The Genetic Epidemiology of Age-Related Maculopathy

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KEY WORDS Macular degeneration; blindness; senescence; multifactorial inheritance.

ABSTRACT Age-related maculopathy is a leading cause of blindness in the elderly. It is a major public health issue of increasing importance as populations become older. Currently considered untreatable, it is a complex disease associated with both genetic and environment factors. As currently reviewed, the importance of genetics in the etiology of age-related macular degeneration has been demonstrated by family studies, twin studies and segregation analysis. Ongoing research at the molecular level is endeavoring to isolate genes involved in the pathogenesis of this complex disease with the goal of identifying those individuals who are susceptible to impairment of visual function prior to overt manifestation of disease. The ultimate aim of this research is to identify molecular targets for appropriate and early therapeutic intervention.

INTRODUCTION

Age-related maculopathy (ARM) is an important cause of severe visual impairment (Tielsch 1995; Klein et al. 1995). Currently, there is no treatment to prevent visual loss for most eyes that develop late stages of this condition. While the natural history of ARM is beginning to become better understood, its pathogenesis remains largely unknown. Reduced vascular flow, atherosclerosis, photo-oxidative damage, inflammation, and increased growth factors are hypothesized pathogenetic mechanisms for this disease (Young 1987; Vingerling et al. 1995a; Friedman 2000). Data from most studies of this condition have shown a strong genetic component (Weeks et al. 2000; Small et al. 1998, 1999; Klein (ML) et al. 1998; Hoyng et al. 1996; Meyers et al. 1995; Meyers 1994; Stone et al. 1992; Small et al. 1992b; Dosso and Bovet 1992; Meyers and Zachary 1988). The purpose of this paper is to review briefly the natural history, pathology, and epidemiology of ARM with emphasis on its genetic epidemiology.

NATURAL HISTORY AND HISTOPATHOLOGY

Early ARM

Drusen vary a great deal in appearance, merging at one end of the spectrum with the normal fundus background and at the other end with diffuse degeneration of the retinal pigment epithelium (RPE). Typical small drusen usually appear as individual round, flat spots in the plane of the RPE. They may be a pale yellowish-white color that contrasts sharply with the surrounding RPE, or only slightly more pale than the RPE and easily overlooked. Soft drusen may be distributed as individual spots or may appear to merge with adjacent drusen. Soft drusen usually first appear in the 5th decade of life. Soft drusen consist of proteinaceous material containing lipofuscin, phospholipids, collagen types I, III, IV, and V, laminin, heparan sulfate proteoglycans, and vitronectin (Green and Harland 1999; Hageman et al. 1999; Mullins et al. 2000). While soft drusen usually increase in

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size, number, and become confluent with age, they may regress and disappear (about 20% over a 5-year period) [Klein et al. 1997b]. The central vision is usually unaffected or minimally affected when retinal soft drusen are the only manifestations of ARM (Klein et al. 1995).

Progression of ARM, in the presence of soft drusen, is marked by disruption of the RPE cells in the outer layer of the retina. These changes are characterized on ophthalmoscopy by the appearance of grayish-blackish deposits in the deep retina accompanied by depigmentation of the RPE. Histopathologically, the RPE is attenuated or atrophic and contains pigment clumps (Green and Harland 1999). If the central part of the retina, the fovea, is involved, visual acuity may decrease and straight lines may appear distorted or have missing areas. Bruch’s membrane, which separates the choroid and retina, may contain lipid deposits, and may be thicker than in eyes of similar aged persons without early signs of ARM (Pauleikhoff et al. 1990; Curcio et al. 2000). Data from one study has suggested that normal hydraulic flow between the choroid and the RPE may be altered (Moore et al. 1995). Soft drusen with or without pigmentary abnormalities in the absence of signs of late ARM (described below) characterize the early stages of ARM. The choroidal blood vessels usually appear normal during the early stages of ARM. Normally the condition involves both eyes; a lack of symmetry may indicate a different underlying cause of the drusen than aging.

Late ARM

Geographic atrophy, a sign of late ARM, is usually characterized by a sharply defined, round area of dropout of the outer segments of the retinal rod and cone photoreceptors, the RPE, and the choriocapillaris (the small blood vessels in the choroid that normally supply the outer layers of the retina), exposing larger choroidal blood vessels. Initially, the atrophic areas may surround the center of the fovea, the area of the retina responsible for fine vision, sparing central reading vision; however, as the condition progresses, the atrophic areas become confluent and involve the fovea, resulting in a loss of central vision. The visual acuity may vary between 20/80 to 5/200 or worse and a large scotoma, or blind spot, is apparent to the patient. This condition has also been labeled “dry” age-related macular degeneration.

