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Focal segmental glomerulosclerosis in a patient homozygous for a CD2AP mutation

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Focal segmental glomerulosclerosis (FSGS) is a histologic diagnosis in several kidney diseases characterized by proteinuria and a severe decrease in kidney function. Mutations in several genes were found in patients with primary FSGS, one of which is a CD2-associated protein *CD2AP* (originally referred to as CMS). This gene encodes an adaptor protein that plays a role in endocytosis, cell motility, and cell survival. Mice deficient in Cd2ap (the mouse homolog) die due to kidney failure, while heterozygous mice develop lesions similar to those of FSGS patients. In the kidney, CD2AP regulates the actin cytoskeleton. The only previously described patient with *CD2AP* mutation had a severely truncated protein. In this study, we describe a patient with a novel mutation resulting in a premature stop codon yielding a protein truncated by only 4%. This shortened CD2AP protein displays a significantly decreased F-actin binding efficiency *in vitro* with no expression of the mutated allele in the patient's lymphocytes. Heterozygous expression of the CD2AP mutation in both parents did not lead to any kidney pathology, as both have normal glomerular filtration rates and no proteinuria.

Kidney International (2007) **72**, 1198–1203; doi:10.1038/sj.ki.5002469; published online 22 August 2007

KEYWORDS: focal segmental glomerulosclerosis; CD2AP; mutation; actin interaction

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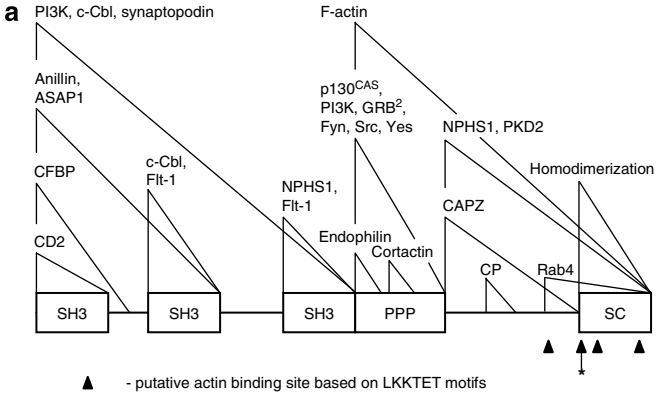
Received 15 March 2007; revised 24 May 2007; accepted 19 June 2007; published online 22 August 2007

Increasing numbers of patients, even in non-familial cases, are reported with focal segmental glomerulosclerosis (FSGS) owing to mutations in well-characterized genes. One of these genes is *CD2AP* (CD2-associated protein, originally named CMS).

Cd2ap, the mouse homolog of CD2AP, was first found in a yeast two-hybrid screen as a protein binding to the mouse T-cell membrane protein CD2, where it plays a role in clustering CD2 and polarizing the T cell.[1] The role of Cd2ap in the kidney became apparent when Cd2ap knockout mice developed severe kidney problems and Cd2ap haploinsufficiency led to lesions resembling the human condition FSGS.[2,3]

The human homolog CD2AP was found to directly bind $p130^{Cas}$, a docking protein, which appears to play a role in the integrin-mediated cell adhesion to the extracellular matrix.[4] Over the years, more and more proteins were found to interact directly with CD2AP/Cd2ap and its function in several cellular systems became apparent (Figure 1a and Table 1). The direct interaction of the COOH terminus of Cd2ap with F-actin suggests that Cd2ap is involved in the regulation of the actin cytoskeleton.[15] Indeed, several studies showed interaction of CD2AP/Cd2ap with proteins involved in cytoskeletal remodeling and cell motility.[7–9,20] More recently it became apparent that CD2AP/Cd2ap plays a role in endocytosis,[10–14] in the transforming growth factor-β-induced apoptosis, and in the phosphatidylinositol 3-kinase/AKT survival pathway as well.[19,21,22]

The CD2AP protein consists of 639 amino acids (aa) encoded by 18 exons (chromosome 6) and has a molecular mass of approximately 75 kDa. The protein structure includes several protein-binding domains and is highly homologous to the mouse Cd2ap (Figure 1b). At the NH_2 terminus, three SRC homology 3 (SH3) domains are localized followed by a proline-rich region containing SH3-binding domains. Kirsch *et al.*[4] found four putative actin-binding sites at the COOH terminus (aa 534–538/599–603/610–614 and 631–635) similar to the LKKTET motifs found in a number of actin-binding partners. Direct interaction between F-actin and Cd2ap was proven by actin filament precipitation assays. The



