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Heritability of Choroidal Thickness in the Amish

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Purpose: To evaluate the heritability of choroidal thickness and its relationship to age-related macular degeneration (AMD).

Design: Cohort study.

Participants: Six hundred eighty-nine individuals from Amish families with early or intermediate AMD.

Methods: Ocular coherence tomography was used to quantify choroidal thickness, and fundus photography was used to classify eyes into categories using a modified Clinical Age-Related Maculopathy Staging (CARMS) system. Repeatability and heritability of choroidal thickness and its phenotypic and genetic correlations with the AMD phenotype (CARMS category) were estimated using a generalized linear mixed model (GLMM) approach that accounted for relatedness, repeated measures (left and right eyes), and the effects of age, gender, and refraction.

Main Outcome Measures: Heritability of choroidal thickness and its phenotypic and genetic correlation with the AMD phenotype (CARMS category).

Results: Phenotypic correlation between choroidal thickness and CARMS category was moderate (Spearman's rank correlation, $r_s = -0.24$; n = 1313 eyes) and significant (GLMM posterior mean, −4.27; 95% credible interval [CI], −7.88 to −0.79; $P = 0.02$) after controlling for relatedness, age, gender, and refraction. Eyes with advanced AMD had thinner choroids than eyes without AMD (posterior mean, −73.8; 95% CI, −94.7 to −54.6; $P < 0.001$; n = 1178 eyes). Choroidal thickness was highly repeatable within individuals (repeatability, 0.78; 95% CI, 0.68 to 0.89) and moderately heritable (heritability, 0.40; 95% CI, 0.14 to 0.51), but did not show significant genetic correlation with CARMS category, although the effect size was moderate (genetic correlation, −0.18; 95% CI, −0.49 to 0.16). Choroidal thickness also varied with age, gender, and refraction. The CARMS category showed moderate heritability (heritability, 0.49; 95% CI, 0.26 to 0.72).

Conclusions: We quantify the heritability of choroidal thickness for the first time, highlighting a heritable, quantitative trait that is measurable in all individuals regardless of AMD affection status, and moderately phenotypically correlated with AMD severity. Choroidal thickness therefore may capture variation not captured by the CARMS system. However, because the genetic correlation between choroidal thickness and AMD severity was not significant in our data set, genes associated with the 2 traits may not overlap substantially. Future studies should therefore test for genetic variation associated with choroidal thickness to determine the overlap in genetic basis with AMD. *Ophthalmology 2016;123:2537-2544 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).*

Age-related macular degeneration (AMD) is a major cause of blindness in older adults.[1] Both demographic and environmental factors, including advanced age, gender, smoking history, and diet, contribute to the risk of AMD developing.[2–4] Intermediate and advanced AMD are also heritable (heritability [the proportion of phenotypic variation that is explained by genetic differences], 0.44−0.71[5,6]), with several common and rare genetic risk factors.[4,7] Although identified genetic variants explain a relatively large proportion (40%−60%) of the heritability of advanced disease, a substantial portion remains unexplained.[6,7] Progression of AMD also is poorly understood and highly variable.[8] In addition to unidentified rare variants or interaction effects, residual variation in disease risk, heritability, and progression may be partly a reflection of the currently used classification for AMD.

Despite the complexity of the AMD phenotype, eyes usually are classified into discrete categories using the Age-Related Eye Disease Study (AREDS)[9,10] or simplified Clinical Age-Related Maculopathy Staging (CARMS) classification systems,[11] which are based largely on the presence and size of key hallmarks of AMD, such as drusen or retinal pigmentation. Furthermore, most studies of genetic association compare individuals with no or few signs of AMD (controls or CARMS categories 1 and 2) with those with late-stage disease (CARMS categories 4 and 5),

