Case Report: A Novel Heterozygous Mutation of CD2AP in a Chinese Family With Proteinuria Leads to Focal Segmental Glomerulosclerosis

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Idiopathic focal segmental glomerulosclerosis (FSGS) is a relatively frequent kidney disorder that manifests clinically as proteinuria and progressive loss of renal function. Genetic factors play a dominant role in the occurrence of FSGS. CD2-associated protein (CD2AP) is an adapter molecule and is essential for the slit-diaphragm assembly and function. Mutations in the CD2AP gene can contribute to FSGS development. Here, we describe a Chinese family of four generations with unexplained proteinuria. The proband, a 12-year-old boy, was diagnosed as FSGS. Whole-exome sequencing (WES) revealed an unknown frameshift insertion mutation (p.K579Efs*7) of CD2AP gene that leads to a truncation of CD2AP protein. Bioinformatics strategies predicted that the novel mutation was pathogenic. The mutation was absent in either healthy family members or our 200 healthy controls. In summary, we used WES to explore the genetic lesion of FSGS patients and identified a novel mutation in CD2AP gene. This work broadens the mutation spectrum of CD2AP gene and provides data for genetic counseling to additional FSGS patients.

Keywords: FSGS, CD2AP, mutation, heterozygote, whole-exome sequencing

INTRODUCTION
Idiopathic focal segmental glomerulosclerosis (FSGS) is a relatively frequent kidney disorder that manifests clinically as proteinuria and progressive deterioration of renal function. FSGS is histologically characterized by focal and segmental glomerular sclerosis and foot-process effacement (1). As a leading cause of steroid-resistant nephrotic syndrome (SRNS), FSGS makes up about three quarters of the SRNS in children and adults and frequently leads to end-stage renal disease (2).

Genetic factors play a dominant role in the occurrence and development of FSGS. As a kind of podocytopathy, many FSGS-causing genes have been identified and are mainly expressed in glomerular podocytes. The proteins encoded by these genes are crucial for the maintenance of podocyte structure and function, including protein assembly of glomerular basement membrane (GBM) and podocyte skeleton (1). Over the past decades, at least 60 genes have been linked to...
FIGURE 1 | The clinic and sequencing data of this FSGS family. (A) The pedigree of this FSGS family. Squares indicate male family members; circles, female members; black symbols, the affected members; white symbols, unaffected members; arrow, proband. (B) HE staining. (C) Masson staining for renal biopsy of the proband (IV-1). (D) Sanger DNA sequencing chromatogram demonstrates a heterozygous mutation (c.1734_1735insG/p.K579Efs*7) of CD2AP gene in affected members. (E) Alignment of multiple CD2AP protein sequences across species. Letters in red show the K579 site is evolutionarily conserved.
was described in this research (The Chinese family with four generations including 13 persons). Clinical Features

Case Presentation

Three living cases (IV1, III-3, and III-7) among the seven patients were enrolled in this family. Two hundred unrelated healthy subjects were collected as control subjects to exclude polymorphisms. The genetic etiology of primary FSGS remains obscure in many cases.

Herein, we investigate a Chinese family with unexplained proteinuria. The proband was diagnosed as FSGS. Using whole-exome sequencing (WES) technology in combined with bioinformatics strategies, we detected a previously unreported heterozygous mutation in CD2AP.

Case Presentation

Clinical Features

The Chinese family with four generations including 13 persons was described in this research (Figure 1A). Three living cases (IV1, III-3, and III-7) among the seven patients were enrolled in this family. Two hundred unrelated healthy subjects were collected as control subjects to exclude polymorphisms. The information of the healthy controls group has been provided in our previous study (16).

The proband (IV1) was a 12-year-old boy from Hunan Province in China. He visited our hospital because of an abnormal urine test. Physical examination showed lower extremity edema and hypertension. Laboratory examination showed high proteinuria (2+), high serum creatinine (149 μmol/L), blood urea nitrogen (8.81 mmol/L), and uric acid (478.6 μmol/L). Microscopic urine analysis indicated microscopic hematuria (1+). Since persistent proteinuria after 6 weeks of prednisone treatment (60 mg/m²/day), the patient was considered as steroid resistant. Renal biopsy was carried out and revealed glomerulomegaly, segmental podocytes proliferation, and hypertrophic. The GBM characterized segmental thickening. Masson staining mesangial area showed mesophilic deposition (Figures 1B,C). Thus, the patient was diagnosed as FSGS. Family history survey showed his father (III-7) also suffered from proteinuria. One aunt (III-3) investigated proteinuria and had a similar 3-year history of lower extremity edema and high blood pressure. The proband’s mother refused further medical examination because of divorce. The relevant clinical data of the patients in this family are provided in Table 1.

