Messenger RNA expression of B7-1 and NPHS1 in urinary sediment could be useful to differentiate between minimal-change disease and focal segmental glomerulosclerosis in adult patients

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Abstract

Background. Podocyte proteins are involved in the pathogenesis of glomerular kidney disease (GKD). However, there is little information on messenger RNA (mRNA) expression patterns of B7-1 and NPHS1 in urinary sediment of patients with GKD. The objective of this study was to analyse the gene expression of B7-1 in urinary sediment and correlate it with the expression of podocyte-specific genes in patients with GKD.

Methods. Adult patients with proliferative and non-proliferative GKD, proteinuria and stable renal function, were included. A group of healthy subjects was used to determine normal levels of urinary markers and to obtain reference RNA. Biochemical, clinical and experimental procedures included measurement of creatinine level and total urinary protein, renal biopsy, identification of urinary podocytes, gene expression analysis of B7-1, NPHS1, NPHS2 and SyNPO genes and urinary B7-1 protein analysis by enzyme-linked immunosorbent assay.

Results. Between June 2006 and November 2009, 69 patients with GKD (median age: 46 ± 15 years, 64% men) and 14 healthy subjects (median age: 34 ± 12 years, 43% men) were included. In both groups, urinary mRNA levels of B7-1 and NPHS1 were significantly higher in patients with GKD compared to healthy subjects (P = 0.050 and P = 0.008, respectively). Regarding GKD subtypes, patients with focal segmental glomerulosclerosis (FSGS), but not patients with minimal change disease (MCD), had a significantly higher mRNA expression of B7-1 and NPHS1 than healthy subjects (P = 0.012 and P = 0.030, respectively). Patients with MCD had a significantly lower NPHS1 mRNA expression than patients with FSGS (P = 0.012). The B7-1:NPHS1 urinary mRNA ratio was significantly higher in patients with MCD compared with patients with FSGS (P = 0.027).

Conclusion. mRNA expression analysis of B7-1 and NPHS1 in urinary sediment may be useful to differentiate between different histologic subtypes of GKD, particularly between MCD and FSGS.

Keywords: B7-1; gene expression; glomerular kidney disease; nephrin; podocyte

Introduction

Glomerular kidney disease (GKD) comprises a group of disorders affecting the glomerulus. Glomerulonephritis, one of the two major categories of GKD, develops due to many conditions, which are broadly grouped into proliferative and non-proliferative histologic types. The first group includes IgA nephropathy (IgAN; Berger’s disease) and membranoproliferative glomerulonephritis (MPGN), and the latter group includes focal segmental glomerulosclerosis (FSGS), minimal change disease (MCD) and membranous glomerulonephritis (MGN).

The podocyte, one of the components of the glomerular filtration barrier, is a well-differentiated epithelial cell with a distinctive morphology that exerts a major regulatory role in the glomerulus by acting as a final barrier to leakage of proteins into the urine. Thus, injury to the podocyte may ultimately lead to proteinuria, the hallmark of GKD [1–3].

Recently, it has been suggested that the podocyte may act as an antigen-presenting cell, as it has been shown to express the cell membrane protein B7-1, also known as CD80 [4]. B7-1 is a type I membrane glycoprotein belonging to the immunoglobulin superfamily. It participates in the co-stimulatory signal essential for T-lymphocyte activation through binding of CD28 or CTLA-4 to this receptor [5, 6].

In vitro and in vivo studies have shown an increased expression of B7-1 in glomerular epithelial cells from different
glomerular diseases [7–10]. Therefore, B7-1 represents an interesting molecule for the study of different forms of GKD as well as an attractive therapeutic target [11, 12].

Regardless of the mechanism involved, whether it is detachment of viable podocytes or increased apoptosis, podocyte number decreases in most GKD [13]. Podocyte depletion leads to proteinuria and glomerulosclerosis and ultimately to impaired renal function [1, 2]. Therefore, it is of great importance to recognize podocyte injury at an early stage of the disease to prevent further progression of the glomerular damage to overt renal failure [14].

Although renal biopsy remains the gold standard for the diagnosis of GKD, the recognition of podocyte proteins as key elements in the pathogenesis of GKD opens up a new field of study regarding the development of non-invasive diagnostic tools and the search for novel therapeutic targets [13, 15–18]. Hence, the main objective of this study was to analyse the gene expression of B7-1 in the urinary sediment and associate it with expression of podocyte-specific genes, namely nephrin (NPHS1), podocin (NPHS2) and synaptopodin (SyNPO), in patients with proliferative GKD, non-proliferative GKD and in healthy subjects. Finally, we sought to determine whether the expression pattern of B7-1 and of these podocyte-specific genes varied according to proliferative and non-proliferative forms of GKD.

