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Association of a Novel Complement Factor H Mutation With Severe Crescentic and Necrotizing Glomerulonephritis

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Abstract

Severe crescentic and necrotizing glomerulonephritis is typically associated with anti-glomerular basement membrane (anti-GBM) antibodies or anti-neutrophil cytoplasmic antibodies (ANCA). In this report, we describe a 23-year old man with severe crescentic and necrotizing glomerulonephritis. Both anti-GBM and ANCA titers were negative. Kidney biopsy showed bright C3 staining in the mesangium and along capillary walls and no staining for immunoglobulins. Electron microscopy showed waxy deposits (many mesangial; few intramembranous or subendothelial), prompting an evaluation of the alternative pathway of complement. alternative pathway evaluation revealed a novel mutation in short consensus repeat (SCR) 19 of complement factor H (CFH). In addition, the patient carried CFH and C3 risk alleles. Prompt treatment with intravenous steroids followed by oral steroids resulted in symptom alleviation and improved kidney function. This case shows what is to our knowledge a unique and previously unpublished cause of severe crescentic and necrotizing glomerulonephritis. Furthermore, the case demonstrates an expanding spectrum of complement-mediated glomerulonephritis and shows that crescentic and necrotizing glomerulonephritis with solely complement deposits should be evaluated for abnormalities in the alternative pathway of complement.

Crescentic and necrotizing glomerulonephritis (GN) is the most severe form of kidney injury. In the majority of cases the pathologic process is due to injury resulting from circulating anti-glomerular basement membrane (anti-GBM) antibodies, immune complex deposition, or anti-neutrophil cytoplasmic antibodies (ANCA). These forms of

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glomerulonephritis are often classified as type I, type II, and type III (pauci-immune crescentic GN), respectively.(1) Immune-complex mediated GN with crescents include entities such as lupus nephritis and IgA nephropathy.

In this manuscript we report the case of a patient with severe crescentic and necrotizing GN associated with a novel mutation in the complement factor H gene (*CFH*), a key component in the regulation of the alternative pathway of complement.(2) We discuss the implications of these findings and suggest evaluation of the alternative pathway in certain cases of crescentic and necrotizing GN.

CASE REPORT

A 23-year old previously healthy man presented with myalgias, loss of appetite, vomiting, fever and joint pains following extensive physical exertion. He noted that his urine was brownish-red. He denied a sore throat, skin rash or dysuria. He also had not experienced any chest pain, syncope, or hemoptysis. Initial examination revealed a fever of 99°F, blood pressure 130/75, pulse 76/minute, and respiration rate of 20/minute. Cardiovascular, lung, and neurological examinations were unremarkable. He was active as a player on a college football team, and used non-steroidal analgesic drugs (NSAIDs) prior to and after games. There was no family history of kidney disease. Initial laboratory evaluation showed a serum creatinine of 1.8 mg/dL (159.12 μ mol/L) corresponding to an eGFR of 51 ml/min/1.73m² (.85 mL/s/1.73m²) as determined using the Modification of Diet in Renal Disease (MDRD) Study equation with no schistocytes on peripheral smear. Urinalysis was significant for hematuria. The laboratory data are shown in table 1. The clinical impression was that of acute tubular necrosis secondary to dehydration or rhabdomyolysis, acute interstitial nephritis due to NSAID use, and a post-infectious GN due to the active urinary sediment. A kidney biopsy was performed to determine the cause of the kidney failure and the active urinary sediment.