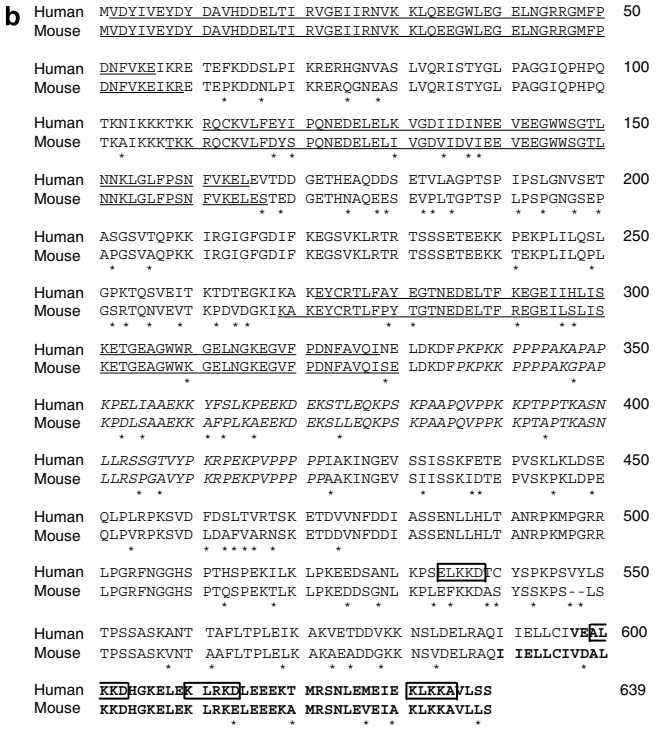


Figure 1 | Schematic representation of the CD2AP/Cd2ap protein and its interacting partners. (**a**) CD2AP/Cd2ap contains three SRC homology 3 (SH3) domains followed by a proline-rich region (P). At the COOH terminus, a super-coiled domain (SC) is found. CD2AP/Cd2ap uses this domain for homodimerization.[4] The putative actin-binding sites[4] and the location of the mutation described in this paper are shown. The defined proteins are proven to interact *in vitro* with CD2AP and/or Cd2ap. (**b**) Alignment of human CD2AP[4] and mouse Cd2ap[5] showing high homology. The asterisks indicate non-matching amino acids. The different domains are shown (SH3 domain underlined, proline-rich region in italic, and the SC domain in bold), and the putative actin-binding sites are in rectangles.

COOH-terminal domain (aa 331–637) of Cd2ap was found to bind to F-actin directly.[15] Finally, CD2AP also contains a super-coiled domain (SC, aa 597–639) through which the protein forms homodimers.[4]

As yet, only one heterozygous CD2AP mutation detected in two patients with primary FSGS has been reported. In these patients, the nucleotide changes (GC > CT) affected the splice acceptor of exon 7 leading to a reduced expression level of CD2AP in lymphocytes.[3] In this report, a novel homozygous mutation is presented. Moreover, we were able to show that the CD2AP interaction with filamentous (F-actin) was affected by this homozygous mutation and that the mutated allele is not expressed in lymphocytes.

Table 1 | CD2AP/Cd2ap interacting proteins

Protein	Function	Reference
Anillin	Actin-binding protein involved in cytokinesis	Monzo *et al.*[6]
ASAP1	Membrane trafficking and cytoskeletal remodeling	Liu *et al.*[7]
CAPZ/CP	Regulates the growth of the actin filament	Hutchings *et al.*[8] and Bruck *et al.*[9]
c-Cbl	Negative regulator of the tyrosine kinase signaling pathway	Kirsch *et al.*,[10] Cormont *et al.*,[11] and Kobayashi *et al.*[12]
CD2	T-cell polarization	Dustin *et al.*[1]
CFBP	Internalization and downregulation of the EGF receptor	Konishi *et al.*[13]
Cortactin	Regulation of receptor-mediated endocytosis	Lynch *et al.*[14]
Endophilin	Growth factor receptor endocytosis	Lynch *et al.*[14]
F-actin	Major component of the cell cytoskeleton	Lehtonen *et al.*[15]
Flt-1 (VEGFR-1)	VEGF receptor 1, involved in angiogenesis	Kobayashi *et al.*[12]
Grb^2	Regulates complex formation of proteins involved in growth signaling pathways	Kirsch *et al.*[4]
NPHS1	Key component of the podocyte slit diaphragm	Palmen *et al.*[16] and Shih *et al.*[17]
NPHS2	*Establishment of the podocyte slit diaphragm*	Schwarz *et al.*[18]
p130^{Cas}	Linking the actin cytoskeleton to the extracellular matrix	Kirsch *et al.*[4]
PI3K	Lipid kinase that controls complex cellular programs	Kirsch *et al.*[4] and Huber *et al.*[19]
PKD2	Involved in preserving the kidney tubular epithelial cells	Lehtonen *et al.*[5]
Rab4	Involved in early endocytosis	Cormont *et al.*[11]
Synaptopodin	Cell shape and motility	Huber *et al.*[20]
Fyn, Scr, Yes	Tyrosine kinases	Kirsch *et al.*[4]

