

Expression profile of nephrin, podocin, and CD2AP in Chinese children with MCNS and IgA nephropathy

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Received: 27 February 2006 / Revised: 2 May 2006 / Accepted: 3 May 2006 / Published online: 29 August 2006
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Abstract There have been many exciting advances in our understanding of genetic causes of nephrotic syndrome since 1998 when nephrin was first found. The mRNA expressions of nephrin and CD2AP were studied by quantitative real-time polymerase chain reaction (PCR) in aspirated renal biopsy tissues from 9 subjects with minimal change nephrotic syndrome (MCNS), 6 with primary IgA nephropathy (IgAN), and 15 controls. Protein expression of nephrin, podocin, and CD2AP were analyzed by immunohistochemistry, indirect immunofluorescence, and laser confocal microscope. Compared with controls, the CD2AP mRNA level was significantly downregulated in renal samples from MCNS and IgAN patients ($p=0.001$ in MCNS, $p=0.046$ in IgAN), though no significant down-regulation was found in the mRNA level of nephrin ($p=0.346$ in MCNS, $p=0.311$ in IgAN). The expression levels of protein CD2AP and nephrin were significantly reduced in MCNS and IgAN (MCNS: nephrin, $p=0.034$, CD2AP, $p=0.005$; IgAN: nephrin, $p=0.021$, CD2AP,

$p=0.025$). The podocin staining did not differ significantly between controls and disease groups (p value 0.340 and 0.787, respectively). The results suggest that transcript and translation expression changes of nephrin and CD2AP may have pathogenetic roles in some patients with MCNS and IgAN in Chinese, though no correlation was found in podocin with proteinuria in this study.

Keywords Glomerular slit diaphragm · MCNS · IgAN · Nephrin · Podocin · CD2AP

Introduction

Nephrotic syndrome (NS) is a clinical diagnosis defined by the presence of massive proteinuria, hypoalbuminemia, hyperlipidemia, and edema. According to its etiology, NS can be classified as secondary, primary, and congenital nephrotic syndrome. Often as the target of injury in NS patients, the glomerular filtration barrier consists of glomerular visceral epithelial cells (podocytes), fenestrated capillary endothelial cells, and glomerular basement membrane. The podocyte is the final layer of the glomerular filtration barrier with cells forming primary and secondary processes that interdigitate and encircle the glomerular basement membrane and endothelium. These foot processes are separated by narrow spaces which are bridged by a thin membrane, the slit diaphragm (SD).

There have been many exciting advances in our understanding of genetic causes of nephrotic syndrome since 1998 when nephrin was found to be encoded by the *NPHS1* gene and mutated in severe congenital nephrotic syndrome of the Finnish type [1, 2]. Podocin is another important molecule located at the slit diaphragm encoded by gene *NPHS2*, the mutation of which can cause

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autosomal recessive steroid-resistant nephrotic syndrome. The identification of nephrin and podocin as genes that underlie two forms of nephrotic syndrome has provided a critical molecular foothold into podocyte biology [3]. CD2-associated protein (CD2AP) was first identified in 1998 as an 80-kDa protein with SH3 domains that is critical for stabilizing the contact between T lymphocytes and antigen-presenting cells [4]. Interestingly, Shaw and colleagues reported in 1999 that CD2AP knockout mice died at 6–7 weeks of age from nephrotic syndrome and renal failure. Mice with CD2AP haploinsufficiency also developed glomerular change at 9 months of age and had increased susceptibility to glomerular injury suggesting an impairment of the intracellular degradation pathway [5], implicating CD2AP as a determinant of human susceptibility to glomerular disease.

These results suggested that an intact SD is essential for normal glomerular filtration, and alterations of only one of its key components, nephrin, CD2AP, or podocin, may lead to the impairment of the structural integrity of the glomerular filtration barrier and massive proteinuria [6–13]. In the present study, the mRNA and protein expression profiles of nephrin, podocin, and CD2AP in aspirated renal tissues were investigated in Chinese children with minimal change nephrotic syndrome (MCNS) and IgA nephropathy (IgAN) with the aim of disclosing the possible roles of these molecules in the development of proteinuria and primary nephrotic syndrome.

Materials and methods

Patients and controls

Aspirated renal biopsy tissues from 15 patients (9 males and 6 females, 9 with MCNS and 6 with IgAN) with a clinical and renal immunopathologic diagnosis of MCNS or primary IgAN were studied. None of them received previous steroid or immunosuppressive therapy and previous treatment with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin I receptor antagonists, though all MCNS patients were steroid-sensitive nephrotic patients as proved after a standard course of prednisone therapy. The patients were recruited into the study within 3–22 days after the onset of disease. In each instance, informed consent was obtained from the patients and/or their parents for the use of blood and/or renal tissue samples for experimental purposes.

Effacement of visceral epithelial cell foot processes was observed in all nine MCNS patients by electron microscopy with no evidence by light, immunofluorescence (IF), and electron microscopy for any other glomerular disease. In six IgAN patients, the presence of predominant IgA deposits in

the mesangium were observed by immunofluorescence microscopy, associated with focal or diffuse mesangial cell proliferation and mesangial matrix expansion in electron microscopy. Epithelial foot process effacement was also observed in these patients.

