REVIEW ARTICLE

The molecular genetic basis of age-related macular degeneration: an overview

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Abstract

Age-related macular degeneration (AMD) is a complex disorder of the eye and the third leading cause of blindness worldwide. With a multifactorial etiology, AMD results in progressive loss of central vision affecting the macular region of the eye in elderly. While the prevalence is relatively higher in the Caucasian populations, it has gradually become a major public health issue among the non-Caucasian populations (including Indians) as well due to senescence, rapidly changing demographics and life-style factors. Recent genome-wide association studies (GWAS) on large case–control cohorts have helped in mapping genes in the complement cascade that are involved in the regulation of innate immunity with AMD susceptibility. Genes involved with mitochondrial oxidative stress and extracellular matrix regulation also play a role in AMD pathogenesis. Majority of the associations observed in complement (*CFH, CFB, C2* and *C3*) and other (*ARMS2* and *HTRA1*) genes have been replicated in diverse populations worldwide. Gene–gene (*CFH* with *ARMS2* and *HTRA1*) interactions and correlations with environmental traits (smoking and body mass index) have been established as significant covariates in AMD pathology. In this review, we have provided an overview on the underlying molecular genetic mechanisms in AMD worldwide and highlight the AMD-associated-candidate genes and their potential role in disease pathogenesis.

[Katta S., Kaur I. and Chakrabarti S. 2009 The molecular genetic basis of age-related macular degeneration: an overview. *J. Genet*. **88**, 425–449]

Introduction

Age-related macular degeneration (AMD) is a late-onset, complex disorder of the eye with a multi-factorial etiology in elderly (Hawkins *et al*. 1999). Being the third leading cause of blindness worldwide, it accounts for 8.7% of blind persons globally (Resnikoff *et al*. 2004). In India, the prevalence of AMD ranges between 1.4%–1.8% in different epidemiological studies (Nirmalan *et al*. 2004; Krishnaiah *et al*. 2005; Gupta *et al*. 2007). AMD results in progressive and irreversible loss of central vision affecting the macula of the eye and involves the retinal pigment epithelium (RPE), Bruch's membrane (BM) and choriocapillaries (Fine *et al*. 2000; Stone *et al*. 2001). Prior to the onset of AMD, visual defects such as reduced contrast sensitivity, central visual field loss and spatiotemporal sensitivity (delayed macular recovery) are experienced by patients causing difficulty with daily tasks (Stangos *et al*. 1995; Hageman *et al*. 2001).

The prevalence of AMD varies widely across different ethnic groups worldwide (see table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/). It is estimated that by the year 2020, at least 80 million people will be affected by AMD globally (Clemons *et al*. 2005). While the prevalence of AMD is relatively higher in Western (Caucasian) populations, it is increasingly becoming a public health issue among the Oriental populations (including Indians) due to the rapidly changing demographics, senescence and life-style factors (Krishnaiah *et al*. 2005; Gupta *et al*. 2007).

AMD, being a complex genetic disorder, is attributed to multiple genes with varying magnitudes of effect (Edwards and Malek 2007). Along with genetic variants, environmental factors such as age, smoking, gender and body mass index

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Keywords. AMD; epidemiology; genes; polymorphism; risk.

(BMI) play a major role in the disease pathogenesis (Age-Related Eye Disease Study Group 2000; van Newkirk *et al*. 2000). Gene–gene and gene–environment interactions are the hallmarks of AMD susceptibility. In this review, we provide an overview on the epidemiology of AMD and its associated risk factors. We briefly focus on the epidemiology of this disease, its associated risk factors and provide some insights on the roles of some major genes associated with AMD and their involvement and interactions with other genes in different populations.

The AMD phenotype

Different clinical types of AMD

Phenotypically, AMD is classified as 'early' and 'late' based on specific clinical features (Caswell *et al*. 1985; Hageman *et al*. 2001). Early AMD is characterized by the presence of a large number of drusens ($\leq$ 10) with pigmentary changes such as hypopigmentation or hyperpigmentation. Late AMD is further sub-classified into 'dry' and 'wet' categories (Caswell *et al*. 1985; Hageman *et al*. 2001), wherein 'dry AMD' accounts for 80%–90%, and 'wet AMD for 10%–20% of all cases. The majority of vision loss (~90%) is attributed to 'wet AMD', which is the most severe form of the disease (Bressler 2002). The classification of dry and wet AMD is based on the following features as described in table 1. Although, AMD phenotypes are clinically distinct, the presence of drusens along with hypopigmentation and/or hyperpigmentation of the RPE are common features associated with both dry and wet AMD (Nowak 2006).

Grading the AMD phenotype

AMD being a complex disease requires a universal and uniform definition to understand the disease etiology comprehensively, predict risk and progression, identification of common risk factors and also for comparison across different studies (Bird *et al*. 1995). The most commonly used system of classification is based on the Age-Related Eye Disease Study (AREDS) criteria (Age-Related Eye Disease Study Group 2001), which uses fundus photographs for extensive grading of different sub-stages of the disease.

While the AREDS grading system is widely used, other grading systems were introduced (based on AREDS criteria) for simpler and rapid diagnosis and classification of AMD (Ferris *et al*. 2005; Seddon *et al*. 2006a). The clinical age-related maculopathy staging system (CARMS) is based on a five-stage clinical scale. The CARMS classification provides a simpler demarcation of different stages of progressive AMD. This is widely used as it requires relatively lesser time and expertise compared to other systems (Seddon *et al*. 2006a).

The macula and drusen formation in AMD

The macula is a specialized region of the retina, which is 5–6 mm in diameter; the centre of the macula is called the fovea with maximum number of cone photoreceptor cells. Unlike rod cells, which permit night vision, cone photoreceptor cells are responsible for colour perception and visual acuity (Ronald *et al*. 1996).

Drusen formation in the macular region is the hallmark of all AMD phenotypes. Drusens are pathological deposits that form between the basal lamina of RPE and the inner collagenous layer of the BM and are visible as translucent punctate dots under slit-lamp biomicroscopy (Ambati *et al*. 2003; Theodore and Ahmad 2007). They are associated with advanced age and with other chorioretinal pathologies (Hageman and Mullins 1999a; Hageman *et al*. 1999b). The size, number and confluency of drusen are common risk factors for AMD (Pauleikhoff *et al*. 1990). Morphologically, they are classified as hard (<63 μm in diameter) and soft (>63 μm in diameter with indistinct edges) drusens. Presence of a few hard drusens may not be a risk factor, but numerous hard drusens could independently result in loss of vision in AMD (Pauleikhoff *et al*. 1990; Ambati *et al*. 2003). Soft drusens have a tendency to become confluent and when several of them coalesce, it results in detachments of the overlying RPE (Sarks *et al*. 1999).

The epidemiology of AMD

Epidemiological studies in non-Indian populations

Epidemiological studies in large population cohorts have provided prevalence data in different populations worldwide (see table 1 in electronic supplementary material). The Beaver Dam Eye Study (BDES) from US reported a 15 year cumulative incidence of AMD as 8% for people aged >75

Table 1. Phenotypic features of different forms of classical AMD.

Major types of AMD	Subtypes of AMD	Clinical features
Dry AMD	Drusen	Large number of drusens with pigmentary changes, diffuse, irregular patches of hyper-autofluorescence
	Geographic atrophy	Confluent round or oval areas of RPE atrophy (175 μm in diameter) with clearly visible choroidal vessels and photoreceptor cell death (figure 1)
Wet AMD	Choroidal neovascularization	New blood vessels formation in choroid that breaches the RPE and enters the sub-retinal space
	Disciform scar	CNV leaks that finally lead to the formation of a scar (figure 2)

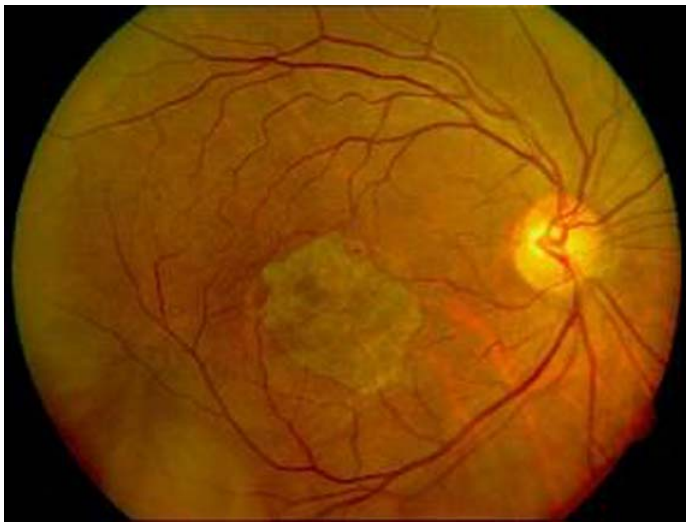

Figure 1. Fundus photograph showing geographic atrophy (courtesy: Dr Nazimul Hussain, L.V. Prasad Eye Institute, Hyderabad, India).

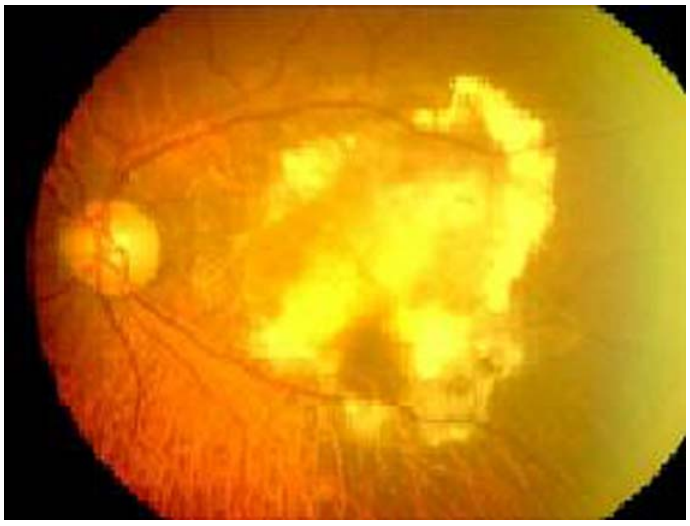

Figure 2. Fundus photograph showing choroidal neovascularization (courtesy: Dr Nazimul Hussain, L.V. Prasad Eye Institute, Hyderabad, India).

years, and 3.1% for people in the age group of 43–54 years (Klein *et al*. 1992). The Blue Mountains Eye Study (BMES) conducted in the elderly urban populations in Australia was based on the grading of fundus photographs using WARMGS (Wisconsin Age-Related Maculopathy Grading System) (Mitchell *et al*. 1995). The prevalence of AMD was observed to be 1.94% (95%CI, 1.49–2.39) and age was strongly associated with the disease prevalence ($P < 0.001$). Age and gender-specific prevalence of AMD increased from 1.3% among those aged < 55 years to 28% in subjects aged > 85 years ($P < 0.001$) (Mitchell *et al*. 1995). Another Australian study, the Visual Impairment Project (VIP) reported an overall cumulative 5 year incidence of early and late AMD as 0% and 13% for people aged 60 years, while it was 20% and 6.3% in the age group of 70–79 years, respectively (van Newkirk *et al*. 2000; Mukesh *et al*. 2004).

