Sunlight Exposure, Pigmentation, and Incident Age-Related Macular Degeneration

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Purpose. Examine potential effects of sunlight exposure, hair color, eye color, and selected gene single-nucleotide polymorphisms (SNPs) on incidence of AMD.

Methods. Subjects participated in up to five examinations over a 20-year period. Eye color, self-reported hair color as a teenager, and sunlight exposure were ascertained at the baseline examination. Presence and severity of AMD and its lesions were determined via fundus photographs. Genetic data were available on a subset of participants. The SNPs *CFH* Y402H rs1061170 and *ARMS2* A69S rs10490924 were used to analyze genetic risk of AMD; *OCA2* rs4778241 and *HERC2* rs12913832 represented genetic determinants of eye color.

Results. Incidence of early AMD was higher in blond/red-haired persons compared with brown/black-haired persons (hazard ratio [HR] 1.25, $P = 0.02$) and in persons with high sun exposure in their thirties (HR 1.41, $P = 0.02$). However, neither was significant after adjustment for multiple comparisons. Eye (HR 1.36, $P = 0.006$) and hair color (HR 1.42, $P = 0.003$) were associated with incidence of any retinal pigmentary abnormalities (RPAs). Both remained significant after adjustment for multiple comparisons. Neither presence of alleles for light-colored eyes nor those associated with high risk of late AMD altered the association of eye or hair color with early AMD. None of the characteristics studied were significantly associated with late AMD.

Conclusions. Modest associations of eye color, hair color, and *HERC2* genotype with any RPAs were found. Genes for AMD did not affect these associations. Eye color phenotype was more strongly associated with outcomes than *HERC2* or *OCA2* genotype.

Keywords: sunlight exposure, hair color, eye color, age-related macular degeneration, pigmentation

Few environmental and personal risk factors, aside from cigarette smoking[1,2] and genetic factors,[3,4] have been identified for age-related macular degeneration. Some studies suggest that exposure to sunlight or ultraviolet radiation may cause changes in the RPE similar to those seen during the development of AMD.[5–9] However, epidemiologic evidence of an association between sunlight exposure and AMD has been inconsistent.[10–14]

At the Beaver Dam Eye Study (BDES) baseline examination in 1988 to 1990, the amount of leisure time spent outdoors in summer was related to the prevalence of increased retinal pigment in men and late AMD in the whole population.[15] An inverse association was found between the level of protection from sunlight via use of brimmed hats and sunglasses and the prevalence of soft indistinct drusen in men. Cruickshanks et al.[16] reported that leisure time spent outdoors while persons were teenagers and in their thirties was significantly associated with 5-year incidence of early AMD (odds ratio [OR] 2.09, 95% confidence interval [CI] 1.19, 3.65) but estimated ultraviolet-B exposure and historical markers of sun sensitivity were not. Persons with blond or red hair were more likely to develop early AMD but this was not statistically significant. In 10-year incidence data, both iris and hair color were associated with the development of retinal pigmentary abnormalities (RPAs).[17] Because the aging of the BDES cohort has led to more cases of AMD and because the potential effects of selected genetic markers of eye color may better indicate susceptibility to the effects of light exposure, we investigated the potential effects of hair and eye color, measures of sun exposure, and modifying effects of AMD risk genes on the incidence of early and late AMD in this population.

Methods

Population

A private census of Beaver Dam, Wisconsin, was performed in 1987 and 1988 to identify all eligible residents. Of these, 4926

persons aged 43 to 86 years were seen at the baseline examination in 1988 to 1990. At the four follow-up examinations 5, 10, 15, and 20 years after baseline 3722, 2962, 2375, and 1913 participants, respectively, were examined.[18–22] Data were collected with Institutional Review Board approval from the University of Wisconsin-Madison in conformity with federal and state law. The work was compliant with the Health Insurance Portability and Accountability Act. The study adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from every participant at each examination. Participants were examined at the study site, a nursing home, or their home.

