Extremely Discordant Sib-Pair Study Design to Determine Risk Factors for Neovascular Age-Related Macular Degeneration

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Objective: To search for factors that contribute to the development of neovascular age-related macular degeneration (AMD).

Methods: In a matched-pair case-control study, we studied sib pairs in which the index sibling had neovascular AMD in at least 1 eye and the unaffected sibling had normal maculae (or at most only a few small drusen) and was past the age at which the index case was diagnosed. Factors studied included sex, iris color, education, alcohol consumption, body mass index, vitamin use, smoking history, hypercholesterolemia, aspirin use, hypertension, other cardiovascular disease, any autoimmune disease, and non–insulin-dependent diabetes mellitus. Conditional logistic regression was performed to identify predictors of neovascular AMD.

Results: On the basis of 73 sib pairs, multivariate regression analysis revealed a statistically significant 2% increase in risk of neovascular AMD with each pack-year of smoking (odds ratio, 1.02; 95% confidence interval, 1.01-1.04; \( P = .007 \)). Suggestive but nonsignificant associations were also observed for mean lifetime alcohol consumption, adult lifetime body mass index, and hypertension in multivariate regression analyses.

Conclusion: Using extremely discordant sib pairs to study risk factors for AMD, a novel approach in epidemiological design, we found evidence that smoking is a risk factor for neovascular AMD.


RESULTS OF PREVIOUS STUDIES of environmental, constitutional, medical, and social factors have not been in agreement as to predictors of age-related macular degeneration (AMD). Cigarette smoking appears to be the only risk factor consistently associated with an increased risk for AMD. However, this factor has not been detected in some studies of the neovascular form of the disease.\(^1\)\(^-\)\(^3\) Given the multifactorial and heterogeneous nature of AMD, the use of epidemiological methods to detect weak to moderate associations may be required to identify modifiable risk factors.

Mathematical analyses indicate that the evaluation of sib pairs who are extremely discordant for a quantitative multifactorial trait can be a powerful method for identifying the genes that govern the trait.\(^4\) The prototype of an “extreme” sib pair consists of 1 member with a trait value in the top 10% and the other member in the bottom 10% of the population distribution.\(^5\) Sib pairs composed of the top 10% and bottom 30% can be almost as valuable.\(^6\) Such sib pairs are more likely than pairs of siblings with intermediate trait values to differ at many of the multiple genetic loci that govern a multifactorial trait. By extension, extremely discordant sib pairs would also be expected to report different exposures to any environmental factors that influence the trait. Thus, epidemiological studies of multifactorial traits might also benefit from the analysis of extremely discordant sib pairs.

We studied extremely discordant sib pairs to search for epidemiological risk factors associated with AMD. To do this, we needed to measure macular degeneration as a quantitative trait. Some groups have proposed staging schemes that in effect provide that quantification;\(^6\)\(^-\)\(^7\) (Mathew Davis, MD, unpublished data, May 2003). Regardless of the quantification scheme, it seems clear that patients with neovascular AMD or atrophic AMD involving the fovea (ie, geographic atrophy) are at the severe end of the spectrum of macular aging changes. Data from epidemiological studies of populations in the Netherlands, Australia, and Wisconsin\(^8\) indicate that the prevalence of neovascular AMD is about 4% and of geographic atrophy is about 2%, for a combined total of 6% in those older than 75 years.
For this study, we considered patients with these forms of AMD to be in the top (ie, most severe) decile. Even fewer younger individuals have neovascularization or geographic atrophy, so individuals younger than 75 years with these severe forms of AMD also would be in the top decile. Patients with no aging changes in the fundus would represent the opposite end of the AMD spectrum; they would have the lowest trait values in any AMD grading scheme. Drusen are present in at least 1 eye of about 95% of those older than 43 years, so it would be reasonable to place individuals with no drusen or other macular aging changes in the bottom decile. Among individuals aged 75 years or older in Beaver Dam, Wis, about 30% have no drusen greater than 63 µm in diameter, no retinal pigmentary abnormalities, no geographic atrophy, and no neovascularization. On the basis of these results, we considered such individuals to be in the bottom 30% of the spectrum of macular aging changes.

