Familial C3 glomerulonephritis associated with mutations in the gene for complement factor B

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ABSTRACT

We report the first case of familial C3 glomerulonephritis (C3GN) associated with mutations in the gene for complement factor B (CFB). A 12-year-old girl was diagnosed with biopsy-proven C3GN. Her mother had a history of treatment for membrano-proliferative glomerulonephritis, and her brother had hypo-complementemia without urinary abnormalities. DNA analysis revealed heterozygosity for CFB p.S367R in the patient, mother and brother. Evaluation of the structure–function relationship supports that this mutation has gain-of-function effects in CFB. The present case suggests that CFB has an important role in the etiology of C3GN and provides a new insight into anticomplement therapy approaches.

Keywords: C3 glomerulonephritis, complement alternative pathway, complement factor B, genetic mutation

BACKGROUND

C3 glomerulonephritis (C3GN) is a recently described disorder that results from dysregulation of the complement alternative pathway (AP) and is typically characterized by dominant C3 deposition with an absence or paucity of immunoglobulin deposition measured by immunofluorescence (IF) [1–6].

The continuous low-level activation of AP in plasma is tightly regulated by activating and regulatory complement proteins such as complement factor B (CFB), complement factor H (CFH), complement factor I (CFI) and membrane cofactor protein (MCP). CFB, a key molecule that activates the early stages of AP, is cleaved by complement factor D (CFD) into two fragments: Ba and Bb. Bb, a serine protease, then combines with C3b to generate C3 convertase, leading to the generation of a membrane attack complex. The causes of AP dysregulation in previous reported cases of C3GN include mutations in complement genes and autoantibodies that stabilize C3 convertase, or autoantibodies that affect pathway inhibition [4–6].

Here, we report a case of familial C3GN associated with mutations in the CFB genes. To the best of our knowledge, this is the first case report that demonstrates the involvement of CFB in the etiology of C3GN.

CASE

A 9-year-old girl was found to have proteinuria and hematuria during the Japanese school urinary screening system in May 2009. At the initial visit to a local hospital, her serum C3 level was low at 5 mg/dL (normal range, 65–135 mg/dL). In April 2012, at the age of 12 years, she developed massive proteinuria with gross hematuria and was referred to our hospital.

She had no symptoms at admission, and physical examination revealed no edema. Laboratory investigation on admission was as follows: serum total protein, 5.05 g/dL; serum albumin, 2.30 g/dL and serum creatinine, 0.43 mg/dL. By urinalysis, protein excretion was 2.1 g/day. Immunological evaluation was as follows: C3, 15 mg/dL; C4, 16 mg/dL (normal range, 13–35 mg/dL); hemolytic complement activity (CH50), 19 U/mL (normal range, 30–45 U/mL) and antinuclear antibody titer, <20-fold. Soluble C5b-9 was markedly elevated at 1.61 mg/L compared with normal healthy controls (0.87 ± 0.22 mg/L, n = 5).

Levels of soluble C5b-9 were determined by using a BD OptEIA™ human C5b-9 ELISA Set (BD Biosciences, San Diego, CA).

A kidney biopsy was performed and light microscopy showed membranoproliferative glomerulonephritis (MPGN); global mesangial proliferation and segmental endocapillary proliferation with lobular formation and remarkable double

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contour of glomerular basement membrane (Figure 1). IF analysis revealed dominant C3 deposition with an absence of immunoglobulin deposition, and electron microscopy demonstrated subendothelial, mesangial and intramembranous electron-dense deposits. She has been treated with drugs including steroid and cyclosporine for 2 years, and has now achieved partial remission with u-P/Cr of 0.2 g/g Cre and no hematuria despite persistent low C3 levels.

Regarding the family history, her mother was diagnosed with MPGN type I (IF findings were not available) in her teenage years, but she had discontinued periodic examination 18 years ago. Recent urinalysis of the mother showed moderate proteinuria with u-P/Cr of 0.5 g/g Cre without hematuria, and laboratory investigation showed hypocomplementemia (C3, 15 mg/dL) with normal kidney function. Her elder brother also had hypocomplementemia (C3, 24 mg/dL), but no urine abnormalities. Her father and younger sister had normal urinalysis and complement levels.

**DNA ANALYSIS**

DNA analysis was performed under written informed consent and approval of the ethics committee of Nara Prefectural Medical University, Nara, Japan, and the National Cerebral and Cardiovascular Center, Osaka, Japan. All exons of genes that encode the molecules regulating AP, C3, CFB, CFH, CFI, MCP and THBD were analyzed by direct sequencing of amplified genomic DNA obtained from a whole blood sample of the patient [7]. DNA analysis demonstrated heterozygous p.S367R in the CFB gene, p.R201S in the CFI gene and p.V916I in the C3 gene (Figure 2A). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the family members revealed CFB p.S367R in the patient’s mother and elder brother, CFI p.R201S in her mother and younger sister and C3 p.V916I in her father (Figure 2B).

