

Biomarkers of Cardiovascular Disease as Risk Factors for Age-Related Macular Degeneration

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Purpose: To measure and contrast 2 biomarkers of cardiovascular disease, C-reactive protein (CRP) and plasma homocysteine, in individuals with age-related macular degeneration (AMD) and control individuals without AMD.

Design: Case-control study.

Participants: Seventy-nine affected individuals and 77 unaffected individuals from the AMD Genetic Study Group returned to obtain CRP and homocysteine levels.

Methods: Both affected and unaffected individuals underwent testing for CRP and homocysteine. A detailed cardiovascular history was taken.

Main Outcome Measures: Mean CRP and homocysteine levels in affected and unaffected individuals.

Results: Mean CRP levels for affected and unaffected individuals were 3.42 and 2.30 mg/l, respectively ($P = 0.03$). Mean homocysteine levels for affected and unaffected individuals were 11.72 and 8.88 $\mu\text{mol/l}$, respectively ($P < 0.0001$). In logistic regression models, older age, higher CRP, and higher homocysteine were risk factors for AMD. There were no significant differences between cases and controls in terms of gender, diabetes, hypertension, use of statin drugs, and smoking. The control group was significantly younger and had a lower rate of vitamin usage than the affected group.

Conclusions: Elevated CRP and homocysteine levels are associated with AMD and implicate the role of chronic inflammation and atherosclerosis. *Ophthalmology* 2005;112:2076-2080 © 2005 by the American Academy of Ophthalmology.

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss and blindness¹ in the United States. It is a complex multifactorial disease whose pathogenesis is poorly understood. Epidemiological studies have implicated an interaction of genetic and environmental factors in the pathogenesis of AMD. Population studies²⁻⁴ have suggested familial aggregation of AMD and genetic predisposition to disease pathogenesis. Numerous epidemiological studies⁵⁻¹⁴ have tried to elucidate modifiable envi-

ronmental risk factors for macular degeneration. Many of these documented risk factors represent a cardiovascular risk profile. Smoking,^{5,10} obesity,^{6,10} and elevated plasma fibrinogen¹⁰ increase the risk of AMD. The Eye Disease Case-Control Study⁷ demonstrated 4-fold increased odds of having neovascular AMD in the group with the highest total serum cholesterol level. The Age-Related Macular Degeneration Risk Factors Study Group⁹ reported that moderate to severe hypertension was linked to neovascular AMD. Plaques in the carotid bifurcation and lower-extremity arterial disease⁸ are also associated with AMD.

These epidemiological studies have evaluated risk factors that are common to both cardiovascular disease and AMD and suggest that the diseases may have similar etiological mechanisms. Evaluating new biomarkers of cardiovascular disease may provide insight into the etiological mechanisms of AMD. Two novel markers of cardiovascular disease are particularly interesting: high-sensitivity C-reactive protein (CRP) and homocysteine. High-sensitivity CRP is an ultrasensitive assay of CRP, a marker of systemic inflammation,¹⁵ and has been shown to be an independent risk factor and predictor of cardiovascular disease.^{16,17} Plasma homocysteine, a sulfur-containing amino acid formed during the metabolism of methionine,¹⁸ is an independent risk factor¹⁹ for cardiovascular disease, similar to smoking or hyperlipidemia. Evaluation of these 2 biomarkers of inflammation

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and cardiovascular disease in patients with AMD may provide insights into etiological mechanisms involved in AMD.

Materials and Methods

The University of Michigan Kellogg Eye Center previously established an AMD Genetic Study Group consisting of over 1400 individuals with documented AMD who agreed to provide data and blood samples for DNA analysis. The AMD Genetic Study Group has also recruited ≥ 65 -year-old individuals with no evidence of AMD for similar testing. These individuals have all undergone a complete ophthalmic examination. Affected individuals were classified by staff ophthalmologists as having macular drusen, geographic atrophy, or neovascular macular degeneration. In this case-control study, which had institutional review board approval, affected individuals were sent a letter requesting that they return for CRP and homocysteine testing. Nonaffected individuals with similar ages were also sent a letter to return for the tests. Sample size estimation showed that 86 individuals in each group could detect a difference of the magnitude of 3.0 $\mu\text{mol/l}$ of homocysteine and 0.35 mg/l of CRP with 90% power. An extensive medical and drug history was retaken at the time of the testing. All individuals were provided with the test results, and if either biomarker was elevated, they were instructed to share this information with their primary care physician.

