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Association of Rare Predicted Loss-of-Function Variants in Cellular Pathways with Sub-Phenotypes in Age-Related Macular Degeneration

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Purpose: To investigate the association of rare predicted loss-of-function (pLoF) variants within age-related macular degeneration (AMD) risk loci and AMD sub-phenotypes.

Design: Case-control study.

Participants: Participants of AREDS, AREDS2, and Michigan Genomics Initiative.

Methods: Whole genome sequencing data were analyzed for rare pLoF variants (frequency <0.1%) in the regions of previously identified 52 independent risk variants known to be associated with AMD. Frequency of the rare pLoF variants in cases with intermediate or advanced AMD was compared with controls. Variants were assigned to the complement, extracellular matrix (ECM), lipid, cell survival, immune system, metabolism, or unknown/other pathway. Associations of rare pLoF variant pathways with AMD sub-phenotypes were analyzed using logistic and linear regression, and Cox proportional hazards models.

Main Outcome Measures: Differences in rare pLoF variant pathway burden and association of rare pLoF variant pathways with sub-phenotypes within the population with AMD were evaluated.

Results: Rare pLoF variants were found in 298 of 1689 cases (17.6%) and 237 of 1518 controls (15.6%) (odds ratio [OR], 1.11; 95% confidence interval [CI], 0.91−1.36; $P = 0.310$). An enrichment of rare pLoF variants in the complement pathway in cases versus controls (OR, 2.94; 95% CI, 1.49−5.79; $P = 0.002$) was observed. Within cases, associations between all rare pLoF variants and choroidal neovascularization (CNV) (OR, 1.34; 95% CI, 1.04−1.73; $P = 0.023$), calcified drusen (OR, 1.33; 95% CI, 1.04−1.72; $P = 0.025$), higher scores on the AREDS Extended AMD Severity Scale (Standardized Coefficient Beta (β)=0.346 [0.086−0.605], $P = 0.009$), and progression to advanced disease (hazard ratio, 1.25; 95% CI, 1.01−1.55; $P = 0.042$) were observed. At the pathway level, there were associations between the complement pathway and geographic atrophy (GA) (OR, 2.17; 95% CI, 1.12−4.24; $P = 0.023$), the complement pathway and calcified drusen (OR, 3.75; 95% CI, 1.79−7.86; $P < 0.001$), and the ECM pathway and more severe levels in the AREDS Extended AMD Severity Scale ($\beta = 0.62$; 95% CI, 0.04−1.20; $P = 0.035$).

Conclusions: Rare pLoF variants are associated with disease progression. Variants in the complement pathway modify the clinical course of AMD and increase the risk of developing specific sub-phenotypes. *Ophthalmology 2017;■:1−9 Published by Elsevier on behalf of the American Academy of Ophthalmology*

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Age-related macular degeneration (AMD) is a multifactorial neurodegenerative disease that is the most common cause of incurable blindness worldwide.[1] The population prevalence of AMD increases to 13% in individuals older than 85 years of age,[2] and thus poses an increasing burden to the healthcare system.[3] The strongest nongenetic risk factors include advanced age and smoking status.[4] Early AMD is present when medium-sized drusen (≥63 μm and <125 μm) are detected in the retina. The presence of large drusen (≥125 μm) indicates intermediate AMD. This may be accompanied by retinal pigment epithelial changes in the retina.[5] Drusen are focal extracellular accumulations of debris between the retinal pigment epithelium (RPE) and Bruch's membrane. The composition of drusen is similar to atherosclerotic plaques, consisting of apolipoproteins, cholesterol, amyloid, and crystallins.[6,7] AMD progresses to 2 late forms, including geographic atrophy (GA), which is characterized by loss of photoreceptors and RPE, resulting in a large area of depigmentation and visible choroidal vessels, and choroidal neovascularization (CNV), which is characterized by aberrant vessel growth and leakage of fluid into the retina, leading to RPE or sensory

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retinal detachment, subretinal fibrosis, and atrophy.[8] The Age-Related Eye Disease Study (AREDS) AMD scale is often used to classify disease and risk of progression.[9] Clinical heterogeneity in AMD lends itself to the study of sub-phenotypes that mark progression through various stages of disease.

