ability of dialysis facilities, the need for early detection and preventative measures with regard to renal disease in our area is evident.

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References


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Functional analysis of promoter mutations in the ACTN4 and SYNPO genes in focal segmental glomerulosclerosis

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The first two authors contributed equally to this work.

Abstract

Background. To investigate the promoter mutations of ACTN4 and SYNPO genes in patients with idiopathic focal segmental glomerulosclerosis (FGS), and to provide functional analysis of these mutations in the role of FSGS occurrence.

Methods. The study consisted of 82 Chinese idiopathic FGS patients (55 patients had nephrotic syndrome: NS) and 90 healthy individuals. Genomic DNA extracted from peripheral leukocytes of patients of healthy individuals were used to analyse the ACTN4 and SYNPO gene promoter mutations by polymerase chain reaction (PCR) and direct sequencing. Mutations were matched with GenBank and TRANSFAC software database (www.genome-tix.de; www.gene-regulation.com). A dual luciferase assay system was used to analyse the effects of mutations based on PGL3-Basic vector, pRL-SV40 vector, a PC12 cell line and podocytes in vitro. Kidney alpha-actinin-4 and synaptopodin expression of mutated patients and genomic DNA of their parents were investigated.

Results. The study detected the ACTN4 gene promoter 1–34C>T, 1–590delA and (1–1044delT)+(1–797T>C)+...
(1–769A>G) heterozygous mutations in three patients, respectively, and the SYNPO gene promoter 1–24G>A and 1–851C>T heterozygous mutations in two patients, respectively (with adenine of translation start site ATG naming +1). The same mutations were not found in the control group of 90 healthy people. Excluding one patient with an ACTN4 gene promoter mutation who inherited her parents’ 1–1044delT and 1–797T>C mutated chromosome, respectively, the same mutations were not found in patients’ parents. Alpha-actinin-4 and synaptopodin protein expression are reduced in mutated patients’ kidneys. Dual luciferase assays show that compared to the normal group (with the exception of the 1–1044delT group), luciferase activity in mutated groups decreased for the most part. (1–1044delT)+(1–797T>C)+(1–769A>G) mutations are associated with poor clinical outcomes, and patients with these mutations progress to end-stage renal failure.

**Conclusion.** The study detected heterozygous mutations in the promoters of the ACTN4 and SYNPO genes in patients with idiopathic FSGS. These mutations affected gene transcription in vitro and may affect protein translation in vivo. So we presumed that the ACTN4 and SYNPO promoter mutations might also contribute to pathophysiology of idiopathic FSGS.

**Keywords:** ACTN4; focal segmental glomerulosclerosis; mutation; promoter; SYNPO

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**Introduction**

Focal segmental glomerulosclerosis (FSGS) is characterized as segmental sclerosis in a proportion of glomeruli. FSGS patients present with variable levels of proteinuria and a progressive loss of renal function. It is a common form of glomerular lesion and a significant cause of end-stage renal disease (ESRD) [1,2]. The incidence of FSGS has risen significantly in the past 20 years, and accounts for 9–25% of the total kidney biopsies in adult patients, and FSGS is the most common primary glomerulonephritis leading to ESRD in the USA [3,4]. The genetic contribution to the aetiology of FSGS has been found in podocyte proteins including nephrin, podocin, alpha-actinin-4, CD2AP and TRPC6 [5–10].

Alpha-actinin-4 and synaptopodin are both actin-bundling proteins coded by the ACTN4 and SYNPO genes, respectively. The function of these proteins is to cross-link and bundle F-actin filaments, and also play a role in signalling conduction and regulation of podocyte mobility [11–13]. They interact with each other through tight junction protein MAGI-1 [13]. Alpha-actinin-4 is expressed widely in different cell types, while alpha-actinin-4 is the only actinin subtype that is expressed in the kidney [9]. Synaptopodin is expressed mainly in neuronal dendrites, where it is found at the postsynaptic density and the spine apparatus in a subset of telencephalic neurons [14]. Mundle et al. [15,16] detected it in differentiated renal podocytes using the monoclonal antibody mAb G1. Data show the expression level of synaptopodin in glomeruli as a tool to discriminate diagnosis between MCD and FSGS, and allow a proper regimen of steroid treatment [17–19]. Recently, functional variants in the NPHS2 promoter have been identified in a large cohort of patients with nephrotic syndrome (NS) and FSGS, which shows that promoter functionality plays a key role in FSGS processes [20]. Whether the ACTN4 and SYNPO gene promoter variants or mutations involving functional activity are part of the FSGS process is unclear.