Measurements and Definitions

The same protocols for measurements relevant to this investigation were used at every examination. Date of birth and sex were recorded. Standard questionnaires were used to record self-reported medical and lifestyle histories. Smoking status was categorized as current, past (>100 cigarettes in lifetime, but not currently smoking), or never. Women were asked whether they had ever used or were currently using hormone replacement therapy (HRT). Such use was categorized as never, past, or current.

Eye color was obtained by a trained examiner using a hand light comparing a given participant's iris to standard photographs and was defined as gray/blue, yellow/green, or tan/brown. Hair color was the participant's self-reported natural hair color as a teenager, reported at the baseline examination as blond, red, brown, or black. For the purposes of this report, eye color was divided into two groups: light (gray/blue) and dark (yellow/green or tan/brown). Hair color was also divided into two groups: light (blond/red) and dark (brown/black).

Self-report of current sun exposure was obtained at the baseline examination. Participants reported how much of their day was spent outside in the summer when not working (none, less than a quarter of a day, half a day, greater than three-quarters of a day); how much of the day they wore a hat with a brim or visor when outdoors (never, seldom, half the time, usually, always); and how much of the day they wore sunglasses when outdoors (never, seldom, half the time, usually, always). At the second examination, participants were asked similar questions regarding sun exposure in their teenage years and thirties. Participants were asked how many hours per day they spent outdoors in the summer (<2, 2–4, or ≥5 hours); how often they wore a hat with a brim or visor in the summer (rarely, half the time, usually); and how often they wore sunglasses in the summer (rarely, half the time, usually).

Sun exposure (time outside) and sun protection (wearing a hat, visor, or sunglasses) factors were further combined to create variables which reflected high, medium, or low sun exposure in a participant's teenage years, thirties, and at the baseline examination. A participant was considered to have high sun exposure if she/he spent many hours outdoors (greater than three-quarters of a day at baseline, ≥5 h/d in teenage years or thirties) and did not use a hat or sunglasses (never or seldom at baseline, rarely in teenage years or thirties). A participant was considered to have low exposure if she/he spent small amounts of time outdoors in the summer (none or a quarter of day at baseline, <2 h/d in teenage years or thirties), regardless of protective factors. All other persons were considered to have medium level exposure.

Genetic Measurements

Two common single nucleotide polymorphisms (SNPs) associated with AMD, *CFH* Y402H rs1061170, and *ARMS2* A69S rs10490924, were used in this study. We genotyped *ARMS2* A69S using two different platforms, a PCR assay (TaqMan; Applied Biosystems, Foster City, CA, USA) and a commercial array (Illumina, Inc., San Diego, CA, USA), in 2248 and 2940 samples, respectively. Of 588 samples genotyped with both platforms, a genotype concordance of 99.7% was observed.

We genotyped *CFH* Y402H using a PCR assay (Applied Biosystems) in 3015 samples in the BDES.[23,24] This variant was also imputed in 2940 samples based on 70 common SNPs (minor allele frequency > 0.05), which were genotyped using a commercial array (Illumina, Inc.) in the *CFH* locus using haplotyper software (MACH 1.0; University of Michigan, Ann Arbor, MI, USA).[25] A concordance rate of 99.8% was observed among 1476 samples for which both genotyped and imputed data were available.

A custom panel (iSelect; Illumina, Inc.) was designed that included selected SNPs in the *OCA2-HERC2* region of chromosome 15. These SNPs were assayed in 2965 persons in the BDES cohort. Following analysis by Donnelly et al.,[26] we chose rs4778241 from the *OCA2* gene and rs12913832 from the *HERC2* gene to represent genetic determinants of eye color.