Another form of late ARM, labeled neovascular or “wet” ARM, involves the new growth of small choroidal blood vessels that grow through Bruch’s membrane into the subretinal space. The early stages of neovascular age-related macular degeneration may not be apparent if there is no other disturbance of the retina. However, these vessels may leak, resulting in the detachment of the RPE and the sensory retina causing a disturbance of vision. Lipid exudate may appear in the deep retina and hemorrhage may appear in the subretinal space and the retina. In the later stages, sheets or mounds of white fibrous or fibrovascular tissue may appear in the central retina. This scar tissue, if it involves the fovea, results in a profound loss of central vision. The peripheral retina and vision is usually spared in eyes with late ARM.

Other conditions, which may resemble ARM, include various dystrophies such as Best’s disease, Sorsby’s, North Carolina, and pattern dystrophies, fundus flavimaculatus and Stargaard’s disease (Gass 1987). These conditions have clinical characteristics that usually permit them to be distinguished from ARM. There are other causes of neovascular macular degeneration (e.g., myopic degeneration, choroidal rupture due to trauma, Paget’s disease, angiod streaks) that must be differentiated from neovascular ARM (Gass 1987). In epidemiological and genetic studies, careful examination by a retinal specialist along with a detailed history is important to avoid misclassifying neovascular macular degeneration due to these conditions as late ARM.

Classification

Earlier clinical studies of ARM relied on detection of this condition by direct and indirect ophthalmoscopy. These approaches are not optimal for epidemiological studies and clinical trials because they require uniformly trained observers, do not provide a permanent record, and make it difficult to compare findings from center to center. Most epidemiological studies now use stereoscopic color fundus photographs of the macula, which provides an objective,
sensitive, and reproducible method for documenting ARM (Klein et al. 1991a; Bird et al. 1995). Fundus photography is rapid, noninvasive and usually accepted by subjects. The photographs are graded using standardized protocols to detect and classify the presence and severity of ARM lesions.

Various classification schemes are available. One such scheme has been published and has become widely used. In this system the presence and severity of various component lesions of ARM (e.g., soft drusen, their size, type, area of the retina covered, and confluence and pigmentary abnormalities) are graded using a standard sized grid to define the macula and other grids to define the size and area of the retina covered by the ARM lesions (Klein et al. 1991a; Bird et al. 1995). Computer-assisted grading approaches are being developed for quantitating ARM lesions from color fundus photographs (Shin and Berger 1999).

To date, there is no widely accepted severity scale for ARM. Because early stages of ARM (and even late stages of the disease, if not involving the fovea) may be found in the presence of normal visual acuity, the latter is not used to classify the presence of this condition in epidemiological studies.

Fluorescein angiography is an approach to detect the presence, type, severity, and progression of choroidal new vessels in clinical trials (Pieramici and Bressler 1999). However, angiography has not been used in epidemiological studies because it is a costly invasive technique that is associated with allergic side effects, including anaphylaxis and death. Other angiographic tests, such as indocyanine green, are also used to detect subretinal new vessels in patient care but not in epidemiological studies. There are no other standardized clinical tests that characterize ARM.

**EPIDEMIOLOGY**

**Prevalence and Incidence**

Data from population-based studies have provided information on the prevalence, incidence, and rates of progression of ARM (Klein et al. 1997b; Kahn et al. 1977a, b; Klein and Klein 1982; Klein et al. 1992; Klein et al. 1999a, b; Schachat et al. 1995; Vingerling et al. 1995a; Mitchell et al. 1995; Bressler et al. 1989; Cruickshanks et al. 1997; Bressler et al. 1995; Gibson et al. 1985; Sparrow et al. 1997). One such study was done in a large (n=4,926) white (99%) largely Western European population of persons 44-86 years of age, living in Beaver Dam, WI. (Klein et al. 1997b; Klein et al. 1992)

The age-specific prevalence and 5-year incidence of early and late ARM in the population, as determined from 30º color stereoscopic fundus photographs, are shown in Figures 1 and 2. The prevalence of late ARM in Beaver Dam is very similar to that found in the Rotterdam (1.2%), Blue Mountains (1.4%), and Framingham (1.5%) Eye Studies in persons less than 86 years of age (Kahn et al. 1977; Vingerling et al. 1995a; Mitchell et al. 1995; Bressler et al. 1989).

The prevalence of early and late ARM increases significantly with age in all epidemiological studies. In Beaver Dam, persons 75 years of age or older had a prevalence of late ARM of 7.8% and a five-year incidence of 5.4% (Klein et al. 1997b; Klein et al. 1992). Over the first five years of the Beaver Dam study, persons 75 years of age or older were six times as likely to develop early or late ARM as persons 43 to 54 years of age at baseline.

