A case report of CRB2 mutation identified in a Chinese boy with focal segmental glomerulosclerosis

Jiaojiao Fan, BS, Rong Fu, MD*, Fuxian Ren, MD, Junjie He, BS, Shujing Wang, MS, Mengfan Gou, BS

Abstract
Rationale: Focal segmental glomerulosclerosis (FSGS) is a common disease resulting in end-stage renal disease. The incidence of FSGS is increasing in Western countries. The clinical manifestations include proteinuria, hypoproteinemia, oedema, and hypertension. Single-gene heritable mutations are considered to be the source of FSGS pathogenicity according to recent in-depth studies on the pathogenesis. Here, we first reported the case of a Chinese boy whose histology presented with FSGS caused by a compound heterozygous mutation.

Patient concerns: A 7-year-old Chinese boy was repeatedly admitted to our hospital for fever, cough, and proteinuria since he was 1.6 years old.

Diagnoses: FSGS was identified by renal biopsy. Whole exome sequencing (WES) showed that a novel mutation of crumbs homolog 2 (CRB2) was identified in a Chinese boy with FSGS.

Interventions: Patient was treated with low-dose corticosteroid and mycophenolate mofetil for maintenance therapy.

Outcomes: At last follow-up, protein (+++) was observed in his urinalysis.

Lessons: We identified a novel mutation of CRB2 in a Chinese boy with FSGS that had never been described in a previous report. These findings suggested that mutations in recessive disease genes are more frequent among early-onset disease.

Abbreviations: CRB2 = crumbs homolog 2, CRP = c-reactive protein, CT = computed tomography, FSGS = focal segmental glomerulosclerosis, MMF = mycophenolate mofetil, MRI = magnetic resonance imaging, NS = nephrotic syndrome, RBC = red blood cell, UPE = urinary protein excretion, WBC = white blood cell, WES = whole exome sequencing.

Keywords: crumbs homolog 2, focal segmental glomerulosclerosis, mutation, whole exome sequencing

1. Introduction
Nephrotic syndrome (NS), one of the most common glomerular diseases in children,[1] can be clinically divided into steroid resistant and steroid sensitive on the basis of response to corticosteroid therapy.[2] Approximately 85% of patients with SRNS exhibit renal pathology of FSGS; it is a heterogeneous disorder with the renal glomerular filtration barrier that leads to the impairment of glomerular permselectivity.[3,4] Human FSGS is a primary podocytopathy resulted from podocyte-specific gene mutations and points to defects in glomerular epithelial cells.

More than 30 single-gene heterogenous mutations, including INF2, ACTN4, TRPC6, CD2AP, LAMB2, WT-1, NPHS1, and NPHS2, have been discovered.[5] A report published by WES in 2015 first described 4 different families with sequence variants in crumbs homolog-2 (CRB2) mutations that were associated with FSGS.[2] To date, we were able to find a few clinical findings and reviewed earlier, similar studies of cases affected by SRNS resulting from CRB2 mutation. In this study, we confirmed a CRB2 gene mutation in a Chinese patient with FSGS that had never been described in a previous report.

2. Case report
The study was approved by the institutional review board of Puyang Oilfield General Hospital Affiliated with Xinxiang Medical University. Informed consent was obtained from the patient for publication of this case report and accompanying images. A 1.6-year-old Chinese boy was admitted to our Department of Paediatrics for fever and cough for 2 days. Laboratory test showed elevated CRP (c-reactive protein) levels (12.8 mg/L) and normal WBC, neutrophil and lymphocyte counts (9.4 × 10^9/L, 62.9% and 31.2%, respectively), and urinalysis revealed protein ++, occult blood +++, and RBC count 21.1/HP. Acute upper respiratory infection was diagnosed as a primary diagnosis. His fever and cough disappeared after treatment with an antibiotic. Re-examination of urine routine showed negative protein and elevated 24-hour urinary protein excretion (UPE) (262.4 mg/d). Six days later, the patient was discharged from the hospital. Eight-week follow-up urine analysis without drug...
therapy revealed protein (+++) and 24-hour UPE 150 to 250 mg/d. However, his glomerular filtration rate (108.9 mL/min) and anti-O (44.3 IU/L) were in normal range. Both the father and the mother showed normal urinalysis results. Parental consanguinity was denied. The other members of the family had no history of chronic kidney disease or autoimmune disease.

