CD2AP mutations are associated with sporadic nephrotic syndrome and focal segmental glomerulosclerosis (FSGS)

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Abstract

Background. CD2-associated protein (CD2AP) is a crucial protein for the slit-diaphragm assembly and function. In spite of the fact that CD2AP knockout causes nephrotic syndrome in mice and the heterozygous +/- mouse is prone to proteinuria, little is known about the relevance of this molecule in human renal pathology.

Methods. A total of 80 Italian patients with idiopathic nephrotic syndrome were enrolled and screened for changes in the CD2AP gene. A normal control group of 200 healthy donors was also studied. The coding region of the CD2AP gene was analysed by polymerase chain reaction, denaturing high-performance liquid chromatography and sequencing. Peripheral blood mononuclear cells from patients with CD2AP mutations and from healthy donors were isolated by the Ficoll–Hypaque gradient, and the CD2/CD2AP interaction was studied on T-lymphocytes by confocal laser scanning microscopy analysis. The expression levels of CD2AP, nephrin and podocin proteins were evaluated by indirect immunofluorescence on renal biopsies from a patient with p.delGlu525 mutation and from control subjects. Moreover, the effect of the p.K301M mutation on cell viability was evaluated by flow cytometry and annexin V/propidium iodide staining.

Results. Three heterozygous mutations (c.904A>T; c.1120A>G; c.1573delAGA) producing respectively aminoacidic changes (p.K301M, p.T374A) or a deletion in functional domains (p.delGlu525) were found in three unrelated patients. One (p.K301M) produced a lysine to methionine change in the third interactive SH3 domain (position 301) and resulted in the defective CD2–CD2AP interaction and clustering; the other (c.1573delAGA) caused the deletion of the glutamic acid in position 525 in the COOH-terminal region of binding with nephrin and was associated with down-modulation of CD2AP, podocin and nephrin glomerular expression.

Conclusions. Our findings suggest that CD2AP mutations modify the interaction with CD2 in lymphocytes and alter the expression of the renal slit diaphragm.

Keywords: CD2AP; mutations; nephrotic syndrome

Introduction

CD2-associated protein (CD2AP) is an adapter molecule originally identified as a ligand for the T-cell-adhesion protein CD2. It is an 80 kDa cytoplasmic protein expressed in all tissues except the brain. CD2AP contains three Src homology 3 (SH3) domains at the NH₂ terminus followed by proline-rich sequences and a coiled-coil domain at the COOH terminus [1]. CD2AP plays a key role in the kidney where it is necessary for the ultrafiltration functions of the slit-diaphragm network. In fact, it is expressed primarily in glomerular podocytes at the cytoplasmic face of the slit-diaphragm (SD) domain where it interacts with nephrin and podocin [2,3]. Lack of the glomerular expression of CD2AP in animals produces mesangial cell proliferation with extracellular matrix deposition, glomerulosclerosis and extensive foot-process effacements [4] that are correlated with the entity of the defect. Accordingly, CD2AP −/− mice develop severe nephrotic syndrome and die of massive proteinuria shortly after birth. CD2AP +/+ heterozygous mice present, instead, a glomerular disease at 9 months with a histology pattern that mimics human focal segmental glomerulosclerosis (FSGS) [5], and also show deposition of immunoglobulins into the basement membranes that is reminiscent of an immunologic condition. It is relevant that the renal phenotype of CD2AP −/− deficient animals is rescued by podocyte transfection with CD2AP that strongly supports the concept that the kidney dysfunction is primary due to a podocyte anomaly [6].

In spite of the clear association of CD2AP defects with a glomerular pathology reminiscent of idiopathic FSGS in animal models, little is known in human beings. Kim et al. [5] described one heterozygous nucleotide variant resulting in an aberrant CD2AP splicing in two patients with idiopathic FSGS out of 30 African Americans with FSGS and 15 African Americans with FSGS associated with HIV infection. More recently, Löwik et al. described a patient affected by early-onset nephrotic syndrome with a CD2AP homozygous mutation resulting in a premature stop codon yielding a slightly truncated protein (by only 4%). This mutation reduced the expression of CD2AP by lymphocytes and the binding with F-actin [7]. Overall, the
mutilations described by Kim and by Löwik remain unique, so it is still uncertain whether CD2AP mutations have a real clinical impact as an inherited form of FSGS.

