Published in final edited form as:

Am J Ophthalmol. 2011 December ; 152(6): 1005–1013.e1. doi:10.1016/j.ajo.2011.05.016.

Evaluation of New and Established Age-Related Macular Degeneration Susceptibility Genes in the Women's Health Initiative Sight Exam (WHI-SE) Study

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Abstract

PURPOSE—To assess whether established and newly reported genetic variants, independent of known lifestyle factors, are associated with the risk of age-related macular degeneration (AMD) among women participating in the Women's Health Initiative Sight Exam (WHI-SE) Genetic Ancillary Study.

DESIGN—Multicenter case-control study.

METHODS—One hundred and forty-six women with intermediate and late stages of AMD and 1269 subjects without AMD underwent ocular examinations and fundus photography to determine stage of AMD. Fourteen polymorphisms at or near 11 genes, including previously confirmed genes *CFH*, *ARMS2/HTRA1*, *C2*, *C3*, and *CFI*; recently reported AMD genes in the high-density lipoprotein cholesterol (HDL) pathway *LIPC*, *ABCA1*, *CETP*, and *LPL*; *TIMP3/SYN3*, a known ocular gene recently linked with AMD; and *APOE*, were assessed using logistic regression analysis.

RESULTS—After adjustment for demographic, behavioral, and other genetic factors, a protective effect was detected among TT carriers compared with non-carriers for the HDL pathway gene, *LIPC* rs493258, for intermediate and late AMD (OR [95% confidence interval]: 0.3 [0.2–0.7], $P = .003$). Variants in *CFH* rs1410996, *ARMS2/HTRA1* A69S, and *C3* R102G were significantly associated with an increased risk of AMD. Individuals with the homozygous *CFI* rs10033900 TT genotype had a 2.9 [1.2–7.2]-fold increased risk, and those with the *CFH* Y402H GG genotype

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Supplemental Material available at AJO.com.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF Interest and none were reported.

had a 2.2 [1.0–4.8]-fold higher risk of developing AMD compared with non-carriers. *APOE4* carriers may have a reduced risk of intermediate/late AMD (OR = 0.5 [0.3–0.9], $P = .015$). Suggestive associations were seen between AMD and the HDL pathway genes CETP and LPL.

CONCLUSION—In this unique national cohort of women, we found associations with established AMD-related genetic factors and the recently reported *LIPC* gene in the HDL pathway. These findings may help develop novel therapeutic targets to treat or delay the onset of the disease.

AGE-RELATED MACULAR DEGENERATION (AMD) IS the leading cause of irreversible blindness among elderly individuals worldwide. The public health impact of this disease is rising since the elderly population is growing substantially. It has been forecast that cases of early AMD will increase from 9.1 million in 2010 to 17.8 million in 2050.¹ Healthy lifestyles can reduce the prevalence and incidence of AMD. Modifiable risk factors linked to AMD over the past 2 decades include cigarette smoking, lack of physical activity, excessive weight, and insufficient nutritional antioxidant dietary and supplement intake.^{2–7}

There has also been compelling evidence for the role of genetic factors in AMD pathogenesis.^{8,9} Genetic variants associated with the prevalence of the disease have been identified in the complement pathway (*CFH*,^{10–14} *C2/CFB*,^{10,15} *C3*,^{16,17} and *CFI*¹⁸). There is also an association with a region containing several tightly linked genes on chromosome 10 (*LOC387715*, *HTRA1*, *ARMS2*),^{19–22} although the function of those genes and variants is not fully understood. We have shown that a combination of these demographic, ocular, behavioral, and multi-loci genetic factors are highly predictive of AMD and its progression to the advanced stages, with a predictive ability of over 80% to discriminate progressors from non-progressors.^{10,23}

Recent genome-wide association (GWA) studies examining much larger numbers of individuals, and therefore with more statistical power, have confirmed all of the above AMD variants, and also have identified additional pathways associated with the disease, including one involved in the high-density lipoprotein cholesterol or reverse cholesterol transport pathway. Specifically, the hepatic lipase (*LIPC*) gene was discovered to affect AMD susceptibility,²⁴ which was corroborated by an independent GWA study.²⁵ Both GWA studies implicate a number of other genes in high-density lipoprotein (HDL) metabolism. Furthermore, a susceptibility locus near the *TIPM3* gene, a metalloproteinase involved in degradation of the extracellular matrix, previously shown to be mutated in cases of Sorsby fundus dystrophy, an early-onset maculopathy, has been implicated²⁵ and corroborated.²⁴ The apolipoprotein E (*APOE*) gene may also be associated with AMD but results are inconsistent, possibly because of the strong negative association of the E4 allele with longevity and increasing age.²⁶

We evaluated the relative contribution of novel and established genetic factors toward AMD susceptibility in our Genetic Ancillary Study involving a cohort of women enrolled in the Women's Health Initiative Sight Exam (WHI-SE) study. This is a study within a set of clinical trials and an observational study, which was designed to test the effects of postmenopausal hormone therapy, diet modification, and calcium and vitamin D supplements on heart disease, fractures, and breast and colorectal cancer.²⁷ The findings of

the initial study have shown that treatment with conjugated equine estrogens (CEE) alone and CEE with progestin (CEE+P) does not affect early- or late-stage AMD.²⁸ The WHI-SE study cohort provides a unique opportunity to study AMD in a large population of women who were examined and uniformly assessed with regard to their AMD status. Detection of AMD-associated genetic markers expands the understanding of mechanisms related to AMD, can help identify individuals who are at high risk of developing AMD, and could lead to novel targets for preventive and therapeutic interventions.