The AREDS conducted in US observed the prevalence of intermediate and advanced AMD to be 26.4% and 43%, respectively. Age (OR = 3.12) and smoking (OR = 1.61) were the major risk factors for the development of GA, while neovascular AMD was found to be associated with white race (OR = 4.22), increasing age (OR = 4.11), hyperopia (OR = 2.31), smoking (OR = 1.91), hypertension (OR = 1.45), increased BMI (OR = 1.43), and presence of

lens opacity (OR = 1.32) (Age-Related Eye Disease Study Group 2000).

Epidemiological studies in Indian populations

Retinal disorders have been implicated to be an important cause of blindness in India (Nirmalan *et al*. 2004). It is estimated that by the year 2050, there would be 244 million people (14.9% of the population) of > 65 years of age in India, which may lead to an increased prevalence of age-related disorders like AMD (Nirmalan *et al*. 2004). Thus, with an increase in proportion of elderly population, limited treatment options and lack of appropriate modalities of intervention to prevent disease progression, AMD would likely become a major public health problem.

Three epidemiological studies have been conducted across different geographical regions of India to estimate the prevalence of AMD. These are the Aravind Comprehensive Eye Study (ACES) from Tamil Nadu (Nirmalan *et al*. 2004), and the Andhra Pradesh Eye Disease Study (APEDS) from Andhra Pradesh (Krishnaiah *et al*. 2005) in southern India, and the INDEYE study from Haryana (Gupta *et al*. 2007) in north India. The prevalence of AMD ranged between 1.4% and 1.8% across these studies (Nirmalan *et al*. 2004; Krishnaiah *et al*. 2005). An increased prevalence of late AMD from 0.5% (50–59 years) to 4.6% (≥70 years) was observed in the INDEYE study (Gupta *et al*. 2007). This increased prevalence was attributed to more number of participants in the higher age group. While, there was no major difference in the disease prevalence between north and south Indian populations, the subtle differences could be attributed to ethnic differences, study design and methodology, clinical parameters and the definition of disease status.

Risk factors for AMD

Based on the epidemiological studies, several risk factors have been identified with the altered risk of AMD. These include age, race, smoking, light exposure, greater BMI, hypertension, cataract surgery and genetic predisposition.

Age

Age was shown to be a consistent risk factor for AMD in various epidemiological studies (see table 1 in electronic supplementary material). Subjects aged between 60–80 years had a three-fold greater risk of developing advanced AMD compared to those < 60 years (Friedman *et al*. 1999; van Newkirk *et al*. 2000; Muñoz *et al*. 2005). In the Indian context, the APEDS and ACES study also demonstrated a higher odds (OR = 3.55, 95%CI, 1.61–7.82 and OR = 2.80, 95%CI, 1.69–4.66, respectively) in subjects > 60 years of age for developing AMD (Nirmalan *et al*. 2004; Krishnaiah *et al*. 2005).

Ethnicity

The prevalence of AMD varied greatly among different ethnic populations (see table 1 in electronic supplementary material). All forms of AMD were relatively more prevalent in the white populations (1.91%–3.5%) compared to the non-whites (0.19%–1.4%) (Friedman *et al*. 1999; Bressler *et al*. 2008). While the underlying mechanism is yet unknown, it was suggested that melanin may perhaps protect against the formation of lipofuscin, which is a marker for cellular senescence and promotes oxidative damage (Kawasaki *et al*. 2008). However, a recent study observed no differences in macular or melanin pigment densities between eyes with and without early AMD (Kayatz *et al*. 2001).

Smoking

Many population-based studies have demonstrated the association of smoking with increased risk of AMD (Delcourt *et al*. 1998; Age-Related Eye Disease Study Research Group 2000; Berendschot *et al*. 2002) and demonstrated that prior and current smokers are prone to develop AMD at least 5–10 years before nonsmokers. A risk ratio of two or higher was observed for neovascular AMD (Klein *et al*. 1998a). Although the exact mechanism by which smoking would affect the retina, RPE, or choroid is unknown, it is hypothesized that AMD would result due to repeated oxidative insults to the outer retina that affects the anti-oxidant metabolism. Smoking is also known to lower the levels of circulating antioxidants (Berendschot *et al*. 2002). Stryker *et al*. (1988) reported that men who smoked one pack of cigarettes per day had only 72% of the plasma β-carotene levels of nonsmokers, even after adjusting for dietary differences between smokers and nonsmokers. In the Indian context, smoking as a risk factor was assessed in terms of smokers and nonsmokers, and light and heavy smokers (with respect to pack years). The APEDS study reported an odds ratio of 3.29 (95%CI, 1.42–7.57) for AMD in individuals with history of cigar smoking (Krishnaiah *et al*. 2005).

Light exposure

There have been conflicting reports on association of ultraviolet or visible light with AMD. Cruickshanks *et al*. (1993) observed that light exposure was not associated with early age-related maculopathy in women but was associated with increased retinal pigment in men after adjusting for age and the amount of time spent outdoors (OR = 1.44, 95%CI, 1.01–2.04). The amount of leisure time spent outdoors in summer was significantly associated with exudative macular degeneration (OR = 2.26, 95%CI, 1.06–4.81) and late maculopathy (OR = 2.19, 95%CI, 1.12–4.25) (Stryker *et al*. 1988). There was no association between estimated ambient UV–B exposure and age-related maculopathy (Stryker *et al*. 1988). Darzins *et al*. (1997) found no association between sunlight exposure and AMD that was also replicated in a population-based study from UK (Khan *et al*. 2006).

Hypertension

Systemic hypertension was found to be associated with neovascular AMD in some studies. Hyman *et al*. (2000) ob-

served that neovascular AMD was associated with diastolic blood pressure >95 mm Hg (OR = 4.4, 95%CI, 1.4–14.2), while non-neovascular AMD showed no association. The association of untreated hypertension with advanced AMD was also reported in the AREDS study (OR = 1.34 for GA and 1.27 for neovascular AMD) (Clemons *et al*. 2005).

Greater BMI

The association of greater BMI with AMD has been reported in some studies (Klein *et al*. 2003; van Leeuwen *et al*. 2003; Johnson 2005). Johnson (2005) reported that the risk of obesity with AMD could be related to the physiologic changes, such as increased oxidative stress, changes in the lipoprotein profile, and increased inflammation resulting in increased cellular destruction and decreased delivery of lutein and zeaxanthin to the macula.

Cataract surgery

There are few studies, which reported cataract surgery as a risk factor for AMD. Wang *et al*. (2003) studied the association of cataract surgery and 5-year incidence of late-stage age-related maculopathy in the patients from the BDES and BMES and observed that either neovascular AMD or GA developed in 6.0%–7.5% of nonphakic eyes compared to 0.7% of phakic eyes. The data from the APEDS indicated a significant association of prior cataract surgery with increased prevalence of AMD (adjusted OR = 3.79, 95%CI, 2.1–6.78) (Nirmalan *et al*. 2004).

Genetic predisposition

Genetic predisposition to AMD was initially indicated by case–control association studies (Hyman *et al*. 1983). This was further supported by evidences of familial aggregation of the disease (Klein *et al*. 1994; Silvestri *et al*. 1994; Seddon *et al*. 1997; Klaver *et al*. 1998a), segregation analysis (Heiba *et al*. 1994; Yates and Moore 2000), twin studies (Meyers *et al*. 1995; Hammond *et al*. 2002) and classical linkage studies (Klein *et al*. 1998a; Weeks *et al*. 2000; Majewski *et al*. 2003; Schick *et al*. 2003; Seddon *et al*. 2003). A higher concordance of AMD was reported among monozygotic than dizygotic twins (Meyers *et al*. 1995; Hammond *et al*. 2002) and a higher disease risk among the first-degree relatives of AMD probands provided further evidence that genetic factors might play a key role in AMD pathogenesis (Klein *et al*. 1994; Silvestri *et al*. 1994; Seddon *et al*. 1997; Klaver *et al*. 1998a).

Genetics of AMD

Several strategies have been undertaken to identify the precise genomic regions involved in AMD pathogenesis. The initial strategies involved screening genes that were functionally relevant to hereditary macular dystrophies, which exhibit certain commonalities with AMD (Stone *et al*. 2001). But majority of these genes failed to show any significant association with AMD (table 2).

Table 2. Candidate genes screened in AMD and their association status in various studies.