Interacting domains of CD2AP/Cd2ap with the proteins enlisted above are illustrated in Figure 1, with the exception of NPHS2, of which the specific domain is not known. The interaction between these proteins and CD2AP/Cd2ap are proven by *in vitro* interaction studies.

RESULTS

Case report

A male child of consanguineous parents of Mediterranean ancestry with no family history of kidney disease was born prematurely at gestational age of 35.3 weeks by caesarean section because of fetal distress. His birth weight was 2746 g and Apgar score was 9/10.

At the age of 10 months, the patient was evaluated because of failure to thrive (height 68 cm (−2.5 s.d.), weight 6120 g (weight to height < −2.5 s.d.)). His clinical examination revealed a pale skin color, enlarged liver (2 cm below the right costal margin), and no peripheral edema. Blood pressure was

elevated (124/57 mm Hg). Laboratory examination showed microcytic anemia (hemoglobin 5.8 mmol/l and mean corpuscular volume 78 fl), normal serum creatinine (30 μmol/l), and low serum albumin 18 g/l. Anemia was successfully treated with iron and erythropoietin. Microscopic urine analysis showed microscopic hematuria (red blood cells > 30/h.p.f. with > 60% dysmorphic red blood cells) and the presence of hyaline cylinders. Urinary protein excretion was severely elevated (total protein 10.5 g/l, protein/creatinine 60 g:10 mmol). Laboratory values during follow-ups are summarized in Figure 2a. Renal biopsy was performed and showed global glomerular sclerosis in 4/10 glomeruli; 1/10 glomerulus showed a palisade conformation of the visceral epithelium, suggestive of collapsing-type glomerulosclerosis; the other 5/10 glomeruli showed mesangial proliferation and matrix expansion and hypertrophic visceral epithelium. Electron microscopic examination of two glomeruli showed mesangial matrix expansion and mild effacement of the podocyte foot processes (Figure 2b and c). Immunosuppressive therapy was deemed to be unuseful, considering the lesions observed in the renal biopsy. Antiproteinuric therapy with enalapril and diuretics resulted in an initial reduction of proteinuria by > 50%. Over the next year, a progressive deterioration of the glomerular filtration rate was noted. At 2 years and 10 months, the glomerular filtration rate estimated by the Schwartz formula was 24 ml/min/1.73 m^2, blood pressure was well controlled, and the clinical condition of the patient was satisfactory. At the age of 3 years, the patient suddenly presented with severe hypertension, acute respiratory insufficiency, cardiac decompensation, and acute renal failure. This episode was preceded by several days of diarrhea and fever. Temporary ventilatory support was instigated, and peritoneal dialysis was commenced. Blood culture revealed *Salmonella Enteritidis*. Renal failure did not recover. At the age of 5 years, the patient has undergone a successful post-mortal renal transplantation. No recurrence of proteinuria or anemia was observed. Blood analysis showed no lymphopenia. Before renal transplantation, the patient was treated with recombinant growth hormone to improve his growth velocity; however, after renal transplantation, his growth is still insufficient despite normal renal function and low steroid dose.

Urine examination of both parents revealed no proteinuria.

Molecular genetic study

We analyzed a group of 20 histologically proven FSGS patients for the presence of CD2AP mutations. In one patient, we found a mutation in exon 18 (Figure 3). It concerns a homozygous substitution at position 1834 C > T (R612Stop), resulting in a premature stop codon situated at the COOH terminus. This premature stop codon leads to a truncation of approximately 4% compared to the native CD2AP protein. Both parents were proven to be heterozygous for this mutation. The mutation was not found in our control group of 50 healthy individuals.

