IJKD KIDNEY DISEASES

C3 Glomerulonephritis With Multiple Mutations in Complement Factor H

Nooshin Dalili,[1] Babak Behnam,[2,3] Farzaneh Vali,[2] Mahmoud Parvin,[4] Peyman Torbati,[4] Nakisa Rasaii,[1] Fariba Samadian,[1] Pedram Ahmadpoor[1]

[1]Chronic Kidney Disease Research Center, Department of Nephrology, Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3]NIH Undiagnosed Diseases Program, Common Fund, Office of the Director, NIH; Office of the Clinical Director, NHGRI, National Institutes of Health; Bethesda, Maryland, USA
[4]Department of Pathology, Labbafinejad Hospital, Shahid Beheshti Medical University, Tehran, Iran

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Complement C3 glomerulopathy refers to a disease process in which abnormal control of complement activation or degradation results in predominant C3 fragment deposition within the glomerulus and causes glomerular damage. Abnormal control of the complement alternative pathway is a well-established risk factor for the occurrence of C3 glomerulonephritis. It is the first reported case in Iran with multiple mutations in complement factor H, with one of these mutations we have expected in hemolytic uremic syndrome rather than C3 glomerulopathy. Genetic analysis showed that the molecular abnormalities of factor H led to complement factor H malfunction that were polymorphous and not restricted to the C-terminal domains of the protein.

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Case Report

INTRODUCTION

The complement system is a set of over 30 different proteins and protein fragments, always found in the blood. With an infection, this system of molecules is activated, and a cascade of events follows, in which each step leads to the next. At the center of the cascade are steps in which the proteolysis of a complement protein leads to a smaller protein and a peptide. The smaller protein remains bound to the complex at the surface of the microorganism, while the peptide diffuses away leading to destroying the pathogen and eliminating the infection. Although the complement system is known as part of innate immunity, the adaptive immune system also can be recruited.[1,2] Complement has a central role in host defense and at the same time should be regulated precisely, otherwise overactivated complement can lead to injury in different tissues.[3] The alternative pathway is continuously activated at a low level, as a result of spontaneous C3 hydrolysis due to the breakdown of the internal bond. This pathway does not rely on pathogen-binding antibodies like the other pathways and is regulated by several membrane-bound and fluid-phase proteins. Among them, factor H is a plasma regulator that restricts the activity of the C3 convertase C3bBb both on the cell surface and in the fluid phase.[4-6] We report a case of primary glomerulonephritis with isolated C3 deposits which shares known mutations of hemolytic uremic syndrome (HUS).

CASE REPORT

A 44-year-old woman with no relevant past

medical history presented with hematuria and subnephrotic proteinuria (1200 mg/24 h) detected in urinalysis incidentally. Physical examination did not reveal peripheral lymphadenopathy, hepatosplenomegaly, or edema. Because of decreased kidney function with a serum creatinine of 2.5 mg/dL (estimated glomerular filtration rate, 25 mL/min/1.73 m^2) and normal-sized kidneys on ultrasonography, a renal biopsy was taken. Light microscopic examination revealed 15 glomeruli with mild mesangial matrix expansion and segmental hypercellularity, mild glomerular basement membrane thickening and endocapillary hypercellularity. Immunofluorescence findings were negative for immunoglobulin A, immunoglobulin G, C1q, C4c, fibrinogen, and albumin, but there were strongly positive depositions of C3c along the glomerular basement membrane and mesengium, consistent with membranoproliferative glomerulonephritis (MPGN). Kappa and lambda light chain staining was uniformly negative (Figure 1).

According to the result of kidney biopsy additional workup was done, the results of which are summarized in Table 1. Prednisolone, 60 mg/d, was started. In the next follow-up visit, partial remission was achieved and corticosteroid was tapered to 20 mg/d after 6 months during which serum creatinine reached to 1.2 mg/dL and proteinuria dropped to 800 mg/d. A few months later, the patient experienced disease progression and was admitted with 2250 mg/d of proteinuria accompanied by rising serum creatinine up to 3.2 mg/dL. On this admission, a second renal biopsy was taken (Figure 2). This time according to dominant C3c deposition along glomerular basement membrane in immunofluorescence microscopic findings and diffuse extracapillary proliferative glomerulonephritis with MPGN pattern, the patient was diagnosed with C3 glomerulopathy versus dense deposition disease and biopsy specimen was sent for electron microscopy study, results of which were consistent with C3 glomerulonephritis. (Figure 3).