Genetic Analysis

WES yielded 9.13 Gb of data with 99.7% coverage of the target region and 99.1% of the target covered over 10×. In total, about 4,995 variants were detected in the proband. Data filtering were performed as our previous study (17). A set of 11 variants in 11 genes were detected (Table 2) and were further analyzed. Information including the inheritance pattern, OMIM clinical phenotypes, Toppgene function (18), and American College of Medical Genetics Classification (19) of these 11 genes has been shown in Table 2. No variants in other known FSGS-related genes were detected. Sanger sequencing was carried out in all family members. Co-segregation analysis shown that only a previously not described heterozygous mutation (c.1734_1735insG/p.K579Efs*7) in exon 16 of the CD2AP gene was observed in all three affected patients (III-3, III-7, and IV-1) and excluded in the healthy members (Figure 1D). Family screening showed that the frameshift mutation was inherited via the paternal allele (Figure 1A). The newly identified mutation was absent in our 200 healthy controls. Alignment of CD2AP amino acid sequences revealed the affected amino acid was evolutionarily conserved (Figure 1E). In addition, Swiss-Model software (https://swissmodel.expasy.org/interactive) was utilized to explore the spatial configuration of this CD2AP mutation. As the results showed, a loss of almost all of the C-terminal in the K579Efs*7 mutated CD2AP protein was observed, in comparison with the wild type, as marked by the red frame in the figure (Figure 2A).

| Subjects | IV-1 (proband) | III-3 | III-7 | Normal |
|----------|----------------|-------|-------|--------|
| Sex | M | F | M | / |
| Age (years) | 12 | 49 | 40 | / |
| Microscopic hematuria | 1+ | - | - | - |
| Proteinuria | 2+ | 1+ | 1+ | - |
| Ureaemia | No | No | No | / |
| Blood creatinine (μmol/L) | 149.0 | 128.3 | 168.5 | M:<106; F:<86 |
| Blood urea nitrogen (mmol/L) | 8.81 | 11.04 | 8.52 | 1.8-7.1 |
| Uric acid (μmol/L) | 478.6 | 520.5 | 444.3 | M:149-416; F:<89-357 |

F: female; M: male.
# TABLE 2 | Variants identified by WES in this family.

| Gene   | Transcript variant | Protein variant | SIFT | Polyphen-2 | Mutationtaster | GnomAD  | OMIM clinical phenotype                                                                 | ToppGene function                                                                 | American college of medical genetics classification |
|--------|--------------------|-----------------|------|------------|----------------|---------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|----------------------------------------------------|
| SLC28A1 | NM_004213.4; c.1-16C>G | -               | -    | -          | D              | 0.0189213 | AR, uridine-cytidineuria                                                               | Purine nucleobase transport                                                                 | pvs1, pm3, pp3                                     |
| NEUROD1 | NM_002500.4; c.34G>C  | p.G12R          | T    | B          | D              | 0.0015766 | AD, type 2 diabetes mellitus                                                             | Pancreatic A cell fate commitment                                                         | ps1, pm2                                           |
| TGM6   | NM_198994.2; c.1171G>A | p.V391M         | D    | B          | D              | 0.0096227 | AD, spinocerebellar ataxia                                                               | Peptide cross-linking                                                                     | ps1, pm1, pp3                                     |
| CIDEC  | NM_022094.3; c.457C>T  | p.Q153*         | -    | -          | D              | -        | AR, Lipodystrophy                                                                       | Lipid droplet organization                                                                | ps1, pm1, pm2                                     |
| TREM2  | NM_018965.3; c.574G>A  | p.A192T         | T    | B          | P              | 0.000435114| AR, Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy         | C-C chemokine receptor CCR7 signaling pathway                                            | ps1, pm1, pm2                                     |
| HOXA10 | NM_018961.3; c.170A>G   | p.Y57C          | T    | D          | D              | 0.00121275| -                                                                                       | Proximal/distal pattern formation                                                         | ps1, pm2                                           |
| CFTR   | NM_000492.3; c.1666A>G  | p.I556V         | T    | B          | D              | 0.0471606 | AR, Cystic fibrosis                                                                     | Regulation of cyclic nucleotide-gated ion channel activity                                | ps1, pm1                                           |
| PYGM   | NM_005609.3; c.1860del  | p.I621Sfs*37    | -    | -          | D              | -        | AR, McArdle disease                                                                     | Glucan catabolic process                                                                   | ps1, pm2                                           |
| MCTP2  | NM_018349.3; c.239del   | p.S80Tfs*17     | -    | -          | D              | -        | AR, McArdle disease                                                                     | Calcium-dependent phospholipid binding                                                   | ps1, pm2                                           |
| XIRP2  | NM_152381.5; c.3318_3319del | p.Y1107*      | -    | -          | D              | -        | -                                                                                       | Muscle tissue morphogenesis                                                              | ps1, pm2                                           |
| CD2AP  | NM_012120.2; c.1734dup  | p.K579Efs*7     | -    | -          | D              | -        | AD, Glomerulosclerosis, focal segment                                                  | Transforming growth factor beta1 production                                              | ps1, pm2                                           |