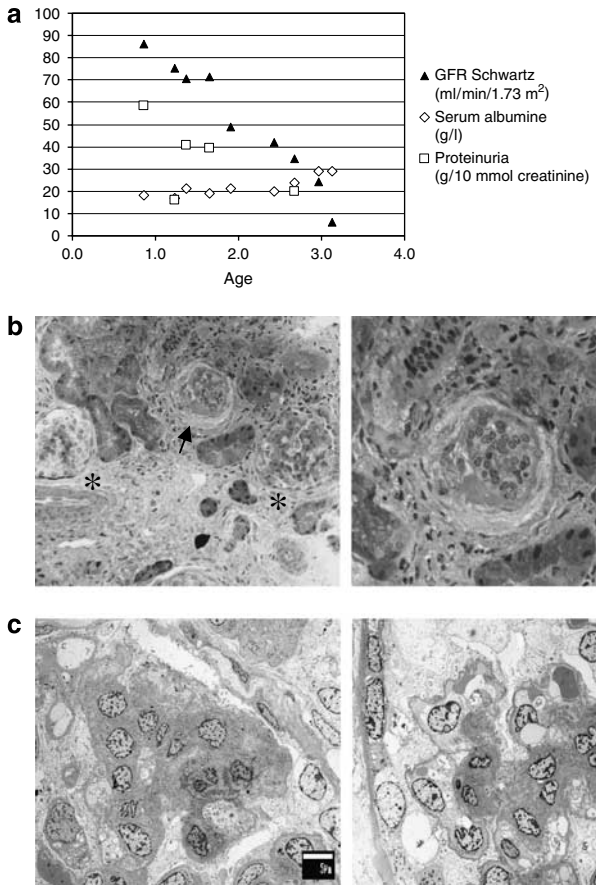


Figure 2 | Clinical data and renal biopsy of the patient with the homozygous CD2AP mutation. (**a**) Clinical data during follow-up showing a decline of glomerular filtration rate Schwartz and proteinuria and a slight elevation of serum albumin. (**b**) Light microscopy (toluidine blue staining of semi-thin-sectioned plastic embedded biopsy). In the centre of the left panel, one glomerulus shows a segmental lesion (arrow), whereas the two other glomeruli show several degrees of mesangial proliferation (asterisks). The right panel displays a higher magnification of the glomerulus with segmental lesion (original magnification × 20 (left) and × 40 (right)). (**c**) Electron microscopy. Left panel shows a segmental sclerosis lesion. In the right panel, an example of mesangial proliferation accompanied by mild increase of mesangial matrix is shown.

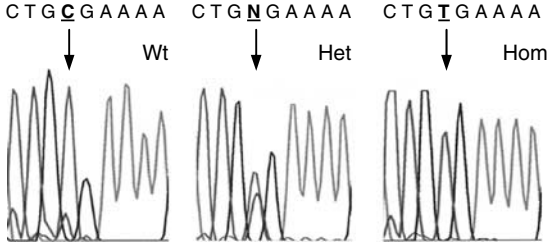


Figure 3 | Sequence chromatogram of the CD2AP mutant. The arrow shows the 1834 C > T substitution of the patient (Hom) and in the parents (Het) in comparison with the wild-type (Wt).

Actin-binding assay

To investigate whether the detected CD2AP mutation affects the interaction with actin, we performed an actin-binding assay. *In vitro*-translated CD2AP wild type and mutant

proteins were incubated with polymerized F-actin. After incubation, F-actin and the possible bound proteins were pelleted by centrifugation.

Western blot analysis shows a successful polymerization of globular-actin (G-actin) to F-actin, since a fewer amount of G-actin is present in the supernatant. Non-polymerized G-actin (incubated in water) stays in solution after centrifugation (Figure 4a). In the absence of actin, the *in vitro* translated CD2AP protein also stays in the supernatant after centrifugation (Figure 4b).

Densitometric analysis of the wild-type CD2AP revealed an almost equal distribution between the supernatant (not bound) and the pelleted (bound) fraction. More accurately, the amount of wild-type CD2AP, which was bound to F-actin and formed a pellet, was approximately 40% (Figure 4c).

The mutant R612Stop protein shows, as predicted, a lower protein band (estimated molecular mass of approximately 70 kDa) compared with the wild type (75 kDa). The amount of the R612Stop mutant protein bound to actin was reduced to approximately 6%. Consequently, a higher amount of the mutant protein is seen in the supernatant (Figure 4c). This result provides evidence that the 4% truncation of the CD2AP protein at the COOH terminus owing to the mutation results in a disturbed interaction with F-actin. The actin-binding assay was performed in duplicate and showed identical results.