**Table 1. Clinical characteristics**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Sex (male/female)</th>
<th>HP* (yes/no)</th>
<th>24-h urinary protein excretion (g)</th>
<th>Serum albumin (g/l)</th>
<th>Serum creatinine (μmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS</td>
<td>82</td>
<td>43/39</td>
<td>32/50</td>
<td>5.08 ± 4.14</td>
<td>27.57 ± 11.82</td>
<td>125.44 ± 110.71</td>
</tr>
<tr>
<td>NS</td>
<td>55</td>
<td>37/18</td>
<td>22/33</td>
<td>6.99 ± 3.76</td>
<td>16.96 ± 6.64</td>
<td>120.71 ± 112.87</td>
</tr>
<tr>
<td>Non-NS</td>
<td>27</td>
<td>6/21</td>
<td>10/17</td>
<td>1.20 ± 0.89</td>
<td>37.00 ± 5.76</td>
<td>135.07 ± 107.62</td>
</tr>
</tbody>
</table>

Occurrence of HP and levels of urinary protein, serum albumin and serum creatinine in 82 FSGS patients. HP, hypertension; NS: nephrotic syndrome.

**Table 2. Primers for ACTN4 and SYNPO genes promoter PCR and sequencing**

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Forward primer (5′→3′)</th>
<th>Reverse primer (5′→3′)</th>
<th>Sequencing primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>ACCTGAGGTCGGGGGATTTGA</td>
<td>GCCATCGGACCTGAACCA</td>
<td>ACGCTAGGGAGAGTTGA</td>
</tr>
<tr>
<td>A2</td>
<td>ACCTGAGGTCGGGGGATTTGA</td>
<td>GCCATCGGACCTGAACCA</td>
<td>GCCATCGGACCTGAACCA</td>
</tr>
<tr>
<td>A3</td>
<td>TGTTTCAGGTCGGGATTTGA</td>
<td>ACGCTAGGCTACCCACA</td>
<td>ACGCTAGGCTACCCACA</td>
</tr>
<tr>
<td>S1</td>
<td>ACCGAAAGTGAAGCGATAGAG</td>
<td>ACTCACAGGCTAGCAGAAA</td>
<td>CACAGCTGCTAAGTCTATA</td>
</tr>
<tr>
<td>S2</td>
<td>ACCGAAAGTGAAGCGATAGAG</td>
<td>ACTCACAGGCTAGCAGAAA</td>
<td>CACAGCTGCTAAGTCTATA</td>
</tr>
<tr>
<td>S3</td>
<td>TAATCAGGGTGGCGTAGAC</td>
<td>TAGCTGAGGTTGATACCTTGT</td>
<td>TAATCAGGGTGGCGTAGAC</td>
</tr>
</tbody>
</table>

Three pairs of PCR forward and reverse primers were used to amplify each gene promoter. Each sequence fragment was sequenced by one specific sequencing primer. A: ACTN4, S: SYNPO.
Fig. 1. Schematic diagram of promoter constructs subclone. (a) pACTN4 construct subclone ACTN4 gene promoter 1366 bp from −1115 upstream to +251 downstream of translation start site (ATG). (b) pSYNPO1 construct subclone SYNPO gene promoter 741 bp from −939 upstream of translation start site (ATG) to +133 downstream of first exon and pSYNPO2 construct subclone SYNPO gene promoter 600 bp from −173 upstream of second exon to +95 downstream of translation start site (ATG).

