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CLINICAL STUDY

Age-related macular degeneration: a perspective on genetic studies

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Abstract

Aim **Age-related macular degeneration (AMD) is a common macular disease in the developed world and recent studies have shown that specific genes may be associated with it and may contribute to a higher risk of developing AMD.**

Objective **Our objective was to review systematically recent publications related to the genetics of AMD and provide relevant information that would help both scientists and clinicians in advising patients.**

Method **A systematic search was performed on PubMed, Medline, and National Library of Medicine as well as ARVO abstracts using key words relevant to the genetic associations of AMD.**

Results **The most important genetic associations in AMD involved the complement factor H (*CFH*) gene, which showed that possession of the variant Y402H polymorphism significantly increases the risk for AMD. Protective genes have also been identified such as those on either factor B (*BF* or complement factor B (CFB)) or complement component 2 (*C2*) genes. The genes involved in inherited macular dystrophies such as ATP-binding cassette, subfamily A (ABC1), member 4 (*ABCA4*), vitelliform macular dystrophy (*VMD2*), tissue inhibitor of matrix metalloproteinase-3 (*TIMP3*), and *EFEMP1* have yielded some important information but further confirmatory work has yet to establish a clear association with AMD.**

Conclusion **Patients with AMD possess specific genetic variants of the *CFH* gene, which put them at a higher risk of developing the disease. Other unaffected individuals may possess certain protective genetic variants, which could prevent them from developing AMD. Further research will no doubt shed light on other such mechanisms and these will be useful in identifying possible direct targets for drugs or indirectly through modulation of the genes responsible for disease presentation.**

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Keywords: genetics; age-related macular degeneration; systemic review; complement factor H

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Introduction

Age-related macular degeneration (AMD) is a common macular disease affecting about 30% of adults aged 75 or older who have some signs of maculopathy, and about 6–8% of the individuals in this age group are afflicted with the advanced stages of this disease which causes the most severe visual loss.[1] As the disease primarily impacts on central vision, it is the leading cause of blindness in people above 60 years of age and accounts for about 50% of blind registration.[2,3]

A number of epidemiological studies have implicated AMD as an inherited disease showing that family members are at increased risk of the disease.[4,5] Results from twin and family-based studies have provided compelling evidence for a genetic basis for AMD.[6–8] As it is a condition prevalent at old age, finding well-established family pedigrees is difficult. The inheritance is thought to be polygenic and environmental factors such as smoking, hypertension, and chronic inflammation are thought to play major roles in its pathogenesis. The emphasis on the genetic basis of AMD has increased in recent years. This systematic review was performed to look at the role of genes in AMD and to evaluate which particular genes play important roles in the inheritance of the disease so that accurate information can be displayed in a logical pattern for both clinicians and scientists. The information could be used to

identify high-risk patients so that lifestyle behaviour such as smoking can be modified.

Methods

A detailed search using, PubMed, MEDLINE (1985 to August 2006), and EMBASE (1985 to August 2006) was performed. Conference abstracts (sessions related to AMD genetics and Inherited Macular Dystrophies) in ARVO 1975 to 2006 were analysed in detail looking for relevant published articles in this field.

Discussion

A number of studies including familial, twin-based, and other analyses have shown a strong genetic component in AMD. The work done by Seddon *et al*[9] showed that prevalence among relatives was significantly higher compared to controls (23.7% *vs* 11.6%). Similar work by Klaver *et al*[10] showed that the odds ratios for siblings and offsprings of AMD patients were higher compared to siblings of controls. Several independent groups have examined studies comparing concordance rates in monozygotic *vs* dizygotic twins.[11,12] These two larger studies showed that there is a substantial heritable component to AMD; however unique environmental factors also play an important role.

Researchers have carried out a number of linkage studies in an attempt to identify the genomic regions containing susceptibility loci for AMD. These studies were performed to evaluate whether genetic marker loci segregated independently of disease status within family pedigrees. In the last decade, the study of hereditary macular dystrophies such as Best's, Stargardt's, Sorsby's fundus, autosomal-dominant drusen- and peripherin/*RDS*-related dystrophy has provided some clues to the pathogenesis of AMD. Many investigators have proposed that inherited macular dystrophies could be potential models for AMD. Once positional loci and function of the various genes from some of these inherited macular diseases were confirmed, more specific analysis was performed on AMD patients. Tables 1 and 2 show the various candidate genes tested with negative findings in the former and positive findings in the latter.

