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Plasma biomarkers of oxidative stress and genetic variants in age-related macular degeneration

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Abstract

Purpose—To compare plasma levels of oxidative stress biomarkers in patients with age-related macular degeneration (AMD) and controls and to evaluate a potential relationship between biochemical markers of oxidative stress and AMD susceptibility genotypes.

Design—Prospective case-control study

Methods—Plasma levels of oxidative stress biomarkers were determined in 77 AMD patients and 75 controls recruited from a clinical practice. Cysteine (Cys), cystine (CySS), glutathione (GSH), isoprostane (IsoP), and isofuran (IsoF) were measured, and participants were genotyped for polymorphisms in the complement factor H (*CFH*) and age-related maculopathy susceptibility 2 (*ARMS2*) genes.

Results—CySS was elevated in cases compared to controls ($p = 0.013$). After adjustment for age, gender, and smoking, this association was not significant. In all participants, CySS levels were associated with the *CFH* polymorphism rs3753394 ($p = 0.028$) as well as an eight-allele *CFH* haplotype ($p = 0.029$) after correction for age, gender, and smoking. None of the other plasma markers was related to AMD status in our cohort.

Conclusions—Our investigation of the gene/environment interaction involved in AMD revealed a relationship between a plasma biomarker of oxidative stress (CySS) and *CFH* genotype. These data suggest a potential association between inflammatory regulators and redox status in AMD pathogenesis.

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All procedures were prospectively approved by the local Institutional Review Board, the Vanderbilt University Human Research Protection Program. Research adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with Health Insurance Portability and Accountability Act regulations. Informed consent was obtained from all participants upon study enrollment.

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Introduction

Age-related macular degeneration (AMD) is a leading cause of irreversible blindness in older individuals in the Western world. Approximately 1.75 million people in the United States over the age of 40 have the sight-threatening advanced stages of the disease, and this number is projected to approach 3 million by 2020.¹

Oxidative stress appears to play a role in the pathogenesis of AMD. Several demographic and environmental risk factors for AMD, such as aging, smoking, and light exposure, have been linked to increased production of reactive oxygen species (ROS) and thus cumulative cellular oxidative injury.² Supplementation with antioxidants (vitamin C, vitamin E, and β -carotene) and zinc was shown to slow AMD progression in the Age-Related Eye Disease Study (AREDS), a multicenter, randomized clinical trial,³ and high dietary intake of antioxidants (particularly carotenoids) has been correlated with lower AMD prevalence⁴ and incidence.⁵ Additionally, plasma biomarkers of oxidative stress, such as the lipid peroxidation product carboxymethyl pyrrole (CEP)^{6, 7} and homocysteine,^{8, 9} have been associated with AMD.

Quantification of oxidative stress by the measurement of cysteine thiol/disulfide couples and lipid peroxidation products in plasma may be an effective tool for identifying patients at risk of developing AMD and/or progressing to later stages of the disease. The thiolated amino acid cysteine (Cys) and the Cys-derived antioxidant glutathione (GSH) are oxidized to their respective disulfides, cystine (CySS) and glutathione disulfide (GSSG). The redox potentials (E_h) of Cys/CySS and GSH/GSSG couples reflect the body's overall redox status. In our previous studies, E_h (Cys/CySS) and E_h (GSH/GSSG) showed increased oxidation in association with the AMD risk factors age¹⁰ and smoking.¹¹ Altered plasma redox status of these thiol metabolites has also been demonstrated in a range of human diseases, including Alzheimer's disease,^{12, 13} cystic fibrosis,¹⁴ diabetes mellitus,^{15, 16} and cardiovascular disease.^{17, 18} Levels of these thiol metabolites in plasma have not yet been compared in AMD patients versus controls.

Increased plasma levels of the lipid peroxidation metabolites F₂-isoprostanes (F₂-IsoPs) and isofurans (IsoFs), produced by the non-enzymatic free radical-catalyzed peroxidation of arachidonic acid, indicate impaired clearance of lipid peroxidation products throughout the body. Abnormal lipid metabolism may be detrimental to the retina/RPE complex, which is particularly susceptible to oxidative damage given the high rate of phagocytosis in RPE cells and the high prevalence of readily-oxidized polyunsaturated fatty acids in photoreceptor outer segments. Increased plasma levels of IsoPs have been associated with disease risk factors such as smoking¹⁹ as well as multiple systemic diseases.^{20, 21} As an environment high in oxygen favors the production of IsoFs over IsoPs, the combined measurement of these two metabolites offers a comprehensive and reliable approach to evaluating oxidative stress status *in vivo*.^{22, 23} Small-scale studies have linked increased IsoFs, but not F₂-IsoPs, with diseases involving mitochondrial dysfunction and/or abnormal oxygen levels: Parkinson's disease,²³ hypoxic lung injury,²³ and bronchopulmonary dysplasia.²⁴ The potential relationship of IsoPs and IsoFs with age-related eye diseases has not been fully investigated.