**RISK FACTORS**

**Sex**

In Beaver Dam, women 75 years of age or older had twice the incidence of early and more than seven times the incidence of late ARM as similarly aged men (Klein et al. 1997b). In the Blue Mountains, women had consistently higher prevalence of ARM than men although the differences were not statistically significant (Smith et al. 1997). No sex differences have been found in other population-based studies (Kahn et al. 1977; Klein and Klein 1982; Schachat et al. 1995; Vingerling et al. 1995a).

**Race**

Similar frequencies of soft drusen have been found in Mexican Americans, blacks and whites (Klein et al. 1999a, b; Schachat et al. 1995). However, more severe stages of early ARM
Fig. 1. The prevalence of early and late age-related maculopathy by age and sex in the Beaver Dam Eye Study (1988-1990)

Fig. 2. The 5-year incidence of early and late age-related maculopathy by age and sex in the Beaver Dam Eye Study
manifest by pigmentary abnormalities and late ARM were found to be less frequent in Mexican Americans and blacks compared to whites. The reason for these differences among racial/ethnic groups is not known. It is not explained by differences in known risk factors (e.g., cigarette smoking, hypertension) among the racial/ethnic groups (Klein et al. 1999a, b).

If pigmentation is causally associated (protective) with late stages of ARM, what might be the mechanism? One proposed physiologic explanation for a protective effect of pigment is that melanin in the RPE and choroid protects the retina from free radicals associated with photooxidation and light absorption (Jampol 1992). Other support for a protective role of pigmentation in the pathogenesis of neovascular macular degeneration comes from recent observations in transgenic mice (Rohan et al. 2000). Rohan et al. (2000) reported a higher rate of iris neovascularization and hyphema after implantation of corneal pellets of bFGF in albino mice (C57BL/6J-Tyro2j and 129/SV mice) compared to their pigmented relatives (C57BL/6J and 129/SylmJ mice, respectively). It is also possible that there is no protective effect of pigment and that genes associated with ARM are linked to those affecting pigment.

**Cardiovascular Disease and Its Risk Factors**

Epidemiological data regarding atherosclerosis and vascular factors as risk factors for ARM have not been consistent except for an association of cigarette smoking (Klein, 1998). Two possible biological explanations for this association is that smoking leads to a decrease in choroidal blood flow and depresses serum antioxidant levels, both of which have been postulated to cause ARM (Pauleikhoff et al. 1990; Berman et al. 1958; Hammond et al. 1996). Hyman et al. (1983) showed a significant association between cigarette smoking and ARM in men (odds ratio (OR) 2.6, 95% confidence interval (CI) 1.15 to 5.75) but not in women (OR 1.2, 95% CI 0.80 to 1.89). In the Eye Disease Case Control Study, smoking history was significantly associated with the presence of exudative macular degeneration (ex-smokers versus never smokers [OR 1.5 95% CI 1.2 to 2.1]; current smokers versus never smokers [OR 2.2, 95% CI 1.4 to 3.5]) [Anonymous, 1992]. Cross-sectional data from three population-based studies also show significant relationships between smoking status and odds of late ARM (Klein et al. 1993; Smith et al. 1996; Vingerling et al. 1996). In these studies, the OR for current smoking and exudative macular degeneration varied from 2.5 to 5.6. A prospective study of nurses 50 years of age or older showed a significant relationship between pack-years smoked and cumulative risk of self-reported physician-confirmed ARM assessed 12 years later (Seddon et al. 1996). The Physicians’ Health Study reported similar findings in men (Christen et al. 1996). In Beaver Dam, smoking at baseline was associated with increased odds of developing early ARM but not late ARM. The 5-year incidence of late ARM may have been too low to determine a difference between smoking status and late ARM in that study (Klein et al. 1998b). In addition, persons at greatest risk for developing late ARM (those in the oldest age group) were least likely to be current smokers at baseline. Smoking was not found to be associated with increased risk of developing choroidal new vessels in a clinical trial, the Macular Photocoagulation Study (Anonymous 1997). Further studies in larger populations are necessary to determine the relation between smoking and late ARM.