Table 1. Secondary Workup Results

Parameter	Result
C3	0.4 g/L (0.85 g/l to 1.85 g/L)
C4	Normal
CH50	Normal
Serum levels of factor H	Normal
Serum levels of factor I	Normal
Serum levels of factor B	120 µg/mL (170 µg/mL to 258 µg/mL)
ANA, Anti-dsDNA, ANCA -C, ANCA-P	Normal
HIV-Ab, HBs Ag, HCV-PCR, HBV-DNA PCR	Negative
Total CD46 expression by flowcytometry	Normal
Serum protein electrophoresis	Normal

Genomic DNA was extracted from patient's peripheral blood cells, using a standard phenol-chloroform protocol. All 22 coding exons of the *CFH* gene, including intron-exon boundaries, were amplified by polymerase chain reaction,

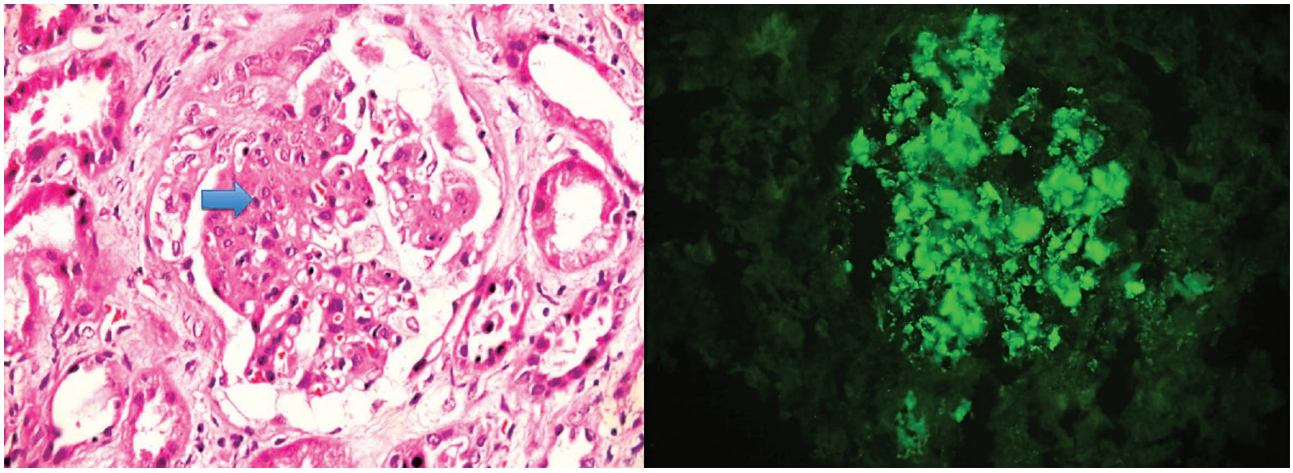

Figure 1. Light microscopy. Left, Mesangial hypercellularity (arrow), glomerular basement membrane thickening; some of the tubules showed resorptive changes and contained erythrocytes and casts. Interstitial fibrosis and tubular atrophy was seen in 10% of cortical area and mild hyaline arteriolopathy was noticeable with no evidence of vasculitis. Right, Deposition of C3c along glomerular basement membrane and mesengium.

