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Et al.

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PURPOSE. The purpose of this study was to determine associations between macular drusen parameters derived from an automatic optical coherence tomography (OCT) algorithm, nonadvanced age-related macular degeneration (AMD) stage, and genetic variants.

METHODS. Eyes classified as early or intermediate AMD with OCT imaging and genetic data were selected ($n = 239$ eyes). Drusen area and volume measurements were estimated using the Zeiss Cirrus advanced retinal pigment epithelium analysis algorithm in a perifoveal zone centered on the fovea. Associations between drusen measurements and common genetic variants in the complement and high-density lipoprotein (HDL) lipid pathways and the *ARMS2/HTRA1* variant were calculated using generalized estimating equations and linear mixed models adjusting for age, sex, smoking, body mass index, and education.

RESULTS. Drusen area ≥ the median was independently associated with a higher number of risk alleles for *CFH* risk score and risk variants in *C3* and *ARMS2/HTRA1* compared with eyes with no measurable drusen. Similar results were obtained for drusen volume. When all genes were analyzed in the same model, only *CFH* score and *ARMS2/HTRA1* were associated with drusen measurements. HDL pathway genes were not significantly related to drusen parameters. Nonadvanced AMD stages were associated with OCT-derived drusen area and volume.

CONCLUSIONS. Variants in *CFH* and *ARMS2/HTRA1*, commonly associated with advanced AMD, were independently associated with an increase in drusen burden determined by OCT in an allele dose dependent manner, in eyes with early and intermediate AMD. Biomarkers such as a quantitative classification of nonadvanced AMD and other OCT-derived subphenotypes could provide earlier anatomic endpoints for clinical trials and facilitate the development of new therapies for AMD.

Keywords: age-related macular degeneration, drusen, genetic diseases

Age-related macular degeneration (AMD) is a leading cause of vision loss and irreversible blindness in adults older than age 60.[1] The etiology of AMD is multifactorial, and both behavioral and genetic risk factors contribute to personal risk.[2,3] The genetic component of AMD risk is particularly well documented with regard to progression to advanced disease, including transitions to both advanced subtypes: geographic atrophy (GA) and neovascular disease (NV).[4–7] Each advanced subtype is generally preceded by early and intermediate stages of disease that are primarily characterized by the formation of drusen between the retinal pigment epithelium (RPE) and Bruch's membrane. The mechanisms by which an individual might develop GA or NV are not fully understood; however, it is clear that drusen development is predictive of progression to both forms of advanced disease.[8,9] Management of the growing burden of advanced AMD remains a significant challenge. Therefore, identification of therapeutic targets for earlier, high-risk stages of the disease is needed to lower the rate of progression to advanced stages and to preserve vision.

With advances involving in vivo imaging, spectral domain–optical coherence tomography (SD-OCT) devices are capable of noninvasive visualization and discrimination of the retinal and choroidal layers. Dysfunction of the RPE, including area and volume abnormalities at locations where drusen develop, can be directly visualized and measured using OCT. Prior studies have indicated that OCT-derived drusen area and volume and retinal features such as hyper-reflective foci and choroidal parameters are informative in determining the likelihood of progression from early or intermediate to advanced AMD.[10–15]

However, knowledge about the association between OCT derived retina parameters and genetic variants related to AMD is sparse.[16–18] We previously reported the effects of genes on progression to different stages of AMD.[19] In that study, genes in the complement pathway were associated with a higher risk of progression from small and intermediate to large size drusen and from large size drusen to GA and NV. A novel association was also found between the *ABCA1* gene in the HDL pathway

that reduced the risk of progression from normal to intermediate drusen and from intermediate to large drusen. Protective effects for transitions to advanced disease and larger drusen size were also observed in other pathways.[20,21] These assessments provide a framework to evaluate the role of genetics as they relate to drusen measurements based on OCT.

We evaluated the association between genetic risk and OCT derived drusen area and volume in a clinical cohort of patients with early and intermediate AMD. Understanding these relationships, in addition to other OCT parameters,[10-15,22] may lead to clinical use of these measurements with identification of earlier high-risk phenotypes and better stratification of risk of progression. Further characterization of drusen morphology on OCT may lead to the identification of disease biomarkers that could serve as anatomic end points for clinical trials. Our study aimed to evaluate the associations between a subset of genes implicated in risk of advanced AMD, and drusen area and volume measurements based on OCT in eyes with clinically diagnosed early and intermediate AMD. Preliminary results were presented at AAO in 2017[23] and at ARVO in 2019 (Widjajahakim et al. *IOVS* 2019;60:ARVO E-Abstract 1164).