MATERIAL AND METHODS

STUDY POPULATION

In the WHI-SE, 4288 women 65 years and older underwent fundus photography for the determination of signs of AMD.²⁸ Participants were recruited from April 2000 to June 2002 at 21 clinical sites. In the WHI Hormone Therapy Trial, women with a uterus were randomized to treatment with CEE+P or placebo. Women without a uterus were randomized to treatment with unopposed CEE or placebo. The present study is restricted to individuals of European descent. Non-white individuals were excluded because of the small sample size and because the distribution of advanced AMD in other populations differs considerably from that among white populations.²⁹

PHENOTYPE DEFINITIONS

Fundus photography was performed using a standard protocol as described elsewhere.^{28,30,31} The sample of 1717 women was selected based on availability of adequate ocular photographs. AMD severity was defined as no AMD (N = 1269), minimal early AMD (N = 25), moderate early (N = 277) and severe early AMD (herein called intermediate; N = 98), and advanced AMD (geographic atrophy or dry, N = 13; and neovascular or wet, N = 35), as described elsewhere.²⁸ Mild, moderate, and advanced cases were age-matched to controls originally, but mild cases were excluded from the analyses to evaluate more clinically and visually significant disease, so the final sample sets were not matched. We analyzed late AMD as 1 case group and the combined phenotypes of intermediate and late AMD as another group. Self-administered questionnaires were used to collect information on race, age, education, smoking status, and diabetes status, as well as lipid-lowering, anti hypertensive, and diabetes medication history. At the first screening examination, body weight and height were measured to assess body mass index (BMI), calculated as the weight in kilograms divided by the square of the height in meters.

SINGLE NUCLEOTIDE POLYMORPHISM SELECTION AND GENOTYPING

We reviewed genome-wide association and candidate gene studies to select 14 single nucleotide polymorphisms (SNPs) at or near 11 genes previously associated with AMD in populations of European ancestry (Table 1). Genotyping was performed with Taqman assays (Applied Biosystems; Supplemental Table, available at AJO.com) on a 7900HT (Applied Biosystems, Carlsbad, California, USA) at Tufts Clinical and Translational Science Institute and Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University. The *CFH* polymorphism rs1061170 was genotyped using a custom assay (forward primer 59'-GGTCCTTAGGAAAATGT-TATTTTCCTTATTTGG-39', reverse primer 59'-

GGCAGGCAACGTCTATAGATTTACC-39', G allele probe (VIC): 59'-TTTCTTCCATGATTTTG-39', and A allele probe (FAM): 59'-TTCTTCCATAAATTTTG-39). The minimum call rate was 96.9% and the average consensus rate from 20 duplicates was 100%. Seven samples were sequenced and the concordance with the initial genotype calls was 100%. All SNPs conformed to Hardy-Weinberg equilibrium. The allelic variants derived from the 2 SNPs within *APOE*, rs429358 and rs7412, and referred to as E2, E3, and E4, are differentiated on the basis of cysteine-arginine residue interchanges at positions 112 and 158 in the amino acid sequence and give rise to 6 bi-allelic haplotypes (E3/E3, E3/E4, E2/E3, E4/E4, E2/E4, and E2/E2).

STATISTICAL ANALYSIS

Participants with intermediate and advanced AMD were individually compared to the control group of subjects with no AMD. First, univariate analysis was performed for each variant. For common variants, tests for trend for the number of risk alleles (0, 1, or 2) were calculated; for rare variants, carriers of 1 or 2 alleles were compared to non-carriers (dominant model). Then, multivariate logistic regression analysis was conducted to identify significant factors collectively associated with disease status, including age (continuous), education (high school diploma or less vs college or higher), cigarette smoking (never, past, current), treatment assignment in the clinical trial (CEE, CEE+P, or placebo for CEE or CEE+P), BMI (continuous), and 12 genetic variants. To account for potential survival effect of the *APOE* genotype an *APOE*-age interaction term was included in the fully adjusted model. A 1-tailed $P < .05$ was considered statistically significant. All analyses were performed using SAS/STAT and SAS/Genetics software version 9.1 (SAS Institute, Inc, Cary, North Carolina, USA).

RESULTS

THE MEAN AGES OF THE SUBJECTS AND CONTROLS WERE 74.5 ± 4.7 and 73.6 ± 4.7 , respectively. Table 2 displays the unadjusted associations with intermediate and late AMD. Controls were more likely to have education beyond high school. There was a nonsignificant trend for cases to be older, to be former or current smokers, and to have higher BMI. Risk genotypes for *C3* R102G, *CFH* rs1410996 and Y402H, *CFI* rs10033900, and *ARMS2/HTRA1* A69S were significantly higher in cases, while the frequency of the homozygous TT genotype for *LIPC* rs493258 and rs10468017 (in strong linkage disequilibrium: $D' = 0.95$, $r^2 = 0.63$) was higher in controls. For *APOE*, the E4 allele tended to be higher in controls for the combined case group.