Materials and methods

Study design

This was an observational prospective study carried out between June 2006 and November 2009 at the Nephrology Department of the Hospital Universitari Germans Trias i Pujol (Barcelona, Spain). All study procedures were performed at the start of corticosteroids or any other immunosuppressive treatment. To be included, patients had to be ≥18 years of age show clinical signs of renal disease such as proteinuria, with or without haematuria, or nephrotic syndrome with stable renal function, and in whom renal biopsy was indicated for clinical diagnostic purposes. Only patients with primary GKD were included. Patients with other forms of GKD, such as those associated with nephron mass reduction, infections, drugs or inflammatory diseases, were excluded. Patients with proliferative GKD, non-proliferative GKD and in healthy subjects. Finally, we sought to determine whether the expression pattern of B7-1 and of these podocyte-specific genes varied according to proliferative and non-proliferative forms of GKD.

Study procedures

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Measurement of serum creatinine and total urinary protein

Determination of serum creatinine levels was carried out using the modified Jaffé method (Roche Diagnostics, Basel, Switzerland). Total urinary protein was measured spectrophotometrically from a 24-h urine sample using a cobas u 711 analyser (Roche Diagnostics) according to the manufacturer’s instructions.

Renal biopsy

We obtained a single renal biopsy specimen from each patient for diagnostic purposes. All biopsies were performed at the beginning of the study, before the start of corticosteroids or any other immunosuppressive treatment. Percutaneous renal biopsies were performed using a Bard® Monopty® Disposable Core biopsy Instrument (Bard Biopsy Systems, Tempe, AZ) following standard procedures. Renal tissues were routinely processed for light, immunofluorescence and electron microscopy, according to standard procedures. Light microscopy stains included haematoxylin/eosin, Schiff’s periodic acid, methenamine silver, Masson’s trichrome and Congo red.

Immunofluorescence assays were carried out by incubating cryostat sections with polyclonal fluorescein isothiocyanate-conjugated secondary antibodies against IgG, IgM, IgA, C3 fraction, C1q, C4, kappa and lambda chains and fibrinogen (Dako Corporation, Copenhagen, Denmark).

Isolation of peripheral blood mononuclear cells

Nine milliliters of heparinized venous blood were used for isolation of peripheral blood mononuclear cell (PBMC) to obtain reference RNA for normalizing gene expression of B7-1. The blood sample was shaken by inversion (×2–3) and diluted in phosphate buffered saline (PBS) or Hank’s balanced salt solution at a 1:1 ratio, followed by a Ficoll density gradient to obtain the lymphocyte pellet. The sample was centrifuged at 1000 g for 30 min at room temperature and the PBMC-containing layer was carefully aspirated. PBMCs were washed with PBS and centrifuged at 240 g for 5 min (×3) and finally stored at −80°C.

Preparation of urinary sediment and identification of urinary podocytes

Fresh first morning urine samples were collected from participants on the same day that the renal biopsy was performed. These specimens were used to identify podocytes and for gene expression analysis. Briefly, urine samples were centrifuged at 2100 g for 30 min at 4°C and the supernatant urine was saved for later use.

Urinary podocytes were identified according to the method described by Hara et al. [19], modified according to Vogelman et al. [13]. Nucleated podocytaxy-positive cells were considered to be podocytes.

Isolation of messenger RNA from urinary sediment and PBMCs

Urine and lymphocyte pellets were lyzed with digestion buffer containing 4 M thiocyanate guanidine, 25 mM trisodium citrate, 0.5% Sarkosyl and 7 μL 2–β-mercaptoethanol per mL of digestion buffer. Water was then added to obtain a final volume of 50 mL. Total RNA was extracted using the phenol–chloroform method as described previously [18, 20]. Samples were treated with deoxyribonuclease 1 (Invitrogen; Life Technologies, Carlsbad, CA) to digest contaminating chromosomal DNA and stored at −80°C until use. RNA purity was determined using the ratio of absorbance at 260 and 280 nm using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE).

