46], and ubiquitously expressed. Mutations in ACTN4 lead to an inheritable form of glomerulosclerosis [46]. In alpha-actinin-4 mutant mice, Nephrin mRNA and protein levels were significantly reduced [47]. *In vitro*, silencing of Nephrin, using RNAi, did not change the expression of alpha-actinin-4, but silencing of alpha-actinin-4 downregulated Nephrin and upregulated podocin and CD2AP expression [48].

3.2 Nephrin-mediated intracellular signaling pathways

Besides the important role in scaffolding of SD, Nephrin also serves as a "signaling node" in the SD by transmitting extracellular domain from the SD to the intracellular actin cytoskeleton [27,49,50]. Nephrin has the nine tyrosine residues in the intracellular domain, some of which are phosphorylated after ligand binding [51] and could serve as docking sites for SH2 domain-containing kinase and adaptor proteins.

3.2.1 PI3K/AKT pathway

The phosphorylated Nephrin can bind to the p85 regulatory subunit of phosphoinositide 3-OH kinase (PI3K), allowing the catalytic subunit p110 to act on the phospholipids of inner leaflet of the cell membrane, then stimulating the serine–threonine kinase AKT, which controls cell growth, migration, and survival [22]. Bad is one of the downstream target proteins of AKT. Bad interacts with pro-survival Bcl2 family members to promote apoptosis. Phosphorylation of Bad protects podocytes against detachment-induced podocyte apoptosis [22].

3.2.2 Nck

Nephrin can bind to the Nck adaptor proteins after the phosphorylation by the Src-family tyrosine kinase Fyn. Nephrin-Nck interaction regulates FP morphology and actin dynamics and is important for podocyte FP development and repair after injury. Nck proteins contain one SH2 and three SH3 domains. The SH2 domain of Nck binds to phosphotyrosine residues of Nephrin, and the SH3 domain recruits other proteins involved in actin cytoskeleton regulation. Recently it has been shown that inducible deletion of Nck1 and 2 in the podocytes of adult mice results in proteinuria and reduces phosphorylation of Nephrin [36,52].

3.2.3 PKC

PKCα is a Nephrin binding protein, and sequence analysis of the Nephrin intracellular domain predicted a PKC recognition motif. In diabetic nephropathy, Nephrin internalization is accelerated through regulation of the β-arrestin2-Nephrin interaction by PKCα. PKCα can induce the binding of phosphorylated threonine residues 1120 and 1125 of Nephrin with β-arrestin2 [53,54]. Recently, Satoh and colleagues [55] have shown that the turnover rates of Nephrin was up-regulated by atypical protein kinase C (aPKC).

3.2.4 Calcium signalling

Nephrin plays a crucial role in the regulation of podocyte

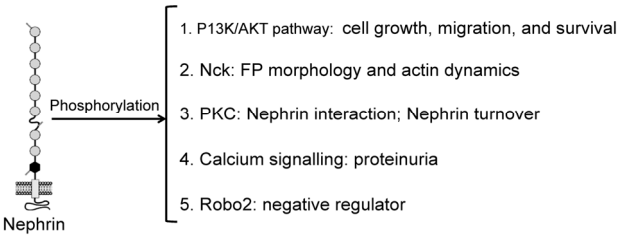

Figure 2 Nephrin-mediated intracellular signaling pathways.

calcium homeostasis. Phosphorylation of Nephrin at Tyr-1204 can recruit and activate phospholipase C-γ 1 (PLC-γ1), and then triggers a rapid $[Ca^{2+}]_i$ influx in podocyte [56].

3.2.5 Others

Roundabout (Robo) family proteins are cell surface receptors. Recently Robo2 has been shown to be expressed at the basal surface of podocytes and co-localizes with Nephrin and podocin. Robo2 signaling acts as a negative regulator on Nephrin signaling. Robo2 interacts directly with adaptor protein Nck and forms a complex with Nephrin, thereby inhibiting actin polymerization induced by Nephrin [57].