In the multivariate analysis controlling for all demographic, environmental, and genetic factors among subjects with complete data for all variables (Table 3), age and BMI were related to AMD, and subjects were more likely to be smokers (not significant). Genetic polymorphisms that remained significant after the adjustment for all demographic, lifestyle, and genetic factors included *CFH* rs1410996, *ARMS2/HTRA1* A69S, and *C3* R102G regardless of disease severity. In addition, a protective effect was seen for the minor allele (T) of *LIPC* rs493258 with the lower AMD risk seen for carriers of 2 minor alleles compared to non-carriers (odds ratio [OR], 95% confidence interval [CI]: 0.3, 0.2–0.7, $P = .$

003 for individuals with intermediate and late AMD and OR, 95% CI: 0.3, 0.1–1.1, $P = .06$ for individuals with late wet and dry AMD). Also, *CFI* rs10033900 TT carriers had 2.9-fold higher odds of developing late AMD (95% CI 1.2–7.2; $P = .02$), whereas *CFH* Y402H GG carriers had a 2.2-fold higher odds of developing intermediate or late AMD (95% CI 1.0–4.8; $P = .04$) compared to non-carriers. In addition, *CFH* rs1410996 was significant in the multiple degree of freedom test for the overall effect ($P = .02$ and $P = .002$ for advanced and intermediate and late AMD, respectively) independent of *CFH* Y402H. *APOE* E4 carriers were less likely to be diagnosed with intermediate and late AMD when compared to non-carriers (OR, 95% CI: 0.53, 0.32–0.89, $P = .015$), and the *APOE* E4-age interaction term was not significant. A suggestive association was observed between *LPL* rs12678919 and late AMD; however, this was not statistically significant (OR 0.5, 95% CI: 0.2–1.2, $P = .10$) for carriers of 1 or 2 G alleles compared to non-carriers. A nonsignificant association was suggested for the A allele of *CETP* (OR 2.3 for AA vs CC) comparing late AMD to controls.

DISCUSSION

THIS ARTICLE UNDERSCORES THE COMBINED IMPACT OF common environmental and genetic risk factors, including newly identified genetic variants, on prevalence of AMD in women of European descent enrolled in the WHI-SE Genetic Ancillary Study. This study expands upon previous reports by adding new AMD susceptibility loci and evaluating the role of several genes among women. After the adjustment for age, smoking and BMI, established susceptibility loci in the complement factor genes *CFH*, *CFI*, and *C3*, as well as the *ARMS2/HTRA1* region, were independently associated with a 2.7-fold to 13.1-fold increased risk of late AMD in homozygote carriers compared to non-carriers. When intermediate and late cases were combined, smaller but significant associations remained for *CFH*, *C3*, and *ARMS2/HTRA1*.

For the first time in an independent cohort of women, we confirmed the genetic association between AMD and a gene involved in HDL metabolism, hepatic lipase, or *LIPC*.²⁴ Our results also suggest an association between AMD and the lipoprotein lipase gene, *LPL*. The T allele of *LIPC* and the G allele of *LPL* both increase serum HDL levels and are associated with a reduced risk of AMD. Common variations in these genes have been associated with triglyceride and HDL levels by numerous candidate gene and GWA studies.^{32–36} The trends observed in the recent GWA studies for other genetic loci in the HDL pathway, *ABCA1* rs1883025 and *CETP* rs3764261,^{24,25} were also seen, but were not significant in the present multivariate analysis. The exact mechanism underlying the relationship between these HDL-related pathway genes and AMD risk is unknown. We have shown in a previous study that *LIPC* and serum HDL levels are independently associated with AMD.³⁷ This finding, together with the observation that the HDL pathway genes do not all have the same direction of effect on AMD relative to the HDL raising or lowering allele, suggests that there may be mechanisms involved other than the effect on serum HDL levels. AMD development may be associated with highly toxic oxidized cholesterol formed in photoreceptors as a photo-oxidative byproduct of the visual cycle.³⁸ Accumulation of cholesterol in Bruch's membrane is a potent attractor of macrophages, which may help explain the link between drusen accumulation and subsequent breaks in Bruch's membrane with the development of choroidal neovascularization.³⁸

Results also suggest a protective effect of the *APOE4* allele on AMD. The *APOE* gene is involved in the triglyceride-rich and low-density lipoprotein metabolism.³⁹ *APOE*-null mice have demonstrated lipid deposits in Bruch's membrane, while *APOE2* transgenic mice had lipid accumulation in retinal pigment epithelium, both similar to those observed in AMD.^{40,41} The effect of the *APOE* genotype on AMD risk, however, has been questioned because of inconsistent results in the literature and the known reduction in the frequency of the *APOE4* allele with increasing age.⁴² Since individuals with the *APOE4* allele are less likely to live to advanced ages, lack of careful matching on age between cases with an age-related disorder and corresponding controls can lead to bias related to differential survival in the 2 groups.