As controls, 4 specimens of normal kidney tissue were obtained from an intact pole of kidney removed for a circumscribed tumor and 11 aspirated renal biopsy tissues from isolated hematuria patients clinically and non-IgA mesangial proliferative glomerulonephritis (MsPGN) histologically with no evidence of podocyte process effacement and alteration in glomerular basement membrane under electromicroscopy were studied. Table 1 summarizes the clinical features of the patients included in the study.

Reagents

Rabbit polyclonal anti-human nephrin, podocin, and CD2AP IgG antibody against the carboxy terminus of protein of human origin was purchased from Santa Cruz Biotechnology (nephrin: sc-28192, podocin: sc-21009, CD2AP: sc-9137, Santa Cruz, CA, USA). The primers and probes used in real-time polymerase chain reaction (PCR) were designed by the software Primer3 and synthesized by Invitrogen (Invitrogen, Shanghai, China). The sequences of the primers and probes are shown in Table 2. The primers for nephrin, CD2AP, and β -actin were cDNA specific, not amplifying genomic DNA. Fluorescein isothiocyanate (FITC)-conjugated sheep anti-rabbit IgG was purchased from Dako (Glostrup, Denmark).

Glomeruli microdissection

Renal biopsies were taken with a 16-gauge needle. The cortical parts of the biopsies were transferred to a Petri dish containing ice-cold phosphate-buffered saline (PBS) and cut into pieces approximately 2 mm long. The tissue was micro-

Table 1 Characteristics of patients and controls in study. Values are expressed as mean \pm SD. MCNS minimal change nephrotic syndrome, IgAN IgA nephropathy

Characteristics	MCNS (n=9)	IgAN (n=6)	Controls (n=15)
Males/females	5/4	4/2	9/6
Age (years)	7.5 \pm 2.6	8.6 \pm 2.3	8.2 \pm 3.1
Plasma albumin (g/l)	18.2 \pm 3.7	15.3 \pm 3.4	42.2 \pm 15.2
Proteinuria (g/day)	5.2 \pm 1.7	4.9 \pm 2.0	Negative
Serum creatinine (mg/dl)	1.0 \pm 0.3	0.9 \pm 0.4	1.0 \pm 0.3
Serum cholesterol (mmol/l)	8.8 \pm 2.4	7.7 \pm 2.2	2.2 \pm 0.9

Table 2 The primers and probes used in real-time PCR of β -actin, nephrin, and CD2AP. *FAM* 6-carboxyfluorescein, *TAMRA* 6-carboxy-tetramethylrhodamine

	β -actin	Nephrin	CD2AP
Forward primer	5'-TCACCCACACTGTGCC CATCTACGA-3'	5'-CCAGCTTCCCATCACTACCC-3'	5'-CACATCCACAAACCAAAAACATT-3'
Reverse primer	5'-CAGCGGAACCGCTCATTG CCAATGG-3'	5'-CCCGACACAGGAGGCATTG-3'	5'-CTCCACCAGCCTTCTTCTACC-3'
Product size	300 bp	160 bp	151 bp
Probe	5'FAM-ATGCCCTCCCC ATGCCATCCTGCGT- 3'TAMRA	5'FAM-AAGCCCCCAAG AGCGAACA- 3'TAMRA	5'FAM-ACCAAGAAGCGTCAGTGTA AGTT- 3'TAMRA

dissected by two needles (Microlance, Becton Dickinson, Franklin Lakes, NJ, USA) using a stereo microscope (Zeiss, Oberkochen, Germany) to separate the glomeruli from the surrounding interstitial tissue. The glomeruli were transferred to a second dish containing ice-cold PBS and washed free of surrounding tissue debris. The glomeruli were then put into tubes containing 40 U of RNase inhibitor (rRNasin) (Promega, Madison, WI, USA) and used for the RNA extraction experiments described below.

mRNA isolation, cDNA synthesis, and real-time PCR

Four frozen microdissected glomeruli were homogenized and total RNA was extracted according to the TRIzol method and used for cDNA synthesis with the aid of the Sensiscript RT Kit (Qiagen, Westburg BV, Leusden, The Netherlands). RNA samples were treated with RNase-free DNase. The total RNA extracted from each patient was used to synthesize cDNA with the Superscript First-Strand synthesis system for reverse transcription (RT)-PCR. Gene expression was analyzed by real-time quantitative PCR using the TaqMan system based on real-time detection of the accumulated fluorescence (ABI Prism 7700, Perkin-Elmer Inc., Foster City, CA, USA). Fluorescence for each cycle was quantitatively analyzed by an ABI Prism 7700 Sequence Detection system.

PCR amplification was done in a 25 μ l volume, including 1 \times TaqMan buffer, 200 μ mol/l dNTP mixture, 100 nmol/l fluorogenic probe, 200 nmol/l each of forward and reverse primers, 1.25 U DNA polymerase (PE Biosystems, Foster City, CA, USA), and 100 ng sample cDNA. The amplification was performed with the following time course: 50°C, 2 min and 10 min at 95°C; and 40 cycles of 94°C, 20 s, 60°C, 1 min. All measurements were performed in duplicate. To control for variation in the amount of cDNA available for PCR in the different samples, gene expression of the target sequence was normalized in relation to the expression of an endogenous control, β -actin, a constitutively expressed gene. Controls consisting of bidistilled water were negative in all runs.

