Published in final edited form as:

Prog Retin Eye Res. 2014 May ; 40: 1–15. doi:10.1016/j.preteyeres.2013.12.004.

Nature and nurture- genes and environment- predict onset and progression of macular degeneration

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Abstract

Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in the developed world. Both environmental and genetic factors contribute to the development of disease. Among environmental factors, smoking, obesity and dietary factors including antioxidants and dietary fat intake most consistently affect initiation and progression of AMD. There are also several lines of evidence that link both cardiovascular and inflammatory biomarkers to AMD. The genetic etiology of AMD has been and continues to be an intense and fruitful area of investigation. Genome-wide association studies have revealed many common variants associated with AMD and sequencing is increasing our knowledge of how rare variants impact disease. Evidence for specific interactions between environmental, therapeutic and genetic factors is emerging and elucidating the mechanisms of this interplay remains a major challenge in the field. The knowledge of non-genetic, modifiable risk factors along with information about heritability and genetic risk variants for this disease acquired over the past 25 years have greatly improved patient management and our ability to predict which patients will develop or progress to advanced forms of AMD.

Keywords

Age-related macular degeneration; Epidemiology; Genetics; Risk factors

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¹Percentage of work contributed by each author in the production of the manuscript is as follows: Lucia Sobrin: 50%; Johanna Seddon: 50%.

Conflicts of interest

Tufts Medical Center: P.

Uncited reference

Age-Related et al., 2000; Age-Related et al., 2013; Eye et al., 1992; Eye, Disease, Case-Control, Study and Group, 1993.

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the United States (Friedman et al., 2004; Seddon and Sobrin, 2013). The etiology of AMD is multifactorial: both environmental and genetic factors contribute to the development of disease. Smoking is the most consistently identified modifiable risk factor. (Seddon et al., 1996; Tomany et al., 2004) Overall and abdominal obesity and dietary factors including antioxidants and dietary fat intake also affect AMD incidence and progression. (Cho et al., 2001; 2004, 1993; Mares-Perlman et al., 1995a; Mares-Perlman et al., 1995b; Seddon et al., 1994a, 2003a, 2003b, 1994b, 2003a, 2003b; 2001b) A lifestyle that includes a healthy diet, physical activity, weight control and smoking avoidance, can reduce the risk of AMD. (Cho et al., 2001; 2004, 1993; Mares-Perlman et al., 1995a; Mares-Perlman et al., 1995b; Mares et al., 2011; Seddon et al., 1994a, 2003a, 2003b, 1994b, 2003a, 2003b; Seddon and Hennekens, 1994; Seddon et al., 2001b) There has also been great progress in identifying the genetic variants that impact risk of AMD. (Chen et al., 2010; Dewan et al., 2006; Edwards et al., 2005; Fritsche et al., 2013; Hageman et al., 2005; Haines et al., 2005; Jakobsdottir et al., 2005; Klein et al., 2005; Maller et al., 2006, 2007; Neale et al., 2010; Raychaudhuri et al., 2011; Seddon et al., 2013d; Yang et al., 2006; Yu et al., 2011) The knowledge of genetic risk variants for the disease coupled with knowledge of non-genetic risk factors over the past two and a half decades have improved both the ability to manage and advise patients as well as the ability to predict which patients will develop advanced forms of the disease. (Seddon et al., 2009a; 2011b; 2013c) Understanding the interplay between environmental, therapeutic and genetic factors will lead to new preventive and therapeutic strategies in the evolving field of personalized medicine.

1.1. Prevalence and impact of AMD

Population-based studies have provided information on the prevalence and incidence of AMD within the US. The Beaver Dam Eye Study (BDES) was a census of the population of Beaver Dam, Wisconsin. (Klein et al., 1992) Incidence of early AMD increased from 3.9% in individuals aged 43–54 years to 22.8% in persons 75 years of age and older. Persons 75 years of age or older had a 5.4% incidence rate and a 7.1% prevalence rate of late AMD, defined as choroidal neovascularization (CNV) and/or geographic atrophy (GA). Similarly, the Visual Impairment Project in Australia found that the 5-year incidence of AMD was 6.3% in those age 80 years and older at baseline. (Mukesh et al., 2004) The Blue Mountains Eye Study (BMES) in Australia found that end-stage AMD was present in 1.9% of the Caucasian population, rising from 0% among people younger than 55 years of age to 18.5% among those 85 years of age or older. (Mitchell et al., 1995) Prevalence of early and late AMD in an Asian Malay population was similar to that reported in the BMES. (Kawasaki et al., 2008) The recent National Health and Nutrition Examination Survey (NHANES), conducted from 2005 to 2008, sampled approximately 5500 persons. (Klein et al., 2011b) The total prevalence of any AMD in this civilian, noninstitutionalized US population aged 40 years or older was 6.5% (7.2 million people), and 809,000 persons were estimated to have the late stages of AMD. (Klein et al., 2011b) While some data suggest that the incidence of advanced AMD in the USA may be on the decline, due in part to changes in lifestyle habits of the American public over the last 40 years, (Klein et al., 2008b) the

prevalence of AMD is still expected to increase by 97% by the year 2050. (Klein and Klein, 2009)

Ophthalmologists rarely observe visual loss caused by CNV among US ethnic minority groups. In the Baltimore Eye Survey, AMD accounted for 30% of bilateral blindness among whites and for 0% among African Americans. (Sommer et al., 1991) Data from a population-based study of blacks in Barbados, West Indies, (Leske et al., 2004; Schachat et al., 1995) revealed that incidence of AMD and signs of AMD changes occurred commonly but at a lower frequency than in predominantly white populations in other studies. Hispanics also have a lower prevalence of advanced AMD than non-Hispanics. The Los Angeles Latino Eye Study indicates Latinos have a relatively high rate of early AMD but not late AMD. (Varma et al., 2004) Overall, the literature to date suggests that early AMD is common among blacks and Hispanics, although less common than among non-Hispanic whites, whereas advanced AMD is much less common in these groups compared with non-Hispanic whites.

AMD adversely affects quality of life and activities of daily living, causing many affected individuals to lose their independence in their retirement years. Patients with vision loss resulting from AMD often report AMD as their worst medical problem and have a diminished quality of life. (Alexander et al., 1988; Mangione et al., 1999) In one study of well-being, patients with AMD had lower quality of life scores than patients with chronic obstructive pulmonary disease and acquired immunodeficiency syndrome; the lower quality of life in patients with AMD was related to greater emotional distress, worse self-reported general health, and greater difficulty carrying out daily activities. (Williams et al., 1998)

1.2. Proportion of disease attributable to nature vs nurture: twin studies

Twin studies have allowed dissection of the relative contribution of genetic and environmental factors in AMD. In the first large population-based twin study of AMD including over 12,000 WWII veterans in the National Academy of Sciences-National Research Council Twin Registry, the roles of environment and heredity were quantified by studying both monozygotic and dizygotic twins and their AMD concordance rates using complex twin analyses and including all stages of AMD. After screening, 840 of these WWII veterans were examined and photographed. Genetic factors explained 46%–71% of the variation in the overall severity of the disease, whereas unique environmental exposures accounted for 19%–37% of the variance. (Seddon et al., 2005a, 1997b) The higher heritability estimate was associated with more advanced stages of AMD. A British survey of early AMD among female twins showed a concordance of 0.37 in monozygotic twins vs. 0.19 in dizygotic twins with a heritability estimate for early AMD of about 45%. (Hammond et al., 2002)

The concordance for AMD in monozygotic twins has also been demonstrated in several small case series. (Gottfredsdottir et al., 1999; Klein et al., 1994; Meyers et al., 1995; Seddon et al., 2005a) One series reported eight of nine monozygotic twins were concordant for AMD. (Klein et al., 1994) Another compared monozygotic and dizygotic twins and demonstrated 100% concordance in 25 monozygotic twins compared to 42% concordance in

12 dizygotic twins. (Meyers et al., 1995) A third study case series study found 90% concordance in 50 monozygotic twins. (Gottfredsdottir et al., 1999)