Chromosomal region	Gene	Function	Total number of studies	Association with AMD	References	No association with AMD	References
1p22.1-21	*ABCR*	Transports all-trans-retinylidene phosphatidylenalamine to the disc across the disc membrane	12	3	Allikmets *et al*. 1997; Allikmets *et al*. 2000; Shroyer *et al*. 2001	9	Kuroiwa *et al*. 1999; De La Paz *et al*. 1999; Fuse *et al*. 2000; Rivera *et al*. 2000; Souied *et al*. 2000; Guymer *et al*. 2001; Webster *et al*. 2001; Berstein *et al*. 2002; Baum *et al*. 2003;
1q21-q23	*CRP*	Acute phase reactant protein and inflammatory marker	3	0		3	Despriet *et al*. 2006; Schaumberg *et al*. 2006; Kim *et al*. 2008;
1q25-31	*RGS16*	The gene product is a regulator of 'G protein signaling family	1	0		1	Conley *et al*. 2005
1q25-31	*OCLM*	Involved in the function of trabecular mesh work	1	0		1	Conley *et al*. 2005
1q25-31	*PRELP*	Encodes a protein which is a component of extracellular matrix connective tisssue	1	0		1	Conley *et al*. 2005

Table 2 *(contd)*

Chromosomal region	Gene	Function	Total number of studies	Association with AMD	References	No association with AMD	References
1q25-31	*LAMC1, LAMC2, LAMC3*	Extracellular matrix glycoproteins, major constituents of basement membranes	2	0		2	Hayashi *et al*. 2004; Conley *et al*. 2005
1q25.3-q31.1	*HMCN1*	Extracellular matrix protein	8	2	Schultz *et al*. 2003; Thompson *et al*. 2008	6	Abecasis *et al*. 2004; McKay *et al*. 2004; Bojanowski *et al*. 2005; Fuse *et al*. 2006; Fisher *et al*. 2007; Seitsonen *et al*. 2006
1q31.2-q31.3	*GLRX2*	Participates in cellular redox reactions	1	0		1	Conley *et al*. 2005
1q32	*CFH*	Regulates the alternate complement pathway	43	43	As in table 3	0	0
1q41	*TGFB2*	Growth factor involved in various cellular pathways	1	0		1	Conley *et al*. 2005
1q41-42	*ADPRT1*	Poly (ADP-ribosyl) transeferase enzyme which modifies nuclear proteins	1	0		1	Esfandiary *et al*. 2005
1q42.1	*EPHX1*	Activation and detoxification of epoxides	1	0		1	Esfandiary *et al*. 2005
2p16	*GPR75*	Member of G protein coupled receptor family	1	0		1	Sauer *et al*. 2001
2p	*EFEMP1*	Extracellular matrix protein	3	0		3	Stone *et al*. 1999; Guymer *et al*. 2002; Iyengar *et al*. 2004
2q14	*ILIA*	Member of interleukin 1 cytokine family	1	0		1	Haines *et al*. 2006
3p25.1	*FIBULIN 2*	Encodes an extracellular matrix protein	1	0		1	Stone *et al*. 2004
3p21.3	*GPX1*	Member of glutathione peroxidase family and functions in the detoxification of hydrogen peroxide	1	0		1	Esfandiary *et al*. 2005
3q12.2-q12.3	*IMPG2*	Interphotoreceptor matrix proteoglycan-2, occupying the inerface between the retinal pigment epithelium and photoreceptor cells	1	0		1	Kuehn *et al*. 2001
4q35	*TLR3*	Pathogen recognition and activation of innate immunity	3	2	Edwards *et al*. 2008; Yang *et al*. 2008	1	Cho *et al*. 2009
6p21.2-p12.3	*RDS*	Encodes a transmembrane protein which mediates signal transduction	1	0		1	Shastry *et al*. 1999

Table 2 *(contd)*

Chromosomal region	Gene	Function	Total number of studies	Association with AMD	References	No association with AMD	References
6p21.3	*BF*	Component of alternate complement pathway	8	8	Gold *et al*. 2006; Maller *et al*. 2006; McKay *et al*. 2009; Spencer *et al*. 2007; Jakobsdottir *et al*. 2008; Kaur *et al*. 2009; Francis *et al*. 2009; Richardson *et al*. 2009; Sawitzke *et al*. 2009;	0	
6p21.3	*C2*	Component of alternate complement pathway	8	8	As in table 4	0	
6p12	*VEGF*	Mitogen for endothelial cells, induces angiogeneis and vasculogenesis	5	3	Churchill *et al*. 2006; Haines *et al*. 2006; Lin *et al*. 2008	2	Boekhoorn *et al*. 2008; Richardson *et al*. 2007
6q14	*ELOVL4*	Participates in the biosynthesis of fatty acids	2	0		2	Ayyagari *et al*. 2001; Seitsonen *et al*. 2006
7p15	*AhR*	Encodes a ligand-activated transcription factor involved in the regulation of biological responses to aromatic hydrocarbons	0	0		1	Esfandiary *et al*. 2005
8p22	*NAT2*	Encodes an enzyme that functions to both activate and deactivate arylamine and hydrazine drugs and carcinogens	1	0		1	Esfandiary *et al*. 2005
9q32-q33	*TLR4*	Pathogen recognition and activation of innate immunity	4	1	Zareparsi *et al*. 2005	3	Kaur *et al*. 2006; Despreit *et al*. 2008; Edwards *et al*. 2008;
9q33-34	*C5*	Plays an important role in inflammatory and cell killings processes	1	1	Yates *et al*. 2007	0	
10q26	*ARMS2*	Unknown function, the protein is localized to mitochondrial outer membrane	27	27	As in table 5	0	
10q26	*HTRA1*	Serine protease which gets activated during cellular stress	20	20	As in table 6	0	
10q24.3-qter	*CYP2E1*	Encodes a member of the cytochrome P450 superfamily of enzymes	1	0		1	Esfandiary *et al*. 2005
11p13	*CAT*	Encode antioxidant enzymes which protect cells from toxic effect	1	0		1	Esfandiary *et al*. 2005

Table 2 *(contd)*

Chromosomal region	Gene	Function	Total number of studies	Association with AMD	References	No association with AMD	References
11q13	*VMD2*	Involved in ion channel formation and transport of bicarbonate ions	5	0		5	Allikmets *et al*. 1999; Kramer *et al*. 2000; Lotery *et al*. 2000; Akimoto *et al*. 2001; Seddon *et al*. 2001
12p13.3-p12.3	*A2M*	Protease inhibitor and cytokine transporter	1	0		1	Haines *et al*. 2006
12p12.3-p12.1	*MGST1*	Encodes a protein that catalyzes the conjugation of glutathione to electrophiles and the reduction of lipid hydroperoxides	1	0		1	Haines *et al*. 2006
14q32	*CKB*	Encodes a cytoplasmic enzyme involved in energy homeostasis	1	0		1	Haines *et al*. 2006
15q24.1	*CYP1A1*	Encodes a member of the cytochrome P450 superfamily of enzymes	1	0		1	Esfandiary *et al*. 2005
15q24.1	*CYP1A2*	Encodes a member of the cytochrome P450 superfamily of enzymes	1	0		1	Esfandiary *et al*. 2005
17q23-qter	*APOH*	Involved in lipoprotein metabolism, coagulation, and the production of antiphospholipid autoantibodies	1	0		1	Conley *et al*. 2005
17q25	*ITGB4*	Transmembrane receptor involved with maintenance of basal cell-matrix adhesion and binding of laminins	1	0		1	Conley *et al*. 2005
19p13.3-p13.2	*C3*	Activator of classical and alternate complement pathway	7	7	Maller *et al*. 2006; Yates *et al*. 2007; Spencer *et al*. 2008; Despriet *et al*. 2009; Francis *et al*. 2009; Park *et al*. 2009; Sawitze *et al*. 2009;	0	
19q	*APOE*	Plays a key role in cholesterol metabolism	11	6	Klaver *et al*. 1998; Souied *et al*. 1998; Simonelli *et al*. 2001; Baird *et al*. 2004; Bojanowski *et al*. 2006; Sanchez *et al*. 2006;	5	Schmidt *et al*. 2000; Gotoh *et al*. 2004; Zareparsi *et al*. 2004; Kaur *et al*. 2006; Wong *et al*. 2006;
22q12.3	*TIMP3*	Inibitor of matrixmetalloproteinases	1	0		1	Felbor *et al*. 1997

Table 2 *(contd)*

Chromosomal region	Gene	Function	Total number of studies	Association with AMD	References	No association with AMD	References
22q13.1	*CYP2D6*	Gene encodes a member of the cytochrome P450 superfamily of enzymes	1	0		1	Esfandiary *et al*. 2005

Studies involving classical linkage analysis were not very successful in a complex disease like AMD due to the late age at onset, phenotypic variability and unavailability of large affected families. Nonetheless, whole genome scans in AMD led to mapping of > 31 loci across multiple chromosomes (Haddad *et al*. 2006; Swaroop *et al*. 2009). A schematic representation of these loci is available in a recent review by Swaroop and colleagues (Swaroop *et al*. 2009). Among the mapped loci, two regions on 1q32 and 10q26 have been replicated across multiple studies (Klein *et al*. 1998a; Seddon *et al*. 2003; Kenealy *et al*. 2004; Iyengar *et al*. 2004; Weeks *et al*. 2004) and were further validated through a meta analysis of multiple whole genome scans (Fisher *et al*. 2005).

Recent advances in genotyping technologies have paved the way for gene identification in complex diseases. Genome-wide association studies (GWAS) in large cohorts with high density markers like SNPs on microarray platforms have revealed several susceptible loci in complex diseases like diabetes (Scott *et al*. 2007) rheumatoid arthritis (The Wellcome Trust Case Control Consortium 2007), SLE (Hom *et al*. 2008) and AMD (Klein *et al*. 2005). These technologies backed up by robust statistical methodologies have helped in identifying precise disease intervals on chromosomes that have been largely replicated in multiple ethnic groups worldwide (Seng and Chia 2008).

Candidate genes in AMD

The complement factor H (CFH)

The first major gene to be associated in AMD was the complement factor H (*CFH*) on 1q32 that was independently characterized in three different cohorts (Edwards *et al*. 2005; Haines *et al*. 2005; Klein *et al*. 2005). This was perhaps the first success story of mapping a gene using GWAS. A coding variant Y402H (rs1061170, T>C) in exon 9 of *CFH* was significantly associated with AMD. This association was widely replicated in multiple ethnic groups worldwide, except in the Japanese (table 3). Individuals with the risk allele 'C' exhibited an increased risk of AMD with OR ranging between 2.4 and 4.6 for a single copy and 3.3–7.4 for two copies of the variant allele in these studies that was further confirmed through a meta analysis (Thakkinstian *et al*. 2006). Haplotype analysis with intragenic SNPs flanking rs1061170 indicated a risk haplotype in the background of the risk allele 'C' in multiple studies (see table 2 in electronic supplementary material).