Immunofluorescence and laser scanning confocal microscope

For immunofluorescence, the slides were thawed in PBS, fixed in a mixture of 50% alcohol and 50% acetone for 5 min and subsequently in 100% alcohol for 10 min, and washed in PBS. The slides were then incubated overnight with the primary antibody at room temperature (rabbit anti-nephrin 1:100, rabbit anti-podocin 1:200, and rabbit anti-CD2AP 1:200) and thereafter washed in PBS. The slides were incubated with the FITC-conjugated anti-rabbit IgG antibody (1:40) for 30 min, washed in PBS 4 times, and covered with glycerol and VECTASHIELD (Vector Laboratories, Burlingame, CA, USA). For each antibody, all samples were stained in one session. Specimen images were viewed with a confocal laser scanning microscope with laser 488 nm and 568 nm excitation (TCS-SP, Leica Microsystems, Heidelberg, Germany). For controls, sections were incubated with nonimmune rabbit IgG instead of a primary antibody.

All sections were observed by a blinded reader (not knowing the clinical and histological diagnosis) in the department of the confocal laser scanning microscope. Of the immunofluorescence-stained samples, digital image analysis of all of the glomeruli in the section were taken at a \times 400 magnification using the quantify software equipped with the confocal laser scanning microscope (TCS-SP, Leica Microsystems, Heidelberg, Germany). Three glomeruli for each antibody and each case were taken randomly and recorded in the computer for digital image analysis. The glomerular area stained was calculated by drawing a region of interest around the glomerulus in which the amount of staining within a color spectrum specific for the FITC staining and above a fixed intensity threshold was determined. The average immunofluorescence intensity of three glomeruli from one patient was determined.

Immunohistochemistry

Three-micrometer cryostat sections were cut at -20°C , transferred to Starfrost slides, air dried, and stored at -20°C

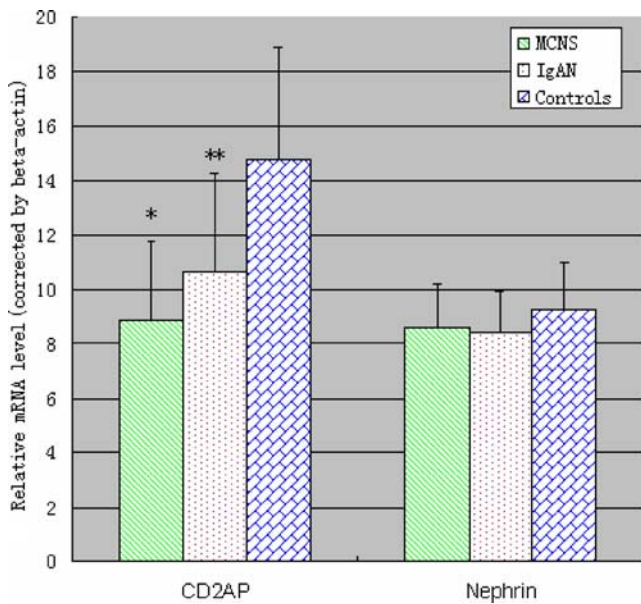


Fig. 1 Glomerular mRNA level of nephrin and CD2AP using real-time PCR. CD2AP mRNA level was significantly altered in the diseased state, * $p=0.001$ and ** $p=0.046$. The gene expression of the target sequences was normalized in relation to the expression of β -actins

until use. Endogenous peroxidase was inactivated using 0.1% hydrogen peroxide in PBS for 10 min, and then sections were incubated with a protein blocking agent for 20 min. For immunohistochemistry (IHC), the slides were deparaffinized with xylene and iodine, and rehydrated in a graded series of alcohol. The sections were treated with Target Retrieval Solution (Dako, Carpinteria, CA, USA) in a streamer at 90–95°C for 20 min and then cooled for 15 min. The sections were then washed in PBS and incubated for 1 h at room temperature with the primary antibody diluted in 1% bovine serum albumin (BSA) in PBS (rabbit anti-nephrin 1:1,000, rabbit anti-podocin 1:2,000, and rabbit anti-CD2AP 1:2,000). The slides were then washed in PBS and incubated for 30 min with horseradish peroxidase-conjugated anti-rabbit Envision (1:1). The slides were again washed in PBS, and the staining was developed with diaminobenzidine. The color was enhanced by rinsing the slides in 0.5% CuSO_4 solution for 5 min. After counterstaining with hematoxylin, the slides were dehydrated and mounted. Distribution and expression patterns of nephrin, CD2AP, and podocin in glomeruli were assessed independently by pathologists in a blinded manner.