Studies of Sorsby's fundus dystrophy and the identification of the gene mutation for a tissue inhibitor of matrix metalloproteinase-3 (*TIMP3*) has not correlated with AMD.[13] The identification of the ATP-binding cassette, subfamily A (ABC1), member 4 (*ABCA4*) gene that encodes for a retinal rod/cone photoreceptor protein and its deletion results in Stargardt's macula dystrophy. Allikmets *et al*[14] showed the presence of *ABCA4* polymorphisms in 3.4% of their total screened patients compared to 0.95% of controls. It has also been suggested that heterozygotes for *ABCA4* mutations were predisposed to AMD but these results have been disputed in later studies.[14,15] The role of *ABCA4* remains uncertain in AMD and the possibility that this gene causes a small number of AMD cases cannot be excluded. The protein bestrophin and its gene mutation is responsible for vitelliform macular dystrophy (*VMD2*, Best's disease), an early onset, autosomal, dominant macular degeneration characterized by the deposition of lipofuscin-like material within and below the retinal pigment epithelium but studies have shown a small, non-significant correlation with AMD.[16,17]

Malattia leventinese or Doyne honeycomb retinal dystrophy shares similar clinical and histological features to AMD and the gene (EFEMPI) responsible for the disease encodes for a member of the fibulin family (fibulin-3/SI-5/FBNL), a newly recognized group of extracellular matrix proteins.[18] A recent study has shown that missense variations in the fibulin 5 gene were found in 1.7% of patients with AMD with many variations in other fibulin genes being found in these patients.[19] This

Table 1 Negative candidate gene screening in patients with AMD to date

Site	*Authors*	*Loci*
Chromosome 1q	Esfandiary *et al*[55]	EPHX1, ADPRT1
	Hayashi *et al*[56]	LXR2, LAMC1
	Conley *et al*[57]	LAMC2, LAMB3, OCLM, RGS19, TGFB2
Chromosome 2p	Stone *et al*[58]	*EFEMP1*
	Guymer *et al*[59]	
Chromosome 2q	Haines *et al*[52]	IL1A
	Stone *et al*[19]	*FIBULIN 2*
Chromosome 3p	Esfandiary *et al*[55]	GPX1
Chromosome 3q	Kuehn *et al*[60]	IMPG2
Chromosome 6p	Shastry *et al*[61]	*RDS*
Chromosome 7	Esfandiary *et al*[55]	AhR
Chromosome 8p	Esfandiary *et al*[55]	NAT2
Chromosome 10q	Esfandiary *et al*[55]	CYP2E1
Chromosome 11p	Esfandiary *et al*[55]	CAT
Chromosome 11q	Stone *et al*[19]	*FIBULIN 4*
	Lotery *et al*[17]	*VMD2*
	Akimoto *et al*[62]	
	Seddon *et al*[63]	
	Allikmets *et al*[16]	
	Kramer *et al*[64]	
Chromosome 12p	Haines *et al*[52]	A2M, MSGT1
Chromosome 14q	Haines *et al*[52]	CKB
Chromosome 15q	Esfandiary *et al*[55]	CYP1A1, CY1A2
Chromosome 17q	Conley *et al*[57]	APOH, ITGB4
Chromosome 22q	Esfandiary *et al*[55]	CYP2D6
	Stone *et al*[19]	*FIBULIN 1*
	De La Paz *et al*[13]	TIMP-3

Abbreviations: AMD, age-related macular degeneration; *TIMP3*, tissue inhibitor of matrix metalloproteinase-3; *VMD2*, vitelliform macular dystrophy.