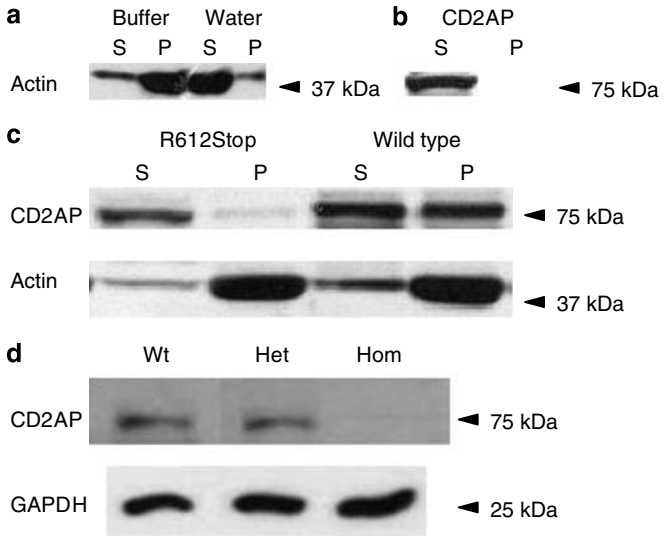


Figure 4 | COOH-terminal truncation of 4% results in a disturbed interaction of CD2AP with F-actin and no CD2AP expression in lymphocytes. (**a**) G-actin polymerizes successfully to F-actin under physiological conditions (lanes marked buffer) and forms a pellet (P) after centrifugation. In water, non-polymerized G-actin stays in the supernatant (S) fraction, and pellets at a low level (lanes marked water). (**b**) *In vitro* translated CD2AP (5 of 25 μl, incubated in polymerization buffer without actin) is found in the supernatant fraction. (**c**) *In vitro* translated wild-type CD2AP shows an almost equal distribution between the fractions, with 40% of the protein co-precipitated with F-actin (upper panel, lanes marked wild type). Protein R612Stop exhibits a slightly lower molecular mass because of the premature stop codon, and is found in the supernatant with only a negligible amount in the pellet (6%). The lower panel shows a successful polymerization of F-actin. (**d**) CD2AP levels in a control sample (Wt) and one of the parents carrying the mutation R612Stop heterozygously (Het). Cell lysates from lymphocytes were immunoblotted with anti-CD2AP (upper panel) and with anti-GAPDH as a control for protein loading.

CD2AP expression lymphocytes

The patients with FSGS described by Kim *et al.*[3] showed a heterozygous CD2AP mutation with a reduced expression level of CD2AP in immortalized B-lymphocytes. To determine the *in vivo* expression of CD2AP in the patient and his heterozygously mutated parents, we performed Western blot analysis on lysates from lymphocytes and compared the level of CD2AP expression with lymphocyte lysates from a control individual. Membranes were incubated with a polyclonal rabbit anti-CD2AP antibody and the results are seen in Figure 4d.

The immunoblot shows no CD2AP expression in the patient carrying the R612Stop mutation homozygously (Hom). Furthermore, there is no difference in CD2AP expression between the parent and the control individual given the equal GAPDH presence. Both the control (Wt) and the parent (Het) show a band of the same molecular mass, whereas the truncated CD2AP protein has a predicted molecular mass of approximately 70 kDa. These results indicate that the mutated allele is not expressed.

DISCUSSION

In this report, we describe a novel homozygous mutation in the adaptor-type protein CD2AP in a patient with FSGS. The mutation (R612Stop) results in a premature stop codon and a truncation of 4%, such that aas 613–639 of the protein are lacking. An actin-binding assay, in which the *in vitro* translated mutated proteins were incubated with filamentous actin, showed a dramatic reduction of actin binding due to the R612stop mutation.