Gene expression analysis

Reverse transcription was carried out in 20 μL of a solution containing MMLV reverse transcriptase, 3 μg of total RNA and random hexadeoxynucleotidic primers (Invitrogen; Life Technologies). Relative messenger RNA (mRNA) abundance was quantified using a LightCycler® 480 Real-Time PCR System (Roche Applied Science, Penzberg, Germany). Primer and probe sequences of target genes are listed in Table 1. All primer pairs spanned exon boundaries. Quantitative real-time polymerase chain reactions (RT-PCR) reactions were performed in 10 μL reaction mixture containing the complementary DNA, LightCycler® 480 Probes Master reaction mix (Roche Applied Science) and each forward and reverse primers. All samples were performed in triplicate.

Relative mRNA levels of each target gene were quantified with the comparative threshold cycle method (2−ΔΔCt) using 18S mRNA as housekeeping gene. Human kidney reference RNA (Applied Biosystems/Ambion; Austin, TX) and lymphocyte RNA were used as calibrator samples (exogenous controls). Normalized expression values (ΔACp) were determined as follows: 2 −(ΔCt, sample−ΔCt, calibrator) where ΔCt values were calculated by subtracting the cycle threshold (the cycle that denotes a significant accumulation of target DNA over the background threshold) of the target gene from the housekeeping gene. Before relative quantification by RT-PCR, we optimized and evaluated the primers and probes designed to target genes of interest (B7-1, NPHS1, NPHS2 and SyNPO) as well as the endogenous gene (18S), as described in Table 1. The absolute polymerase chain reaction cycles of the samples analysed were within the optimum range (Supplementary Figure 1).

The intra-assay coefficient of variation of <25% was accepted for this assay, and an inter-assay coefficient of variation between 3.7 and 7.1% of urinary mRNA levels was observed.

Analysis of urinary B7-1 (CD80) protein levels

Protein levels of B7-1 in the urinary supernatant were assessed by enzyme-linked immunosorbent assay [10] using a Human sCD80 Instant ELISA® kit (BMS291INST; Bender MedSystems, Burlingame, CA). The intra-assay
and inter-assay coefficients of variation of the kit were 7.2 and 9.2%, respectively.

Statistical analysis

Normality was assessed with the Kolmogorov–Smirnov test. Continuous variables are expressed as mean ± SD, median and range and were compared using the Mann–Whitney U-test or Kruskal–Wallis test, as appropriate. Categorical variables were analysed using chi-square or Fisher’s exact probability test. Associations among variables were assessed using the Spearman’s correlation coefficient. The diagnostic performance of urinary biomarkers was evaluated by calculating their sensitivity and specificity using the Receiver Operating Characteristics (ROC) curves. The area under the ROC curve (AUC), 95% confidence intervals and cut-off points were calculated using the non-parametric method. A multiple correspondence analysis was carried out to evaluate the relationship between B7-1 and NPHS1 urinary mRNA expression. To perform this analysis, we created dummy variables of B7-1 and NPHS1 using the results of the ROC curves as cutoffs. Glomerular subtypes were used as supplementary variables [21]. Statistical analyses were carried out using SPSS software (version 15.0; SPSS Inc., Chicago, IL) and SAS (version 9.2; SAS Institute Inc., Cary, NC). A two-tailed P-value of <0.05 was considered statistically significant.

Results

Patient characteristics

Sixty-nine patients with GKD in whom renal biopsy was available were included in this study. Median age was 46 ± 15 years and 64% were men. The control group comprised 14 healthy subjects with a median age of 34 ± 12 years, of whom 43% were men. Baseline characteristics of patients with GKD and healthy subjects are shown in Table 2. Significant differences between groups were observed regarding age and renal function, but not gender.

Table 1. Nucleotide sequences of primers and probes used for quantitative real-time PCR analysis of mRNA expression of human NPHS1, NPHS2, SyNPO and B7-1 genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Internal probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPHS1</td>
<td>Forward</td>
<td>aggagacgggacacacgaga</td>
<td>cctcaggtgcaccaaacagagc</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>cctcaggtgcaccaaacagagc</td>
<td></td>
</tr>
<tr>
<td>NPHS2</td>
<td>Forward</td>
<td>tctcagggagtctcaacctgga</td>
<td>atcgcccaaggtcaaggggcc</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>atcgcccaaggtcaaggggcc</td>
<td></td>
</tr>
<tr>
<td>SyNPO</td>
<td>Forward</td>
<td>ggtgcacattcctcccggtge</td>
<td>tccagggcagtctcccg</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>tccagggcagtctcccg</td>
<td></td>
</tr>
<tr>
<td>B7-1</td>
<td>Forward</td>
<td>tgggtgggctgggcttcct</td>
<td>acctcggtcaggtgttatccacgtgacaa</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>acctcggtcaggtgttatccacgtgacaa</td>
<td></td>
</tr>
<tr>
<td>18S</td>
<td>Forward</td>
<td>ggtgcacattcctcccggtge</td>
<td>cctcaggtgcaccaaacagagc</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>cctcaggtgcaccaaacagagc</td>
<td></td>
</tr>
</tbody>
</table>

*aHousekeeping gene.