2. Non-genetic risk factors

2.1. Smoking

Smoking is an important, independent, avoidable risk factor for AMD. The preponderance of epidemiologic evidence indicates a strong positive association between all forms of AMD and smoking. In the prospective cohort study reported in 1996, women who currently smoked 25 or more cigarettes per day had a relative risk (RR) of 2.4, and women who were past smokers had an RR of 2.0 for developing AMD compared with women who never smoked. (Seddon et al., 1996) There was a dose-response relationship between incident AMD and pack-years of smoking, and risk remained elevated for many years after smoking cessation. Results were consistent for various definitions of AMD, including CNV and dry AMD, with different levels of vision loss, and for different definitions of smoking. It was estimated that 29% of the AMD cases among women in that study could be attributable to smoking. These results were supported by a prospective study among men. (Christen et al., 1996) Several other studies also support an increased risk for AMD among smokers. (2000, Delcourt et al., 1998; Klein et al., 2002; Tomany et al., 2004)

Smoking may increase the risk of developing AMD by adversely affecting blood flow, decreasing levels of high-density lipoprotein (HDL), increasing platelet aggregability and fibrinogen, increasing oxidative stress and lipid peroxidation, reducing plasma levels of antioxidants, and raising levels of inflammation and inflammatory cytokines. (Seddon et al., 1996) In animal models, nicotine has been shown to increase the size and severity of experimental CNV, suggesting that nonneuronal nicotinic receptors may also play a part in the effect of smoking on advanced AMD. (Suner et al., 2004)

2.2. Antioxidants, vitamins, and minerals

The first study launched to systematically evaluate the relationship between dietary intake, nutritional factors and AMD was initiated in 1986 by JMS and incorporated into the Eye Disease Case-Control Study (EDCCS) as an ancillary study. The hypothesis for this Dietary Intake Study for AMD, that antioxidant dietary and nutritional factors could influence AMD, was based on the known influence of daily insults on free radical formation and oxidation, and that the retina was a set up for these oxidative processes due to the abundance of polyunsaturated fatty acids in the photoreceptor outer segment membranes. (Anderson et al., 1984; Young, 1987) The deposit of oxidized compounds in healthy tissue could result in cell death because they are indigestible by cellular enzymes. (Anderson et al., 1984; Young, 1987) Theoretically this could lead to impaired function of the retinal pigment epithelium (RPE) and eventually to degeneration involving the macula. We proposed that dietary antioxidants could potentially block this damaging effect of oxidation and scavenge, decompose, or reduce the formation of these harmful compounds in the macula.

A standardized dietary questionnaire was designed for use by elderly individuals with visual impairment and it was shown to be a reliable and reproducible questionnaire for use in

ocular research. (Ajani et al., 1994) The results published in 1994 showed an inverse association between neovascular AMD and dietary intake of carotenoids from foods. (Seddon et al., 1994b) Those in the highest quintile of lutein and zeaxanthin intake had a 43% lower risk for AMD compared with those in the lowest quintile (OR = 0.57, 95% CI = 0.35–0.92, P for trend = 0.02). The median intake of this highest quintile was 6 mg lutein, which has become a standard dose in numerous ocular nutraceuticals on the market. In contrast, higher dietary intake of beta-carotene did not reduce the risk of advanced AMD. We then tested the associations between AMD and foods rich in lutein and zeaxanthin and found that higher frequency of intake of spinach and collard greens (5 times per week as compared with <once per month) was associated with a substantially lower risk for AMD (OR = 0.12, 95% CI = 0.01–0.09, P for trend = 0.001). A separate prospective study showed that fruit intake, a rich source of antioxidant nutrients, was inversely associated with neovascular AMD, and participants who consumed three or more servings of fresh fruit per day had an RR of 0.64 compared to those who consumed less than 1.5 servings per day. (Cho et al., 2004)

Other studies have shown similar effects for early AMD. The Pathologies Oculaires Liees an l'Age (POLA) Study in 2006 suggested a protective role of the xanthophylls, in particular zeaxanthin, against early AMD. (Delcourt et al., 2006) In a British study of 380 men and women in 2003, lower plasma levels of zeaxanthin were also found to be associated with an increased risk of early AMD. (Gale et al., 2003) A randomized, double-masked, placebo-controlled clinical trial of an oral preparation containing lutein, zeaxanthin, vitamin C, vitamin E, copper, and zinc vs. placebo showed functional benefits in persons with early AMD. (Beatty et al., 2013) The differential between active and placebo groups increased steadily, with average visual acuity in the former being approximately 4.8 letters better than the latter for those who had 36 months of follow-up ($P = 0.04$). In the longitudinal analysis, for a 1-log-unit increase in serum lutein, visual acuity was better by 1.4 letters (95% CI, 0.3–2.5; $P = 0.01$), and a slower progression along a morphologic severity scale ($P = 0.014$) was observed. Another trial randomly assigned early AMD patients to receive lutein, lutein plus zeaxanthin, or placebo. (Ma et al., 2012) Early functional abnormalities of the central retina as measured by multifocal electroretinography and diminished macular pigment optical densities in these early AMD patients could be improved by lutein and zeaxanthin intake. Xanthophyll supplementation may have benefits even at the earliest stages of AMD.

The Age-Related Eye Disease Study (AREDS)(2001) was a double-blind clinical trial in 11 centers around the US to test the effect of supplement use. Subjects were randomly assigning 3640 participants to take daily oral supplements of antioxidants, zinc, antioxidants and zinc, or placebo to test the hypothesis generated previously by other studies. Both zinc alone and antioxidants and zinc together significantly reduced the odds of developing advanced AMD in participants with intermediate signs of AMD in at least one eye. The zinc supplement included zinc (80 mg) as zinc oxide and copper (2 mg) as cupric oxide; the antioxidant supplement included vitamin C (500 mg), vitamin E (400 IU), and beta-carotene (15 mg). If the AREDS formulation were used to treat the 8 million individuals in the USA who are at risk for developing advanced AMD, the AREDS study estimated that more than 300,000 would avoid advanced AMD and the associated vision loss during the following 5 years. (Bressler et al., 2003) AREDS supplements are a cost-effective way of reducing visual

acuity due to the progression of AMD, (Hopley et al., 2004) although the effect of antioxidant supplements on the incidence of early AMD was not shown in this study.

AREDS2 was a follow-up, randomized, double-masked, placebo-controlled clinical trial to determine whether adding supplements containing lutein plus zeaxanthin, docosahexaenoic acid (DHA) plus eicosapentaenoic acid (EPA), or both to the AREDS formulation decreases the risk of developing advanced AMD and to evaluate the effect of eliminating supplements with beta-carotene, lowering zinc doses, or both in the AREDS formulation. (2013) Participants were randomized to receive lutein (10 mg) plus zeaxanthin (2 mg), DHA (350 mg) plus EPA (650 mg), lutein plus zeaxanthin and DHA plus EPA, or none of these. All participants were also asked to take the original AREDS formulation or accept a secondary randomization to four variations of the AREDS formulation, including elimination of beta-carotene, lowering the zinc dose, or both. Compared to the original or modified AREDS formula referred to as “placebo” in the primary analyses, there was no statistically significant reduction in progression to advanced AMD (hazard ratio [HR], 0.90 [98.7% CI, 0.76–1.07]; $p = 0.12$ for lutein plus zeaxanthin; 0.97 [98.7% CI, 0.82–1.16]; $p = 0.70$ for DHA plus EPA; 0.89 [98.7% CI, 0.75–1.06]; $p = 0.10$ for lutein plus zeaxanthin and DHA plus EPA). In subgroup analyses, there was a statistically significant reduced risk of progression to advanced AMD for lutein and zeaxanthin supplements among participants with low dietary lutein and zeaxanthin intake. There was no apparent effect of beta-carotene elimination or lower-dose zinc on progression to advanced AMD. More lung cancers were noted in the beta-carotene vs no beta-carotene group (23 [2.0%] vs. 11 [0.9%], nominal $p = 0.04$), mostly in former smokers. In another subgroup analysis, lutein plus zeaxanthin appeared to be beneficial in reducing progression to advanced AMD, particularly CNV, when specifically comparing participants who received lutein plus zeaxanthin but no beta-carotene with those who received beta-carotene but no lutein plus zeaxanthin. Considering all of the above, the clinical recommendation that has emerged from the AREDS group is that lutein plus zeaxanthin supplements are an appropriate substitute for the beta-carotene supplement in the original AREDS formula.