4 Clinic features of Nephrin deficiency

4.1 Nephrin in congenital nephrotic syndrome of Finnish type

Mutations in the *NPHS1* gene cause congenital nephrotic syndrome of the Finnish type (CNF). The nephrotic syndrome progresses rapidly after birth and patients die within the first two years of life. CNF has an incidence of 1:10,000 births in Finland, and an autosomal recessive mode of inheritance. Two mutations, named Fin_{major} and Fin_{minor}, were found in over 90% of CNF patients. The Fin_{major} is a deletion (CT) in exon 2, which causes a frameshift and the formation of stop codon at the end of exon 2. This mutation leads to a complete lack of Nephrin protein. Fin_{minor} is a nonsense mutation(C→T) in exon 26, leading to a truncated nephrin protein with 1109-residues [1,2].

The most classic clinic manifestation of CNF is heavy proteinuria, which begins in utero, along with hypoproteinemia and edema. The other abnormal findings include prematurity and large placenta. The clinical features of patients with Fin_{major} and Fin_{minor} mutations are similar. Therapies with agents to reduce the glomerular filtration pressure have not been successful in reducing the proteinuria. Children with CNF are currently treated with bilateral nephrectomy and dialysis, followed by renal transplantation [58,59].

Under light microscopy, the kidney from patients with CNF is characterized by marked hyperplasia of mesangial cells, hyperlobulated capillary tufts, microcystic dilation of both proximal and distal tubuli, interstitial fibrosis and inflammation [45,60]. Diffuse podocyte foot process effacement, narrowing of the filtration slits and absence of slit-diaphragms are observed on electron microscopy, indicating that Nephrin is essential for the development of the SDs.

Recurrence of CNF occurs in 20%−25% of the patients after renal transplantation [61,62]. The recurrence of NS in CNF children appears only in patients with a Fin_{major}/Fin_{major} homozygous genotype and in patients with high levels of serum anti-Nephrin antibodies, suggesting an autoimmune reaction against Nephrin.

4.2 Nephrin expression in acquired kidney diseases

Nephrin expression has been shown to be down-regulated in many human glomerular disease and animal models [63]. In addition, it has been noted in several human biopsy and animal studies that Nephrin is localized away from the slit diaphragm and changes from a linear capillary loop pattern to a granular cytoplasmic pattern in nephrotic disease.

4.2.1 Primary nephrotic syndrome

Furness et al. compared the expression of Nephrin in human samples of six normal kidneys and four specimens of nephrotic syndrome and found that Nephrin mRNA decreased in adult minimal change patients with nephrotic syndrome [64,65]. Nephrin staining is negative in sclerotic lesions in FSGS glomeruli, and in MN glomeruli with mesangial expansion. The expression of Nephrin was decreased only in the areas where the foot processes were effaced [66]. In the rat model of puromycin nephrosis, the glomerular Nephrin mRNA expression was diminished by 30% and 50% at days 3 and 8 after puromycin injection [8,67]. Nephrin expression was also reduced in the kidney of passive Heymann nephritis rats model [68], and in a PAN rat model [65,69].

Nephrin expression in podocyte is correlated inversely with extent of glomerulosclerosis, but not with the amount of proteinuria. Urinary neph/AQP is increased in adult nephrotic syndrome (NS) patients, with the highest levels in FSGS patients [70].

However, some studies reported that expression of Nephrin did not change in proteinuric kidney disease [65,71]. Patrakka et al. [71] found that Nephrin mRNA and protein levels were similar between pediatric MCN patients and controls. Expression of Nephrin did not change in children proteinuric kidney disease such as MCN, FSGS, and MN. These studies suggest that the reduction of nephrin is not necessarily the underlying mechanism of proteinuria in these diseases.