Expression of B7-1, NPHS1, NPHS2 and SyNPO in urinary sediment

The observation of podocalyxin-positive cells in the urinary sediment of patients with GKD confirmed the presence of podocytes. A positive correlation was observed between NPHS1 and SyNPO (r = 0.583, P < 0.001), NPHS2 and SyNPO (r = 0.723, P < 0.001) and NPHS1 and NPHS2 (r = 0.297, P = 0.028) mRNA expression levels in urinary sediment.

Several cells within the kidney may express B7-1, such as podocytes and tubular epithelial cells. Therefore, in order to better identify the source of B7-1 in the urinary sediment, we discarded urinary samples from patients in whom tubular damage due to viral infections, acute tubular necrosis or drug toxicity were suspected. We detected few tubular epithelial cells in four cases, of which two...
corresponded to MCD and the other two to MGN. Moreover, no associations were observed between urinary B7-1 mRNA expression and tubular epithelial cells.

Presence of B7-1 in urinary sediment was related to the presence of urinary podocytes, as reflected by a significant correlation between the expression of B7-1 and both NPHS1 ($r = 0.268$, $P = 0.022$), NPHS2 ($r = 0.317$, $P = 0.019$) and SyNPO ($r = 0.385$, $P = 0.002$). Moreover, significant correlations were observed between serum creatinine levels and urinary mRNA levels of NPHS1 ($r = 0.317$, $P = 0.006$), NPHS2 ($r = 0.284$, $P = 0.042$) and SyNPO ($r = 0.390$, $P = 0.001$), whereas there was a trend toward significance between proteinuria and the urinary expression of B7-1 ($r = 0.222$, $P = 0.059$) and NPHS2 ($r = 0.254$, $P = 0.067$). No associations were observed between age and the urinary mRNA expression of any of the genes.

As shown in Figure 1, urinary mRNA expression of B7-1 and NPHS1 was significantly higher in patients with GKD when compared with healthy subjects ($P = 0.005$ and $P = 0.05$, respectively). NPHS2 and SyNPO mRNA expression in the urinary sediment of patients with GKD was also increased, but this increase did not reach statistical significance.

We observed that the urinary mRNA expression of B7-1 and NPHS1 was significantly higher in patients with GKD than in healthy subjects ($P = 0.050$ and $P = 0.008$, respectively). However, when proliferative and non-proliferative GKD types were analyzed separately, significant differences in the urinary mRNA expression of NPHS1, but not of B7-1, were observed between patients with proliferative and non-proliferative types ($P = 0.017$ and $P = 0.487$, respectively) (Figure 2).

Validation of the diagnostic accuracy of B7-1 and NPHS1 urinary sediment mRNA levels by ROC curves and correspondence analysis

In order to discriminate patients with GKD from healthy subjects, we calculated the AUC and cut-off points for B7-1 and NPHS1. Those with the best equilibrium between sensitivity and specificity were chosen as the best markers. Urinary mRNA expression of B7-1 and NPHS1 showed a high discriminatory power for GKD (Figure 3).

As described above, based on B7-1 and NPHS1 urinary mRNA expression points, we were able to differentiate between healthy subjects and patients with GKD (Figure 3). However, the separate analysis of B7-1 or NPHS1 did not yield enough information to distinguish among glomerular subtypes.

With the use of dummy variables for B7-1 and NPHS1, we observed that the most frequent combination was the presence of high mRNA expression levels of both B7-1 and NPHS1, present in 62% of the samples. In the remaining combinations, a high mRNA expression level of B7-1 and a low mRNA expression level of NPHS1 was present in 23% of the samples.
of samples, a low mRNA expression level of B7-1 and a high mRNA expression level of NPHS1 was present in 12% of the samples and low expression levels of both NPHS1 and B7-1 were present in 3% of the samples.