Grading of Fundus Photographs for AMD

Photographs of the retina were taken after pupil dilation and graded in masked fashion by experienced graders using the Wisconsin Age-Related Maculopathy Grading System.[27,28] Circles printed on clear acetate with diameters of 63, 125, 175, 250, 325, 350, and 650 μm were used to estimate drusen size, areas involved by drusen, increased retinal pigment, and RPE depigmentation. Quality assurance procedures were employed throughout the study.[29]

Statistical Analyses

We examined the relationship between eye color and natural hair color during teenage years and sun exposure with risk of incident AMD over 20 years of follow-up adjusting for known risk factors. The presence of AMD lesions was determined by combining data from both eyes. If either eye had a given lesion, then the lesion was considered present. If neither eye had the lesion, or if one eye was free from the lesion and data from the other eye was missing, then it was considered absent. To be included in analyses, a participant must have had complete data for the presence or absence of a given lesion at both the beginning and the end of an interval and have had complete data for risk factors (age, sex, smoking status, hair and eye color, and sun exposure in teenage years, thirties, and at baseline) at the initial visit in the interval. Overall, there were 2728 participants with 7573 person-visits (intervals) contributing data for incidence analysis of at least one AMD outcome.

Time to incidence of AMD was modeled using a discrete-time hazard model with a complementary log-log link function and time-updating predictors.[30] Sex, hair color, eye color, genetic factors, and sun exposure variables remained constant across all visits. Age, smoking status, and use of hormone replacement therapy were updated at each visit.

For the primary analysis, we examined the associations of hair color, eye color, sun exposure, genetic markers of eye color, and use of hormone replacement therapy individually with risk of incident AMD or AMD lesions in a model adjusted for age, sex, and smoking status. The effect of each risk factor was adjusted for multiple comparisons using Bonferroni correction. Each risk factor was adjusted for nine independent tests with each of the nine outcomes of interest.

We performed secondary analyses to investigate the possibility of interactive effects. A maximally adjusted model for each AMD outcome was constructed including age, sex,

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smoking status, and any hair color, eye color, or sun exposure variable that was significant in the primary models, as well as any interactions between hair and eye color phenotypes and between hair or eye color and sun exposure variables that were statistically significant. Backwards selection, with inclusion criteria of 0.05, was used to establish the most parsimonious models.

Subset analyses were performed to examine the effect of hormone replacement therapy in women at risk of incidence of RPAs because of previous findings of a protective effect of this exposure in our data.[31] Overall, 4725 person-visits contributed to these analyses.

Subset analyses were also performed to examine the effect of specific SNPs in the *HERC2*, *OCA2*, *CFH*, and *ARMS2* genes and their relationship with incidence of AMD or its individual lesions. Overall, 4915, 4911, 7020, and 7230 person-visits contributed to subset analyses for *HERC2*, *OCA2*, *CFH*, and *ARMS2* genes, respectively.

Results

Eye color was not significantly associated with the incidence of early AMD (Table 1). Light hair color (versus dark: HR = 1.25, $P = 0.02$) and high sun exposure in one's thirties (versus low: HR = 1.41, $P = 0.02$) were significantly associated with early AMD. Having medium level sun exposure (versus low: HR = 1.22, $P = 0.08$) was marginally associated with incidence of early AMD. None of these associations were significant after adjustment for multiple comparisons. No eye color, hair color, sun exposure, or eye color genotype variables were significantly associated with incident late AMD (Table 1) or either of its subtypes (data not shown).

Light eye color (versus dark, HR = 1.36, $P = 0.006$); light hair color (HR = 1.43, $P = 0.003$); and *HERC2* (GG versus AA/AG: HR = 1.53, $P = 0.005$) were associated with incident RPA (Table 2). These associations remained significant after adjustment for multiple comparisons.

No factors were significantly associated with incidence of soft indistinct drusen. Light hair color (versus dark: HR = 1.24, $P = 0.03$), high sun exposure in the teenage years (versus low exposure: HR = 1.38, $P = 0.04$), high sun exposure in the thirties (versus low: HR = 1.38, $P = 0.03$), and high sun exposure at baseline (high versus low: HR = 1.58, $P = 0.02$) were associated with risk of drusen $\geq$125 μm in diameter (Table 2). None of these associations remained significant after adjustment for multiple comparisons.