The patient underwent renal biopsy at other hospital. The results of the report were as follows: light microscopy—findings: global glomerulosclerosis observed in a total of 30 glomeruli; and a glomerular capillary loop could be seen in the other proximal tubules. Glomerular podocyte swelling was easily seen; there were no abnormalities in the interstitium and tubules. Immunofluorescence study showed IgM ± and negative IgG, C3, C4, C1q, Fn, and CD80 detection. On electron microscopy, mild swelling of renal tubular epithelial cells and segmental effacement of foot processes were present in 9 glomeruli. Glomerular basement membrane was normal, and there were no electronic dense deposits. Therefore, FSGS was identified in September 2012. At that time, physical examination on admission was as follows: BP, 100/50 mmHg; weight, 16 kg; and height, 94 cm. There were no obvious abnormalities. In addition, there was no oedema in bilateral eyelids or bilateral lower limbs. Laboratory test at this hospital is provided in Table 1. As shown by the urine analysis, apart from his urine being ++ for protein and 6.35 mg/dL for 24-hour UPE, higher than the normal range, other tests showed no specific findings. Furthermore, as revealed by ultrasound (US) scan, the right kidney size was 7.2x3.1 cm, and the left kidney size was 6.8x3.9 cm, indicating the kidneys were normal. Computed tomography (CT) and magnetic resonance imaging (MRI) scan of the brain and chest did not reveal any substantial abnormality. He was treated with high-dose methylprednisolone pulses and cyclosporine A. His renal function improved with no occasional proteinuria, indicating that the drug treatment was effective. Three years later, unfortunately, the proteinuria increased to ++++; we discontinued the cyclosporine A, and mycophenolate mofetil (MMF) was added. At last follow-up, protein (+++) was observed in his urinalysis with low-dose corticosteroid and MMF for maintenance therapy. The boy was 26 kg in weight at 7 years old (normal range 20.2–26.5 kg), and he looked healthy and oedematous compared to other nephrotic patients. However, he was repeatedly admitted to our hospital with fever and cough. Ultimately, the patient and his parents underwent gene testing for diagnostic evaluation.

With the subjects’ informed consent, samples of their blood were obtained for genetic analysis. Genomic DNA was obtained from blood samples using BloodGen Midi Kit (CWBIO, China) following the manufacturer’s guidelines. WES was performed on exome targets isolated by capture using NimbleGen with 200-bp interrupted by the Covaris instrument. The elution product was amplified by 10 rounds of LM-PCR. The final products were sequenced with Illumina, and we analyzed these data. In addition, we performed Sanger sequencing: DNA was denatured at 95°C for 5 minutes followed by 30 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 60°C, and a final extension for 10 minutes at 72°C (ABI 3730XL).

We found that the Chinese boy with FSGS carried 3 novel heterozygous mutations of CRB2 in exon 10. WES confirmed that c.3190C>T (predicting p.Pro1064Ser) and c.2705C>T (predicting p.Thr902Met) were inherited from his healthy father, and c.445G>T (predicting p.Glu149X, 1137) was inherited from his healthy mother (Fig. 1). We believed the CRB2 mutation is consistent with autosomal dominant inheritance; these mutations had not been described previously. Moreover, further molecular diagnostics analysis of the CRB2 gene detected 2 missense mutations, c.3190C>T and c.2705C>T, resulting in replacement of a cysteine by a threonine, and a nonsense mutation with nucleotide substitution of G to T at position 445 (c.445G>T), leading to premature translational termination at amino acid position 149 (Glu149X). However, it is unclear that the mechanism leads to cellular and molecular changes, as there are only a handful of reports.

3. Discussion

The family of crumbs proteins including CRB1 and CRB3, is human homologs of drosophila CRB and plays a key role in the establishment and the maintenance of epithelial polarity. Using bioinformatics, Masuko Katoh first identified and characterized a novel crumbs family gene, CRB2, localized on 9q33.3, consisting of 13 exons and encoding a 1285 amino acid transmembrane protein that is essential for the maintenance of epithelial cell polarity and in the organization of epithelia derived from ectoderm, containing large extracellular domains with epidermal growth factor (EGF). Podocytes are highly polarized, specialized epithelial cells that are important in renal glomerular filtration through their intermediate foot processes connected by slit diaphragms. Mutations in several podocyte genes have been considered to be the cause of FSGS. An earlier study of animal models reported