Carotenoids are relevant to AMD because of their physiologic functions and their location in the retina. Lutein and zeaxanthin, in particular, comprise the macular pigment. (Bone et al., 2003; Krinsky et al., 2003) Trace minerals such as zinc and copper may also be involved in antioxidant functions of the retina. Evidence is not as strong for other minerals like manganese and selenium. (Seddon and Hennekens, 1994)

The role of dietary antioxidants and nutritional factors in the prevention of AMD has evolved from speculation, hypothesis generation and testing beginning in the 1980s, to evidence based on observational data in the study published in 1994 regarding lutein and zeaxanthin derived from foods, (Seddon et al., 1994b) and then randomized trial data based on AREDS a decade later regarding the use of supplements. (Age-related et al., 2001)

2.3. Dietary fat and Omega-3 fatty acids

Higher total dietary fat intake increases risk of AMD. This was first shown in the Dietary Ancillary Study of EDCCS in which the RR of progression to advanced AMD was 2.9 in the group with the highest dietary fat intake compared to the group with the lowest intake.

(Seddon et al., 1994a, 2001b) In the BDES, individuals with greater saturated fat and cholesterol intake also had increased risk for early AMD. (Mares-Perlman et al., 1995b) There are reported associations between risk of AMD and increased intake of vegetable, monounsaturated, and polyunsaturated fats and linoleic acid. (Delcourt et al., 2007; Seddon et al., 2001b)

However, not all fats are harmful. The protective effects of omega-3 fatty acids, which are found in high quantities in fatty fish and nuts, are of increasing interest. (Christen et al., 2011; Querques et al., 2009; Seddon et al., 1994a, 2006c, 2001b, 2003b, 2006c, 2001b) Over the past twenty years, investigations with a variety of different study designs have consistently show a protective effect of omega-3 fatty acid intake with an AMD risk reduction between 30 and 50%. An inverse association between omega-3 fatty acid intake and AMD was initially found in the Dietary Ancillary Study of EDCCS, (Seddon et al., 2001b) and higher intake was also found to reduce rates of progression from intermediate to advanced disease in a prospective study. (Seddon et al., 2003b) Higher fish intake reduced the risk of AMD progression when linoleic acid intake was low, and higher nut intake also decreased the risk of progression. (Seddon et al., 2003b, 2001b) In the US Twin Study of AMD, consuming fish two or more times per week was associated with a decreased AMD risk, and increased overall omega-3 fatty acid intake almost halved the risk of AMD. (Seddon et al., 2006c) Increased self-reported dietary intake of omega-3 long chain polyunsaturated fatty acids (DHA and EPA) has been specifically associated with reduced risk of developing GA. (Reynolds et al., 2013) AREDS2, however, did not find a benefit to omega-3 fatty acids. One possible explanation for this inconsistency may be that the effect is modified by underlying genetic risk factors. This has been observed in other conditions that are associated with AMD and in which omega-3 fatty acids may play a role. In a large atherosclerosis cohort study, for example, *APOE* genotype modified the association between omega-3-fatty acid intake and plasma lipids. (Liang et al., 2013) Similarly, genotype may modify the effect of omega-3 fatty acids in AMD. (Reynolds et al., 2013) Another possible reason for different findings is the amounts of DHA and EPA varied between studies. It is possible that there is an optimal level of supplementation with omega-3 fatty acids and that supplementation with amounts higher than the optimal level could be ineffective or detrimental.

The proposed mechanisms through which omega-3 fatty acids, such as found in fish and nuts, exert a protective effect on macular degeneration include antioxidative, anti-inflammatory and antiangiogenic effects. (Hughes and Pinder, 1996; Hughes et al., 1996; Luostarinen and Saldeen, 1996; Yang et al., 1998) DHA is a main structural lipid of retinal photoreceptor outer segments. (Anderson and Penn, 2004) In animal models, DHA has been shown to prolong survival of photoreceptors and has a protective effect on signs of apoptosis such as fragmented photoreceptor nuclei and mitochondrial dysfunction. (Rotstein et al., 1997) Thus, omega-3 fatty acids may attenuate the effects of environmental insults to photoreceptors such as ischemia, light exposure, inflammation, and aging. Omega-3 fatty acids have been shown to have multiple anti-inflammatory effects such as decreasing monocyte cell surface antigen presentation, TNF-alpha and IL-1beta expression, neutrophil superoxide presentation, natural killer lymphocyte activation, and lymphocyte presentation. (Calder, 2001) Finally, omega-3 fatty acids diminish pathologic angiogenesis in different cell

and animal models through their influence on multiple angiogenic factors including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). (Hida et al., 2003; Mukutmoni-Norris et al., 2000)

2.4. Obesity

There is an association between AMD and obesity as well as abdominal adiposity and anthropomorphic measures. (Delcourt et al., 2001; Klein et al., 2001; Schaumberg et al., 2001; Seddon et al., 2003a; Smith et al., 1998) In the prospective cohort study of 261 examined individuals with some sign of nonadvanced AMD in at least one eye at baseline, (Seddon et al., 2003a) individuals with a body mass index (BMI) between 25 and 29 had an RR of 2.32 for progression of AMD to the advanced stages when compared to those with a BMI of less than 25. Those with a BMI of at least 30 had an RR of 2.35 compared to the lowest category (BMI < 25), after controlling for other known risk factors. Similarly, the highest tertile of waist circumference had a two-fold increased risk compared to the lowest tertile, and the highest tertile of waist-to-hip ratio had an RR of 1.84 compared to the lowest tertile. Thus, both overall and abdominal obesity were related to AMD progression. Vigorous physical activity three times a week reduced the risk of AMD progression by 25% compared to no physical activity. (Seddon et al., 2003a) Obesity and physical activity are modifiable factors that may alter an individual's risk of AMD incidence and progression.

3. Cardiovascular and inflammatory biomarkers associated with AMD

3.1. Cardiovascular disease risk factors and AMD

In 1999 we reviewed the evidence that AMD and cardiovascular disease (CVD) share common antecedent risk factors including smoking, dietary fat intake, physical activity, and obesity (Snow and Seddon, 1999) and proposed that biomarkers associated with CVD, such as those associated with systemic inflammation, may be associated with AMD. The presence of atherosclerotic lesions, determined by ultrasound, was examined in relation to risk of AMD in a large population-based study conducted in the Netherlands. (Vingerling et al., 1995) Results obtained from this cross-sectional study showed a 4.5-fold increased risk of late AMD (defined as GA or CNV) associated with plaques in the carotid bifurcation and a two-fold increased risk associated with plaques in the common carotid artery. Lower-extremity arterial disease (as measured by the ratio of the systolic blood pressure level of the ankle to the arm) was also associated with a 2.5-times increased risk of AMD. Other studies of the association between AMD and CVD risk factors in 2003 showed associations between a higher pulse pressure, higher systolic blood pressure and increased carotid wall thickness and incident AMD. (Klein et al., 2003; van Leeuwen et al., 2003)

3.2. Cholesterol and AMD

Cholesterol level is a well-established biomarker for CVD. (Gordon et al., 1981) Parallels between cholesterol deposition in arterial intima and Bruch's membrane, as well as the observation that drusen contain abundant lipids including cholesterol, (Curcio et al., 2005) have supported the evaluation of the relationship between cholesterol and AMD. There is some evidence linking cholesterol level to AMD, but the results have been inconsistent. The EDCCS reported a statistically significant four-fold increased risk of CNV associated with

the highest serum cholesterol level (>4.88 mmol/l) and a two-fold increased risk in the middle-cholesterol- level group, compared with the lowest-cholesterol level group, controlling for other factors. (1992) A positive association was found between risk of AMD and increasing HDL levels in both the population-based POLA study (Delcourt et al., 2001) and the Rotterdam Study. (van Leeuwen et al., 2004) A case-control study showed the mean level of HDL was lower and mean level of low density lipoprotein (LDL) was higher in cases of advanced AMD compared with controls. (Reynolds et al., 2010) Higher total cholesterol and LDL levels were associated with increased risk of advanced AMD, with P for trend = 0.01 for both total cholesterol and LDL, in models controlling for both environmental and genetic covariates. (Reynolds et al., 2010) In contrast, in a study of early AMD without adjustment for genetic covariates, early AMD was related to low total serum cholesterol levels in women and men older than 75 years and men with early AMD had higher HDL and lower total cholesterol-to-HDL ratios. (Klein et al., 1993, 1997) When evaluating the apolipoprotein components of HDL and LDL that best indicate CVD risk, no association with AMD was found. (Dashti et al., 2006) One possible explanation for the different results is that cholesterol found in aged Bruch's membrane and drusen may also be derived from a lipoprotein of intraocular origin and, therefore, an association with plasma lipoproteins might not be detected. (Curcio et al., 2005)

The genes involved in the HDL cholesterol pathway that have been associated with AMD have had discordant effects between the HDL-increasing alleles and their protective or risk effects for AMD. The HDL-raising allele of the *LIPC* gene was associated with a reduced risk of AMD whereas the HDL-increasing alleles of *CETP* and *ABCA1* increased risk for AMD. (Chen et al., 2010; Neale et al., 2010) Furthermore, we found that the *LIPC* association with AMD was independent of HDL level, suggesting that *LIPC*, a gene in the HDL pathway exerts its effect on AMD in ways not related directly to HDL level. (Reynolds et al., 2010) The mechanisms through which genetic variants in the HDL cholesterol pathway as well as HDL level exert their effect on AMD risk require further investigation.

