Case report and literature review: A de novo pathogenic missense variant in ACTN4 gene caused rapid progression to end-stage renal disease

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Background: Focal segmental glomerulosclerosis (FSGS) is a histopathological diagnosis of the sclerosis of glomeruli and the damage to renal podocytes. FSGS affects the filtration function of the kidneys and results in nephrotic syndrome (NS) in children and adults. FSGS is a clinically and genetically heterogeneous disorder. FSGS-1 [OMIM #603278] is one of the progressive hereditary renal diseases. It is caused by heterozygous variants of the actinin alpha 4 (ACTN4) [OMIM∗604638] gene on chromosome 19q13.2 in a dominant inheritance (AD) manner. With the recent development of whole-exome sequencing (WES), 22 (including our case) pathogenic or likely pathogenic variants have been identified in ACTN4 gene.

Case presentation: We reported a 17-year-old Chinese girl who was hospitalized with foamy urine, nausea and vomiting. Laboratory tests revealed increased levels of serum creatinine and urea nitrogen. Ultrasonography demonstrated bilaterally reduced size of kidneys. The primary diagnoses were NS and chronic kidney disease stage 5 (CKD5). The hemodialysis was initiated in 48 h after admission. After 4 months of treatment, the patient received an allogeneic kidney transplantation from her father. A novel heterozygous missense variant c.494C>T (p.A165V) in the ACTN4 gene was found by WES in the patient. This variant was confirmed by Sanger sequencing. The computational simulation of the stability of mutant protein (p.A165V) was decreased. Interatomic interactions of the p.A165V site were increased, and it might be associated with the increased ubiquitylation in the vicinity of the mutant site.

Conclusion: As per the guidelines of the American College of Medical Genetics and Genomics for interpreting sequence variants, the novel heterozygous missense variant was pathogenic...
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(PS2 + PM1 + PM2 + PP3 + PP4). It should be noted that the early onset of severe proteinuria with a poor prognosis is an important and universal symptom for most genetic FSGS. If necessary, genetic screening is recommended.

KEYWORDS
focal segmental glomerulosclerosis, end-stage renal disease, proteinuria, ACTN4, de novo

Introduction

Focal segmental glomerulosclerosis (FSGS) is a pathological feature of glomerular diseases that presents clinically with proteinuria and a progressive decline in renal function. Patients with FSGS may have initial symptoms of nephrotic syndrome (NS), such as massive proteinuria, hypoalbuminemia, hyperlipidemia, hypertension, and sepsis. FSGS1–FSGS10 are genetically heterogeneous disorders. They are caused by variants in ACTN4 (AD FSGS1 [OMIM #603278]), TRPC6 (AD FSGS2 [OMIM #603965]), CD2AP (susceptibility to FSGS3 [OMIM #607832]), APOL1 (susceptibility to FSGS4 [OMIM #612551]), INF2 (susceptibility to FSGS5 [OMIM #613237]), MYO1E (AR FSGS6 [OMIM #614131]), PAX2 (AD FSGS7 [OMIM #616002]), ANLN (AD FSGS8 [OMIM #616032]), CRB2 (AD FSGS9 [OMIM #616220]), and LMX1B (AD FSGS10 [OMIM #256020]), respectively. Pathogenic variants in the ACTN4 gene are rarely detected in individuals with NS or severe proteinuria. Sadowski et al. (1) showed that disease-causing ACTN4 variants were not found in 2016 individuals with steroid-resistant nephrotic syndrome (SRNS). In addition, Wang et al. (2) also did not detect ACTN4 variants in 110 Chinese children with SRNS. Nagano et al. (3) found that only 2 of 230 Japanese patients with severe proteinuria had ACTN4 variants. Following a review of literature, only 21 pathogenic or likely pathogenic ACTN4 variants were identified. Most variants within the actin binding domain (ABD) of ACTN4 protein contributed to cytoskeleton and podocytes via increasing the binding affinity of ACTN4 to filamentous-actin (F-actin) (4).