In secondary analyses, we evaluated the effects of eye color, hair color, and sunlight exposure on incidence of early AMD or RPAs while accounting for interaction effects. For persons with light colored eyes (gray/blue), high level sunlight exposure at baseline was associated with increased risk of early AMD compared with low exposure (HR = 1.94, $P = 0.01$). For persons with light colored hair (blond/red), incidence of early AMD was significantly higher in persons with medium or high sun exposure in the teenage years compared with those with low sun exposure (medium versus low: HR = 3.25, $P = 0.003$; high versus low: HR = 2.80, $P = 0.01$). Other interaction effects we tested were not significant.

In a subset analysis, we sought to determine the effects of adding hormone replacement therapy to the models for incidence of any RPA, as use of hormone replacement therapy in women had previously been found to be protective. Model results did not change significantly when restricting the analysis to women only or when adding hormone replacement therapy status to the model (data not shown).

To explore the effects of eye color genes, we substituted examiner reported eye color with representative genotypes using a dominant genetic model for *HERC2* rs12913832 (AA/AG versus GG) and *OCA2* rs4778241 (AA/AC versus CC). The *HERC2* SNP was associated with incidence of any RPA in the age, sex, and smoking adjusted model, which was similar to what was found for phenotypic eye color (Table 2). When replacing eye color with the genetic markers in the multivariable models, neither eye color gene was significant nor were any interactions of the genes with sunlight exposure measures associated with incident AMD lesions when using examiner reported eye color. Adding the *CFH* and *ARMS2* genotype risk factors did not significantly alter the findings from the most parsimonious models (data not shown).

Discussion

We found modest associations of eye color and hair color with the incidence of early AMD and its lesions. Cruickshanks et al.[16] reported a borderline significant increased risk of incident early AMD in persons with light hair. Our current analyses are consistent with the findings of Cruickshanks et al.[15] on the association of leisure time spent outside in the teenage years and in the thirties with incidence of early AMD. We found joint effects of hair color and sunlight exposure during the teenage years associated with RPAs. In the Los Angeles Latino Eye Study, light eye color was cross-sectionally associated with geographic atrophy.[32] This association may be compatible with our finding if there is a small effect of light exposure and is influenced by variability of expression. Mitchell et al.[13] found an association between light iris color and AMD, but they reviewed several other studies in which the association varied from no association to a very strong association. It is possible that persons with light irises are relatively deficient in melanin in the choroid and retina, and that would serve to make such eyes less protected from the negative effects of sun exposure. In our analyses adjusted for age, sex, and smoking status, we found that the homozygous light eye color in *HERC2* rs12913832 was significantly associated with incidence of any RPA, with an HR of approximately 1.5. However, there was no apparent effect of this genotype when it was included in models adjusting for other risk factors (data not shown). This may be due to small sample size and subsequently decreased power to detect interactions in the more complex models.

Retinal pigmentary abnormalities have been linked to a locus on chromosome 1q25 near the *CFH* locus in findings by Thompson et al.[33] Their data suggest that this lesion is a step in the severity pathway leading to geographic atrophy. In the current analysis, we did not have enough power to evaluate whether such an effect is also associated with the incidence of geographic atrophy. Including *CFH* and *ARMS2* gene SNPs in our models did not influence the findings. Collaborations with studies that have a larger number of cases of geographic atrophy may further elucidate this relationship.