4.2.2 Nephrin expression in diabetic nephropathy

Aaltonen et al. [72] showed that glomerular Nephrin expression was increased by 50% in the STZ rats 4 weeks after induction of diabetes. Nephrin was found in the urine of the STZ-rats 4 to 6 weeks after induction of diabetes. However, in advanced DN, Nephrin expression seems to be

reduced. Bonnet et al. [73] showed that induction of diabetes in spontaneously hypertensive rats by injection of STZ caused advanced DN together with significant reduction in glomerular Nephrin mRNA and protein levels. In human DN, Nephrin mRNA was reduced in renal biopsy samples of T2DM patients compared to healthy controls by 62% [74,75]. Also a reduction in Nephrin protein levels has been reported in diabetic patients with microalbuminuria and nephrotic syndrome [76].

Nephrin expression is a marker of normal podocyte and the loss of podocytes correlating closely with disease progression [77]. It remains unclear whether reduction of nephrin in DN is second to loss of podocyte number or reduction of nephrin expression per podocyte.

4.2.3 Other systemic and metabolic diseases

Nephrin expression was absent in the sclerotic lesions and glomerular crescents of kidneys from patients with Henoch-Schonlein nephritis, lupus nephritis and some membranoproliferative glomerulonephritis. In lupus nephritis (LN), the reduction of Nephrin expression started from the early stage in the kidney of NZB/W LN mice. In patients with LN, nephrin was decreased particularly in diffuse proliferative LN and Nephrin expression correlates with disease severity in histology [78]. The levels of urinary nephrin excretion were increased in rheumatoid arthritis (RA) patients with nephropathie, and had a positive correlation with urinary protein concentrations [79].

5 Nephrin deficient animal model for study

5.1 Nephrin knock-out (KO) mice model

Nephrin KO mice were born at an expected Mendelian ratio, and seemingly normal at birth. But consistent with the human CNF disease phenotype, Nephrin homozygous KO mice develop edema and heavy non-selective proteinuria immediately after births, and die within 24 h. The kidneys of the KO mice lacked Nephrin expression at both protein and mRNA levels. Similar to those in the glomeruli of CNF patients, these mice develop enlarged Bowman's spaces, dilated tubules, and effacement of podocyte foot process with the absence of SD. The glomerular ultrastructure of the heterozygotes Nephrin KO mice was identical to normal wild-type mice, indicating that one functional allele is enough to maintain the normal structure and function of podocyte SD [13].

In addition, podocyte-specific, doxycycline-induced transgenic expression of rat Nephrin in Nephrin KO mice successfully rescued the phenotype from perinatal death to normal morphology of podocytes and architecture of the SD [80]. Furthermore, KO of Nephrin in mice do not significantly affect the glomerular morphogenesis, podocyte viability or expression of other FPs and SD protein complex genes during development [19]. These data suggest that nephrin is critical for maintaining normal SD structure and prevent proteinuria. However, nephrin is not required for podocyte viability.

5.2 Nephrin TRAP mice

Rantanen and their colleagues generated a mutant Nphs1 mouse line by gene-trapping. Nephrin$^{trap/trap}$ mutants show typical features of proteinuric disease and die soon after birth. Fibrotic glomeruli with distorted structures and cystic tubular lesions were found in the kidney. CD2AP and ZO-1 appeared unchanged as compared with the wild-type (wt) and Nephrin$^{wt/trap}$. Electron microscopy revealed that >90% of the podocyte foot processes were fused and slit diaphragms were missing. In Nephrin$^{wt/trap}$, approximately 1/3 of the FP were fused and Nephrin mRNA level decrease more than 60% [81].

5.3 Anti-Nephrin antibody causes proteinuria in rats

Injection of anti-Nephrin antibody (monoclonal antibody 5-1-6) in rats results in immediate proteinuria and foot process effacement. This antibody was recently shown to recognize an epitope of the extracellular domain of Nephrin [9]. The complement activation and leukocyte recruitment are absent in these rats. The glomerular histology remains intact except for partial retraction of the podocyte foot processes. The immediacy of proteinuria with relatively normal glomerular morphology in this rat model suggests that proteinuria is likely caused by binding of the antibody to the extracellular domain of Nephrin, thereby disrupting the molecular rearrangement of the slit diaphragm and permselectivity of the glomerular filtration barrier. The binding of this antibody can induce ligand endocytosis resulting in a loss of function or alteration of intracellular signaling mediated by Nephrin. The exact mechanism through which anti-Nephrin antibodies cause proteinuria in the mAb 5-1-6 nephropathy model remains to be determined [82].