In this study, we reported a Chinese girl with a novel pathogenic missense ACTN4 variant affected with end-stage renal disease (ESRD). We conducted a systematic literature review to summarize previously reported clinical phenotypes and ACTN4 variants. Most cases are affected by severe proteinuria at early onset, hypertension, and podocytopathy features.

Case presentation

A 17-year-old girl self-reported nocturia that progressed for half a year. Before being hospitalized, she had noticed foamy urine for 3 weeks. She was admitted following 5 days of nausea and vomiting. Blood tests revealed increased serum creatinine of 18.02 mg/dl, high urea nitrogen, low hemoglobin of 75 g/L, low serum albumin of 28.2 g/L, and severely increased parathyroid hormone (PTH) of 606.0 pg/ml. A lipid panel blood test revealed that the levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were normal, and the level of very-low-density lipoprotein (VLDL) was decreased a bit. A urine analysis showed an increased level of proteinuria (>9,000 pg/ml). In the meantime, there was no significant change in her weight. She did not have mental development delay. Ultrasonography revealed the reduced size of bilateral kidneys with hyperechoic reflections inside the renal parenchyma. A kidney biopsy was offered, but it was refused by her parents. Echocardiography indicated small accumulations of pericardial fluid, the mildly regurgitant tricuspid valve, and a slightly enlarged left ventricle. The primary diagnoses were NS, CKD5, renal anemia, and secondary hyperparathyroidism. After admission, the hemodialysis was initiated in 48 h and continued for 4 months. Then, the patient received an allogeneic kidney transplant from her father.

The patient was the third child of phenotypically normal Chinese parents. The other two siblings were healthy and proteinuria was not found (Figure 1). Her parents did not have consanguineous relation and family history was not remarkable. She was naturally conceived with an uneventful perinatal period. She was born at term at 40 weeks of gestational age. Based on the fact that disease-causing gene variants have been identified in adolescents with idiopathic NS, genetic screening in the patient's family for inherited diseases was recommended.

Genetic testing

The patient and her parents signed informed consent for genetic analysis. Our legal ethics committee approved this genetic study. Genomic DNA was extracted from the peripheral blood of the patient and phenotypically normal parents for
WES. Sanger sequencing was used for further verification. We identified a \textit{de novo} heterozygous missense variant c.494C $>$ T in the \textit{ACTN4} gene (NM_004924.6). This variant has not been registered in population databases (1,000 Genomes Project, gnomAD, and dbSNP) or reported in disease databases (ClinVar, Human Gene Mutation Database, OMIM).

A cosegregation analysis was performed among family members. This variant was \textit{de novo} and verified by Sanger sequencing (Figure 2). Silico predictive algorithms (SIFT, PolyPhen-2, REVEL, MutPred, MutationTaster, PROVEAN, and MVP) of pathogenicity all showed damage. The analysis of conserved sequences suggested this variant was located in highly conserved sequences across several mammalian species (Figure 3). The variant c.494C $>$ T (p.A165V) was located in a critical and well-established functional domain (the calponin-homology domain of \textit{ACTN4} protein) without benign variants (Figure 4). Summing up the above, according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) for interpreting sequence variants, the novel missense variant was pathogenic (PS2 + PM1 + PM2 + PP3 + PP4).

The computational simulation of the stability and interatomic interactions of mutant protein

DynaMut (a web server) (5) is a well-established normal mode approach. We used it to visualize and assess the stability and interatomic interactions of mutant proteins. We put information into DynaMut as follows: wild-type structure (PDB accession code: 6O31), mutation detail (A165V and chain A). The prediction outcome of stability was $\Delta \Delta G$: $-0.087$ kcal/mol (destabilizing). In comparison with p.A165V, we put p.G195D into DynaMut. The prediction outcome of stability of p.G195D was $\Delta \Delta G$: $-1.349$ kcal/mol (destabilizing). The residues 165 and 195 in the wild-type were observed to form residue interactions (colored in light green) with their surrounding residues, whereas some interactions were seen to be increased in mutant sites (p.A165V and p.G195D) (Figure 5).