Twenty glomeruli were present for light microscopic evaluation, one of which was globally sclerosed. Two glomeruli showed segmental scars with adhesion of the scarred segments to the Bowman's capsule. Nine of the remaining 17 glomeruli showed large circumferential cellular crescents (Figure 1A and B), with many areas of segmental fibrinoid necrosis (necrotizing lesions). Few intracapillary neutrophils were also noted, but most of the glomeruli not within the region of the crescents or necrosis appeared unremarkable and did not show any proliferative or exudative features. There was no interstitial inflammation present. Tubules contained RBCs and RBC casts. There was no significant tubular atrophy or interstitial fibrosis present. Vessels were unremarkable. Immunofluorescence studies revealed 7 glomeruli, one of which was globally sclerosed. The glomeruli showed bright granular 3+ mesangial and capillary wall staining for C3 (Figure 1C). In the glomeruli, IgG, IgA, IgM, C1q, kappa and lambda light chains were undetectable. Electron microscopy revealed mesangial and paramesangial electron dense deposits (Figure 1E). A few subepithelial "hump-like" deposits (Figure 1F) and a few small subendothelial and intramembranous deposits were also present (Figure 1G). Sausage-shaped large electron dense deposits were not present along the glomerular basement membranes. Based on these

findings, the initial kidney biopsy diagnosis was of a post-infectious GN, with crescents and necrosis.

A search for an infectious etiology found no indication of a triggering event of this nature. Multiple blood and urine cultures gave negative results. Anti-streptolysin O titers were not elevated. ANCA and anti-GBM titers were negative. Due to the severe crescentic and necrotizing lesions on the kidney biopsy, the patient was initially treated with bolus intravenous steroids for 3 days. He was then switched to oral prednisone and the dose was tapered down to 20 mg/day over 6 weeks. His serum creatinine returned to 1.2 mg/dL (106.08 $\mu\text{mol/L}$) corresponding to an eGFR $> 60 \text{ mL/min/1.73 m}^2$ ($> 1 \text{ mL/s/1.73 m}^2$). However, when the prednisone was stopped the symptoms, including back pain, returned and the patient started feeling worse. The serum creatinine started to rise again and urinalysis showed hematuria. The patient was restarted on oral prednisone. He also developed hypertension for which he was started on amlodipine. At this time he was referred to the Mayo Clinic.

A review of the biopsy showed some features that were atypical for postinfectious GN, but instead were suggestive of a unique and severe crescentic and necrotizing GN resulting from an abnormality in the alternate pathway of complement (alternative pathway).(3) These included the following: 1) Many glomeruli did not show endocapillary proliferation or mesangial proliferation; 2) Immunofluorescence studies showed bright 3+ staining for C3 in the mesangium and along capillary walls and the absence of immunoglobulin staining; and 3) Many mesangial and paramesangial deposits, few subendothelial and subepithelial deposits, and few waxy intramembranous deposits were noted on electron microscopy. In our experience, the strong 3+ staining for C3, absence of immunoglobulin staining, intramembranous and occasional subepithelial deposits are good markers for GN resulting from dysregulation of the alternative pathway. Thus, the alternative pathway was evaluated and this led to rather surprising results.

Direct sequence analysis of the coding region of *CFH* including analysis of intron/exon boundaries revealed a heterozygous single-nucleotide polymorphism, a guanine to adenine change at nucleotide 3,350 of the *CFH* complementary DNA (c.3350A>G; corresponding to an asparagine to serine change at amino acid 1,117 [p.Asn1117Ser]), which occurs in short consensus repeat (SCR) 19 (figure 2). This substitution has, to our knowledge, not been previously described. The consequence score is 5 exposed (1, low; 9, high) and PolyPhen, a tool that predicts the potential effects of an amino acid substitution on a protein of interest (available at genetics.bwh.harvard.edu/pph/), suggests that this change is possibly damaging. In addition, risk alleles that were identified included 2 copies of the *CFH* risk polymorphism H402 (reference single-nucleotide polymorphism (rs) number 1061170; corresponding to a tyrosine to histidine change at amino acid 402 in SCR7), two copies of the C3 risk allele G102 (an arginine to glycine substitution at amino acid 102), and 1 copy of the C3 risk allele L314 (a proline to leucine substitution at amino acid 314). The *CFH* risk allele I62 (rs800292), by contrast, was not present. Moreover, sequence analysis of the genes for complement factors B (*CFB*) and I (*CFI*), membrane cofactor protein (*CD46*), *CFH*-related protein 5 (*CFHR5*), and the *CFHR3-CFHR1* region (by multiplex ligation-dependent probe amplification) revealed the patient was homozygous for the wild-type alleles. Antibodies to

complement regulating proteins, including C3 nephritic factor (C3NeF), CFH, and CFB, were also undetectable (table 2).