ACTN4 and SYNPO promoter mutations in FSGS

Table 3. Mutations and potential binding transcription factor

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Potential transcription factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–34C&gt;T</td>
<td>Stimulating protein 1, SP1</td>
</tr>
<tr>
<td>1–590delA</td>
<td>Myogenic enhancer factor 2, MEF2</td>
</tr>
<tr>
<td>1–769A&gt;G</td>
<td>Carbohydrate response element binding protein (CHREBP)</td>
</tr>
<tr>
<td>1–797T&gt;C</td>
<td>Hepatic nuclear factor 4, HNF4</td>
</tr>
<tr>
<td>1–1044delT</td>
<td>Brn-2, POU-III protein class</td>
</tr>
<tr>
<td>1–24G&gt;A</td>
<td>Olfactory neuron-specific factor</td>
</tr>
<tr>
<td>1–851C&gt;T</td>
<td>TGF beta-inducible early gene, TIEG</td>
</tr>
</tbody>
</table>

Potential disease contributing mutations were selected by the promoter transcription factor binding prediction software (www.genometrix.de; www.gene-regulation.com). Transcription factors binding to mutation sites are listed.

To address this question, we carried out a sequencing study of the promoter regions of the ACTN4 and SYNPO genes from −1100 to ATG and −1370 to ATG, respectively, looking for variants or mutations that could affect alpha-actinin-4 and synaptopodin gene expression in idiopathic FSGS patients. In this study, we reported for the first time the presence of mutations in the promoters of the ACTN4 and SYNPO genes that reduced alpha-actinin-4 and synaptopodin expression and correlated with a poor disease outcome.

Materials and methods

Patients and control normal volunteers

We selected 82 cases with biopsy-proven FSGS patients (age range: 12–76 years, 43 males and 39 females). Among them, 55 cases had NS. Secondary and inherited FSGS were excluded based on clinical presentation and histology (Table 1).

Nineti unready healthy individuals (aged 24–55 years), without history of renal disease or abnormal urinary findings, were also studied as controls. Informed consent was given in accordance with a protocol approved by the Institutional Review Board at the Ruijin Hospital.

ACTN4 and SYNPO gene coding region sequence

Genomic DNA was extracted and purified from peripheral leukocytes in whole blood samples and from hair follicles according to the standard procedures. The ACTN4 and SYNPO gene sequences were obtained from GenBank (NC_000019, NC_000005). Exons were amplified by polymerase chain reaction (PCR) using flanking intronic primers and subjected to automatic sequencing and analysis that are shown below.

ACTN4 and SYNPO gene promoter potential mutation screening

Promoter region-sequencing primers were designed using the primer 5 and Oligo 6.44 software (Table 2). PCR reactions were performed in a total volume of 25 μl containing 20 ng/μl genomic DNA, 10 pg of each primer, 2.5 μl MgCl2 (25 mM), 2.0 μl dNTP mixture (2.5 mM each), 2.5 μl of 10× reaction buffer and 0.2–0.4 unit of thermostable DNA polymerase. DNA was denatured at 95°C for 2 min, followed by 35 cycles of denaturation of 30 s at 95°C, annealing for 45 s at 60–64°C, extension of 45–60 s at 72°C, and final extension of 10 min at 72°C.

Aliquots (3 μl) of the PCR product were electrophoresed on 1% agarose gels and visualized under UV illumination after staining with ethidium bromide. The PCR products were purified using a Millipore PCR plate or a QIA quick PCR Purification Kit (Qiagen, USA), PCR-amplified with a Big dye terminator or ET-terminator, cycle sequencing and sedimentation. The cycle sequencing product was then run through an ABI 3730 automated sequencer (Perkin Elmer, Applied Biosystems, USA) according to the manufacturer's protocol.

The screening of potential mutations was performed by comparing the output nucleotide sequence with the published sequence of the ACTN4 and SYNPO genes (GenBank database) using Clustaw and naked eye check by the Chromas software. Then, potential disease contributing mutations were selected by the promoter transcription factor binding predict software (www.genometrix.de; www.gene-regulation.com).

Cell culture

The rat pheochromocytoma PC12 cells were maintained in an RPMI 1640 medium with GlutaMAX-I (Invitrogen, USA) supplemented with 10% horse serum (Invitrogen, USA) and 5% fetal bovine serum. Conditionally immortalized murine podocytes proliferated under permissive conditions (gamma interferon at 33°C) but differentiated under non-permissive conditions (37°C). Prior to the experiments, cells were grown at 37°C on type I collagen-coated dishes for 10 days to inactivate the temperature-sensitive T antigen and to allow for differentiation. In all cases, culture medium was supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin, and cells were grown in a humidified atmosphere containing 5% CO2 at 37°C. The medium was replaced every 2–3 days.