Assessment of these two genes using multiple correspondence analyses resulted in two main groups in the biplot graph (Figure 4A). Regarding glomerular subtypes, 63% of healthy subjects were associated with low mRNA expression levels of both B7-1 and NPHS1. Twenty percent of patients with MCD also had low levels of both B7-1 and NPHS1, and 40% had low levels of NPHS1. Analysis of the other glomerular subtypes showed a higher percentage of patients with high mRNA expression levels of both B7-1 and NPHS1: MPGN (86%), FSGS (80%), IgAN (76%) or MGN (59%). Figure 4B shows the results of an analysis carried out after excluding the control population (healthy subjects) in order to discriminate among types of GKD.

**Urinary gene expression analysis of B7-1 and NPHS1 in MCD and FSGS**

No differences were observed in B7-1 and NPHS1 mRNA expression in patients with MCD compared to healthy subjects. However, patients with FSGS had a significantly higher B7-1 and NPHS1 mRNA expression than healthy subjects (P = 0.012 and P = 0.030, respectively).

On the other hand, patients with MCD had a significantly lower NPHS1 mRNA expression than patients with FSGS (P = 0.012), but a trend toward higher B7-1 mRNA expression that did not reach statistical significance (Figure 2). Finally, we observed that the B7-1:NPHS1 urinary mRNA ratio was significantly higher in patients with MCD compared to patients with FSGS (P = 0.027) (Figure 5A), allowing us to discern between patients with MCD and FSGS. This finding was further confirmed with the ROC curve analysis, which showed a high discriminatory power for positive MCD (Figure 5B).
cell regulation by binding to CD28 and CTLA-4 [4–6].

Glomerular expression of B7-1 on podocytes has been recently described in experimental models and in human systemic lupus erythematosus [7, 8]. In our study, we confirm that B7-1 mRNA expression is upregulated in urinary podocytes of adult patients with GKD. Interestingly, this upregulation was not specifically related to any particular histological type [22–24]. Because the loss of podocytes in the urine could be reflecting a decrease in the total number of glomerular podocytes [25], it is important to standardize mRNA B7-1 expression by using a podocyte-specific gene, thus facilitating comparisons across samples from different histologic types [15, 16].

Although levels of urinary sediment mRNA of B7-1 did not allow us to discriminate among the different types of GKD, when analysed in conjunction with mRNA expression of NPHS1, the discriminatory power increased substantially, especially in the group of patients with non-proliferative diseases, resulting in a clear distinction between disease subtypes. In glomerular diseases that tend to proliferate, such as IgAN and MPGN, we found low B7-1 mRNA expression and a high NPHS1 mRNA expression in urinary sediment. We found more NPHS1 mRNA expression in patients with FSGS than in patients with the other non-proliferative histologic types (i.e. MCD and MGN) consistent with the increased podocyturia observed in FSGS [13, 26, 27]. Thus, correlating the expression of B7-1 mRNA and NPHS1 mRNA allows us to differentiate between MCD and FSGS, which is otherwise difficult to do without a confirmatory renal biopsy.

The upregulation of B7-1 mRNA expression observed in urinary podocytes in patients with MCD is consistent with results reported by Garin et al. [9, 10], who suggested that urinary B7-1 excretion could be a useful marker to differentiate between MCD and FSGS. They found an increase in B7-1 protein level in MCD patients in relapse as compared to patients with FSGS. In our study, we observed an increase in the B7-1:NPHS1 mRNA expression ratio in urinary podocytes, demonstrating that upregulation of B7-1 is detected both at protein level, as reported by Garin et al., and at mRNA level, as our results have shown. The fact that we found an increase in the B7-1:NPHS1 mRNA expression ratio in urinary sediment but not in urine supernatant supports the hypothesis that any B7-1 detected in urine is derived from the podocyte cell membrane rather than a soluble form. B7-1, as well as other membrane proteins, has been described both in its membrane-associated and soluble forms [28, 29]. From the work by Garin et al. [9, 10], one can conclude that the increased levels of B7-1 in urine observed in patients with MCD relapse correspond to the soluble form of the B7-1 receptor, whereas the increased levels of B7-1 detected using western blot (by precipitating proteins present in lysates of the cell fraction of urine) would correspond to the membrane-associated form. The method used in our study to determine levels of B7-1 (gene expression analysis) does not allow us to discriminate between soluble and membrane-associated forms. However, we did not detect the soluble form of B7-1 at protein level. Thus, we could speculate that the increased levels of B7-1 we detected corresponded to the membrane-associated form. Even though our results are consistent with those of Garin et al. [10], we observed that analysis of B7-1 mRNA expression alone cannot distinguish between MCD and FSGS and that to distinguish between both GKD subtypes, it must be associated with data regarding NPHS1 mRNA expression.