Literature review

We searched the PubMed database, the Human Gene Variant Database (HGMD), and Online Mendelian Inheritance in Man (OMIM) using “focal segmental glomerulosclerosis” and “ACTN4” as keywords. The search time was from the establishment of the databases to April 1, 2022. Previous studies with \textit{ACTN4} variants and clinical characteristics were reviewed. In total, fifteen documents were retrieved. Although 41 mutations were registered in HGMD, we reclassified the pathogenicity of these variants according to standards and guidelines for the interpretation of sequence variants. A total of 22 pathogenic or likely pathogenic \textit{ACTN4} variants and related phenotypes are summarized in Table 1 and Figure 4. Three heterozygous variants [c.1-34C $>$ T, c.1-590delA, and (1-1044delT) + (1-797T $>$ C) + (1-769A $>$ G)] in the promoter of the \textit{ACTN4} gene have been reported in patients with FSGS. The functional analysis of these promoter variants indicated that they might also contribute to the pathophysiology of idiopathic FSGS (6). The remaining 17 known variants are shown in Table 2. More importantly, variants in the \textit{ACTN4} gene were missense and occurred \textit{de novo}. Most patients with \textit{ACTN4} variants had proteinuria at adolescent onset and rapidly progressed to ESRD.

Discussion

The human \textit{ACTN4} gene is located on chromosome 19q13.2. The transcript of \textit{ACTN4} (NM_004924.6) has 21 exons, a transcript length of 4,990 base pairs, and a translation length of 911 amino acids. The alpha-actinin-4 protein (ACTN4) [UniProtKB-O43707] is one member of four actin-binding proteins. ACTN4 is ubiquitously expressed in different tissues and highly expressed in the placenta and kidney (20). ACTN4 was prominently distributed in podocytes via immunostaining followed by confocal microscopy (21).

Actinin alpha 4 has three main domains: (1) the N-terminal highly conserved ABD consists of two calponin homology (CH1/2); (2) spectrin 1-4; and (3) the C-terminal domain consists of EF-hand 1/2 (Figure 4).

Most of the pathogenic or likely pathogenic variants were missense and located within ABD. \textit{ACTN4} variants in ABD damaged the podocyte foot process architecture and function through a “gain-of-function” mechanism (increased the binding of \textit{ACTN4} to F-actin). It was a “dominant effect” that mutant \textit{ACTN4} protein unconventionally aggregated in the cell and
the binding affinity of ACTN4 to F-actin was increased (22). Bartram et al. (12) identified p.G195D in a patient with juvenile onset of proteinuria and ESRD. A functional analysis of p.G195D showed that ACTN4 was the significantly reduced protein in the patient’s primary renal epithelial cells. Further analysis revealed a “dominant effect” in which the decreased stability of mutant protein was associated with the increased ubiquitylation in the vicinity of p.G195D site. The aggregated unstable mutant protein led to the disturbance of the renal cell cytoskeleton. The p.G195D and p.A165V were all located within CH2 domain of ACTN4. So we hypothesized that p.A165V may have the same pathogenic mechanism as p.G195D. The
FIGURE 4
The schematic diagram of ACTN4 variants and ACTN4 protein's domains (UniProtKB-O43707). The patient’s ACTN4 variant was marked with a red box.

FIGURE 5
Prediction of interatomic interactions of p.A165V (A,B) and p.G195D (C,D). Residues 165 and 195 in the wild-type and mutant proteins (p.A165V and p.G195D) are colored in light green and are shown as sticks. The respective chemical interactions are labeled as dotted lines and colored as follows: hydrogen bonds—(red), weak hydrogen bonds—(orange), hydrophobic contacts—(green), amide-amide contacts—(blue), and ionic interactions—(gold). Amino acid residues are also colored according to type, namely: nitrogen (blue), oxygen (red), and sulfur (yellow). In comparison with wild-type sites, some interactions were observed to be added in mutant sites.
### TABLE 1  Genotypes and phenotypes of patients with ACTN4 variants previously reported.