In this study, we report the mutational screening for the CD2AP gene in 80 Italian patients with sporadic FSGS and 200 healthy blood donors. Three heterozygous novel mutations that had functional consequences for the CD2/CD2AP interaction on T-lymphocytes and/or CD2AP expression in glomeruli were found in the coding region of the CD2AP gene in three unrelated patients.

Our findings suggest that CD2AP mutations are significant in FSGS patients, even though the true pathogenic mechanism involved appears unclear and so requires further investigation.

Methods

Patients

This study was approved by the local institutional review board and informed consent for genetic studies was obtained from each patient or legal tutor in the case of minors.

A total of 80 Italian patients with idiopathic nephrotic syndrome were enrolled in the study. Overall, all patients presented steroid resistance, 50 were younger than 18 years. Steroid resistance was considered when proteinuria persisted after 2 months of therapy. Based on immune-histological findings, all patients were classified as FSGS for the presence of at least one segmental area of glomerulosclerosis associated with diffuse mesangial IgM deposition. All patients were searched for mutations of relevant genes involved in inherited FSGS, such as NPHS2 and exons 8 and 9 of WT1 (typically affected in WT1-associated nephrotic syndrome), and were found negative. A normal control group of 200 healthy Italian donors (400 chromosomes) was also studied.

Mutational screening

Genomic DNA was extracted from peripheral blood samples using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Molecular analyses of the 17 exons of the CD2AP gene (NM 012120.2; GI 125987597) were performed by polymerase chain reaction (PCR), denaturing high-performance liquid chromatography (DHPLC) and direct sequencing. CD2AP-flanking intronic primers were selected based on published sequences or designed using the primer3 programme (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The PCR reactions were run in a total volume of 50 μl, with a final MgCl2 concentration of 1.5 mM and containing 100 ng of template DNA, 1 x reaction buffer, 0.2 mM of each nucleotide, 30 pmoles of primers and 0.3 U of DNA polymerase (TaqGold, Applied Biosystems, CA 94404, USA). The PCR reactions were carried out for 30–35 cycles with denaturation at 94°C for 30 s, annealing at ~55–60°C for 30 s and extension at 72°C for 30 s. All PCR programmes include an initial denaturation of 10 min at 94°C and the extension lasted 7 min in the last cycle.

DHPLC analysis was carried out on a WAVE DNA Fragment analysis system (Transgenomic™, Crewe, UK) equipped with a DNAsep® Column (Transgenomic™, Crewe, UK). PCR products for each exon were pooled, denatured for 5 min at 95°C and loaded on the DNAsep column. DNA was eluted at a flow rate of 0.9 ml/min within a linear acetonitrile gradient consisting of buffer A (0.1 M triethylammonium acetate, TEAA) and buffer B (0.1 M TEAA, 25% acetonitrile). Optimal DHPLC column temperatures for mutation detection were selected by WaveMaker 4.1 software (Transgenomic™, Crewe, UK) or by software available at http://insertion.Stanford.edu/melt.html. Data were analysed using the WAVE DNA Fragment Analysis System (Transgenomic™, Crewe, UK).

PCR products showing an abnormal elution profile were re-amplified, purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and subjected to automatic sequence analysis (Automated sequencer ABI 3130; Applied Biosystems, CA 94404, USA) by BigDye terminator reaction according to the supplier’s instructions (ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kits Version 2.0; Applied Biosystems, CA 94404, USA). Sequences were analysed by Seqscape program (Applied Biosystems, CA 94404, USA). CD2AP novel missense mutations were confirmed on the DNA of 200 healthy controls by DHPLC analysis. As a second indirect demonstration, a comparative analysis in nine different species (human, orangutan, mouse, rat, bovine, chicken, xenopus laevis, drosophila melanogaster, caenorhabditis elegans) of CD2AP amino acid sequences was done by ClustalW software. The patients with heterozygous CD2AP mutations were tested for NPHS1, ACTN4 and TRPC6 mutations by PCR and direct sequencing.