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whereas only a few studies have considered the genetics of early or intermediate AMD or specific AMD subtypes.[6,12,13] Such broad-scale classification of disease stages may not adequately represent the biological basis of the disease and may mask important subphenotypes that are linked more directly to the underlying disease process. Features found in AMD cases also may overlap with other retinal diseases that have a distinct genetic basis, confounding our ability to predict disease risk. We hypothesized that parsing the complex AMD phenotype into heritable finer-scale retinal traits that are easily measurable in all individuals and that each have a relatively simple genetic basis (endophenotypes[14]) will increase our understanding of the biological basis of AMD, enabling better prediction of disease risk and progression, and aiding the discovery of novel drug targets.[15,16] For example, an endophenotype approach was used recently to identify ocular traits and genes associated with glaucoma[15,17] and myopia.[18]

Because of recent technological advances, spectral-domain (SD) ocular coherence tomography (OCT) now allows detailed cross-sectional imaging of the retina's ultrastructure, offering enhanced detection, measurement, and analysis of retinal traits beyond those offered by traditional fundus photography.[19] Therefore, SD OCT may aid the identification of AMD endophenotypes or biomarkers that can be used to predict risk or progression to advanced stages.[20,21] Traits such as choroidal thickness,[22,23] drusen volume,[20] and the presence of reticular pseudodrusen[20,24] have been linked previously to AMD disease status and progression and may define AMD endophenotypes. For example, choroidal thickness was found to decrease with increasing AMD severity (AREDS categories 1–4).[23] However, most studies have measured only the overall phenotypic correlation between retinal traits and AMD, but phenotypic correlation may result from genetic correction (overlapping genes), environmental correlation, or both. If environmental factors drive the correlation between retinal traits and AMD, rather than the same genes, then performing genetic association analyses on these fine-scale retinal traits may not be informative for AMD. Therefore, the relationship between retinal features, AMD risk and progression, and genetics is unclear and requires further investigation. Specifically, for a trait to be useful as an AMD endophenotype requires that the trait is shown to be heritable and genetically correlated, to some extent, with the AMD phenotype, that is, that there is some shared genetic basis between the quantitative trait and the disease.[14,15,25] Such analyses can be performed by measuring the phenotypic similarity and relatedness between family members in a pedigree or twin study because this allows phenotypic variation to be separated into genetic variation, environmental variation, and individual-level variation (repeatability).

To assess the use of choroidal thickness as an AMD endophenotype for future genetic studies, we examined whether the trait is heritable (i.e., whether a significant proportion of the phenotypic variation is explained by genetic variation) and phenotypically and genetically correlated with the AMD phenotype (CARMS category) using families from the Amish Eye Study. The Amish are genetically and culturally isolated, and experience a relatively uniform environment, reducing genetic diversity and variance in disease risk. Additionally, their large extended families provide a powerful tool for heritability analyses. The frequency of smoking (a key environmental risk factor for AMD[2]) also is low. The Amish therefore provide an excellent opportunity to examine the genetic architecture of complex disease.

Methods

Study Population and Data Collection

Participants were recruited from Amish populations in Lancaster County, Pennsylvania; Holmes County, Ohio; and Elkhart and LaGrange Counties, Indiana. Informed consent was obtained from all individuals. Institutional review board approval was obtained, and research complied with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki. Individuals and their siblings were recruited from families with at least 2 affected individuals with early or intermediate AMD. Recruited families varied in size from nuclear families of up to 13 siblings to extended families of up to 30 individuals.

At each clinical center (Indiana, Ohio, Pennsylvania) participants underwent a health history and ophthalmologic examination that included color fundus photography and SD OCT volume scans for both eyes where possible. For choroidal thickness assessments, SD OCT imaging was performed with the Spectralis OCT device (Heidelberg Engineering, Inc., Heidelberg, Germany) using a $20^\circ \times 20^\circ$ field of view centered on the fovea with 97 B-scans each comprising 512 A-scans. Images were exported to the Doheny Image Reading Center and the choroidal thickness was measured at the foveal center, from the lower border of the retinal pigment epithelium–Bruch's membrane band to the choroidal–scleral junction, using the caliper tool in the HEYEX (Heidelberg, Germany) software, in accordance with previous reports from the reading center.[26] Eyes were classified by a modified CARMS classification (categories 0–5) at the Doheny Center from color fundus photographs (Table 1). The CARMS system grades eyes from 1 to 5[11] and considers eyes with no drusen and few small drusen as category 1. To achieve a more granular phenotype, for this analysis, eyes with no drusen were assigned to a new category of 0, whereas only those with a few small drusen were included in category 1. Category 2 included eyes with many small drusen or a few medium drusen, and thus included eyes both without AMD and with early AMD (using the convention that medium drusen constitute the minimum criteria for AMD[27]). Category 3 included eyes with intermediate AMD, and categories 4 and 5 included eyes with advanced AMD, as in the CARMS system[11] (Table 1).

