Rare Variants in Complement Genes May Not Be That Rare After All

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The complement system is an ancient and conserved effector system involved in the defense against pathogens and host homeostasis, the activation of which occurs through the classical, lectin, and/or alternative pathway. The pathways converge to the C3 convertase and activation of C5, including the terminal complement pathway, with the formation of the membrane attack complex (i.e., C5b9). In the appropriate setting, complement activation leads to opsonization, inflammation, and/or lysis. The alternative pathway is continuously active through spontaneous hydrolysis of (H2O)C3. The host's endothelium is in permanent contact with the complement and, in physiological conditions, protected from harmful effects by tight regulation. Alternative pathway dysregulation and extensive formation of C5b9 on the microvascular endothelium, often related to variants in the complement genes, such as, *CFH* (factor H), *CFI* (factor I), *CD46* (membrane cofactor protein), *C3*, and *CFB* (factor B), characterize primary atypical hemolytic uremic syndrome (hereafter referred to as complement-mediated [C-]thrombotic microangiopathy [TMA]).[1] Most patients with C-TMA phenotypically present with consumptive thrombocytopenia, microangiopathic hemolytic anemia, and ischemic kidney disease. The penetrance of C-TMA, however, is incomplete in carriers of (likely) pathogenic variants in complement genes, and a second hit, such as, uncontrolled hypertension, is needed to lower the threshold for the disease to manifest. In addition, "common" variants in the complement genes, although of weaker clinical significance as compared with (likely) pathogenic variants, have been found to affect the penetrance of disease.[2]

C-TMA and, in particular, those cases related to variants in the complement genes may present as an acute and recurrent form of the disease, whereas recurrent TMA is considered rare in non-carriers.[1]

Screening for variants in the complement genes has therefore become routine clinical practice. Knowledge on genotype-phenotype correlations, indeed, is important for clinical decisions. For example, a pathogenic variant in *C3* (i.e., c.481C>T results in the replacement of arginine by tryptophan at amino acid position 161 [p.R161W] and a direct gain-of-function C3) is associated with nephrotic proteinuria and "smoldering" TMA after kidney transplantation, often limited to the donor kidney.[3] Recipients with "smoldering" TMA on the background of this particular variant may present with proteinuric kidney disease without profound hematologic abnormalities. Thus, a kidney biopsy is often needed to detect and treat the TMA at the earliest possible stage of disease, having major impact on prognosis. To date, hundreds of variants in the complement genes have been identified in patients with C-TMA.[4] These variants should be classified as (likely) pathogenic, uncertain significance (i.e., VUS), or (likely) benign according to international standards, including functional studies, clinical testing, and data on minor allele frequency among other criteria. Functional studies revealing a deleterious effect are considered "strong" but not closing evidence for (likely) pathogenicity. In this issue of *Kidney International Reports*, Nobile *et al.*[5] studied the phenotype of a variant in *CFI*, that is, c.1246A>C using the BIOMNIS databank, including 3122 patients who underwent whole exome sequencing, either for an "unknown cause of kidney disease" or not. Factor I is a serine protease that cleaves and inactivates the complement in the presence of

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cofactor proteins (e.g., factor H, membrane cofactor protein) by attenuating activation of C3 and C5 within the alternative pathway and, thus, preventing the formation of C5b9 on, for example, the microvascular endothelium. *CFI* c.1246A>C results in the replacement of isoleucine by leucine at amino acid position 416 (p.I416L) and a quantitative defect *in vitro*.[6] The authors therefore hypothesized that carriers of this particular variant are at risk for C-TMA.

Of 1340 patients with an "unknown cause of kidney disease," 13 (~1.0%) carried *CFI* c.1246A>C, all of whom identified their selves as African; data from 12 patients were available for clinical testing. Many carriers presented with (thrombotic) microangiopathy and co-existing uncontrolled hypertension, including hypertensive emergency, preeclampsia, and the syndrome of hemolysis, elevated liver enzyme levels, and low platelet count. None of the patients had been treated with therapeutic complement inhibition, and end-stage kidney disease was common. The clinical course of the disease may suggest C-TMA as the key causative factor of end-stage kidney disease.[1,7] Of note, the other 5 patients with an "unknown cause of kidney disease" did not present with (thrombotic) microangiopathy on peripheral blood smear despite co-existing (uncontrolled) hypertension. The latter patients invariably progressed to chronic rather than end-stage kidney disease, resembling the clinical course of hypertensive kidney disease in African patients who lack *APOL1* risk variants (i.e., arteriolar nephrosclerosis). Morphologic features on kidney biopsy and long-term follow-up data would have informed the phenotype of these particular patients who likely represent a mixture of distinct causes linked to "smoldering"

vasculopathies. Furthermore, data on kidney presentation and outcome measures from carriers of *CFI* c.1246A>C who underwent whole exome sequencing for reasons unknown would have further informed the phenotype.