Although we had above 80% statistical power to detect the magnitude of the effect previously reported for the *TIMP3/SYN3* locus (pooled effect size from the discovery and replication samples OR 1.41, CI: 1.27–1.57),²⁵ no association between this locus and AMD status was found in the present study. We can speculate that this relationship is less pronounced in women, but other reasons for nonreplication may include population substructure within European ancestry, phenotypic heterogeneity, or a chance finding.

The study cohort consisted of female subjects of European descent, and to the best of our knowledge women have not been previously analyzed separately for associations between AMD and these genes. It is possible that there are different mechanisms related to AMD development and progression between men and women. There are some potential limitations of our study. Results may not be generalizable to men, as well as to women from other ethnic backgrounds. Although there are no reported major differences in the incidence and prevalence of AMD between men and women controlling for age, population-based studies have revealed significant differences in the incidence and prevalence of AMD in different ethnic and racial groups. Individuals of European ancestry have a higher risk of developing the disease than those of African or Asian descent.⁴³⁻⁴⁵ Moreover, the relatively small sample size may have limited our ability to detect some statistically significant associations. Also, we cannot rule out that in addition to *APOE*, there might be other genes that are associated with longevity or other competing risks and have differential frequency in the AMD cases compared with controls.

In summary, in our WHI-SE Genetic Ancillary Study, we found an important role for the previously established AMD-related genetic risk factors among women. We replicated novel associations pointing to the important involvement of the HDL and reverse cholesterol transport pathway in disease pathogenesis. With the increasing awareness of the involvement of modifiable risk factors in the development of AMD, early screening, which includes assessment of genetic profiles, may in the future help identify high-risk individuals who would particularly benefit from healthy lifestyles known to affect AMD prevalence and progression.²³ This includes maintaining optimal weight, exercising, not smoking, eating an antioxidant-rich diet, taking nutritional supplements related to ocular health, and adhering to heart-healthy guidelines for cholesterol and blood pressure control. Identification and confirmation of new genetic loci, including results reported here, expand our knowledge of the mechanisms related to AMD and may help lead to novel therapeutic targets to treat or delay the progression of the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Publication of this article was supported by the Russo Dual-Investigator Grant, Tufts University School of Medicine, Boston, Massachusetts; Grant RO1-EY11309 from the National Institutes of Health, Bethesda, Maryland; Massachusetts Lions Eye Research Fund, Inc, New Bedford, Massachusetts; Research to Prevent Blindness, Inc, New York, New York; and the Macular Degeneration Research Fund of the Ophthalmic Epidemiology and Genetics Service, New England Eye Center, Tufts Medical Center, Tufts University School of Medicine, Boston, Massachusetts. WHI-SE was funded by Wyeth-Ayerst Laboratories, Inc, Collegeville, Pennsylvania; and WHI was funded by National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland. Tufts Medical Center has filed a patent application for some materials related to this work. Involved in design and conduct of the study (I.P., M.H., J.M.S.); collection and interpretation of the data (I.P., G.S.H., J.M.O., M.H., J.M.S.); and preparation, review, or approval of the manuscript (I.P., G.S.H., J.M.O., M.H., J.M.S.). This ancillary study was approved by the Institutional Review Board of Tufts University School of Medicine and Tufts Medical Center and the WHI ancillary study subcommittee.

REFERENCES

1. Rein DB, Wittenborn JS, Zhang X, Honeycutt AA, Lesesne SB, Saaddine J. Forecasting age-related macular degeneration through the year 2050: the potential impact of new treatments. *Arch Ophthalmol*. 2009; 127:533–540. [PubMed: 19365036]
2. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. *Arch Ophthalmol*. 2006; 124:995–1001. [PubMed: 16832023]
3. Seddon JM, Cote J, Davis N, Rosner B. Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio. *Arch Ophthalmol*. 2003; 121:785–792. [PubMed: 12796248]
4. Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transun-saturated fat, nuts, and fish intake. *Arch Ophthalmol*. 2003; 121:1728–1737. [PubMed: 14662593]
5. Seddon JM, Willett WC, Speizer FE, Hankinson SE. A prospective study of cigarette smoking and age-related macular degeneration in women. *JAMA*. 1996; 276:1141–1146. [PubMed: 8827966]
6. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 1994; 272:1413–1420. [PubMed: 7933422]
7. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no.8. *Arch Ophthalmol*. 2001; 119:1417–1436. [PubMed: 11594942]
8. Haddad S, Chen CA, Santangelo SL, Seddon JM. The genetics of age-related macular degeneration: a review of progress to date. *Surv Ophthalmol*. 2006; 51:316–363. [PubMed: 16818082]
9. Peter I, Seddon JM. Genetic epidemiology: successes and challenges of genome-wide association studies using the example of age-related macular degeneration. *Am J Ophthalmol*. 2010; 150:450–452. [PubMed: 20863920]
10. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. *Nat Genet*. 2006; 38:1055–1059. [PubMed: 16936732]
11. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005; 308:421–424. [PubMed: 15761121]
12. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005; 308:419–421. [PubMed: 15761120]

13. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2005; 102:7227–7232. [PubMed: 15870199]
14. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005; 308:385–389. [PubMed: 15761122]
15. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet*. 2006; 38:458–462. [PubMed: 16518403]
16. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. *Nat Genet*. 2007; 39:1200–1201. [PubMed: 17767156]
17. Yates JR, Sepp T, Matharu BK, et al. Complement C3 variant and the risk of age-related macular degeneration. *N Engl J Med*. 2007; 357:553–561. [PubMed: 17634448]
18. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. *Eur J Hum Genet*. 2009; 17:100–104. [PubMed: 18685559]
19. Yang Z, Camp NJ, Sun H, et al. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science*. 2006; 314:992–993. [PubMed: 17053109]
20. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am J Hum Genet*. 2005; 77:389–407. [PubMed: 16080115]
21. Dewan A, Liu M, Hartman S, et al. HTRA1 promoter polymorphism in wet age-related macular degeneration. *Science*. 2006; 314:989–992. [PubMed: 17053108]
22. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet*. 2005; 14:3227–3236. [PubMed: 16174643]
23. Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci*. 2009; 50:2044–2053. [PubMed: 19117936]
24. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (*LIPC*). *Proc Natl Acad Sci U S A*. 2010; 107:7395–7400. [PubMed: 20385826]
25. Chen W, Stambolian D, Edwards AO, et al. Genetic variants near TIMP3 and high-density lipoprotein associated loci influence susceptibility to age-related macular degeneration. *Proc Natl Acad Sci U S A*. 2010; 107:7401–7406. [PubMed: 20385819]
26. Panza F, D'Introno A, Capurso C, et al. Lipoproteins, vascular-related genetic factors, and human longevity. *Rejuvenation Res*. 2007; 10:441–458. [PubMed: 17990970]
27. Anderson GL, Manson J, Wallace R, et al. Implementation of the Women's Health Initiative study design. *Ann Epidemiol*. 2003; 13:S5–17. [PubMed: 14575938]
28. Haan MN, Klein R, Klein BE, et al. Hormone therapy and age-related macular degeneration: the Women's Health Initiative Sight Exam Study. *Arch Ophthalmol*. 2006; 124:988–992. [PubMed: 16832022]
29. Seddon, J.; Sobrin, L. Epidemiology of age-related macular degeneration. In: Albert, DM.; Miller, J., editors. *The Principles and Practice of Ophthalmology*. 3rd ed. W.B. Saunders; Philadelphia: 2007. p. 413–422.
30. Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study. *Ophthalmology*. 2002; 109:1767–1779. [PubMed: 12359593]
31. Klein R, Davis MD, Magli YL, Segal P, Klein BE, Hubbard L. The Wisconsin age-related maculopathy grading system. *Ophthalmology*. 1991; 98:1128–1134. [PubMed: 1843453]
32. Aulchenko YS, Ripatti S, Lindqvist I, et al. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nat Genet*. 2009; 41:47–55. [PubMed: 19060911]

33. Kooner JS, Chambers JC, Aguilar-Salinas CA, et al. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. *Nat Genet.* 2008; 40:149–151. [PubMed: 18193046]
34. Kathiresan S, Willer CJ, Peloso GM, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet.* 2009; 41:56–65. [PubMed: 19060906]
35. Willer CJ, Sanna S, Jackson AU, et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet.* 2008; 40:161–169. [PubMed: 18193043]
36. Saxena R, Voight BF, Lyssenko V, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science.* 2007; 316:1331–1336. [PubMed: 17463246]
37. Reynolds R, Rosner B, Seddon J. Serum lipid biomarkers and hepatic lipase gene associations with age-related macular degeneration. *Ophthalmology.* 2010; 117:1989–1995. [PubMed: 20888482]
38. Curcio CA, Johnson M, Huang JD, Rudolf M. Aging, age-related macular degeneration, and the response-to-retention of apolipoprotein B-containing lipoproteins. *Prog Retin Eye Res.* 2009; 28:393–422. [PubMed: 19698799]
39. Mahley RW, Innerarity TL, Rall SC Jr. Weisgraber KH. Plasma lipoproteins: apolipoprotein structure and function. *J Lipid Res.* 1984; 25:1277–1294. [PubMed: 6099394]
40. Lee SJ, Kim JH, Chung MJ, et al. Human apolipoprotein E2 transgenic mice show lipid accumulation in retinal pigment epithelium and altered expression of VEGF and bFGF in the eyes. *J Microbiol Biotechnol.* 2007; 17:1024–1030. [PubMed: 18050922]
41. Ishida T, Choi SY, Kundu RK, et al. Endothelial lipase modulates susceptibility to atherosclerosis in apolipoprotein-E-deficient mice. *J Biol Chem.* 2004; 279:45085–45092. [PubMed: 15304490]
42. Lewis SJ, Brunner EJ. Methodological problems in genetic association studies of longevity—the apolipoprotein E gene as an example. *Int J Epidemiol.* 2004; 33:962–970. [PubMed: 15319409]
43. Friedman DS, O’Colmain BJ, Munoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol.* 2004; 122:564–572. [PubMed: 15078675]
44. Li Y, Xu L, Jonas JB, Yang H, Ma Y, Li J. Prevalence of age-related maculopathy in the adult population in China: the Beijing eye study. *Am J Ophthalmol.* 2006; 142:788–793. [PubMed: 16989759]
45. Oshima Y, Ishibashi T, Murata T, Tahara Y, Kiyohara Y, Kubota T. Prevalence of age related maculopathy in a representative Japanese population: the Hisayama study. *Br J Ophthalmol.* 2001; 85:1153–1157. [PubMed: 11567955]