Statistical Analysis

To assess the use of choroidal thickness as an AMD endophenotype we quantified (1) its overall phenotypic correlation with the

Table 1. Modified Clinical Age-Related Maculopathy Staging Classification System[11]

Category	Description
0	No drusen
1	<20 Hard drusen
2	>20 Hard drusen or some medium drusen
3	>20 Medium drusen or a single large drusen
4	Foveal geographic atrophy
5	Choroidal neovascularization

Table 2. Demographic Parameters for 689 Participants

Parameter	Pennsylvania	Indiana	Ohio	All
Gender	193 women, 122 men	142 women, 106 men	82 women, 44 men	417 women, 272 men
Age, yrs (mean ± SD)	65.6±10.5	65.5±11.4	70.1±9.9	66.4±10.9

SD = standard deviation.

AMD phenotype, (2) its heritability, and (3) its genetic correlation with the AMD phenotype to assess the extent to which genetic variation underlying the 2 traits overlapped. Our primary analyses treated the AMD phenotype as an ordinal trait, CARMS category (0–5), because this approach was more powerful than dichotomizing the phenotype as a binary trait (presence or absence of AMD). However, in a secondary analysis we reanalyzed data treating the AMD phenotype as a binary trait where possible.

Broad-sense heritability (the proportion of phenotypic variance that is explained by genetic variance) of both choroidal thickness and CARMS category and their correlation were quantified in a generalized linear mixed model (GLMM) framework. A GLMM approach enabled the use of repeated measures (both left and right eyes per subject) and hence an estimate of the proportion of phenotypic variance in each trait that was explained by individual identity (repeatability), the inclusion of covariates such as age, and it maximized the use of relatedness information from a pedigree.[28]

Analyses were run using the R-package MCMCglmm (available at https://cran.r-project.org/web/packages/MCMCglmm/index.html) that fits models in a Bayesian framework using Markov chain Monte Carlo methods.[29] First, a univariate GLMM of choroidal thickness was used to test whether choroidal thickness varied with AMD severity (CARMS category) while controlling for age, gender, spherical equivalent refraction (sphere plus half the cylinder), relatedness, and repeated measures (left and right eyes). A bivariate GLMM with a 2-trait response variable then was fit with CARMS category (0–5) specified as an ordinal (threshold) trait with probit link and choroidal thickness specified as a Gaussian trait to quantify the heritability of each trait and their genetic correlation while controlling for age, gender, and spherical equivalent refraction (as a covariate for choroidal thickness only). A pedigree (participant, mother, father) was used to estimate a genetic variance–covariance matrix, and a random effect of individual identification was fit to account for repeated measures per person (left and right eyes), allowing phenotypic variance to be partitioned into genetic, individual-level, and residual variance. Shared environmental effects between family members were not accounted for and may conflate our estimate of genetic variance, but because AMD is a late-onset disease, it was unclear whether accounting for a shared sibship environment was relevant.[5] Eyes that were missing 1 of the 2 traits (CARMS category or choroidal thickness; n = 61) were included in analyses because bivariate GLMMs can handle missing data in the response variable. In the bivariate model, random effect and residual variances were specified using the us(trait) structure, thereby allowing the variance and covariance to vary between the 2 traits. Default priors were used for fixed effects. Priors on variance components for residual terms were inverse Wishart distributed with variance of 1 and a low degree of belief (nu = 0.002), with variance fixed at 1 for the ordinal trait.[29] For random effects, we used parameter-expanded priors to facilitate mixing with a mean (mu) of 0 and variance (V) of 1000 for choroidal thickness and 100 for CARMS category.[29] Variance estimates were similar when models were run with alternative priors. Models were run for 10 500 000 iterations with a burn-in interval of 500 000 and thin of 2000 to give an effective sample size of approximately 5000 and autocorrelation less than 0.1 between consecutive samples. Model convergence and mixing were assessed by visual inspection of plots and by using the heidel.diag function in the Coda package.[30] All analyses were conducted in R version 3.0.1 (available at: http://www.cran.r-project.org). The heritability of choroidal thickness was quantitatively similar when run in a (restricted) maximum likelihood framework using the R package pedigreemm.