Cell cultures

Human peripheral blood mononuclear cells (PBMCs) were obtained by whole blood from two patients with CD2AP mutations and from healthy donors by density gradient centrifugation over Ficoll-Paque Plus (Amersham Biosciences, Milan, Italy). The culture medium was AIM-V (Invitrogen-Life Technology, Karlsruhe, Germany). PBMCs were washed, resuspended in the AIM-V medium and allowed to differentially adhere to a 75 cm2 tissue culture flask (Costar Corning, NY, USA) by culturing 90 × 10⁶ cells in 10 ml of AIM-V medium, for 90 min at 37°C, 5% CO2. Non-adherent cells (T-lymphocytes) were then removed by gentle rinsing and used for subsequent experiments.

Confocal laser scanning microscopy and tissue immunofluorescence

The expression and interaction of CD2 and CD2AP were evaluated in vitro on T-lymphocytes of two patients with CD2AP coding mutations and healthy donors in the presence or absence of phytohaemagglutinin (PHA; 0.2 mg/ml; Sigma-Aldrich, St Louis, USA), an activator of T-cell proliferation. We performed a double-fluorescence immunolabelling to evaluate the expression and the co-localization of the proteins. The cells were analysed by confocal laser scanning microscopy Leica TCS SP2 (Leica, Wetzlar, Germany). For this purpose, PBMCs (5 × 10⁶/slide) were seeded on coverslips (S-lens, Sigma-Aldrich, St Louis, USA) and incubated with secondary antibodies (goat anti-mouse IgG-Alexa Fluor 488 conjugate, goat anti-rabbit IgG-Alexa Fluor 488 conjugate antibody, rabbit anti-goat IgG-Alexa Fluor 488 conjugate antibody, goat anti-rabbit IgG-Alexa Fluor 488 conjugate antibody and chicken anti-mouse IgG-Alexa Fluor 488 conjugate antibody) and chicken anti-mouse IgG-Alexa Fluor 488 conjugate antibody (Molecular Probes, Eugene, OR, USA) for 1 h at room temperature. The slides were mounted in Gel/Mount (Biomedica, Foster City, CA) and sealed.

The expression levels of CD2AP, podocin, nephrin and synaptopodin proteins were evaluated by indirect immunofluorescence and confocal microscopy analysis on renal biopsy from a patient with p.delGlu525 mutation and from control subjects. The slides were incubated with the primary antibodies at room temperature (mouse anti-CD2AP antibody, Santa Cruz Biotechnology, 1:100; goat anti-podocin antibody, Santa Cruz Biotechnology, 1:200; rabbit anti-nephrin antibody, previously described [8], 1:150; mouse anti-synaptopodin antibody, Progen Biotechnik, ready to use), washed in PBS and then incubated with the secondary antibody chicken anti-mouse IgG-Alexa Fluor 488 conjugate antibody, rabbit anti-goat IgG-Alexa Fluor 488 conjugate antibody, goat anti-rabbit IgG-Alexa Fluor 488 conjugate antibody and chicken anti-mouse IgG-Alexa Fluor 488 conjugate antibody (Molecular Probes, Eugene, OR, USA), respectively. The slides were mounted with an anti-fading aqueous medium (Gel/Mount, Biomedica, Foster City, CA), were examined under a fluorescence microscope equipped with appropriate filters (Leica TCS SP2). Confocal images were taken at 590 nm intervals along the z-axis of the section, encompassing a total of 4 μm in depth. Images from individual optical planes and multiple serial optical sections were analysed, and the images were sequentially scanned. Image analysis was performed on all acquired fields. Negative secondary antibody controls were processed in parallel. Each experiment was carried out three times.