The functional consequences of CD2AP dysfunction/absence have been investigated by studying a mouse model.[2] The Cd2ap$^{-/-}$ mice (the mouse homolog of CD2AP) die at an age of 6–7 weeks because of severe kidney disease involving the glomerulus. Already in 1-week-old knockout mice, glomeruli show an increase in size and cellularity, and electron microscopic examination showed foot process effacement. At 2 weeks of age, almost all glomeruli were affected and mesangial deposits were detected. By 4 weeks, glomeruli were sclerotic, capillary loops were extended, and an increase of mesangial deposits was seen.[2] Cd2ap$^{+/-}$ mice did not exhibit proteinuria, but showed glomerular lesions at 9 months of age, similar to the ones in 3 to 4-weeks-old Cd2ap$^{-/-}$ mice. Some lesions were similar to FSGS.[3]

Because of the phenotype of Cd2ap$^{+/-}$ mice, primary FSGS patients have been tested for CD2AP mutations. One mutation reported in the literature affects the splice acceptor of exon 7 on one allele that replaces two nucleotides, GC with CT. This change results in aberrant splicing between exons 4 and 18, and the predicted protein product would lack more

than 80% of the CD2AP protein. This mutation yields to reduced CD2AP expression, and no expression of the truncated allele, as investigated by immunoblotting CD2AP isolated from lymphocytes. In these patients, CD2AP haploinsufficiency resulted in FSGS.[3]

In this study, we present a homozygous mutation in CD2AP (R612Stop), not described before. The predicted mutated protein would lack only 4% of the COOH-terminal end of CD2AP. This effect of the mutation in the heterozygous state is subtle, since the heterozygous parents are not clinically affected. In the heterozygous state, the CD2AP expression level is similar compared with the wild-type expression level, as shown by immunoblotting (Figure 4d). The presence of the wild-type-sized and not the truncated sized CD2AP protein in the heterozygous parents strongly suggests that the mutated allele hardly influences expression from the wild-type allele. In the homozygous state, the effect of the R612Stop mutation is more dramatic. Analysis of the *in vivo* expression of CD2AP in the lymphocytes of the patient showed a complete absence of CD2AP expression (Figure 4d). Furthermore, we clearly showed that the COOH-terminal truncation of the CD2AP protein has serious consequences on binding F-actin as showed by the *in vitro* actin-binding system: only 6% of the truncated CD2AP protein was bound to F-actin compared with 40% of the wild-type protein. Direct interaction between F-actin and the COOH terminus of Cd2ap (aa 331–637) has been proven by precipitation assays before,[15] and in CD2AP, four putative actin-binding sites were found similar to the LKKTET motifs found in a number of actin-binding partners.[4] The homozygous R612Stop mutation is located in the second putative actin-binding site in the super-coiled domain. The disturbance in actin binding, as showed by the actin-binding assay, provides evidence that the 27 amino acids COOH-terminal tail that is lacking due to the stop mutation is required for actin binding.

The R612Stop mutation may also affect (1) homodimerization that takes places via the super-coiled domain of CD2AP or (2) binding to other proteins like NPHS1, Rab4, or PKD2 (Figure 1 and Table 1). These proteins all bind CD2AP at a specific domain in which the homozygous mutation is situated. The R612Stop mutation has a subtle effect in the heterozygous state, in contrast to the exon 7 splice acceptor mutation GC>CT (Kim *et al.*)[3] that has severe consequences when found heterozygous. This difference is most probably caused by the level of truncation of the CD2AP protein. The 80% truncation owing to the splice acceptor mutation will have a more dramatic effect on CD2AP expression and function than the 4% truncation resulting from the R612Stop mutation. Apparently, for this reason, the effect of the R612Stop mutation is only observed when both alleles are affected.

In this paper, we have described a novel *CD2AP* mutation (R612Stop Hom) in a patient with primary FSGS. Although the first described mutation in *CD2AP* by Kim *et al.* affected only one allele in two FSGS patients, we would like to stress that not all heterozygous *CD2AP* mutations cause kidney diseases as shown in our family. Development of FSGS clearly depends on the severity of the mutation.

MATERIALS AND METHODS

Study population

The molecular genetic study was performed in a group of 20 non-familial histologically proven FSGS patients. The study was performed with informed consent of the patients and their family members. The clinical data of the patient with the homozygous *CD2AP* mutation are listed under results.

Molecular genetic study

Genomic DNA was isolated from peripheral blood leukocytes using a salting-out method. Amplification of the *CD2AP* gene (GenBank accession nos NM_001767 and NT_011109) was performed by PCR using primers in the intron regions flanking the exons. The PCR products were analyzed by DNA sequencing (Dye Terminator Cycle Sequencing, PE Applied Biosystems, Foster City, CA, USA). The genomic DNA from 50 healthy controls was used to confirm mutations and to exclude DNA polymorphisms.