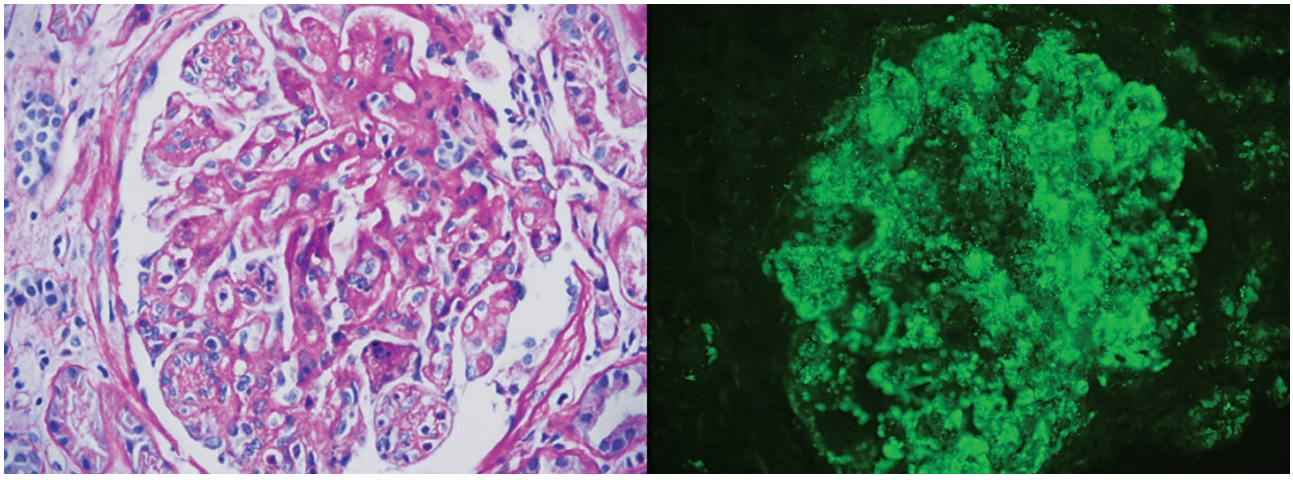

Figure 2. Light microscopy findings including diffuse extracapillary proliferative glomerulonephritis with membranoproliferative glomerulonephritis pattern in the second renal biopsy and dominant C3c deposits along glomerular basement membrane and mesengium.

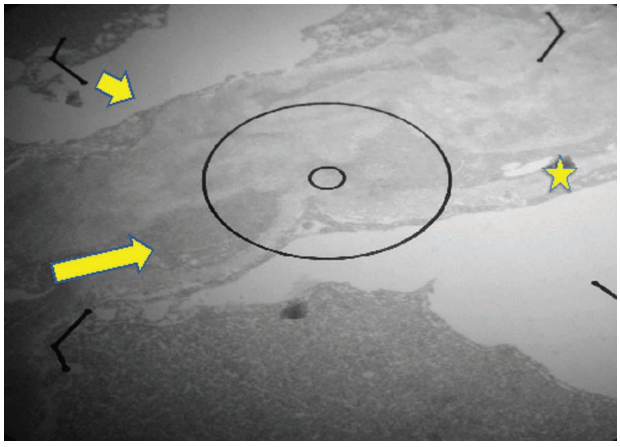

Figure 3. Electron microscopy findings of renal biopsy. Diffuse foot process effacement (short arrow), GM thickening and multilayering plus sub endothelial dense deposits (long arrow) with no intramembranous depositions (asterisk) consistent with the diagnosis of C3 glomerulopathy.

utilizing the primers listed in Table 2. Single-strand sequencing was performed using standard ABI3730 system (Applied Biosystems, Macrogen, South Korea) with both forward and reverse primers. Sequencing results were analyzed using Chromas version 2.4.1 software, and were aligned to the published template (ENST00000367429) using Clustal Omega software (EMBL-EBI). Results showed a splice site mutation (IVS9-3 T > C) in the intron spanning the start of exon 9 in heterozygote state (Figures 4A and 4B). Also it was associated respectively with a known homozygote mutation (c.1204C > T; p. H402Y), and a novel heterozygote variation (c.1207G > C; p.G403R), in the exon 9 of the *CFH* gene (Figures 4A and 4C). It interprets