Apoptosis detection

The effect of the p.K301M mutation on cell viability was determined by flow cytometry and annexin V/propidium iodide staining (Beckman Coulter-Instrumentation Laboratory, Milano, Italy). Briefly, PBMCs from patient and control subject were seeded in six-well dishes (2 × 10⁶ cells/well) in the AIM-V medium supplemented with AB human serum 5% and incubated for 18 h at 37°C 5% CO2. The cells were cultured in the absence (basal condition) and in the presence of H2O2 (0.5 mM)
Table 1. CD2AP detected SNPs

<table>
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<tr>
<th>Region</th>
<th>Position</th>
<th>dbsNP allele</th>
<th>Protein residue</th>
<th>Amino acid position</th>
</tr>
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<tbody>
<tr>
<td>5′UTR</td>
<td>−54</td>
<td>A/T</td>
<td></td>
<td></td>
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<tr>
<td>5′UTR</td>
<td>−35insGGA</td>
<td>−/−/GGA</td>
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<tr>
<td>IVS 3</td>
<td>c.319 + 79</td>
<td>T/C</td>
<td></td>
<td></td>
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<tr>
<td>IVS 3</td>
<td>c.320 − 113</td>
<td>C/T</td>
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<td>c.421 − 25</td>
<td>G/A</td>
<td></td>
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<tr>
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<td>c.696</td>
<td>C/T</td>
<td>Ser/ser</td>
<td>232</td>
</tr>
<tr>
<td>IVS 6</td>
<td>c.730 − 55delA</td>
<td>−/−/A</td>
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<td>T/C</td>
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<tr>
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<td>c.1275 − 20</td>
<td>G/A</td>
<td></td>
<td></td>
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<tr>
<td>IVS 12</td>
<td>c.1275 − 55</td>
<td>A/T</td>
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<td>c.1274 + 36</td>
<td>T/G</td>
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<td>IVS 14</td>
<td>c.1531 − 33</td>
<td>A/G</td>
<td></td>
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<tr>
<td>IVS 14</td>
<td>c.1632 + 8</td>
<td>G/T</td>
<td></td>
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Table 2. Clinical features of FSGS patients and CD2AP mutations

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Age at onset (years)</th>
<th>Therapy sensitivity</th>
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<tbody>
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<td>1</td>
<td>F</td>
<td>c.904A &gt; T</td>
<td>p.K301M</td>
<td>23</td>
<td>Resistant</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>c.1120A &gt; G</td>
<td>p.T374A</td>
<td>2</td>
<td>Resistant</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>c.1573delAGA</td>
<td>p.delGlu525</td>
<td>2</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

M, male; F, female.

Fig. 1. DHPLC profiles and electropherograms of CD2AP mutations.

Results

Mutational screening

Mutational analysis of CD2AP done in 80 Italian patients with idiopathic FSGS revealed the presence of polymorphisms and intronic changes (Table 1) and three heterozygous mutations (c.904A > T; c.1120A > G; c.1573delAGA) in important functional domains of three unrelated patients (Table 2).

The first mutation (Figure 1A) consisted of an A-to-T base change (c.904A > T) in exon 8, resulting in the substitution of lysine 301 with methionine (p.K301M). Comparative analysis in nine different species (human, orangutan, mouse, rat, bovine, chicken, xenopus laevis, drosophila melanogaster, caenorhabditis elegans) of amino acid CD2AP sequences showed a high conservation of Lys 301 (Figure 2A) through the evolutionary lineages with the only exception being the drosophilia sequence. The p.K301M mutation is localized in the region coding for the third SH3 domain between the residues 269 and 330, that is involved in the interaction of CD2AP with other podocyte proteins and/or with CD2 in T cells.

The second mutation (c.1120A > G) produced an A-to-G nucleotide change in exon 12, resulting in the substitution of threolnine 374 with alanine (p.T374A) (Figure 1B). It is a missense mutation localized in the prolline-rich domain containing three core PXKP motifs that act as binding sites for the SH3 domain of other podocyte proteins. Multiple alignment of amino acid sequences showed conservation of Thr 374 among primates (Figure 2B).

The third mutation (c.1573delAGA) in exon 15, consisted in a three nucleotide deletion coding for the glutamic acid in position 525 (p.delGlu525). This mutation is endowed in the CD2AP COOH-terminal region (Figure 1C) that is deputed to the binding with nephrin [2]. Comparative analysis of amino acid CD2AP sequences revealed evolutionary conservation of Glu 525 (Figure 2C) among vertebrates.

All CD2AP coding mutations were absent in the control population of 200 healthy blood donors.