Actin-binding assay

The actin-binding assay was performed as described before by Kaplan *et al.*[23] In short, we developed expression constructs holding the entire coding region of CD2AP for *in vitro* transcription. The wild-type cDNA of CD2AP was cloned into pBluescript II SK (+) (Stratagene, La Jolla, CA, USA) by PCR, and mutant was created using the QuickChange II Site-Directed Mutagenesis kit (Stratagene).

In vitro translation of the wild-type and mutant CD2AP was accomplished according to the user's manual of the TnT-Coupled Reticulocyte Lysate kit (Promega, Madison, WI, USA). To study the interaction of the non-labeled *in vitro*-translated products with F-actin, we first polymerized G-actin under the following conditions: incubating G-actin (1.3 μM) in a 40 μl reaction buffer containing (in mM): KCl (100), $MgCl_2$ (2), ATP (0.5), DTT (dithiothreitol) (0.5), and Tris (10, pH 7.4) for 1 h at room temperature. Later, we added 5 μl (out of 25) of the *in vitro*-translated products and incubated for an additional hour. The samples were subsequently centrifuged at 100 000 g for 60 min at 21°C. The supernatant was removed and used for gel electrophoresis. The pellet was resuspended in the initial volume of 40 μl and also used for gel electrophoresis on a 10% acrylamide gel. The samples were transferred to Immobilon Transfer Membranes (Millipore, Billerica, MA, USA) and probed by a rabbit polyclonal anti-CD2AP antibody (diluted 1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and by a mouse monoclonal anti-actin antibody (diluted 1:10 000; MP Biomedicals, Illkirch, France). Secondary antibodies were AP-conjugated goat anti-rabbit IgG and goat anti-mouse IgG (diluted 1:10 000; Tropix, Applied Biosystems, Foster City, CA, USA) and protein bands were visualized using CDP-*Star* chemiluminescent substrate (Tropix, Applied Biosystems). Finally, bands were quantified by an image analysis system (Leika, Cambridge, UK) to determine the percentage of binding protein.

CD2AP expression in lymphocytes

Lymphocytes were isolated from whole blood 4.5 × diluted with phosphate-buffered saline using Ficoll Paque (1.078 g/ml; Pharmacia, Piscataway, NJ, USA) and centrifugation at 540 g for 30 min. The

interface was removed and washed four times with phosphate-buffered saline for 15 min at 300 g. The cells were lysed on ice for 30 min in RIPA buffer containing protease inhibitors PMSG (pregnant mare serum gonadotrophin) (100 μg/ml), aprotinin (5 μg/ml), and sodium orthovanadate (1 mM). The cells were subsequently sonificated three times for 10 s and centrifuged at 13 000 g and 4°C for 20 min.

Twelve microgram of total protein was separated on a 12% acrylamide gel and transferred to Protran Nitrocellulose Membranes (Schleicher & Schuell, Dassel, Germany). The membranes were probed by a rabbit polyclonal anti-CD2AP antibody (diluted 1:200; Santa Cruz Biotechnology) followed by a horseradish peroxidase-conjugated secondary antibody goat anti-rabbit IgG (diluted 1:2000; DakoCytomation, Glostrup, Denmark). As a control for protein loading, a mouse monoclonal anti-GAPDH (diluted 1:10 000, Abcam, Cambridge, UK) was used followed by a horseradish peroxidase-conjugated secondary antibody goat anti-mouse IgG (diluted 1:1000; DakoCytomation). Protein bands were visualized using the ECL+ detection reagents (Amersham Biosciences, Buckinghamshire, UK).

ACKNOWLEDGMENTS

We thank the families and their physicians for their participation in this study. This work was supported by a grant of the Dutch Kidney Foundation (project no. C.98-1764).