Western blot

The PC12 cells grown to confluence were washed twice with cold PBS and harvested in a membrane lysis buffer (30 mM Tris, pH 8.0, 10 mM NaCl, 5 mM EDTA, 10 g/l polyoxylethylene-8-lauryl ether, 1 mM 0-p-nitroaniline, 1 mM iodoacetamide, 10 mM NaF, 5 mM orthovanadate and 10 mM sodium pyrophosphate). The cells were immediately frozen in liquid nitrogen, lysed and subjected to Western blot analysis using an anti-synaptopodin (Biosdesign, USA) antibody.

RT-PCR

The PC12 cells were transferred to a 15 ml Falcon tube and collected by centrifugation. The cells were washed twice and resuspended in 100 μl

Fig. 3. Sequence of (1–797T>C)+(769A>G)+(1–1044delT) mutated patient's parents. (a) Sequence of 1–797T>C mutation in the patient's father. (b) Sequence of 1–1044delT mutation in the patient's mother (N—normal, M—mutation, arrow show mutated site).
PBS. Total RNA was collected with the RNA SafeKit (Bio 101, Vista, CA, USA). Poly(A+) mRNA was then isolated with the mRNA Kit Oligo [dT]30 (Bio 101, Vista). The concentration of mRNA was determined by spectrophotometry. cDNA was prepared from 0.2 μg PC12 mRNA (Clontech, Palo Alto, CA, USA) using the ThermoScript RT-PCR Kit (Life Technologies, Rockville, MD) with oligo [dT] primers. PCR was performed with SYNPO gene primers, sense: tactcagaggaggctagctt and anti-sense: tgtgccattagatgggagtt. PCR products were sequenced with a sense primer.

Generation of promoter constructs

The ACTN4 and SYNPO promoter fragment of about 1366 bp (pACTN4), 741 bp (pSYNPO1) and 600 bp (pSYNPO2) upstream from the translation start site was generated from genomic DNA of mononuclear cells from patients and a healthy control group by PCR (Figure 1). The sequences of sense and antisense primers to amplify this fragment were ACTN4: 5′-gctAGATCTacctgaggtcggga-3′ and 5′-catTAAGCT-Tacgctccagtatcctttca-3; SYNPO01: 5′-taaGGTACCtctaggta ggccggaaat-3′ and 5′-catAGATCTcaggctggcagaaatcac-3′; SYNPO02: 5′-cgAGATCT-cagggctgattacgaagtga-3′ and 5′-cgcTAAGCTTtcctttag ataaaccaccag-3′, respectively. The PCR products were subcloned into a pGEM-T-easy vector and were sequenced to ensure that no misincorporated mutations were introduced into the PCR products during amplification. The luciferase reporter recombinant pACTN4-luc, pSYNPO1-luc and pSYNPO1-luc encoding a modified firefly luciferase gene (or the empty pGL3 vector) co-transfected with 12.5 ng of pRL-SV40 (Promega, USA) encoding the Renilla luciferase gene. After 48 h under normoxic conditions, the cells were assayed for luciferase activity in a Packard Lumicount™ using the DualGlow™ Luciferase Assay System (Promega, USA) according to the manufacturer's instructions. Promoter activities were expressed as a ratio of firefly luciferase to Renilla luciferase luminescence in each well. Results were given as arbitrary units (AU) [21]. The paired t-test was utilized to compare luciferase expression by different constructs. Each experiment was conducted using at least nine replicates. The values of P < 0.05 were regarded as significant.

Immunofluorescence

Fresh kidney tissue was embedded in Tissue-Tek OCT compound, frozen in liquid nitrogen and sectioned at 4 μm. Slides were fixed in cold 100% acetone and then air dried. The sections were then incubated with 1% BSA at room temperature to block non-specific binding. They were incubated with mouse anti-alpha-actinin (AT6.172, Chemicon, USA) and mouse anti-synaptopodin (Biodesign, USA) at room temperature for 1 h, rinsed with PBS, and then incubated with an FITC-labelled anti-mouse IgG antibody (KPL, USA) at room temperature for 1 h, and mounted with a VECTASHIELD medium. Staining was analysed by LSM-510 confocal laser scanning microscopy (ZEISS, German). Laser power, pinhole size
and detector gain were the same for all samples. Confocal images were analysed with the MetaMorph imaging analysis software. Fluorescence intensity of glomeruli was obtained after subtraction of the background intensity. Six glomeruli were quantified in each sample (n=6), and one patient (n=6) compared to three controls (n=18).