Results

A total of 689 participants (417 women and 272 men) from Indiana (n = 248), Pennsylvania (n = 315), and Ohio (n = 126) were recruited and examined between August 2013 and November 2015 (n = 1378 eyes; Table 2). Mean baseline age of participants was 66.4±10.9 years (range, 33–99 years). Considering the most severely affected eye per individual, approximately 42.1% of participants were CARMS category 0, 27.1% were CARMS category 1, 11.6% were CARMS category 2, 11.5% were CARMS category 3, 5.4% had advanced AMD (CARMS categories 4 or 5), and 2.3% were not graded because of a fundus camera malfunction at 1 site (Table 3). Mean spherical equivalent refraction was 0.55±1.62 diopters for right eyes and 0.56±1.65 diopters for left eyes.

Mean choroidal thickness of 679 right eyes was 253.9±70.6 μm and that of 670 left eyes was 253.5±69.4 μm (Fig 1). Choroidal thickness was strongly correlated between left and right eyes (Pearson's correlation coefficient, $r = 0.82$; n = 660; Fig 2). As expected, CARMS category also was strongly correlated between eyes (Spearman's correlation coefficient, $r_s = 0.84$; n = 665; Fig 2); therefore, eyes tended to be at the

Table 3. Number of Participants by Modified Clinical Age-Related Maculopathy Staging Classification System Category (Most Advanced Eyes)

	Clinical Age-Related Maculopathy Staging Classification System Category							
Population	0	1	2	3	4	5	Not Graded	All
Pennsylvania	131	82	43	36	7	1	15	315
Indiana	114	75	25	21	8	4	1	248
Ohio	45	30	12	22	17	0	0	126
Total	290	187	80	79	32	5	16	689

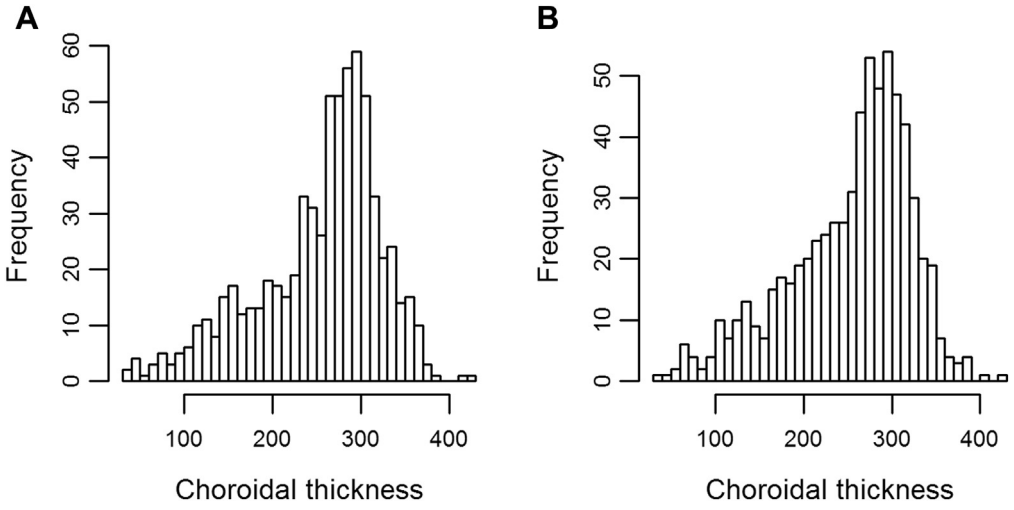

Figure 1. Bar graphs showing the distribution of subfoveal choroidal thickness (in micrometers) for (**A**) right eyes and (**B**) left eyes.