All the carriers of the three above mentioned mutations presented overt proteinuria with nephrotic syndrome at variable ages (patient 1 had adult onset disease; the other two had paediatric onset), and FSGS was the corresponding pathological finding (Table 2). In all cases, a high degree of resistance to steroids was reported, but definitive data on progression to end-stage renal failure were not available. Parents’ blood samples were not available for the patient with p.K301M mutation because they died before the enrolment. However, a genetic analysis of the patient’s
CD2AP mutations and focal segmental glomerulosclerosis

Fig. 2. Comparative analysis in nine different species (human, orangutan, mouse, rat, bovine, chicken, xenopus laevis, drosophila melanogaster, caenorhabditis elegans) of amino acid CD2AP sequences showing the evolutionary conservation of mutated amino acid (Lys 301; Thr 374; Glu 525).

relatives was performed: the same mutation was found in the 6-year-old child who was reported as normal. The adult age of the onset of the mother (Table 2) could explain this genetic data and the absence of disease in the children. The parents of a patient with p.T374A mutation were not analysed because she was adopted. The patient with amino acid deletion (p.delGlu525) inherited his mutation from the mother, who did not seem to be affected. The patients with heterozygous CD2AP mutations were also analysed for the NPHS1, ACTN4 and TRPC6 genes. No mutations were found in the ACTN4 and TRPC6 genes, while the screening of the NPHS1 gene revealed in the patient with p.delGlu525 mutation the p.E117K variant in homozygous status. Both parents were heterozygous for this variant.

Confocal laser scanning microscopy, tissue immunofluorescence and apoptosis assay

The functional effects of the above mutations were analysed with two different approaches depending on the availability of fresh blood for studying the CD2AP–CD2 interaction on T-lymphocytes or renal tissue for determining the glomerular expression of the components of the slit diaphragm. The CD2AP–CD2 interaction was studied by confocal laser scanning microscopy on T-lymphocytes of the two patients with p.K301M and p.delGlu525 mutations. The glomerular expression of both CD2AP and podocin was instead determined in the case of p.delGlu525.

As shown in Figure 3F, the PHA treatment of normal lymphocytes strongly enhanced the CD2/CD2AP interaction and clustering. Instead, CD2/CD2AP double staining on T-lymphocytes of the patient with p.K301M mutation (Figure 3N) revealed no CD2 clustering after the PHA treatment that is consistent with the localization of the mutation in the region of binding with CD2. In the patient with the p.delGlu525 mutation, confocal microscopy revealed a normal CD2/CD2AP interaction and CD2 clustering after PHA treatment (Figure 3T) that is consistent with the site of the defect not involving any CD2 interacting region. Since the p.delGlu525 mutation is localized in the carboxy-terminal region that is the site of interaction with nephrin and podocin, we hypothesized a major effect on the composition of the slit-diaphragm network. The glomerular expression of CD2AP, podocin, nephrin and synaptopodin in control subjects and in the carrier of p.delGlu525 is shown in Figure 4; in contrast to the intense immunofluorescence observed in normal kidneys (Figure 4A, C and E), a significant downregulation was observed for the CD2AP signal (Figure 4B) and a faint and irregular distribution along the capillary loops was appreciated for podocin and nephrin proteins (Figure 4D, F). The synaptopodin signal (Figure 4G and H) was used as a podocyte marker and internal control. These findings strongly suggest that p.delGlu525 determines the rearrangement of the slit-diaphragm composition and integrity that can be considered the cause of proteinuria.

The effect of the p.K301M mutation on cell viability was evaluated by flow cytometry and Annexin V/Propidium Iodide staining on PBMCs of a patient in basal condition. As shown in Figure 5, basal apoptosis rate in the FSGS patient was significantly elevated compared to normal control cells.
Fig. 3. Confocal laser scanning microscopy of a double staining for CD2AP and CD2 on T-lymphocytes of the two patients with p.K301M (G–N) and p.delGlu525 (O–T) mutations in comparison with normal lymphocytes (A–F). (F) PHA treatment strongly enhanced the CD2/CD2AP interaction and clustering in normal lymphocytes. (N) No CD2 clustering after PHA treatment was revealed on T-lymphocytes of the patient with p.K301M mutation. (T) Normal CD2/CD2AP interaction and CD2 clustering after PHA treatment on T-lymphocytes of the patient with the p.delGlu525 mutation.

(60% versus 43%), suggesting that the germline p.K301M mutation is associated with a premature cell death.