Atherosclerosis of the choroidal vasculature has been hypothesized to result in ARM by affecting choroidal blood flow (Friedman 2000; Pauleikhoff et al. 1990). Lipid deposition in Bruch’s membrane, associated with atherosclerosis, has also been hypothesized to decrease the hydraulic conductivity of the membrane resulting in RPE and outer segment dysfunction and upregulation of vascular endothelial growth factor (Friedman 2000; Moore et al. 1995). These changes are postulated to cause further degeneration of Bruch’s membrane. However, few atherosclerotic risk factors have consistently been found to be associated with incident early and late ARM (Klein 1998). High blood pressure levels and uncontrolled hypertension have been found in some but not other studies to be associated with prevalent ARM (Klein 1998). Data from Beaver Dam showed that persons with uncontrolled hypertension (systolic blood
pressure of 160 mm Hg or more or diastolic blood pressure of 95 mm Hg or more while taking antihypertensive medications) had a higher 5-year incidence of RPE depigmentation (after controlling for age and sex, OR 1.98 (95% CI 1.00 to 3.94) (Klein et al. 1997a). Higher serum cholesterol was associated with exudative macular degeneration in the Eye Disease Case Control Study (Anonymous 1992). However, serum total cholesterol and HDL-cholesterol have not been found to be associated with prevalent or incident ARM in most population-based epidemiological studies (Klein 1998). Contrary to expectations, most of these studies show either no association or a direct association of HDL-cholesterol with early and late ARM. The reason for a direct association of prevalent and incident ARM with HDL-cholesterol is not known.

Clinical evidence of cardiovascular disease (e.g., myocardial infarction or angina) is not associated with either early or late ARM in most studies (Klein 1998). However, markers of subclinical atherosclerotic disease, such as pulse pressure and carotid artery plaques as determined by ultrasonography, are. In the Beaver Dam Eye Study, while controlling for age and sex, higher pulse pressure was associated with a 30% increased 5-year incidence (95% CI 1.02 to 1.65 per 10 mm Hg of pulse pressure) and a 25% increased 5-year progression (95% CI 1.01 to 1.53 per 10 mm Hg of pulse pressure) of exudative macular degeneration in persons 65 years of age or older (Klein et al. 1997a). Vingerling et al. (1995b) reported that of persons younger than 85 years of age who had participated in the Rotterdam Study, those with plaques at the carotid bifurcation were 4.7 times more likely to have late ARM (95% CI 1.8 to 12.2) as those without these plaques. Plaques in the carotid artery were associated with exudative macular degeneration in the Cardiovascular Health Study (Klein R et al. unpublished data). Higher incidence of early and late ARM in people with stroke was found in Beaver Dam; however these associations failed to reach statistical significance (Klein et al. 1997a). These data suggest that large vessel disease (e.g., carotid artery, ophthalmic artery) may have a role in the pathogenesis of ARM.

Environmental

While animal study data show that exposure to intense bright sunlight or ultraviolet radiation may result in RPE damage similar to that seen in ARM, most data from epidemiological studies have not shown a relation of cumulative UV exposure to ARM (Klein 1998; Young 1988; Tso 1973). This may reflect the difficulty in measuring lifetime UV-B exposure from questionnaires. With the exception of the Chesapeake Bay watermen and the Beaver Dam Eye Study, most epidemiological studies have also not shown an association of light exposure with ARM (Klein 1998; Taylor et al. 1992; Cruickshanks et al. 1993). In the Chesapeake Bay watermen, cumulative ocular exposure to blue light or all wavelengths in the visible spectrum within the 20 years before examination was associated with an increased prevalence of late ARM (OR 1.35, 95% CI 1.00 to 1.81) [Taylor et al. 1992]. In cross-sectional data from Beaver Dam, light exposure was not associated with early ARM in women (Cruickshanks et al. 1993). In men, after adjusting for age, there was a relationship between the amount of leisure time spent outdoors in the summer and the prevalence of increased retinal pigment (OR 1.44, 95% CI 1.01 to 2.04 for three fourths of leisure time spent outdoors in the summer compared with one fourth of leisure time spent outdoors in the summer). The amount of time spent outdoors in summer was significantly associated with the presence of exudative macular degeneration (OR 2.26, 95% CI 1.06 to 4.81). Weak relationships of sunlight exposure to incident ARM were also reported in Beaver Dam (Cruickshanks et al. 2001).

Ocular Factors

No ocular factor (e.g., cataract, cataract surgery, iris color, and refractive error) has been consistently found to be associated with ARM (Klein 1998). Brown irides, hyperopia, and nuclear sclerosis have been reported to decrease, increase or not affect the risk of early and late ARM in various studies that have examined these relationships. For example, the presence of nuclear sclerotic cataract has been found to be inversely associated with ARM in some studies.
This has been thought to be due to a protective effect of the cataract for UV light. In other studies nuclear sclerosis was associated with an increased risk, where it has been postulated to be a result of commonly shared genetics or environmental factors, such as diet or light exposure. In Beaver Dam, there were no associations between nuclear sclerosis and the 5-year incidence of early or late ARM or progression of ARM (Klein et al. 1998a). However, in Beaver Dam, eyes that had undergone cataract surgery were more likely to have progression of ARM (OR 2.71, 95% CI 1.69 to 4.35) and develop signs of late ARM (OR 2.80, 95% CI 1.03 to 7.63) compared with phakic eyes. This association may be due to photic retinal injury during the surgery, easier detection of the ARM lesions after surgery, inflammatory changes after surgery, or to chance.