REFERENCES

1. Dustin ML, Olszowy MW, Holdorf AD *et al.* A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* 1998; **94**: 667–677.
2. Shih NY, Li J, Karpitskii V *et al.* Congenital nephrotic syndrome in mice lacking CD2-associated protein. *Science* 1999; **286**: 312–315.
3. Kim JM, Wu H, Green G *et al.* CD2-associated protein haploinsufficiency is linked to glomerular disease susceptibility. *Science* 2003; **300**: 1298–1300.
4. Kirsch KH, Georgescu MM, Ishimaru S *et al.* CMS: an adapter molecule involved in cytoskeletal rearrangements. *Proc Natl Acad Sci USA* 1999; **96**: 6211–6216.
5. Lehtonen S, Ora A, Olkkonen VM *et al. In vivo* interaction of the adapter protein CD2-associated protein with the type 2 polycystic kidney disease protein, polycystin-2. *J Biol Chem* 2000; **275**: 32888–32893.
6. Monzo P, Gauthier NC, Keslair F *et al.* Clues to CD2-associated protein involvement in cytokinesis. *Mol Biol Cell* 2005; **16**: 2891–2902.
7. Liu Y, Yerushalmi GM, Grigera PR *et al.* Mislocalization or reduced expression of Arf GTPase-activating protein ASAP1 inhibits cell spreading and migration by influencing Arf1 GTPase cycling. *J Biol Chem* 2005; **280**: 8884–8892.
8. Hutchings NJ, Clarkson N, Chalkley R *et al.* Linking the T cell surface protein CD2 to the actin-capping protein CAPZ via CMS and CIN85. *J Biol Chem* 2003; **278**: 22396–22403.
9. Bruck S, Huber TB, Ingham RJ *et al.* Identification of a novel inhibitory actin-capping protein binding motif in CD2-associated protein. *J Biol Chem* 2006; **281**: 19196–19203.
10. Kirsch KH, Georgescu MM, Shishido T *et al.* The adapter type protein CMS/CD2AP binds to the proto-oncogenic protein c-Cbl through a tyrosine phosphorylation-regulated Src homology 3 domain interaction. *J Biol Chem* 2001; **276**: 4957–4963.
11. Cormont M, Meton I, Mari M *et al.* CD2AP/CMS regulates endosome morphology and traffic to the degradative pathway through its interaction with Rab4 and c-Cbl. *Traffic* 2003; **4**: 97–112.
12. Kobayashi S, Sawano A, Nojima Y *et al.* The c-Cbl/CD2AP complex regulates VEGF-induced endocytosis and degradation of Flt-1 (VEGFR-1). *FASEB J* 2004; **18**: 929–931.
13. Konishi H, Tashiro K, Murata Y *et al.* CFBP is a novel tyrosine-phosphorylated protein that might function as a regulator of CIN85/CD2AP. *J Biol Chem* 2006; **281**: 28919–28931.
14. Lynch DK, Winata SC, Lyons RJ *et al.* A cortactin-CD2-associated protein (CD2AP) complex provides a novel link between epidermal growth factor receptor endocytosis and the actin cytoskeleton. *J Biol Chem* 2003; **278**: 21805–21813.
15. Lehtonen S, Zhao F, Lehtonen E. CD2-associated protein directly interacts with the actin cytoskeleton. *Am J Physiol Renal Physiol* 2002; **283**: 734–743.
16. Palmen T, Lehtonen S, Ora A *et al.* Interaction of endogenous nephrin and CD2-associated protein in mouse epithelial M-1 cell line. *J Am Soc Nephrol* 2002; **13**: 1766–1772.
17. Shih NY, Li J, Cotran R *et al.* CD2AP localizes to the slit diaphragm and binds to nephrin via a novel C-terminal domain. *Am J Pathol* 2001; **159**: 2303–2908.
18. Schwarz K, Simons M, Reiser J *et al.* Podocin, a raft-associated component of the glomerular slit diaphragm, interacts with CD2AP and nephrin. *J Clin Invest* 2001; **108**: 1621–1629.
19. Huber TB, Hartleben B, Kim J *et al.* Nephrin and CD2AP associate with phosphoinositide 3-OH kinase and stimulate AKT-dependent signaling. *Mol Cell Biol* 2003; **23**: 4917–4928.
20. Huber TB, Kwoh C, Wu H *et al.* Bigenic mouse models of focal segmental glomerulosclerosis involving pairwise interaction of CD2AP, Fyn, and synaptopodin. *J Clin Invest* 2006; **116**: 1337–1345.
21. Schiffer M, Mundel P, Shaw AS *et al.* A novel role for the adaptor molecule CD2-associated protein in transforming growth factor-beta-induced apoptosis. *J Biol Chem* 2004; **279**: 37004–37012.
22. Peters I, Tossidou I, Achenbach J *et al.* IGF-binding protein-3 modulates TGF-beta/BMP-signaling in glomerular podocytes. *J Am Soc Nephrol* 2006; **17**: 1644–1656.
23. Kaplan JM, Kim SH, North KN *et al.* Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet* 2000; **24**: 251–256.