| References         | Ethnicity          | Gender | Family history | Age of onset | Initial symptoms or diagnosis | Histopathology diagnosis | Time to ESKD | Age of kidney transplant | ACTN4 (NM_004924.6) | Origin of variant | Protein domain | Evidence of pathogenicity | Pathogenicity   |
|--------------------|--------------------|--------|----------------|--------------|-----------------------------|--------------------------|--------------|--------------------------|----------------------|--------------------|-----------------|-------------------------|------------------|
| Weins et al. (7)   | Western European   | M      | FH             | 5            | Proteinuria                 | FSGS                     | 3 y          | 10                       | c.175T > C (p.W59R) | de novo            | ABD             | PS2, PS3, PM1, PM2, PP3, PP4 | Pathogenic       |
| Dai et al. (8)     | Chinese            | ND     | S              | ND           | ND                          | FSGS                     | ND           | ND                       | c.184T > A (p.S62T) | de novo            | ABD             | PS2, PM1, PM2, PP3, PP4 | Pathogenic       |
| Weins et al. (7)   | ND                 | ND     | ND             | ND           | Proteinuria                 | FSGS                     | ND           | ND                       | c.214G > C (p.E71G) | ND                 | ABD             | PM1, PM2, PP3, PP4 | Likely pathogenic |
| Rodrigues et al. (5)| Colombian          | ND     | ND             | ND           | ND                          | FSGS                     | ND           | ND                       | c.448_450delATC (p.I150del) | ND                 | ABD             | PM1, PM2, PP3, PP4 | Likely pathogenic |
| Barua et al. (9)   | ND                 | ND     | ND             | ND           | Proteinuria                 | FSGS                     | ND           | ND                       | c.457T > C (p.F153L) | ND                 | ABD             | PM1, PM2, PP3, PP4 | Likely pathogenic |
| Giglio et al. (10) | Caucasian          | F      | S              | 3            | SRNS                        | FSGS                     | ND           | ND                       | c.464T > C (p.I155T) | de novo            | ABD             | PS2, PM1, PM2, PP3, PP4 | Pathogenic       |
| Bezdička et al. (11)| Czech              | F      | ND             | 15.1         | SRNS                        | ND                       | ND           | ND                       | c.475 T > C (p.I159P) | ND                 | ABD             | PM1, PM2, PP3, PP4 | Likely pathogenic |
| Batram et al. (12) | German             | F      | S              | 13           | ESKD                        | ND                       | ND           | 14                       | c.584G > A (p.G195D) | de novo            | ABD             | PS2, PS3, PM1, PM2, PP3, PP4 | Pathogenic       |
| Nagano et al. (3)  | Japanese           | M      | ND             | 6            | Proteinuria                 | FSGS                     | 7 y          | ND                       | c.671T > C (p.L224P) | de novo            | ABD             | PS2, PM1, PM2, PP3, PP4 | Pathogenic       |
| Kaplan et al. (13) | Oklahoma           | ND     | ND             | ND           | ND                          | FSGS                     | ND           | ND                       | c.763A > G (p.K255E) | ND                 | ABD             | PS3, PM1, PM2, PP3, PP4 | Pathogenic       |
| Batram et al. (12) | Canary Islands     | ND     | ND             | ND           | FSGS                        | ND                       | ND           | ND                       | c.776C > T (p.T259T) | ND                 | ABD             | PS3, PM1, PM2, PP3, PP4 | Pathogenic       |