Cells were fixed in 4% paraformaldehyde for 15 min at room temperature. They were then permeabilized with 0.2% TritonX-100 in 10% goat serum and incubated with primary antibodies in (PBS) overnight at 4°C. They underwent five washes with PBS, and were then incubated with fluorescent-conjugated secondary antibodies for 1 h at room temperature and mounted with a VECTASHIELD medium after a PBS wash. Both stainings were analysed by LSM-510 confocal laser scanning microscopy (ZEISS, German).

Statistical analyses
Data were expressed as the mean ± SD and compared by Student's t test and ANOVA. P < 0.05 was considered statistically significant.

Results
ACTN4 and SYNPO gene promoter sequence results
In total, we identified twelve promoter variants in 82 patients. Seven of those were not present in 90 healthy individuals. In contrast, there were nine variants that were
identified in the control group and could not be identified in patients. The promoter variants were matched with the GenBank (www.ncbi.nlm.nih.gov) and TRANSFAC software databases (www.genometix.de; www.gene-regulation.com). Eventually, seven potential disease-contributing mutations that were identified as regulatory elements were found in the promoter regions (Table 3). Three patients with the 1–34C>T, 1–590delA, 1–1044delT, 1–797T>C and (1–797T>C)+(1–769A>G) heterozygous mutations, respectively, in the ACTN4 gene promoter (Figure 2a–c) and two patients with the 1–24G>A and 1–851C>T heterozygous mutations, respectively, in the SYNPO gene promoter (with adenine of translation start site ATG naming +1) (Figure 2e and f). The patients with 1–851C>T and (1–1044delT)+(1–797T>C)+(1–769A>G) clinically show NS with resistance to steroid treatment, and the latter progress

Fig. 6. pACTN4-luc and pSYNPO-luc constructs confirmed by sequencing. (a, b) Recombinant pACTN4 wild-type construct and constructs include the following five variants of the ACTN4 gene promoter: 1–34C>T, 1–590delA, 1–1044delT, 1–797T>C and (1–797T>C)+(1–769A>G), confirmed by sequencing. (c) Recombinant pSYNPO1 and pSYNPO2 wild-type constructs and constructs include two variants of the SYNPO gene promoter: 1–851C>T and 1–24G>A, confirmed by sequencing.
to ESRD within 8 years after diagnosis. The patients with 1–34C>T, 1–24G>A and 1–851C>T clinically show non-NS proteinuria without progress to ESRD within a 12-year follow-up after diagnosis.

**Screening mutation in patients’ parents**

The 1–34C>T, 1–24G>A and 1–851C>T mutations were not found in the DNA extracted from hair follicles of patients’ parents (data not shown). (1–1044delT)+(1–797T>C)+(1–769A>G) promoter region mutated patient inherited her parents’ 1–1044delT and 1–797T>C mutated chromosomes, respectively. In addition, there was a novel 1–769A>G mutation (Figures 2c and 3a and b).

**PC12 cell line alpha-actinin-4 and synaptopodin expression**

In order to use PC12 cells to analyse the ACTN4 and SYNPO promoter mutation function, we confirmed PC12 cell synaptopodin expression by immunofluorescence, RT-PCR, PCR product sequencing and Western blot (Figure 4a–d). Alpha-actinin-4 expression in PC12 cell line has been reported [12].

**pACTN4-luc and pSYNPO-luc construct confirmation**

In total, we recombined six pACTN4-luc constructs including five mutated and one normal control, which were confirmed by sequencing and restriction enzyme cutting (Figures 5a and 6a and b). We also used four pSYNPO-luc constructs, including two mutated and two normal controls, which were also confirmed by sequencing and restriction enzyme cutting (Figures 5b and 6c).