A parallel study by Hageman *et al*. (2005) explored the other genetic variations in *CFH* using two case–control cohorts from Iowa and Columbia. Several single nucleotide polymorphisms (SNPs) were identified that were significantly associated with AMD and the strongest association was observed with A473A (rs2274700) in the Iowan and IVS10 (rs203674) in the Columbian cohort. Most of these SNPs were located in the functional domains of *CFH* and hence would affect the expression levels and binding efficiencies with its ligands. Real-time PCR using human RPE cell lines confirmed that the transcripts of factor H (*FH* and *FH1*) were abundantly present in the RPE and choroid. Immunohistochemical analysis of human donor eyes with AMD revealed strong immuno reactivity for *CFH* within the macula compared to controls supporting the role of complement activation in drusen formation (Hageman *et al*. 2005).

To understand the potential role of the *CFH* variants, subsequent studies analysed the entire genomic region of *CFH* and its flanking regions to assess their contributions in AMD susceptibility (Li *et al*. 2006; Maller *et al*. 2006). Multiple SNPs in the coding and non-coding regions were observed that contributed to the disease susceptibility. These SNPs exhibited stronger associations compared to the rs1061170 (Y402H), indicating the potential role of other determinants, specially the non-coding variants in AMD susceptibility (Li *et al*. 2006; Maller *et al*. 2006).

Due to the high homology between *CFH* and the flanking *CFH*-related genes (*CFHR*), Hughes and co-workers (2006) genotyped polymorphisms spanning *CFH* and *CFHR* and observed a haplotype that comprised of deletions at *CFHR1* and *CFHR3* with reduced risk of AMD. The gene product could not be detected in the serum of individuals homozygous for this haplotype. The association of deletion at *CFHR1* and *CFHR3* was tested in a Caucasian cohort that accounted towards reduced risk of AMD with certain haplotypes in *CFH* (Spencer *et al*. 2008a). However, the association was no longer significant ($P = 0.07$) after adjusting for smoking, *CFH* (rs1061170) and *LOC387715* (rs10490924) SNPs suggesting other protective variants in this region. Further studies in Caucasian populations (American and British) have exhibited associations in multiple *CFH*-related genes (see table 3 in electronic supplementary material) with AMD that are yet to be replicated in other populations.

The biochemistry of CFH

The complement system is an integral part of innate immunity that can be activated by three pathways; namely,

Table 3. Studies on AMD-associated *CFH* gene variant Y402H in different populations.

Population	Cases (*n*)	Controls (*n*)	Association with *Y402H*	*P* value	Odds ratios (95%CI)	Other associated SNPs	References
American	450	262	Yes	3.70×10^{-8}	2.4 (1.75–3.30)	1 exonic, 4 intronic	Edwards *et al*. 2005
American	96	50	Yes	NA	NA	2 intronic	Klein *et al*. 2005
American	495	185	Yes	0.00006	2.45 (1.41–4.25)	1 exonic, 1 intronic	Haines *et al*. 2005
American	66	58	Yes	1.7×10^{-3}	3.2 (1.52–6.73)	None	Narayanan *et al*. 2007
American	211	183	Yes	<0.0001	2.40 (2.07–2.73)	3 exonic, 3 intronic	Francis *et al*. 2007a
American	1238	934	Yes	1.79×10^{-59}	2.91 (2.44–3.47)	1 exonic, 8 intronic	Hughes *et al*. 2007
German	794	612	Yes	6.7×10^{-29}	6.72 (5.14–8.79)*	None	Rivera *et al*. 2005
American	549	272	Yes	1.64×10^{-13}	2.25 (1.70–2.75)	3 exonic, 4 intronic	Hageman *et al*. 2005
American	111	401	Yes	5×10^{-3}	1.59 (1.04–2.44)	None	Schaumberg *et al*. 2006
American & Icelandic	1330	1265	Yes	3.86×10^{-18}	1.99 (1.7–2.33)	None	Magnusson *et al*. 2006
American	544	268	Yes	$<10^{-25}$	NA	2 promoter, 47 intronic, 2 exonic	Li *et al*. 2006
American	437	1015	Yes	<0.001	2.09 (1.66–2.62)	None	Schaumberg *et al*. 2007
American	616	275	Yes	1×10^{-13}	3.02 (2.24–4.07)	None	Zareparsi *et al*. 2005a
British	443	262	Yes	<0.0005	2.71 (2.17–3.39)	None	Sepp *et al*. 2006
British	173	170	Yes	5.96×10^{-6}	2.01 (1.48–2.73)	2 exonic, 8 intronic	Hughes *et al*. 2006
American	168	108	Yes	8×10^{-7}	3.5 (2.11–5.89)	1 intronic	Conley *et al*. 2005
American	574	280	Yes	4.18×10^{-8}	2.24 (1.67–2.99)	None	Seddon *et al*. 2006
Austrian	179	163	Yes	1×10^{-8}	3.64 (2.32–5.71)	None	Wegscheider *et al*. 2007
American	89	230	Yes	5.4×10^{-6}	3.17 (1.9–5.27)	None	Pulido *et al*. 2007
American	584	248	Yes	3.88×10^{-8}	1.82 (1.35–2.46)	3 exonic, 1 intronic	Spencer *et al*. 2007a
European	242	157	Yes	4.7×10^{-9}	3.49 (2.28–5.36)	None	Weger *et al*. 2007
Japanese	96	89	Yes	1.16×10^{-6}	4.9 (2.51–9.53)	None	Okamoto *et al*. 2006
Japanese	80	192	No	0.25	0.58 (0.24–1.36)	None	Fuse *et al*. 2006
Japanese	63	107	No	0.52	1.3(0.68–2.49)	None	Uka *et al*. 2006
Japanese	188	139	No	0.1	0.59 (0.32–1.11)	2 exonic, 1 intronic	Mori *et al*. 2007
Chinese	163	232	Yes	6.5×10^{-4}	4.24 (1.73–10.34)	None	Lau *et al*. 2006
Chinese	163	244	No	0.2	NA	1 promoter, 1 intronic	Chen *et al*. 2006
Chinese	121	132	No	0.353	1.33 (0.73–2.45)	None	Xu *et al*. 2008
Chinese	163	155	No	NA	NA	2 Promoter, 2 intronic, 4 exonic	Kin Ng *et al*. 2008
Chinese (Taiwan)	133	180	Yes	1×10^{-4}	3.25 (1.76–6.02)	None	Lin *et al*. 2008a
Han Chinese	144	126	Yes	0.003	2.63 (1.36–5.07)	None	Chu *et al*. 2008
Korean	114	187	No	0.071	1.8 (0.96–3.46)	1 promoter, 1 Intronic, 1 Exonic	Kim *et al*. 2008b
French	141	91	Yes	1.64×10^{-4}	2.86 (1.64–4.99)	None	Souied *et al*. 2005
Finnish	154	105	Yes	9.7×10^{-5}	2.73 (1.63–4.5)	None	Seitsonen *et al*. 2006
Indian	100	120	Yes	1.19×10^{-7}	NA	1 intronic, 1 exonic	Kaur *et al*. 2006
Australian	236	144	Yes	<0.001	2.7 (2–3.68)	None	Baird *et al*. 2006
Swiss	425	50	Yes	1×10^{-5}	2.57 (1.66–3.96)	None	Droz *et al*. 2008
Israeli	240	118	Yes	0.0002	1.9 (1.3–2.6)	None	Chowers *et al*. 2008
American	701	175	Yes	3.23×10^{-9}	2.75 (1.95–3.87)	None	Conley *et al*. 2006
American	126	1051	Yes	1.5×10^{-4}	2.02 (1.39–2.94)	None	Conley *et al*. 2006
American	1882	215	Yes	2.4×10^{-14}	2.5 (2.0–3.2)	1 exonic, 1 intronic	Bergeron-Sawitzke *et al*. 2009
Russian	155	151	Yes	0.003	NA	None	Fisher *et al*. 2007
Greeks	100	115	Yes	0.001	NA	None	Marioli *et al*. 2009

NA, data not available.

classical, lectin and alternate (Edwards *et al*. 2005; de Córdoba and de Jorge 2008). These pathways result in the formation of C3 convertase. Subsequent downstream activities in the complement system result in the formation of the membrane attack complex (MAC) and cell lysis (de Córdoba and de Jorge 2008). *CFH* is a regulator of the alternate complement pathway and is composed of 20 repetitive units of 60 amino acids known as short consensus re-

peats (SCR) or complement component modules (Edwards *et al*. 2005; de Córdoba and de Jorge 2008). The Tyr402His (rs1061170) polymorphism lies within SCR7 domain, that has binding sites for heparin, C-reactive protein (CRP) and M protein. The CRP is an activator of the classical complement pathway and helps in the decreased deposition of MAC by binding with *CFH*. Based on *in vitro* evidences, it has been suggested that the Tyr402His change may affect the ligand-binding properties of *CFH* which in turn affects its function resulting in aberrant complement activation leading to destruction of bystander host cells (Laine *et al*. 2007; Sjöberg *et al*. 2007; Ormsby *et al*. 2008). Spatial and temporal expressions of *CFH* have indicated higher level of expression in the ageing ocular tissues like the neural retina (Mandal and Ayyagari 2006). It is yet unclear if this expression is required to protect the tissue from excessive inflammation.

Complement factor B (BF) and complement component 2 (C2)

The discovery of *CFH* provided a significant breakthrough in understanding the role of complement genes and their

Table 4. Distribution of the associated variants in *C2*, *BF* and *C3* genes in AMD in various studies.