5.4 siRNA-mediated inducible depletion of Nephrin model

Early perinatal lethality of conventional Nephrin KO mice makes it impossible to study the role of Nephrin in the adult kidney. We generated transgenic mice with doxycycline-inducible shRNA-mediated *Nphs1* knockdown using an *in vivo* RNA interference approach in our laboratory [83] . Our *in vivo* studies demonstrate that short-term Nephrin knockdown (6 weeks), starting after the completion of kidney development, had no impact on glomerular structure and function. In contrast, mice with chronic Nephrin knockdown (20 weeks) developed mild proteinuria, foot process effacement, filtration slit narrowing, mesangial hypercellularity and sclerosis, glomerular basement membrane thickening, subendothelial zone widening, and podocyte

Table 1 Different Nephrin knock-out or knock-down animal model

Animal model	Species	Generation method	Phenotype
Coventional Nephrin KO	Mice	Homologous recombination	Homozygous mice: massive proteinuria, nephrotic syndrome, death soon after birth Heterozygous mice have no phenotype
Mutant *Nphs1*	mice	Gene-trap	$Nephrin^{trap/trap}$ mutants show characteristic features of proteinuric disease and die soon after birth. $Nephrin^{wt/trap}$ mice have no phenotype
Anti-Nephrin antibody	rat	Inject anti-Nephrin monoclonal antibody mAb 5-1-6	Immediate proteinuria and foot process effacement
siRNA-mediated Nephrin knockdown	mice	siRNA interference approach	Chronic long-time Nephrin knockdown results in mild proteinuria, foot process effacement in adulthood

apoptosis.

When subjected to an acquired glomerular insult induced by either unilateral nephrectomy or Adriamycin, mice with short-term Nephrin knockdown developed more severe glomerular injury compared to those without Nephrin knockdown. AKT phosphorylation was markedly reduced in mice with chronic Nephrin knockdown. Our data provide the first direct experimental evidence suggesting that under the basal condition and in acquired glomerular diseases, Nephrin is required to maintain slit diaphragm integrity and slit diaphragm-mediated signaling to preserve glomerular function in adult mice.

6 Nephrin expression in extra-renal organs

Expression of Nephrin is not limited to the podocyte in kidney. Nephrin expression is also found in the testis, central nervous system (CNS), pancreas, placenta, heart and lymphoid tissue, indicating that Nephrin may have distinct functions in different tissues.

6.1 CNS

Nephrin is expressed in the fourth ventricle, spinal cord, cerebellum, hippocampus and olfactory bulb in the mouse brain during the development [13]. Recently, endogenous nephrin in adult rodent CNS had been found on the pons and corpus callosum and is expressed by granule cells and Purkinje cells of the cerebellum. *In vitro* study showed that nephrin expressed close to synaptic proteins and demonstrate that nephrin interacts with Fyn kinase, glutamate receptors and the scaffolding molecule PSD95 [84]. However, histological analysis did not reveal any apparent morphological changes in the cerebellum of newborn Nephrin KO mice. The role of Nephrin in brain remains unclear. It has been reported that nearly10% of CNF patients have minor neurological abnormalities such as muscular dystonia, ataxia and athetosis. However, these abnormalities may also be a complication of the severe proteinuria or other comorbidities.

6.2 Pancreas

In the pancreas, Nephrin expression is localized to the insulin-producing cells of the islets of Langerhans [13,85], beta cells [85] and islet microendothelia [86]. Although the exact function of Nephrin in pancreas is still unknown, it may serve as a structural protein in islet microendothelium [86]. Glucose levels and insulin secretion are normal in CNF patients as assessed by OGTT test [87].