No functional studies could have been done in the case of the p.T374A mutation since PBMC and frozen renal tissue were not available.

Discussion

It is well established that CD2AP integrity is necessary for a stable functioning of the slit diaphragm and that structural defects involving this protein are associated with proteinuria. Experiments in mice with targeted disruption of CD2AP [4] strongly supported this aspect and put the basis for considering CD2AP as a putative gene in inherited

Fig. 4. Immunofluorescence showing the expression levels of CD2AP (A and B), podocin (C and D), nephrin (E and F) and synaptopodin (G and H) on renal biopsy from control subjects and patient with p.delGlu525 mutation. The glomerular expression of CD2AP, podocin and nephrin is markedly downregulated in the carrier of p.delGlu525 mutation (B, D and F). Synaptopodin (G and H) was used as a podocyte marker. Each experiment was carried out three times. Light microscopy (haematoxylin-eosin staining, ×400 magnification) on renal biopsy from control subjects and a patient with p.delGlu525 mutation, demonstrating the glomerular structure, was shown in panels I and J, respectively.

Fig. 5. Apoptosis assay performed by annexin V/propidium iodide staining exclusion. Apoptosis rate in the FSGS patient was significantly elevated compared to normal control cells (60% versus 43%; *P < 0.05). The reported results are expressed as mean (±SD) values for three independent experiments.
nephrotic syndrome. Functional studies confirmed the key role of CD2AP in the slit-diaphragm assembly. Huber et al. demonstrated that both nephrin and CD2AP interact with the p85 regulatory subunit of phosphoinositide 3-OH kinase (PI3K), recruit PI3K to the plasma membrane, and, together with podocin, stimulate PI3K-dependent AKT signalling in podocytes. AKT is inhibited in CD2AP knockout mice suggesting that both nephrin and CD2AP are involved in this cascade. Moreover, the ‘in vitro’ interaction between nephrin, podocin and CD2AP activates AKT and, in turn, controls gene expression, the remodelling of cytoskeleton, endocytosis and apoptosis of podocytes [9–11]. Podocytes lacking CD2AP are highly susceptible to apoptosis and targeted disruption of CD2AP results in apoptotic cell death of podocytes in vivo [9].

CD2AP mutational screening allowed us to detect polymorphisms and intronic changes with similar frequency in the control population. However, three mutations were found in the coding region of the CD2AP gene in three different unrelated FSGS patients. One of the mutations described here (i.e. p.K301M) is localized in the CD2AP N-terminal portion (amino acids 1–334) that interacts with the p85 regulatory subunit of phosphoinositide 3-OH kinase (PI3K) and together with podocin and nephrin stimulates the activation of the AKT pathway [9]. In lymphocytes, the p.K301M mutant protein failed to interact with CD2 during cell stimulation suggesting a central role of the p85 subunit of PI3K in this interaction; the result is a premature cell death. Moreover, apoptosis assay on PBMCs of a patient with p.K301M mutation showed increased levels of basal apoptosis rate compared to normal control cells.

Besides the regulatory functions on AKT pathways, it is well known that CD2AP directly interacts with nephrin and podocin in vitro and in vivo at the C-terminal region between amino acids 421 and 550 [2,3]. The second mutation reported here (i.e. p.delGlu525) produces the deletion of the conserved Glu525 in this domain with consequent formation of a shorter CD2AP protein. In this case, theglomerular expression and distribution of CD2AP, podocin and nephrin were altered since a significative downregulation was observed for the CD2AP signal and a decreased and irregular distribution was appreciated for podocin and nephrin proteins. In fact, normal kidneys showed a consistently linear staining for nephrin and podocin along the glomerular capillary loop, while in patient glomeruli the linear podocin and nephrin pattern was abolished, being replaced by a granular pattern (Figure 4). Similar alterations have been reported by other authors [12,13] in association with primary nephrotic syndrome and FSGS, suggesting a role of the slit-diaphragm disorganization in proteinuria.

Therefore, at least two mutations described here determined serious functional impairments of CD2AP and modified its interaction with CD2 or with other proteins in the slit-diaphragm assembly with critical consequences for glomerular functions. On clinical grounds, all the three carriers of CD2AP mutations presented nephrotic syndrome implying a direct causative association.