3.3. Inflammation, C-reactive protein and homocysteine

There is substantial evidence that inflammation plays a central role in the pathogenesis of drusen and AMD. (Anderson et al., 2002; Johnson et al., 2001; Mullins et al., 2000; Seddon et al., 2004, 2005b; Snow and Seddon, 1999) Examination of tissue samples has shown that "cellular debris" from RPE cells becomes trapped in the RPE basal lamina and Bruch's membrane, potentially causing a chronic inflammatory response which may prompt drusen formation. (Anderson et al., 2002) Drusen contain proteins that are associated with chronic and acute inflammatory responses (Johnson et al., 2001) and other age-related diseases, including amyloid P component, complement proteins, and carboxyethyl pyrrole adducts which are generated by the oxidation of lipids. (Crabb et al., 2002; Mullins et al., 2000) Inflammation is also associated with angiogenesis and may play a role in the neovascularization seen in the advanced forms of AMD. Inflammatory biomarkers have thus become an area of research interest in AMD.

C-reactive protein (CRP) and homocysteine are two well-established biomarkers for CVD that have been associated with risk of AMD. CRP is a marker for systemic inflammation as

well as cardiovascular disease, and homocysteine is an amino acid that adversely affects the vascular endothelium. (Snow and Seddon, 1999) A study of 930 individuals has shown that CRP serum levels are significantly elevated in individuals with advanced AMD. (Seddon et al., 2004) This study showed that, after adjusting for variables such as age, gender, BMI, and smoking, the odds ratio (OR) for AMD between the highest and lowest quartiles of CRP was 1.65 (P for trend = 0.02). In stratified analyses, the highest levels of CRP were associated with a two-fold increased risk among both smokers and nonsmokers. A subsequent analysis confirmed this association. (Vine et al., 2005) Another study suggested a small, independent association between higher homocysteine levels and AMD. (Seddon et al., 2006b) Homocysteine is a sulfhydryl-containing amino acid that is derived from the demethylation of methionine, which is found mainly in animal protein, and can be influenced by genetic defects, renal impairment, and various drugs and diseases. Median values of homocysteine were higher among advanced AMD cases (9.51 mmol/l) compared with persons with no AMD (8.81 mmol/l; P = 0.01). Values of >12 mmol/l vs. =12 mmol/l were also associated with an increased risk of AMD (P = 0.023), when controlling for other covariates. In a separate study, increased plasma levels of complement activation fragments Bb and C5a were also independently associated with advanced AMD with OR of 3.3 and 3.6, respectively. (Reynolds et al., 2009)

Biomarkers for CVD are also associated with *progression* of AMD. (Seddon et al., 2005b) CRP was associated with progression of AMD from early and intermediate to late stages, with an RR of 2.10 controlling for BMI, smoking and other cardiovascular risk factors. Interleukin-6 (IL-6) is an inflammatory cytokine that also has been associated with cardiovascular disease. (Ridker et al., 2000) IL-6 level was related to AMD progression with a multivariate adjusted RR of 1.81. (Seddon et al., 2005b) Other studies support these associations. (Hong et al., 2011; Schaumberg et al., 2007)

3.4. Kidney function

AMD and chronic kidney disease may have shared risk factors, including cardiovascular disease risk factors. (Seddon and Sobrin, 2013) AMD also shares causal pathways with other kidney diseases. Dense deposit disease (also referred to as type II membranoproliferative glomerulonephritis) and atypical hemolytic uremic syndrome may share a common causal link with AMD in that all of these diseases are associated with polymorphisms in the complement pathway. (Abrera-Abeleda et al., 2006; Dragon-Durey et al., 2004; Warwicker et al., 1998) Therefore, associations between markers of kidney disease and AMD have been explored. A cross-sectional nested case-control study matching on age, sex, and race was performed using data on adult participants from the Third National Health and Nutrition Examination Survey to investigate an association between measures of kidney function and AMD. (Weiner et al., 2011) In conditional logistic regression analyses adjusting for diabetes, hypertension, and total cholesterol, lower estimated glomerular filtration rate (eGFR) was independently associated with late AMD (OR = 3.05, 95% CI 1.51–6.13), while albuminuria was not significant. For any AMD, neither albuminuria nor eGFR were significant in adjusted models. In the Beaver Dam cohort, eGFR less than 60 ml/min/1.73 m² was associated with incident early AMD but was not associated with incident geographic atrophy or progression of AMD. (Klein et al., 2009) In a post hoc analysis of the Blue Mountains

Eye Study, persons with reduced kidney function were three times more likely to develop early AMD than those with intact kidney function (OR = 3.2, 95% CI, 1.8–5.7, $p < 0.0001$). (Liew et al., 2008) The associations between overall renal function and AMD may reflect a common causal pathway or shared risk factors such as hypertension and other CVD risk factors and requires additional study. A possible common causal pathway is supported by emerging evidence of shared genetic risk factors between specific kidney diseases and some forms of AMD (see Genetics section below).

4. Relationships between non-genetic risk factors and inflammation

Relationships between markers of inflammation and non-genetic risk factors provide additional evidence for the role of inflammation in the pathophysiology of AMD (Fig. 1). For example, in a case-control study of 934 people, serum CRP and homocysteine were associated with dietary and behavioral risk factors for AMD. (Seddon et al., 2006a) Higher levels of the serum antioxidants vitamin C and lutein/zeaxanthin, and higher fish intake were associated with lower serum CRP levels, whereas smoking and increased BMI were associated with increased levels of CRP. (Seddon et al., 2006a) Serum vitamin E, serum alpha-carotene, and dietary intake of antioxidants and vitamin B6 were associated with lower levels of plasma homocysteine. (Seddon et al., 2006a) Higher BMI was also related to increased levels of the complement components complement factor B (CFB), complement factor H (CFH), and Complement Component 3 (C3) as well as the plasma complement fragments C3a and iC3b as shown in a study of 120 cases who had progressed to develop either GA or CNV and 60 controls without AMD. (Reynolds et al., 2009) In addition, CRP and IL-6 levels were both significantly associated with higher BMI and current smoking in a prospective study of progression to advanced AMD. (Seddon et al., 2005b) In aggregate, these results support the mechanism that modifiable risk factors, including nutritional factors and smoking, may be altering AMD risk via their effect on systemic inflammatory markers.

Fish can be a rich source of Vitamin D as well as omega-3 fatty acids, and both have anti-inflammatory and anti-angiogenic properties. A correlation between vitamin D and AMD would be further support for an anti-inflammatory, anti-angiogenic mechanism through which fish consumption protects against AMD, and the relationships between dietary Vitamin D intake and serum levels of Vitamin D and AMD have been examined in various studies. Some but not all studies have shown correlations between lower vitamin D levels and higher risk for early AMD and/or CNV. (Golan et al., 2011; Morrison et al., 2011; Parekh et al., 2007; Seddon et al., 2011a) The studies that have shown an association include a twin pair study (Seddon et al., 2011a), a cross-sectional study (Parekh et al., 2007) and a systems biology-based analysis. (Morrison et al., 2011) The cross-sectional study included 7752 individuals for whom AMD was graded based on fundus photographs and an inverse association was found between serum levels of vitamin D and early AMD. (Parekh et al., 2007) This study did not find a correlation between fish intake and serum vitamin D levels, but there are several other sources of vitamin D in the diet which may lead to possible health benefits of this vitamin. (Parekh et al., 2007) A cross-sectional study of over 1000 AMD cases and 8000 controls ascertained using ICD-9 codes rather than fundus photographs or eye examinations did not find an association between vitamin D and any AMD. (Golan et

al., 2011) An association between vitamin D and AMD is biologically plausible and additional studies are underway.

