Supplementary Material

Methods

Similar analyses to those described for the Age-Related Eye Disease Study 2 (AREDS2) in the main section of the manuscript were performed for the AREDS dataset.

Study procedures

The AREDS design has been described previously.[1] In brief, 4,757 participants aged 55 to 80 years were recruited between 1992 and 1998 at 11 retinal specialty clinics in the United States. The participants were randomly assigned to receive placebo, antioxidants, zinc, or the combination. The randomized clinical trial lasted five years and was followed by epidemiologic follow-up for another five years. As in the AREDS2, at baseline and annual visits, eye examinations were performed, and stereoscopic color fundus photographs were captured and graded centrally at the Wisconsin Reading Center. Institutional review board approval was obtained at each site and written informed consent was obtained from all participants. The research was conducted under the tenets of the Declaration of Helsinki.

The definitions of geographic atrophy (GA) and methods to measure GA area and other characteristics on color fundus photographs have been described previously.[2,3] In the AREDS, the minimum size requirement to define GA was grading circle I-1 (1/8 disc diameter or 217 µm), i.e., smaller than in the AREDS2, where the minimum size requirement was grading circle I-2 (1/4 disc diameter or 433 µm). Planimetry tools were used to demarcate the area of GA within the AREDS grid.[3] GA proximity to the central macula was documented in microns.[3]

GA data were available on a large subset of AREDS participants with GA. For most of these eyes, GA area measurements have been described previously[4], including a subset described in AREDS Report 26[3]. The eyes comprised both those with prevalent and incident GA, but excluded those with co-existing neovascular AMD. GA area measurements were not always available at baseline/first GA appearance, so the current analyses considered GA area and the other characteristics (including reticular pseudodrusen (RPD) status) at the first timepoint when GA area was available. Unlike the AREDS2 analyses, data on GA configuration were not available for most eyes, so this variable was excluded from the analyses.

AREDS deep learning algorithm grading of color fundus photographs for reticular pseudodrusen

Reading center grading for RPD presence was not available in the AREDS, owing to the absence of imaging other than color fundus photography (CFP). Grades for RPD presence were therefore obtained by deep learning-based automated grading of the CFP. The algorithm and its performance metrics have been described previously.[5] In brief, a deep learning algorithm was trained by exposing it to over 8000 AREDS2 CFP-fundus autofluorescence (FAF) image pairs from the AREDS2 ancillary FAF study. The ground truth labels for RPD presence/absence in each image pair came from grading of the FAF images (i.e., label transfer). Multimodal multitask training was used, whereby the algorithm first underwent joint training with two other deep learning algorithms (an FAF algorithm and a CFP-FAF algorithm), using a representation shared between the three algorithms. This was followed by additional training separately from the two other algorithms, i.e., fine-tuning training suitable for grading from CFP alone. The benefits of multimodal multitask training are that what is learned by each algorithm from each image modality can improve the training of the other algorithms (by sharing features that are complementary between the image modalities), i.e., the grading from CFP alone benefits from paired FAF images having been present during training. In previous evaluation of the deep learning algorithm on an AREDS2 test set of CFP images, it achieved an area under the receiver operating characteristic

(AUROC) of 0.832; in external validation using an independent test set (Rotterdam Study, Netherlands), it achieved an AUROC of 0.965.[5]

Genetic data

As part of the AREDS, 2889 participants consented to genotype analysis. SNPs were analyzed using a custom Illumina HumanCoreExome array, as described previously.[6]

Study populations and statistical methods

Similar inclusion criteria for the study populations and similar statistical methods were used in the AREDS analyses as those described for the AREDS2 analyses. The study population for analyses of GA enlargement according to RPD status comprised all eyes that had GA present at two or more study visits (without previous or simultaneous neovascular AMD). The study population for analyses of GA enlargement according to both RPD and *ARMS2* genotype comprised the subset that also had genetic data available.