Table 2. Primer Pairs for *CFH*

Primer Name	Primer Sequence	Product Size, bp
CFH-1F	ACCAGCTGCTGATTTGCAC	534
CFH-1R	TCAACAATGTCAAAAGCCACTC	534
CFH-2F	AGGTGTTTTTCCACAGTGAACAT	946
CFH-2R	GGATGACCACCCCTTTTTG	946
CFH-3F	TTCAAAAAGGGGTGGTCATC	564
CFH-3R	GCATACTGTTTTCCCACTCTCC	564
CFH-4F	TCAGTCCATGCACCAAGAAG	564
CFH-4R	GCATTCGTTTTTGGCTTTTG	564
CFH-5F	TTTCATTGTCCACTCCCATAGA	680
CFH-5R	TGATAATCCAGGGCATGTGA	680
CFH-6F	GCGGTCAAGTCAAAACAGAAC	661
CFH-6R	CTGGGCATTAGTGGAGCAAT	661
CFH-7F	TGAGAACATTGCCAGTTATGG	603
CFH-7R	TTTCGGCAACTTCGAAAACT	603
CFH-8F	CCTAGAAACCCTAATGGAATGTG	1075
CFH-9R	GGTCCATTGGTAAAACAAGGTG	1075
CFH-10F	TGTCTTTGGCAACTCTGAGC	558
CFH-10R	AGGAAAAGCCTGAATGGAAAG	558
CFH-11F	TTTTGGGGCTTAAGCAATGA	612
CFH-11R	GGTAGGTGGCCCATAGGAAG	612
CFH-12F	TCTGATGCCCCTCTGTATGA	508
CFH-12R	TGGGAATACTTCCAGCACATC	508
CFH-13F	ACCATTCTTGATTGTTTAGGATGC	728
CFH-14R	GACTGGAAATGTTGAGGCATATC	728
CFH-15F	TTGATGCAATGTGATCAGGAA	620
CFH-15R	AAGCACTGGTCCTCCAACAG	620
CFH-16F	TGAGAATACAAGCCAAAAGTTCT	1159
CFH-17R	CTCAATTATTCCCCTCACTTTG	1159
CFH-18F	CACCAGAAGGCTAGTTTTAGGAA	599
CFH-18R	TGTGCCCTAAGAACAGAGATGA	599
CFH-19F	CACCATCGTGAGAACAGCAT	797
CFH-19R	ATGTCACCATGCCCCACTAT	797
CFH-20F	TTCGTTTACACTGGCTTCCA	654
CFH-20R	TTTGGTCGAATCTTTCTGGA	654
CFH-21F	CCTGGTCTACCATAAGCAGCA	687
CFH-21R	GTTAGCCAGGATGGTCCTCA	687
CFH-22F	CGCAGGGATCCTAAAATGAC	628
CFH-22R	AAGAAGAGAGCCACCGGTCT	628

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CFH(8-9)          GCAAATTTATGTTTCTCATTTACTTTATTTATTTATCATTGTTATGGTCCTTAGGAAAAT
F135,CFH8-9       GCAAATTTATGTTTCTCATTTACTTTATTTATTTATCATTGTTATGGTCCTCAGGAAAAT
                  ************************************************ ********

CFH(8-9)          GTTATTTTCCTTATTTGGAAAATGGATATAATCAAAATCATGGAAGAAAGTTTGTACAGG
F135,CFH8-9       GTTATTTTCCTTATTTGGAAAATGGATATAATCAAAATTATCGAAGAAAGTTTGTACAGG
                  ************************************ ** ******************

CFH(8-9)          GTAAATCTATAGACGTTGCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAG
F135,CFH8-9       GTAAATCTATAGACGTTGCCTGCCATCCTGGCTACGCTCTTCCAAAAGCGCAGACCACAG
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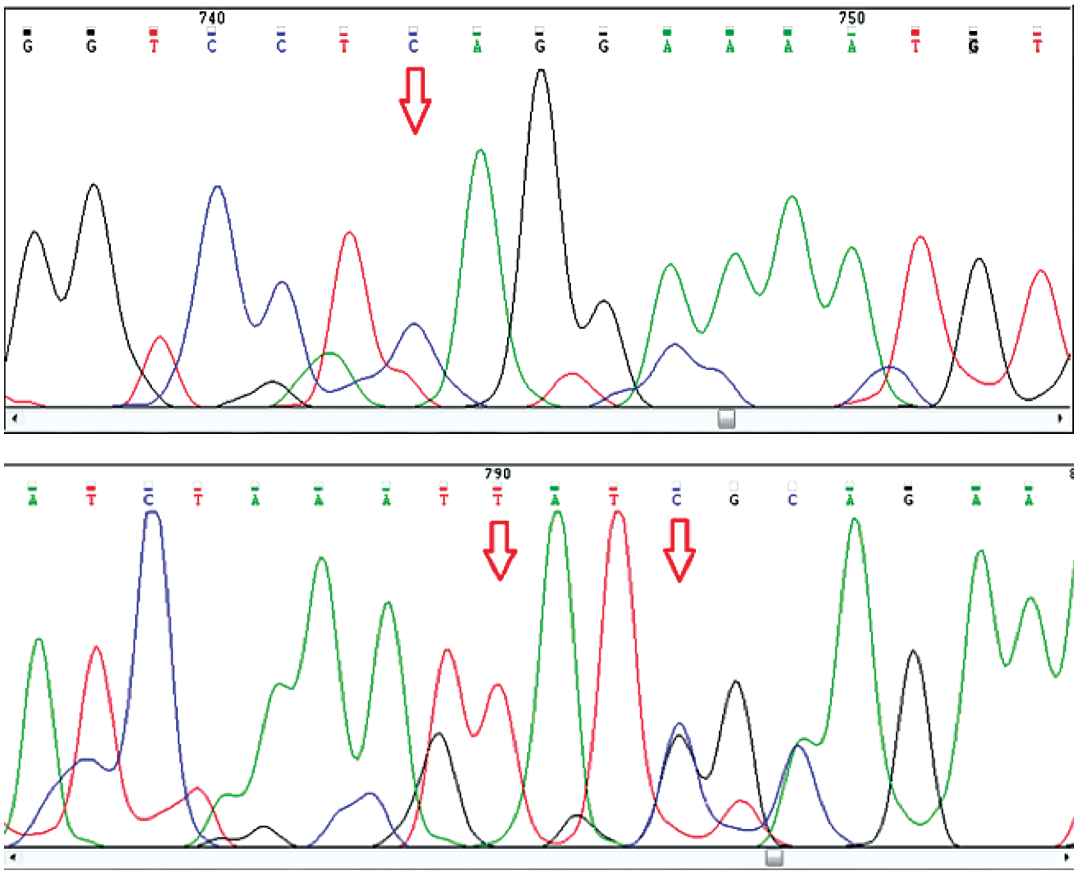