**DISCUSSION**

Here, we describe two cases of C3GN and one case of hypocomplementemia without urine abnormalities that occurred within a family, in which CFB p.S367R was considered to contribute to the dysregulation of AP.

It is highly likely that p.S367R causes a gain of function in CFB through a structure–function relationship. The p.S367R is located in a von Willebrand factor type A (vWfA) domain of the catalytic subunit Bb of CFB. *In vitro* experimental data demonstrated a strong association between mutations in the vWfA domain and gain of function in CFB through the promotion of high-affinity C3 binding [9]. In atypical hemolytic uremic syndrome, which also results from the dysregulation of AP, a recent report of functional analysis of CFB mutations revealed that six genetic changes which were concluded to have relevance to disease were all located in the vWfA domain of CFB, suggesting that this domain plays an important role in CFB function [8]. Additionally, structural evaluation demonstrates that p.S367R is located close to p.K323E/Q, one of the mutations that causes CFB gain of function as demonstrated by surface plasmon resonance analysis (Figure 2C) [8]. The p.K323E/Q showed increased resistance to inactivation of C3 convertase by CFH, and a similar functional consequence is considered for p.S367R [8].

In this study, CFB p.S367R was present concurrent with two other genetic changes, CFI p.R201S and C3 p.V916I, in the patient and in various combinations in family members; however, we speculate that these additional two genetic changes are not associated with potential disease relevance. First, the mutations of p.R201S and p.V916I had no impact on the phenotype of her sister and father, respectively. Second, although p.R201S was detected in the patient and her mother, both of whom had C3GN, p.R201S is a polymorphic allele found in Far East populations, with a frequency of about 0.03 in Japan [10]. However, functional assays of mutant CFB, CFI

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**FIGURE 1**: Histological findings of the patient. Light microscopy shows global mesangial proliferation and segmental endocapillary proliferation with lobular formation and remarkable double contour of glomerular basement (PAM, magnification ×400; left panel). IF shows dominant C3 deposition in the mesangium and along the capillary walls with negative immunoglobulin deposition (middle panel). Electron microscopy shows remarkable subendothelial electron-dense deposits (asterisk) and mesangial electron-dense deposits (black arrows). Mesangial interposition (white arrows), double contour of glomerular basement membrane and endocapillary hypercellularity are observed (right panel). These findings indicate C3GN rather than dense-deposit disease, because electron-dense deposits were mainly observed not within the glomerular basement membrane but in the subendothelial and mesangial areas, while ribbons of electron-dense transformation of glomerular basement membranes are not found.
and C3 to assess their involvement in the activation of AP are required to prove a detailed etiological mechanism.

Some limitations exist in this study. C3NeF and other autoantibodies were not investigated; these autoantibodies may also be associated with our cases, concomitant with CFB mutations. Although low C3 levels and elevated soluble C5b-9 levels indicate continuous activity of the AP, detailed complement investigations (e.g., the measurement of Ba, Bb, C3a, C3d and C5a) are required to clarify the complement activation mechanisms more precisely.

In conclusion, this study suggests that CFB has a critical role in AP in the pathogenesis of C3GN and expands our understanding of the genetic factors conferring predisposition to C3GN and supports the development of anticomplement therapies, including those targeting CFB activation.

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CONFLICTS OF INTEREST STATEMENT

None declared.

FIGURE 2: DNA analysis of the patient and family members and structural evaluation of CFB S367R. (A) Direct sequencing of patient genomic DNA shows heterozygous p.S367R (c.1099 A>C) in exon 8 of the CFB gene, p.R201S (c.603 A>C) in exon 4 of the CFI gene and p. V9161 (c.2746 G>A) in exon 21 of the C3 gene. The Met encoded by the translation initiation site (start codon) is numbered as residue 1. Upper row: mutant type, lower row: wild type. (B) PCR-RFLP analysis of family members shows CFB p.S367R in the mother and elder brother, CFI p.R201S in the mother and younger sister and C3 p.V9161 in the father. P, patient; F, father; M, mother; B, elder brother; S, younger sister; WT, wild type; He, heterozygous. (C) Visualization of the complex of CFB, CFD and C3b. The figure was prepared using PyMOL (www.pymol.org). Each molecule is represented as follows: CFB, gray; CFD, yellow; C3b, cyan and p.S367R, red sphere. p.S367R is not located attached to CFD or C3b but rather on the surface of CFB and close to p.K323E/Q (blue sphere), of which the gain of function of CFB was proven by surface plasmon resonance analysis in a previous report [8]. p.K323E/Q was described as p.K298E/Q in the original article, with the notation using the nomenclature system of mature proteins.

REFERENCES