(Continued)
| References          | Ethnicity  | Gender | Family history | Age of onset | Initial symptoms or diagnosis | Histopathology diagnosis | Time to ESKD | Age of kidney transplant | ACTN4 (NM_004924.6) | Origin of variant | Protein domain | Evidence of pathogenicity | Pathogenicity |
|---------------------|------------|--------|----------------|--------------|-------------------------------|--------------------------|---------------|--------------------------|---------------------|----------------|----------------|---------------------------|---------------|
| Sen et al. (14)     | ND         | M      | ND             | 6            | SRNS                          | FSGS                     | ND            | ND                       | c.776C > A (p.T259N) | ND             | ABD            | PM1, PM2, PM5, PP3, PP4   | Likely pathogenic |
| Dai et al. (8)      | Caucasian  | F      | S              | 7            | SRNS                          | FSGS                     | 8 y, 9 m      | 11                       | c.782T > A (p.V261E) | de novo         | ABD            | PS2, PM1, PM2, PP3, PP4   | Pathogenic    |
| Kakajiwala et al.   | Caucasian  | F      | S              | 5            | NS                            | FSGS                     | 6 m           | 6                        | c.784T > C (p.S262P) | de novo         | ABD            | PS2, PM1, PM2, PP3, PP4   | Pathogenic    |
| Choi et al. (16)    | Korean     | M      | S              | 3            | Proteinuria                   | FSGS                     | ND            | ND                       | c.785T > T (p.S262F) | Germline mosaicism from the father's sperm | ABD    | PM1, PM2, PM5, PP3, PP4   | Likely pathogenic |
| Bierzynska et al.   | White      | F      | 3.7            | Proteinuria  | FSGS                          | ND                       | 1 y, 4 m      | 5.7                      | c.778_786del TATGTGTGC (p.Y260_S262del) | ND             | ABD            | PM1, PM2, PM4, PP3, PP4   | Likely pathogenic |
| Feng et al. (4)     | European American | F          | 14             | NS            | FSGS                          | ND                       | 6 m           | 16                       | c.793T > C (p.Y265H) | ND             | ABD            | PS1, PM1, PM2, PP3, PP4   | Pathogenic    |
| Nagano et al. (3)   | Japanese   | M      | ND             | 8            | Proteinuria                   | FSGS                     | ND            | ND                       | c.912 + 1G > A (p.Y264H) | ND             | -              | PM2, PM6, PP3, PP4        | Likely pathogenic |
| Rodrigues et al.    | Black      | F      | 20             | Proteinuria  | FSGS                          | ND                       | ND            | ND                       | c.1043A > G (p.Q348R) | ND             | Spectrin    | PS3, PM2, PM6, PP3, PP4   | Pathogenic    |
| Schapiro et al.     | Kurd       | M      | ND             | Proteinuria, hematuria         | FSGS                     | ND            | ND                       | c.1606C > A (p.R536S) | ND             | Spectrin   | PM2, PM6 PP3, PP4        | Likely pathogenic |
| Safariková et al.   | Czech      | F      | FH             | 54           | NS                            | FSGS                     | ND            | ND                       | c.2242A > G (p.N748D) | ND             | Spectrin    | PM2, PM6 PP3, PP4        | Likely pathogenic |
| Our patient         | Chinese    | F      | S              | 17           | Proteinuria                   | ND                       | ND            | 17                       | c.494C > T (p.A165V) | de novo         | ABD            | PS2, PM1, PM2, PP3, PP4   | Pathogenic    |

M, male; F, female; y, years; m, month; FH, family history; S, sporadic; ESKD, end-stage kidney disease; ND, not done or no data; ABD, actin binding domain; FSGS, focal segmental glomerulosclerosis; NS, nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; PS2, de novo (both maternity and paternity confirmed) in a patient with the disease and no family history; PS3, well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product; PM1, variants located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation; PM2, variants were absent from controls; PM4, protein length changes as a result of in-frame deletions/insertions in a non-repeat region or stop-loss variants; PM5, novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before; PM6, assumed de novo, but without confirmation of paternity and maternity; PP3, multiple lines of computational evidence support a deleterious effect on the gene or gene product; PP4, patients’ phenotype or family history is highly specific for a disease with a single genetic etiology.
computational simulation of p.G195D seemed to accord closely with the functional analysis of p.G195D. All results showed that the stability of the mutant proteins (p.G195D and p.G195D) was decreased. The stability of the mutant protein might be mediated by the increased ubiquitination. Therefore, it was tempting to speculate that the increased ubiquitination in the vicinity of mutant sites may be connected with increased interatomic interactions of mutant sites. Presumably, p.A165V and p.G195D had a basic similarity in the pathogenic mechanism.