We performed laser microdissection and mass spectrometry to determine the glomerular proteomic profile. (4) There was extensive deposition of fibrinogen α , fibrinogen β , and fibrinogen γ , consistent with crescentic and necrotizing GN. There was also deposition of C3 and CFHR1, and less intense spectra corresponding to proteins identified with lower confidence as C9 and CFHR5 (figure 3). There was no evidence suggesting accumulation of complement factors of the classical complement pathway, such as C1, C2 or C4. In addition, there was no immunoglobulin present.

As a result of these additional investigations, the diagnosis was refined to crescentic and necrotizing GN, severe, associated with dysregulation of the alternative pathway of complement resulting from a mutation and a permissive background in *CFH* and *C3*.

At time of writing, one year after initial presentation, the patient is well and asymptomatic. His serum creatinine is stable at 1.2 mg/dL (eGFR > 60 ml/minute) and he has been maintained on prednisone (15 mg/day) for the last 6 months. Attempts to reduce the dosage have resulted in a return of symptoms.

Discussion

We report a case of young man with a severe crescentic and necrotizing GN associated with a novel mutation in *CFH*, the most important regulator of the alternative complement pathway. Mutations in *CFH* result in dysregulation and uncontrolled activation of the alternative pathway, causing deposition of activated complement factors and complement degradation products in the glomeruli, ultimately leading to proliferative GN.(2) Based on electron microscopy, such lesions are classified as either Dense Deposit Disease (DDD) or C3 GN. (3, 5, 6) In both DDD and C3 GN, the underlying lesion is typically one of a proliferative GN, such as mesangial, endocapillary, or membranoproliferative GN. Crescents and necrotizing lesions can also be present, but the predominant lesion is that of a proliferative GN.(7, 8) Our case was extremely unusual in that the kidney biopsy showed a severe crescentic and necrotizing GN with no significant mesangial or membranoproliferative features. It is likely that the lesion developed acutely with no time for progression and development of mesangial or membranoproliferative features. Treatment with intravenous high-dose steroids followed by oral low-dose steroids for maintenance controlled the disease process by both alleviating symptoms and improving kidney function.

Immunofluorescence microscopy revealed bright C3 staining in the mesangium and along capillary walls and complete absence of Ig staining. In addition, small waxy intramembranous, subendothelial, and subepithelial deposits suggested complement deposits (as opposed to Ig-containing deposits, which appear darker and more sharply demarcated). These findings prompted assessment of the alternative pathway, which to our surprise revealed a polymorphism in the sequence corresponding to SCR19 of CFH (Figure 2), an extremely unusual finding in the setting of crescentic and necrotizing GN since mutations in SCR19 have not been previously described to cause C3 GN or DDD. Evaluation also

revealed 2 copies of the H402 allele of *CFH*, and in *C3*, 2 copies of the G102 allele and 1 copy of the L314 allele. These variants of *CFH* and *C3* are causally associated with increased baseline alternative pathway activity even in healthy controls and are linked to both *C3* GN and DDD.(9–11)