METHODS

Study Population and Classification of AMD Phenotypes

All participants were previously enrolled in our ongoing genetic and epidemiologic studies of AMD beginning in 1985.[24-27] Participants were derived from clinic populations and nationwide referrals and were prospectively followed. The study protocol includes an ocular examination, fundus photography, and OCT imaging, as well as interviews and blood sampling. This research adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board. Written informed consent was obtained for all participants.

AMD phenotypes were based on ocular examination and ocular imaging. Eyes were classified using the Clinical Age-Related Maculopathy Staging (CARMS) system by JMS as previously described.[28] CARMS grades were defined as follows: grade 1 (no AMD, no drusen or only a few small drusen <63 μm); grade 2 (early AMD, RPE irregularities and/or intermediate size drusen 63–124 μm); grade 3 (intermediate AMD, large drusen ≥125 μm); grade 4 (advanced dry AMD, or GA, including both central and noncentral GA); and grade 5 (advanced exudative AMD with choroidal neovascularization).

Risk factors were determined using our standardized questionnaire including demographic information (age, sex, education), anthropomorphic data (height and weight converted to body mass index [BMI]), and smoking.[2,29–31]

Evaluation of Drusen Area and Volume Measurements

Beginning in 2007, OCT scans were included in our study protocols for the purpose of conducting studies to assess OCT parameters associated with various stages of AMD, AMD progression, and genetic factors. In 2016, a retrospective review of Cirrus SD-OCT 4000 and 5000 scans (Carl Zeiss Meditec, Inc., Dublin, CA, USA) was conducted among individuals with one or both eyes with stage 2 or 3. The OCT scanning protocols included high-definition 1-line scan, 5-line scan, and macular cube (512 × 128:128 B-scans and 512 A-scans per B scan) over a 6- × 6-mm square, centered on the fovea.[32] The position of the fovea was determined by the OCT technician to match the known anatomic configuration of the

fovea. Drusen area and volume measurements were determined by the Cirrus Version 6.0 Advanced RPE Analysis algorithm, a fully automated algorithm available using the Cirrus SD-OCT instrument. This algorithm is based on an automatic determination of the RPE floor and measurement of the elevations of the RPE deformations. This yields an automatic quantitative assessment of RPE deformations and calculates measurements of area and volume that are highly reproducible.[33-35] These measurements provide an estimate of drusen burden for a circle with 5 mm in diameter (perifoveal zone) centered on the fovea.[32]

Only eyes with early or intermediate AMD (CARMS grades 2 or 3) at the time of the OCT scan were selected for inclusion in the analyses. We selected participants with at least one OCT macular cube scan in at least one eye and with DNA genotyping data. Scans with signal strength less than 6 of 10 were excluded, similar to what has been previously reported.[14,36] For individuals with multiple scans, the earliest, highest-quality scan was evaluated.

Genotyping and Genetic Data

Enrolled participants provided blood or saliva samples for DNA extraction according to a standard study protocol. Genotypes were determined using array-based and gene sequencing platforms as previously described.[24,25,37,38] All single nucleotide polymorphisms (SNPs) had a high genotype call rate (>98%), and PLINK was used to perform all quality control steps.[39]

Common variants in genes previously associated with drusen and AMD in the complement pathway, HDL pathway, and the gene locus on chromosome 10q26 were selected given their consistent association with advanced disease or biologically plausible relationship to drusen formation. The genetic variants included complement factor H (*CFH*) Y402H rs1061170,[40] *CFH* rs1410996,[37,41] age-related maculopathy susceptibility 2/high-temperature requirement A serine peptidase 1 locus on chromosome 10q26 (*ARMS2* A69S/*HTRA1*) represented by SNP rs10490924,[42,43] complement component 3 (*C3*) R102G rs2230199,[44] and variants in the HDL pathway: hepatic lipase C (*LIPC*) rs10468017,[45] adenosine tri-phosphate binding cassette transporter 1 (*ABCA1*) rs1883025, and cholesteryl ester transfer protein (*CETP*) rs3764261.[19,20,45,46]