As most solicited individuals were elderly, they were instructed to have a small nonprotein breakfast on the day of testing. To ensure that fasting and nonfasting homocysteine levels were comparable, repetitive testing of a small group of individuals in the fasting and nonfasting states (small nonprotein breakfast) was carried out.

For total plasma homocysteine measurement, blood was collected in a tube containing ethylenediaminetetraacetic acid as an anticoagulant and was immediately centrifuged in the clinic to remove plasma from red blood cells. Immediate centrifugation²⁰ prevents the rapid increase in plasma homocysteine when whole blood is left at ambient temperature. The plasma was then transported on ice to the laboratory, where total plasma homocysteine was measured by high-performance liquid chromatography.²¹ At the time of blood collection, none of the individuals had had a fluorescein angiogram, which can interfere²² with homocysteine testing.

C-reactive protein is usually measured in clinical laboratories by either immunonephelometric or immunoturbidimetric assays²³ with a detection limit of 3 to 5 mg/l, which is adequate for the traditional clinical utility of CRP in monitoring infection. In contrast, studies that have shown that CRP can be used to predict the risk of future cardiovascular disease have used a high-sensitivity CRP assay that is capable of measuring CRP at a concentration of 0.007 mg/l. For high-sensitivity CRP, blood was collected in a tube with a clot activator and centrifuged, and the serum aliquot was removed. High-sensitivity CRP was measured by a particle-enhanced nephelometric immunoassay²³ on a BN ProSpec analyzer (Dade Behring Inc., Deerfield, IL). Polystyrene particles coated with monoclonal antibody to CRP are agglutinated when mixed with a patient's sample containing CRP. The amount of light scattered by the complexes formed is proportional to the CRP concentration in the sample.

Results

Two hundred six letters were sent to affected individuals known to be alive at the same address within 30 miles of the eye center, in

the AMD Genetic Study Group. Seventy-nine affected individuals returned for CRP and homocysteine testing. In comparing nonresponders with responders, nonresponders were slightly older (average age, 83 vs. 79 years), had a higher prevalence of diabetes (13% vs. 1%), but were similar in other respects: hypertension (26% vs. 30%), past or active smoking (54% vs. 51%), elevated cholesterol (26% vs. 30%), and vitamin usage (91% vs. 81%). One hundred twenty letters were sent to unaffected individuals in the AMD Genetic Study Group, and 77 individuals returned for testing. In comparing nonresponder unaffected individuals with responders, nonresponders were similar in average age (76 vs. 74 years) but had a higher prevalence of diabetes (26% vs. 2.6%) and hypertension (47% vs. 44%) and higher vitamin usage (77% vs. 60%). Nonresponders were similar to responders in the prevalence of elevated cholesterol (47% vs. 44%) and past or active smoking (53% vs. 51%). None of the nonresponders or responders in the affected or unaffected groups was blood related.

Subsequent analysis was based on the 79 affected individuals and 77 unaffected individuals who returned for CRP and homocysteine testing. Mean ages were 79.1 years (range, 55–93) for affected individuals and 74.3 years (range, 67–85) for unaffected individuals. In both groups, all individuals were Caucasian. Demographic and medical history information is summarized in Table 1. Diabetes was defined as the presence of type 1 or type 2 diabetes as reported by the patient. Statin therapy to reduce cholesterol was defined as the present use of statin therapy, regardless of duration. All patients with elevated cholesterol were on statin therapy. Smoking was defined as active or past smoking. Hypertension was assessed by the current use of antihypertensive medications. For vitamin supplementation, most individuals were taking a multivitamin preparation, but the individual vitamin dosages were not verified. For both affected individuals and controls, nutritional variables and body mass index were not assessed.

Of affected individuals, 50.6% had neovascular macular degeneration and 49.4% had atrophic macular degeneration. In the small group ($n = 4$) who had repetitive testing of homocysteine in fasting and nonfasting states, the difference between mean fasting homocysteine and nonfasting homocysteine was 0.75 $\mu\text{mol/l}$. Mean CRP levels for affected and unaffected individuals were 3.42 and 2.30 mg/l, respectively ($P = 0.03$). Mean homocysteine levels for affected and unaffected individuals were 11.72 and 8.88 $\mu\text{mol/l}$, respectively ($P < 0.0001$). There was no significant difference between mean CRP (3.55 vs. 3.29 mg/l) or mean homocysteine levels (11.53 vs. 11.92 $\mu\text{mol/l}$) for individuals with neovascular macular degeneration versus those with atrophic macular degeneration.