Figure 4. Analysis of CFH exon 9 sequencing. Top, Aligned sequence of the patient with published template (ENST00000367429) in Clustal Omega software. The yellowish trinucleotide shows the starting of exon 9. Middle and Bottom, Corresponding chromatogram (Chromas, V2.4.1) for the region containing alterations. Red arrows show the substituted nucleotide. A splice site mutation (IVS9-3 T > C) has been shown in the intron spanning the start of exon 9 in heterozygote state. Also it was associated with a homozygote mutation (c.1204C > T), and a heterozygote variation (c.1207G > C), in the exon 9 of *CFH* gene.

the patient's involvement to a kind of atypical hemolytic uremic syndrome/C3 glomerulopathy.

Predicted 3D structure models of the *CFH* native (Figure 5) and mutant proteins encoded by wild type, and c.1204C > T (p. H402Y) (Figure 6) and c.1207G > C (p. G403R) (Figure 7) variations, are shown, respectively. Although these mutants have high similarity with the native structures, a secondary structure transformation from alpha helix to beta indicates a major and significant *CFH* structural variation in comparison with wild type.

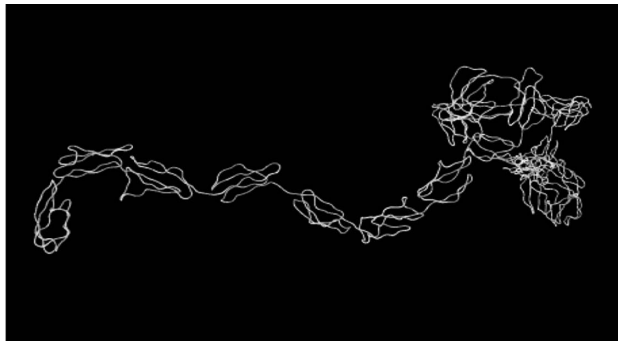

Figure 5. Tertiary structure of a native factor H.

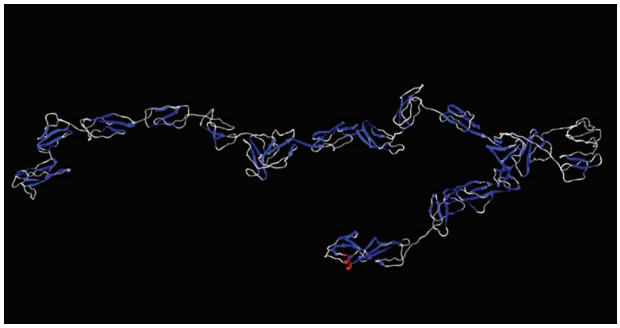

Figure 6. Predicted 3D structure model of mutant protein encoded by c.1204C > T (p. H402Y) variation of complement factor H.