Statistical Methods

A total of 239 eyes among 179 participants were included in these analyses. The distributions of demographic (age [<70, 70–79, ≥80], sex, education [≤high school, >high school]), behavioral (smoking and BMI), ocular (baseline AMD grade), and genetic factors were evaluated for each area and volume measurement. Categorical comparisons were made between eyes with some drusen but <median versus eyes with no measurable drusen, and eyes with some drusen but ≥median versus eyes with no measurable drusen in separate models for each outcome. Univariate associations between each genetic factor and the drusen measurements were evaluated by generalized linear models based on generalized estimating equations (GEEs) using PROC GENMOD of SAS 9.4 with the individual eye as the unit of analysis, using a logistic link and a binomial distribution with a working independence correlation structure to account for the correlation between fellow eyes.[47,48] We only considered eyes without advanced AMD because drusen morphology is altered by the presence of advanced AMD; thus, some subjects contributed two eyes to the analyses, whereas other subjects contributed a single eye if, for example, the fellow eyes had advanced AMD. In addition, a multivariate model included all genetic factors in the same model. All models were adjusted for age, sex, smoking, BMI,

and education. Two distinct outcomes of interest were assessed: drusen area and drusen volume in the perifoveal zone. Genetic variables were defined as having zero, one, or two risk or protective alleles. For the two *CFH* variants that convey different information about AMD risk ($R^2 = 0.44$), we combined them into a risk score category from zero to four, consisting of the total number of alleles in the two variants combined. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated per allele as estimates of effect size.

Because we identified consistent significant associations in categorical analyses between drusen measurements and risk genotypes for *CFH* risk score and *ARMS2/HTRA1*, we looked in more detail at mean area and volume represented as continuous variables for these two genes and assessed the independent effects of each gene on drusen measurements. For these analyses, AMD grade was included as an additional covariate. Associations between continuous drusen measurements and AMD stages and genotypes were evaluated by a linear mixed effects model using PROC MIXED of SAS 9.4 with the individual eye as the unit of analysis. This accounts for the intereye correlation in drusen area and volume between fellow eyes.[48] The LSMEANS option of PROC MIXED was used to compute adjusted means of area and volume measurements for specific genotype categories and AMD grades. In addition, we used the LSMEANS option to compute adjusted means for area and volume for categories of the *CFH* risk score, adjusted for age, sex, smoking, BMI, education, AMD grade, and *ARMS2/HTRA1* genotype (and similarly, LSMEANS for categories of the *ARMS2/HTRA1* genotype were adjusted for *CFH* risk score).

We tested for significant interactions between *CFH* and *ARMS2/HTRA1*, by first creating binary variables for each gene (*CFH* risk score: three or four alleles with reference = 0–2; and *ARMS2/HTRA1* GT and TT as one or two risk alleles with reference = GG as 0), and then creating the cross-product of the two binary variables. Finally, we estimated Pearson correlation between area and volume measurements.[49] Two-sided $P < 0.05$ was considered statistically significant. All analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

Distributions of area and volume for different drusen categories are shown in Supplementary Table S1. More than 50% of the analyzed eyes had measurable drusen, with a wide range in both area and volume among these eyes. The Pearson correlation between area and volume for individual eyes was 0.84, indicating similarity between the two measurements. Relationships between age, sex, education, smoking, BMI, and drusen parameters comparing no measurable drusen versus <median drusen and no measurable drusen versus ≥median drusen, were evaluated as shown in Supplementary Table S2. Older age was associated with greater drusen area and volume, and the other nongenetic factors were not significantly associated with these drusen measurements.

Ocular Images of Drusen Measurements

Representative color fundus photographs and OCTs corresponding to different measurements using the advanced RPE analysis tool are shown in Figure 1 for no measurable drusen, <median, and ≥median drusen measurements.

