

Gene Polymorphism of Complement Factor H in a Turkish Patient With Membranoproliferative Glomerulonephritis Type II

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Membranoproliferative glomerulonephritis (MPGN) is characterized by proliferation of mesangial and endothelial cells and by thickening of the peripheral capillary walls. Type II of the MPGN is associated with complement abnormalities which are factor H deficiencies due to mutations in the complement factor H (*CFH*) gene. We report a 15-year-old boy diagnosed with MPGN II in whom genetic analyses of the *CFH* gene revealed that the patient was heterozygote for a polymorphism in exon 2 of the *CFH* (c.184G>A), heterozygote for a polymorphism in exon 9 of the *CFH* (c.1204C>T), and heterozygote for a polymorphism in exon 10 of the *CFH* (c.1419G>A). These data recapitulate a prototypical complement genetic profile, the presence of major risk factors for MPGN II, which support the hypothesis that these dense deposit diseases have a common pathogenic mechanism involving dysregulation of the alternative pathway of complement activation.

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INTRODUCTION

Membranoproliferative glomerulonephritis (MPGN) mainly affects older children and adolescents, presenting mostly with mild proteinuria and hematuria. Types I and II of MPGN are variants of immune complex-mediated disease; MPGN II, in contrast, has no known association with immune complexes.¹ The MPGN II accounts for 20% of cases of MPGN in children and both sexes are affected equally.¹ The diagnosis usually made in children between the ages of 5 and 15 years who present with nonspecific findings such as hematuria, proteinuria, acute nephritic syndrome, or nephrotic syndrome.¹ The MPGN II is characterized by complement-containing dense deposits within the basement membrane of the glomerular capillary wall, followed by capillary wall thickening, mesangial cell proliferation, and glomerular fibrosis.^{1,2} This morphological hallmark is so characteristic of MPGN II that the disease is more accurately referred to as dense deposit disease.

Patients with MPGN II are positive for serum

complement C3 nephritic factor (C3NeF), an autoantibody directed against C3 convertase of the alternative pathway of the complement cascade (C3bBb).³ Defective complement control is a major cause of the disease.⁴ The absence or defective function of factor H results in unrestricted activity of the alternative C3bBb, leading to complement activation and deposition of activated complement components within the glomerular basement membrane.

Analysis of the genetic defects leading to complete deficiency of complement factor H (CFH) in plasma of patients revealed homozygous or compound heterozygous factor H gene mutations in short consensus residues,^{2,4} which results in nonframework amino acid exchanges or mutations of framework Cys residues, affecting disulphide bond formation within the factor H molecule.^{5,6} More recently, common genetic polymorphisms in different complement genes have been reported to increase the risk or confer protection for both age-related macular degeneration (AMD) and MPGN II.

In particular, the *CFH* His402 variant, in the short consensus repeat 7 of factor H, has consistently been shown to be associated with increased risk of AMD in numerous studies.^{7,8} This has also been associated with MPGN linkage studies.⁹