### Table 1

| Parameters                     | Result   | Reference range |
|-------------------------------|----------|-----------------|
| Blood routine tests           |          |                 |
| Erythrocytes, 10^12/L         | 4.59     | 3.5–5.5         |
| Leucocyte, 10^9/L             | 5.07     | 4–10            |
| Platelet count, 10^9/L        | 264      | 100–300         |
| Haemoglobin, g/L              | 128      | 110–160         |
| Neutrophil, (%)               | 39.7     | 50–70           |
| Lymphocyte (%)                | 46.7     | 20–40           |
| Urine routine tests           |          |                 |
| Specific gravity              | 1.010    | 1.003–1.030     |
| Urine protein                 | ++       |                 |
| Urinary occult blood          | —        |                 |
| Urine glucose                 | +        |                 |
| Urine ketone bodies           | —        |                 |
| Urine creatinine, mmol/L      | 2293.00  |                 |
| 24-hour UPE, mg/d             | 635      | 28–150          |
| Urinary b2-microglobulin, mg/L| 91.74    | 0–300           |
| Immunology                    |          |                 |
| Immunoglobulin A, g/L         | 0.51     | 0.3–1.2         |
| Immunoglobulin G, g/L         | 6.04     | 3.5–10          |
| Immunoglobulin M, g/L         | 1.21     | 0.4–1.4         |
| Complement C3, g/L            | 0.82     | 0.85–1.99       |
| Complement C4, g/L            | 0.150    | 0.120–0.360     |
| Serum chemistry               |          |                 |
| Total protein, g/L            | 61.1     | 60–80           |
| Albumin, g/L                  | 37.9     | 35–55           |
| Globulin, g/L                 | 23.2     | 20–30           |
| Serum creatinine, μmol/L      | 34.3     | 27–130          |
| Blood coagulation tests       |          |                 |
| Prothrombin time              | 11       | 11–13           |
| Activated partial thromboplastin time, seconds | 26.8   | 21–36          |
| Prothrombin time-international normalized ratio | 1 | 0.8–1.2 |
| Fibrinogen, g/L               | 2.84     | 2–4             |

Bold values in result column indicate the abnormal detected valued out of the reference range.
that knock-down of zebrafish CRB2 led to the disappearance of slit diaphragms and reduced the expression of ZO-1 in phagocyte foot processes, damaging the glomerular filtration barrier. In 2015, Ebarasi et al. showed that a mutation in the extracellular 10th EGF-like domain resulted in loss of function, and the miscegenation of phagocyte apical basal polarity in CRB2 was a key factor causing monogenic FSGS in humans. In 2016, an individual with FSGS with compound heterozygous mutations of CRB2 (p.Trp1086ArgfsX64 and p.Asn1184Thr) was reported by Udagawa et al. showing by renal biopsy that a mutation among the 15th EGF-like domain and a frame-shift of CRB2 protein were likely pathogenic. Further histological analysis indicated that CRB2 was critical for the glomerular filtration barrier via regulating the expression and proper targeting of the slit diaphragm proteins, which are different from nephrin, which is apically mis-localized in phagocytes. These data explore some of the mechanisms and impact of the relationship between CRB2 mutations and phagocytes to some extent. In 2018, Watanabe et al. showed a Japanese patient that her renal biopsy specimens in keeping with FSGS, a newly compound heterozygous mutation of CRB2, p.Arg628Cys and p.Gly839Trp located in the 10th and 11th epidermal growth factor-like domains, respectively. These results demonstrate that CRB2 mutations may also occur in Chinese patients.

The CRB2 gene is mainly expressed in human retinal pigment epithelium/choroid, foetal eye, placenta, and lung. According to recent studies, phenotypic variability leads to a severe syndrome in addition to renal disease related to CRB2 mutation. In 2016, Jaron. showed 2 patients with severe obstructive congenital hydrocephalus, uroter-poletic renal malformation, cardiac defects, and lung hypoplasia. Exon sequencing found a missense mutation (c.2400C>T) in CRB2; the associated disease is a new cardiopathy based on clinical features that was not described previously, and few cases currently account for the conclusion. Accordingly, we believe that the Chinese patient with repeated fever and cough suffered from a new ciliopathy syndrome caused by CRB2 gene mutations, and more experimental studies are needed to support this conclusion. Classically, mutations in the CRB2 gene have been distinguished by their onset in childhood, usually after 3 months of age. This was laterly verified by the findings of others, whose patients with CRB2 mutations presented with FSGS exclusively from birth to 3 months. They also suggested that the phenotype of CRB2 mutations in these foetuses was consistent with congenital nephrosis syndrome, akin to the Finnish type, with cerebral ventriculomegaly as well as greatly elevated amniotic fluid alpha-fetoprotein and maternal serum alpha-fetoprotein levels. These results suggested that MRI and CT scans of the brain and chest in the Chinese patient with mutations in the CRB2 gene were required.

FSGS is an important aetiology of end-stage renal disease, and 33% risk of recurrence is estimated after kidney transplantation. Notably, it is one of the most pressing diseases in nephrology, and curative treatment is unavailable for most individuals. Fortunately, in some cases, rare single-gene mutations may be amenable to treatment. For example, 2 patients with recessive mutations in PLCε1 gene responded well after treatment with cyclosporine A or steroids.

Likewise, individuals with CUBN mutations may respond to vitamin B12 treatment, and individuals with ARHGDIA may theoretically respond to eplerenone. However, to date, we failed to find specific drugs for the treatment of CRB2 gene mutations in this boy. Therefore, his proteinuria persisted according to urinalysis.