Association Between OCT Drusen Measurements and AMD Grade

The mean OCT-derived drusen area was significantly associated with grade of AMD based on color photographs and was higher in
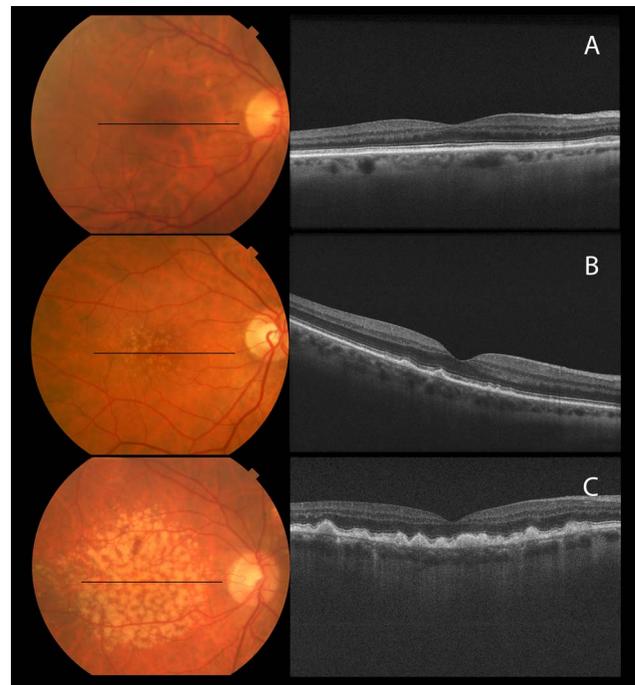

FIGURE 1. Representative color fundus photographs and OCTs corresponding to different measurements using the advanced RPE analysis tool are shown as (**A**) no measurable drusen, (**B**) drusen area and volume <median, and (**C**) drusen area and volume ≥median.

intermediate AMD eyes than in early AMD eyes after adjusting for age, sex, education, smoking, and BMI ($P = 0.008$; Fig. 2A). Similarly, the mean drusen volume was higher in eyes with intermediate AMD compared with early AMD ($P = 0.005$; Fig. 2B). Individual data points are shown in Supplementary Figure S1.

Associations Between Drusen Measurements and Each AMD Genetic Variant

A higher *CFH* score was associated with greater drusen area in the perifoveal 5-mm zone controlling for age, sex, education, smoking, and BMI (Table 1). There was a significant association between drusen area ≥median versus no measurable drusen with OR = 1.79 (95% CI, 1.18–2.71; $P = 0.01$) per category of *CFH* score. Therefore, the OR is 5.74 (equal to 1.79^3) for category 3+ vs. 0. For the variant in another complement pathway gene, *C3* R102G, there was a significant association between drusen area ≥median versus no measurable drusen with OR = 1.80 (95% CI, 1.07–3.03; $P = 0.03$) per risk allele (G). A higher number of risk alleles for the variant in *ARMS2/HTRA1* was also associated with greater drusen area controlling for age, sex, education, smoking, and BMI. For the variant at this locus, there was a significant association between drusen area ≥median versus no measurable drusen with OR = 2.76 (95% CI, 1.57–4.86; $P < 0.001$) per each risk allele (T). Genetic variants in the HDL pathway were not significantly associated with drusen area.