Analyses of GA enlargement were performed using similar methods to those described in the main section of the manuscript. In all cases, the unit of analysis was the eye. Mixed-model repeated-measures regression was performed with square root of GA area as the outcome measure. The square root transformation was used. The models included the variable of interest (i.e., RPD status or *ARMS2* genotype), years from first GA area measurement, and their interaction term. They were adjusted for the same variables as in the AREDS2 analyses, with the exception of GA configuration, i.e., age, sex, smoking status, education, and GA characteristics at first GA area measurement (specifically central involvement and square root of area). RPD status was considered at the first timepoint with GA area measurements available. To account for the correlation between both eyes of the same person and between different visits of the same eye, an unstructured and a first-order autoregressive covariance structure (UN@AR(1)), respectively, was specified.

Mediation analysis

Mediation analysis was performed on the AREDS dataset using the same methods as those described previously for the AREDS2 dataset.

Analyses of geographic atrophy progression to central involvement

Again, similar methods were used as those described previously for the AREDS2 dataset. The study population for analyses of GA progression to central involvement comprised all eyes that had both (i) incident non-central GA (without previous or simultaneous neovascular AMD), with the GA area data available at first appearance, and (ii) at least one subsequent study visit with GA. The unit of analysis was the eye. Kaplan-Meier analyses were performed for the outcome of progression to central involvement. Multivariable repeated-measures proportional hazards regression analyses were performed for the same outcome, according to RPD status (defined at the first timepoint with GA area measurements available). The models were adjusted for the same variables as in the AREDS2 analyses, with the exception of GA configuration, i.e., age, sex, smoking status, education level, and GA characteristics at incidence (specifically square root of area and proximity to central macula). Adjustment for correlation between both eyes of the same person was made by using the robust sandwich estimate for the covariance matrix in the Wald tests.[7] In addition, in the same study population, mixed-model repeated-measures regression analyses were performed for the outcome of GA proximity to the central macula, considered during follow-up, with adjustment for the same variables as those for progression to central involvement.

Results

Geographic atrophy enlargement rate according to reticular pseudodrusen status

The study population for these analyses comprised 578 eyes of 456 participants (Table 1).

Table 1. Demographic, clinical, and genetic characteristics of the study populations.

	Combined cohort (prevalent and incident GA)	Combined cohort with genetic data available
Participants	456 (578 eyes)	287 (362 eyes)
Age (years), mean (SD)	70.7 (5.4)	70.1 (5.1)
Female	250 (54.8%)	161 (56.1%)
Smoking status		
Never	175 (38.4%)	118 (41.1%)
Former	237 (52.0%)	146 (50.9%)
Current	44 (9.6%)	23 (8.0%)
Education level		
High school or less	187 (41.0%)	114 (39.7%)
At least some college	139 (30.5%)	83 (28.9%)
Postgraduate	130 (28.5%)	90 (31.4%)
Follow-up (years), mean (SD)*	4.4 (2.7)	4.8 (2.8)
Genetics data available	287 (62.9%)	-
rs10490924 *ARMS2*		
Unavailable	169	-
GG	85 (29.6%)	85 (29.6%)
GT	137 (47.7%)	137 (47.7%)
TT	65 (22.6%)	65 (22.6%)
Eyes	578	362
Cohort		
Prevalent	99 (17.1%)	44 (12.2%)
Incident	479 (82.9%)	318 (87.8%)
RPD present†	229 (39.6%)	151 (41.7%)
Central/noncentral GA		
Noncentral	401 (69.4%)	249 (68.8%)
Central	177 (30.6%)	113 (31.2%)
Configuration		
Unknown	168	94
Small (single patch <1DA)	223 (54.4%)	149 (55.6%)
Multifocal	89 (21.7%)	56 (20.9%)
Horseshoe or ring	16 (3.9%)	12 (4.5%)
Solid	75 (18.3%)	49 (18.3%)
Indeterminate	7 (1.7%)	2 (0.7%)
Fellow eye with GA		
Unknown	1	1
No	321 (55.6%)	195 (54.0%)
Yes	256 (44.4%)	166 (46.0%)
GA area (mm^2), mean (SD)	3.4 (5.2%)	3.2 (4.7%)
Proximity to fovea (µm), mean (SD)	285.5 (383.6)	283.2 (391.0)

Abbreviations: DA=disc areas; GA=geographic atrophy; RPD=reticular pseudodrusen; SD=standard deviation

* follow-up from baseline (for prevalent GA) or from first GA appearance (for incident GA)

† defined at baseline (for prevalent GA) or at/any time prior to first GA appearance (for incident GA)

For the outcome of square root of GA area, a statistically significant interaction was observed between RPD status and years (P < 0.0001). GA enlargement was significantly faster in eyes with RPD present (Table 2). The enlargement rate was 0.257 mm/year (95% CI 0.243-0.271) in eyes with RPD absence and 0.353 mm/year (95% CI 0.334-0.371) in eyes with RPD presence.