The first complex disease Genome-Wide Association Study (GWAS) success story was the discovery of an association between the Y402H polymorphism in Complement Factor H (*CFH*) and AMD,[10] followed by additional strong association with many noncoding variants in *CFH*.[11] The other major susceptibility gene is *ARMS2*.[12,13] Presence of both *CFH* and *ARMS2* homozygous risk alleles confers a 50-fold increased risk of AMD.[14] The most recent large GWAS study of 16 144 cases and 17 832 controls identified 52 common and rare variants distributed across 34 loci that are associated with AMD.[15] The genes at these loci belong to a variety of biological pathways, including complement, lipid transport, and extracellular matrix (ECM), many of which have been implicated in AMD pathogenesis.[8] The complement pathway is one of the most well-defined pathways involved in AMD, and several of the identified genetic variants are related to the complement pathway. Disruptions in the complement and other pathways are predicted to result in clinical variability observed in patients with AMD. Notably, *CFH* risk variants are reported to increase the risk of GA, whereas *ARMS2* variants enhance the risk of CNV.[16]

Despite the progress in AMD genetics, variants at risk loci explain only approximately 50% of the disease heritability, and most of the common variants (except at *CFH* and *ARMS2* loci) identified through GWAS exert small effect size on their own. Moreover, for many of the different GWAS loci, we are not yet certain which gene is affected because of the linkage disequilibrium region surrounding the variant. One way to investigate which gene is involved is by identifying rare variants (allele frequency $<1\%$) within candidate genes. They will usually have a stronger predisposition for disease than common variants and may imply a functional role of that gene in AMD pathogenesis. These rare variants may also provide further insight into the role of the affected gene in AMD by showing phenotype correlations. Previous studies have identified rare variants in various complement genes and have shown associations with drusen load and GA.[17–23] However, so far, only few rare variants in genes unrelated to the complement pathway have been implicated in AMD.[24] Possibly, this is due to a smaller effect size of the rare variants in other genes compared with variants in complement genes. A step beyond looking at rare variants is investigating rare predicted loss-of-function (pLoF) variants. As the name implies, these variants are usually rare (allele frequency $<0.1\%$) and are predicted to substantially affect the gene function. Therefore, they are expected to have even larger effect sizes. Whole genome sequencing (WGS) allows for the identification of novel rare variants that might have larger effect sizes, and evaluation of AMD-associated regions previously identified by GWAS is a rational approach to identify potential causal genes at different loci because it increases our chances of capturing relevant genes.[25] We report the identification of rare pLoF variants, their association broadly with AMD, and more specifically with AMD sub-phenotypes. Because pLoF variants may be rare and many are present in no more than a single person in the sequenced population, they usually cannot be analyzed individually. Therefore, we collapsed rare variants into 7 biological pathways relevant to disease pathology based on the gene function and examined the increase in risk of developing AMD and its distinct sub-phenotypes. Our studies demonstrate enhanced risk of the development of calcified drusen, GA, and CNV with distinct biological pathways and suggest novel opportunities for diagnosis and treatment of AMD.

Methods

Ethics Statement

The study followed the tenets of the Declaration of Helsinki and complied with the Health Portability and Accountability Act. This study was approved by the local institutional review boards and the local ethics committees at the participating study centers. Written informed consent was obtained from each participant after explanation of the nature and possible consequences of the study.

Population

Age-Related Eye Disease Study (1992–2005). The AREDS, a multicenter, randomized clinical trial of oral supplements of antioxidant vitamins (C, E, β-carotene) and minerals (zinc and copper) for the treatment of AMD and cataract, was also designed to assess the clinical course, prognosis, and risk factors associated with AMD and cataract. Participants (n = 4757) aged 55 to 80 years were described previously.[26] The participants were enrolled on the basis of their baseline AMD severity: from no evidence of AMD to advanced AMD in 1 eye. Participants with WGS and with intermediate AMD or advanced AMD in 1 eye were included as cases (n = 381), whereas participants with no evidence of AMD served as part of our control group (n = 199). Participants were followed every 6 months for at least 7 years. Extensive phenotypic information was gathered, including annual stereoscopic fundus photographs of the macula that were graded by certified and masked graders at a central photograph reading center for AMD severity.

Age-Related Eye Disease Study 2 (2006–2012). The AREDS2 was a multicenter, phase III, randomized, controlled clinical trial enrolling 4203 individuals aged 50 to 85 years with bilateral large drusen or late AMD in 1 eye as described previously.[27] It was designed to evaluate the safety and efficacy of adding supplementation with lutein plus zeaxanthin and omega-3 long-chain polyunsaturated fatty acids to AREDS supplements, as well as changes in the original AREDS supplements (elimination of beta-carotene and reducing the zinc dose) in reducing the risk of developing advanced AMD. Participants were followed for an average of 5 years. Follow-up study visits were scheduled annually and included standardized stereoscopic fundus photographs that were assessed by masked graders at the same reading center. This population is different from AREDS because it only includes individuals at high risk of progression to late disease. For