Results for each genetic factor analyzed separately for association with the other drusen parameter, drusen volume, are shown in Table 2. Higher *CFH* score was associated with ≥median drusen volume compared with no measurable drusen volume (OR = 1.73; 95% CI, 1.13–2.64; $P = 0.01$) per category of *CFH* score. *C3* R102G was also related to drusen volume, with a significant association between number of risk alleles and drusen volume ≥median compared with no drusen volume with OR = 1.92 (95% CI, 1.09–3.41; $P = 0.03$) per risk allele. *ARMS2/HTRA1*
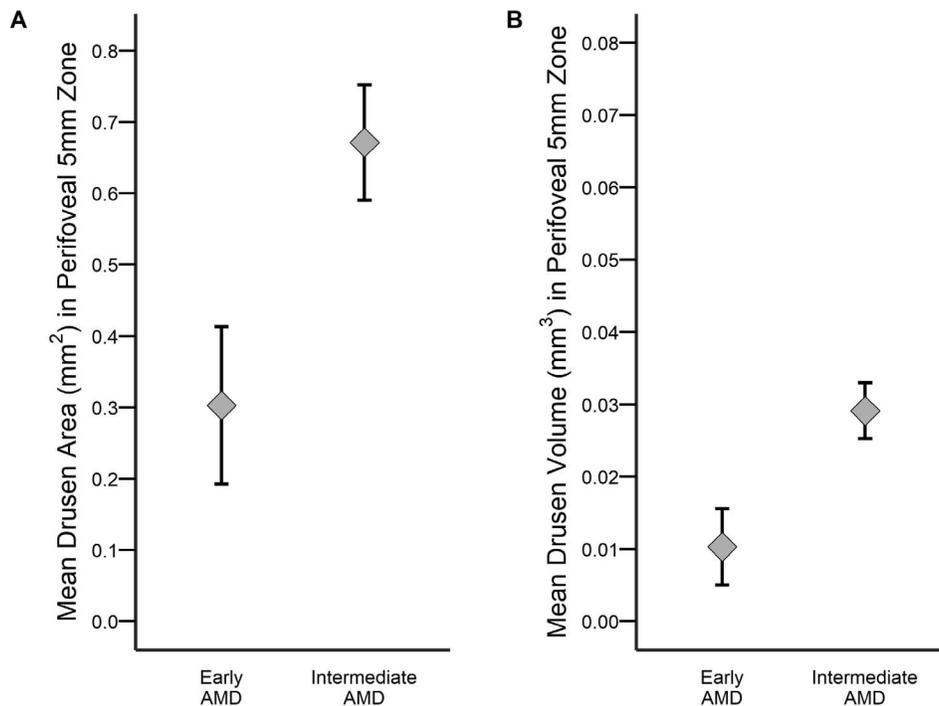
FIGURE 2. Associations between OCT-derived drusen measurements and early and intermediate stages of AMD for (**A**) drusen area ($P = 0.008$) and (**B**) drusen volume ($P = 0.005$). The *diamond* represents the adjusted mean controlling for age, sex, education, smoking, and BMI. The *vertical line* represents ±SE of the adjusted mean.

TABLE 1. Associations Between Drusen Area Measurements and Individual Genetic Loci*

Gene/Variant	No Drusen Measured (N = 111)	Drusen Area < Median (N = 54)		Drusen Area ≥ Median (N = 74)	
		OR (95% CI)†	P Value‡	OR (95% CI)†	P Value‡
CFH score	1.0 (ref)	0.97 (0.65–1.47)	0.90	1.79 (1.18–2.71)	0.01
ARMS2/HTRA1: rs10490924	1.0 (ref)	1.29 (0.77–2.14)	0.33	2.76 (1.57–4.86)	<0.001
C3 R102G: rs2230199	1.0 (ref)	0.99 (0.57–1.72)	0.98	1.80 (1.07–3.03)	0.03
ABCA1: rs1883025	1.0 (ref)	0.66 (0.32–1.37)	0.26	0.67 (0.35–1.31)	0.24
LIPC: rs10468017	1.0 (ref)	1.14 (0.61–2.12)	0.68	0.87 (0.51–1.50)	0.62
CETP: rs3764261	1.0 (ref)	1.15 (0.67–1.98)	0.62	1.49 (0.92–2.40)	0.10

* Each genetic variant assessed in a model, controlling for age, sex, education, body mass index, and smoking.
† OR and CI per risk or protective allele. *CFH* score categorized as 0, 1, 2, 3–4 risk score categories.
‡ Based on GEE using PROC GENMOD of SAS with the eye as the unit of the analysis using a logistic link and a binomial distribution with the working independence model to account for the intereye correlation. Separate analyses were performed (1) comparing eyes with measurable drusen less than the median versus eyes with no drusen and (2) comparing eyes with measurable drusen greater than or equal to the median versus eyes with no drusen. The reference category is no drusen measured.

TABLE 2. Associations Between Drusen Volume Measurements and Individual Genetic Loci*

Gene/Variant	No Drusen Measured (N = 96)	Drusen Volume < Median (N = 66)		Drusen Volume ≥ Median (N = 77)	
		OR (95% CI)†	P Value‡	OR (95% CI)†	P Value‡
CFH score	1.0 (ref)	0.99 (0.66–1.48)	0.96	1.73 (1.13–2.64)	0.01
ARMS2/HTRA1: rs10490924	1.0 (ref)	1.29 (0.77–2.16)	0.34	2.72 (1.48–5.01)	<0.001
C3 R102G: rs2230199	1.0 (ref)	0.99 (0.55–1.79)	0.98	1.92 (1.09–3.41)	0.03
ABCA1: rs1883025	1.0 (ref)	0.69 (0.36–1.34)	0.28	0.62 (0.32–1.22)	0.17
LIPC: rs10468017	1.0 (ref)	0.94 (0.53–1.66)	0.82	0.88 (0.50–1.53)	0.65
CETP: rs3764261	1.0 (ref)	1.17 (0.69–1.99)	0.56	1.63 (0.99–2.68)	0.06

