Separating causal from confounding associations with disease has been a long-standing problem in epidemiology. The situation improved when it became feasible to perform genome-wide association studies (GWAS): genotyping of large case-control cohorts at several million single-nucleotide polymorphisms (SNPs) spread across the genome, in an unbiased screen for association (i.e., with no prior hypothesis). A key factor in their success was the use of stringent statistical thresholds to control for the numerous tests. Because genotypes are randomly distributed at meiosis, predate most traits, and remain stable throughout life, they are not generally subject to confounding influences and have provided robust and causal associations. GWAS also confirmed classic genetic theory stating that the majority of complex traits are influenced by innumerable variants with tiny individual effects. A recent extension of this theory, the “omnigenic” model, proposes that most traits are influenced by variants in a limited set of “core” genes, with direct and biologically interpretable effects, alongside more numerous “peripheral” genes, mostly with very small effects and acting through interconnected regulatory networks. In fact, peripheral rather than core variants account for most trait heritability because, despite their small effects, there are over 100 times more of them. Peripheral variants lie outside coding regions and individually provide limited insights into trait biology. Indeed, most are only detectable collectively. As an example, >100,000 SNPs are estimated to show independent causal effects on human height, the majority exerting only tiny “peripheral” effects and providing limited biological insights. Given these considerations, what insights have been gained from GWAS in age-related macular degeneration (AMD)?

In this issue, Lores-Motta et al describe 2 new GWAS associations that confirm a core pathway and illuminate AMD pathogenesis (see http://www.aaojournal.org/article/S0161-6420(17)32520-4/fulltext). Genetic associations in AMD were among the first fruits of the GWAS approach, helped by uncharacteristically strong effects. One of these was attributed to an SNP (rs1061170) in the complement factor H gene (CFH), causing a Tyr402His substitution. This may compromise the ability of its product, the soluble glycoprotein complement factor H (CFH), to suppress activation of complement on the surfaces of host (self) tissues while allowing complement to proceed unchecked on foreign surfaces (Fig 1). Additional independent CFH missense mutations that likely affect function were also reported. Other AMD-associated variants lay in noncoding, presumed regulatory, regions. Rare but more highly penetrant variants of CFH were later found in small subsets of AMD subjects. Unfortunately, although GWAS associations are statistically robust and indicative of causal associations, they do not always indicate the precise nucleotides responsible. This is a particular problem in the CFH region, where there is a strong tendency for neighboring nucleotides to be co-inherited in blocks only rarely separated by recombination (this is called “linkage disequilibrium”).

A dysfunctional complement pathway in AMD was independently supported by immunohistochemistry showing that CFH and other complement components were present in drusen, a hallmark of AMD. Subsequently, other complement encoding genes (C3, factor B, factor I, C9, vitronectin) were associated with AMD in further and bigger GWAS, the largest of which included >16,000 advanced AMD cases and approximately 18,000 controls. Complement pathway variants collectively accounted for approximately one third of AMD risk. When the collective effect of genome-wide SNPs in AMD was partitioned into functional subsets, the most significant contribution came from approximately 1300 SNPs in and around genes influencing complement biology. Together, the results pointed to a causal role for increased complement activation in AMD.

These exciting findings stimulated a high level of translational and commercial activity, but uncertainty remains over the relative importance of systemic versus local complement activation in AMD. Although most complement genes are strongly expressed by the liver and encode proteins that circulate at high levels in the blood, other sources of complement proteins include the cells lying on either side of Bruch’s membrane (Fig 1). This raises the key question of where to target therapeutic intervention. Although systemic activation could damage the choriocapillary endothelium, local retinal or choroidal dysregulation might contribute most to retinal pigment epithelial (RPE) damage or drusen formation.