Further support for the hypothesis of selective sun sensitivity based on skin or iris pigmentation can be found in examining the pathobiology of damaging effects of light exposure to the skin. Eumelanin and pheomelanin occur in human skin melanosomes. Eumelanin in particular is thought to be photoprotective.[34] Persons with fair skin have few melanosomes, which is associated with increased susceptibility to the damaging effects of ultraviolet radiation.[35] Persons with light-colored irises have less melanin, especially eumelanin.[36] It is speculated that such a mechanism may render persons with light irises more susceptible to AMD.[37]

Another mechanism of phototoxic effects of light on the retina comes from the finding that a specific chromophore in the retina, when oxidized by light, is toxic to the retina.[38] However, it is uncertain whether this phenomenon occurs

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Table 1. Associations of Participant Characteristics With Early and Late AMD in Minimally Adjusted Models

	Early AMD						Late AMD					
			Primary Model*						Primary Model*			
Factor	At Risk, *n*	Incidents, *n* (%)	Incidents, % (95% CI)	HR	*P* Value	Adjusted *P* Value†	At Risk, *n*	Incidents, *n* (%)	Incident, % (95% CI)	HR	*P* Value	Adjusted *P* Value†
Eye color												
Green/brown	2952	244 (8.3)	6.8 (5.9, 7.7)	Ref			3735	44 (1.2)	0.5 (0.4, 0.8)			
Gray/blue	2909	287 (9.9)	7.6 (6.8, 8.6)	1.14	0.13	>0.99	3769	67 (1.8)	0.7 (0.5, 1.0)	1.32	0.15	>0.99
OCA2 genotype												
AA/AC	1144	106 (9.3)	7.7 (6.4, 9.3)	Ref			1453	16 (1.1)	0.4 (0.2, 0.8)			
CC (blue eyes)	2678	235 (8.8)	6.8 (5.9, 7.9)	0.88	0.27	>0.99	3415	56 (1.6)	0.6 (0.4, 1.0)	1.43	0.21	>0.99
HERC2 genotype												
AA/AG	1355	113 (8.3)	6.9 (5.7, 8.3)	Ref			1727	20 (1.2)	0.5 (0.3, 0.8)			
GG (blue eyes)	2471	228 (9.2)	7.2 (6.2, 8.3)	1.05	0.66	>0.99	3145	52 (1.7)	0.6 (0.4, 1.0)	1.32	0.30	>0.99
Hair color												
Brown/black	4544	386 (8.5)	6.9 (6.2, 7.7)	Ref			5783	81 (1.4)	0.6 (0.4, 0.8)			
Blond/red	1317	145 (11.0)	8.4 (7.2, 9.9)	1.25	0.02	0.21	1721	30 (1.7)	0.7 (0.4, 1.1)	1.17	0.46	>0.99
Sun exposure												
Teenage years												
Low	664	57 (8.6)	6.0 (4.6, 7.8)	Ref			837	13 (1.6)	0.5 (0.3, 1.0)			
Medium	2943	285 (9.7)	7.5 (6.6, 8.5)	1.26	0.11	0.99	3802	55 (1.4)	0.5 (0.4, 0.8)	1.04	0.89	>0.99
High	2254	189 (8.4)	7.2 (6.2, 8.2)	1.20	0.23	>0.99	2865	43 (1.5)	0.7 (0.5, 1.1)	1.39	0.30	>0.99
Thirties												
Low	1350	107 (7.9)	6.1 (5.0, 7.4)	Ref			1716	21 (1.2)	0.5 (0.3, 0.8)			
Medium	3765	349 (9.3)	7.3 (6.5, 8.2)	1.22	0.08	0.71	4786	73 (1.5)	0.6 (0.4, 0.9)	1.38	0.19	>0.99
High	746	75 (10.1)	8.4 (6.8, 10.4)	1.41	0.02	0.22	1002	17 (1.7)	0.8 (0.5, 1.3)	1.71	0.11	0.96
Study baseline												
Low	3575	339 (9.5)	7.3 (6.4, 8.2)	Ref			4603	72 (1.6)	0.6 (0.4, 0.9)			
Medium	2019	166 (8.2)	6.9 (5.9, 8.0)	0.94	0.56	>0.99	2535	32 (1.3)	0.6 (0.4, 0.9)	1.03	0.91	>0.99
High	267	26 (9.7)	9.1 (6.4, 12.9)	1.28	0.23	>0.99	366	7 (1.9)	1.0 (0.5, 2.2)	1.71	0.19	>0.99
HRT (women only)												
Never	2174	198 (9.1)	7.1 (6.1, 8.2)	Ref			2801	49 (1.7)	0.7 (0.5, 1.1)			
Past	793	90 (11.3)	6.9 (5.6, 8.6)	0.98	0.89	>0.99	1061	19 (1.8)	0.6 (0.3, 1.0)	0.81	0.43	>0.99
Current	697	53 (7.6)	7.2 (5.6, 9.3)	1.02	0.89	>0.99	816	7 (0.9)	0.6 (0.3, 1.4)	0.87	0.74	>0.99