* Each genetic variant assessed in a model, controlling for age, sex, education, body mass index, and smoking.
† OR and CI per risk or protective allele. *CFH* score categorized as 0, 1, 2, 3–4 risk score categories.
‡ Based on GEE using PROC GENMOD of SAS with the eye as the unit of the analysis using a logistic link and a binomial distribution with the working independence model to account for the intereye correlation. Separate analyses were performed (1) comparing eyes with measurable drusen less than the median versus eyes with no drusen and (2) comparing eyes with measurable drusen greater than or equal to the median versus eyes with no drusen. The reference category is no drusen measured.

TABLE 3. Multivariate Analyses of Associations Between Drusen Area and Genetic Loci*

Gene/Variant	No Drusen Measured (N = 111)	Drusen Area < Median (N = 54)		Drusen Area ≥ Median (N = 74)	
		OR (95% CI)†	P Value‡	OR (95% CI)†	P Value‡
CFH score	1.0 (ref)	0.93 (0.61–1.41)	0.73	1.58 (1.01–2.46)	0.04
ARMS2/HTRA1: rs10490924	1.0 (ref)	1.24 (0.72–2.15)	0.44	2.45 (1.35–4.45)	<0.001
C3 R102G: rs2230199	1.0 (ref)	1.00 (0.55–1.82)	0.99	1.57 (0.87–2.85)	0.14
ABCA1: rs1883025	1.0 (ref)	0.65 (0.31–1.38)	0.27	0.84 (0.41–1.73)	0.65
LIPC: rs10468017	1.0 (ref)	1.14 (0.61–2.12)	0.69	0.76 (0.43–1.36)	0.36
CETP: rs3764261	1.0 (ref)	1.17 (0.67–2.04)	0.57	1.28 (0.78–2.12)	0.33

* Each genetic variant assessed in a model, controlling for age, sex, education, body mass index, and smoking.
† OR and CI per risk or protective allele. CFH score categorized as 0, 1, 2, 3–4 risk score categories.
‡ Based on GEE using PROC GENMOD of SAS with the eye as the unit of the analysis using a logistic link and a binomial distribution with the working independence model to account for the intereye correlation. Separate analyses were performed (1) comparing eyes with measurable drusen less than the median versus eyes with no drusen and (2) comparing eyes with measurable drusen greater than or equal to the median versus eyes with no drusen. The reference category is no drusen measured.

risk was associated with greater drusen volume compared with no drusen volume (OR = 2.72; 95% CI, 1.48–5.01; $P < 0.001$). The variant in the HDL gene, *CETP,* was borderline associated with higher drusen volume compared with no drusen volume with OR = 1.63 (95% CI, 0.99–2.68; $P = 0.06$).

Multivariate Analyses of Associations Between Drusen and Genetic Variants

When analyzing each genetic variable while controlling for all of the other variants, only two genetic variables, *CFH* risk score and the *ARMS2/HTRA1* variant, remained independently associated with a significantly higher drusen area (≥median compared with no measurable drusen) as shown in Table 3. For *CFH* score, the OR was 1.58 (95% CI, 1.01–2.46; $P = 0.04$), and for the *ARMS2/HTRA1* variant, the OR was 2.45 (95% CI, 1.35–4.45; $P < 0.001$).

Multivariate associations between genes and drusen volume controlling for all genes are shown in Table 4. Comparing drusen volume ≥median to no measurable drusen, *CFH* score had an OR of 1.54 (95% CI, 0.97–2.45; $P = 0.07$). *ARMS2/HTRA1* remained significant with an OR = 2.49 (95% CI, 1.29–4.80; $P = 0.01$). The *C3* and *CETP* variants were not associated with higher drusen volume ($P = 0.09$).