We confined this study to patients with neovascular AMD and excluded patients with geographic atrophy because patients with neovascular AMD were readily available for recruitment at the Massachusetts Eye and Ear Infirmary, Boston. More important, because some risk factors may specifically predispose patients to this severe subtype of AMD, an analysis of only those with neovascular AMD might have more power. For epidemiological evaluation, we recruited sib pairs in which the index sibling had the neovascular form of AMD, and the matching unaffected sibling had either no macular aging changes or at most only a few small drusen at an age equal to or older than the age at which the index sibling had neovascular AMD diagnosed.

Methods

The protocol was reviewed and approved by the institutional review board at the Massachusetts Eye and Ear Infirmary and conforms to the tenets of the Declaration of Helsinki. Eligible patients were enrolled in this study after they provided informed consent either in person, on the telephone, or through the mail.

Patient Population and Recruitment

Recruitment for this study occurred from September 1998 to June 2003. Index patients with neovascular AMD were recruited from the Retina Service of the Massachusetts Eye and Ear Infirmary. Each recruited index patient was asked if he or she had a sibling with no history of AMD and who was past the age at which the index patient first had neovascular AMD diagnosed. After obtaining informed consent from the index patient, we contacted those siblings, asked for their consent to enter the study, and then reviewed their ocular history. If a sibling was apparently eligible as an unaffected sibling, we sought to document that status by obtaining fundus photographs or by arranging to have copies of pre-existing fundus photographs sent to us for review. A few unaffected siblings were unwilling to come to an ophthalmologist’s office for fundus photographs, or they were too frail to do so, but they were amenable to a home visit by 1 of the investigators (T.P.D.) for a dilated fundus examination.

Validation That Sib Pairs Are Likely Full Siblings

Blood samples from index and unaffected siblings were collected. The DNA purified from these samples was analyzed with 3 highly polymorphic microsatellite markers (D2S428, D3S186, D7S796) with a heterozygosity of 94%.

Clinical Evaluation of Index Patients and Control Subjects

All index patients were aged 50 years or older and had the neovascular form of AMD in at least 1 eye; neovascular AMD was defined as subretinal hemorrhage, fibrosis, or fluorescein angiographic presence of neovascularization documented at the time of or before enrollment in the study. Patients whose only exudative finding was a retinal pigment epithelium detachment were excluded because this finding may not represent definite neovascular AMD and, therefore, the severe phenotype we sought. Patients with signs of pathologic myopia, presumed ocular histoplasmosis syndrome, angiod streaks, or choroidal rupture were also excluded.

The unaffected siblings had normal maculae at an age older than that at which the index patient had neovascular AMD diagnosed. Unaffected siblings had maculae (defined as the zone centered at the foveola and extending 2 disc diameters, or 3000 µm, in radius) fulfilling the following criteria: 0 to 5 small drusen (all less than 63 µm in diameter), no pigment abnormalities, no geographic atrophy, and no neovascularization as defined previously. Disease status of every participant was confirmed by means of fundus photography or fluorescein angiography by at least 2 investigators (T.P.D. and J.W.M.), except in 4 cases in which 1 of the investigators (T.P.D.) conducted a home examination.

Data Collection and Ascertainment of Exposures

Interviews of participants were conducted in person or by telephone. After a sib pair was deemed eligible (Figure), we administered a standardized questionnaire to all participants to ascertain potential risk factors for AMD. The factors studied were sex, iris color, education, alcohol consumption, body mass index (BMI), multivitamin use, vitamin C use, vitamin E use, smoking history, hypercholesterolemia, aspirin use, hypertension, other cardiovascular risk factors (angina, bypass surgery, myocardial infarction, stroke, or transient ischemic attack), autoimmune disease (thyroid disease, psoriasis or eczema, systemic lupus, multiple sclerosis, rheumatoid arthritis), and non-insulin-dependent diabetes mellitus.