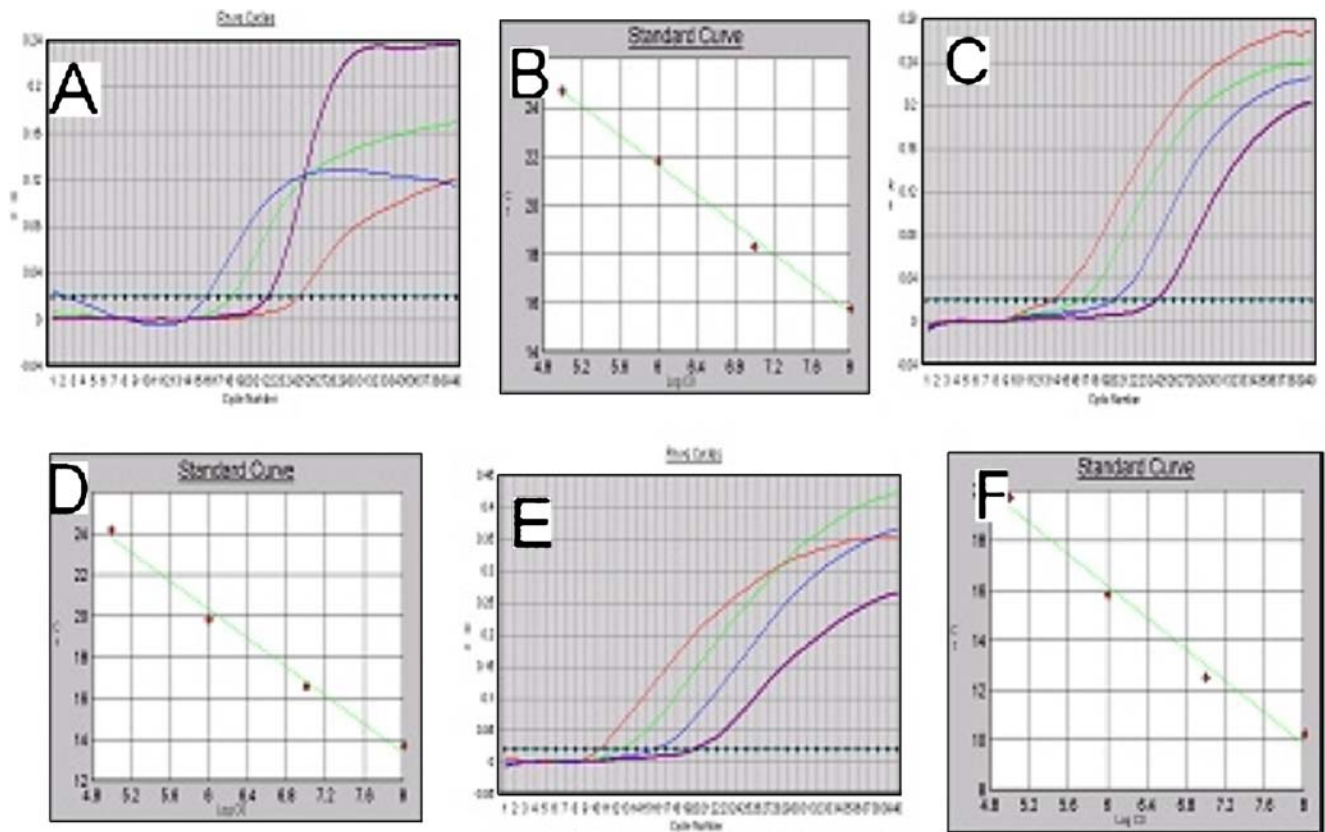


Fig. 2 Standard curve of β -actin (a, b), CD2AP (c, d), and nephrin (e, f) in aspirated glomeruli by real-time PCR. Gene expression of the target sequence was normalized in relation to the expression of β -actin. In b, $r = -0.998403$; in d, $r = -0.995427$; in f, $r = -0.993463$. The

PCR has three phases: exponential phase, linear phase, and plateau phase. The exponential phase is the earliest segment. The linear phase is characterized by a linear increase in product as PCR reagents become limited. Eventually the PCR will reach the plateau phase

Statistical analysis

Data are shown as mean±SD. Results of the mRNA level by quantitative real-time PCR and the protein level by immunofluorescence (IF) were analyzed by independent sample *t*-test using SPSS software. The correlations between protein levels of nephrin or CD2AP and plasma albumin concentration in the patient group were analyzed by Pearson's correlation test. A *p* value of less than 0.05 was viewed as statistically significant.

Results

Real-time PCR

The corrected mRNA level for the molecules of nephrin and CD2AP are depicted in Fig. 1. In general, the mRNA level of CD2AP was decreased in MCNS ($p=0.001$) and IgAN ($p=0.046$) patients compared with controls. There was no significant difference in the mRNA level of nephrin in MCNS ($p=0.346$) and IgAN patients ($p=0.311$) compared with the controls. Standard curves of β -actin, CD2AP, and nephrin are shown in Fig. 2.

Immunofluorescence and laser scanning confocal microscope

In normal glomeruli, nephrin, podocin, and CD2AP showed intense epithelial staining along the peripheral capillary loops of the glomeruli. In glomeruli of diseased kidneys, the staining of nephrin and CD2AP was weaker, and in some patients the staining showed a more granular appearance. Five MCNS patients and four IgAN patients showed weaker staining intensity and the granular pattern of distribution of nephrin and CD2AP. Figures 3, 4, and 5 give a graphical overview of the staining intensity and pattern for the different molecules in control and diseased kidneys.

Quantification of the staining using digital image analysis showed that the stained glomerular surface for nephrin and CD2AP was significantly diminished in nephrotic syndrome patients with MCNS and IgAN compared with controls (Fig. 6, in MCNS: nephrin, $p=0.034$, CD2AP, $p=0.005$; in IgAN: nephrin, $p=0.021$, CD2AP, $p=0.025$). The glomerular fluorescence intensity of the podocin staining did not differ significantly between controls and disease groups (*p* value 0.340 and 0.787, respectively). Data shown in Fig. 6 are the relative values of pixel gray value per 3 glomeruli.