Independent Associations Between Drusen Measurements and *CFH* Score and *ARMS2/HTRA1*

As shown in Table 5, both mean perifoveal drusen area and volume increased as the *CFH* score increased, and the trends

for increasing drusen burden as the *CFH* score increased were significant after adjusting for age, sex, education, smoking, BMI, and AMD grades (P trend = 0.004 for area and P trend = 0.002 for volume). Carriers of two risk alleles versus zero or one had higher drusen area ($P = 0.03$) and drusen volume ($P = 0.04$), and carriers of three or four risk alleles also had significantly higher drusen area ($P < 0.001$) and volume ($P < 0.001$) compared with having zero or one risk alleles. Similar comparisons were assessed for the *ARMS2/HTRA1* variant: mean drusen area and volume increased as the number of risk alleles increased, after adjustment for other variables (P trend < 0.001 for both drusen area and volume). Carrying two risk alleles versus none was significantly related to higher drusen area and volume ($P = 0.008$ and 0.004, respectively). These associations between OCT-derived perifoveal drusen measurements and genetic factors are also depicted in Figure 3.

When both genes were adjusted for each other (bivariate analyses shown in Table 5), the associations between drusen measurements and *CFH* score were somewhat reduced. However, the trend for higher drusen area and volume with higher score remained significant (P trend = 0.01 for area; P trend = 0.005 for volume). When the *ARMS2/HTRA1* genetic variant was adjusted for the *CFH* score, results were essentially unchanged from the univariate analysis as above, and trends for increasing drusen area and volume with increasing number of *ARMS2/HTRA1* risk alleles were significant (P trend < 0.001 and 0.001 for area and volume, respectively).

All tests of interaction between *CFH* score and *ARMS2/ HTRA1* were not significant. When comparing drusen area or volume measurements ≥median versus no measurable drusen,

TABLE 4. Multivariate Analyses of Associations Between Drusen Volume and Genetic Loci*

Gene/Variant	No Drusen Measured (N = 96)	Drusen Volume < Median (N = 66)		Drusen Volume ≥ Median (N = 77)	
		OR (95% CI)†	P Value‡	OR (95% CI)†	P Value‡
CFH score	1.0 (ref)	0.95 (0.64–1.42)	0.81	1.54 (0.97–2.45)	0.07
ARMS2/HTRA1: rs10490924	1.0 (ref)	1.27 (0.74–2.18)	0.39	2.49 (1.29–4.80)	0.01
C3 R102G: rs2230199	1.0 (ref)	1.00 (0.53–1.90)	1.00	1.78 (0.92–3.45)	0.09
ABCA1: rs1883025	1.0 (ref)	0.69 (0.36–1.35)	0.28	0.76 (0.37–1.57)	0.45
LIPC: rs10468017	1.0 (ref)	0.91 (0.51–1.62)	0.75	0.73 (0.41–1.32)	0.30
CETP: rs3764261	1.0 (ref)	1.19 (0.69–2.04)	0.53	1.54 (0.93–2.54)	0.09

* Each genetic variant assessed in a model, controlling for age, sex, education, body mass index, and smoking.
† OR and CI per risk or protective allele. CFH score categorized as 0, 1, 2, 3–4 risk score categories.
‡ Based on GEE using PROC GENMOD of SAS with the eye as the unit of the analysis using a logistic link and a binomial distribution with the working independence model to account for the intereye correlation. Separate analyses were performed (1) comparing eyes with measurable drusen less than the median versus eyes with no drusen and (2) comparing eyes with measurable drusen greater than or equal to the median versus eyes with no drusen. The reference category is no drusen measured.