Recent studies have established genetic variation as an important factor in assessing AMD risk. Polymorphisms in the complement factor H gene (*CFH*)^{25–28} and the age-related maculopathy susceptibility 2 (*ARMS2*) high temperature requirement factor A-1 (*HTRA1*)^{29–32} locus have been consistently linked with AMD. The majority of AMD-associated genes are related to the complement cascade,^{33–38} and current evidence suggests

that the interaction between oxidative stress and inflammation may play a key role in AMD pathophysiology.^{39–42}

The purpose of the present study was to determine identifiable differences between AMD patients and controls using two independent measures of oxidative stress: thiol redox metabolites and lipid peroxidation products. We also aimed to evaluate a potential relationship between these biochemical markers of oxidative stress and AMD susceptibility genotypes.

Methods

Study Participants

For this prospective case-control study, blood was drawn from 152 individuals (77 AMD patients and 75 non-AMD controls) for measurement of plasma biomarkers of oxidative stress and genotyping. Individuals over the age of 55 were recruited from the Retina Division at the Vanderbilt Eye Institute. Cases were diagnosed with intermediate or advanced AMD (AREDS categories 3 or 4) and were required to have at least intermediate drusen in both eyes. Controls had no clinical signs of AMD. Exclusion criteria included the presence of any retinopathy other than AMD, active uveitis or ocular infection, and any ocular surgery within the 60 days prior to enrollment. Patients with diabetes mellitus were excluded given the potential role of ROS in the pathogenesis of diabetic complications.^{15, 43} Disease status was confirmed by high-resolution fundus photography. Fifty-degree fundus images were examined by a masked retina specialist (PS) for the presence or absence of the following AMD-related findings: drusen, RPE changes, neurosensory retinal detachment, pigment epithelial detachment, sub- and/or intra-retinal exudation (hemorrhage and/or lipid), choroidal neovascularization, and fibrovascular tissue. AMD patients were then classified by disease stage using the AREDS criteria.³ For phenotypic analysis, patients with intermediate AMD had intermediate drusen (AREDS category 3) in both eyes, and patients with advanced AMD had either geographic atrophy or neovascular AMD (AREDS category 4) in at least one eye. Patients with neovascular AMD in one or both eyes constituted the neovascular AMD group. Smoking history was obtained from all participants.

Blood Sample Collection and Plasma Biomarker Measurement

At the time of study enrollment, blood was collected from each participant using a 23-gauge butterfly needle. For the measurement of plasma thiol metabolites, 1.5 ml blood was immediately transferred to a microcentrifuge tube containing 0.5 ml of serine-borate preservation solution, which has been demonstrated to protect against auto-oxidation.⁴⁴ Following centrifugation to remove blood cells, 200 μ l of supernatant was transferred to another microcentrifuge tube containing 200 μ l of 10% perchloric acid, 0.2 M boric acid, and 10 μ M γ -glutamyl-glutamate (internal standard). Samples were frozen at -80°C for no more than 6–8 weeks until derivatization with dansyl chloride. Plasma Cys, CySS, and GSH were measured by high-performance liquid chromatography (HPLC).⁴⁴ Levels of GSSG were below the detection limit for the majority of specimens. All patients with available biomarker measurements were included in data analyses.

For the measurement of lipid peroxidation metabolites, 8 ml blood was immediately transferred to two 4 ml blood collection tubes containing 7.2 mg K_2 EDTA each. These tubes were centrifuged at 4°C to remove blood cells, and 2 ml supernatant from each tube was transferred to one of two 15 ml conical tubes. Plasma was immediately frozen at -80°C and not thawed prior to analysis, which took place within 4–6 months.^{45, 46} Samples were analyzed for F_2 -IsoP and IsoF concentration by the Vanderbilt University Eicosanoid Core

Laboratory using gas chromatography/negative-ion chemical ionization mass spectrometry (GC/NICI-MS) as described below.⁴⁷

