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Familial C3 glomerulonephritis caused by a novel *CFHR5-CFHR2* fusion gene

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ABSTRACT

C3 glomerulopathy (C3G) is an ultra-rare complement-mediated renal disease characterized histologically by the predominance of C3 deposition within in the glomerulus. Familial cases of C3G are extremely uncommon and offer unique insight into the genetic drivers of complement dysregulation. In this report, we describe a patient who presented with C3G. Because a relative carried the same diagnosis, we sought an underlying genetic commonality to explain the phenotype. As part of a comprehension genetic screen, we completed multiplex ligation-dependent probe amplification across the complement factor H related region and identified amplification alterations consistent with a genomic rearrangement. Using comparative genomic hybridization, we narrowed and then cloned the rearrangement breakpoints thereby defining a novel fusion gene that is translated into a serum protein comprised of factor H related-5 (short consensus repeats 1 and 2) and factor H-related-2 (short consensus repeats 1–4). These data highlight the role of factor H related proteins in the control of complement activity and illustrate how perturbation of that control leads to C3G.

1. Introduction

C3 glomerulopathy (C3G) is an ultra-rare complement-mediated renal disease with a prevalence of ~2 per million (Medjeral-Thomas et al., 2014b). It can be diagnosed at any age (Lu et al., 2012; Medjeral-Thomas et al., 2014b; Servais et al., 2007, 2012; Sethi et al., 2012) and shows no sex predilection (Lu et al., 2012; Medjeral-Thomas et al., 2014b; Servais et al., 2007; Sethi et al., 2012). Patients typically present as nephritic nephrotics, with hematuria (~80% (Lu et al., 2012; Medjeral-Thomas et al., 2014b)), hypertension (~50% (Lu et al., 2012; Medjeral-Thomas et al., 2014b; Servais et al., 2007)), heavy proteinuria (~90% (Lu et al., 2012; Medjeral-Thomas et al., 2014b; Servais et al., 2007)), and edema (~40% (Lu et al., 2012)). In a few reported cases, retinal drusen is identified at presentation by ophthalmoscopy (Colville et al., 2003; Leys et al., 1991; Lu et al., 2012; Montes et al., 2008).

Two C3G subtypes are widely recognized, dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). These subtypes are distinct from other glomerulopathies by virtue of C3-dominant immunofluorescence (IF) in the absence, or near absence, of staining for other immunoreactants, particularly immunoglobulin (C3 staining must be at least 2 orders of magnitude greater than any other immunoreactant) (Pickering et al., 2013). DDD and C3GN can be distinguished from each other by electron microscopy (EM). In DDD, the lamina densa of the glomerular basement membrane (GBM) appears by EM to be thickened, often massively, with

Abbreviations: C3G, C3 glomerulopathy; C3GN, C3 glomerulonephritis; C3Nefs, C3 nephritic factors; DDD, dense deposit disease; EM, electron microscopy; FHR, complement factor H-related proteins; GBM, glomerular basement membrane; IF, immunofluorescence; MLPA, multiplex ligation-dependent probe amplification; RCA, regulators-of-complement-activation; SCRs, short consensus repeats.

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extremely dark, ribbon- or sausage-like discontinuous amorphous deposits (Medjeral-Thomas et al., 2014b; Pickering et al., 2013). In C3GN, by comparison, the deposits are lighter and typically located in subendothelial, subepithelial and mesangial regions (Medjeral-Thomas et al., 2014b; Servais et al., 2013). The glomerular deposits in both DDD and C3GN have been shown by laser capture with mass spectroscopy to be accumulated complement proteins and their split products (Sethi et al., 2012, 2009). This finding is consistent with the IF picture of C3 dominance and also supports the hypothesis that complement dysregulation is causally important in disease pathogenesis (Servais et al., 2013), an association further substantiated by studies demonstrating that compared to controls, C3G patients have significant abnormalities in serum biomarkers of complement activity (Zhang et al., 2014).

Drivers of complement dysregulation in C3G can be acquired or genetic. The most commonly identified acquired factors are C3 nephritic factors (C3Nefs), which are autoantibodies to the C3 convertase, C3bBb (Zhang et al., 2012). The most common genetic drivers of C3G disease are specific alleles of several complement genes that in aggregate define 'at-risk' complement haplotypes (Abrera-Abeleda et al., 2011, 2006; Servais et al., 2012). Disease-causing mutations are less often reported, although rare and novel variants are more frequently found in C3 convertase genes (*C3* and *CFB*) in C3G patients as compared to controls (Bu et al., 2015; Servais et al., 2012). Few families segregate multiple cases of C3G suggesting that single genetic factors *alone* are seldom sufficient to cause the disease and strengthening the presumption that C3G is genetically complex, with both genetic and acquired factors driving its pathogenesis.