6.3 Testis

Nephrin is also expressed in Sertoli cells of the mouse testis [88]. Sertoli cells provide mechanical support, protection and nutrition to the developing germ cells, and formation of the blood-testis barrier. Nephrin seems to have an important role in these functions. Nephrin KO male mice were infertile, with undescended testicles and impaired seminal vesicle formation. Abnormal pubertal development and testicular function have also been reported in male CNF patients [87] .

6.4 Lymphoid tissue

Nephrin can be found in the spleen of the rat [8]. Aström et al. [89] demonstrated Nephrin expression in human tonsil, adenoid and lymph node. Nephrin-positive cells were detected in the germinal centers of the lymphoid follicles in a staining pattern typical for dentritic cells. Dendritic cells maintain the framework in the lymphoid follicle and offer a stable network for proliferation and differentiation of B lymphocytes [90]. Therefore, Nephrin may act as a cell junction adhesion molecule in lymphoid tissue, similarly to its function in the SD.

6.5 Heart

Nephrin is also required for cardiovascular development. Nephrin is expressed in the epicardium and coronary vessels during human and mouse embryonic development. Nephrin KO showed abnormal epicardial cell morphology and a reduced number of coronary vessels, and cardiac fibrosis [91].

7 Conclusion

Taken together, Nephrin is a key scaffolding protein in po-

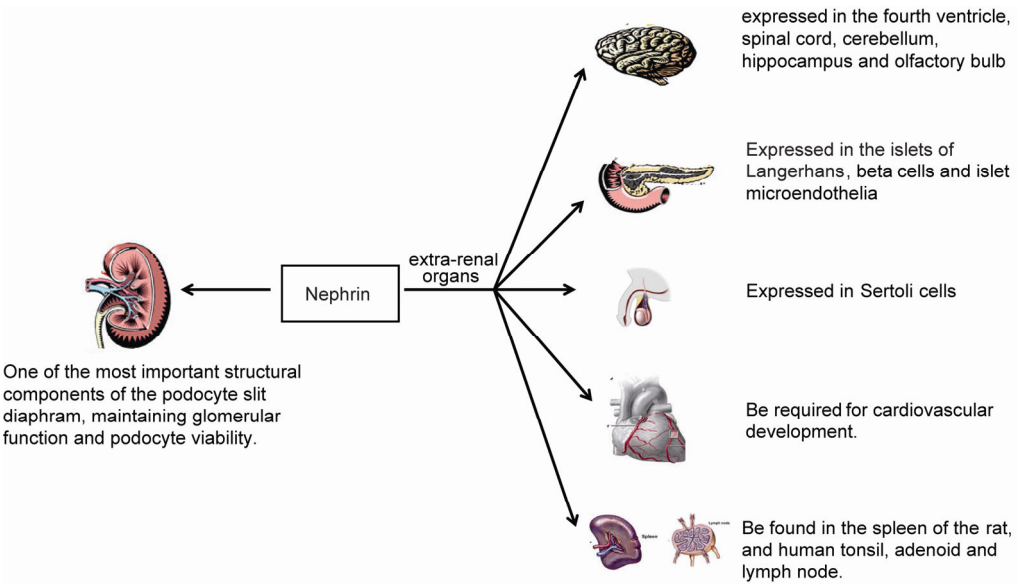

Figure 3 Nephrin in different organs.

docyte SD. Nephrin is required for maintaining SD integrity and SD-mediated signaling to preserve glomerular function and podocyte viability. Reduced expression or abnormal distribution of Nephrin has been observed in many glomerular diseases. Future studies are required to confirm whether Nephrin expression could be used as a biomarker for prediction of the progression of glomerular disease or Nephrin could be developed as a potential drug target for treatment of glomerular disease. In addition, it would be interesting to further explore the function of Nephrin outside of the kidney.

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