Upregulation of B7-1 in patients with MCD may explain some of the abnormalities seen in T-cell function
and in levels of circulating factors that lead to increased glomerular permeability [6, 12, 30–34]. As the disease progresses and phenotypic changes resembling a FSGS form occur, mRNA expression levels of B7-1 in podocytes decrease. Therefore, podocyte damage is not only due to T-cell dysfunction but also due to structural changes in the podocyte.

It is known that several cells within the kidney may express B7-1, such as podocytes and tubular epithelial cells. Therefore, in order to better identify the source of B7-1 in the urinary sediment, we discarded urinary samples from patients in whom tubular damage due to viral infections, acute tubular necrosis or drug toxicity were suspected. We detected few tubular epithelial cells in only four cases, of which two corresponded to MCD and the other two to MGN, and they were independent from the expression of B7-1. Also, the detection of podocalyxin-positive cells in the urinary sediment of patients with

![Multiple Correspondence Analysis](image-url)
GKD, and the fact that the increase in B7-1:NPHS1 mRNA expression ratio was observed in urinary sediment but not in urine supernatant, supports the hypothesis that all B7-1 detected in urine was derived from podocytes.

Counting of urinary podocytes based on urine cytology has been used as a non-invasive method [15, 27, 35]; however, it involves the participation of experienced cytologists and is time consuming. Very recently, a novel method for isolating podocytes was described by Murakami et al. [36]. The main advantage of this method is that it enables the complete enrichment of podocytes and subjects them to further analysis within 6 h after removal of a mouse kidney. Therefore, it has potential utility for various downstream applications such as genomic analysis, proteomics and
transcriptomics to elucidate molecular profiling of podocyte biology in vivo. However, it requires a high cell number $(1 \times 10^6)$. In our study, performing this cell sorting approach would have been difficult because there were few urine sediment cells. However, we report an alternative approach, the quantification of mRNA expression of podocyte-specific markers, such as NPHS1 (a transmembrane protein exclusively expressed by glomerular podocytes) in the urinary sediment by quantitative RT-PCR, which may be a valuable method for detecting urinary podocytes, and therefore to analyse B7-1-podocyte relationship.

Our results also suggest that NPHS1 could be a useful marker to assess podocyte B7-1 mRNA expression in the urinary sediment. Previous studies have regarded nephrituria as a possible marker of glomerular damage in acquired diseases [25, 35, 37, 38]. In our study, detection of both B7-1 and NPHS1 mRNA expression showed a good discriminatory power in GKD.

Additionally, we assessed the mRNA expression of other podocyte markers, namely NPHS2 and SyNPO. Despite the fact that we observed an increase in the gene expression of these two markers in the urinary sediment of patients with GKD compared to healthy subjects, subsequent analyses revealed that neither were as sensitive as NPHS1 in the GKD compared to healthy subjects, subsequent analyses revealed that neither were as sensitive as NPHS1 in the identification of adult patients with GKD. Nonetheless, previous studies have attempted to differentiate between types of glomerular diseases using SyNPO as housekeeping gene to normalize for NPHS1 and NPHS2 expression [20, 39, 40]. Currently, it is known that calcineurin induces dephosphorylation of synaptopodin, leading to disruption of the podocyte actin cytoskeleton; thus, SyNPO is not considered an appropriate housekeeping gene [20, 38, 41, 42].

Despite the fact that B7-1 mRNA expression and other podocyte-associated markers have been linked to the pathogenesis of GKD [1, 2], we did not observe a link between B7-1 mRNA expression and proteinuria per se in glomerular patients. A possible explanation for this finding is that we did not evaluate podocyturia levels for different degrees of proteinuria and according to the subtype of glomerular disease. In consequence, we found it difficult to assess the relationship between proteinuria and an increase in B7-1 and/or NPHS1 mRNA expression.

In summary, non-proliferative GKD, and in particular MCD, showed an increased urinary mRNA expression of B7-1 associated to a low podocyturia. We believe that the combination of data related to B7-1 and NPHS1 mRNA expression levels could be useful to differentiate between MCD and FSGS forms in association with other known glomerulosclerosis-related histologic patterns. Further studies with larger patient samples are warranted in order to confirm our results.

Supplementary data

Supplementary Figure 1 is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

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B7-1 and NPHS1 urinary expression in glomerular disease


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