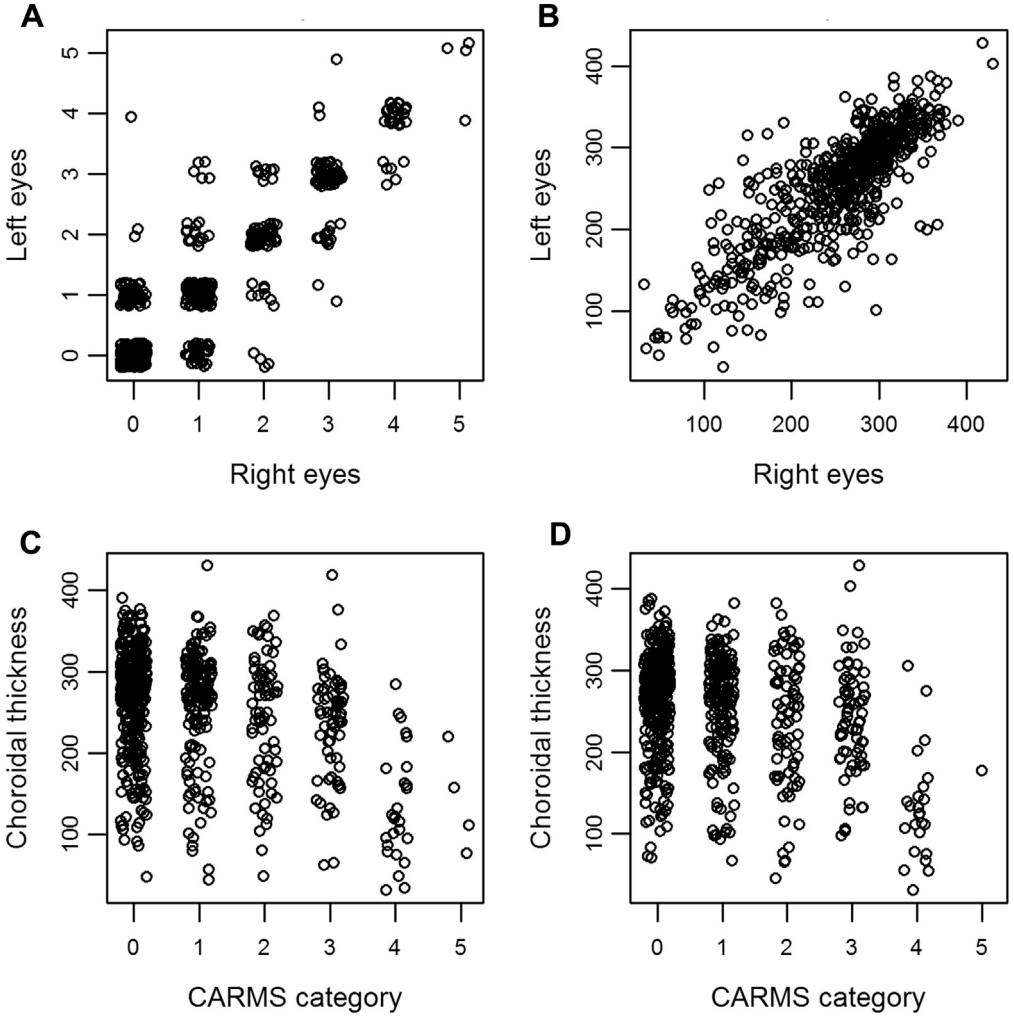

Figure 2. Scatterplots showing the phenotypic correlation of (**A**) Clinical Age-Related Maculopathy Staging (CARMS) category and (**B**) subfoveal choroidal thickness between left and right eyes and the correlation between subfoveal choroidal thickness and CARMS category for (**C**) right eyes and (**D**) left eyes. Points have been jittered for visualization.