CFH has 20 SCRs, each of which is about 60 amino acids long. SCRs 1–4 are at the amino terminus, bind *C3b*, and are important for fluid-phase regulation of the *C3* convertase. Mutations in these domains have been reported in DDD and *C3* GN. (5, 6) SCRs 19–20 at the carboxy terminus are essential for *CFH* binding to host surfaces, and also interact with the *C3b* degradation products *iC3b* and *C3d*.(12) The importance of these two SCRs has been verified in experiments using a recombinant protein containing only SCR 19–20 of *CFH*; this truncated protein competes with full-length *CFH* for *C3b* binding sites and host polyanions, resulting in complement activation on host surfaces in vitro. (13) Mutations in SCR19–20 are causally associated with atypical hemolytic uremic syndrome (aHUS) (14, 15) but have not been reported in the setting of a crescentic and necrotizing GN. Thus, this case demonstrates a new pattern/manifestation of severe injury, i.e., crescentic and necrotizing GN, associated with a polymorphism in SCR19 of *CFH* and, we suggest, a permissive environment resulting from the presence of the H402 variant of *CFH* and the G102 and L314 variants of *C3*.

Severe crescentic and necrotizing GN in the absence of significant mesangial or membranoproliferative features is typically seen in anti-glomerular basement membrane GN and ANCA-associated pauci-immune GN. At the same time, severe crescentic and necrotizing GN due to a *CFH* mutation has not been reported. To our knowledge this is the first case to link a severe crescentic and necrotizing GN to a *CFH* mutation. This also expands the spectrum of kidney lesions that occur due to abnormalities of the alternative pathway. Although this case could fall under the umbrella of *C3* glomerulopathy and *C3* GN (given the bright *C3* staining on immunofluorescence studies and mesangial and capillary wall deposits on electron microscopy), the morphologic pattern of severe injury, i.e., crescentic and necrotizing GN, is extremely unusual. Laser microdissection–mass spectrometry data was consistent with a necrotizing GN associated with complement deposition because large signals corresponding to fibrinogen and *C3* were noted in the glomeruli along with the absence of any detectable immunoglobulin deposition (figure 3).

It is possible that some cases currently labeled as pauci-immune crescentic and necrotizing GN may be a result of alternative pathway dysfunction, particularly those belonging to the 15% that are ANCA-negative. The finding of glomerular *C3* staining (in the absence of Ig staining) is sometimes dismissed as non-specific in the setting of a pauci-immune crescentic and necrotizing GN. Based on the case described in this report, we postulate that some of these cases may be associated with alternative pathway dysfunction.

Finally, this case is noteworthy because aHUS, DDD, and *C3* GN are all complement-mediated diseases. The p.Asn117Ser mutation were described herein lies in SCR19, a commonly mutated SCR in aHUS (Figure 2), but the resulting phenotype in this case was severe crescentic and necrotizing GN and not aHUS, suggesting that other modifying genetic changes may be present in this person. Distinguishing DDD and *C3* GN at the

genetic and biomarker levels is more challenging, but again, this case offers some insights. Fluid-phase dysregulation of the C3 convertase alone is sufficient to result in DDD as Martínez-Barricarte and colleagues demonstrated in a small nuclear family in which a mother and her two identical twin boys segregated a two amino-acid deletion in the MG7 domain of C3 (the loss of the aspartate and glycine residues at amino acids 923 and 924, respectively).(16) The association of severe crescentic and necrotizing GN with a polymorphism in SCR19 of *CFH* suggests that dysregulation of the C5 convertase may also be important in some cases of complement-mediated GN.(17)

To summarize we present a case of severe crescentic and necrotizing GN associated with a novel mutation in *CFH* and the presence of 2 copies of the H402 allele of *CFH* and 2 copies of the G102 and 1 copy of the L314 alleles of *C3*. A crescentic and necrotizing GN with extensive glomerular C3 staining on immunofluorescence studies warrants an in depth evaluation of the alternative pathway.

