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The complement system in age-related macular degeneration: A review of rare genetic variants and implications for personalized treatment

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ABSTRACT

Age-related macular degeneration (AMD) is a progressive retinal disease and the major cause of irreversible vision loss in the elderly. Numerous studies have found both common and rare genetic variants in the complement pathway to play a role in the pathogenesis of AMD. In this review we provide an overview of rare variants identified in AMD patients, and summarize the functional consequences of rare genetic variation in complement genes on the complement system. Finally, we discuss the relevance of this work in light of ongoing clinical trials that study the effectiveness of complement inhibitors against AMD.

1. Clinical characteristics of age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss among the elderly, accounting for 8.7% of blindness worldwide. AMD is most prevalent in populations of European ancestry with approximately 1–3% of the total population suffering from an advanced form of AMD (Chakravarthy et al., 2010b; Tomany et al., 2004; Wong et al., 2014). Globally, the total number of patients with any type of AMD is expected to increase over the next 25 years to 288 million affected individuals (Wong et al., 2014).

The disease is characterized by a gradual loss of central vision due to photoreceptor cell degeneration in the centre of the retina at the back of the eye, known as the macula. Photoreceptors are in close contact with a layer of cells called the retinal pigment epithelium (RPE). RPE cells support the function of the photoreceptors and play an important role in maintaining retinal homeostasis. In AMD, this natural function of the RPE is disturbed, resulting in the accumulation of retinal waste products called drusen underneath the RPE. Drusen are the tell tale sign of AMD and are easily recognized by ophthalmologists.

AMD is a progressive retinal disease is which the early stage is characterized by relatively few small drusen within the macula. When AMD progresses, drusen size and number increase, eventually leading towards more advanced stages of AMD. Two forms of advanced AMD are distinguished. The first form, neovascular AMD, is characterized by infiltration of abnormal blood vessels into the retina. These newly formed vessels are fragile and when they break, the leakage of blood constituents in the retina leads to sudden vision loss. The second form of advanced AMD, geographic atrophy, is the result of gradual degeneration of the RPE and photoreceptors cells. Although neovascularization occurs in only 15–20% of AMD cases, it is responsible for the vast majority of vision loss caused by AMD. Drugs targeting vascular endothelial growth factor (VEGF), one of the central molecules in neovascularization, have proven to be very successful in neovascular AMD. However, no treatment is available for the remaining majority of early, intermediate or geographic atrophy AMD cases, and furthermore there are no effective means of preventing progression of early to advanced stages (Chakravarthy et al., 2010a; Jager et al., 2008).

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2. The complement system plays a central role in the etiology of AMD

2.1. Research on the etiology of AMD: a historical perspective

Today it is known that AMD is the result of a complex interaction of environmental and genetic risk factors. Pooled evidence from numerous studies has demonstrated that environmental factors like aging itself, smoking behavior, and body mass index (BMI) are strong risk factors for AMD. In addition, cataract surgery, cardiovascular disease and family history are also strongly associated (Chakravarthy et al., 2010b). Before any specific gene or biological pathway had been conclusively linked to AMD, studies into the molecular constituents of drusen had already suggested that AMD may have an immunological component. This suggestion arose after proteins involved in inflammation and/or other immune-associated responses, including components of the complement system, were found within drusen (Hageman et al., 2001; Johnson et al., 2001; Mullins et al., 2001).

Evidence for a strong genetic component in AMD arose from twin and family studies. Twin studies observed a high concordance of AMD between monozygotic pairs, even double compared to dizygotic pairs, and estimated that the heritability of AMD may be as high as 45 to 70% (Hammond et al., 2002; Meyers et al., 1995; Seddon et al., 2005). These findings were in line with familial aggregation analyses that observed a higher prevalence of AMD characteristics and an earlier onset of disease symptoms among relatives of patients compared to control families (Klaver et al., 1998; Seddon et al., 1997).

2.2. Genetic evidence for a role of the complement system in AMD

In search for genomic regions implicated in AMD, genetic linkage analyses were done in large family-based studies (Abecasis et al., 2004; Iyengar et al., 2004; Majewski et al., 2003; Seddon et al., 2003; Weeks et al., 2004). Among a few other regions, the findings from these studies strongly and consistently implicated a region on chromosome 1 in the disease. When the first genome-wide association study (GWAS) for AMD was performed in 2005, it identified that same genomic region, which lead to the discovery of a highly associated genetic variant in complement factor H (*CFH*; Tyr402His) (Klein et al., 2005). These findings were corroborated by three additional studies (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005).