**Functional implication of ACTN4 and SYNPO promoter mutations**

Functional implications of mutations in the ACTN4 and SYNPO gene promoters were studied following consolidated protocols based on subcloning in pGL3 reporter vector and transient transfection experiments in PC12 cells and podocytes. A reduction of luciferase expression by PC12 and podocytes compared to the wild-type sequence was determined in all mutations except 1–1044delT. The (1–797T>C)+(1–769A>G) and 1–851C>T mutated groups produced >50% reduction (Figure 7a and b).

**Alpha-actinin-4 and synaptopodin immunofluorescence in patients with mutations**

In normal kidney, alpha-actinin-4 was distributed on the glomerular capillary. Alpha-actinin-4 level in the kidney was similar between the normal group and the FSGS group (379.67 ± 84.44 versus 398.11 ± 48.01, P > 0.05). In patients with the (1–1044delT)+(1–797T>C)+(1–769A>G) mutation, alpha-actinin-4 expression was reduced significantly as compared to normal or FSGS patients without these mutations (130.00 ± 13.33 versus 398.11 ± 48.01, P < 0.01) (Figure 8). Although synaptopodin protein expression reduction can be found in NS-FSGS patients compared to the normal group (514.00 ± 31.21 versus 798.56 ± 43.14, P < 0.05), much more reduction of synaptopodin expression can still be found in 1–24G>A and 1–851C>T mutated patients compared to NS-FSGS patients (321.33 ± 18.01, 373.3 ± 30 versus 514.00 ± 31.21, P < 0.05) (Figure 9).

**Discussion**

The pathogenesis of FSGS may be heterogeneous, and the clinicopathologic syndrome has been defined as either primary (idiopathic or sporadic), secondary or familial. In recent years, defective podocyte proteins were recognized as the most important factor contributing to FSGS [22]. Several mutations have been found in podocyte proteins including nephrin, podocin, alpha-actinin-4, CD2AP and TRPC6 [5–10]. These mutations have typically been involved in affecting normal amino acid coding and defective protein structure. More than 50 different NPHS2 mutations have been reported from studies of NPHS2 exon mutations involving familial and idiopathic NS or FSGS [23–27]. Similar exon mutations have also been found in the ACTN4 and SYNPO genes in idiopathic Chinese FSGS patients (our unpublished data).
In 2000, Kaplan et al. [9] reported that heterozygous ACTN4 exon mutations caused the dominant hereditary family FSGS, which presents usually as adult onset, non-nephrotic range proteinuria, with a slow decline of renal function. Although the above mutation sites do not cause alpha-actinin-4 structural changes, their pathogenetic functions were proven in animal models [28,29], and by in vitro studies using a podocyte culture [30–32]. These in vivo and in vitro studies support the important role of alpha-actinin-4 in maintaining normal glomerular function and in the pathogenesis of glomerular diseases. SYNPO gene knockout in mice causes neurons to lack spine apparatus and show deficits in synaptic plasticity [33,34]. In addition, heterozygosity for synaptopodin is sufficient to promote FSGS-like disease in CD2AP heterozygous mice [35]. Although there are not as much data show that the defects in synaptopodin cause nephritic syndrome or FSGS as alpha-actinin-4, its change was found in nephritic
syndrome patients. In the puromycin minimal change rat model, the podocyte cytoskeleton structure, as synaptopodin, reduces initially and recovers along with the elimination of proteinuria. Yang [17] found that synaptopodin is reduced in collapsed FSGS- and HIV-associated kidney disease while the same change could not be viewed in minimal change or membrane nephropathy. Kemeny [18] also found the same result in primary FSGS. Because of the limitation of quantification in the above studies, Srivastava [19] performed further quantitative exams in patients with MCD, DMH and FSGS, and observed that there was a statistically significant difference of synaptopodin expression between FSGS and MCD. Moreover, synaptopodin expression was reduced with recurrence of proteinuria after transplantation and recovered with the treatment of steroid [36]. So, synaptopotin is a protective

Fig. 9. Immunofluorescence of synaptopodin in patients with 1–24G>A and 1–851C>T mutations and in controls. Fresh kidneys tissue frozen section incubated with anti-synaptopodin followed by FITC-labelled anti-mouse IgG antibody. Staining was analysed by LSM-510 confocal laser scanning microscopy. (a) Representative glomeruli of normal synaptopodin were distributed on glomerular capillary. (b) Representative glomeruli of non-NS FSGS group, synaptopodin distribution and density were similar to the normal group. (c) Representative glomeruli of the NS FSGS group, synaptopodin level lower than the normal group. (d, e) Representative glomeruli of patients with 1–24G>A and 1–851C>T mutations, synaptopodin expression reduced significantly. (f) Negative control of immunofluorescence without an anti-synaptopodin first antibody (magnifications ×400).
factor to proteinuria [37], and investigation of synaptopodin can provide a means to discriminate diagnosis between MCD and FSGS, and cast an index to the outcome of steroid treatment.