TABLE 1

Single Nucleotide Polymorphisms Assessed for Age-Related Macular Degeneration

dbSNP Identifier ^a	Gene	Chromosomal Position	Nucleotide Substitution	Amino Acid Substitution
Established genetic variants				
rs1061170	<i>CFH</i>	1q32	CT	Y402H
rs1410996	<i>CFH</i>	1q32	CT	
rs10490924	<i>ARMS2/HTRA1</i>	10q26.13	GT	A69S
rs9332739	<i>C2</i>	6p21.3	GC	E318D
rs2230199	<i>C3</i>	19p13.3	CG	R102G
rs10033900	<i>CFI</i>	4q25	CT	
Newly reported AMD variants				
rs493258	<i>LIPC</i>	15q21–q23	CT	
rs10468017	<i>LIPC</i>	15q21–q23	CT	
rs9621532	<i>TIMP3, SYN3</i>	22q12.3	AC	
Suggestive genetic variants				
rs429358	<i>APOE</i>	19q13.2	CT	C130R
rs7412	<i>APOE</i>	19q13.2	CT	R176C
Potential HDL pathway variants				
rs1883025	<i>ABCA1</i>	9q31.1	CT	
rs3764261	<i>CETP</i>	16q21	CA	
rs12678919	<i>LPL</i>	8p22	AG	

ABCA = ATP-binding cassette; *APOE* = apolipoprotein E; *ARMS2/HTRA1* = age-related maculopathy susceptibility 2/ HtrA serine peptidase 1; *C2* = complement component 2; *C3* = complement component 3; *CETP* = cholesteryl ester transfer protein; *CFH* = complement factor H; *CFI* = complement factor I; *LIPC* = hepatic lipase C; *LPL* = lipoprotein lipase; *SYN3* = synapsin III; *TIMP* = tissue inhibitor of metalloproteinase 3.

^aDatabase of single nucleotide polymorphisms (SNPs): <http://www.ncbi.nlm.nih.gov/projects/SNP/>.

TABLE 2

Univariate Associations Between Age-Related Macular Degeneration and Baseline Demographic, Environmental, and Genetic Variables

	Control,N (%)	Late AMD			Intermediate and Late AMD		
		N (%)	OR (95% CI)	P	N (%)	OR (95% CI)	P
Total patients	1269	48			146		
Age (years)							
<70	275 (21.7)	6 (12.5)	1.0		22 (15.1)	1.0	
70+	994 (78.3)	42 (87.5)	1.9 (0.8–4.6)	.13	124 (84.9)	1.6 (0.97–2.5)	.07
Education							
High school	500 (39.6)	26 (54.2)	1.0		67 (46.2)	1.0	
>High school	762 (60.4)	22 (45.8)	0.6 (0.3–0.99)	.046	78 (53.8)	0.8 (0.5–1.1)	.13
Smoking							
Never	709 (56.5)	22 (46.8)	1.0		81 (56.6)	1.0	
Past	471 (37.5)	22 (46.8)	1.5 (0.8–2.8)	.18	53 (37.1)	1.0 (0.7–1.4)	.94
Current	75 (6.0)	3 (6.4)	1.3 (0.4–4.4)	.69	9 (6.3)	1.1 (0.5–2.2)	.89
BMI							
>25	348 (27.6)	11 (22.9)	1.0		39 (26.7)	1.0	
25–29	454 (36.0)	17 (35.4)	1.2 (0.5–2.6)	.62	52 (35.6)	1.0 (0.7–1.6)	.83
30	458 (36.4)	20 (41.7)	1.4 (0.7–3.0)	.36	55 (37.7)	1.1 (0.7–1.7)	.67
Treatment group ^b							
Placebo for CEE + P	378 (29.8)	18 (37.5)	1.0		46 (31.5)	1.0	
CEE + P	433 (34.1)	11 (22.9)	0.5 (0.2–1.1)	.11	38 (26.0)	0.7 (0.5–1.1)	.16
CEE	216 (17.0)	10 (20.8)	1.0 (0.4–2.1)	.94	31 (21.2)	1.2 (0.7–1.9)	.50
Placebo for CEE	242 (19.1)	9 (18.8)	0.8 (0.3–1.8)	.55	31 (21.2)	1.1 (0.6–1.7)	.84
ESTABLISHED GENETIC VARIANTS							
<i>CFH</i> :rs1061170 (Y402H)							
TT	503 (40.0)	8 (16.7)	1.0		29 (19.9)	1.0	
CT	592 (47.1)	28 (58.3)	3.0 (1.3–6.6)		70 (48.0)	2.1 (1.3–3.2)	
CC	163 (13.0)	12 (25.0)	4.6 (1.9–11.5)	.0005 ^a	47 (32.2)	5.0 (3.0–8.2) <.0001 ^a	
<i>CFH</i> :rs1410996							
TT	226 (18.0)	3 (6.3)	1.0		13 (8.9)	1.0	
CT	631 (50.2)	14 (29.2)	1.7 (0.5–5.9)	39 (26.7)	1.1 (0.6–2.1)		
CC	399 (31.8)	31 (64.6)	5.8 (1.8–19.4)	<.0001 ^a	94 (64.4)	4.1 (2.2–7.5)	<.0001 ^a
<i>ARMS2/HTRA1</i> rs10490924 (A69S)							
GG	806 (63.9)	17 (35.4)	1.0		59 (40.4)	1.0	
GT	406 (32.2)	23 (47.9)	2.7 (1.4–5.1)		74 (50.7)	2.5 (1.7–3.6)	
TT	49 (3.9)	8 (16.7)	7.7 (3.2–18.9)	<.0001 ^a	13 (8.9)	3.6 (1.9–7.1)	<.0001 ^a
<i>C2</i> :rs9332739 (E318D)							
GG	1163 (92.4)	43 (89.6)	1.0		140 (95.9)	1.0	
CC+CG	96 (7.6)	5 (10.4)	1.4 (0.5–3.6)	.48	6 (4.1)	0.5 (0.2–1.2)	.13