4. Conclusions

To summarize, we reported the case of a Chinese patient with FSGS carrying 2 missense mutations (c.3190C>T and c.2705C>T) and a nonsense mutation (c.445G>T); genetic testing revealed these mutations in CRB2. These findings are consistent with the notion that mutations in recessive disease genes are more frequent in early-onset disease. Further studies are urgently needed to develop novel and curative treatment approaches for all patients.
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Author contributions
Conceptualization: Rong Fu.
Data curation: Fuxian Ren, Junjie He.
Formal analysis: Mengfan Gou.
Project administration: Shujing Wang.
Writing – original draft: Jiaojiao Fan.

References
[1] Iorember F, Aviles D. Anemia in nephrotic syndrome: approach to evaluation and treatment. Pediatr Nephrol 2017;32:1323–30.
[2] Ebarasi L, Ashraf S, Bierzynska A, et al. Defects of CRB2 cause steroid-resistant nephrotic syndrome. Am J Hum Genet 2015;96:153–61.
[3] Voskarides K, Makarious C, Papagregoriou G, et al. NPHS2 screening with SURVEYOR in Hellenic children with steroid-resistant nephrotic syndrome. Pediatr Nephrol 2008;23:1375–5.
[4] Bierzynska A, Soderquest K, Dean P, et al. MAGI2 mutations cause congenital nephrotic syndrome. J Am Soc Nephrol 2017;28:1614–21.
[5] Chen YM, Liapis H. Focal segmental glomerulosclerosis: molecular genetics and targeted therapies. BMC Nephrol 2015;16:101.
[6] Slavotinek AM. The family of crumbs genes and human disease. Mol Syndromol 2016;7:274–81.
[7] Tepass U, Knust E. Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in Drosophila melanogaster. Dev Biol 1993;159:311–26.
[8] Katoh M, Katoh M. Identification and characterization of Crumbs homolog 2 gene at human chromosome 9q33.3. Int J Oncol 2004;24:743–9.
[9] van den Hurk JA, Rashbass P, Roepman R, et al. Characterization of the Crumbs homolog 2 (CRB2) gene and analysis of its role in retinitis pigmentosa and Leber congenital amaurosis. Mol Vis 2005;11:263–73.
[10] New LA, Martin CE, Jones N. Advances in slit diaphragm signaling. Curr Opin Nephrol Hypertens 2014;23:420–30.
[11] Moller CC, Flesche J, Reiser J. Sensitizing the slit diaphragm with TRPC6 ion channels. J Am Soc Nephrol 2009;20:950–3.
[12] Ebarasi L, He L, Hulthen K, et al. A reverse genetic screen in the zebrafish identifies crb2b as a regulator of the glomerular filtration barrier. Dev Biol 2009;334:1–9.
[13] Udagawa T, Jo T, Yanagihara T, et al. Altered expression of Crb2 in podocytes expands a variation of CRB2 mutations in steroid-resistant nephrotic syndrome. Pediatr Nephrol 2017;32:801–9.
[14] Watanabe S, Aizawa T, Tsukaguchi H, et al. Long-term clinicopathologic observation in a case of steroid-resistant nephrotic syndrome caused by a novel Crumbs homolog 2 mutation. Nephrology (Carlton) 2018;23:697–702.
[15] Xiao Z, Patrakka J, Nukui M, et al. Deficiency in Crumbs homolog 2 (Crb2) affects gastrulation and results in embryonic lethality in mice. Dev Dyn 2011;240:2646–56.
[16] Jaron R, Rosenfeld N, Zahdeh F, et al. Expanding the phenotype of CRB2 mutations—a new ciliopathy syndrome? Clin Genet 2016;90:540–4.
[17] Philippe A, Nevo F, Esquivel EL, et al. Nephrin mutations can cause childhood-onset steroid-resistant nephrotic syndrome. J Am Soc Nephrol 2008;19:1871–8.
[18] Slavotinek A, Kaylor J, Pierce H, et al. CRB2 mutations produce a phenotype resembling congenital nephrosis, Finnish type, with cerebral ventriculomegaly and raised alpha-fetoprotein. Am J Hum Genet 2015;96:162–9.
[19] Shimizu A, Higo S, Fujita E, et al. Focal segmental glomerulosclerosis after renal transplantation. Clin Transplant 2013;27(suppl 23):6–14.
[20] Hinkes B, Wiggim RG, Ghadegesin R, et al. Positional cloning uncovers mutations in PLCE1 responsible for a nephrotic syndrome variant that may be reversible. Nat Genet 2006;38:1397–405.
[21] Sadowski CE, Lovric S, Ashraf S, et al. A single-gene cause in 29.5% of cases of steroid-resistant nephrotic syndrome. J Am Soc Nephrol 2015;26:1279–89.