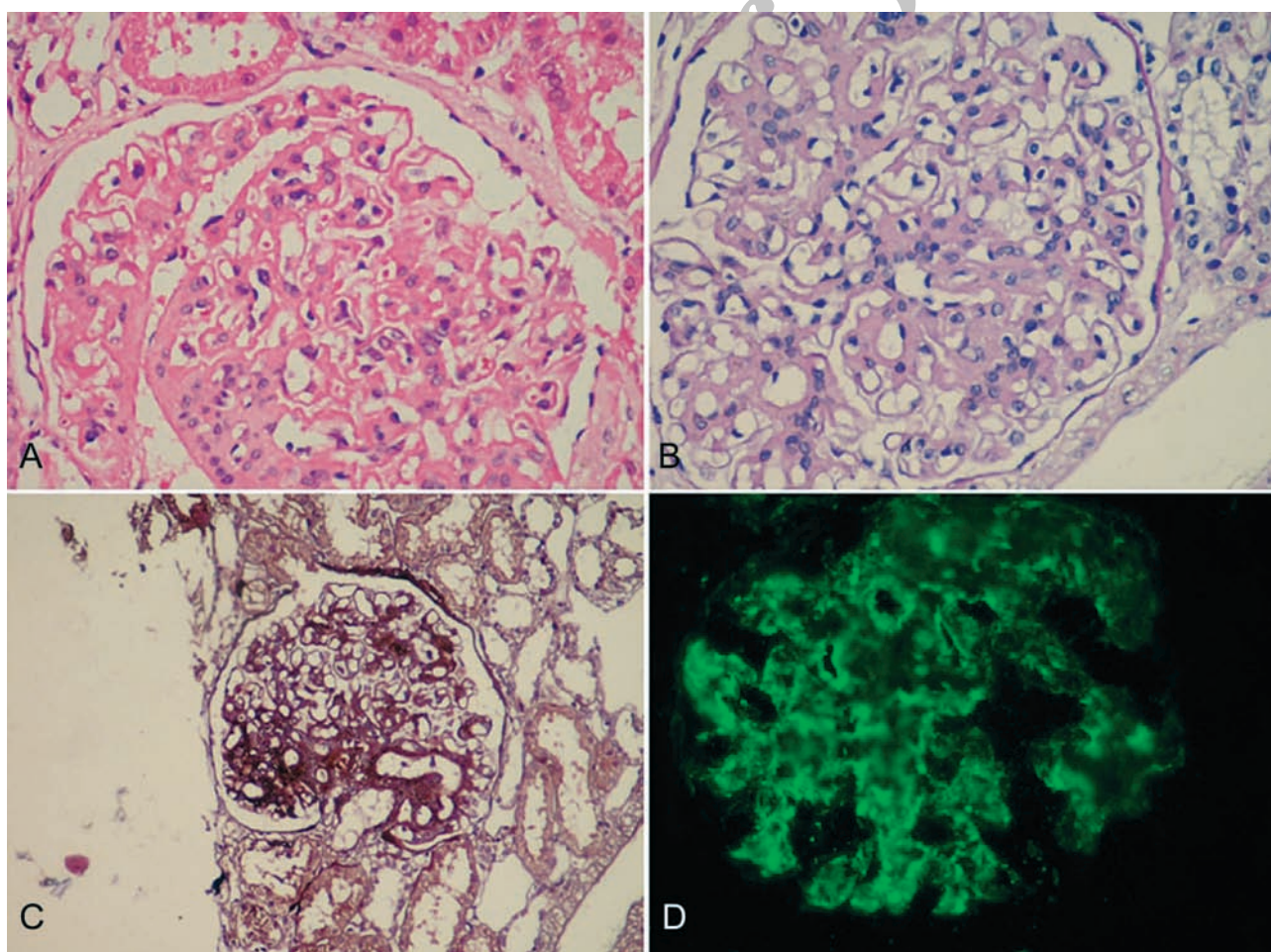
We reported a Turkish patient who developed MPGN II with mutation in the *CFH* gene. The patient presented persistent low levels of C3, further supporting that dysregulation of the complement alternative pathway is a pathogenic mechanism for MPGN.

CASE REPORT

A 15-year-old boy with a history of arterial hypertension presented in 2002 (at the age of 11 years old) with renal insufficiency (serum creatinine, 0.3 mg/dL; serum urea, 23 mg/dL; urea protein excretion, 23 mg/m²/h; and microhematuria). His

blood pressure was 166/80 mm Hg (stage 2) and his pulse rate was 74 per minute at the onset of the disease. His laboratory results were as follows: hematocrit, 34%; leukocyte count, 7.6×10^6 /L; platelet count, 260×10^6 /L; serum albumin, 1.4 g/dL; serum cholesterol, 339 mg/dL; serum C3, 21 mg/dL (reference range, 75 mg/dL to 140 mg/dL); and serum C4, 24 mg/dL (reference range, 11 mg/dL to 61 mg/dL). The presence of C3 nephritic factor was not determined. Immunoglobulins G, A, and M levels were within normal ranges. Serological studies for antineutrophil cytoplasmic antibodies directed against myeloperoxidase and proteinase 3 and the anti-double-stranded DNA antibodies were negative. Viral studies for antibodies to hepatitis C and B were negative. His urinalysis showed proteinuria (45 mg/m²/h) and hematuria.

