C3 Glomerulonephritis Associated with a Missense Mutation in the Factor H Gene

Keisuke Sugimoto,1 Shinsuke Fujita,1 Kouhei Miyazaki,1 Mitsuru Okada1 and Tsukasa Takemura1

1Department of Pediatrics, Kinki University School of Medicine, Osaka, Japan

The complement system, the major component of the innate immune functions resisting microbial infection, includes the classical complement pathway, the alternate pathway, and the mannose-binding lectin pathway. All of these merge at the level of complement component (C) 3. Complement factor H (CFH), a soluble complement mediator in blood, regulates alternate pathway activation; a conformational change of C3 molecules by C3 convertases leads to an enzyme complex formation resulting in opsonization and cell lysis. Clinical manifestations arising from CFH gene (CFH) abnormalities include hemolytic uremic syndrome and membranoproliferative glomerulonephritis. We encountered a 24-year-old woman initially diagnosed with C3 glomerulonephritis associated with persistently low circulating C3. Definitive diagnosis of C3 glomerulonephritis was made from immunohistologic demonstration of isolated mesangial C3 deposits. The biopsy specimen showed moderately increased mesangial proliferation, without thickening of the glomerular capillary walls. Genetic analysis disclosed a homozygous CFH missense mutation, a G-to-T transversion at nucleotide 3,048 in exon 18, resulting in substitution of Asp for Glu at position 936. A low serum CFH concentration (110 μg/mL) might reflect the consequences of this CFH mutation. C3 glomerulonephritis is associated with a CFH mutation, the mutation of which results in the unexpected activation of alternate pathway complement with clinical laboratory fluctuations, such as varying reduction of serum CFH and C3. The finding of a patient with a CFH mutation associated with C3 glomerulonephritis represents an opportunity to expand the phenotypic spectrum of the CFH mutations.

Keywords: atypical hemolytic uremic syndrome; complement factor H; C3 glomerulonephritis; dense deposit disease; membranoproliferative glomerulonephritis

The complement system is the major component of the innate immune system, which defends against microbial infection by activating inflammation, lysis of organisms, opsonization, and immune clearance. The 3 different complement pathways, the classical complement pathway, the alternative pathway, and the mannose-binding lectin pathway, merge at the level of complement component (C) 3 (Collard et al. 1999). The alternative pathway is initiated by spontaneous hydrolysis of C3. Complement regulators located both in the fluid phase and on the cell surface act to prevent inappropriate complement activation that can damage host tissues (Walport 2001a, 2001b; Rodríguez et al. 2004). Uncontrolled complement activation may be associated with many disorders, including renal diseases (Pickering and Cook 2011). Recent investigation of abnormal complement activation has focused on the alternate complement pathway, which functions independently of antibodies. Complement factor H (CFH), a soluble complement mediator and a crucial regulator of complement system activation, is the regulatory protein of the alternative pathway (Ault 2000). CFH is a single polypeptide glycoprotein with the molecular mass of 150 kDa; it consists of 1,213 amino acid residues, that make up 20 complement control protein units, termed short consensus repeats (SCR). Each SCR is a highly conserved sequence of about 60 amino acids (Kristensen and Tack 1986; Ripoche et al. 1988).

Impaired CFH functions are associated with various renal diseases such as atypical hemolytic-uremic syndrome and C3 glomerulopathy (Józsi and Zipfel 2008; Pickering and Cook 2011). Distinct forms of C3 glomerulopathy include C3 glomerulonephritis, dense deposit disease, and complement factor H-related 5 nephropathy (Sethi et al. 2009). Some patients with these disorders display the glomerular morphologic pattern of membranoproliferative glomerulonephritis (MPGN), while others show a different pattern such as glomerulonephritis without mesangial proliferation or capillary wall thickening (Sethi et al. 2009;...
Complement gene mutations including \textit{CFH} mutations have been associated with atypical hemolytic-uremic syndrome (Levy et al. 1986; Dragon-Durey et al. 2004; Licht et al. 2006; Vaziri-Sani et al. 2006; Servais et al. 2011). Servais et al. (2007) reported that only 13 of 19 patients with C3 glomerulonephritis showed pathologic findings of MPGN. When those authors tested for gene mutations including genes for CFH, complement factor I (CFI), and CD46, 3 of the 19 patients were heterozygous for mutations in the \textit{CFH} gene, although how heterozygosity for the \textit{CFH} mutations might be related to C3 glomerulus was unknown. Habbig et al. (2009) reported 2 patients diagnosed with C3-deposition glomerulonephritis who had a \textit{CFH} mutation, a deletion of a single amino acid; treatment using fresh frozen plasma (FFP) infusion to supply CFH was successful. Here we report a patient with a homozygous \textit{CFH} mutation that causes C3 glomerulonephritis in childhood.