Pathologic studies showed that the expression of podocyte proteins is regulated in the presence of proteinuria or pathologic injury. Such pathologic studies indicate that the molecular scaffold of the slit diaphragm dissembles in animals and humans with proteinuria [25,38,39], and in these cases, some podocyte proteins are diminished in glomeruli [17,18,40,41]. It indicates that in addition to the presence of structurally functioning proteins in podocytes, a proper quantitative equilibrium among different components is required for the maintenance of podocyte morphology and the barrier functional integrity. This appears to be a dynamic process in which regulatory mechanisms of different proteins play a role. These data indicate that either the gene mutations that affect the protein translation and structure formation or the factors that affect podocyte protein expression can contribute to the pathogenesis of FSGS. As we know, the most important factor that regulates protein expression is promoter function. Although there are few studies on the podocyte protein coding gene promoter in NS or FSGS patients, functional variants in the NPHS2 gene promoter have been identified as a large cohort of patients with NS and FSGS, which is associated with a poor clinical outcome and evolution to end-stage renal failure [20].

In order to broaden our knowledge on promoter function contribution in FSGS process, we firstly report a study on two F-actin related genes: ACTN4 and SYNPO promoter mutations and function in 82 idiopathic Chinese FSGS patients. Combined analysis of mutations with a TRANSFAC software database predictor and a dual luciferase promoter function assay, we detected potential disease causing mutations in 1–34C>T, 1–590delA and (1–1044delT)+(1–797T>C)+(1–769A>G) in the ACTN4 gene promoter in three patients respectively, and 1–24G>A and 1–851C>T in the SYNPO gene promoter in two patients respectively. A significant reduction (−50%; \( P < 0.01 \)) of luciferase expression compared to the wild-type sequence were determined in the (1–797T>C)+(1–769A>G) and 1–851C>T mutated groups. They were associated with significantly reduced alpha-actinin-4 or synaptopodin protein expression in kidney. Except for one patient who inherited her parents’ mutated chromosome, respectively, in addition to her novel mutated site, the same variants were not found in the parents, indicating the possibility of germ cell mutation. Although (1–1044delT)+(1–797T>C)+(1–769A>G) mutated patients have poor clinical outcome and evolution to end-stage renal failure, whether and how down-regulation of podocyte protein influences the extent and outcome of FSGS is a matter of debate. While the finding of low alpha-actinin-4 and synaptopodin expression in glomeruli leads us to conclude a potential correlation between mutations in the ACTN4 or SYNPO gene promoters and alpha-actinin-4 or synaptopodin expression in renal biopsies, it also allows to speculate on the pathogenesis of proteinuria because it is possible that low regulation lasting over years could represent a reason for progression to renal failure.

In summary, original results of a sequencing screening study on the ACTN4 and SYNPO gene promoters demonstrates for the first time that heterozygous carriers of mutations may contribute to the pathogenesis of idiopathic FSGS in some patients. Functional studies and case reviews demonstrated that some of these variants are associated with a poor clinical outcome with progression to end-stage renal failure. Thus, even if not sufficient to cause the disease per se, these mutations could represent factors for severity and/or progression of the disease in FSGS and/or in other proteinuric diseases.

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Conflict of interest statement. None declared.

References

Similar renal decline in diabetic and non-diabetic patients with comparable levels of albuminuria

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Abstract

Background. Diabetes is the main cause of ESRD, and albuminuria is a major determinant of adverse renal outcome. Likewise, albuminuria is an intermediate risk factor of chronic kidney disease (CKD) progression in diabetic patients. Our aim was to compare the rate of renal decline in diabetic and non-diabetic CKD patients (GFR < 50 ml/min) with comparable levels of albuminuria.

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