Histological analysis of kidney biopsies from



Pathologic examination of the first kidney biopsy specimen. **A to C**, In light microscopy (A to C), the glomeruli show moderate mesangial widening and thickening of the capillary walls. There is relatively little cellularity (A, hematoxylin-eosin, $\times 40$; B, periodic acid-Schiff, $\times 40$; C, silver stain, $\times 20$). **D**, Immunofluorescence microscopy shows granular deposits of complement C3 in the mesangium and capillary walls (C3 immunofluorescence stain; $\times 20$).

the patient demonstrated features consistent with a diagnosis of MPGN II. These included intense glomerular hypercellularity, thickening of the capillary walls, and increased amounts of mesangial matrix visible at the light microscopic level. In addition, immunofluorescence analysis showed a strong deposition of C3 (4+) in the glomerular basement membrane with absence of immunoglobulin. No electron microscopy data was available from the first kidney biopsy of the patient (Figure). Ophthalmoscopic analysis revealed that both retinas of the patient were intact. In addition, the patient did not carry mutation in C3 gene.

A final diagnosis of MPGN II was made and he was treated with pulse methylprednisolone, 30 mg/kg every other day; prednisolone, 60 mg/d; and an angiotensin receptor blocker. During the follow-up period, he received cyclophosphamide, 2 mg/kg/d for 12 weeks, and cyclosporine A, 3 mg/kg/d, because proteinuria and hypocomplementemia were persistent. At the 3rd year of his follow-up, he had a 2nd kidney biopsy which was consistent with the previous study. In addition, it showed glomerular sclerotic lesions in 1 of 40 glomeruli and diffuse dense deposits of the lamina densa of the glomerular basement membrane on electron microscopy. He was treated with intermittent plasmapheresis. During that time, he was taking cyclosporine A. His serum C3 levels was low throughout the course (27 mg/dL to 54 mg/dL), and his kidney function was normal.

A blood sample was drawn for factor H mutation screening by the bidirectional sequencing of all coding exons. The patient was screened for mutations and polymorphisms in the *CFH* gene by automatic DNA sequencing of polymerase chain reaction amplified fragments. Genomic DNA was prepared from peripheral blood cells according to the standard procedures. Each exon of the *CFH* gene was amplified from the genomic DNA by using specific primers derived from the 5' and 3' intronic sequences as described. Genetic analyses of the *CFH* gene revealed that he was compound heterozygote for the V62 allele and Y402 allele variants of factor H (rs# 800292, exon 2; an isoleucine-to-valine change, and rs# 1061170, exon 9; a tyrosine-to-histidine change). Also, he was heterozygote for a polymorphism in exon 10 of the *CFH* (c.1419G>A) that results in an Ala473Ala (rs#2274700) substitution in short

Mutations Identified from the Complement Factor H Gene in Patients with Membranoproliferative Glomerulonephritis Type II*

Mutation	Nucleotide	Domain	Exon	References
Ile62Val	c.184G>A	SCR1	2	This study, 9
His402Try	c.1204C>T	SCR7	9	This study, 9, 19
Ala473Ala	c.1419G>A	SCR8	10	This study, 9
K224 del	d.743-745	SCR4	6	20

*SCR indicates short consensus repeat.

consensus repeat 8 of the factor H protein. These results are summarized in the Table.