**Clinical findings**

A girl, now 24 years old, was admitted to our hospital at the age of 12 years because of hematuria, proteinuria, and hypocomplementemia. Her parents were consanguineous, but no family members had exhibited similar symptoms or end-stage renal failure except for one elder brother with microscopic hematuria. Hypertension, oliguria, and edema were absent. She had no physical findings of lipodystrophy such as symmetric loss of adipose tissue from the face, arms, or upper portions of the trunk. Serum creatinine and blood urea nitrogen (BUN) were normal, as was creatinine clearance (125.6 mL/min). Serologic analysis for antinuclear antibody was positive, and anti-DNA antibody was slightly elevated (80 IU/L; normal range, below 80 IU/L). Serum C3 (10 mg/dL; normal range, 82 to 145 mg/dL); C4 (18 mg/dL; normal range, 12 to 33 mg/dL); and CH50 (14.3 U/mL; normal range, 24.2 to 52.8 mg/dL) all were low. C3d (138 mU/L; normal range, upper limit, 130 mU/L), and circulating immune complex (CIC; 3.67 µg/mL; normal range: below 3.0 µg/mL) were slightly increased. No specific focus of infection such as chronic tonsillitis was demonstrated in this patient, however she occasionally showed transient hypocomplementemia during upper respiratory infections.

Urinary abnormalities and hypocomplementemia persisted after admission, and then renal biopsy was performed. The sample included 13 glomeruli, and showed moderately increased mesangial matrix and mesangial proliferation (Fig. 1a, arrow). Diffuse thickening of glomerular capillary walls was absent, as were tubular atrophy, and significant interstitial mononuclear infiltrates. Immunofluorescence showed intense C3 reactivity in portions of the mesangium (Fig. 1b), but no reactivity for IgG, IgA (Fig. 1c) and electron-dense deposits were observed mainly in mesangial and paramesangial areas (d, indicated by arrows, original magnification, × 3,000).

![Fig. 1](image-url)
C3 Glomerulonephritis and Factor H Gene

1c), IgM, or other complement components such as C4 or C1q. Electron microscopy disclosed nodular deposits in mesangial and paramesangial areas (Fig. 1d, indicated by arrows). No hump formation representing acute glomerulonephritis was observed. Taken together, these findings confirmed a diagnosis of C3 glomerulonephritis. Treatment with an angiotensin-converting enzyme inhibitor to lower glomerular arteriolar resistance was begun after diagnosis.

Serum CFH, measured by enzyme-linked immunosorbent assay (ELISA; Hycult Biotech, Plymouth Meeting, PA), was 110 μg/mL, significantly below the normal range (284 to 428 in adults) (Licht et al. 2006). Genetic analysis revealed a homozygous CFH mutation causing a G-to-T transversion at nucleotide 3,048 in exon 18, which resulting in substitution of D (GAT) for E (GAG) at the amino acid position 936 (E936D).