In the 79 AMD cases, the correlation coefficient between CRP and homocysteine was +0.17 ($P = 0.13$). In the 77 controls, the correlation coefficient between CRP and homocysteine was -0.01 ($P = 0.90$).

Table 1. Demographic and Medical History Information

Variable	Cases (n = 79)	Controls (n = 77)	P Value
Age (yrs) (mean)	79.1	74.3	<0.001
Gender (% male)	41.8	44.2	0.86
Diabetic (%)	1	2.6	0.54
Statin drugs* (%)	30.4	44	0.86
Vitamin usage (%)	81	60	0.006
Smokers [†] (%)	51	63.6	0.19
Hypertension (%)	58	43	0.06

*3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors for elevated cholesterol.

[†]Past or active cigarette smokers.

Table 2. Factors Associated with Age-Related Macular Degeneration Based on Logistic Regression

Factor	Adjusted OR	95% CI	P Value
Model including CRP			
Age (in 1-yr increments)	1.12	1.06, 1.19	<0.001
CRP	4.00	1.05, 15.33	0.043
Gender	0.92	0.44, 1.92	0.833
Smoking	1.50	0.73, 3.11	0.271
Statin use	1.46	0.72, 2.96	0.294
Model including homocysteine			
Age (in 1-yr increments)	1.09	1.03, 1.16	0.003
Homocysteine	1.18	1.05, 1.33	0.006
Gender	1.26	0.60, 2.62	0.541
Smoking	1.53	0.73, 3.19	0.254
Statin use	1.74	0.84, 3.57	0.133
Model including both CRP and homocysteine			
Age (in 1-yr increments)	1.10	1.03, 1.17	0.003
Homocysteine	1.16	1.03, 1.31	0.012
CRP	3.12	0.81, 11.98	0.098
Gender	1.11	0.52, 2.37	0.785
Smoking	1.68	0.79, 3.53	0.175
Statin use	1.59	0.76, 3.32	0.214

CI = confidence interval; CRP = C-reactive protein; OR = odds ratio. n = 156, with data available on all factors.

Several logistic regression models using SPSS 12.0 statistical software (SPSS, Inc., Chicago, IL) were carried out (Table 2). In a model that included age, gender, smoking status, statin use, and CRP level, only age ($P < 0.001$) and CRP level ($P = 0.043$) showed significant associations with AMD. The adjusted odds ratio (OR) for age was 1.12 (95% confidence interval [CI], 1.06–1.19), and the adjusted OR for CRP level was 4.00 (95% CI, 1.05–15.33). In a model that included age, gender, smoking status, statin use, and homocysteine level, only age ($P = 0.003$) and homocysteine level ($P = 0.006$) showed significant associations with AMD. The adjusted OR for age was 1.09 (95% CI, 1.03–1.16), and the adjusted OR for homocysteine level was 1.18 (95% CI, 1.05–1.33). A model including both CRP and homocysteine showed significant associations of age and homocysteine with AMD, and an association of borderline significance with CRP. Separate analyses of patients with atrophic AMD and patients with neovascular AMD yielded findings supportive of these associations. The sample size was insufficient to compare the 2 groups as quintiles or tertiles.

Discussion

Our study shows that levels of CRP and homocysteine are elevated significantly in individuals with AMD relative to similar unaffected controls, and these factors remain associated with AMD upon adjustment for covariates of potential influence. Low-grade chronic inflammation is now recognized to play a pivotal role in the pathogenesis of numerous degenerative diseases, including atherosclerosis and Alzheimer's. Our data support the role of chronic inflammation in the development of AMD. Cellular remnants and debris from degenerate retinal pigment epithelial cells, sequestered between the retinal pigment epithelium basal lamina and Bruch's membrane, may constitute a chronic

inflammatory stimulus.²⁴ High levels of monocyte activation, an early participant in the inflammatory cascade, correlate with a 5-fold risk of developing neovascular AMD.²⁵ Aspirin therapy,²⁶ an antiinflammatory drug, and statin therapy,²⁶ which decreases CRP,²⁷ are associated with decreased rates of choroidal neovascularization in patients with AMD.