First, 1.0 ng of the internal standard [²H₄]-15-F_{2t}-IsoP (8-iso-PGF_{2α}) was added to each plasma sample. Samples were applied to a C₁₈ Sep-Pak cartridge (Waters, Milford, MA) and eluted with ethyl acetate:heptane (50:50, v/v). The eluate was applied to a silica Sep-Pak cartridge (Waters) and eluted with ethyl acetate:methanol (50:50, v/v). The resulting eluate was dried under nitrogen, converted to pentafluorobenzyl esters by the addition of pentafluorobenzyl bromide and diisopropylethanolamine in acetonitrile, and incubated at 37°C for 30 min. Products were dried under nitrogen and reconstituted in 30 μl chloroform and 20 μl methanol. The initial separation of IsoPs, PGF_{2α}, and IsoFs from other lipid metabolites was achieved by thin layer chromatography (TLC) in a solvent system of chloroform:methanol (93:7, v/v). The methyl ester of PGF_{2α} was used as a standard on a separate lane and visualized by staining with 10% phosphomolybdic acid in ethanol followed by heating. The R_f of PGF_{2α} methyl ester in this solvent system is 0.15. Compounds migrating in the region ± 1 cm of the PGF_{2α} standard were collected from the TLC plate, extracted with 1 ml ethyl acetate, and dried under nitrogen.

Following TLC purification, compounds were converted to trimethylsilyl (TMS) ether derivatives by addition of 20 μl N,O-bis(trimethylsilyl)trifluoroacetamide and 10 μl dimethylformamide and dried under nitrogen. The residue was dissolved for GC/MS analysis in 20 μl undecane that had been stored over a bed of calcium hydride. GC/NICI-MS was carried out on an Agilent 5973 Inert Mass Selective Detector coupled with an Agilent 6890n Network GC system (Agilent Labs, Torrance, CA) and interfaced with an Agilent computer. GC was performed using a 15 m by 0.25 μm (film thickness) DB-1701-fused silica capillary column (J and W Scientific, Folsom, CA). The column temperature was programmed to increase from 190°C to 300°C at 20°C per minute. The major ion generated in the NICI mass spectrum of the PFB ester, TMS ether derivative of F₂-IsoPs was the m/z 569 carboxylate anion [M-181 (M-CH₂C₆F₅)], and the major ion for IsoFs was the m/z 585 carboxylate anion [M-181 (M-CH₂C₆F₅)]. The corresponding ion generated by the deuterated internal standard [²H₄]-15-F_{2t}-IsoP was m/z 573. Levels of endogenous F₂-IsoPs and IsoFs in each biological sample were calculated from the ratio of intensities of the ions m/z 569 (IsoPs) or m/z 585 (IsoFs) to m/z 573. Employing this assay, the lower limit of detection of F₂-IsoPs and IsoFs is in the range of 4 pg, using an internal standard with a blank of 3 parts per thousand. Validation of this assay has shown precision of ±6% and accuracy of 94% in biological fluids.⁴⁵⁻⁴⁷

Genotyping

Genomic DNA was isolated from whole blood using the PureGene system (Gentra Systems, Minneapolis, MN), and all Caucasian participants were genotyped for AMD-associated single nucleotide polymorphisms (SNPs) in *CFH* and *ARMS2*. Genotyping was conducted by the DNA Resources Core in the Center for Human Genetics Research at Vanderbilt University using the Sequenom MassARRAY platform. All patients with available genotypes were included in data analyses.

Data Analysis

Descriptive statistics for all demographic and clinical variables were calculated, and comparisons between cases and controls were made using the two sample t-test for continuous data (e.g., age) and the chi-squared test for categorical data (e.g., gender, race, and smoking). Comparisons of the biomarker levels between cases and controls were made using t-test and Wilcoxon rank sum test. Logistic regression models were fitted to evaluate the effect of plasma biomarkers on AMD risk while adjusting for the effects of age, gender,

and smoking. Stratified analysis was performed for multiple AMD severity levels, including intermediate, neovascular, and advanced. The Hardy-Weinberg equilibrium (HWE) test was carried out for all SNPs in the control group. The influence of individual *CFH* and *ARMS2* genotypes on CySS levels was evaluated by linear regression. Haplotype analysis was employed to test the association between plasma CySS and each *CFH* haplotype. When appropriate, adjustments were made for the potential confounders age, gender, and smoking. All analyses were performed with R (www.r-project.org). The haplotype analysis was performed through the R package haplo.stats, in which the haplo.score program yielded both global and haplotype-specific scores and p-values while allowing for covariate adjustment.⁴⁸ For all statistical analyses, $p < 0.05$ was considered to be significant.