**Familial Aggregation in Macular Degeneration**

As described earlier, ARM is quite prevalent in older populations and is associated with a number of risk factors. However, these factors do not sufficiently account for the high prevalence of ARM and several studies suggest that genetic determinants play a role in the development of ARM. In an early report of familial aggregation, Streiff and Babel (1963) documented ARM in an 80-year-old mother and her 50-year-old affected daughter and suggested that, because of the late onset of the disease, a dominant form of inheritance was most likely. Meyers and Zachary (1988) subsequently reported on a pair of 85-year-old female concordant monozygotic twin sisters and suggested that genetic factors are important in some cases of ARM (Table 1). These twins lost central vision in both eyes within three years of each other. They also confirmed that two of three deceased affected siblings had ARM with both disciform degeneration and atrophic maculopathy prior to death, but five additional siblings failed to display any signs of the disease. Nine offspring of the affected twins, whose ages ranged from 51 to 62 years, failed to show symptoms of the disease but may not have reached the age-at-onset. Likewise, Dosso and Bovet (1992) reported on the development of severe ARM in monozygotic twin brothers, who exhibited similar symptoms (Table 1). Over three to four years, both developed a neovascular membrane in the left eye and later in the right eye. Interestingly, the location of the neovascular membrane was identical in both brothers.

A twin study carried out by Meyers (1994) on 83 monozygotic pairs, 28 dizygotic pairs, and one triplet set suggested a genetic predisposition to ARM (Table 1). A follow-up study analyzed 134 twin pairs and two triplet sets and found a statistically significant higher concordance of ARM in monozygotic than in dizygotic twin pairs, which again suggested the importance of genetic factors (Table 1) [Meyers et al. 1995]. In a report of familial clustering, Seddon et al. (1997) examined 119 cases and 72 controls and reported that the occurrence of ARM was greater among first-degree relatives of patients with ARM than among first-degree relatives without ARM. De La Paz (1997) concluded that the phenotypic appearance of the macula in families with multiple affected individuals is heterogeneous and characteristic of macular findings typically associated with ARM.

**Other Types of Macular Degeneration**

An early report of familial aggregation was described by Best in 1905 (Best 1905). A study performed by Small et al. (1992a) reported on

Table 1: Concordance rates found in significant twin-based maculopathy studies

<table>
<thead>
<tr>
<th>Twin Pairs</th>
<th>ARM Affected Twin Pairs</th>
<th>Concordance Rates (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Monozygotic (Sisters)</td>
<td>1 Monozygotic (Sisters)</td>
<td>100</td>
<td>Meyers and Zachary, 1988</td>
</tr>
<tr>
<td>1 Monozygotic (Brothers)</td>
<td>1 Monozygotic (Brothers)</td>
<td>100</td>
<td>Dosso and Bovet, 1992</td>
</tr>
<tr>
<td>83 Monozygotic</td>
<td>23 Monozygotic</td>
<td>100</td>
<td>Meyers, 1994</td>
</tr>
<tr>
<td>28 Dizygotic</td>
<td>8 Dizygotic</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>1 Triplet Set</td>
<td>25 Monozygotic</td>
<td>100</td>
<td>Meyers et al., 1995</td>
</tr>
<tr>
<td>134 Twin Sets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Triplet Sets</td>
<td>12 Dizygotic P</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

*Exhibited similar ophthalmological characteristics*
an autosomal dominant form of macular dystrophy known as North Carolina Macular Dystrophy (NCMD). By examining courthouse and census records, these researchers were able to trace all known affected individuals with this disease to three founding brothers who lived in Spartanburg, South Carolina, in 1790 and migrated into North Carolina during the 1820’s. Their efforts resulted in a family pedigree for NCMD consisting of more than 2000 individuals.

To characterize the genetic component for another type of macular degeneration, Edwards et al. (1999) characterized the phenotypes of two families that spanned 10 generations with an autosomal dominant form of Stargardt’s disease linked to chromosome 6q14 and determined that they shared a common ancestry.