Recent meta-analyses^{28,29} of prospective studies have confirmed a significant association between elevated plasma homocysteine and cardiovascular disease and stroke. Homocysteine may induce vascular damage³⁰ by a variety of mechanisms, including platelet activation, oxidative stress, hypercoagulability, endothelial dysfunction, and vascular smooth muscle cell proliferation. Whether homocysteine is causative in the pathogenesis of atherosclerosis or is a marker of existing vascular disease will be addressed in large controlled trials^{31,32} evaluating the effect of lowering homocysteine on cardiovascular end points. Our study provides further support for a cardiovascular role in the pathogenesis of AMD. The presence of cholesterol, apolipoprotein B, and apolipoprotein E in drusen³³ and basal linear deposits of retinal pigment epithelial cells links AMD with lipoproteins involved in the pathogenesis of atherosclerosis. Allelic variations³⁴ in apolipoprotein E have been linked to susceptibility to AMD.

As both homocysteine and CRP are associated with the risk of cardiovascular disease, it may be that these 2 biomarkers reflect the same underlying cardiovascular disease status. In our study of AMD cases and AMD-free controls, however, there was no evidence of a strong or significant linear relationship between CRP and homocysteine, which would indicate that these factors may play separate roles in the risk of AMD.

A small number of previous studies have produced dissimilar results regarding homocysteine and CRP in age-related maculopathy (ARM). The National Health and Nutrition Examination Survey³⁵ found no association with homocysteine and AMD. Axer-Siegel et al³⁶ found that homocysteine levels were significantly elevated in neovascular ARM relative to dry maculopathy or controls. Our study found that homocysteine was significantly elevated in individuals with AMD relative to controls, but there was no difference in mean homocysteine levels between individuals with neovascular AMD and those with atrophic AMD. In the Cardiovascular Health Study,³⁷ there was no association between CRP and AMD, but this study also found no association with smoking, which has been found to be a major risk factor in other studies.^{5,10} In contrast, data from the Age-Related Eye Disease Study³⁸ showed that CRP was an independent risk factor for AMD.

In this case-control study, CRP and, particularly, homocysteine are associated with AMD. As both total plasma homocysteine and CRP correlate with the degree of cardiovascular disease in any individual, it is imperative that the extent of cardiovascular disease be similar in the affected and control populations. The 2 groups are very similar in many features. There are no significant differences between affected and control groups in gender, diabetes, hypertension, elevated cholesterol requiring statin therapy, and smoking (Table 1). The affected group, however, is signif-

icantly younger (74.3 vs. 79.1 years) and has a lower rate of vitamin supplementation (60% vs. 81%). The higher vitamin usage in the affected group would mainly bias the results in favor of the affected group having lower homocysteine values. Vitamin supplementation with folic acid or vitamin B₁₂ decreases plasma homocysteine.¹⁸ The older age in the affected group, however, would bias the results in favor of the cases having higher homocysteine levels, as plasma homocysteine increases with age.

This case-control study suffers from certain inherent limitations. Affected and control individuals were not individually matched. The control group is significantly younger and has a lower rate of vitamin usage. It is possible that the individuals who agreed to return for testing were not representative of the entire group of affected individuals with macular degeneration. In the comparison of nonresponders to responders who returned for the testing, there is a suggestion that responders in both affected and unaffected groups tended to be healthier, as the prevalence of diabetes was greater in the nonresponder individuals. The CRP level is very stable³⁹ within any individual and is unaffected by fasting conditions. For both affected and control individuals, total plasma homocysteine was not measured in the fasting state but after a small nonprotein breakfast. Plasma homocysteine potentially can be affected by a large protein meal rich in methionine, but it is controversial whether accurate sampling requires fasting conditions. After a breakfast containing 15 to 18 g of protein, Guttormsen et al⁴⁰ found a brief elevation of free plasma homocysteine, but total homocysteine remained essentially stable. They and others⁴¹ concluded that there is no difference between samples from fasting and nonfasting subjects. In 4 individuals who had repeat testing under fasting and nonfasting conditions, total plasma homocysteine levels were very similar.

The present study shows that elevated CRP and homocysteine levels are associated with AMD, and supports the role of inflammation and atherosclerosis in the pathogenesis of AMD. Would reducing CRP and homocysteine reduce the incidence of AMD? C-reactive protein potentially can be reduced⁴² by physical activity, weight loss, and statin therapy. Studies assessing the effect of statin therapy, which reduces CRP, on AMD have had inconsistent results^{26,43-47} and have failed to show that statin therapy reduces or prevents AMD. Large-scale studies^{31,32} evaluating homocysteine reduction with cardiovascular end points are underway. Further studies are needed to differentiate which common disease mechanisms of chronic inflammation and atherosclerosis contribute to the pathogenesis of AMD.

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