5. Genetic factors

Our understanding and approach to discovering the genetic underpinnings of AMD has evolved over the last twenty years. We now know that AMD is a common, polygenic disease in which multiple genetic variants, each adding a small to moderate amount of increased risk, contribute to disease in addition to environmental factors. (Lim et al., 2012; Liu et al., 2012) Multiple types of genetic investigations including familial aggregation studies, twin studies, linkage analyses, candidate gene association studies, and genome-wide association studies (GWAS) and more recent sequencing studies have contributed to our concepts of the role of genetics in AMD.

5.1. Evolution of genetic understanding of AMD

Familial aggregation studies supported the role of genetic and shared environmental factors on risk of AMD. The familial aggregation study for AMD reported in 1997 found the prevalence of AMD to be significantly higher among the relatives of the cases with AMD compared with the relatives of controls (23.7% vs. 11.6%) with an odds ratio of 2.4. (Seddon et al., 1997a) Among relatives of the cases with exudative disease, the odds ratio was higher at 3.1, indicating a 3-fold increased risk of developing AMD for family members of advanced cases. Klaver et al. later studied siblings and offspring of patients with late AMD and compared them to siblings and offspring of control subjects. (Klaver et al., 1998) After adjusting for age, sex, smoking and atherosclerosis, the prevalence odds ratio for early AMD for siblings of cases vs. controls was 4.8 and for offspring of cases vs. controls was 6.6.

Twin studies have provided direct evidence of AMD heritability by comparing disease concordance rates in monozygotic vs. dizygotic twins. The large US population-based twin survey of all stages of AMD yielded heritability estimates of 0.46 and 0.71 for overall and advanced AMD, respectively, which implied that genetic factors explain between 46% and 71% of AMD variation. (Seddon et al., 2005a, 1997b)

A number of genetic linkage studies attempted to find the genomic regions containing susceptibility loci for AMD using linkage analyses. In a study of 158 families including 511 affected sibling pairs and 97 other pairs, linkage to several chromosomes including 1q and 10q was found. (Seddon et al., 2003c) An extremely discordant sib-pair linkage analysis implicated chromosomes 1q, 2q, 6q, 19p, and 20q in the development of AMD. (Santangelo et al., 2005) Meta-analysis of AMD genome linkage results from several studies revealed that the most replicated findings were on chromosomes 1q25-31 and 10q26 (Fisher et al., 2005) and peaks in each of these regions were reported by at least five independent study groups involved in this meta-analysis. The importance of these two regions for estimating AMD genetic risk has been validated by results from GWAS (see below).

Prior to the era of GWAS, multiple candidate gene association studies were undertaken. In particular, genes associated with Mendelian macular diseases were investigated for a possible role in AMD. Several causative mutations in the vitelliform macular dystrophy 2

(*VMD2*) gene have been identified in Best's disease, an early onset, autosomal dominant macular degeneration. (White et al., 2000) However, *VMD2* mutations were not found to play a significant role in the predisposition to AMD. (Allikmets et al., 1999; Seddon et al., 2001a) Mutations in a photoreceptor cell-specific factor involved in the elongation of very long chain fatty acids (*ELOVL4*) have been associated with Stargardt-like macular degeneration and autosomal dominant macular degeneration. However, no statistically significant associations were observed between sequence variants in *ELOVL4* and susceptibility to AMD. (Ayyagari et al., 2001) ATP-binding cassette sub-family A member 4 (*ABCA4*), a gene mutated in Stargardt's disease, (Allikmets et al., 1997) has been examined in AMD by multiple studies with mixed results, (Bernstein et al., 2002; Haddad et al., 2006) and GWAS have not borne out a clear association for this gene in AMD. Finally, Tissue Inhibitor of Metalloproteinase 3 (*TIMP3*) is a gene mutated in Sorsby's fundus dystrophy. (Barbazetto et al., 2005) No evidence was found for either linkage or association for three markers at this locus as part of a small candidate gene study. (De La Paz et al., 1997) Genome-wide association studies with very large sample sizes have subsequently shown that a common variant in this gene is associated with AMD. (Chen et al., 2010; Neale et al., 2010)

5.2. Current understanding of AMD genetics: GWAS and beyond

Since 2005, several genetic variants have been consistently associated with AMD (Table 1 and Fig. 1). GWAS have been instrumental in identifying these variants. Starting with the common coding variant Y402H in the *CFH* gene on chromosome 1 found by GWAS, (Klein et al., 2005) odds ratios associated with the homozygous risk genotype varied between 2.45 and 3.33. (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Klein et al., 2005) Several other genes in the alternative complement cascade have also been consistently shown to impact AMD risk. These include an intron in *CFH*, (Maller et al., 2006) Factor B (*BF*)/Complement Component 2 (*C2*), (Gold et al., 2006; Maller et al., 2006) *C3*, (Maller et al., 2007; Yates et al., 2007) and Complement Factor I (*CFI*). (Fagerness et al., 2009)

The existence of multiple, complement-related AMD risk alleles has lent further support to the inflammatory pathogenesis theory for AMD and has shed light on the role of uncontrolled alternative complement pathway activation in this disease. *CFH* inhibits the alternative complement pathway by blocking formation of and accelerating the decay of alternative pathway C3 convertases; it also serves as a cofactor for the factor-1 mediated cleavage and inactivation of C3b. (Soames and Sim, 1997) The Y402H single nucleotide polymorphism (SNP), consistently associated with AMD, is within the *CFH* binding site for heparin and C-reactive protein. Binding to these sites increases the affinity of *CFH* for C3b, which in turn increases the ability of *CFH* to inhibit complement's effects. The genes *CFB*, *C2*, *C3* and *CFI* are all involved in this complement and immune pathway.

Several genes not involved in the complement cascade have also been implicated. Variation in the age-related maculopathy susceptibility 2/HtrA serine peptidase (*ARMS2/HTRA1*) locus on chromosome 10 has been convincingly associated with AMD (Dewan et al., 2006; Jakobsdottir et al., 2005; Yang et al., 2006) The function of this gene is not completely understood but there is evidence from animal models that it may be involved in

angiogenesis, extracellular matrix mineralization, and transforming growth factor-beta signaling. (Canfield et al., 2007; Jones et al., 2011) Genes in the high density lipoprotein cholesterol (HDL-C) pathway were shown to be associated with AMD by GWAS including: Lipase C (*LIPC*), cholesterol ester transfer protein (*CETP*), and ATP-binding cassette sub-family A member 1 (*ABCA1*). (Chen et al., 2010; Neale et al., 2010) *TIMP3* was also shown to be associated with AMD in GWAS. (Chen et al., 2010; Neale et al., 2010) Variants near collagen type X alpha 1 precursor/fyn related kinase (*COL10A1/FRK*) and collagen type XIII alpha 1 (*COL8A1*) were first reported in a large AMD GWAS and a meta-GWAS, supporting a role for extracellular matrix biology in AMD, (Neale et al., 2010; Yu et al., 2011) and these results were confirmed by a larger meta-GWAS. (Fritsche et al., 2013) GWAS associations with vascular endothelial growth factor A (*VEGFA*) (Yu et al., 2011) and transforming growth factor beta receptor 1 (*TGFBR1*) (Fritsche et al., 2013) emphasize the importance of angiogenesis in AMD. Other loci that have reached genome-wide significance in recent analyses are *TNFRSF10A*, *REST-c4orf14-POLR2B-IGFBP7*, *IER3/DDR1*, *SLC16A8*, *RAD51B*, *ADAMTS9*, *MIR548A2*, and *B3GALTL*. (Arakawa et al., 2011; Fritsche et al., 2013) The implications for pathophysiology of these latter associations are still unclear but roles for oxidative stress and DNA repair in AMD are supported by the *SLC16A8* and *RAD51B* findings, respectively (Fig. 1).