Ref, referent category.

* Adjusted for age, sex, and smoking status.

† Further adjusted for multiple comparisons.

Table 2. Associations of Participant Sunlight Exposure and Pigmentation Characteristics With Incidence of Any Retinal Pigmentary Abnormality and Drusen ≥ 125 μm in Diameter

	Any Retinal Pigmentary Abnormality						Drusen ≥125 μm Diameter					
			Primary Model*						Primary Model*			
Characteristics	At Risk, *n*	Incidents, *n* (%)	Incidents, % (95% CI)	HR	*P* Value	Adjusted *P* Value†	Risk, *n*	Incidents, *n* (%)	Incidents, % (95% CI)	HR	*P* Value	Adjusted *P* Value†
Eye color												
Green/brown	3399	147 (4.3)	3.1 (2.5, 3.7)	Ref			3074	250 (8.1)	6.9 (6.1, 7.8)	Ref		
Gray/blue	3235	196 (6.1)	4.1 (3.5, 4.9)	1.36	0.006	0.05	3153	289 (9.2)	7.2 (6.4, 8.1)	1.04	0.63	>0.99
OCA2 genotype												
AA/AC	1320	57 (4.3)	3.2 (2.4, 4.2)	Ref			1199	104 (8.7)	7.3 (6.0, 8.8)	Ref		
CC (blue eyes)	2978	161 (5.4)	3.9 (3.2, 4.7)	1.21	0.22	>0.99	2880	248 (8.6)	6.8 (5.9, 7.8)	0.93	0.56	>0.99
HERC2 genotype												
AA/AG	1574	59 (3.7)	2.8 (2.1, 3.6)	Ref			1408	118 (8.4)	7.1 (5.9, 8.4)	Ref		
GG (blue eyes)	2728	159 (5.8)	4.2 (3.4, 5.1)	1.53	0.005	0.05	2675	234 (8.7)	6.9 (6.0, 7.9)	0.98	0.82	>0.99
Hair color												
Brown/black	5124	239 (4.7)	3.3 (2.8, 3.9)	Ref			4812	390 (8.1)	6.7 (6.1, 7.5)	Ref		
Blond/red	1510	104 (6.9)	4.6 (3.7, 5.7)	1.42	0.003	0.03	1415	149 (10.5)	8.2 (7.0, 9.6)	1.24	0.03	0.24
Sun exposure												
Teenage years												
Low	739	34 (4.6)	2.8 (1.9, 3.9)	Ref			698	53 (7.6)	5.6 (4.3, 7.2)	Ref		
Medium	3367	194 (5.8)	3.8 (3.2, 4.5)	1.39	0.08	0.69	3125	279 (8.9)	7.0 (6.1, 7.9)	1.27	0.11	0.97
High	2528	115 (4.5)	3.5 (2.9, 4.3)	1.29	0.20	>0.99	2404	207 (8.6)	7.5 (6.6, 8.6)	1.38	0.04	0.36
Thirties												
Low	1519	71 (4.7)	3.1 (2.4, 4.0)	Ref			1434	113 (7.9)	6.2 (5.1, 7.5)	Ref		
Medium	4270	224 (5.2)	3.6 (3.1, 4.3)	1.18	0.23	>0.99	3980	346 (8.7)	7.0 (6.3, 7.9)	1.15	0.21	>0.99
High	845	48 (5.7)	4.2 (3.2, 5.6)	1.38	0.09	0.80	813	80 (9.8)	8.4 (6.8, 10.3)	1.38	0.03	0.27
Study baseline												
Low	4068	228 (5.6)	3.7 (3.1, 4.4)	Ref			3778	323 (8.5)	6.7 (5.9, 7.5)	Ref		
Medium	2249	98 (4.4)	3.3 (2.7, 4.1)	0.89	0.36	>0.99	2163	185 (8.6)	7.2 (6.2, 8.3)	1.08	0.40	>0.99
High	317	17 (5.4)	4.3 (2.7, 6.8)	1.16	0.56	>0.99	286	31 (10.8)	10.1 (7.3, 13.9)	1.58	0.02	0.16
HRT (women only)												
Never	2491	144 (5.8)	3.8 (3.1, 4.7)	Ref			2300	190 (8.3)	6.4 (5.5, 7.5)	Ref		
Past	934	64 (6.9)	3.7 (2.7, 4.9)	0.96	0.77	>0.99	829	93 (11.2)	7.0 (5.6, 8.7)	1.09	0.48	>0.99
Current	757	24 (3.2)	2.9 (2.0, 4.3)	0.76	0.22	>0.99	727	51 (7.0)	6.7 (5.2, 8.7)	1.05	0.77	>0.99