**Segregation Analyses For Age-Related Maculopathy**

Segregation analysis is the statistical methodology used to determine from family data the mode of inheritance of a particular phenotype, especially with a view to elucidating single gene effects (Elston 1981). Heiba et al. (1994) performed complex segregation analysis, using Bonney’s (1984) regressive models, to determine if a quantitative measure for ARM showed evidence for major gene segregation in sibships from the Beaver Dam Eye Study. The quantitative trait was measured using a standardized protocol, the Wisconsin Age-Related Maculopathy Grading scheme (Klein et al. 1991a, b, 1992) that was adjusted for age and sex. Heiba et al. examined four modes of transmission, parameterized by the transmission probabilities $t_u$, the probabilities that a parent of type $u$, for $u = AA, AB$ or $BB$, transmits a factor $A$ to offspring. These modes of transmission correspond to: (1) no parent-offspring transmission, with homogeneity of the trait distribution across generations and allowing the presence of a major random environmental effect; (2) mendelian inheritance, in which case $A$ is an allele; (3) arbitrary transmission of a major effect from an $AB$ individual, which corresponds to $t_{AA} = 1, 0 \leq t_{AB} \leq 1, t_{BB} = 0$; (4) the arbitrary transmission of a major effect with all three $t$’s freely estimated between 0 and 1. The results were consistent with a major effect accounting for 62% and 59%, in the right and left eyes, respectively, of the determination of age-related maculopathy when using the t’s free model. Under an assumption of Mendelian inheritance, about 55% and 57% of the total variability, in the right and left eyes, respectively, could be attributed to single gene segregation. Thus, Mendelian inheritance accounted for 89% and 97% of the variability due to the major effect for the right and left eyes, respectively. The maximum likelihood parameter estimates (gene frequency, genotype-specific means and variances) for both eyes were very similar. While other evidence is available demonstrating that early-onset forms of ARM are heritable, this study was the first to establish that a single major gene could account for ARM inheritance in the general population. Following up on evidence from this segregation analysis, we are currently performing a genomewide-linkage scan on a sample from the Beaver Dam Eye Study to identify predisposing loci for ARM, which should enable us to further understand the etiology of ARM.

**Linkage and Candidate Gene Studies for Macular Degeneration**

Linkage studies have been conducted on families that demonstrate autosomal dominant or recessive modes of inheritance, compatible with a single major gene, for macular degeneration. For example, Small et al. (1992b) localized North Carolina Macular Dystrophy (NCMD or MCRD1) to three microsatellites located in the 6q13-q21 region of chromosome 6. Following the original report of linkage on 6q, Small (1998, 1999) examined 13 more families of various ethnic and geographic origins with NCMD for linkage with markers on chromosome 6q. Multipoint linkage analysis and haplotype analysis indicated the MCRD1 gene is in a 1.1 cM region between markers D6S249 and D6S1671 (maximum lod score = 40.03; Table 2).

Similarly, Stone et al. (1992) mapped Best’s vitelliform dystrophy, an early-onset, autosomal dominant form of macular degeneration, to chromosome 11q13 (Table 2) in a five-generation family comprising 57 members. Multipoint analysis generated a maximum lod score of 9.3 in the interval between markers INT2 and D11S871. This gene was cloned by Petrukhin et
al. (1998), is retina-specific and was designated VMD2. While mutations in this gene have been identified in Best disease families, examination of two large series of patients with ARM suggests that VMD2 does not play a major role in this common disorder (Kramer et al. 1999; Allikmets et al. 1999). However, Lotery et al. (2000) have suggested that a small fraction of patients diagnosed with ARM may actually have a late-onset variant of Best disease. Gorin et al. (1995) screened three families affected with an autosomal dominant form of peripheral and macular degeneration that included extensive geographic atrophy, pigment epithelial changes, and drusen. This group discovered a proline to arginine mutation in codon 210 of peripherin/RDS in affected family members but not in the non-affected family members. A broad variation in expression was noted in the largest family included in this study. The authors suggested that photoreceptor genes should be considered potential factors in the etiology of ARM. Kaplan et al. (1993) mapped the disease gene for Stargardt’s disease (STGD), a juvenile form of ARM, to 1p21-p13 (Table 2). Linkage studies revealed that STGD was allelic to fundus flavimaculatus, despite differences in age at onset, clinical course and severity (Gerber et al. 1995). The gene for STGD was cloned by Allikmets et al. (1997b) and encoded a retina-specific ATP-binding cassette transporter (ABCR).