The above described variants are common (minor allele frequency ~5%) and together explain, at best, 65% of the total genetic contribution to AMD. (Fritsche et al., 2013) Some of the missing heritability may be explained by rare (minor allele frequency <5%), highly penetrant mutations. We reported that a rare, high-risk CFH haplotype with a missense mutation, R1210C, was associated with earlier onset of disease and advanced stages. (Raychaudhuri et al., 2011) Genotyping R1210C in 2423 AMD cases and 1122 controls demonstrated high penetrance (present in 40 cases versus 1 control, $p = 7.0 \times 10^{-6}$) and six year earlier onset of disease ($p = 2.3 \times 10^{-6}$). This mutant CFH protein compromises C-terminal CFH function and exhibits defective binding to C3b, C3d, heparin and endothelial cells, so this result suggests that loss of function alleles at CFH likely drive AMD risk. This same missense mutation is associated with atypical hemolytic uremic syndrome. (Martinez-Barricarte et al., 2008) This finding was one of the first instances in which a common complex disease variant led to the discovery of a rare penetrant mutation and the first one reported for AMD.

Subsequently, additional rare variants associated with AMD have emerged. Our large targeted sequencing study to define the role of rare variants in advanced AMD explored the exons of 681 genes within all known AMD-associated common genetic loci and related pathways in 2493 cases and controls. (Seddon et al., 2013d) We identified 59 rare *CFI* variants, and 7.8% of AMD cases compared to 2.3% of controls were carriers of rare missense *CFI* variants ($OR = 3.6$, $p = 2 \times 10^{-8}$). There was a predominance of dysfunctional *CFI* variants in cases compared to controls. When individual variants in other genes were tested, there was significant association between AMD and rare missense alleles in *C3* and *C9*. Genotyping in 5115 independent samples confirmed associations between AMD and a K155Q allele in *C3* (joint $p = 5.2 \times 10^{-9}$, $OR = 3.8$; replication $p = 3.5 \times 10^{-5}$, $OR = 2.8$) and a P167S allele in *C9* (joint $p = 6.5 \times 10^{-7}$, $OR = 2.2$; replication $p = 2.4 \times 10^{-5}$, $OR = 2.2$). The K155Q allele in *C3* resulted in resistance to proteolytic inactivation by CFH and CFI.

These results implicate loss of C3 protein regulation and excessive alternative complement activation in AMD pathogenesis. This study also underscores the importance of multiple rare variants (in *CFH*, *C3*, *CFI*, and *C9*) in the etiology of AMD.

One of these many rare, highly penetrant missense mutations in *CFI* encoding a Gly199Arg substitution was also found to confer a high risk of AMD ($p = 3.79 \times 10^{-6}$) in another study. (van de Ven et al., 2013) This was identified by sequencing *CFI* coding exons from the genomic DNA of 84 unrelated cases with AMD. Three cases carried the Gly119Arg substitution. A fourth case carried a Gly188Ala substitution. Neither of these variants was identified in 192 ancestry- and age-matched controls. The Gly188Ala substitution was also detected in 3 affected family members of the fourth case, although it was not found in 809 unrelated AMD cases.

By definition, rare variants are only found in a minority of the population. Therefore, rare variants only explain higher AMD risk in a small subset of patients affected by AMD and do not account for a significant proportion of AMD risk in the overall affected population. However, these rare variants have a strong effect on AMD risk in certain individuals and families, are more likely to be causal and have functional impact compared to common variants, and can offer greater insights into disease pathophysiology.

5.3. Genetic risk for AMD progression, subtypes, and peripheral findings in AMD

Genes are related not only to the occurrence of AMD in case-control studies but also to progression of the disease. The *CFH* and *ARMS2/HTRA1* loci were each independently associated to progression from early or intermediate stages to advanced stages of AMD, controlling for other known AMD risk factors. (Seddon et al., 2007). In a study of transitions from one stage to another using a Markov model, controlling for demographic and behavioral factors, variation in *LIPC* was associated with decreased risk of progression from large drusen to choroidal neovascularization and tended to reduce the risk of progression from a normal macula to intermediate drusen. (Yu et al., 2012) A risk variant in *ABCA1* was associated with decreased risk of progression from normal to intermediate drusen and from intermediate drusen to large drusen, and *CFH*, *C3*, *CFB*, and *ARMS2/HTRA1* were associated with progression from intermediate drusen to large drusen and from large drusen to GA or CNV. (Yu et al., 2012)

There are differences between how genetic variants influence risk for the two advanced subtypes of AMD, choroidal neovascularization and geographic atrophy. (Seddon et al., 2007) Two siblings are more likely to have the same subtype of advanced AMD than would be expected by chance alone. (Sobrin et al., 2012) The *ARMS2/HTRA1* locus is the first to be identified as explaining a portion of this heritability; the risk variant confers a 38% greater chance of developing CNV compared with GA. (Sobrin et al., 2010) No other variants associated with overall advanced AMD were found to have a similar differential effect in a GWAS of AMD subtypes. (Fritsche et al., 2013; Sobrin et al., 2012)

Peripheral retinal drusen and reticular pigment changes have been observed at higher rates in patients with advanced AMD. (Seddon et al., 2009b) Both peripheral phenotypes have also been associated with variation in *CFH*. (Seddon et al., 2009b) Reticular pigment was related

to *CFHY402H* with an OR of 2.0 for the CC genotype vs. TT (p for trend <0.001 , for increase in pigment with each risk allele) and to rs1410996 in *CFH* (p for trend = 0.006). For peripheral drusen, the OR was 2.8 for the CC genotype vs. TT (p for trend <0.001 , with increase in peripheral drusen with each risk allele). Similar results were seen for *CFH* rs1410996. No associations between peripheral retinal phenotypes and *ARMS2/HTRA1*, *CFB*, *C2* or *C3* were identified. Peripheral drusen were associated with homozygosity for the *CFHY402H* risk allele by an additional group. (Droz et al., 2008) Peripheral reticular pigmentary change was associated with *CFHY402H* ($p = 0.0006$) in another clinic-based case series but peripheral drusen were not associated with *CFH* genotype, (Shuler et al., 2008a) and no association was found between *ARMS2/HTRA1* and either peripheral phenotype. (Shuler et al., 2008b) To determine the independent association between AMD genes and peripheral phenotypes, controlling for the presence of AMD or adjusting for AMD grade is recommended. (Seddon et al., 2009b)

6. Gene-environment-treatment associations and interactions

6.1. CFH - environment

Gene environment studies of the *CFH* locus provide evidence that modifiable factors can alter genetic susceptibility. In one study in 2006, the susceptibility to advanced AMD associated with *CFHY402H* was modified by BMI, and both BMI and smoking increased risk of advanced AMD within the same genotype. (Seddon et al., 2006d) The association between AMD and BMI varied depending on genotype (P interaction = 0.006 for the CT vs. TT genotype). The CC genotype plus higher BMI (OR 5.9) or smoking (OR 10.2) conferred the greatest risks. There is also some evidence that an individual's response to AREDS supplements may also be related to *CFH* genotype. A treatment interaction was observed between the *CFHY402H* genotype and supplementation with antioxidants plus zinc (CC; $P = 0.03$). (Klein et al., 2008a) Supplementation was less beneficial for the *CFHY402H* homozygous risk genotype. Analyses suggested that this interaction may be related primarily to the zinc component of the supplement. Another study found an interaction between AREDS vitamin-mineral treatment and *CFHY402H*, after controlling for all AMD-related genotypes. (Seddon et al., 2009a) In the 2011 Rotterdam Study, high dietary intake of nutrients with antioxidant properties reduced the risk of early AMD even in those at high genetic risk at the *CFH* and *ARMS2/HTRA1* loci. (Ho et al., 2011) Although these early studies indicate some differential effect of supplementation based on genotype, all groups showed some benefit. There is insufficient evidence to vary recommendations regarding AREDS supplement use based on a patient's genotype.