		Late AMD				Intermediate and Late AMD		
	Control,N (%)	N (%)	OR (95% CI)	P		N (%)	OR (95% CI)	P
C3:rs2230199 (R102G)								
CC	808 (64.1)	26 (54.2)	1.0			79 (54.1)	1.0	
CG	398 (31.6)	14 (29.2)	1.1 (1.6–2.1)			52 (35.6)	1.3 (0.9–1.9)	
GG	54 (4.3)	8 (16.7)	4.6 (2.0–10.7)	.01 ^a		15 (10.3)	2.8 (1.5–5.3)	.002 ^a
CFI:rs10033900								
CC	348 (27.6)	9 (18.8)	1.0			34 (23.3)	1.0	
CT	623 (49.4)	20 (41.7)	1.2 (0.6–2.8)			68 (46.6)	1.1 (0.7–1.7)	
TT	289 (22.9)	19 (39.6)	2.5 (1.1–5.7)	.016 ^a		44 (30.1)	1.6 (1.0–2.5)	.06 ^a
NEWLY REPORTED AMD VARIANTS								
LIPC:rs493258								
CC	354 (28.8)	19 (39.6)	1.0			50 (35.0)	1.0	
CT	597 (48.6)	26 (54.2)	0.8 (0.4–1.5)			80 (56.0)	0.9 (0.7–1.4)	
TT	277 (22.6)	3 (6.3)	0.2 (0.1–0.7)	.01 ^a		13 (9.1)	0.3 (0.2–0.6)	.002 ^a
LIPC:rs10468017								
CC	650 (52.7)	34 (70.8)	1.0			89 (63.1)	1.0	
CT	487 (39.5)	11 (22.9)	0.4 (0.2–0.9)			46 (32.6)	0.7 (0.5–1.0)	
TT	97 (7.9)	3 (6.3)	0.6 (0.2–2.0)	.04 ^a		6 (4.3)	0.5 (0.2–1.1)	.01 ^a
TIMP3/SYN3:rs9621532								
AC+CC	131 (10.5)	5 (10.4)	1.0			16 (11.1)	1.0	
AA	1114 (89.5)	43 (89.6)	1.0 (0.4–2.6)	.98		128 (88.9)	0.9 (0.5–1.6)	.83
SUGGESTIVE GENETIC VARIANT								
APOE								
E2E2+E2E3+E3E3	928 (73.7)	39 (81.3)	1.0			120 (82.2)	1.0	
E2E4+E3E4+E4E4	331 (26.3)	9 (18.8)	0.6 (0.3–1.4)	.25		26 (17.8)	0.6 (0.4–0.9)	.03
POTENTIAL HDL PATHWAY VARIANTS								
ABCA1:rs1883025								
CC	647 (53.0)	29 (60.4)	1.0			77 (55.0)		
CT+TT	573 (47.0)	19 (39.6)	0.7 (0.4–1.3)	.32		63 (45.0)	0.9 (0.7–1.3)	.66
CETP:rs3764261								
CC	571 (45.8)	20 (41.7)	1.0			58 (40.6)	1.0	
CA	544 (43.6)	21 (43.8)	1.1 (0.6–2.1)			67 (46.9)	1.2 (0.8–1.8)	
AA	132 (10.6)	7 (14.6)	1.5 (0.6–3.7)	.41 ^a		18 (12.6)	1.3 (0.8–2.4)	.22 ^a
LPL:rs12678919								
AA	1009 (81.3)	42 (87.5)	1.0			120 (84.5)	1.0	
AG+GG	232 (18.7)	6 (12.5)	0.6 (0.3–1.5)	.28		22 (15.5)	0.8 (0.5–1.3)	.35

AMD = age-related macular degeneration; BMI = body mass index; CI = confidence interval; OR = odds ratio.