Table 2. Geographic atrophy enlargement rates according to reticular pseudodrusen status.

RPD status*	Estimate† (mm/year)	95% CI (mm/year)	P‡
RPD absence	0.257	0.243-0.271	<0.0001
RPD presence	0.353	0.334-0.371	

Abbreviations: CI=confidence interval; RPD=reticular pseudodrusen
* RPD status defined at the same timepoint as the first geographic atrophy (GA) area measurement available (i.e., at or near study baseline for eyes with prevalent GA and at or near first GA appearance for eyes with incident GA)
† Mixed-model, repeated-measures regression with the square root of GA area as the dependent variable
‡ P value for interaction between RPD status and years

Geographic atrophy enlargement rate according to ARMS2 genotype

For the outcome of square root of GA area, a statistically significant interaction was observed between *ARMS2* genotype and years (P < 0.0001). GA enlargement was significantly faster in individuals with at least one versus no risk alleles at *ARMS2* (Table 3).

Table 3. Geographic atrophy enlargement rates according to *ARMS2* genotype.

ARMS2 risk alleles	Estimate* (mm/year)	95% CI (mm/year)	P†
0 (GG)	0.237	0.212-0.263	<0.0001
1 (GT)	0.320	0.300-0.340	
2 (TT)	0.308	0.281-0.335	

Abbreviations: CI=confidence interval
* Mixed-model, repeated-measures regression with the square root of geographic atrophy area as the dependent variable
† P value for interaction between *ARMS2* risk alleles and years

Geographic atrophy enlargement according to reticular pseudodrusen status and ARMS2 genotype considered simultaneously

The study population for these analyses comprised 362 eyes of 287 participants (Table 1). For the outcome of square root of GA area, in a model including both interaction terms (i.e., one between RPD status and year and one between *ARMS2* genotype and year), both were statistically significant (P < 0.0001, each). In this model, the enlargement rate remained significantly faster in eyes with RPD present

(i.e., while adjusting for *ARMS2* genotype), and was also significantly faster in eyes of individuals with *ARMS2* risk alleles (i.e., while adjusting for RPD status). The estimates and confidence intervals are shown in Table 4.

Table 4. Geographic atrophy enlargement rates according to reticular pseudodrusen status and *ARMS2* genotype, considered simultaneously.

RPD status or *ARMS2* risk alleles*	Estimate† (mm/year)	95% CI (mm/year)	P‡
RPD absence	0.257	0.240-0.274	<0.0001
RPD presence	0.345	0.323-0.367	
0 (GG)	0.253	0.227-0.278	<0.0001
1 (GT)	0.333	0.313-0.353	
2 (TT)	0.318	0.291-0.345	

Abbreviations: CI=confidence interval; RPD=reticular pseudodrusen
* RPD status defined at the same timepoint as the first geographic atrophy (GA) area measurement available (i.e., at or near study baseline for eyes with prevalent GA and at or near first GA appearance for eyes with incident GA)
† Mixed-model, repeated-measures regression with the square root of GA area as the dependent variable
‡ P value for interaction between characteristic (RPD status or *ARMS2* risk alleles) and years

Subsequent analyses were performed that considered eyes in several groups, based on RPD status and *ARMS2* genotype simultaneously. The GA enlargement rate was lowest in those with RPD absence and no *ARMS2* risk alleles, intermediate in those with RPD absence and *ARMS2* risk alleles, and highest in those with RPD presence (particularly in those with *ARMS2* risk alleles) (Table 5).

Table 5. Geographic atrophy enlargement rates according to reticular pseudodrusen status and *ARMS2* genotype, considered simultaneously in groups.