Smoking and increased BMI are directly related to higher levels of inflammatory cytokines including plasma complement markers and serum CRP. (Reynolds et al., 2009; Seddon et al., 2006a) In a study to explore the associations between serum CRP and two major genetic loci, high-sensitivity CRP and SNPs in the *CFH* gene were independently associated with risk of AMD, and the combined effects of genetic susceptibility and higher CRP levels increased risk above the individual risks attributed to each factor alone. (Seddon et al., 2010a)

6.2. ARMS2/HTRA1 – environment and treatment

As with *CFH* variants, *ARMS2/HTRA1* risk variants combined with higher CRP levels confer greater AMD risk than would be expected from additive, independent effects. (Seddon et al., 2010a) Presence of both the highest level of CRP together with risk genotypes for both *CFH* and *ARMS2/HTRA1* produces an OR for AMD of 5.4 (95% CI, 1.4–21.1). (Seddon et al., 2010a) Unlike with *CFH*, however, there have not been any significant treatment interactions observed with *ARMS2/HTRA1* and AREDS supplementation. (Klein et al., 2008a; Seddon et al., 2009a) Overall, smoking increases risk for all *ARMS2/HTRA1* genotypes. Evidence for statistical interaction between smoking and *ARMS2/HTRA1* has not been shown in all studies. (Chakravarthy et al., 2013; Francis et al., 2007) Increased DHA intake has been shown to significantly reduce risk of progression to GA among individuals with the *ARMS2/HTRA1* homozygous risk genotype but not the non-risk *ARMS2* genotype, suggesting an interaction between DHA intake and *ARMS2/HTRA1* (P for interaction between gene and fat intake = 0.05) (Reynolds et al., 2013)

Within the Comparison of AMD Treatments Trials, a genetic substudy did not find that AMD risk alleles predicted response to anti-VEGF therapy. (Hagstrom et al., 2013) 834 participants were genotyped for SNPs in *CFH*, *ARMS2*, *HTRA1*, and *C3*. Genotypic frequencies were compared with clinical measures of response to intravitreal anti-VEGF therapy with ranibizumab or bevacizumab. No statistically significant differences in response by genotype were identified for any of the clinical measures studied including final visual acuity or change in visual acuity, the degree of anatomic response, or the number of injections required. Furthermore, a stepwise analysis failed to show a significant epistatic interaction among the variants analyzed; that is, response did not vary by the number of risk alleles present. The lack of association was similar whether patients were treated with ranibizumab or bevacizumab or whether they received monthly or as needed dosing.

6.3. LIPC – environment

Possible gene–environment interactions with the *LIPC* locus are of particular interest. *LIPC* is part of the HDL pathway, and HDL is a major transporter of lutein and zeaxanthin in the body. It is possible that changes in HDL metabolism driven by *LIPC* genotype influence how effectively these carotenoids are transported to the macula. However, thus far the gene *LIPC* and the behavioral factors smoking, BMI and dietary lutein are independently related to AMD and no interactions have been detected. (Seddon et al., 2010b)

6.4. Epigenetic effects

Epigenetics refers to the study of changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence. Examples of such modifications are DNA methylation and histone modification. Epigenetic effects of smoking and diet are beginning to emerge. Smoking as well as dietary methionine, choline and betaine have been shown to have epigenetic effects via DNA methylation. (Poulsen et al., 2007) In monozygotic twins discordant for AMD signs and severity, the number of pack-years of smoking was higher for the twin with the more advanced stage of AMD ($P = 0.05$). (Seddon et al., 2011a) Dietary intakes of betaine and methionine were also significantly higher in twins with lower stage of AMD ($P = 0.009$) and smaller drusen area ($P = 0.03$), respectively. (Seddon et al., 2011a)

These modifiable factors may induce epigenetic changes that modify gene expression, leading to different phenotypes in genetically identical individuals. The results suggest that smoking and nutritional factors induce epigenetic changes and these mechanisms are also involved in the etiology of AMD, in addition to genetic susceptibility.

A study of methylation changes at the genome-wide level in three AMD-discordant twin pairs identified many significant hypo and hyper-methylation differences in transcriptionally active sites within 231 gene promoters in the examined AMD twins. (Wei et al., 2012) These genes were identified as belonging to different pathways, including some that have been previously implicated in eye disease such as *ELOV4*. None of the differentially methylated genes identified in this study overlapped with the most significantly associated genes identified through GWAS. (Fritsche et al., 2013) A functional study on the promoter region of one of the genes identified, *IL17RC*, suggested immune alteration. There is evidence that an aged immune system is hyper-responsive to inflammatory stimuli; two inflammatory mediators, IL-17 and IL-22, are found to be elevated in AMD patients. (Liu et al., 2011) Both of these cytokines can induce demethylation changes like those found in the promoter of *IL17RC*. Therefore, differential methylation in AMD-discordant twin pairs of the *IL17RC* promoter could be mediated by a chronic inflammatory response in the diseased twin in response to particular elements of their environment.

7. Predictive models

Our expanded knowledge of the environmental and genetic factors that influence AMD risk have led to a steady increase in our ability to predict AMD development (Table 2). A means of assessing the predictive ability of a model is to calculate the sensitivity and specificity of the model for different risk thresholds. A plot of the sensitivity on the y -axis and (1-specificity) on the x -axis is called a receiver operating characteristic (ROC) curve and the Area Under Curve (AUC), or C statistic, is an overall measure of the model's predictive ability with predictive ability improving as the C statistic approaches 1. In the ROC for an initial model of AMD risk from case-control data that included *CFH* genotype along with age, education, smoking, BMI and vitamin supplement use, the C statistic was 0.70; this combination of factors accounted for about 62% of the variance in AMD development. (Seddon et al., 2006d) When baseline AMD grade and *ARMS2/HTRA1* genotype were added and applied to a progression model, the AUC and the attributable risk increased to 0.78 and 81%, respectively. (Seddon et al., 2007) Incorporation of *C2*, *CFB* and *C3* variation further improved the predictive ability for progression to advanced AMD, generating a model with an AUC of 0.83. (Seddon et al., 2009a) In a predictive model which included plasma complement components and activation fragment levels in addition to age, BMI, smoking, and seven genetic variants, the C statistic was 0.94 ± 0.20 . (Reynolds et al., 2009)

An expanded model with longer follow-up time that also employed time-varying analyses of both eyes, the AUC for progression at 10 years in the model with genetic factors, baseline drusen size in each eye, AMD stage in each eye, and environmental covariates was 0.91 in the test sample. (Seddon et al., 2011b) This model differentiated low, medium and high rates for individuals at the same stage of AMD according to their demographic, environmental and genetic risk factors. For example, an individual with large drusen in both eyes with a

high risk profile for the various variables has a 60% chance of progressing based on the predictive algorithm to advanced AMD at 10 years whereas a person with the same fundus findings and a low risk profile has a 20% chance of progression. (Seddon et al., 2011b)

A model for prediction of progression among different stages of AMD and progression to advanced AMD used a multi-state Markov model and included known genetic variants in 12 genes including the genetic loci in the HDL-C pathway, and adjusted for age, gender, education, smoking, BMI, and antioxidant treatment. (Yu et al., 2012) This study yielded AUCs for 5- and 10-year progression of 0.88 and 0.90, respectively. (Yu et al., 2012) A model that includes only the genetic information across the 19 loci that have been associated with AMD via GWAS can distinguish cases and controls relatively well with an AUC = 0.73, (Fritsche et al., 2013) but not as well as models that also include non-genetic factors. (Seddon et al., 2009a,, 2011b; 2013c) Validation of the predictive model for AMD progression that included demographic and environmental factors, 6 variants in 5 genes, and baseline AMD grades in both eyes yielded *C* statistics of 0.858 and 0.750 at 5 years and 0.884 and 0.809 at 10 years for the independent derivation and validation samples, respectively, which indicates the model can be useful for identifying high-risk subjects for participation in clinical trials. (Seddon et al., 2013c) For unilateral or bilateral intermediate AMD, 5-year cumulative incidence rates of progression to advanced AMD were 10% with the low-risk score and 50% with the high-risk score; for unilateral advanced disease, the progression rates were 22% and 80% for the fellow eye. (Seddon et al., 2013c)

When we add recently discovered variants into our predictive model, we found that the rare variant *CFHR1210C* and common variants *COL8A1* and *RAD51B* were also independently associated with incident advanced AMD. (Seddon et al., 2013b) The rare variant R1210C in *CFH* (HR 2.5, 95% confidence interval [CI] 1.2–5.3, $P = 0.01$), and common variants *COL8A1* (HR 2.0, 95% CI 1.1–3.5, $P = 0.02$) and *RAD51B* (HR 0.8, 95% CI 0.60–0.97, $P = 0.03$) modified risk of incident advanced AMD. A 9 genetic loci model including these variants contributes more predictive power than a model without genes and adds more information among individuals with high risk scores when compared with a model with only 6 genes. The adjusted odds ratios (OR) for progression within 5 years per one quintile increase in the predictive risk score were 2.7 (95% CI 1.7–4.4, $P < 0.001$) for the 9 vs. 6 genetic loci model, and 3.5 (95% CI 2.6–4.6, $P < 0.001$) for the 9 vs.0 genetic loci model.