There are, however, five well-documented familial cases of C3G associated with four different *CFHR* gene rearrangements (Chen et al., 2014; Gale et al., 2010; Malik et al., 2012; Medjeral-Thomas et al., 2014a; Tortajada et al., 2013). This paper describes the sixth familial case and the fifth *CFHR* gene rearrangement. In this family, the renal disease phenotype is associated with a novel fusion protein in which the first two short consensus repeats (SCRs) of *CFHR5* are fused to the four SCRs of *CFHR2* to render FHR-$5^{1,2}$FHR-2^{1-4} (superscripts denote SCRs). Collectively, these six familial cases strongly invoke a role for complement factor H-related proteins (FHRs) in controlling complement regulation in the glomerular microenvironment.

2. Materials and methods

2.1. Patient

C3G was diagnosed in the European-American family described in Fig. 1 by renal biopsy. The human research Institutional Review Board at the University of Iowa approved all procedures. Consents were received from all participants prior to inclusion in this study.

2.2. Complement studies

Functional assays of the complement system were completed as described (Sethi et al., 2010). Amplification and bi-directional Sanger sequencing of the coding regions and intron-exon boundaries of *CFH* (MIM#134370; NM_000186), *CFI* MIM#217030; NM_000204.3), *MCP* (MIM#120920; NM_002389.3), *CFB* (MIM#138470; NM_001710.5), *CFHR5* (MIM#608593; NM_030787.3) *C3* (MIM#120700; NM_000064.2) were completed using reported primers and conditions (Zhang et al., 2012).

2.3. Copy number variation

Copy-number variants were identified by multiplex ligation-dependent probe amplification (MLPA) using SALSA MLPA EK5-FAM kit (MRC Holland, Amsterdam, the Netherlands) and in-house designed probe pairs. Each MLPA assay met specific criteria validated by screening a subset of controls and patients in triplicate. Copy numbers in this subset were determined by comparing data to normalized peak areas generated in five control samples. A sample was considered wild type if peak area ratios were between 0.7 and 1.33. A single deletion generated ratios between 0.25 and 0.6; no copies of the target sequence generated ratios between 0 and 0.2; duplications resulted in ratios between 1.35 and 1.8. MLPA probes that did not confirm to these criteria were discarded and replaced with alternate probes. Samples were re-assayed if values were outside these parameters. All samples were studied in duplicate and unusual results were studied in triplicate.

2.4. Comparative genomic hybridization

Comparative genomic hybridization was completed using a custom-designed high-density (median resolution of 110 bp) 15k microarray (Agilent Technologies, Palo Alto, CA, USA) spanning the regulators-of-complement-activation (RCA) gene cluster region on chr1q32. DNA samples were labeled, mixed with DNA from a normal control fully characterized at the RCA gene cluster region, and hybridized to the microarray according to the manufacturer's protocols. Microarray data were extracted and visualized using Feature Extraction Software v8.1 and CGH Analytics v3.2.25 (Agilent Technologies, Valencia, Spain). Data analysis was performed using the ADM-1 algorithm, as supplied within 'Genomic Workbench Standard Edition 7.0' (Agilent Corp, Santa Clara, CA, USA).

2.5. Western blotting

10 μl of serum diluted in PBS (1:40) and denatured at 100 °C for 10 min was separated in a 10% SDS/PAGE gel at 80 V for 2½ hours and transferred to a nitrocellulous membrane. After an over-night incubation at 4 °C in 5% milk in TBST buffer (20 mM Tris-HCl, 136 mM NaCl, 0.15% Tween-20, pH 7.4), the membrane was hybridized with rabbit anti-FHR-1/-2 antibody (1:500, in-house) for 1 h and then washed x3 in TBST buffer for 45 min. After 1-h incubation with goat-anti-rabbit antibody (1:5000), the membrane was washed x3 in TBST and developed using an ECL Western Blotting Substrate Kit (Abcam Inc., Cambridge, MA, USA).

2.6. Factor H-dependent hemolytic assay

The capacity of the FHRs to impact FH-mediated control of the alternative complement pathway on cell surfaces was assessed with a hemolytic assay using guinea pig erythrocytes. In brief, guinea pig erythrocytes (TCS Biosciences, Buckingham, UK) in AP buffer (0.5% packed cell volume), veronal buffer saline (2.5 mM barbital, 1.5 mM sodium barbital, 144 mM NaCl, pH 7.4) with 5 mM $MgCl_2$, 8 mM ethylene glycol tetraacetic acid (EGTA) and 0.1% gelatin, were incubated with a sufficient amount of 10% serum (previously depleted of FHR-1, FHR-2 and FHR-5 proteins using MBC125 affinity column (Tortajada et al., 2013)) in AP buffer and increasing amounts of FH for 1 h at 37 °C. After centrifugation, supernatants were read at 414 nm. Titration of FH before each experiment determined conditions for the competition assay. The *minimal* concentration of FH giving 50% lysis was mixed with different amounts of FHR proteins to measure their capacity to compete with FH. Serum without added FH was taken as 100% lysis and ery-