**Discussion**

CFH is the initial and principal regulatory factor of the alternate pathway of complement activation. CFH or CFI deficiency may lower plasma C3 concentrations, since functional impairment of CFH can result in abnormalities of the alternate pathway (Botto et al. 2009). CFI also is a key factor in development of renal diseases, such as MPGN, dense deposit disease, and atypical hemolytic-uremic syndrome (Kavanagh et al. 2005; Rose et al. 2008); normally, CFI acts as a cofactor of C4bp and CFH in inactivation of C3b and C4b. In a summary by Servais et al. (2007) of genetic risk factors in 19 patients diagnosed with primary C3 glomerulonephritis, C3 glomerulonephritis without MPGN had risk factors related to mutations affecting CFH, CFI, and membrane cofactor protein, in contrast with C3 glomerulonephritis patient showing the histologic abnormalities of MPGN. C3 nephritic factor (C3Nef) was detected more frequently in the latter group, which also tended to have normal serum C3 concentrations. Periodic plasma infusions were required to avert severe renal disease with persistent C3 decrease caused by CFH deficiency (Habbig et al. 2009), a finding in agreement with observations in CFH-deficient mice (Fakhouri et al. 2010).

SCR of CFH have been identified as “hot spots” for mutation in several diseases (Prodinger et al. 1998). Structure-function analysis had determined a C3b-binding site to be located within N-terminal SCR (Alsenz et al. 1984; Gordon et al. 1995). CFH has at least 3 distinct binding regions for C3: N-terminal sites that bind to C3b; a second site that binds to the C3c fragment; and a site located within SCR 19 and 20 that binds to the C3d region. In binding C3b, these sites take part in regulating complement activation (Sharma and Pangburn 1996). C3b- and polyanion-binding sites in SCR 19 to 20 are important for CFH interaction with host surfaces (Jokiranta et al. 2005; Ferreira et al. 2006). E-to-D substitution at amino acid position 936, as in our patient has been reported as a disease-associated polymorphism (Saunders et al. 2007). SCR 16, where our patient’s gene mutation occurred, does not encompass principal binding sites as do SCR 19 to 20, although clinical severity and disease phenotype presently are uncertain for mutation at any site. In our patient, typical pathologic findings of MPGN were absent, even though she showed complement component C3 deposition in glomeruli together with low serum complement concentrations. Additionally, the patient lacked C3Nef and overt features of lipodystrophy. Her clinical condition and laboratory values including serum complement have been stable during monotherapy with an angiotensin-converting enzyme inhibitor, and clinical manifestations of disease have not progressed in our patient. One reason might be that her specific polymorphism is in sufficient for ongoing systemic activation. Severity of clinical features may depend partly on specific CFH sites at which pathogens bind, and on which SCR locations are affected by mutation. C3Nef often causes uncontrolled systemic alternative complement pathway activation (Servais et al. 2007). We suppose that another reason for a stable clinical course except when infection occurs might be absence of detectable abnormalities of C3Nef.
In summary, our patient had C3 glomerulonephritis associated with low CFH concentrations caused by a CFH mutation. The clinical course was not progressive during treatment with only an angiotensin-converting enzyme inhibitor. Predictive factors for transient worsening of this disorder in our patient were only increased proteinuria accompanied by decreased serum CFH activity. Differences in clinical phenotypes between our patient and others might depend on variants in the CFH gene. E936D could impair complement regulatory activity of CFH as was observed in our patient. As for changes in serum CFH activity during the clinical course, amino acid substitution caused by the CFH mutation would be unlikely to cause complete inactivation of serum CFH activity. However, direct influence of this gene abnormality upon our patient’s renal condition remains unclear, so functional analysis should be conducted in the future. Our patient’s homozygous missense mutation associated with C3 glomerulonephritis represents an opportunity to expand the spectrum of this disease.

Acknowledgments

The study was supported partly by a Grant-in-Aid for Scientific Research from Morinaga Hoshikai to T.T. (from 2010 to 2011). We thank Naomi Jinno for assistance in manuscript preparation.

Specific Remarks

This manuscript has been seen and approved by all authors, and is not under consideration for publication elsewhere in a similar form, in any language, except in abstract form. Genetic analyses and tissue staining for renal specimens were performed after we received approval from the Ethics Committee at Kinki University and obtained written informed consent from the patient’s parents or guardian.

Conflict of Interest

We have no conflicting interest affecting the present study.

References


Servais, A., Frémeaux-Bacchi, V., Lequintrec, M., Salomon, R.,