B, begin; D, disease-causing; P, polymorphism; T, tolerated; AD, autosomal dominant; AR, autosomal recessive; bp, pathogenic benign; pp, pathogenic supporting; ps, pathogenic strong; pvs, pathogenic very strong; pm, pathogenic moderate.
highly considered that the mutation was responsible for the FSGS in this family.

FSGS is clinically characterized by proteinuria and progressive renal failure (1). In our study, all living patients (III-3, III7, and IV-1) in this family presented proteinuria, but definitive data on progression to end-stage renal failure were not available. In a previous report, Gigante et al. screened for changes in the CD2AP gene in a total of 80 Italian patients with idiopathic nephrotic syndrome. Three heterozygous mutations in CD2AP gene were found in three unrelated patients while there were no definitive data on renal failure in the reported patients (20). For the different molecular and pathogenesis bases of genetically associated FSGS and SRNS, the manifestation and prognosis are different (1). Thus, the genotype-phenotype relation between CD2AP gene and FSGS needs to be further investigated. Since most FSGS/SRNS patients with genetic factors do not respond to common treatment and show poor prognosis (1), the patients in this family were given the cyclosporine treatment according to the KDIGO 2012 guideline (21). And a follow-up visit has been scheduled in a few months to ensure the patients benefit from treatment.

CD2AP is prominently expressed in glomerular podocytes. It is an 80 kDa cytoplasmic protein which consists of four domains: three Src homology 3 (SH3) domains at the NH2 terminus and one coiled-coil domain at the COOH terminus (22). CD2AP was initially identified as a ligand for the T-cell-adhesion protein CD2. And it was also shown to bind to nephrin and podocin, thereby acting as an important component of the slit-diaphragm (SD) network (23). The three SH3 domains are essential for the interaction of CD2AP protein with CD2 (20). Recent reports
exploring that phosphorylation of tyrosine residues within the SH3–1 domain may modify interactions between CD2AP and its binding partners, including nephrin (24). Furthermore, it has been demonstrated that CD2AP directly interacts with nephrin at the C-terminal region between amino acids 428 and 600 (25, 26). As shown in Figures 2A, B, compared with 639 amino acids in the wild type, the resulting truncated protein is 580 amino acids in length with an abnormal binding domain of nephrin, and lacks the coiled–coil domain that promotes homodimerization. In this case, the truncated CD2AP protein in our study would fail to bind to nephrin. Similarly, Gigante et al. reported a frameshift mutation (p.delE525) in CD2AP, which is localized in the same region, affects the ultrafiltration functions of the SD network and might lead to proteinuria (20). Thus, we indicate that the frameshift mutation (p.K579Efs*7) identified in CD2AP gene may be a potential candidate factor for the development of FSGS, consistent with the previous research.

CD2AP was a strong candidate gene for nephrotic syndrome (NS). Animal studies have shown that CD2AP knockout mice suffer from severe NS and die of massive proteinuria in infancy (27). Moreover, the CD2AP heterozygous mouse is prone to proteinuria and presents a glomerular disease at old age with a histology pattern that is similar to human FSGS (28). The relevance of CD2AP in human renal pathology still remains largely unknown and the detailed molecule mechanisms involved requires further investigation (20). So far, ~10 mutations of CD2AP have been reported in FSGS or NS patients. A brief review of these reported mutations was shown in Figure 2B, which may help for the genetic counseling and prenatal diagnosis of FSGS associated with mutation in the CD2AP gene. Although the pathogenic mechanism involved still requires further investigation, our findings offer more evidence that CD2AP gene variant is significant in FSGS. Remarkably, the mutation (c.1734_1735insG/p.K579Efs*7) identified in this study has not been published and, therefore, is considered novel.

CONCLUSION

We applied WES to explore the genetic lesion in a Chinese FSGS family. A novel heterozygous (c.1734_1735insG/p.K579Efs*7) of CD2AP was identified. Our study broadens the mutation spectrum of CD2AP gene and provides data for the clinical management and genetic counseling respect to FSGS.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI BioSample; PRJNA739264.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Third Xiangya Hospital of Central South University. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)’ legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

Y-XL and A-QZ enrolled the family members. F-ML and YS performed genetic analysis. C-YW and YD performed DNA isolation and sanger sequencing. Y-XL and LF wrote the manuscript. LL supported the project. All authors reviewed the manuscript.

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