Results

Plasma levels of thiol metabolites (Cys, CySS, and GSH) and lipid peroxidation products (IsoP and IsoF) were measured in 152 participants, 77 AMD patients and 75 controls. The demographics of all participants are presented in Table 1. Age and gender were significantly different between cases and controls.

In our study population, the mean plasma level of CySS was 9.1% higher in AMD patients than controls ($p = 0.013$). After adjusting for age, gender, and smoking, the association was no longer significant ($p = 0.108$). None of the other oxidative stress markers was associated with AMD status. Mean levels of IsoFs were 47% higher in AMD patients (0.22 ng/ml) than in controls (0.15 ng/ml), but this difference did not reach significance in our study population ($p = 0.087$). Levels for all measured biomarkers in AMD patients and controls, with both unadjusted and adjusted p-values, are reported in Table 2.

To determine if the severity of disease affected the association of CySS levels with AMD, plasma biomarker levels were compared in patients with varying stages of AMD versus controls. Of the AMD patients, 26 had intermediate AMD (AREDS category 3) in both eyes, 9 had geographic atrophy in one or both eyes and no neovascular AMD, and 37 had neovascular AMD in one or both eyes. The 46 patients with either geographic atrophy or neovascular AMD (AREDS category 4) in at least one eye were considered advanced AMD patients. Plasma CySS levels were significantly different between neovascular AMD patients and controls ($p = 0.048$) as well as between advanced AMD patients and controls ($p = 0.016$). These differences were no longer significant after adjustment for age, gender, and smoking. In contrast, plasma CySS levels were not significantly different between intermediate AMD patients and controls (Table 3). As in the study population at large, no differences between any of the AMD severity stages and controls were found for Cys, GSH, IsoP, or IsoF.

All Caucasian participants (75 cases, 73 controls) were genotyped for AMD-associated SNPs in *CFH* and *ARMS2* to identify any potential genotype-biomarker relationships. No significant departure from HWE was detected for any of the tested SNPs. CySS levels in all subjects were found to be associated with *CFH* SNP rs3753394 ($p = 0.028$) and an eight-allele *CFH* haplotype ($p = 0.029$) after correction for age, gender, and smoking. SNP rs3753394 was also associated with CySS levels in AMD patients ($p = 0.024$) and controls ($p = 0.040$) when considered independently. Results for all tested SNPs are given in Table 4, and haplotype data are presented in Table 5.

Discussion

The results of the present study support the hypothesis that increased oxidative stress contributes to AMD. It appears that there is a relationship between this oxidative stress and

innate immunity/inflammation, although the exact mechanism of this interaction remains to be defined.

The detected association between plasma CySS and AMD suggests that the Cys/CySS pool is involved in AMD or linked with established AMD risk factors. Maintaining a physiologic Cys/CySS redox state is critical to proper cellular function, and oxidation of extracellular Cys/CySS has been shown to directly influence RPE vitality.⁴⁹ Interestingly, evaluation of plasma CySS levels in patients with AMD of varying severities revealed significant associations for neovascular and advanced AMD patients but not for intermediate AMD patients, suggesting that the detected association between CySS and AMD is driven largely by the patients with advanced AMD. In this study, the difference in plasma CySS levels between AMD patients and controls was no longer significant after adjusting for age alone ($p = 0.130$), and a positive linear correlation was observed between CySS levels and age ($p < 0.001$), indicating that plasma CySS levels may be more related to aging than AMD. In our cohort, plasma thiol metabolites or lipid peroxidation products alone were not sufficient biomarkers for identifying individuals at risk for AMD.