The heterogeneous phenotype therefore questions the significance of *CFI* c.1246A>C in the pathogenesis of C-TMA. Of note, (likely) pathogenicity of genetic variants increases with rarity and, thus, a lower minor allele frequency. Therefore, variants in the complement genes with a minor allele frequency greater than or equal to 0.1% should not be considered (likely) pathogenic unless functional studies suggest otherwise.[8] Most studies that focus on genotype-phenotype correlations of variants in the complement genes have been conducted in Whites rather than Africans and therefore lack the diversity required to represent the global human population, similar to previous genome-wide association studies. Most of the human genetic diversity, however, is found in African ethnolinguistic populations. Rare genetic variants, that is, variants with a minor allele frequency of less than 0.1%, in Whites, indeed, may be common among Africans.[9] *CFI* c.1246A>C's minor allele frequency is less than 0.1% in Whites but increases to approximately 1.2% in the "healthy" African (American) population according to the Genome Aggregation Database assessed on July 1, 2023. Thus, the prevalence of this variant in the African (American) population is too high for an orphan disease such as C-TMA, with an estimated annual incidence of less than 1 per 1,000,000 persons.[4]

Functional studies linked *CFI* c.1246A>C to a quantitative defect *in vitro*, and although heterozygous patients with C-TMA may present with normal concentrations of

factor I, a subtotal factor I deficiency has been reported in a single homozygous patient with C-TMA.[6] The latter underscores this *CFI* variant's effect on factor I secretion *in vivo*, with "weaker" effects on complement regulation in heterozygous carriers. To the best of our knowledge, recurrent disease has not been reported in patients with C-TMA who carried *CFI* c.1246A>C with no other variants in the complement genes, including those classified as (likely) pathogenic or VUS. Previous studies revealed that variants in *CFI* are associated with a less severe clinical phenotype as compared with patients with variants in *CFH* and/or *C3*.[S1] Moreover, the penetrance of C-TMA in families with variants in *CFI* identified, including those associated with a quantitative defect,[S2] is rather low.[S1] We identified 8 patients with TMA and a rare variant in *CFI* ([likely] pathogenic, $n = 5$; VUS, $n = 3$), either with
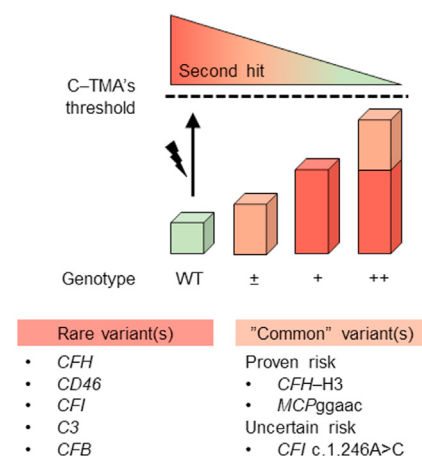
Figure 1. The multiple hit hypothesis of C-TMA. The onset of C-TMA depends on the genetic fingerprint and coexisting (or triggering) conditions. Rare variants in complement genes and, in particular, those classified as (likely) pathogenic, lower the threshold for the disease, whilst "common" variants, such as, *CFH*-H3, *MCP*ggaac, and (perhaps) *CFI* c.1,246A>C, may affect the penetrance of disease. The green bar, orange bars, and red bars indicate wild type (WT), "common" variants in complement genes, and rare variants in complement genes, respectively.

co-existing conditions or not; recurrent TMA was noted in 2 patients, both of whom had other variants identified. These observations suggest that *CFI* c.1246A>C may somewhat lower the threshold for (unrestrained) complement activation on the endothelium and, thus, be an additional risk factor for C-TMA to manifest rather than the cause of disease (Figure 1), akin to the at-risk haplotypes *CFH*-H3 and *MCP*ggaac.[2] Yet, this premise remains to be studied and, in particular, in carriers of other variants in complement genes who, *per definition*, are at risk of recurrent disease. Similar studies are needed for other "common" variants, including but not limited to *C3* c.463A>C that results in the replacement of a lysine by glutamine at amino acid position 155 and a direct gain-of-function in C3.[S3]

Altogether, when classifying variants in the complement genes, the minor allele frequency in non-White populations should be accounted for. Nobile *et al.*[5] provide important data on *CFI* c.1246A>C's phenotype and, in our opinion, underscore the need for exome and genome sequencing of diverse populations, which is essential for the correct interpretation of genetic variants linked to C-TMA and other hereditary (kidney) diseases with reduced penetrance. On the basis of clinical testing, minor allele frequency in the African (American) population, and

functional studies, *CFI* c.1246A>C may be a risk factor for C-TMA rather than the cause of the disease.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)
Supplementary References.
STROBE Statement.

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