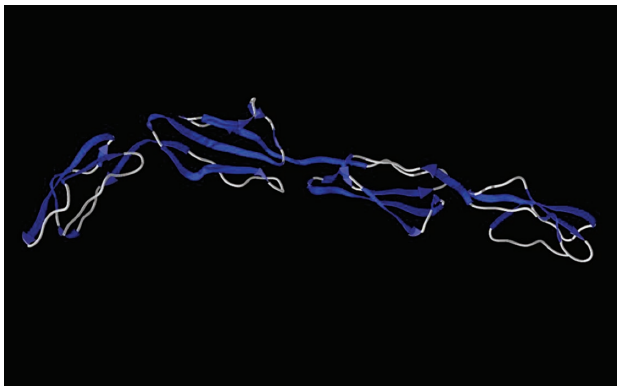

Figure 7. Predicted 3D structure model of the mutant protein encoded by c.1207G > C (p. G403R) variation of complement factor H.

DISCUSSION

Hemolytic uremic syndrome and C3 glomerulonephritis share common genetic risk factors. Constitutional or acquired dysregulation of the complement alternative pathway is probably associated with a wide spectrum of diseases, ranging from HUS to C3 glomerulonephritis or MPGN. Dense deposit disease (DDD) and C3 glomerulonephritis are rare forms of glomerulonephritis that affect both children and young adults. Like DDD, C3 glomerulonephritis is characterized by isolated deposits of C3 on immunofluorescence, but instead of dense intramembranous deposits as in DDD, electron microscopy reveals subendothelial and mesangial electron-dense deposits. In patients with DDD and C3 glomerulonephritis, the activity of C3 convertase can be increased by one or both of the following mechanisms: Generation of a C3 convertase stabilizing autoantibody called C3 nephritic factor, usually of the immunoglobulin G class, or loss of functional factor H activity. The reason why some individuals with homozygous or heterozygous factor H deficiency develop HUS or MPGN, and why some heterozygous factor H-deficient people remains free of apparent disease, is still unclear. The CFH has two terminals, a C terminal (the surface binding end) and an N terminal (the C3 regulatory end). The mutation or deficiency of the C terminal end leads to atypical hemolytic uremic syndrome while that of the N terminal end leads to C3 glomerulopathy.

In HUS, heterozygous factor H mutations have been described in the C-terminal of this factor that may produce normal antigenic levels of factor H with no alternative pathway activation. However, there are healthy factor H-deficient subjects as well as healthy mutated factor H carriers, which indicates other genetic or environmental factors as additional culprits in the initiation or the progression of the disease.

If immunofluorescence studies show predominantly C3 and negative or lower intensity Ig deposits, study of the alternative pathway of complement should be done. Low C3 and normal C4 serum levels also point toward alternative pathway dysfunction. If these tests are positive, mutation screening of complement genes and assays for autoantibodies to complement regulating proteins should be done. Evaluating the alternative pathway in C3 glomerulonephritis is important because it may change the treatment options. The presence of autoantibodies may suggest a role for rituximab, and in cases where uncontrolled terminal complement activity is detected with no autoantibodies; complement-inhibiting drugs like eculizumab may be more beneficial.

This report emphasizes the variability of the kidney diseases progression with factor H deficiencies. Based on ACMG criteria, p.H402Y is classified as a benign variation in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/variation/294490/). However, association of this variant with atypical hemolytic uremic syndrome, mesangiocapillary glomerulonephritis (type II), and macular degeneration has been also reported once each.

Kidney biopsy showed a predominantly MPGN pattern of injury, although crescentic glomerulonephritis were also present. Genetic analysis showed that the molecular abnormalities of factor H malfunctions are polymorphous and not restricted to the C-terminal domains of the

protein. Studies comparing the H402 and Y402 variants of factor H have shown that the former is associated with poorer complement system control secondary to decreased binding to both endothelial cells and lipid peroxidation products like malondialdehyde, which accumulates in many pathophysiological processes.[8]

CONFLCIT OF INTEREST

None declared.

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Correspondence to:
Pedram Ahmadpoor, MD
Department of Nephrology, Labbafinejad Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
E-mail: pedram.ahmadpoor@gmail.com

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