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A Deep Phenotype Association Study Reveals Specific Phenotype Associations with Genetic Variants in Age-related Macular Degeneration

Age-Related Eye Disease Study 2 (AREDS2) Report No. 14

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Purpose: Age-related macular degeneration (AMD), a multifactorial disease with variable phenotypic presentation, was associated with 52 single nucleotide polymorphisms (SNPs) at 34 loci in a genome-wide association study (GWAS). These genetic variants could modulate different biological pathways involved in AMD, contributing to phenotypic variability. To better understand the effects of these SNPs, we performed a deep phenotype association study (DeePAS) in the Age-Related Eye Disease Study 2 (AREDS2), followed by replication using AREDS participants, to identify genotype associations with AMD and non-AMD ocular and systemic phenotypes.

Design: Cohort study.

Participants: AREDS and AREDS2 participants.

Methods: AREDS2 participants (discovery cohort) had detailed phenotyping for AMD; other eye conditions; cardiovascular, neurologic, gastrointestinal, and endocrine disease; cognitive function; serum nutrient levels; and others (total of 139 AMD and non-AMD phenotypes). Genotypes of the 52 GWAS SNPs were obtained. The DeePAS was performed by correlating the 52 SNPs to all phenotypes using logistic and linear regression models. Associations that reached Bonferroni-corrected statistical significance were replicated in AREDS.

Main Outcome Measures: Genotype–phenotype associations.

Results: A total of 1776 AREDS2 participants had 5 years follow-up; 1435 AREDS participants had 10 years. The DeePAS revealed a significant association of the rs3750846 SNP at the *ARMS2/HTRA1* locus with subretinal/sub–retinal pigment epithelial (RPE) hemorrhage related to neovascular AMD (odds ratio 1.55 [95% confidence interval 1.31–1.84], $P = 2.67 \times 10^{-7}$). This novel association remained significant after conditioning on participants with neovascular AMD ($P = 2.42 \times 10^{-4}$). Carriers of rs3750846 had poorer visual acuity during follow-up ($P = 6.82 \times 10^{-7}$) and were more likely to have a first-degree relative with AMD ($P = 5.38 \times 10^{-6}$). Two SNPs at the *CFH* locus, rs10922109 and rs570618, were associated with the drusen area in the Early Treatment Diabetic Retinopathy Study Report (ETDRS) grid ($P = 2.29 \times 10^{-11}$ and $P = 3.20 \times 10^{-9}$, respectively) and the center subfield ($P = 1.24 \times 10^{-9}$ and $P = 6.68 \times 10^{-8}$, respectively). SNP rs570618 was additionally associated with the presence of calcified drusen ($P = 5.38 \times 10^{-6}$). Except for positive family history of AMD with rs3750846, all genotype–phenotype associations were significantly replicated in AREDS. No pleiotropic associations were identified.

Conclusions: The association of the SNP at the *ARMS2/HTRA1* locus with subretinal/sub-RPE hemorrhage and poorer visual acuity and of SNPs at the *CFH* locus with drusen area may provide new insights in pathophysiological pathways underlying different stages of AMD. *Ophthalmology 2017;■:1–10 Published by Elsevier on behalf of the American Academy of Ophthalmology*

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Age-related macular degeneration (AMD) is a common retinal disease affecting millions of people worldwide.[1] Drusen are considered the hallmark feature of AMD, but may be accompanied by other characteristics such as pigment abnormalities, atrophic changes, or neovascularization. Drusen themselves present in various shapes and sizes,

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varying from small to large and from reticular pseudodrusen to calcified drusen.[2] Altogether, phenotypic presentation of AMD is highly variable and multiple distinct AMD features can be recognized.

AMD is a multifactorial disease in which different environmental and genetic risk factors contribute to clinical manifestations. Genetic predisposition is thought to account for 40% to 70% of disease development,[3] and a great deal of effort and resources have resulted in the identification of susceptibility loci. Single nucleotide polymorphisms (SNPs) in complement genes, such as *CFH*, *C2/CFB*, *CFI*, and *C3*, have been consistently associated with AMD and have implicated the complement pathway as a major contributor to AMD.[4–7] SNPs at other loci suggest the involvement of additional biological pathways, such as lipid and extracellular matrix, in AMD pathology.[8] The most recent genome-wide association study (GWAS), including 16 144 late AMD-patients and 17 832 controls, has identified 52 SNPs in 34 loci, augmenting our understanding and identification of pathways that might impact AMD phenotypes.[9] Nonetheless, how genetic variants at these loci influence AMD is unclear. Exploring the relationship of SNPs at genetic loci to AMD and non-AMD phenotypes can lead to novel insights into disease pathology.