The case for systemic complement activation was strengthened by studies of the “complotype” or joint effects
of common functional variants in the \( CFH \), \( CFB \), and \( C3 \) genes.\(^7\) When combined, these showed up to 6-fold variation in complement activation measured in vitro. Complotype is thus likely to influence an individual’s systemic complement activation. However, a similar effect of complotype might be manifested in the eye, depending on the local levels of the various proteins, which remain to be established. Those liver transplant recipients who develop AMD reportedly carry the recipient rather than donor \( CFH \) Y402H risk allele, suggesting local complement activation in the eye trumps systemic activation.\(^8\)

Other evidence supported the primacy of systemic complement activation based on the finding that complement activation end-products are elevated in patients’ blood.\(^9\) C3d is a stable proteolytic fragment of C3 and the end point of successive cleavages beginning with C3 cleavage to C3b, the key step in complement cascade activation (Fig 1).\(^10\) C3b’s short-lived thioester group either hydrolyses or binds covalently to nearby surfaces. C3b, in fluid phase or on surfaces, is eventually inactivated by cleavage to iC3b and then to C3dg and finally C3d. Surface-tethered C3d can remain resident for an extended time. The ratio between serum concentrations of C3d and C3 is used as a proxy for systemic complement activation.\(^11\) Note that this ratio takes no account of potentially large amounts of C3d bound to cellular and other surfaces. Thus, it reflects C3 consumption and fluid-phase activation but does not report directly on surface complement activation.

Lores-Motta et al\(^12\) address the role of systemic complement activation by reporting a GWAS of serum C3d/C3 ratios. The authors studied 717 AMD cases and 831 controls in a discovery cohort and confirmed their findings in a smaller replication cohort. No genetic variants outside a cluster of genes on chromosome 1, which includes \( CFH \) and 5 protein-coding CFH-related genes (CFHR1-5), showed significant association with C3d/C3. Of the 2 independent variants that did show genome-wide significant associations with C3d/C3, one (the strongest) was a coding variant in exon 14 of the \( CFH \) gene (rs3753396) that did not change the amino acid sequence. The other was a noncoding SNP (rs6685931) in the CFHR4 gene. Like other CFH-related proteins, CFHR4 antagonizes the action of CFH, thus potentially promoting complement activation,\(^13\) although its serum levels are 10- to 30-fold lower than those of CFH. Of note, deletion of CFHR1 and CFHR3 is protective in AMD, but this may be due to linkage disequilibrium (discussed previously) with causal variants in the neighboring \( CFH \) gene.\(^14\) The article by Lores-Motta et al\(^12\) reports that a CFHR4 SNP, also in strong linkage disequilibrium with CFH SNPs, is associated with systemic complement activation, which is an intriguing possibility, despite the difficulty of disentangling causal SNPs in the region.

Because a CFHR4 SNP (rs6685931) and a CFH SNP (rs3753396) are both associated with systemic complement activation, the question arises as to whether or not they are also associated with AMD, because this would imply the presence or absence, respectively, of a causal connection. The observed result, namely that rs6685931 is associated with AMD and s3753396 is not associated, is potentially confusing. However, there may be a prosaic explanation for this apparent discrepancy. The \( CFH \) “risk” allele is at substantially lower population frequency than the CFHR4 risk SNP. In this case, the study may simply have lacked the statistical power to show association of the \( CFH \) variant in a relatively small AMD cohort. Moreover, AMD is a more complex trait than the C3d/C3 ratio, so SNP effects on the disease are likely to be smaller and harder to detect. Alternatively, there could be a disconnect between systemic complement activation and AMD.

This study highlights some key considerations for those developing complement pathway therapeutics. First, although complement activation is not the only genetically influenced pathway in AMD, it is further confirmed as a
major player. Second, the association of a CFHR4 variant with both AMD and increased systemic complement activation merits attention, although teasing apart the causal variant(s) will be challenging. Third, systemic complement activation surely has a role in AMD, but may influence only part of a complex disease process operating on both sides of Bruch’s membrane (Fig 1). Fourth, genetic effects that influence circulating or ocular complement activation may be correlated, in which case easily measured systemic markers such as C3d/C3 would be useful for stratifying patients in clinical trials.

In conclusion, several complement pathway SNPs are uncharacteristically common in the general population, considering that they exert large effects on complement function. This may reflect past evolutionary pressure to resist infectious diseases. This factor has certainly enabled the detection of core genes and causal pathways in AMD, which emerges as a paradigm for using the power of GWAS to elucidate disease.

References


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