Table 4. Posterior Means (and 95% credible intervals) for Variance and Covariance Components Estimated from a Bivariate Generalized Linear Mixed Model of Choroidal Thickness and Modified Clinical Age-Related Maculopathy Staging Classification System Category

Trait	Genetic Variance (V_A)	Permanent Individual Variance (V_{pe})	Residual Variance (V_e)	Heritability (H^2)	Genetic Covariance ($Cov_{1,2}$)	Genetic Correlation (r_A)
Choroidal thickness	1540.85 (850.90–2273.01)	1479.79 (889.49–2031.82)	857.21 (769.19–951.88)	0.40 (0.24–0.56)	−18.43 (−53.02 to 16.48)	−0.18 (−0.49 to 0.16)
CARMS classification system category	7.00 (3.01–11.12)	6.08 (2.77–9.26)	1 (fixed)	0.49 (0.26–0.72)		

CARMS = Clinical Age-Related Maculopathy Staging.
Repeatability ($R = V_A + V_{pe}/(V_A + V_{pe} + V_e)$), heritability ($H^2 = V_A/(V_A + V_{pe} + V_e)$), and genetic correlation between choroidal thickness and CARMS category ($r_A = Cov_{1,2}/\sqrt{(V_{A1} \times V_{A2})}$) are shown, where V_A is genetic variance, V_{pe} is permanent environmental variance, V_e is the residual variance, and Cov_{12} is the genetic covariance between traits.

same disease stage. However, correlation coefficients suggested that there was sufficient variation to justify the inclusion of both eyes in future analyses. Choroidal thickness showed moderate negative phenotypic correlation with CARMS category ($r_s = -0.24$; n = 1313 eyes; Fig 2); eyes more severely affected with AMD had thinner choroids. This correlation was significant in a univariate GLMM of choroidal thickness controlling for relatedness, repeated measures (left and right eyes), age, gender, and refraction (posterior mean, −4.27; 95% credible interval [CI], −7.88 to −0.79; MCMC P value = 0.02). Secondary analyses defining AMD severity as a binary trait showed that choroidal thickness was marginally thinner in eyes with AMD (categories 3, 4, and 5; n = 190 eyes; posterior mean, −10.7; 95% CI, −20.87 to 0.15; MCMC P value = 0.05) compared with eyes with no AMD (CARMS categories 0, 1, and 2; n = 1123 eyes). Interestingly, this difference in choroidal thickness between affected and unaffected individuals was substantially stronger and significant if category 3 (intermediate AMD) eyes were excluded (posterior mean, −73.8; 95% CI, −94.7 to −54.6; MCMC P value < 0.001; n = 1178 eyes); therefore, eyes with advanced AMD had significantly thinner choroids than those without AMD. However, note that the sample size for this secondary analysis was small (n = 37 individuals with at least 1 advanced AMD eye).