Gene	SNPs	Population	Cases	Controls	*P* value	Odds ratio (95%CI)	References
C3	rs2250656	English	437	254	0.12	1.53 (1.1–2.13)	Yates *et al*. 2007
	rs2230199	European	2712	934	4.51×10^{-12}	1.66	Maller *et al*. 2007
		English	590	346	5.9×10^{-5}	1.55 (1.13–2.14)	Yates *et al*. 2007
		Caucasian	701	286	0.001	1.53 (1.1–2.13)	Spencer *et al*. 2008
		Danish	357	173	NA	NA	Despreit *et al*. 2009
		Caucasian	202	184	0.64	1.43 (0.90–2.29)	Spencer *et al*. 2008
		Caucasian	438	298	0.008	1.65(1.15–2.63)	Spencer *et al*. 2008
		American	421	215	0.012	1.67 (1.11–2.52)	Bergeron-Sawitzke *et al*. 2009
	rs2230203	English	350	602	0.007	0.65 (0.46–0.91)	Yates *et al*. 2007
	rs2230204		444	266	0.31	0.86 (0.62–1.2)	
	rs2277984		427	254	0.89	0.95(0.70–1.30)	
	rs3745568	European	2712	934	0.83	0.98	Maller *et al*. 2007
	rs344542	English	389	259	0.53	1.08 (0.78–1.50)	Yates *et al*. 2007
		Caucasian	433	294	0.99	1.0 (0.73–1.35)	Spencer *et al*. 2008
	rs2241393	European	2712	934	0.04	0.88	Maller *et al*. 2007
		English	436	261	0.06	0.80 (0.58–1.09)	Yates *et al*. 2007
	rs344550	European	2712	934	0.30	1.07	Maller *et al*. 2007
		English	323	243	0.73	1.03(0.71–1.47)	Yates *et al*. 2007
C2	rs9332739	American	897	381	4.14×10^{-6}	0.36 (0.23–0.56)	Gold *et al*. 2006
		European	1298	934	$1.16x10^{-6}$	NA	Maller *et al*. 2006
		Caucasian	698	282	0.02	0.48 (0.24–0.99)	Spencer *et al*. 2007b
		American	187	168	0.665	0.22(0.10–0.48)	Jakobsdottir *et al*. 2007
		British	318	243	0.04	0.52(0.25–1.04)	McKay *et al*. 2009
		Caucasian	211	187	NA	NA	Francis *et al*. 2009
		Australian	565	204	0.24	1.0 (0.76–2.94)	Richardson *et al*. 2009
		Indian	177	175	0.223	0.66(0.34–1.30)	Kaur *et al*. 2009
	rs547154	American	894	382	8.45×10^{-8}	0.44(0.33–0.60)	Gold *et al*. 2006
		European	1298	934	1.30×10^{-7}	NA	Maller *et al*. 2006
		Caucasian	698	282	9.2×10^{-6}	NA	Spencer *et al*. 2007b
		American	187	168	0.00011	NA	Jakobsdottir *et al*. 2007
		Australian	565	204	9.1×10^{-5}	2.28 (1.52–3.4)	Richardson *et al*. 2009
		Indian	177	175	5.4×10^{-11}	0.24 (0.16–0.38)	Kaur *et al*. 2009
BF	rs4151667	American	1092	546	NA	NA	Gold *et al*. 2006
		European	1298	934	0.0002	NA	Maller *et al*. 2006
		American	187	168	0.666	NA	Jakobsdottir *et al*. 2007
		British	318	243	0.16	0.63 (0.32–1.23)	McKay *et al*. 2009
		Australian	565	204	0.17	1.59(0.82–3.09)	Richardson *et al*. 2009
		Indian	177	175	0.16	0.61(0.31–1.22)	Kaur *et al*. 2009
	rs12614	American	1096	550	NA	NA	Gold *et al*. 2006
		Caucasian	698	282	0.75	NA	Spencer *et al*. 2007b
		Indian	177	175	0.75	0.92 (0.59–1.44)	Kaur *et al*. 2009
	rs641153	American	1096	550	NA	NA	Gold *et al*. 2006

Table 4 *(contd)*

Gene	SNPs	Population	Cases	Controls	*P* value	Odds ratio (95%CI)	References
		European	1298	934	5.5×10^{-13}	NA	Maller *et al*. 2006
		Caucasian	698	282	2.3×10^{-5}	NA	Spencer *et al*. 2007b
		British	318	243	0.00008	0.40 (0.24–0.65)	McKay *et al*. 2009
		Australian	565	204	7.0×10^{-5}	2.31 (1.55–3.45)	Richardson *et al*. 2009
		Indian	177	175	2.2×10^{-7}	0.28 (0.17–0.46)	Kaur *et al*. 2009
	rs1048709	Caucasian	698	282	0.75	NA	Spencer *et al*. 2007b
		British	318	243	0.11	0.79 (0.59–1.07)	McKay *et al*. 2009
		Australian	565	204	0.64	1.07 (0.80–1.43)	Richardson *et al*. 2009
		Indian	177	175	0.41	0.85 (0.59–1.24)	Kaur *et al*. 2009
	rs4151669	American	184	184	NA	NA	Gold *et al*. 2006
		Indian	177	175	0.11	0.57(0.28–1.44)	Kaur *et al*. 2009
	rs4151670	American	184	184	NA	NA	Gold *et al*. 2006
		Indian	177	175	0.99	0.99 (0.06–15.87)	Kaur *et al*. 2009
	rs4151651	American	184	184	NA	NA	Gold *et al*. 2006
		Indian	177	175	0.57	1.98 (0.18–21.97)	Kaur *et al*. 2009
		British	318	243	0.67	1.13 (0.62–2.10)	McKay *et al*. 2009
	rs45600936	American	184	184	NA	NA	Gold *et al*. 2006
		Indian	177	175	0.99	0.99(0.06–15.87)	Kaur *et al*. 2009
	rs4151659	American	182	184	NA	NA	Gold *et al*. 2006
		Australian	565	204	0.35	1.64 (0.54–4.93)	Richardson *et al*. 2009
	rs2072633	Caucasian	698	282	0.59	NA	Spencer *et al*. 2007b
		American	187	168	0.09	NA	Jakobsdottir *et al*. 2007
		British	318	243	0.05	1.27 (0.99–1.67)	McKay *et al*. 2009
		Australian	565	204	0.37	1.12 (0.87–1.44)	Richardson *et al*. 2009
		Indian	177	175	2.0×10^{-4}	0.53 (0.38–0.75)	Kaur *et al*. 2009

NA, data not available.

biological mechanism in AMD susceptibility (Swaroop *et al*. 2009). Following this, other genes involved in the complement cascade were explored; some of which, exhibited very strong association with AMD (table 4). Two paralogous genes, viz. the complement factor B (*BF*) and complement component C2 (*C2*) located on 6p21 in the major histocompatibility complex III were found to be associated with AMD (Gold *et al*. 2006). The *BF* and *C2* are activators of alternate and classical complement pathways, respectively. Among the variations initially observed in *BF*, two SNPs (rs4151667 (L9H) and rs641153 (R32Q)) exhibited a higher frequency in the normal controls (Gold *et al*. 2006). Further screening of haplotype tagging SNPs in an additional cohort identified four SNPs which were significantly associated with AMD: rs4151667 (L9H), rs641153 (R32Q) in *BF* gene and rs547154 (IVS10) and rs9332739 (E318D) in *C2* gene. The L9H and R32Q variants of *BF* were in complete linkage disequilibrium (LD) with E318 and IVS10 variants of *C2*, respectively, and were shown to be protective for AMD (Gold *et al*. 2006). This was further supported by haplotype analysis wherein, haplotypes tagged by R32Q ($P = 2.1 \times 10^{-7}$) and those containing *C2* E318D/*BF* L9H ($P = 3.4 \times 10^{-6}$) were highly protective for AMD. The common haplotype with the normal alleles at these four SNP loci conferred risk for AMD (OR = 1.32; $P = 0.0013$). Further analysis with the Y402H variant (*CFH*) indicated that 56% of unaffected controls had a protective allele either at Y402H or *C2/BF* loci and 74% of AMD cases did not bear any protective allele (Gold *et al*. 2006).

The associated variants in *BF* and *C2* were further replicated in Caucasian cohorts from US (Maller *et al*. 2006; Spencer *et al*. 2007b), Australia (Richardson *et al*. 2009) and UK (McKay *et al*. 2009). The study by Spencer *et al*. (2007b) identified new variants in *BF* associated with AMD (R150R, IVS17 and R32W) in a family based and case–control data set. Unlike previous reports, this study did not indicate a strong LD ($r^2 = 0.002$) between *BF* R32Q and *C2* E318D and these SNPs were shown to be independently associated with AMD (Spencer *et al*. 2007b). When smoking was considered after controlling for age, Y402H (*CFH*) and A69S (*ARMS2*), the association of R32Q was unaffected ($P < 0.0001$, OR = 0.21; 95%CI, 0.11–0.39) but the effect of E318D was diminished ($P = 0.26$, OR = 0.60, 95%CI, 0.25–1.47) (Spencer *et al*. 2007b). The IVS10 and R32Q variants were also protective in an Australian case–control cohort with AMD (Richardson *et al*. 2009). When these SNPs were screened in family-based and case–control datasets of whites, IVS10 was the most significantly associated SNP in both the cohorts (Jakobsdottir *et al*. 2008). The variants rs9332739 and rs4151667 were also analysed to assess disease progression in Caucasians by looking at the disease stage at the end of a two year follow up period. The minor alleles of these

SNPs exhibited reduced rate of progression (OR = 0.35, 95%CI, 0.2–0.6, $P = 0.0001$) (Francis *et al*. 2009).

A real time PCR analysis of BF and C2 proteins with neural retina, RPE and choroids from donor eyes of AMD donors and controls indicated a higher immunoreactivity for BF protein in ocular drusen and bruch's membrane compared to choroidal stroma (Gold *et al*. 2006). A functional basis of protection conferred by the R32Q variant in *BF* was indicated based on a reduced activity and lower binding affinity of the 32Q with C3b to produce the MAC (Lokki and Koskimies 1991; Montes *et al*. 2009). Coupled with the genetic data, the abnormalities in *BF* strongly indicate an unregulated activation of the alternative pathway of which *BF* is a critical enzyme and not the classical pathway and *C2*.