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Figure 1. Kidney biopsy findings

(A) Low power view of 4 glomeruli, 3 with crescents and 1 unremarkable (Jones stain 10×). (B) High power image of a glomerulus with a large circumferential crescent and necrotizing lesion. Note absence of endocapillary proliferation, or mesangial or membranoproliferative features (Jones stain, 40×). Immunofluorescence studies reveal (C) bright 3+ staining for C3 in the mesangium and along capillary walls (40×), and (D) bright fibrinogen staining in the glomerular tufts indicating fibrinoid necrosis (necrotizing lesion). Electron microscopy reveals (E) mesangial and paramesangial deposits with a fuzzy/waxy character (black arrows point to deposits) (5800×); (F) a subepithelial hump (7400×); and (G) few subendothelial (white arrow) and intramembranous deposits (black arrow)(7400×).

Figure 2. Schematic of complement factor H (CFH) and relevant mutations

CFH contains 20 short consensus repeats (SCRs; indicated by circles). SCR19, the location of the polymorphism described in this case, is shown with an arrow. Dark blue circles represent C3b binding sites (SCR 1–4, SCR 7–15, SCR 19–20). Mutations in SCR1–4 are usually associated with dense deposit disease/C3 glomerulonephritis (DDD/C3 GN), while mutations in SCR 19–20 are associated with atypical hemolytic uremic syndrome (aHUS). Some cases of DDD/C3 GN have also reported in association with mutations in SCR 7–15.

			Probability Legend:		Accession Number	Molecular Weight	Protein Grouping Ambiguity	PreBlank	Sample 1	Sample 2							
			over 95%	80% to 94%													
			50% to 79%	20% to 49%													
			0% to 19%														
Bio View:																	
Identified Proteins (256)																	
#	Visible?	Starred?															
1	☒	☒	Fibrinogen alpha chain OS=Homo sapiens GN=FGA PE=1 SV=2 (sp) (... FIBA_HUMAN								95 kDa		36	57			
2	☒	☒	Fibrinogen beta chain OS=Homo sapiens GN=FGB PE=1 SV=2 (sp) (P... FIBB_HUMAN								56 kDa		27	57			
3	☒	☒	Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3 (sp)... FIBG_HUMAN								52 kDa		25	54			
4	☒	☒	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2 (sp) (P01024) CO3_HUMAN								187 kDa		8	4			
5	☒	☒	Complement factor H-related protein 1 OS=Homo sapiens GN=CFHR... FHR1_HU...								38 kDa	★	4	2			
6	☒	☒	Complement component C9 OS=Homo sapiens GN=C9 PE=1 SV=2 (s... CO9_HUMAN								63 kDa			2			
7	☒	☒	Complement factor H-related protein 5 OS=Homo sapiens GN=CFHR... FHR5_HU...								64 kDa	★		1			
8	☒	☒	Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1 (sp) (P... ACTB_HU...								42 kDa	★		52	43		
9	☒	☒	Uncharacterized protein KIAA0552 OS=Homo sapiens GN=KIAA0552 ...K0552_HU...								72 kDa		2	2	6		
10	☒	☒	(CONTAMINANT) Keratin 1 [Homo sapiens] CONTAM_...								66 kDa	★		65			
11	☒	☒	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV...								62 kDa	★		50			
12	☒	☒	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4 (sp) (P08670) VIME_HUM...								54 kDa	★		26	20		
13	☒	☒	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2 (sp) (P02768) ALBU_HU...								69 kDa			28	12		
14	☒	☒	Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2 (sp)...HBB_HUMAN								16 kDa	★		19	12		
15	☒	☒	Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2 (... HBA_HUMAN								15 kDa			8	11		
16	☒	☒	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4 (sp) (P35579) MYH9_HU...								227 kDa			9	8		
17	☒	☒	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2 (sp) (O43... ACTN4_H...								105 kDa			13	4		
18	☒	☒	(CONTAMINANT) Keratin 10 [Homo sapiens] CONTAM_...								59 kDa	★		17			
19	☒	☒	Katanin p60 ATPase-containing subunit A-like 1 OS=Homo sapiens G... KATL1_HU...								55 kDa			5	2		

Figure 3. Laser microdissection and mass spectrometry

(A) glomeruli marked for microdissection. (B) The top 19 proteins detected by mass spectrometry. The proteomic data show accumulation of fibrinogen α , β , and γ , C3, and CFHR1 (yellow-starred records) based on >95% probability of a positive match of mass spectrometric data with protein databases. Lower-probably matches were seen for C9 and CFHR5.