The relationship between plasma CySS and *CFH* genotype seen in this study supports a link between oxidative stress and inflammation in AMD. Immunologic and inflammatory processes (e.g., drusen formation, complement activation) have been independently linked to AMD pathogenesis.³⁹ To date, the most compelling evidence for genetic effects on AMD susceptibility have been demonstrated for complement genes, including *CFH*, complement factor B (*BF*)/complement component 2 (*C2*), complement component 3 (*C3*), complement factor I (*CFI*), complement factor H-related 1 (*CFHR1*), and complement factor H-related 3 (*CFHR3*).^{25–28, 33–38} Furthermore, the complex interaction between oxidative stress and inflammation has been implicated in AMD pathogenesis by recent RPE cell culture^{40, 41} and animal⁴² studies. The CySS-associated SNP in this study, rs3753394, is a C-to-T polymorphism near the 5' region of the *CFH* gene. This SNP has been previously associated with neovascular AMD or polypoidal choroidal vasculopathy (PCV) in Chinese^{50–54} and Japanese⁵⁵ study populations. The CySS-associated haplotype identified in this study seems to be driven largely by the effects of this SNP (rs3753394). Our findings add to the body of work demonstrating interactions between plasma biomarkers of AMD and AMD risk genotypes. Specifically, elevated plasma levels of the inflammatory marker C-reactive protein (CRP) in combination with the *CFH* Y402H risk genotype conferred an additive risk of developing AMD.⁵⁶ Also, increased plasma markers of CEP, a product of lipid peroxidation, were associated with AMD-associated risk genotypes in *ARMS2* and *HTRA1*.⁷

The potential association of IsoPs and IsoFs with AMD seemed plausible, as the retina/RPE complex is highly vulnerable to oxidative damage by radical-catalyzed lipid peroxidation.^{2, 23, 57} Altered levels of these lipid peroxidation products have been associated with a range of human diseases,^{20, 21, 23, 24} including other neurodegenerative diseases such as Parkinson's²³ and Alzheimer's disease.^{58, 59} Although mean plasma IsoF levels were 47% greater in AMD patients (0.22 ng/ml) compared to controls (0.15 ng/ml) in our study population, this difference was not significant given the high variation among individuals ($p = 0.087$, Wilcoxon Rank Sum). In contrast, the isoprostane measurements were clearly unaffected by AMD status (0.051 ng/ml in AMD patients and 0.059 ng/ml in controls, $p = 0.372$ by t-test and $p = 0.850$ by Wilcoxon Rank Sum). The observed trend of increased IsoFs (but not IsoPs) in AMD patients suggests a possible role for oxygen tension in AMD pathogenesis. We found no linear correlation between CySS and IsoF levels in the study cohort ($R^2 = 0.01$), indicating that any contribution of IsoF and CySS to AMD risk likely act by independent mechanisms.

This study was limited by its sample size. Since plasma biomarker levels often vary considerably among normal individuals, it is challenging to obtain enough power to identify small but clinically-significant differences between cases and controls. Future longitudinal studies comparing biomarker levels in the same individuals at different time points may minimize variation in biomarker levels and yield interesting results. The sample size also limited the power of the SNP analysis. We did not correct for multiple comparisons as it would have been difficult to achieve an accurate correction given the various levels of correlation among SNPs. The calculated effect size for variant alleles in rs3753394 suggests that the influence of this genetic variant on plasma CySS levels may be clinically meaningful.

In conclusion, it appears that these biomarkers may be more closely associated with AMD risk factors than with AMD itself. We have previously reported that long-term zinc supplementation decreased plasma CySS levels in a subset of the AREDS study population.⁶⁰ The results of this study suggest that the positive response of AMD patients to zinc treatment may be due to offsetting increased oxidative stress caused by aging. Additionally, our investigation of the gene/environment interaction involved in AMD revealed a relationship between a blood plasma biomarker of redox status, CySS, and *CFH* genotype. These data suggest a potential relationship between inflammatory regulators and the role of redox status in the pathogenesis of AMD and should be added to the body of evidence linking oxidative stress and inflammation to AMD.

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Biography

Dr. Milam A. Brantley, Jr. is an Assistant Professor and Director of the Center for Ocular Pharmacogenomics at the Vanderbilt Eye Institute. He earned a BA from Austin College, Sherman, Texas, and a MD/PhD in Molecular and Human Genetics from Baylor College of Medicine, Houston, Texas. He completed his Ophthalmology training at Washington University School of Medicine, St. Louis, Missouri and Moorfields Eye Hospital, London, UK. His clinical specialty is inherited retinal diseases, and his research investigates how genetics influences disease susceptibility and response to treatment.