A positive correlation was found between patients' plasma albumin concentration and nephrin protein level in

Fig. 3 Staining pattern of nephrin in normal and diseased glomeruli by IF and LCM. Original magnification, $\times 200$. Nephrin showed a podocyte-like staining pattern in normal glomeruli (a). In diseased situations, the staining of nephrin is less intense with a more granular staining pattern (b, c, and d)

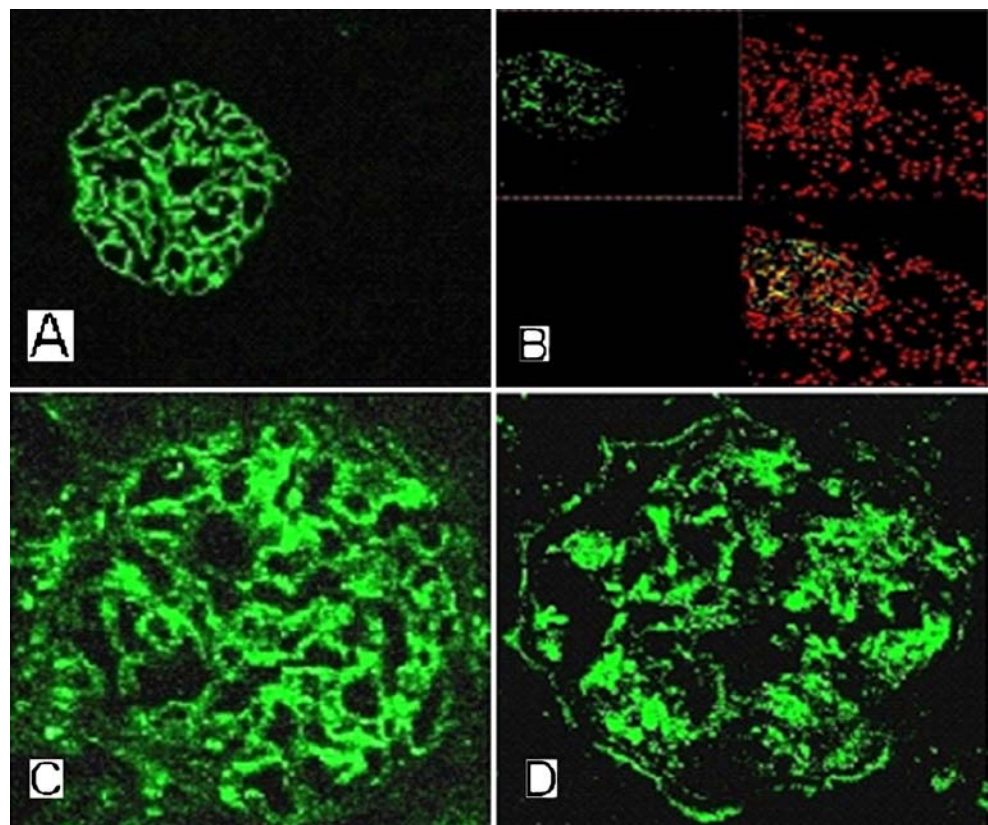
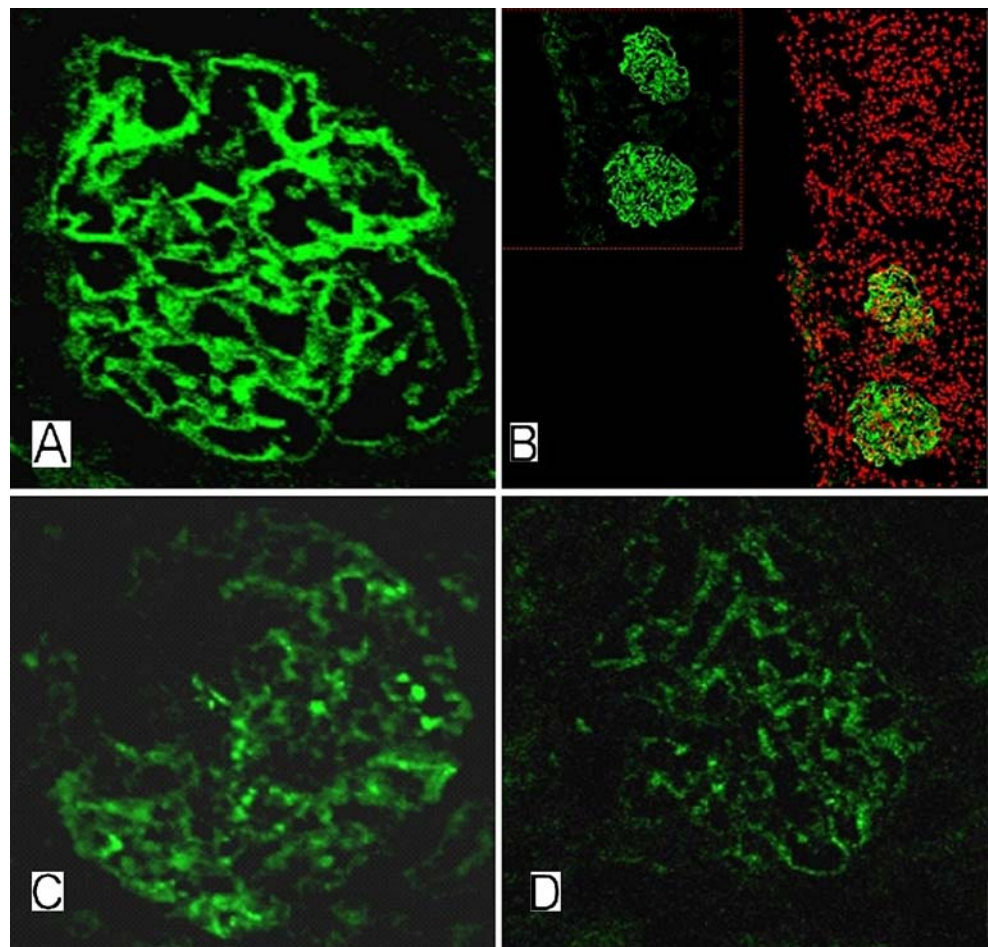