Table 2 Positive candidate gene screening for AMD genes

Site	*Authors*	*Loci*
Chromosome 1p	Allikmets *et al*[14] Kuroiwa *et al*[65] Allikmets[66] Fuse *et al*[67] Rivera *et al*[68] Souied *et al*[69] Guymer *et al*[70] Shroyer *et al*[71] Webster *et al*[72] Bernstein *et al*[73] Baum *et al*[74] Schmidt *et al*[75]	*ABCA4 (ABCR)*
Chromosome 1q	Edwards *et al*[76] Haines *et al*[31] Klein *et al*[77] Hageman *et al*[78] Zareparsi *et al*[29] Conley *et al*[30] Jakobsdottir *et al*[35] Souied *et al*[79] Magnusson *et al*[80]	*CFH*
	Schultz *et al*[81] Abecasis *et al*[82] Hayashi *et al*[56] Iyengar *et al*[83] Stone *et al*[19] Conley *et al*[57]	HEMICENTIN (*FIBULIN 6*)
Chromosome 3p	Tuo *et al*[84]	CX3CR1
Chromosome 6p	Gold *et al*[34] Goverdhan *et al*[48] Haines *et al*[52] Churchill *et al*[85]	*BF* and *C2* *HLA* *VEGF*
Chromosome 6q	Ayyagari *et al*[86] Conley *et al*[57] Kimura *et al*[87] Esfandiary *et al*[55]	*ELOVL4* SOD2
Chromosome 7q	Ikeda *et al*[88] Baird *et al*[42] Esfandiary *et al*[55]	PON1
Chromosome 9p	Conley *et al*[57] Haines *et al*[52]	VLDLR
Chromosome 9q	Zareparsi *et al*[29]	TLR4
Chromosome 10q	Jakobsdottir *et al*[35] Rivera *et al*[36]	GRK5/PLEKHA1/ *LOC387715*
Chromosome 12p	Haines *et al*[52]	LRP6
Chromosome 14q	Stone *et al*[19]	*FIBULIN 5*
Chromosome 17q	Hamdi *et al*[89] Conley *et al*[57] Haines *et al*[52] Seddon *et al*[90]	ACE
	Souied *et al*[40] Pang *et al*[91] Simonelli *et al*[92] Schmidt *et al*[93]	
Chromosome 19q	Baird *et al*[94] Gotoh *et al*[45] Zareparsi *et al*[43] Conley *et al*[57] Wong *et al*[95]	*APOE*
Chromosome 20p	Zurdel *et al*[96]	CST3
Chromosome 20q	Fiotti *et al*[97]	MMP9

Abbreviations: *ABCA4*, ATP-binding cassette, subfamily A (ABC1), member 4; AMD, age-related macular degeneration; *APOE*, apolipoprotein E; *BF*, factor B; *C2*, complement component 2; *CFH*, complement factor H; *HLA*, human leucocyte antigen; *VEGF*, vascular endothelial growth factor.

finding may be relevant to AMD pathogenesis as misfolding of the FBNL-5 protein may result in dysfunction at the extracellular matrix in Bruch's membrane. Reduced secretion of the mutant FBLN-5 protein seems to be important for AMD to develop in these patients.[20] This could result in abnormal deposition as seen in drusen and consequent disinhibition of vascular endothelial growth factor (*VEGF*) resulting in CNV pathogenesis.[21]

The immune system has been implicated in pathogenesis of AMD for years from the work of Hageman and colleagues.[22–24] Histological studies that have showed the presence of complement components both within drusen and along the RPE-choroid interface in AMD eyes.[22] Its role in the pathogenesis of AMD has been confirmed recently by the identification of polymorphisms in the gene that encodes for complement factor H (*CFH*) in AMD patients. The complement system is part of the innate defence mechanism consisting of over 30 serum proteins involved as a cascade that results in the rapid delivery of activated enzymes which create micropores within foreign objects such as microbes resulting in cellular lysis and inflammation.

The *CFH* is part of the alternative pathway and it is specifically involved with the inhibition of the cascade in combination with other proteins. It is thought that for some reason in AMD patients, abnormal regulation of the complement cascade occurs at a local level within Bruch's membrane and adjacent retinal pigment epithelial cells resulting in uncontrolled compliment activation and consequent drusen formation.[25] Recent

studies has shown that the presence of the at-risk haplotype increased the risk of AMD 2.7-fold and accounted for 50% of the attributable risk of AMD in that group.[26,27] Another study has shown that individuals homozygous for the risk alleles (representing a tyrosine–histidine change at amino acid 402) have a 7.4-fold increased likelihood of AMD.[28]

These studies confirm an initial hypothesis, which established that chronic inflammation involving the complement pathway could contribute to disease progression. Using candidate gene screening, several different groups have found an association between the *CFH* gene and AMD in large numbers of patients.[29,30] Another study has shown that possession of the variant Y402 H polymorphism significantly increases the risk for AMD with odds ratios between 2.45 and 5.57 and could explain approximately 43% of AMD in older adults.[31] Table 3 shows a summary of the recent global papers on *CFH* and AMD containing the odds ratio and confidence intervals with each polymorphism.