Complement component 3

The complement component 3 is an important plasma protein, whose activity is critical for the formation of the terminal MAC that leads to cell lysis. The association of SNPs across the *C3* gene (table 4) was tested in two independent case–control cohorts of English (discovery) and Scottish (replication) participants (Yates *et al*. 2007). Among the 10 *C3* variants selected from the HapMap database, the rs2230199, which is a functional polymorphism resulting in the substitution of arginine to glycine (R102G), was shown to be strongly associated with the English ($P = 5.9 \times 10^{-5}$) and the Scottish cohort ($P = 5 \times 10^{-5}$). The glycine variant, referred to as fast (*C3*F) based on the electrophoretic mobility exhibited a higher OR for the homozygotes (OR = 2.6, 95%CI, 1.6–4.1) and heterozygotes (OR = 1.7, 95%CI, 1.3–2.1) respectively, compared to arginine homozygotes (refered to as slow, *C3*S). Since the positively charged arginine is replaced with a neutral glycine, it was proposed that variants of this SNP may result in different functional properties of the gene (Yates *et al*. 2007). The previously associated non-synonymous SNP rs1047286 exhibited a weak association to rs2230199. Logistic regression indicated that rs1047286 did not predict risk for AMD and it was solely contributed by rs2230199 (Yates *et al*. 2007).

Subsequent studies in Caucasians (Maller *et al*. 2007; Bergeron-Sawitzke *et al*. 2009; Francis *et al*. 2009) replicated the initial findings that the casual variant associated was rs2300199. Some studies (Spencer *et al*. 2008b; Despriet *et al*. 2009) however, observed that the rs1047286, which was in LD with rs2300199, exhibited significant association with AMD. Park *et al*. (2009) observed a strong association with rs2230199 ($P = 9.2 \times 10^{-5}$) and rs1047286 ($P = 4.1 \times 10^{-5}$). Conditional analysis further indicated that either rs2230199 or rs1047286 that were in LD ($r^2 = 0.85$), were the only associated SNPs with AMD (Park *et al*. 2009).

ARMS2 and HTRA1

The linkage to the second major AMD susceptibility locus on 10q26 was identified by Weeks *et al*. (2000), that was further confirmed in other studies (Majewski *et al*. 2003; Seddon *et al*. 2003; Iyengar *et al*. 2004; Kenealy *et al*. 2004). This was also validated by a meta-analysis of six genome-wide scans that provided the strongest evidence for an AMD susceptibility locus on 10q26 with a significant linkage (Fisher *et al*. 2005).

The 10q26 was subjected to high-density custom SNP genotyping using 199 SNPs over a 13.4 Mb region. Several two point non-parametric LOD scores were observed of which, the highest HLOD was seen at the region harbouring *PLEKHA1/LOC387715/PRSS11* gene cluster. Further genotyping with SNPs overlying these genes indicated association, but could not implicate any specific gene (Jakobsdottir *et al*. 2005).

Subsequent studies strongly suggested *LOC387715* (now known as *ARMS2*) as the AMD susceptibility gene (Rivera *et al*. 2005; Schmidt *et al*. 2006). Association analysis with 93 SNPS over a 22 Mb region covering the *PLEKHA1* and *ARMS2* genes in two independent case–control German cohorts (Rivera *et al*. 2005), indicated a region of strong LD region of ~60 kb between these genes. To identify the risk-associated variants, AMD cases ($n = 24$) and controls ($n = 8$) were resequenced that indicated the association of rs1045216 ($P = 1.2 \times 10^{-7}$) in exon 12 of *PLEKHA1* and rs10490924 ($P = 3.9 \times 10^{-34}$) in exon 1 of *ARMS2* with AMD (Rivera *et al*. 2005). Conditional modelling indicated that rs10490924 contributed to the disease risk that was further validated in an American cohort using family-based and case–control datasets (Schmidt *et al*. 2006). The interaction between smoking (number of pack years) and genotypes at rs10490924 (A69S) revealed that the relative frequency of the risk genotype 'TT' linearly increased with increasing pack-years of smoking irrespective of age and gender ($P = 0.05$) (Schmidt *et al*. 2006). Case–control studies indicated a similar profile for the risk allele of rs10490924 across Caucasians, Americans, Russian and Asians (table 5).

In a separate study, the association of rs10490924 with AMD was demonstrated to be consistent in three case–control cohorts viz. National Eye Institute (NEI), Age Related Eye Disease Study (AREDS), from the United states and the Blue Mountain Eye Study (BMES) from Australia (Ross *et al*. 2007). This study reported slightly lower ORs in the BMES cohort due to the presence of early AMD cases unlike in the US-based cohorts. The study has also assessed the combined risk of smoking and rs10490924 SNP. It was also observed that among the subjects with the homozygous risk genotype, history of smoking was associated with a higher risk of AMD (OR = 23.3, 95%CI, 6.92–78.6) in the NEI and AREDS cohort, compared to the non-smokers (OR = 6.34, 95%CI, 2.84–14.2). However, there was no such difference in the BMES cohort ($P = 0.953$) (Ross *et al*. 2007).

A novel 443-bp indel polymorphism in the 3'UTR of *ARMS2* was shown to be associated with unstable *ARMS2* mRNA (Fritsche *et al*. 2008). The ARMS2 protein was immunolocalized to the mitochondria-enriched ellipsoid region of the inner segments of rods and cones. This indel removed

the polyadenylation signal and led to the insertion of a 54-bp AU rich element, which is known to control mRNA decay in transcripts. The indel was significantly associated with AMD ($P = 4.1 \times 10^{-29}$). Functionally, subjects homozygous for the indel variant lacked the ARMS2 protein expression, which could be due to an unstable mRNA, thereby affecting mitochondrial homeostasis (Fritsche *et al*. 2008). A case–control study in the Japanese cohort replicated the association of this indel variant (Gotoh *et al*. 2009). Haplotype bearing the T allele of rs10490924 and the indel variant showed an OR of 3.14 ($P = 7.8 \times 10^{-6}$).

Parallel studies on GWAS indicated *HTRA1* as one of the potential AMD susceptible genes on 10q26. Based on the location of the *ARMS2* variant rs10490924 between two genes *PLEKHA1* and *HTRA1*, and the fact that *ARMS2* had a low sequence homology across species, it was speculated that this SNP could be in LD with a putative variant in a different gene (DeWan *et al*. 2006). Resequencing of *PLEKHA1* and *HTRA1* in a case–control cohort, identified an SNP (rs11200638) in *HTRA1* located 512-bp upstream of the *HTRA1* putative transcriptional start site that was in complete LD ($D' = 0.99$) with rs10490924; both these SNPs exhibited similar genotype frequencies ($P = 8.2 \times 10^{-12}$). The *HTRA1* promoter sequence predictions based on computational analysis revealed that the wild-type allele at rs11200638 is a part of CpG island within the putative binding site for the transcription factors adaptor related protein 2α (AP2α) and serum response factor (SRF). The risk allele of *HTRA1* altered the binding affinity of AP2α and SRF to the *HTRA1* promoter. Chromatin immunoprecipitation (ChIp) and quantitative real time PCR (qPCR) also confirmed the binding of transcription factors, AP2α and SRF to the *HTRA1* promoter. ARPE-19 and HeLaS3 cell lines when transfected with plasmid constructs having the *HTRA1* promoter variant resulted in higher gene expression for the risk genotype compared to wild-type genotype.

The implication of the *HTRA1* gene was further confirmed in a Chinese cohort (Yang *et al*. 2006), wherein, immunolabelling with HTRA1 antibodies showed intense staining for HTRA1 in the drusen of AMD patients. Also, a higher *HTRA1* mRNA expression was observed for the risk allele of rs11200638 in the lymphocytes and RPE of AMD patients (Yang *et al*. 2006). The association of rs11200638 was replicated across various ethnic groups (table 6). The disease risk conferred by this SNP was reported to be higher

Table 5. The association of rs10490924 (A69S) SNP in AMD in various studies

Populations	Cases (*n*)	Controls (*n*)	*P* value	Odds ratio (95%CI)	Other associated variants	References
South East Asians	96	130	4.08×10^{-12}	NA		DeWan *et al*. 2006
Americans	323	117	< 0.00001	NA	1 exonic	Jakobsdottir *et al*. 2005
Americans	374	336	< 0.0001	NA		Jakobsdottir *et al*. 2005
Americans	466	280	5.3×10^{-30}	NA	1 exonic, 2 intronic	Kanda *et al*. 2007
Americans	457	1071	< 0.001	1.47 (0.77–2.81)		Schaumberg *et al*. 2007
Americans (AREDS)	701	175	< 0.00001	NA		Conley *et al*. 2006
Americans (CHS)	126	1051	< 0.00001	NA		Conley *et al*. 2006
Americans	399	329	1.80×10^{-18}	2.88 (2.26–3.67)		Ross *et al*. 2007
Americans	530	280	1.28×10^{-11}	3.13 (2.23–4.40)		Francis *et al*. 2007
Americans	1882	215	0.04	0.70 (0.49–1.0)	1 exonic	Bergeron-Sawitzke *et al*. 2009
Japanese	73	94	1.4×10^{-6}	3.0 (1.61–5.75)		Kondo *et al*. 2007
Japanese	95	99	3.23×10^{-6}	2.63 (1.74–3.96)		Tanimoto *et al*. 2007
Japanese	88	97	4.74×10^{-11}	NA		Yoshida *et al*. 2007
Japanese	56	77	1.0×10^{-3}	3.13 (1.52–6.45)	3′UTR indel	Gotoh *et al*. 2009
French	188	116	< 0.0001	4.41 (2.60–7.40)		Leveziel *et al*. 2007
Israeli	255	119	< 0.0001	3.10 (2.20–4.50)		Chowers *et al*. 2008
Finnish	332	455	3.75×10^{-9}	NA		Seitsonen *et al*. 2008
Indian	228	152	5.34×10^{-12}	2.92 (2.17–3.97)	1 exonic	Kaur *et al*. 2008
German	794	612	3.9×10^{-34}	2.97 (2.32–3.81)		Rivera *et al*. 2005
German	760	549	2.8×10^{-29}	2.86 (2.38–3.44)	1 exonic, 1 indel	Fritsche *et al*. 2008
European	401	266	1.02×10^{-25}	3.71 (2.86–4.81)		Hughes *et al*. 2007
American	610	259	3.13×10^{-8}	NA	3 intronic	Schmidt *et al*. 2006
Greek	100	115	< 0.04	NA		Marioli *et al*. 2009
Chinese	159	140	2.68×10^{-6}	3.19 (2.28–4.46)		Jiang *et al*. 2009
Northern Chinese	121	132	< 0.001	2.43 (1.70–3.48)		Xu *et al*. 2008
Chinese (Taiwan)	95	90	9.2×10^{-6}	2.73 (1.77–4.21)		Lin *et al*. 2008
Russian	155	151	0.007	NA		Fisher *et al*. 2007

NA, data not available.

among Asians (Kaur *et al*. 2008; Lin *et al*. 2008b; Tam *et al*. 2008; Jiang *et al*. 2009) compared to Caucasians (Yang *et al*. 2006; Chen *et al*. 2008). A meta-analysis on the *HTRA1* promoter polymorphism based on 14 case–control studies (seven Caucasian and seven Asian) indicated a strong association with AMD ($P < 0.0001$) with a high risk in subjects homozygous for the risk allele (OR = 7.46, 95%CI, 6.16–9.04) (Tang *et al*. 2009).