Through an interview with the index patient, we established the age at which he or she was first told by an ophthalmologist that he or she had AMD; in most cases, AMD and neovascular AMD were diagnosed simultaneously. This age was the reference age for the index patient. The reference age for all siblings of the index patient was the reference age of the index patient. In sibships in which more than 1 sibling was affected (n=4), the affected sibling with the earliest age of diagnosis was used as the reference age for all siblings.

Measurement of Variables

Iris color was self-reported by the patient, with brown defined as dark and nonbrown defined as blue, gray, green, or hazel. Education was self-reported and categorized into 3 groups: college graduate and/or advanced degree (ie, master’s, legal, medical, doctorate) vs high school graduate with or without some college vs no high school diploma. Regular use of alcoholic beverages was measured as grams of alcohol consumed per week, with 1 can, glass, or bottle of beer equal to 12.8 g of ethanol; 1 4-oz glass of wine equal to 11.0 g of ethanol; and 1 drink or shot of liquor equal to 14.0 g of ethanol.
The mean number of drinks per week was calculated per decade from the 20s until the decade of the reference age for each sib pair. Weight in pounds was recorded decade by decade and then converted to kilograms, excluding years of pregnancy or lactation, from the 20s until the decade of the reference age. Adult lifetime BMI was calculated as the mean weight in kilograms across these decades divided by the square of the height in meters at age 25 years.

Use of multivitamins, vitamin C, vitamin E, or aspirin was scored as positive if it was regularly used (ie, taken at least twice per week for at least 6 months before AMD diagnosis). Participants were categorized regarding their smoking history as “never smoked,” “past smoker,” or “current smoker.” A past or current smoker was defined as having smoked 100 or more cigarettes in the course of his or her entire life. If the patient was a past or current smoker, the age when the individual started smoking was recorded. If an individual was a past smoker, the age when the individual quit was recorded. The number of pack-years of cigarettes smoked was estimated for each past and current smoker. A pack-year was defined as 1 pack of cigarettes a day for 1 year, with 20 cigarettes equaling 1 pack.

For medical conditions including hypercholesterolemia, hypertension, non–insulin-dependent diabetes, any autoimmune disease, and angina, the regular use of medication as reported by the individual for at least 6 months before the reference age was necessary to define the presence of the condition. The regular use of medication was defined as at least twice a week for 6 months or more.

DATA ANALYSES

This was a matched-pair case-control study, so conditional logistic regression with commercially available software (StataCorp, College Station, Tex) was performed to identify risk factors for neovascular AMD. Exposures as defined earlier were evaluated initially by means of univariate analysis. Where appropriate, exposures were entered into separate models as either continuous or categorical variables, including alcohol consumption, BMI, pack-years of smoking, and duration of certain chronic conditions. A multivariate model was built by using the risk factors from the univariate model that appeared to be associated with neovascular AMD; $P < .1$ was considered to indicate statistical significance. Nonsignificant variables were then dropped from the multivariate model to create the most parsimonious statistical model predictive of neovascular AMD.

RESULTS

RECRUITMENT AND DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

We recruited 81 extremely discordant sib pairs derived from 64 index cases (ie, some index patients had more than 1 matching sibling). The 64 index cases were identified after approaching 533 patients who had neovascular AMD. We obtained epidemiological data by completing standardized questionnaires in 73 (90%) of 81 extremely discordant sib pairs. The remaining 8 sib pairs were not enrolled because 1 sibling in each pair had severely deteriorating health or had died during the course of the study, which precluded completion of the questionnaire.

The 73 enrolled sib pairs were derived from 50 sibships. Eight of the 50 sibships had more than 1 unaf-
The mean and median interview ages for unaffected siblings were 75.8 and 77.0 years, respectively, whereas the mean and median interview ages for affected siblings were 74.5 and 73.8 years (range, 56.5-88.5 years). The mean age of the 63 unaffected siblings at the time of AMD diagnosis was 69.1 years (range, 44.7-86.5 years). The median age was 70.8 years (range, 44.7-86.5 years).