Fig. 4 Staining pattern of CD2AP in normal and diseased kidney sections by IF and LCM. Original magnification, $\times 200$. CD2AP showed intense and linear or slightly granular staining pattern in normal glomeruli (a, b). In diseased samples, the staining of CD2AP is less intense with a more granular staining pattern (c, d)



glomeruli ($\gamma=0.522$, $p=0.046$), and the positive correlation was also found between the patients' plasma albumin concentration and CD2AP protein level in glomeruli ($\gamma=0.556$, $p=0.031$) (Fig. 7).

Immunohistochemistry

In glomeruli of diseased kidneys, the staining of nephrin and CD2AP was also weaker than the staining in normal glomeruli of control kidneys. Staining of podocin was not significantly altered in nephrotic syndrome patients with MCNS and IgAN compared with controls. No quantification analysis was taken in this study (data not shown).

Discussion

Congenital nephrotic syndrome (CNS) is rare in China. Resulting from the identification of the important role that podocyte-associated molecules play in maintaining the glomerular filtration barrier in the CNS, the hypothesis emerged that these molecules might also be involved in acquired nephrotic syndromes. The present results demon-

strated that, in Chinese patients with MCNS and IgAN, the protein expressions of nephrin and CD2AP in intensity and distribution were significantly changed; in addition, a decrease in glomerular expression of CD2AP mRNA was also observed in this study, suggesting that nephrin and CD2AP may be the target of injury in MCNS and IgAN, implying the role of nephrin and CD2AP in the pathogenesis of proteinuric diseases in Chinese children.

Several reports have investigated the expression of these podocyte molecules in acquired human glomerular disease, though results among these reports were not always consistent (see Table 3). In summary, Koop et al. [14], Furness et al. [15], Huh et al. [16], Srivastava et al. [17], Wang et al. [18], and Doublier et al. [19] reported reduced nephrin expression at protein and/or mRNA level in patients with MCNS, focal segmental glomerular sclerosis (FSGS), membranoproliferative glomerulonephritis (MPGN), IgAN, and membranous nephropathy (MN), respectively, whereas, as reported by Patrakka et al. [20], Guan et al. [21], and Hingorani et al. [22], no significant changes were seen in expression of nephrin at all. Meanwhile, Koop et al. [14] identified a striking discrepancy between an increase in mRNA expression levels of