A bivariate GLMM estimated that the repeatability (proportion of phenotypic variation that was explained by an individual's identity) of choroidal thickness was high (0.78; 95% CI, 0.75 to 0.81; n = 1378 eyes) and that the heritability of choroidal thickness was moderate (0.40; 95% CI, 0.24 to 0.56; Table 4). The CARMS category also was highly repeatable (0.93; 95% CI, 0.91 to 0.94; Table 4) and moderately heritable (heritability, 0.49 on the liability scale; 95% CI, 0.26 to 0.72), similar to that estimated by a twin study (heritability, 0.46[5]). Choroidal thickness ($r = -0.46$; Markov chain Monte Carlo [MCMC] P value < 0.001; Fig 3) and CARMS category ($r = -0.38$; MCMC P value < 0.001; Fig 3) were significantly negatively correlated with age, but only choroidal thickness varied with gender; men had slightly thinner choroidal thickness (MCMC P value = 0.04; Table 5). Choroidal thickness also was correlated positively with refraction (Table 5). Genetic correlation between choroidal thickness and CARMS category was −0.18, but 95% CIs overlapped 0 (−0.49 to 0.16); therefore, the correlation was not statistically significant (Table 4). The bivariate model also estimated that the overall phenotypic correlation between choroidal thickness and CARMS category was moderate, negative, and significant (−0.14; 95% CI, −0.22 to −0.06). We did not test for the genetic correlation between choroidal thickness and the AMD phenotype as a binary trait because the complexity of a bivariate model and the relatively small number of advanced AMD cases (62 vs. 1137 eyes with no AMD) prevented model convergence despite long run time.

Discussion

Decomposing disease phenotypes into heritable subcomponents may be especially useful for unravelling the complex genetic basis of multifactorial diseases such as AMD. Endophenotypes also may be useful as biomarkers of disease risk or progression, thereby influencing clinical decision making and allowing for intervention to alter disease progression.[16] Genetic studies of heritable, novel AMD phenotypes also may provide additional biological pathways and therapeutic targets for early or intermediate

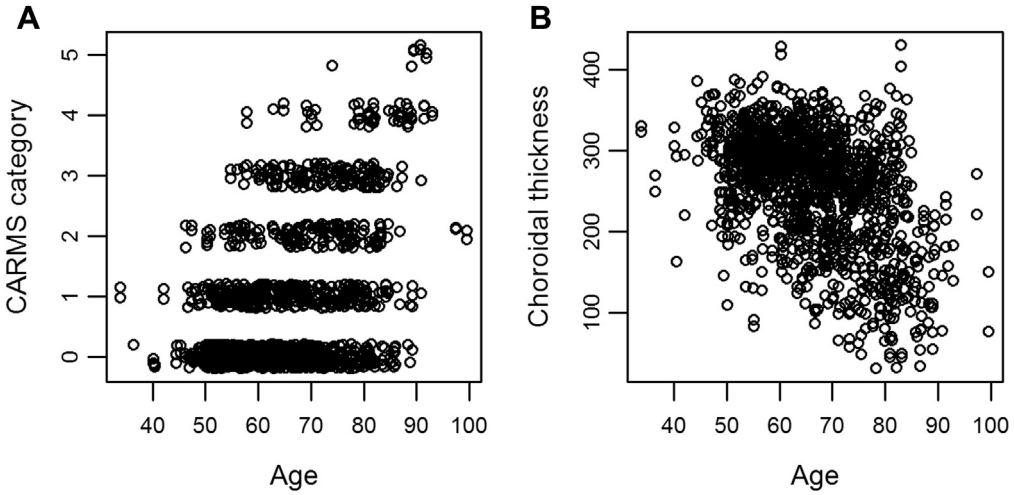

Figure 3. Scatterplots showing the correlation between (**A**) Clinical Age-Related Maculopathy Staging (CARMS) category and age and (**B**) subfoveal choroidal thickness and age. Points are jittered for visualization.

AMD. One retinal trait that is quantifiable in all individuals using OCT imaging, regardless of AMD disease status, is choroidal thickness. The choroid performs many of the retina's essential metabolic functions, and thinning of the choroid previously was associated with age and AMD; older individuals[31–33] and those with AMD[22,34–39] had thinner choroids, although some studies did not find a difference with AMD status.[33,40–42]

Here we show that, after controlling for age, gender, and refraction, choroidal thickness is phenotypically negatively correlated with CARMS category and hence the severity of AMD. Eyes with AMD also had marginally thinner choroids than those without AMD. Phenotypic but not genetic correlation between choroidal thickness and AMD has been tested previously. Several studies found that advanced AMD cases had thinner choroids than controls.[34,38,39] Some studies also found a difference in choroidal thickness between early AMD cases and controls,[35–37] whereas others did not,[22,23,41] and some found that the strength of the correlation depends on the AMD subtype.[39,43] We found a stronger difference between AMD cases and controls when eyes with intermediate AMD were excluded, albeit with a small sample of advanced cases, which suggested that the correlation between choroidal thickness and AMD severity is driven primarily by the considerable decrease in choroidal thickness in advanced AMD cases (Fig 2).