RPD status and *ARMS2* risk alleles*	Estimate† (mm/year)	95% CI (mm/year)	P‡
RPD absence / 0 (GG)	0.204	0.174-0.234	<0.0001
RPD absence / 1-2 (GT+TT)	0.286	0.266-0.306	
RPD presence / 0 (GG)	0.307	0.266-0.349	
RPD presence / 1-2 (GT+TT)	0.367	0.342-0.393	

Abbreviations: CI=confidence interval; RPD=reticular pseudodrusen
* RPD status defined at the same timepoint as the first geographic atrophy (GA) area measurement available (i.e., at or near study baseline for eyes with prevalent GA and at or near first GA appearance for eyes with incident GA)
† Mixed-model, repeated-measures regression with the square root of GA area as the dependent variable
‡ P value for interaction between characteristic (RPD status/*ARMS2* risk alleles, in 4 levels) and years

Mediation analysis

The study population for these analyses comprised the same 362 eyes of 287 participants as in the previous analyses. The direct effect of the *ARMS2* genotype (1-2 risk alleles, versus none) on the GA enlargement rate was not significantly different from 0 (P = 0.078), though the P-value was close to nominal significance, with an estimate of 0.051 mm/year (95% CI -0.005-0.108; Table 6). The indirect effect of the *ARMS2* genotype (i.e., via the potential mediator of RPD status) on the GA enlargement rate was not significantly different from 0 (P = 0.21), with an estimate of 0.009 mm/year (95% CI -0.005-0.023).

Table 6. Results of mediation analysis for the outcome of geographic atrophy enlargement rate, with *ARMS2* genotype as exposure and reticular pseudodrusen status as potential mediator.

	Estimate† (mm/year)	95% CI (mm/year)	P
Natural direct effect of *ARMS2* genotype on enlargement rate*	0.051	-0.006 to 0.108	0.078
Natural indirect effect of *ARMS2* genotype on enlargement rate (i.e., mediated by RPD status)*	0.009	-0.005 to 0.023	0.21
Marginal total effect of *ARMS2* genotype on enlargement rate (sum of direct and indirect effects)*	0.060	-0.005 to 0.125	0.069

Abbreviations: CI=confidence interval; RPD=reticular pseudodrusen

* RPD status defined at the same timepoint as the first geographic atrophy (GA) area measurement available (i.e., at or near study baseline for eyes with prevalent GA and at or near first GA appearance for eyes with incident GA); *ARMS2* genotype considered in two levels (0 risk alleles/GG as reference and 1-2 risk alleles/GT+TT as other level)

† slope of change in square root of GA area over time (mm/year), based on a single value per eye

Progression from non-central geographic atrophy to central involvement according to reticular pseudodrusen status

The study population for these analyses comprised 279 eyes (of 244 participants) with incident non-central GA, considered at first GA appearance. Over mean follow-up time of 4.0 years (SD 2.4), the proportion of eyes that progressed to central involvement was 136 (48.7%). In proportional hazards regression analyses for the outcome of progression to central involvement, eyes with RPD present were significantly more likely to progress to central involvement (P = 0.023). The hazard ratio associated with RPD presence was 1.54 (95% CI 1.06-2.24). In regression analyses for the outcome of GA proximity to the central macula, the rate of change in proximity was significantly faster in eyes with RPD present (P < 0.0001). The estimates for change in proximity per year were 69 µm (95% CI 60-79) and 39 µm (95% CI 31-47) in eyes with RPD present and absent, respectively, i.e., faster progression towards the central macula in eyes with RPD (Table 7).

Table 7. Rate of change of geographic atrophy proximity to central macula per year, according to reticular pseudodrusen status, in eyes with incident non-central geographic atrophy.

RPD status*	Estimate† (µm/year)	95% CI (µm/year)	P‡
RPD absence	39	31-47	<0.0001
RPD presence	69	60-79	

Abbreviations: CI=confidence interval; RPD=reticular pseudodrusen

* RPD status defined at first geographic atrophy (GA) appearance

† Mixed-model, repeated-measures regression with proximity to central macula as the dependent variable

‡ P value for interaction between RPD status and years

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