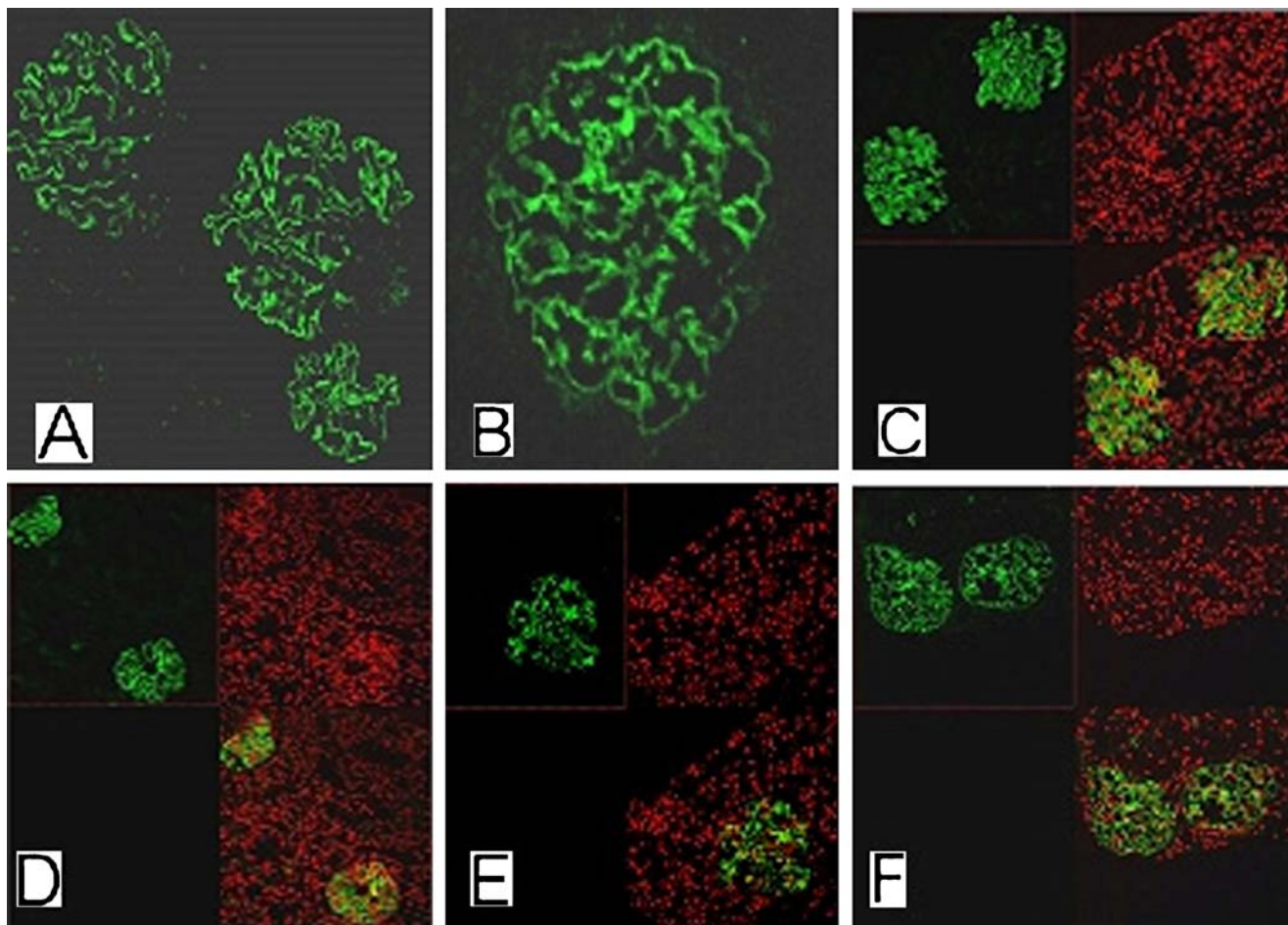


Fig. 5 Podocyte podocin expression in glomeruli in normal and diseased kidney sections by IF and LCM. No difference in the staining patterns was seen in diseased glomeruli (b–f) as compared with the glomeruli from normal kidney sections (a)

podocyte-associated molecules and a decrease in protein expression. This suggests that the alterations in the expression of podocyte-associated molecules represent a

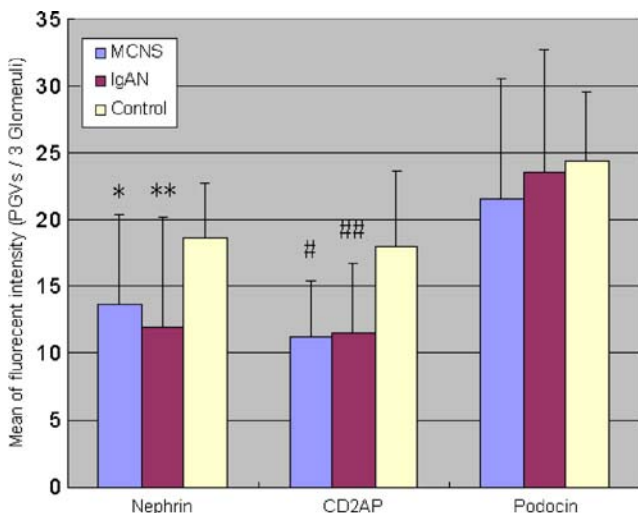


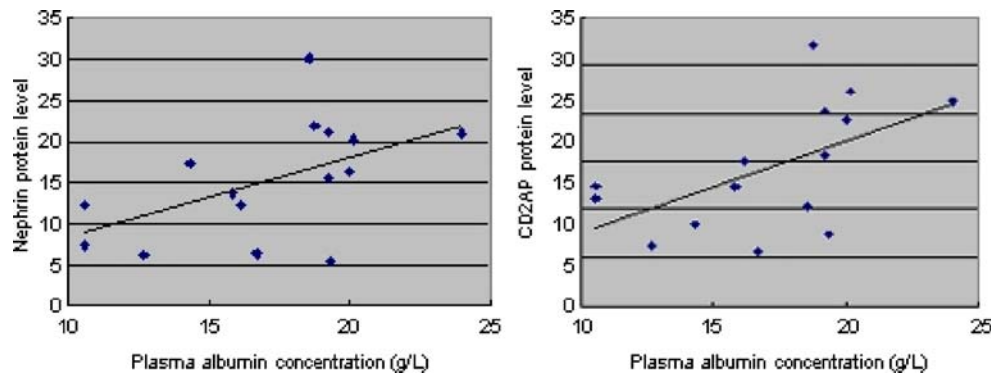
Fig. 6 Fluorescence of glomerular area staining of nephrin, CD2AP, and podocin in MCNS and IgAN patients by laser confocal microscope, * $p=0.034$, ** $p=0.021$, # $p=0.005$, ### $p=0.025$. PGVs (pixel gray value) per 3 glomeruli

compensatory reaction of the podocyte that results from damage associated with proteinuria.

In 2005, Lahdenkari et al. [23] reported that the genes coding for CD2AP were highly conserved and no amino acid substitutions were detected in 38 patients with MCNS, indicating genetics may play a minor role in the etiology of MCNS. This finding was not in conflict with our results, because, even if they do not have a role in the genetic background of MCNS, CD2AP, as a key component of SD, may function as an adapter protein to anchoring the C-terminal cytoplasmic domain of nephrin and/or podocin to the actin cytoskeleton of the podocytes. Furthermore, CD2AP also along with nephrin and podocin participates in cell signaling pathways. So it is reasonable that CD2AP expressions might be reduced and its functions be impaired in non-congenital nephrotic syndrome patients without mutation in gene coding CD2AP.