^aTest for linear trend.

^b Treatment randomization groups defined as: Placebo for CEE + P = placebo for estrogen replacement therapy (ERT) + progestin; CEE + P = ERT + progestin; CEE = ERT only; Placebo for CEE = placebo for ERT only.

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TABLE 3

Multivariate Analysis for Associations Between Age-Related Macular Degeneration and Demographic, Environmental, and Genetic Risk Factors

Variable	Late AMD		Intermediate and Late AMD	
	OR (95% CI)	P ^a	OR (95% CI)	P ^a
Case/control (n)	47/1121		130/1121	
Age (years) ^b	1.1 (1.1–1.2)	.0005	1.1 (1.0–1.1)	.001
BMI (kg/m ²) ^b	1.1 (1.0–1.1)	.01	1.0 (1.0–1.1)	.07
Education				
College or more vs high school or less	0.6 (0.3–1.1)	.10	0.9 (0.6–1.4)	.63
Smoking				
Current vs never	2.9 (0.7–12.2)	.15	1.7 (0.7–3.8)	.23
Past vs never	1.9 (1.0–3.8)	.07	1.0 (0.7–1.6)	.85
Treatment assignment ^d				
CEE + P vs Placebo for CEE + P	0.6 (0.3–1.5)	.37	0.7 (0.4–1.2)	.23
CEE vs Placebo for CEE + P	1.1 (0.4–2.8)	.81	1.5 (0.8–2.6)	.18
Placebo for CEE vs Placebo for CEE + P	0.7 (0.3–1.7)	.40	1.0 (0.5–1.7)	.89
<i>CFH</i> :rs1061170 (Y402H)				
CT vs CC	2.2 (0.8–6.6)	.15	1.8 (0.9–3.3)	.09
TT vs CC	1.9 (0.5–7.0)	.36	2.2 (1.0–4.8)	.04
<i>CFH</i> :rs1410996		.02^c		.002^c
CT vs CC	1.1 (0.2–5.3)	.88	0.8 (0.4–1.9)	.65
TT vs CC	3.3 (0.7–16.9)	.14	2.1 (0.8–5.1)	.11
<i>ARMS2/HTRA1</i> rs10490924				
GT vs GG	2.6 (1.3–5.3)	.01	2.4 (1.6–3.7)	<.0001
TT vs GG	13.5 (4.7–38.4)	<.0001	4.6 (2.2–9.6)	<.0001
<i>C2</i> :rs9332739 (E318D)				
CC+CG vs GG	1.6 (0.6–4.7)	.38	0.6 (0.2–1.4)	.20
<i>C3</i> :rs2230199 (R102G)				
CG vs CC	1.2 (0.6–2.5)	.68	1.3 (0.8–1.9)	.28
GG vs CC	6.9 (2.5–19.1)	.0002	2.7 (1.3–5.7)	.008
<i>CFE</i> :rs10033900				
CT vs CC	1.0 (0.4–2.4)	.96	1.0 (0.6–1.7)	.95
TT vs CC	2.9 (1.2–7.2)	.02	1.6 (1.0–2.8)	.07
<i>LIPC</i> :rs493258				
CT vs CC	0.9 (0.4–1.7)	.68	1.0 (0.6–1.5)	.83
TT vs CC	0.3 (0.1–1.1)	.06	0.3 (0.2–0.7)	.003
<i>TIMP3/SYN3</i> :rs9621532				
AA vs AC+CC	1.4 (0.5–4.3)	.51	1.1 (0.6–2.1)	.75
<i>APOE</i>				
E4 vs E2+E3	0.5 (0.2–1.2)	.17	0.5 (0.3–0.9)	.015

Variable	Late AMD		Intermediate and Late AMD	
	OR (95% CI)	P ^a	OR (95% CI)	P ^a
<i>ABCA1</i> :rs1883025				
CT + TT vs CC	0.8 (0.4–1.5)	.47	0.9 (0.6–1.4)	.75
<i>CETP</i> :rs3764261				
CA vs CC	1.2 (0.6–2.4)	.68	1.1 (0.7–1.7)	.19
AA vs CC	2.3 (0.8–6.3)	.11	1.5 (0.8–2.9)	.72
<i>LPL</i> :rs12678919				
AG+GG vs AA	0.5 (0.2–1.2)	.10	0.7 (0.4–1.3)	.26

AMD = age-related macular degeneration; BMI = body mass index; CI = confidence interval; OR = odds ratio.

*APOE**Age interaction not significant.

^aWhen all other variables are included in the model. Significant P values are in bold.

^bModeled as a continuous variable.

^cWald χ^2 P value (the multiple degree of freedom test for the overall effect of the 2 CFH variables).

^dTreatment randomization groups defined as: Placebo for CEE + P = placebo for estrogen replacement therapy (ERT) + progestin; CEE + P = ERT + progestin; CEE = ERT only; Placebo for CEE = placebo for ERT only