* Adjusted for age, sex, and smoking status.
† Further adjusted for multiple comparisons.

naturally when the human eye is exposed to sunlight, and it has not been noted to be specific to eye or hair/fur pigmentation in experimental animals. Also, age-related deposits of lipofuscin are thought to be related to the development of AMD. The age-related increase in both number of lipofuscin granules in human RPE cells and in their photoreactivity may impose a greater risk of photooxidative damage in the aged RPE,[39] suggesting a light sensitive mechanism in the development of AMD. Moreover, photoreactivity of melanosomes related to exposure to blue light increases with age, thus potentially providing a source of reactive oxygen which may also contribute to cellular dysfunction.[40]

Protective effects on RPAs have been found with use of hormone replacement therapy.[41] The use of these preparations did not affect the association of eye or hair color and RPAs in our study. However, few participants were using hormone replacement therapy, and we examined more covariates than in the study by Gao et al.[31]

Limitations of this study include the subjective measures of eye and hair color and the imprecise measures of sunlight exposure. While these historical measures are likely to have increased variability, we doubt that the errors are systematic with regard to outcome as they were assessed prior to incidence of symptomatic lesions of interest. Another limitation is that our genetic information is based on SNPs in only a few candidate loci, which may influence the relationships between exposure and outcome. Thus, there could be other genetic sites that influence these relationships that we could not assess. Additionally, the relative effect size of the loci for eye color that we tested may be small. There may be other genes which have large effects, and are uncommon, that we have not measured. In addition, some of the variables and interactions that we found to be significant were accompanied by wide confidence intervals, suggesting the possibility of a type 1 error. Lastly, we had relatively few cases of incident late AMD leading to relatively low power to detect meaningful relationships for that endpoint. Further studies of these risks and outcomes would be improved by having better measurement parameters of the risk factors and increased sample size.

In conclusion, we have found some evidence to support the hypothesis that light eye or hair color and the presence of these combined with sunlight exposure is associated with increased risk of developing early AMD. Further research in larger populations with greater range of sunlight exposures and measures of skin pigmentation may reveal stronger associations. In addition, a wider range of genetic information may reveal loci that interact with environmental and skin pigmentation exposure to identify groups at high risk of developing early and late AMD.

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