Multivariate analyses were conducted to determine which risk factors might be independently associated with neovascular AMD. For the multivariate analyses, we did not control for age because the reference age for both the unaffected and affected sibling in a pair was the same. The reference age was the same across sibships to reduce bias in measurement of exposure levels between the siblings and to ensure that the variable being studied occurred before the onset of AMD. Our

To determine if there was substantial contamination of our sib pairs with sib pairs who were unknowingly half siblings or who were unrelated, we analyzed DNA from a subset of 48 of the sib pairs with 3 unlinked microsatellite markers (D2S428, D5S816, D7S796) each having a heterozygosity of 94%. At each marker locus, we recorded the number of alleles (0, 1, or 2) shared by each pair of siblings, which is defined as the identity-by-state score. Summing all 3 markers and excluding occasional samples that did not work for 1 or 2 markers, we found 25 instances of an identity-by-state score of 0, 67 of an identity-by-state score of 1, and 41 of an identity-by-state score of 2. This finding was compared with the expected identity-by-state score totals for markers with a heterozygosity of 94% (30, 66, and 37, respectively). There was no statistically significant difference between the observed and expected distributions of identity-by-state scores (χ²=0.67, P>.75). This result suggests that there is no major contamination of our recruited discordant sib pairs with half siblings or unrelated pairs.

**STATISTICAL ANALYSIS**

In univariate analyses, associations were found at P<.1 between neovascular AMD and alcohol consumption of 105 g or more per week, increasing pack-years of smoking, adult lifetime BMI greater than 20 kg/m² and less than 25 kg/m², and hypertension. Additionally, subjects with hypertension diagnosed more than 5 years before AMD diagnosis had increased risk of neovascular AMD, as compared with subjects who were normotensive or who were hypertensive 5 years or less (Table). Tests for trend for increasing alcohol consumption and BMI were not statistically significant (data not shown). No associations were found between neovascular AMD and the use of multivitamin preparations or the use of vitamins C or E individually (Table); this lack of association was also observed when any vitamin use (vitamin C, vitamin E, or multivitamins) was considered as a group (odds ratio [OR], 1.21; 95% confidence interval [CI], 0.52-2.82; P=.66). Furthermore, no protective effect was suggested when we considered participants who used all 3 supplements (OR, 0.98; 95% CI, 0.62-1.59; P=.92). We did not find a statistically significant association between any other studied risk factor and neovascular AMD (Table).
multivariate model included adult lifetime BMI, alcohol consumption, pack-years of smoking, and duration of hypertension (or history of hypertension); these variables from the univariate analyses met our criteria for entry into the multivariate model (ie, significance at P<.1).

We found that alcohol consumption of 105 g or more per week, which was a statistically significant risk factor in univariate analysis, was not associated with neovascular AMD in the multivariate model (OR, 3.52; 95% CI, 0.42-29.77; P = .25). Similarly, duration of hypertension for more than 5 years (OR, 1.84; 95% CI, 0.62-5.48; P = .27) and mean lifetime BMI greater than 20 kg/m² and less than 25 kg/m² (OR, 3.58; 95% CI, 0.83-15.49; P = .09) were not significantly predictive of neovascular AMD after controlling for other factors. A history of hypertension was also not significantly associated with AMD risk in the multivariate model. Only pack-years of smoking was a significant predictor of neovascular AMD. Specifically, each pack-year of smoking was associated with a 2% increase in the risk of neovascular AMD (OR, 1.02; 95% CI, 1.01-1.04; P = .007).

**COMMENT**

Our analyses of extremely discordant sib pairs suggest that smoking increases the risk of neovascular AMD. These results are in agreement with those of previous studies⁸,¹³,¹⁴ of risk factors for neovascular AMD that found that smoking increases risk. However, authors of 1 recent prospective study from Beaver Dam, Wis,³ did not find an association between pack-years of smoking, evaluated in 10-year increments, and neovascular AMD. Furthermore, a retrospective study by Chaine et al² and a prospective study by Vinding et al³ also showed no association between smoking and neovascular AMD. However, the authors of these studies did not use pack-years to analyze smoking but instead used smoker status (eg, past vs present). To our knowledge, in no previous study of AMD was the extremely discordant sib-pair approach used.