Functional implications of the variants at 10q26

The functional implications of the variants rs10490924 (*ARMS2*) and rs11200638 (*HTRA1*) and their association with AMD was studied using cell constructs and RT-PCR analysis (Kanda *et al*. 2007; Fritsche *et al*. 2008). Although the precise function of *ARMS2* is unknown, the protein was shown to localize to the outer membrane of the mitochondria (Kanda *et al*. 2007; Fritsche *et al*. 2008). The stability, expression and localization of the protein was shown to be unaffected by this variant in mammalian cells. It was hypothesized that the A69S (rs10490924) variant might affect the conformation/interaction of the protein that might further affect the functions of mitochondria (Kanda *et al*. 2007). However, more functional studies are required to explore the correlation between rs10490924 and AMD pathogenesis.

On the other hand, the effect of rs11200638 variant on the *HTRA1* promoter activity was analysed using mammalian constructs that were transfected in different cell lines (Kanda *et al*. 2007). In contrast to earlier reports (Yang *et al*. 2006), the wild type and variation in the promoter SNP did not show any statistically significant difference in luciferase reporter expression. No significant difference was noted for the *HTRA1* mRNA expression in the retina of AMD patients and normal controls (Kanda *et al*. 2007). This study suggested that the AMD susceptibility gene at 10q locus was *ARMS2* and not the *HTRA1*. A recent study based on cell culture and transfections of human ARPE-19 and green monkey kidney epithelial COS7 cells with plasmid constructs for *ARMS2* cDNA, indicated that the *ARMS2* was distributed in the cytosol and not in the mitochondrial outer membrane (Wang *et al*. 2009). Hence the risk conferred by *ARMS2* may involve pathways other than the mitochondria.

Other candidate genes in AMD

Genetic studies in AMD were initiated by screening candidate genes involved in retinal dystrophies (*ABCR, TIMP3, RDS, EFEMP1, VMD2* and *ELOVL4*) that had overlapping clinical features (table 2). This strategy was adopted since AMD is a late onset disorder and recruiting large families for linkage analysis was difficult. The genes associated with retinal dystrophies; however, did not provide substantial evidence in understanding AMD pathogenesis. Most of these retinal dystrophy genes were implicated in monogenic disorders with a classical Mendelian inheritance.

Majority of the other genes screened in AMD based on their functions in the biochemical pathways, did not exhibit any association. The choice of these genes were

Table 6. Distribution of *HTRA1* variant (rs11200638) in AMD in various studies.

Populations	Cases (n)	Controls (n)	P value	Odds ratio (95%CI)	Other associated variants	References
South East Asians	50	38	8.24×10^{-12}	NA		DeWan *et al*. 2006
Americans	581	301	1.0×10^{-9}	NA		Yang *et al*. 2006
Americans	774	294	1.20×10^{-7}	2.20 (1.63–2.97)		Chen *et al*. 2008
Chinese	163	183	1.74×10^{-12}	5.20 (3.24–8.35)	2 promoter, 2 exonic	Tam *et al*. 2008
Chinese (Taiwan)	95	52	6.70×10^{-7}	3.05 (1.97–4.70)		Lin *et al*. 2008
Northern Chinese	121	132	< 0.001	2.82 (1.96–4.06)		Xu *et al*. 2008
Chinese	159	140	7.13×10^{-10}	3.93 (2.76–5.58)		Jiang *et al*. 2009
Indian	228	152	4.30×10^{-12}	2.70 (2.03–3.59)	2 promoter	Kaur *et al*. 2008
Israeli	255	119	< 0.0001	2.70 (1.9–4.0)		Chowers *et al*. 2008
French	188	116	< 0.0001	3.34 (2.1–5.31)		Leveziel *et al*. 2007
Japanese	88	97	1.79×10^{-12}	NA		Yoshida *et al*. 2007
Japanese	123	133	7.75×10^{-6}	2.23 (1.57–3.18)		Mori *et al*. 2007
Japanese	73	94	3.40×10^{-7}	NA		Kondo *et al*. 2007
American	52	13	0.04	2.53		Chan *et al*. 2007
American	466	280	3.8×10^{-18}	NA	14 intronic	Kanda *et al*. 2007
European	242	157	1.39×10^{-8}	3.61 (2.29–5.70)		Weger *et al*. 2007
American	658	294	5.30×10^{-15}	NA		Cameron *et al*. 2007
American	134	268	< 0.001	NA	1 5′UTR, 1 exonic	Deangelis *et al*. 2008
American	342	215	1.26×10^{-10}	2.20 (1.63–2.97)	2 5′UTR, 2 exonic, 3 intronic	Gibbs *et al*. 2008
American	1882	215	2.70×10^{-16}	2.40 (3.50–4.50)		Bergeron-Sawitzke *et al*. 2009

NA, Data not available.

based on their role in cell signalling pathways (*RGS16, TGFB2, GPR75* and *RDS*), extracellular matrix (*PRELP, LAMC1, LAMC2, LAMB3, FIBULIN2*, and *ITGB4*), role in biochemical pathways (*GLRX2, ADPRT1, AhR, NAT2, CYP2E1, VMD2, A2M, CKB , CYP1A1, CYP1A2, APOH, CYP2D6, APOE* detoxification pathways (*EPHX1, GPX1, CAT, MGST1*) and inflammatory pathways or immune system (*ILIA* and *TLR4*) (table 2). One of the first genes, which exhibited an involvement with AMD was *ABCR* (ATP binding cassette transporter) that plays a key role in the visual cycle (De La Paz *et al*. 1999). Among the several studies performed to assess the role of *ABCR* in AMD, only three studies reported a possible involvement (table 2).

Variants in other genes like *HMCN1, APOE, TLR3, TLR4* and *VEGF* have yielded mixed findings with marginal or positive association with AMD across different studies, but these could not explain a significant fraction of the disease (table 2). Studies on the association of variants in toll-like receptors (TLR), which forms an integral part of innate immunity, have revealed discordant results across multiple studies. The *VEGF* (vascular endothelial growth factor) gene, which is an important candidate in AMD pathogenesis due to its role in angiogenesis, have exhibited weak association, except one study that demonstrated a strong association of a risk haplotype in *VEGF* (Churchill *et al*. 2006).

Genetic studies in India

To the best of our knowledge, there are a very few published reports on the genetics of AMD from India. All these studies have been undertaken at the L. V. Prasad Eye Institute in Hyderabad, on a large cohort of AMD cases (n = 300; mean age = 68.8 ± 3.1 years) and ethnically matched unaffected normal controls (n = 300; mean age = 64.4 ± 4.8 years). Among the cases (enrolled as per the AREDS criteria), 75% had late stage (wet) AMD and the rest were dry AMD. The phenotypes were confirmed based on an inter-observer agreement based on Kappa statistics ($\kappa = 0.94 \pm 0.06$). Among the risk factors, age (P = 0.003), gender (P = 0.001) and diabetes (P = 0.001) were significantly associated with AMD susceptibility (Kaur *et al*. 2006). There was a significant association of the Y402H SNP in *CFH* ($P = 1.19 \times 10^{7}$), which was consistent after adjusting for the associated variables. The association of the 'risk' and the 'protective' haplotypes were similar to the Caucasian populations (Kaur *et al*. 2006). The variations in *APOE* and *TLR4* were not associated with AMD.

The second major AMD locus on 10q26 was resequenced to determine its association with AMD in the same cohort. A strong association was observed with the *ARMS2* (rs10490924; $P = 5.34 \times 10^{-12}$) and *HTRA1* (rs1120638; $P = 4.32 \times 10^{-12}$) variants in AMD. These two SNPs were in strong LD ($D' = 0.91$, 95%CI 0.85–0.94). Genotype, haplotype and meta-analysis data suggested that the rs10490924 was the most AMD-susceptible SNP in the present cohort (Kaur *et al*. 2008). The combined effect of *CFH* and *ARMS2* risk genotypes exhibited a significant risk in AMD (OR = 73.89, 95%CI, 8.69–628.13).

Using a combination of customized genotyping by Illumina-based golden gate assay and resequencing, it was observed that SNPs in C2 (rs547154 (IVS10); $P = 5.4 \times 10^{-11}$) and CFB (rs641153 (R32Q), $P = 2.2 \times 10^{-7}$) were strongly associated with reduced risk of AMD in the same cohort (I. Kaur, S. Katta, R. Reddy, R. Narayanan, A. Mathai, A. B. Majji and S. Chakrabarthi, unpublished data). The rs547154 and rs641153 were in strong LD (D' = 0.90, 95%CI, 0.81–0.96) and a protective haplotype 'T–A' was observed (OR = 0.10, 95%CI, 0.05–0.20).

Thus, all these studies in the Indian cohort exhibited a similar risk profile for the variants of *CFH* (table 3), *C2* and *BF* (table 4), *ARMS2* (table 5) and *HTRA1* (table 6) individually and in combination. It would be worthwhile to see the involvement of these variants in other geographic regions of India as well. Currently, the results of our GWAS in AMD (stage I) are being validated in a replication cohort (stage II).