The Age-Related Eye Disease Study (AREDS) was a randomized clinical trial that studied the effects of zinc and antioxidant supplements on the progression of AMD in persons ranging from having no AMD to having late AMD in 1 eye. The supplements reduced the progression to late AMD in persons with intermediate AMD or late AMD in 1 eye.[10] After the completion of AREDS, the Age-Related Eye Disease Study 2 (AREDS2) was designed to investigate the effect of omega-3 fatty acids, substitution of beta-carotene with lutein/zeaxanthin, and lowering of the zinc dose on AMD progression.[11] The AREDS2 results led to the substitution of beta-carotene with lutein/zeaxanthin. In contrast to AREDS, AREDS2 enrolled participants having either bilateral intermediate AMD with large drusen or unilateral late AMD. They represent a high-risk group at the far end of the AMD spectrum, yet with highly variable phenotypic presentation. In addition to detailed AMD phenotyping, the AREDS2 participants have been thoroughly screened for other conditions, such as cardiovascular diseases, cognitive function, and other ocular disorders. AREDS2, therefore, presents a unique population to study the relationship between SNPs involved in AMD pathogenesis, phenotypic variability, and pleiotropy.

The phenome-wide association study (PheWAS) was developed to investigate the relationship of 1 genotype with respect to many phenotypes and is thus an ideal method to identify pleiotropy of genetic variants.[12,13] PheWAS phenotypes are usually recorded as a broad range of disease descriptions in International Classification of Diseases 9 (ICD9) codes, thus lacking specific information at a granular level. In AREDS2, emphasis was not on broad phenotyping, but rather on standardized and reproducible detailed phenotyping (deep phenotyping), such as fundus photographic grading of lesion characteristics of AMD. The data collection did not encompass the entire phenome. Therefore, the term PheWAS was not appropriate for this design. To emphasize the use of deep phenotyping, we adopted the term "deep phenotype association study" (DeePAS) for a better description of the methodology.

In this DeePAS, we apply a PheWAS approach to gain insights into the role of reported AMD-related genetic variants in contributing to phenotypic variability and possible disease mechanisms through pleiotropic associations with non-AMD phenotypes.

Methods

Population

The discovery cohort in this study consisted of participants from the AREDS2 trial, which was a randomized, double-masked, placebo-controlled trial that enrolled 4203 participants between 2006 and 2012. A more detailed design of the clinical trial has been described previously.[14] AREDS2 was designed to evaluate the effect of adding omega-3 fatty acids (docohexaenoic acid 350 mg and eicosapentaenoic acid 650 mg) and lutein/zeaxanthin (10 mg/2 mg) to the AREDS formulation on the progression of AMD. Additionally, in a secondary optional randomization step, participants were further assigned to elimination of beta-carotene and/or lowering of the zinc dosage from 80 mg to 25 mg. In the current study, we combined all treatment groups, as AREDS2 did not have a true placebo arm (all participants were given some form of the AREDS supplements) and previous analyses suggested no interaction between genotype and response to treatment.[15] AREDS2 exclusively enrolled people at high risk of progression to late AMD, selecting for those with bilateral large drusen or unilateral large drusen with late AMD in the fellow eye. Participants were followed up to a median of 5 years.

Ethics Statement

Institutional Review Board approval was obtained previously for both the AREDS and AREDS2 populations. All participants provided written informed consent. This research was HIPAA-compliant and adhered to the tenets of the Declaration of Helsinki.

Phenotypes

In a PheWAS, phenotypes will commonly utilize general diagnoses of diseases, such as ICD9 codes for "macular degeneration" or "acute myocardial infarction," but will usually lack possibly important further details. We evaluated the association of several detailed phenotypes within and outside of the AMD spectrum. Therefore, this study design was termed DeePAS. All AREDS2 participants underwent baseline and yearly eye examinations, including stereoscopic fundus photography of the macula and optic nerve. A central reading center performed masked grading of all fundus photographs,[16] as well as fundus autofluorescence images of AREDS2 participants,[17] providing deep AMD phenotyping for each individual. In-depth phenotyping for other characteristics for each participant was provided by a detailed medical history at every study visit and during telephone contacts occurring 6 months after each study visit. Medical history of cardiovascular and neurologic events, as well as hospital admissions or death, were validated by review of medical records by a trained morbidity and mortality committee consisting of cardiologists, neurologists, and internal medical specialists.[18] Additionally, 3741 participants took part in the ancillary cognitive function study and completed at least 1 cognitive function test. The results of a battery of 8 different cognitive function tests were converted into z scores and subsequently into 1 composite z score, where a higher score