Suggestive findings in our study for BMI, alcohol consumption, and hypertension history are supported by some previous research results. Body mass index was linked to risk of neovascular AMD in the Age-Related Eye Disease Study,¹³ and BMI was a significant factor in AMD progression of any type in the progression of AMD study.¹⁶ Findings regarding associations between neovascular AMD and history of hypertension or heavy alcohol consumption have been inconsistent. Results of 2 prior retrospective studies³,¹⁷ supported an association, whereas results of another retrospective study² and a meta-analysis⁸ of 3 prospective studies (Beaver Dam, Rotterdam, and the Blue Mountain Eye Study) showed no independent association between hypertension and neovascular AMD. Klein et al⁸ reported an increased risk of late-stage AMD (cases of neovascular AMD and cases of geographic atrophy) associated with heavy drinking. This finding is in contrast to those of Cho et al¹² and Ajani et al¹⁰ who found no association of AMD with alcohol consumption.

Differences in definition and measurement of these exposures across studies make it difficult to compare and interpret these findings. In addition, these factors, including smoking, are intercorrelated, and confounding of associations in some studies may contribute to the disparate results. In our analysis, ORs changed only minimally in the multivariate-adjusted analysis, but our study lacked the statistical power for a rigorous evaluation of these relationships.

Our study was retrospective and, by design, most unaffected siblings were older at the time of their interviews than were their affected siblings. As a result, recall bias may have been introduced, with index patients reporting exposures more accurately than did their unaffected siblings. However, we believe this bias was minor because the difference in mean interview age between the affected and unaffected siblings was less than 2 years.

It is possible that we underestimated exposure measures of unaffected siblings by using the youngest age of diagnosis as our reference age. This measure, however, is unlikely to have affected our results because most (80% [58 of 73]) of our sib pairs involved unaffected siblings who were older than the affected siblings. Nevertheless, to evaluate this possibility, we performed additional analyses by using the oldest age of diagnosis as our reference age in families with multiple affected siblings. Results of this analysis were similar to those of our original analysis, with smoking identified as a significant predictor of neovascular AMD.

Although analysis of extremely discordant sib pairs can be used to convincingly identify risk factors with relatively small numbers of cases, false-negative results (type II errors) may occur because of high concordance of genetic and demographic factors among siblings. For example, we found a high level of concordance among our sib pairs for iris color, other cardiovascular factors, non–insulin-dependent diabetes mellitus, and level of education. Moreover, with only 73 sib pairs analyzed, the study may be relatively underpowered, and factors significant in univariate analysis failed to achieve significance in the multivariate models. Thus, our study provides only suggestive evidence that other factors, including BMI, hypertension, and alcohol consumption, may have a role in the development of neovascular AMD.

Despite the limitations of our study, the fact that we found an association between neovascular AMD and cigarette smoking with our admittedly modest sample size is strong evidence in favor of the validity of this association. The extremely discordant sib-pair approach may be a valuable tool for understanding the role and interaction of epidemiological and genetic factors in contributing to multifactorial diseases such as AMD.

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REFERENCES


ARCHIVES Web Quiz Winner

Congratulations to the winner of our December quiz, Anmar M. A. Rahman, University of Wisconsin, Madison. The correct answer to our December challenge was amyloidosis. For a complete discussion of this case, see the Clinicopathologic Reports, Case Reports, and Small Case Series section in the January ARCHIVES (Barouch FC, Benson MD, Mukai S. Isolated vitreoretinal amyloidosis in the absence of transthyretin mutations. Arch Ophthalmol. 2004;122:123-125).

Figure 1. A. Preoperative fundus photograph demonstrating white vitreous deposits. B. Postoperative fundus photograph of the peripheral retina demonstrating yellow deposits under the retina.

Be sure to visit the Archives of Ophthalmology Web site (http://www.archophthalmol.com) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month’s print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also be able to choose one of the following books published by AMA Press: Clinical Eye Atlas, Clinical Retina, or Users’ Guides to the Medical Literature.