Table 1

Patient demographics depicting age, gender, race, and smoking status in patients with age-related macular degeneration and controls

Variable	Cases (n = 77)	Controls (n = 75)	p-value
Age, mean yrs (SD)	75.9 (6.4)	71.6 (7.8)	<0.001
Gender, n (% female)	50 (65%)	34 (45%)	0.023
Race, n (% Caucasian)	75 (97%)	73 (97%)	1.000
Current or past smokers, n (%)	69 (91%)	66 (88%)	0.770

Cases = study participants with age-related macular degeneration; Controls = study participants without age-related macular degeneration; n = number; SD = standard deviation

Table 2

Plasma levels of oxidative stress biomarkers in patients with age-related macular degeneration versus controls

Biomarker	Cases (n =)	Con. (n =)	Cases (mean ± SD)	Controls (mean ± SD)	Unadjusted p-value		Adjusted p-value
					T-test	Wilcoxon Rank Sum	
Cys (μM)	69	68	4.18 ± 1.45	4.11 ± 1.39	0.770	0.835	0.632
CySS (μM)	69	68	55.27 ± 11.21	50.66 ± 10.27	0.013	0.025	0.108
GSH (μM)	69	67	1.77 ± 0.65	1.89 ± 0.97	0.396	0.492	0.167
IsoP (ng/ml)	72	67	0.051 ± 0.034	0.059 ± 0.064	0.372	0.850	0.346
IsoF (ng/ml)	72	67	0.22 ± 0.38	0.15 ± 0.18	0.115	0.087	0.194

Cases = patients with age-related macular degeneration; Con. = controls without age-related macular degeneration; n = number of participants with results available for the specified plasma biomarker; Cys = cysteine; CySS = cystine; GSH = glutathione; IsoF = isofuran; IsoP = F₂-isoprostane; Adjusted = adjustment for age, gender, and smoking in multivariate analysis; SD = standard deviation

Table 3

Plasma levels of the oxidative stress biomarker cystine in patients with varying severities of age-related macular degeneration versus controls

AMD Stage	Cases (n =)	Con. (n =)	Cases (mean level, μ M)	Controls (mean level, μ M)	Unadjusted p-value		Adjusted p-value
					T-test	Wilcoxon Rank Sum	
Intermediate	24	68	55.03 $\pm$ 14.21	50.66 $\pm$ 10.27	0.176	0.264	0.205
Neovascular	34	68	54.68 $\pm$ 9.11	50.66 $\pm$ 10.27	0.048	0.063	0.163
Advanced	41	68	55.40 $\pm$ 9.39	50.66 $\pm$ 10.27	0.016	0.022	0.121

AMD = age-related macular degeneration; Cases = patients with AMD; Con. = controls without AMD; Intermediate = AMD patients in AREDS category 3 in both eyes; Neovascular = AMD patients with neovascular AMD in one or both eyes; Advanced = AMD patients with neovascular AMD or geographic atrophy in one or both eyes; n = number of participants with available plasma cystine levels; Adjusted = adjustment for age, gender, and smoking in multivariate analysis; SD = standard deviation

Table 4

Association between plasma levels of the oxidative stress biomarker cystine and variants in genes related to risk of developing age-related macular degeneration

Gene	SNP	n	Unadjusted p-value	Adjusted p-value
<i>CFH</i>	rs3753394	136	0.033	0.028
	rs529825	138	0.061	0.142
	rs800292	138	0.061	0.142
	rs3766404	136	0.773	0.910
	rs1061170	128	0.756	0.745
	rs2274700	136	0.192	0.300
	rs203674	136	0.846	0.836
	rs6677604	136	0.572	0.786
	rs3753396	137	0.259	0.413
	rs412852	138	0.741	0.736
<i>ARMS2</i>	rs10490924	133	0.276	0.360

SNP = single nucleotide polymorphism; *CFH* = complement factor H; *ARMS2* = age-related maculopathy susceptibility 2; n = number of participants with genotype results for the specified polymorphism; Adjusted = adjustment for age, gender, and smoking

Table 5

Association between plasma levels of the oxidative stress biomarker cystine and haplotypes in the age-related macular degeneration-associated gene Complement Factor H

<i>CFH</i> haplotype	rs3753394	rs800292	rs3766404	rs1061170	rs2274700	rs6699604	rs3753396	rs412852	Unadjusted p-value	Adjusted p-value
1	C	T	T	T	T	G	A	T	0.200	0.245
2	C	C	T	C	C	G	A	C	0.466	0.387
3	C	C	C	T	T	A	A	T	0.727	0.610
4	T	C	T	T	T	A	A	T	0.553	0.791
5	T	C	C	T	T	A	A	T	0.715	0.901
6	T	C	T	T	C	G	G	T	0.127	0.301
7	T	C	T	T	C	G	A	C	0.054	0.092
8	T	C	T	C	C	G	A	C	0.137	0.029

CFH = complement factor H; n = 128 participants included in haplotype analysis; Adjusted = adjustment for age, gender, and smoking