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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