Following up on the results of the linkage analysis conducted by Hoyng et al. (1996), Allikmets et al. (1997a, b) examined the hypothesis that mutations in the ABCR gene are associated with an increased risk of ARM in a case control study. However, these data are controversial (Dryja et al. 1998) and several subsequent studies performed by other researchers have been unable to replicate the association observed by Allikmets (Stone et al. 1998; De la Paz et al. 1999). The major cause of controversy in this study concerns inappropriate matching of cases and controls (Dryja et al. 1998). Almost all the variants reported by Allikmets are missense changes that are not known to cause Stargardt’s disease and most of these variants are rare alleles that were found only in one individual each. Further, by screening only 15 of 51 exons in the

### Table 2: Candidate gene regions implicated in ARM

<table>
<thead>
<tr>
<th>Disease</th>
<th>Phenotype</th>
<th>Chromosome (Marker)</th>
<th>P-Value*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARM</td>
<td>Senile Autosomal</td>
<td>1q25-q31 (D1S466-D1S413)</td>
<td>1.07 x 10^-4</td>
<td>Klein (ML) et al. 1998</td>
</tr>
<tr>
<td>ARM</td>
<td>Senile Dominant</td>
<td>10 (D10S1236)</td>
<td></td>
<td>Weeks et al. 2000</td>
</tr>
<tr>
<td>ARM</td>
<td>Recessive</td>
<td>5 (D5S1480) Model C</td>
<td>7.83 x 10^-3</td>
<td></td>
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<tr>
<td>ARM</td>
<td>Recessive</td>
<td>5 (D5S1480) Model C</td>
<td>5.30 x 10^-3</td>
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<td>Best’s Disease</td>
<td>Infantile</td>
<td>11q13 (INT2-D11S871)</td>
<td>8.46 x 10^-3</td>
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<td>NCMD</td>
<td>Senile Dominant</td>
<td>6q13-q21 Marshfield</td>
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<td>Small et al. 1992b</td>
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<td>Autosomal</td>
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<td>Small et al. 1992b</td>
</tr>
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<td>NCMD</td>
<td>Dominant markers:</td>
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<td>7.52 x 10^-6</td>
<td>Small et al. 1992b</td>
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<tr>
<td>NCMD</td>
<td>Dominant markers:</td>
<td>97</td>
<td>2.43 x 10^-10</td>
<td>Small et al. 1992b</td>
</tr>
<tr>
<td>NCMD</td>
<td>Dominant markers:</td>
<td>6SQ16 (D6S249-D6S1671)</td>
<td>3.20 x 10^-16</td>
<td>Small et al. 1998, 1999</td>
</tr>
<tr>
<td>Stargardt’s Disease</td>
<td>Juvenile &amp; Senile</td>
<td>1p21-p13 (D1S406-D1S236)</td>
<td>4.38 x 10^-5</td>
<td>Hoyng et al. 1996</td>
</tr>
<tr>
<td>(ABCR)</td>
<td>Autosomal Recessive</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P-Value = (P-Value for c^2(1dfv)) / 2, where c^2 = 4.6 x (lod score), assuming an asymptotic one-sided chi-square distribution
control group, Allikmets did not evaluate this group equally with the case group and hence biased the study. De la Paz et al. (1999) was unable to replicate the results of the Allikmets study and advised that the ABCR gene is not a major genetic risk factor for ARM even though its involvement in some cases of ARM could not be ruled out. Reasons for the discrepancies between the two studies may be that De La Paz did not consider patients with only small drusen or peripheral drusen to be affected, in contrast to the patients included in the Allikmets study. De La Paz studied a white population from the southeastern United States while Allikmets used data sets from Utah and Boston that were primarily western European in origin. Maugeri et al. (1999), however, screened the ABCR gene in western European patients affected with Stargardt’s disease and proposed the existence of a relatively large pool of mild ABCR variants in the general population that may confer an increased risk of developing ARM. In a later study, Allikmets et al. (2000) presented new data suggesting that the ABCR gene is a susceptibility locus for ARM. This study reports that two sequence changes, G1961E and D2177N, were found in one allele of the ABCR gene. Souied et al. (2000) examined 52 unrelated cases of ARM and observed that only two (4%) demonstrated segregation of the ABCR gene mutations with familial cases of ARM. The controversy concerning ABCR mutations and ARM may be difficult to resolve, given the high rate of polymorphisms in the ABCR gene, the high incidence rate of ARM (Souied et al. 1999), and the fact that all these studies tested for association and not linkage.

A primary characteristic of ARM is its direct relationship with chronological age. Mainly, it is a disease of the last third of life and its incidence increases with each year of age (Young 1987). Because ARM is characterized by the degeneration of the neuroepithelium in the macular region of the eye, Apolipoprotein E (ApoE), a protein associated with neurodegenerative diseases, was proposed to be associated with ARM through retinal membrane renewal (Klaver et al. 1998). This hypothesis has been examined in two case-control studies. Souied et al. (1998) observed a lower relative frequency of the e4 allele in 116 ARM patients compared with 168 age- and sex-matched control subjects, suggesting that the e4 allele has a protective effect. Pang et al. (2000) examined 98 cases and 39 controls from a Chinese population and suggested that the gene for ApoE is not a major factor associated with ARM in Chinese.