Molecule redistribution on the podocyte has been suggested as a potential mechanism of proteinuria in patients with primary acquired nephrotic syndrome. The expression pattern of proteins was also changed from linear in normal kidney sections to granular in glomeruli from

Fig. 7 Relationship between the nephrin or CD2AP expression level in glomeruli and patients' plasma albumin concentration (nephrin $\gamma=0.522$, $p=0.046$; CD2AP $\gamma=0.556$, $p=0.031$)



MCNS and IgAN patients in the present study. These phenomena coincide with the finding reported by Doublier et al. [19] and Wernerson et al. [24]. Wernerson et al. [24] demonstrated that, in normal kidney, immunofluorescence revealed a linear staining along the capillary basement membranes, corresponding to the localization of nephrin in the slit membranes. In the biopsies from patients with MCNS, the nephrin pattern had become granular. The degree of granularization corresponded to the degree of foot process effacement. In contrast, Patrakka et al. [20] and Hingorani et al. [22] reported that, in MCNS, FSGS, and MN patients, the distribution of nephrin (linear) was similar to that in controls. This discrepancy may be partly attributed to the clinical feature of nephrotic syndrome patients, the time point for taking renal biopsy before or after steroid therapy, and the methods used for detecting protein expression (IHC, IF, or immunoelectron microscope, etc.). In the present study, renal biopsy was done

before the patients receiving steroid therapy; furthermore, IHC and IF were used together to measure the nephrin, CD2AP, and podocin expression in glomerular tissues from the same patient in the group, so, to some extent, the results of the variation of protein expression pattern and quantity should be credible and reasonable in this study.

According to the reports by Salant et al. and the others [7–12, 25–29], nephrin and podocin are two crucial proteins in the complex of molecules that assemble and reinforce the slit diaphragm. From these, it is reasonable to suppose that, in the event of maintaining podocyte process morphology and integrity of the slit diaphragm, nephrin (podocin might be also the same) may play a role in upstream, and CD2AP may exert an influence in the downstream of these. Because of the compensatory mechanism, the mRNA level of molecules in upstream (such as nephrin or podocin) might not be significantly altered during podocyte injury and proteinuria status, though the

Table 3 Comparison of the expression of podocyte molecules in different types of human glomerulonephritis. *MCNS* minimal change nephrotic syndrome, *FSGS* focal segmental glomerular sclerosis, *MsPGN* mesangial proliferative glomerulonephritis, *IgAN* IgA nephropathy, *MN* membranous nephropathy, *MPGN* membranoproliferative glomerulonephritis, *LN* lupus nephritis

Presented by	Pediatric or adult	Primary disease and number	Changes in mRNA transcript	Changes in protein expression
Koop et al. [14]	Adult	10 MCNS, 5 FSGS, 6 DN, 7 LN, 2 MPGN, 1 MN	Nephrin, podocin, and podocalyxin were increased	Nephrin, podocin, and podocalyxin were reduced markedly
Patrakka et al. [20]	Pediatric	11 MCNS, 2 FSGS, 5 MN, 6 IgAN	No change in situ hybridization of nephrin	No change of nephrin
Furness et al. [15]	Adult	3 MCNS	Nephrin mRNA reduced	Nephrin expression was lower
Huh et al. [16]	Adult	1 MCNS, 1 MPGN, 1 GN, 1 MN, 4 IgAN		
Srivastava et al. [17]	Pediatric	3 MCNS, 3 FSGS		Nephrin, synaptopodin, and GLEPP1 were reduced
Wang et al. [18]	Unknown	20 MN, 6 IgAN, 40 MPGN		Downregulation of nephrin
Doublier et al. [19]	Adult	13 MN, 7 FSGS, 10 MCNS		Nephrin expression were reduced
Guan et al. [21]	Pediatric	5 MCNS, 4 IgAN, 10 MsPGN		Podocin expression was reduced, no change in nephrin
Wernerson et al. [24]	Pediatric & adult	13 MCNS		Nephrin expression was reduced and redistributed
Hingorani et al. [22]	Pediatric	19 MCNS, 10 FSGS		No changes were seen in nephrin expression

mRNA level of molecules in downstream (such as CD2AP) may be changed markedly at that time. Certainly, at the protein level of expression, these podocyte molecules will inevitably be reduced and downregulated remarkably in immunofluorescence or immunohistochemistry assay in proteinuric status. With this in mind, it is tempting to say that the expression of nephrin and CD2AP is altered in primary glomerular disease and consequently, the slit membrane is disrupted and podocyte foot processes are effaced. However, it is also possible that the altered cytoskeleton of podocytes with effaced foot processes can change or suppress the expression of nephrin. The recent identification of mutation of podocin and α -actinin-4 in familial idiopathic nephrotic syndromes [30] and congenital nephrotic syndrome are examples of the latter possibility. Together, our results revealed that the expressions of nephrin and CD2AP in the podocytes were markedly reduced in Chinese children with MCNS and IgAN, but whether this reflects a pathogenetic role for nephrin and CD2AP in MCNS and IgAN or a phenomenon secondary to other causes of foot process effacement and proteinuria remains to be elucidated in the future.

Acknowledgements This work was supported by the Zhejiang Provincial Natural Science Foundation of China No. 302656 and the Zhejiang provincial Health Science Foundation No. 2004QN014. The authors thank Wu Yedong, Sheng Zheng, and Yu Zhongsheng for their excellent technical assistance and Drs. Xia Yonghui, Huang Yaping, and Dai Yuwen for many helpful clinical comments and advice. The authors are also grateful to Drs. Wu Dehua and Zhang Chong for providing control kidney materials to us.

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