Table 1

Laboratory evaluation

	Laboratory Results		Reference range
	at presentation	After 6 mo	
Hemoglobin (g/dL)	12.5	13.3	14–18.0
Erythrocyte sedimentation rate (mm/h)	55	10	0–22
Platelets ($\times 10^3/\mu\text{L}$)	275	309	150–400
WBC ($\times 10^9/\text{L}$)	13.1	9.7	3.5–10.5
Albumin (g/dL)	3.6	4.1	3.5–4.8
Creatinine phosphokinase (U/L)	58		49–397
Sodium (mEq/L)	137	142	136–144
Potassium (mEq/L)	4.2	4.5	3.6–5.1
Chloride (mEq/L)	99		99–109
Carbon Dioxide (mEq/L)	29		22–32
Serum urea nitrogen (mg/dL)	12	19	4–20
Creatinine (mg/dL)	1.7	1.2	0.6–1.2
eGFR* (mL/min/1.73 m ²)	51	>60	>60
C3 complement (mg/mL)	1.35	0.99	0.79–1.52
C4 complement (mg/mL)	.37	0.31	0.16–0.38
ASO titers (IU/mL)	240		0–250
Hepatitis serology	Negative		
Urinalysis	50–100 RBC/HPF	3–10 RBC/HPF	
Urinary protein (mg/24 h)		800	
Blood and urine cultures	Negative	Negative	
Cryoglobulins		Undetectable	
ANA, dsDNA		Undetectable	
ANCA, PR3 and MPO	Undetectable	Undetectable	

* eGFR calculated using Modification of Diet in Renal Disease (MDRD) Study equation

Note: Conversion factors for units: hemoglobin in g/dL to g/L, $\times 10$; albumin in g/dL to g/L $\times 10$; creatinine in mg/dL to $\mu\text{mol/L}$, $\times 88.4$; eGFR in mL/min/1.73 m² to mL/s/1.73 m² $\times 0.01667$. No conversion is necessary for erythrocyte sedimentation rate, platelets in $\times 10^3/\mu\text{L}$ and $\times 10^9/\text{L}$, WBC in $\times 10^3/\mu\text{L}$ and $\times 10^9/\text{L}$, sodium in mEq/L to mmol/L, potassium in mEq/L to mmol/L, chloride in mEq/L to mmol/L, carbon dioxide in mEq/L to mmol/L, C3 complement in mg/mL to g/L, C4 complement in mg/mL to g/L.

Abbreviations: WBC, white blood cell; eGFR, estimated glomerular filtration rate; ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; PR3, proteinase 3; MPO, myeloperoxidase; RBC, red blood cell; HPF, high-power field; dsDNA, double-stranded DNA

Table 2

Characterization of the alternative pathway via functional assays and antibody detection analysis

Complement Test	Result	Criteria for Negative/Normal Result
CFH Autoantibody	negative	Titer <1:50
Hemolytic Assay	Normal, no hemolysis detected	<3% hemolysis
Alternative Pathway Functional Assay	79.86%	Within reference range of 65%–130%
C3 Nephritic Factor		
ELISA	negative	OD < 2 SD above normal based on 50 healthy controls
Immunofixation electrophoresis	negative	<7.5%
C3 convertase stabilizing assay	negative	<20%
C3 convertase stabilizing assay with properdin	negative	<20%

Abbreviations: CFH, complement factor H; ELISA, enzyme-linked immunosorbent assay; OD, optical density; SD, standard deviation