This is the third report describing the association between CD2AP mutations and FSGS. In a first report, Kim et al. [5] described a heterozygous nucleotide variant (GC→CT) that affected the splice acceptor of exon 7 leading to a reduced expression level of CD2AP in lymphocytes of two patients with idiopathic FSGS and HIV infection out of 45 African Americans. This mutation was localized in a not well-characterized protein region between the second and the third SH3 domains. Recently, Löwik et al. [7,14] described a patient with nephrotic syndrome associated with a CD2AP homozygous mutation (c.1834C>T) in the C-terminal region and causing a truncated protein for a stop codon at amino position 612 (p.R612X). This patient had developed early-onset nephrotic syndrome with a histological pattern overlapping between FSGS and diffuse mesangial proliferation. The functional effect of this truncation was studied by an actin-binding assay showing a decreased binding of CD2AP with F-actin and by an expression assay showing a downregulation of CD2AP in lymphocytes of both the homozygous patient and his heterozygous parent. CD2AP haplo-insufficiency, described by Löwik, was therefore linked with a functional effect but not with proteinuria implying that the mechanism for renal damage is more complicated than expected. This is a critical point since this does not appear to be a Mendelian dominant trait and relatives of the patients described here who carried in two cases the same mutations, did not show signs of overt proteinuria. A possible explanation is a digenic inheritance in which a mutation of another gene affects the slit-diaphragm assembly. Even if in our patients, mutations affecting other genes involved in inherited nephrotic syndrome (NPHS2, WT1, ACTN4, TRPC6) were excluded, the implication of other unknown molecule cannot be ruled out a priori. For example, the patient with p.delGlu525 mutation who had an early onset of proteinuria and steroid resistance was homozygous for p.E117K variant of the NPHS1 gene, and we cannot exclude a modifying effect of this variant. Observations in animals favour the possibility that haplo-insufficiency confers a sort of susceptibility to proteinuria that explains the casual association of CD2AP mutations in humans. First, CD2AP +/- mice develop an altered selectivity to the charge of macromolecules especially of the anionic protein such as albumin that may cause low-level proteinuria and predispose to more severe glomerular damages [5]. Second, there is an increasing emphasis on considering an implication of partial genetic defects of other podocyte genes such as NPHS1 and NPHS2 in the proteinuria pathogenesis. A clear association of heterozygous mutations of NPHS1 and NPHS2 with mild renal phenotypes characterized by proteinuria sensitive to drugs has also been recently reported [15–17]. Overall our data do not contradict this possibility, and CD2AP should be considered among the putative genes whose mutations are associated with nephrotic syndrome. In order to define a more definite clinical picture of this association, molecular screenings should be extended to wider cohorts of patients with nephrotic syndrome.

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Lack of association between TGF-β-1 genotypes and microalbuminuria in essential hypertensive men

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Abstract

Background. Polymorphisms within the gene for transforming growth factor (TGF)-β-1, a pro-fibrogenic cytokine pathophysiologically involved in hypertension and hypertensive target damage, might modulate the biological activity of the encoded protein. Through that mechanism, they might contribute to microalbuminuria, a marker of subclinical renal damage and a correlate of systemic inflammation and endothelial dysfunction in hypertension, a possibility never before tested. For this reason, we assessed the association of four TGF-β-1 polymorphic variants (C-509T, Leu10→Pro, Arg25→Pro, Thr263→Ile) with albuminuria in uncomplicated essential hypertensive men, using (circulating active + acid-activatable latent) TGF-β-1 levels as an indirect index of their in vivo biological activity. Because of the close pathophysiologial link of TGF-β-1 with the renin–angiotensin system, we also tested the behaviour of the angiotensin converting enzyme (ACE) deletion/insertion (D/I) polymorphism.

Methods. Albuminuria (three overnight collections), office and 24-h BP, left ventricular mass index (LVMI), BMI, renal function, glucose, lipids, plasma TGF-β-1 (n = 162, ELISA) were measured in 222 genetically unrelated, never-treated, uncomplicated Caucasian hypertensive men. ACE D/I polymorphisms were analysed by the polymerase chain reaction technique or a

References


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