Through genetic studies that followed over the next decade, the understanding of the genetic basis of AMD increased dramatically with the identification of disease-associated variants across several biological systems (Fritsche et al., 2013). The genetic link between AMD and the complement system was further expanded when genetic variants in or near complement factor I (*CFI*), complement component 3 (*C3*), complement component 2 (*C2*), complement component 9 (*C9*), complement factor B (*CFB*) and vitronectin (*VTN*) were also found to be associated with the disease (Fagerness et al., 2009; Fritsche et al., 2013, 2016; Gold et al., 2006; Maller et al., 2007; Yates et al., 2007) (Table 1). In addition, a common haplotype carrying a deletion of complement factor H related genes *CFHR1* and *CFHR3* was found to be protective for AMD (Hughes et al., 2006).

2.3. The role of rare genetic variants in AMD

Common genetic variants (with a minor allele frequency (MAF) of >5% in the population) near the complement genes *CFH*, *C2/CFB*, *C3* and *CFI* together explain 40–60% of the heritability of AMD (Fritsche et al., 2014). However, a large fraction of the heritability still remains unknown and is referred to as *missing heritability*. One hypothesis states that low frequency and rare genetic variants (with a MAF of <1–5% and <1%, respectively) may explain the remaining fraction of the heritability (Manolio et al., 2009). During the past years, genetic studies in AMD have therefore shifted towards the identification of rare genetic variants. However, a practical problem arises when analyzing rare variants. The number of patients and controls needed for the identification of novel variants increases when variants are more rare, since the sample size requirements increase roughly linearly with the inverse of the allele frequency. Therefore, analyses of very large cohorts are required for a comprehensive understanding of the role of rare genetic variants in AMD.

2.4. Genetic approaches to identify rare genetic variants in AMD

In order to discover rare variants investigators resort to other methods of analyses than those methods yielding insight into common variation. An effective approach that can be used to detect rare disease-associated variants is through a GWAS using exome chips. An exome chip is an array containing both common genetic variants as well as rare exonic variants, and is cost-effective in capturing a specific set of variants in large case-control studies. These chips can be customized and enriched for specific variants of interest. The approach is limited in the sense that it cannot discover new genetic variants other than the ones that the chip captures, but after imputation the chip covers over 12 million variants across the genome (Fritsche et al., 2016). A recent large GWAS using exome chips detected 52 (45 common and 7 rare) variants at 34 genomic regions that are independently associated with AMD. More than one third (19/52) of these variants reside in or near a gene of the complement system: *C2/CFB*, *C3*, *C9*, *CFH*, *CFI*, and *VTN* (Table 1). Besides evaluating the association of single genetic variants with the disease, the cumulative number of rare variants detected across an entire gene can be compared between patients and control individuals using burden tests. Interestingly, a significant burden of rare variants in the *CFH* and *CFI* genes, in addition to two other genes (*TIMP3* and *SLC16A1*), was observed in AMD (Fritsche et al., 2016).

Another approach that is widely used to detect rare variants is sequence analysis of candidate genes in cases and controls. An advantage of this approach above the use of exome chips is that it can discover new genetic variants, thereby allowing a comprehensive analysis of all genetic variation in a candidate gene or a set of candidate genes. With the development of next-generation

Table 1
Genes in the complement system associated with AMD.

Gene/Locus	Approach	Reference[a]
C2/CFB	Candidate gene	Gold et al. (2006)
C3	Candidate gene/WGS	Maller et al. (2007) and Yates et al., 2007)/(Helgason et al. (2013), Seddon et al. (2013) and Zhan et al. (2013)
C9	Candidate gene	Nishiguchi et al. (2012) and Seddon et al. (2013)
CFH	Candidate gene/Linkage/GWAS	Edwards et al. (2005), Hageman et al. (2005), Haines et al. (2005) and Klein et al. (2005)/Raychaudhuri et al. (2011)
CFHR1-CFHR3	Candidate gene	Hughes et al. (2006)
CFI	Candidate gene	Fagerness et al. (2009)/van de Ven et al. (2013)
VTN	GWAS	Fritsche et al. (2016)

[a] Reference of first cited association based on common and/or rare genetic variant.

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