Klein (ML) et al. (1998) performed a two-point linkage analysis on a 21-member family with an unusually high incidence of ARM and reported that ARM segregated in this family as an autosomal dominant trait. The disease locus was mapped to a 9 cM region in the 1q25-q31 region of chromosome 1 (Table 2). A new locus for dominant drusen and macular degeneration has been mapped to chromosome 6q14 in a single family (Kniazeva et al. 2000). This locus is distinct from, but adjacent to, the MCDR1 locus.

In contrast to the previous reports that specifically examined Mendelian forms of ARM, Weeks et al. (2000) performed a full genome scan on 364 families containing relative pairs affected with ARM, in order to test candidate genes as well as to search for any anonymous genetic loci that might contribute to susceptibility for ARM. A total of 422 markers were typed, including 386 evenly spaced markers spanning the full genome, 18 markers located in candidate gene regions, and 18 follow-up markers located near regions of interest on chromosomes 5, 9, 10 and 12. Candidate regions examined included 1p21-p13 but not 1q25-q31. Using three nonparametric diagnostic models, the multipoint GeneHunter-Plus (GHP) lod scores peaked consistently near marker D10S1236 (Table 2). These models included: (1) Model A in which only those individuals definitely affected with ARM were included in the analysis; (2) Model B, which was limited to both those individuals definitely affected with ARM those probably affected with ARM; and (3) Model C, which considered as affected not only those individuals definitely and probably affected with ARM, but those whose diagnosis provided insufficient evidence to rule out another type of macular disease. After error filtration, a peak GHP lod score of 1.27 was obtained under Model A and 1.42 under Model C. Another region of interest for further study was located on
chromosome 5, close to marker D5S1480 near where the candidate gene glutathione peroxidase 3 resides (Table 2). Using a smaller sample of 212 affected sib pairs from 225 ARM families, evidence of linkage was also found on chromosome 9 near D9S301 and on chromosome 10 near D10S1230 with peak GHP lod scores of 1.69 and 1.83, respectively. With the expanded family size, however, the signal for D9S301 vanished while the signal for D10S1230 decreased to a GHP lod score of 1.0. The inconclusive results obtained from this genome scan for ARM are not surprising because this small sample of families may not provide sufficient evidence for linkage in the presence of heterogeneity, multiple underlying trait loci, misclassifications and potential phenocopies.

**DISCUSSION**

Age-related maculopathy is a multifactorial disease, involving both genetic and environmental components. ARM is associated with advanced age and demonstrates a recent increase in prevalence due to growth in the size of the aging population, as suggested by epidemiologic examination of cohorts from several communities. Possible non-genetic risk factors associated with ARM include cigarette smoking, high blood pressure, cardiovascular diseases, skin pigmentation, iris color, and nutritional factors such as Vitamin E or C deficiencies. Although familial aggregation has been established both by family and twin studies, the elucidation of specific genetic mechanisms has been complicated by the late age of onset of ARM. Segregation analysis of a quantitative trait for age-related maculopathy in the Beaver Dam Eye Study suggested that a major gene is involved in its etiology. Recently, several association and linkage studies have been conducted for ARM. Differences in allelic frequencies in the ABCR gene were tested using case-control methodology. The results of these association analyses have been controversial because only a single group has been able to demonstrate significant association between mutant alleles in ABCR and ARM. However, more encouraging results have been obtained from linkage studies. One group was able to demonstrate moderate evidence for linkage of ARM with markers in a 9 cM region on 1q25-q31 within a single large family, and a genome scan of 364 families with ARM demonstrated some weak evidence for linkage on chromosomes 5, 9 and 10. Because of the complexity of the disease phenotype, including the overlap with other macular phenotypes such as Stargardt’s disease, it is important that the results of the genome scan performed by Weeks et al. (2000) be replicated in an independent sample. We are currently in the process of performing a genome scan in sibships from a community-based sample in Beaver Dam, Wisconsin. In contrast to our sample, the sample used by Weeks et al. (2000) comprised large, highly ascertained families. Because of differences in sample ascertainment and phenotyping between the two studies, it is quite possible that results obtained in our study may not duplicate the results obtained by Weeks et al. (2000). Despite segregation analysis suggesting a single major gene predisposes to ARM, the success in identifying genes for ARM is dependent on many factors, including the homogeneity of the sample. If ARM follows the paradigm of retinitis pigmentosa, for which 17 candidate loci have been identified, then a very large sample may be required to identify loci for this disease. Therefore it is important that multiple study designs be utilized to enable identification of ARM genetic components. As compared to other multifactorial diseases, such as asthma or diabetes, the identification of genetic components for ARM is in its infancy. However, the availability of high throughput molecular methodologies and novel statistical methods should eventually permit identification of genes for ARM.

**ACKNOWLEDGMENTS**

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