Three variants (Table 1) occurred in spectrin domains that were known to mediate CLP36-binding. Functional studies showed ACTN4 variants in spectrin domains provided a plausible "loss-of-function" mechanism (decreased the binding of ACTN4 to CLP36). Liu et al. (23) reported that deficiencies of ACTN4 and CLP36 were observed in podocytes of FSGS cases by performing immunohistochemical staining. The mutant protein (p.Q348R) significantly inhibited the activity of ACTN4 to interact with CLP36. Depletion of the level of CLP36 or disruption of the ACTN4-CLP36 complex significantly impeded RhoA activity and the generation of traction force in podocytes but did not alter the actin-binding affinity (12).

The C-terminal EF-hand domain is involved in binding intracellular Ca$^{2+}$. ACTN4 has two isoforms (Ca$^{2+}$-sensitive and Ca$^{2+}$-insensitive). The Ca$^{2+}$-sensitive isoform is broadly expressed, while the Ca$^{2+}$-insensitive isoform is predominantly expressed in the nervous system (24). The binding of Ca$^{2+}$ to EF-hand domains reduced the affinity of F-actin binding (25). Five variants in HGMD occurred in EF-hand domains. Safáříková et al. (19) found p.A784V, p.P787L, p.C793Y, and p.G798D in patients with IgA nephropathy. We reclassified these four variants as uncertain significance. Chatterjee et al. (26) reported p.V801M in a patient with proteinuria. It was reclassified as a variant of uncertain significance. For now, no pathogenic or likely pathogenic variants have been identified in the C-terminal EF-hand domains.

To our knowledge, ACTN4 variants in literature have all occurred de novo (except no data were collected). ACTN4 variants within ABD showed a "gain-of-function" mechanism. The homozygous Actn4 knockout (Actn4 KO) mice developed albuminuria and FSGS at about 10 weeks of age, loss of Actn4 damaged glomerular podocytes and filtration barrier (13). A plausible "loss-of-function" mechanism of ACTN4 variants within spectrin or EF-hand domains also existed. The extent of the reduction of ACTN4 may be closely associated with the severity of proteinuria in FSGS cases. As shown in Table 1, patients (14/16) with ACTN4 variants within ABD were affected with proteinuria before the age of 18 years and developed rapidly progression to ESRD. Patients (2/16) with ACTN4 variants within spectrin domains appeared proteinuria over 18 years old (one case was 54 years old). It needs more cases to verify whether or not two different mechanisms are associated with the age of onset.

In total, 17 known ACTN4 variants were of unknown significance (Table 2). As per the guidelines of ACMG for interpreting sequence variants, although these variants were absent from normal controls, multiple silico predictive algorithms suggested no impact on these variants (conservation, evolutionary, splicing impact, etc.) or reputable source recently reported these variants as likely benign or benign...
variants. The phenotypes of some patients with these variants were not associated with FSGS. This summary (Table 2) might note that phenotypic variability of ACTN4-mediated diseases. Whereas, it is restricted to a small amount of reported cases. So, clinicians should be cautious of dealing with these variants.

The genetic screening is recommended for those juveniles who are accidentally found to have severe proteinuria. Although we are realistic that no treatment is available for ACTN4-mediated FSGS, a genetic screening can help physicians and patients to prepare the kidney transplantation in time. If parents consider to give birth to another child, a definitive genetic diagnosis is essential for prenatal diagnosis.

In conclusion, we described a Chinese patient with a novel pathogenic ACTN4 variant. The genetic screening of congenital proteinuria is necessary for patients. We summarized the pathogenicity of ACTN4 variants in previous literature. The severe proteinuria at early onset and rapid progression to ESRD are universal and important symptoms of ACTN4-mediated FSGS, and those patients are good candidates for the kidney transplantation.

Data availability statement

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of The First Affiliated Hospital, Zhejiang University School of Medicine. 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

KW wrote the main manuscript text and critically revised the manuscript. WX carried out the molecular genetic experimental. ZH and JC prepared the clinical data and imaging data. ZH and KW contributed to the check of revision, genetic evaluation, and genetic databases analysis. All authors reviewed the manuscript and read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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