Other prediction models based on the AREDS dataset have demonstrated that inclusion of genetic risk factors can improve model performance. One risk assessment model for development of advanced AMD included age, smoking history, family history of AMD, phenotype based on a modified AREDS simple scale score and two genetic variants *CFH* Y204H and *ARMS2* A69S. (Klein et al., 2011a) The model had good discrimination with a *C* statistic of 0.872. Another report based on the AREDS cohort examined whether inclusion of genotypes at nine different risk loci improved accuracy in predicting conversion from early-stage AMD to CNV or GA beyond utilizing phenotypic risk factors alone. (Perlee et al., 2013) The CNV prediction models that combined genotypes with phenotypes revealed superior performance (*C* statistic = 0.96) compared with the phenotype model alone which was based on the AREDS simplified severity scales and the presence of CNV in the non-study eye (*C* statistic = 0.9, $p < 0.01$).

In summary, our current knowledge of both environmental and genetic risk factors provides very good risk estimation for the development of AMD among an adult population over 5–10 years. These models can be used to compute genetic load as well as comprehensive risk scores that incorporate demographic, macular, and environmental factors. These risk algorithms are useful for research, clinical trials, and personalized medicine.

8. Clinical recommendations

Over the past three decades, we have come a long way in understanding the origins of AMD, the risk and protective factors and preventive management. Patients with AMD and people with a family history of AMD should be educated about the lifestyle factors that will reduce their risk of developing this legally-blinding disease. The strong role that smoking plays in disease onset and progression should be emphasized and smoking cessation should be encouraged. The role of regular exercise and weight control should be discussed. Patients should be advised that a healthy diet rich in green, leafy vegetables and fish will decrease the risk of AMD. If a patient cannot include enough of these foods in their diet, supplements that include the key ingredients in these foods (antioxidants, carotenoids, and omega-3 fatty acids) could be helpful. If a patient has been diagnosed with intermediate AMD in one or both eyes, AREDS-type supplements with lutein and zeaxanthin could be prescribed along with a healthy lifestyle.

The genetic variants discovered to date explain over half of the classical sibling risk of AMD.

Commercial genetic tests which screen for a few common AMD risk variants are currently available. A risk prediction model that includes demographic, behavioral, and macular phenotype characteristics along with predisposing genetic variants has been shown to be useful for identifying high risk individuals for participation in clinical trials of new therapies, reducing the sample size needed and therefore shortening the duration and lowering the cost of such studies. (Seddon et al., 2011b) Both common and rare genetic variants are predictive of whether a person will develop advanced disease and rare but highly penetrant genetic markers should be considered in future predictive tests. (Raychaudhuri et al., 2011; Seddon et al., 2013a Abstract 6178, Seddon et al., 2013d) For medical management of patients with AMD, there is currently limited value to genetic testing. Ongoing and future studies could reveal that genetic susceptibility plays a role in the effectiveness of specific therapies, and some patients with intermediate levels of AMD should be treated based on their genotypes. Genotyping and sequencing will likely become a useful tool for identifying individuals who are at higher risk for disease and who may therefore benefit from more intense monitoring and/or preventive treatment strategies. Personalized medicine is on the horizon to optimize the AMD prevention and treatment regimen for an individual.

9. Future directions

Our understanding of the contribution of genetics (nature) and environment (nurture) in the development of AMD has advanced significantly over the past two and a half decades. We

now know that AMD is a complex disease with genetic and environmental components including diet, smoking, BMI, exercise as well as systemic CVD biomarkers such as high sensitivity CRP. More than 20 common and rare AMD genetic loci have been confirmed, gene-environment and gene-treatment interactions are emerging, and both the genetic and lifestyle risk factors point to a central role for the inflammatory, immune, lipid, collagen extracellular matrix degradation, and angiogenic pathways in AMD. There are “susceptible genotypes” and manifestation of the disease is modified by behaviors and lifestyles. Risk algorithms combining these factors along with baseline macular status can predict progression to advanced AMD.

The discovery of genetic and environmental mechanisms provides targets for therapies. Various complement-modulating agents, including a C3 inhibitor, an anti-C5 antibody, a C5 inhibitor, a C5a receptor inhibitor, a CFB inhibitor, and a complement factor D antibody are currently in clinical trials for the treatment of AMD. (Gehrs et al., 2010) These results will shed light on whether modification along the complement and inflammatory pathways will be sufficient to alter the disease course. Much still remains to be discovered regarding the interplay of environmental and genetic factors and how they affect inflammation, oxidative stress, and angiogenesis to ultimately lead to disease. New genetic factors have been identified through collaborative efforts combining larger datasets which will also be valuable to examine gene-environment interactions. With increased application of the high-throughput sequencing technology that was used to identify the rare CFH R1210C variant, as well as rare variants in *CFI*, *C3* and *C9*, (Raychaudhuri et al., 2011; Seddon et al., 2013d) other such instances of rare mutations that influence AMD risk may be discovered. Identification of these highly penetrant rare causal variants provides insight into how a gene alters disease risk and identifies targets for novel biomarkers and therapies. Increased understanding of the relationship between nature and nurture in AMD pathogenesis will lead to better preventive and treatment strategies for this blinding disease.

Acknowledgments

This research was supported by grants K12-EY16335 and R01-EY11309 from the National Institutes of Health, Massachusetts Lions Eye Research Fund, Inc.; the Foundation Fighting Blindness; the Macular Vision Research Foundation; Research to Prevent Blindness Career Development Award; Research to Prevent Blindness Challenge Grant to the New England Eye Center, Department of Ophthalmology, Tufts University School of Medicine; American Macular Degeneration Foundation; The Arnold and Mabel Beckman Initiative for Macular Research; and the Macular Degeneration Research Fund of the Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Tufts University School of Medicine.

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Fig. 1. Diagram of interplay between environmental and genetic risk factors that mediate AMD risk.

Table 1

Genes with confirmed common and rare variants associated with age-related macular degeneration.

Common Variants		
<i>CFH</i> – Y402H	<i>LIPC</i>	<i>TNFRSF10A</i>
<i>CFH</i> – rs1410996	<i>CETP</i>	<i>IER3/DDR1</i>
<i>CFB</i>	<i>ABCA1</i>	<i>SLC16A8</i>
<i>C2</i>	<i>TIMP3/SYN3</i>	<i>RAD51B</i>
<i>C3</i>	<i>VEGFA</i>	<i>ADAMTS9</i>
<i>CFI</i>	<i>COL10A1</i>	<i>B3GALT</i>
<i>ARMS2/HTRA1</i>	<i>COL8A1</i>	<i>TGFBR1</i>
Rare Variants		
<i>CFH</i> – R1210C	<i>C3</i> – K155Q	<i>C9</i> – P167S

CFI- increased burden of disease with multiple variants.

Table 2

Development of AMD prediction models: 2006–2013.

Study, reference	Study design or outcome	Number of Genes/Variants	Demographic and environmental factors	Vitamin supplements	Macular phenotypes	Area under curve (AUC)
Maller et al. <i>Nat Genet</i> 2006	Case-Control	3/5	N	N	N	
Seddon et al. <i>Hum Hered</i> 2006	Case-Control	1/1	Y	Y	N	0.70
Seddon et al. <i>JAMA</i> 2007	Progression	2/2	Y	Y	Y	0.78
Seddon et al. <i>IOVS</i> 2009	Progression	5/6	Y	Y	Y	0.83
Reynolds et al. <i>IOVS</i> 2009 ^a	Case-Control	6/7	Y	N	N	0.94
Seddon et al. <i>Ophthalmology</i> 2011 ^b	Progression/ Internal Validation	5/6	Y	Y	Y	0.91
Yu et al. <i>IOVS</i> 2012	Progression	12/12	Y	Y	Y	0.90
Fritsche et al. <i>Nat Genet</i> 2013	Case-Control	19/19	N	N	N	0.73
Seddon et al. <i>JAMA Ophthalmol</i> 2013	Progression/ External Validation	5/6	Y	N	Y	0.88
Seddon et al. <i>PLoS One</i> 2013 ^{b,c}	Progression	7/9	Y	Y	Y	0.91

^aIncluded plasma complement markers.^bIncluded Detailed Macular Drusen Phenotypes.^cIncluded common and rare genetic variants and assessment by reclassification analyses.