A recent Japanese study showed that the *CFH* gene did not appear to be a primary hereditary contributor to AMD suggesting ethnic differences of AMD phenotypes.[32] Most of the studies that showed significant findings consisted of Caucasian populations; however, in Chinese patients, results on AMD patients has shown that the frequency of the Y402H polymorphism is much lower in comparison and is associated with neovascular AMD.[33] Gold *et al*[34] examined two other factors involved in the complement cascade, factor B (*BF*) and complement component 2 (*C2*), for genetic variation in two independent cohorts. The results showed that 56% of the individuals who did not have AMD were in possession of the protective variant of either *CFH*, *BF*, or *C2* gene compared to 74% with AMD who lacked the protective variant. As shown in Table 4, the figures from this study indicate that possession of the specific *C2* and *BF* polymorphisms may have a protective effect against AMD. An absence of these variations on the *C2*/*CFB* genome may otherwise predispose patients to develop AMD. Other genes such as the *LOC387715* on chromosome 10q26 are also thought to confer higher risks to developing CNV independent of the *CFH* gene.[35,36] This gene is located in an extensive region of linkage disequilibrium, which encompasses other genes that show association with AMD and it is postulated that the causative mutation could be in a neighbouring gene[37] (Table 5). Interestingly, Schimdt *et al*[38] have shown that genetic susceptibility (such as a coding change (Ala69Ser) on the *LOC387715* gene) coupled with a modifiable lifestyle factor such as cigarette smoking could confer a significantly higher risk of AMD than either factor alone.

Table 4 Odds risk ratios associated with *BF* and *C2* variants showing a possible protective effect against AMD in those patients who possess these variations in this particular study

Gene (SNP)	*OR (95% CI)*
C2 (rs9332739)	0.36 (0.23–0.56)
C2 (rs547154)	0.44 (0.33–0.60)
BF (rs4151667)	0.36 (0.23–0.56)
BF (rs641153)	0.32 (0.21–0.48)

Abbreviations: AMD, age-related macular degeneration; *BF*, factor B; *C2*, complement component 2; *CI*, confidence interval; *OR*, odds risk ratios.

Table 3 A summary of the odds risk ratios (OR) associated with Y402H in AMD from recent studies

Study	*Genotype*	*Sample size AMD*	*Control*	*OR (95% CI)*
Columbia cohort*	CC/CT	549	272	2.25 (1.79–2.75)
Iowa cohort*	CC/CT	403	131	2.82 (2.11–3.78)
(Massachusetts)*	CC	111	401	2.13(1.10–4.16)
	CT	—	—	1.46 (1.05–2.04)
Magnusson (Iceland)*	CC/CT	581	171	2.39 (1.86–3.07)
	CC/CT	322	203	2.14 (1.66–2.75)*
Souied (France)*	CC	141	91	6.93 (3.11–15.46)
	CT	—	—	3.00 (1.60–5.62)
Italy*	CC	104	131	3.9 (1.9–8.2)
	CT	—	—	1.4 (0.7–2.6)
Zareparsi (Michigan)*	CC	616	275	5.52 (3.54–8.59)
	CT			4.36 (3.13–6.08)
Sepp (UK)*	CC	443	262	6.3 (3.8–10.4)
	CT	—	—	—

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds risk ratios.
It is important to note that the cases differ amongst the studies of all AMD patients including advanced AMD.
*Advanced AMD.
OR calculated using TT as a reference.

Table 5 CNV risk associated with *PLEKHA1/LOC387715* variants (Jakobsdottir *et al*)

Gene (SNP)	*OR (95% CI)*
rs4146894	
Dominant (RR + RN)	2.53 (1.64–3.91)
Recessive (RN)	2.46 (1.63–3.71)
rs10490924	
Dominant (RR + RN)	5.64 (3.52–9.06)
Recessive (RN)	6.18 (2.62–14.59)

Abbreviations: CI, confidence interval; OR, odds risk ratios.
The data show that these two polymorphisms *rs4146894* and *rs10490924* on the LOC gene carry a higher risk of developing end-stage CNV with the latter carrying an almost doubling effect in the dominant and recessive forms.

Table 6 A summary table of the association between AMD showing the protective effect of the Apoε4 against AMD in comparison to Apoε2 with reference to Apoε3

Study	*Allele carrier*	*Sample size AMD*	*Control*	*OR (95% CI)*
Baird	ε2	200	123	1.59 (0.71–3.55)
(Australia)	ε4			0.65 (0.37–1.13)
Schmidt	ε2	230	372	0.99 (0.59–1.66)
(Tennessee, USA)	ε4			0.88 (0.58–1.35)
Klaver	ε2	88	901	1.50 (0.80–2.82)
(Holland)	ε4			0.43 (0.21–0.88)
Zareparsi	ε2	632	206	1.12 (0.63–2.00)[a]
(Michigan, USA)	ε4			0.55 (0.37–0.82)

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds risk ratios.
[a]Unadjusted OR.