Effect of genetic risk factors on AMD

Risk factors associated with CFH

The effect of modifiable risk factors such as BMI and smoking on the *CFH* variant Y402H has been assessed in multiple studies. Seddon *et al*. (2006b) observed that smoking was significantly associated with both geographic (OR = 5.0, 95%CI, 2.3–10.6) and neovascular AMD (OR = 4.1, 95%CI, 1.6–11.0) and BMI (≥ 30) had an increased risk of neovascular AMD (OR = 2.4, 95%CI, 1.5–3.9) compared to GA (OR = 1.6, 95%CI, 0.9–3.0). It was also observed that the risk of AMD increased in the background of the 'CC' genotype for higher BMI (OR = 5.9, 95%CI, 3.1–11.4) and smoking (OR = 10.2, 95%CI, 5.3–19.7) in the subjects. These findings were replicated in another study in a white population (Schaumberg *et al*. 2007).

Current smokers with the risk genotype had a two-fold higher risk of developing AMD (OR = 8.69, 95%CI, 3.86–19.57) compared to nonsmokers (OR = 4.23, 95%CI, 2.86–6.27). The effect of smoking and higher BMI with the genotypes for the major allele (TT or TC) had a relatively lower risk of developing AMD (Schaumberg *et al*. 2007).

Risk factors associated with ARMS2/HTRA1 SNPs

The interactions between smoking and *ARMS2* variant have been widely assessed as a risk factor in AMD. However, the effect of smoking on rs10490924 in AMD susceptibility has been inconsistent. Some studies did not observe any interaction between smoking and rs10490924 (Jakobsdottir *et al*. 2005; Seitsonen *et al*. 2008; Wang *et al*. 2008), while others have indicated a significant risk in individuals homozygous for the risk allele and with a history of smoking (Schmidt *et al*. 2006; Ross *et al*. 2007; Schaumberg *et al*. 2007; Neuner

et al. 2008). Similarly, the effect of smoking and risk genotype at rs10490924 was associated with a decline on visual function status as observed in the Muenster Aging and Retina Study (MARS) (Neuner *et al*. 2008).

Risk of AMD due to the combined effect of CFH and ARMS2/HTRA1 SNPs

Several studies have analysed the combined interaction of the genotypes of rs1061170 (*CFH*) and rs10490924 (*ARMS2*)/rs11200638 (*HTRA1*). Individuals homozygous for the risk alleles at both the loci exhibited an elevated risk of AMD than those at a single locus. Further details are provided in table 7. The two-locus OR based on the combined genotypes for the risk alleles of rs1061170 and rs10490924 ranged from 27–227 (Rivera *et al*. 2005; Schaumberg *et al*. 2007; Francis *et al*. 2008; Kaur *et al*. 2008; Seitsonen *et al*. 2008) and 8–193 for the combined risk of rs1061170 and rs11200638 (Yang *et al*. 2006; Cameron *et al*. 2007; Yoshida *et al*. 2007; Francis *et al*. 2008; Kaur *et al*. 2008). The additive effect of the risk genotypes for rs10490924 and rs11200638 were consistent across multiple studies (Francis *et al*. 2008; Xu *et al*. 2008).

Mechanisms involved in AMD pathogenesis

The ageing eye undergoes several pathological changes that affects the integrity of the RPE-choriocapillaries and photoreceptor cells and leads to AMD pathogenesis. The key factors involved in the disease pathogenesis are RPE injury, mitochondrial dysfunction, oxidative stress, complement activation, inflammation and abnormal extracellular matrix (ECM) modelling, which are detailed as follows:

RPE injury

RPE injury and inflammation together lead to the formation of drusen, resulting in the release of cytokines and RPE debris into BM, some of which may diffuse in to the choroids (Hageman *et al*. 2001). The release of soluble molecules by the injured RPE serve as chemo-attractants for choroidal and blood-borne monocytes that migrate to the site of injury and mature during which their cellular processes pass through the BM and reach the sub-retinal space. The bulbous terminus of these processes forms the core of drusen, which later increases in size due to the release of various inflammatory molecules (Hageman *et al*. 2001).

Oxidative stress

The retina serves as an ideal environment for the generation of reactive oxygen species due to high oxygen consumption, susceptibility to irradiation, high amounts of polyunsaturated fatty acids (PUFAs) in photoreceptor outer segments, presence of photosensitizers in the RPE and neurosensory retina (Beatty *et al*. 2000), and constant phagocytosis of the RPE for self-renewal (Tate *et al*. 1995). Oxidative stress is mainly due to retinal irradiation, lipid peroxidation, photochemical damage of retinal chromophores and respiratory burst (Beatty *et al*. 2000). These processes result in either significant change in the RPE structure and function or photoreceptor cell death.

Complement activation and inflammation

There has been substantial evidence for the role of inflammation and complement activation in AMD pathogenesis as indicated by immunohistochemical studies. Molecular and histological analyses of drusen using donor tissues have identified several acute phase reactant proteins, immune system components and terminal immune complexes (Mullins *et al*. 2000; Hageman *et al*. 2001). The immunoglobulin molecules and terminal complement complexes are distributed at sites of drusen deposition (Johnson *et al*. 2000, 2001). The detection of immunoglobulin G, terminal C5b-9 complement complexes, inhibitors of complement activation and complement activating molecules in drusen and in RPE cells have indicated that drusen biogenesis could be a result of immune response (Johnson *et al*. 2000). Thus molecular components within and around drusen represent degenerating RPE and failure to eliminate these debris stimulate local pro-inflammatory pathways leading to the deposition of complement components and other inflammatory mediators (Hageman *et al*. 2001).

Table 7. Two-locus odds ratios for *CFH* (rs1061170) with *ARMS2* (rs10490924) and *HTRA1* (rs11200638) in various studies.

Population	*ARMS2* vs *CFH* (odds ratios (95%CI))	*HTRA1* vs *CFH* (odds ratios (95%CI))	References
German	57.58 (37.24–89.0)	NA	Rivera *et al*. 2005
White	50.48 (10.77–236.57)	NA	Schaumberg *et al*. 2007
Finnish	27	NA	Seitsonen *et al*. 2008
American (AREDS)	227.57	192.71	Francis *et al*. 2008
Indian	73.89 (8.69–628.13)	45.33 (5.24–392.08)	Kaur *et al*. 2008
Caucasian	NA	31.52 (4.01–247.96)	Yang *et al*. 2006
American	NA	47.57 (5.18–437.27)*	Cameron *et al*. 2007
Japanese	NA	7.92	Yoshida *et al*. 2007

*Wet AMD cases only; NA, data not available.

Mitochondrial dysfunction

Defect in mitochondrial function either due to ageing or oxidative stress results in the impairment of energy metabolism and homeostasis resulting in the activation of apoptotic pathway (Liang and Godley 2003). As the RPE is an active site of metabolism, impaired mitochondrial function may result in the degeneration of RPE and the photoreceptor cells due to insufficient nutrient supply (Wallace 2005). Structural alterations in mitochondria have been observed in the retina of AMD patients compared to retina of normal individuals (Feher *et al*. 2006).

Treatment strategies for AMD

It is estimated that around 1.3 million people are at a higher risk of developing AMD and are likely to develop advanced symptoms without any treatment or intervention (Clemons *et al*. 2005). Various modalities like dietary supplementation of anti-oxidants, laser therapy (thermal photocoagulation, photodynamic therapy and surgery), Anti-VEGF and combination therapy (laser along with anti-VEGF treatment) are the current treatment modalities available in AMD (Joussen and Bornfeld 2009). These treatments are offered depending on the clinical phenotype to retard progression of the disease, as complete reversal is not possible. Majority of the treatment strategies are available for wet AMD. Dry AMD is currently managed with dietary supplementation of anti-oxidants that are primarily given to reduce the risk of AMD development and progression, although few surgical options are under exploration (Joussen and Bornfeld 2009).

Future directions in AMD research

The complexities associated with the etiology of AMD provides a challenging problem in understanding the molecular basis of pathogenesis and its subsequent management. However, the pace of genomic studies based on GWAS has facilitated a better understanding of the molecular genetic basis of AMD (Swaroop *et al*. 2009). The association of genes in the complement cascade (*CFH, C2, BF* and *C3*) along with *ARMS2* and *HTRA1* have indicated significantly strong associations with AMD. Interestingly, the variants within these genes have exhibited a large effect and the associations have been consistent with similar risk profiles across majority of the populations worldwide. Globally about 75% of all AMD could be explained by the combined effect of *CFH* and *ARMS2* based on population-attributable risk per cent for the homozygous risk alleles. Since AMD is attributable to multiple genes with varying magnitudes of effect, it would be interesting to understand the involvement of the genes with smaller effect.

Current GWAS in AMD by our group and colleagues elsewhere have revealed strong indications for genes outside the complement pathway that may be involved in AMD. Since, the risks conferred by variations in these genes are likely to be of lesser magnitude, they would require further validation in multiple ethnic groups. The combination of all the large and small effect changes in the background of the associated risk factors would provide a clearer understanding on AMD susceptibility in different populations. The role of structural variations in AMD is another area that is being pursued by several groups worldwide. The association of both structural changes and genetic variations would provide a holistic view of AMD pathology. Finally, the functional assessments of individual variations are underway and have revealed some interesting findings. Further characterization of the associated SNPs in different genes in combination with the associated risk and protective haplotypes would provide major insights in to the underlying molecular mechanisms leading to AMD pathogenesis.

Acknowledgements

The authors thank all the AMD patients and normal volunteers for their participation in the LVPEI study on the genetics of AMD. We appreciate the help of our clinician colleagues from the Kannuri Santhamma Retina Vitreous Centre in the diagnosis of the subjects and Mr Avid Hussain for some technical support. This study was supported in part by grants from the Department of Biotechnology (BT/PR/9561/ICD/16/759/2006) and the Department of Science and Technology (SR/FT/L-66/2006), Government of India, to Drs Chakrabarti and Kaur, respectively. Ms Saritha Katta acknowledges the receipt of a senior research fellowship from the Council of Scientific and Industrial Research (CSIR), Government of India.

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Received 3 September 2009, in revised form 11 November 2009; 10 December 2009
Published on the Web: 31 December 2009