DISCUSSION

Membranoproliferative glomerulonephritis type II is most frequently diagnosed in children between the ages of 5 and 15 years.¹ Affected patients usually present with heavy proteinuria, hematuria, and renal insufficiency. The prognosis of patients with MPGN II is generally quite poor.^{10,11} Most patients eventually progress to end-stage renal disease, with a 10-year renal survival rate of 50%.¹²⁻¹⁴ The disease is currently classified as an autoimmune disorder primarily because of the frequent finding of circulating IgG antibodies known as C3 nephritic factors (C3NeFs) that are directed against the C3 convertase of the alternate pathway, known as *C3bBb*.¹⁵

Complement factor H is the primary fluid-phase regulator of the alternative pathway of complement. It has decay-accelerating activity, meaning that it facilitates the decay of C3 convertase, competes with factor B for C3b, and functions as a cofactor for factor I-mediated proteolytic inactivation of C3b. Mutations in factor H have been reported in atypical hemolytic uremic syndrome, age-related macular degeneration, and MPGN II.¹⁶⁻¹⁸ The *CFH* His402 triallelic polymorphism has been strongly associated with AMD. The Major allele (tyrosine) is seen in normal populations at 54%. Ninety-four percent of patients with AMD were found to have the histidine allele.¹⁶ This has also been associated with MPGN linkage studies.^{9,19}

The *CFH* gene mutations result in impaired factor H function, as reported by Licht and coworkers,²⁰ who described 2 sisters homozygous for an in-frame deletion of a single amino acid in factor H that resulted in the development of MPGN II. The mutation, the deletion of lysine at position 224 (DK224), was associated with normal serum

levels of factor H, but the mutant protein had decreased activity.

Few MPGN II disease cases have been reported causing mutations in factor H. Abrera-Abeleda and colleagues⁹ identified Ile 62 Val mutation of *CFH* associated with MPGN II. His 402 allele was found in 85% of patients with MPGN.⁹ Prosser and colleagues²¹ recently showed that switching from a tyrosine to a histidine residue at the position 402 of factor H alters the mode of glycosaminoglycan binding by changing the specificity for particular sulfating patterns. In the absence of adequate amounts of factor H, and possibly in the presence of other triggers or genetic factors, complement-mediated damage of the glomerular basement membrane occurs. Also, Ala 473Ala mutation of the *CFH* gene was found associated with hemolytic uremic syndrome and with autosomal dominant AMD.^{17,22}

Ethnic differences were observed in *CFH*-related phenotypes and disease-susceptible genomic alterations. For example, the C allele frequency varies between 0.30% and 0.39% in different Caucasian populations, while its frequency is 0.11% for Japanese people.^{23,24} In Turkish population, the C allele frequency was found in 0.35%.²⁵ Thus, the disease-related C allele has a frequency in Turkey similar to that of Caucasian populations. We think that this allele may be related to MPGN in Turkey. Our patient had heterozygote polymorphisms of factor H associated with MPGN II, the Val62 variant, His 402 variant, and Ala 473 in exon 2, exon 9 and exon 10, respectively. Previous studies are summarized in the Table.

The current treatment strategies of using corticosteroids and immunosuppressive agents in patients with MPGN II are not promising.²³ Recent studies showed that the replacement of factor H by plasma infusion and plasmapheresis are useful for patients with MPGN II due to proven pathogenic mutations of *CFH*.^{1,23} Our patient developed MPGN at age of 15 years old. He has already hypocomplementemic and proteinuric despite treatment with corticosteroid, several immunosuppressive agents, and plasmapheresis.

All of these results show that the aberrant function of factor H likely plays a significant role in the pathogenesis of MPGN II. Also, certain *CFH* polymorphisms are more prevalent in patients with MPGN II. In this case, it is important to

recognize that additional functional studies on these polymorphisms and further genetic studies of other proteins in the complement system are required to better understand the pathophysiology of this disease.

CONFLICT OF INTEREST

None declared.

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