Apolipoprotein E (*ApoE*) genotype was studied initially for its association with Alzheimer disease due to the presence of increase amyloid β-peptides deposition in cerebral cortex.[39] The role of *ApoE* has also been investigated extensively to understand the aetiology of AMD. This polymorphic protein plays an important role in lipid metabolism and central nervous system lipid homeostasis and the presence of lipid in drusen has prompted these studies. Souied *et al*[40] showed a reduced prevalence of the ε4 allele in patients with exudative AMD as compared to a control sample and concluded that the *ApoE* gene is a genetic protective factor identified in AMD. Other studies have shown the presence of *ApoE* protein within AMD-associated deposits in the macula.[41] Some studies have shown that ε4 allele could be protective, or at the very least, delaying the age of diagnosis of disease, whereas the ε2 allele appears to have a modifier effect by bringing forward the mean age of disease diagnosis.[42,43] There are other studies which did not find a significant association between AMD development and *ApoE* allele frequency.[44,45] Table 6 summarizes the findings of the odds ratios in AMD looking at ApoE2 and ApoE4 alleles in reference to ApoE3. The data shows that possession of ApoE4 may have a protective effect in comparison to ApoE2 with regard to development of AMD.

The role of inflammation and genetic predisposition reported in AMD may involve immune response gene such as human leucocyte antigen (*HLA*) polymorphisms that modulate susceptibility to AMD, *HLA* antigens are expressed both normally and in eyes affected with AMD and with intense *HLA*-DR immunoreactivity that has been demonstrated in both soft and hard drusens.[46] Increased *HLA* class II immunoreactivity has been observed in the human retina affected with AMD and related to drusen formation.[47] Genetic analysis of patients with AMD have revealed that there may be relevant positive and negative associations between *HLA* alleles and AMD, and these could influence the development of AMD through modulation of the choroidal immune function.[48]

Conclusion

These studies have confirmed that some affected patients may possess specific genetic variants of the *CFH* gene, which put them at a higher risk of developing the disease. Other unaffected individuals may possess certain protective genetic variants, which could prevent them from developing AMD. Modifiable factors such as smoking and body mass index (BMI) are very important as they confer a higher risk of developing CNV in the presence of high-risk alleles.[49] It is imperative to clarify that the mechanism of disease development can be difficult in complex diseases such as AMD, and these studies have helped in our understanding of pathological findings and genetic predispositions. From previous experience, screening has been important in prevention, early detection, and prompt treatment such as in diabetes. Using the same concept in AMD, we know that genetic factors play an important part as discussed previously and although mass screening in local populations is not indicated in AMD, there may be sufficient evidence to screen familial cases initially using genetic laboratory tests for the *CFH*, *C2*/*BF*, LOC, and *ApoE* polymorphisms. These tests are in fact inexpensive to perform and could be readily made available in local laboratories (actual costs for genotyping a DNA sample by Taqman assay could be as less as 28 pence. Identification of higher risk cases can then be used to inform patients of their potential risk of developing the disease and advised to implement changes in their lifestyle such as lowering BMI or cessation of smoking. It

is important to note here that there may not be sufficient evidence yet to suggest that adjusting these modifiable risk factors will influence future risk of developing AMD in the presence of these genetic polymorphisms.

New treatments of neovascular AMD such as Ranibizumab and Pegaptanib have shown promising results so far.[50,51] It is feasible to assume that patients will respond differently to these new drugs due to inherent genetic influences which may need to be investigated further. These drugs were designed specifically as anti-*VEGF* therapy following extensive laboratory research. (*VEGF*) is an endothelial cell-specific mitogen that promotes angiogenesis and is a potent mediator of vascular permeability. Studies on diseases that involve inflammation and angiogenesis such as cancer, sarcoidosis, and more recently AMD have revealed *VEGF* polymorphisms that could put patients at higher or lower risks of developing the disease.[52–54] Further research would no doubt shed light on other such mechanisms and these would be useful directly in identifying possible direct targets for drugs or indirectly through modulation of the genes responsible for disease presentation.

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