Table 5. Main Effect Parameters (Posterior Mean, 95% Credible Interval, and MCMC *P* Values) from a Bivariate Generalized Linear Mixed Model Investigating Variation in Choroidal Thickness and Modified Clinical Age-Related Maculopathy Staging Category with Respect to Age, Gender, and Refraction

Trait	Posterior Mean	95% Credible Interval	MCMC *P* Value
Choroidal thickness intercept (women)	476.1	446.2 to 504.4	<0.001
CARMS category intercept (women)	−11.3	−13.6 to −9.1	<0.001
Choroidal thickness: age	−3.4	−3.8 to −3.0	<0.001
CARMS category: age	0.17	0.1 to 0.2	<0.001
Choroidal thickness: men	−8.6	−17.1 to −0.5	0.04
CARMS category: men	0	−0.6 to 0.6	0.91
Choroidal thickness: refraction	6.0	3.5 to 8.3	<0.001

CARMS = Clinical Age-Related Maculopathy Staging.

Moreover, we showed for the first time that choroidal thickness is significantly heritable and therefore has a substantial genetic component. Choroidal thickness therefore may define an AMD endophenotype useful for genetic studies. However, for a trait to define an endophenotype, it also should show some (but not perfect) genetic correlation with the disease phenotype. Although the effect size of the genetic correlation between choroidal thickness and CARMS category was moderate (−0.18), suggesting that the phenotypic correlation observed between the 2 traits may reflect some overlap in genetic basis, the genetic correlation was not significantly different from 0. Genetic correlation is the extent to which 2 traits share the same genes, whereas phenotypic correlation also encompasses environmental correlation. It is likely that the absence of significant genetic correlation results from relatively low power to detect correlation with the ordinal trait, CARMS category, because our study was focused primarily on families whose members demonstrated early or intermediate AMD, and therefore our sample consisted of many controls (approximately 70% of individuals were categories 0 or 1) and relatively few individuals with advanced AMD (5%). Any genetic correlation between choroidal thickness and AMD may be more likely to be detected across a sample with a larger proportion of advanced AMD cases or a more variable sample of unrelated individuals using genome-wide trait analysis. Finally, CARMS category is only 1 measure of AMD severity or presence. Indeed, many studies dichotomize AMD scales into

case-control status to study the genetics of advanced AMD risk. Our preliminary analyses of this relatively small sample size suggested that the genetic correlation between these 2 traits was stronger than that for CARMS category. Therefore, the absence of a significant genetic correlation in this data set of patients with early or intermediate disease and their unaffected relatives does not preclude the use of choroidal thickness as an AMD endophenotype.

Because the correlation between choroidal thickness and AMD severity was, at most, moderate, choroidal thickness may capture novel genetic and phenotypic variation and therefore may be especially informative for future studies of AMD. Numerous common and rare variants are associated with advanced AMD,[7] although cumulatively they explain no more than 60% of the heritability of advanced disease, and less for early or intermediate AMD.[6,12] Age-related macular degeneration endophenotypes may be associated with a subset of these known AMD variants, with novel variants, or with both. Cohort studies focusing on families are especially useful for detecting genetic causes of disease, and the Amish Eye Study is such a resource. Finding the genetic causes for choroidal thickness has the potential to uncover novel biology for the progression of early to late AMD and ultimately may lead to better prognostic indicators and treatments to prevent AMD.

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **CARMS** = Clinical Age-Related Maculopathy Staging; **CI** = credible interval; **GLMM** = generalized linear mixed model; **OCT** = ocular coherence tomography; **SD** = spectral-domain.

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