TABLE 5. Associations Between Drusen Area and Volume and *CFH* and *ARMS2/HTRA1*

No. of Risk Alleles	n (Eyes)	Univariate Gene Analysis*				Bivariate Gene Analysis†			
		Drusen Area Mean (mm^2 ± SE)	P Value	Drusen Volume Mean (mm^3 ± SE)	P Value	Drusen Area Mean (mm^2 ± SE)	P Value	Drusen Volume Mean (mm^3 ± SE)	P Value
CFH Risk Score‡									
0–1	39	0.07 ± 0.16	Ref	0.0002 ± 0.008	Ref	0.25 ± 0.17	Ref	0.007 ± 0.008	Ref
2	103	0.49 ± 0.10	0.03	0.019 ± 0.005	0.04	0.57 ± 0.11	0.09	0.022 ± 0.005	0.11
3–4	97	0.66 ± 0.10	<0.001	0.029 ± 0.005	<0.001	0.74 ± 0.11	0.01	0.032 ± 0.005	0.006
P (trend)			0.004		0.002		0.01§		0.005§
ARMS2/HTRA1: rs10490924‖									
0	105	0.19 ± 0.10	Ref	0.007 ± 0.005	Ref	0.15 ± 0.098	Ref	0.005 ± 0.0047	Ref
1	104	0.68 ± 0.10	<0.001	0.028 ± 0.005	0.002	0.60 ± 0.10	0.001	0.024 ± 0.005	0.004
2	30	0.90 ± 0.19	0.008	0.036 ± 0.009	0.004	0.80 ± 0.19	0.002	0.031 ± 0.0089	0.009
P (trend)			<0.001		<0.001		<0.001‖		0.001‖

   * Analyses based on continuous area and volume, adjusting for age, sex, education, body mass index, smoking, and AMD grade using the LSMEANS option of PROC MIXED of SAS. Each genetic factor was analyzed separately.

   † Analyses for *CFH* score are based on continuous area and volume, adjusting for age, sex, education, body mass index, smoking, AMD grade, and number of *ARMS2/HTRA1* risk alleles using the LSMEANS option of PROC MIXED of SAS. Analysis for *ARMS2/HTRA1* was performed similarly, adjusting for *CFH* score.

   ‡ No. of risk alleles for rs1410996 and rs1061170 combined.

   § The *P* trend assessing effect on drusen measurements per one category increase in *CFH* score was obtained after adjusting for number of *ARMS2/HTRA1* risk alleles and other risk factors.

   ‖ The *P* trend assessing effect of increase in *ARMS2/HTRA1* risk alleles was obtained after adjusting for categories of the *CFH* score and other risk factors.

the *P* values were 0.38 for drusen area and 0.33 for drusen volume (data not shown).

DISCUSSION

We evaluated associations between measurements of perifoveal drusen area and volume and common risk and protective alleles known to be related to advanced AMD in patients with early and intermediate AMD. Drusen area and volume measurements were estimated using the advanced RPE analysis algorithm from Zeiss Cirrus OCT. These measurements were related to stage of nonadvanced AMD and were greater for eyes classified as having intermediate AMD compared with early AMD. In addition, among eyes with the same AMD grade (early or intermediate), genetic risk was associated with a higher drusen burden. *CFH* score and *ARMS2/HTRA1* were independently associated with perifoveal drusen area and volume compared with no measurable drusen.

Interestingly, genes in the HDL pathway were not significantly associated with drusen area and volume. Significant associations between drusen parameters and the *C3* variant were seen in univariate analyses but not in the multivariate analyses.

When comparing the adjusted means of the drusen area and volume for *CFH* score and *ARMS2/HTRA1,* we found significant trends for increasing drusen measurements with increasing number of risk alleles for each variant. The trends persisted when both genes were adjusted for each other, as well as when the baseline AMD grade was included as a covariate in the analysis. There was no interaction between these genetic factors when assessing their association with drusen parameters. These two genetic variants contributed independently to drusen burden.

Prior studies investigating the role of genetics and drusen assessments based on OCT are limited and inconsistent. Chavali et al.[17] studied an Amish population with early AMD and found an association between drusen and risk alleles in *CFH* rs12038333 and *SYN3* rs5749482, but not *ARMS2*. Authors reported that the population had a high rate of

homozygous risk allele of *SYN3* and suggested that this population has a unique genetic background. Oeverhaus et al.[50] evaluated 85 patients in Germany for only *CFH* and *ARMS2* and individuals homozygous for each genetic variant had larger amounts of drusen and different types of drusen based on manual OCT measurements, although multivariate analyses and measurements of area and volume of drusen based on an automatic OCT algorithm were not assessed. A study of change in drusen volume over 1 year among 30 patients in Florida who participated in a study of eculizamab showed an association with *CFH* rs1061170 but not *ARMS2/HTRA1*.[18] Drusen volume based on OCT was assessed in the Singapore Eye Disease program, and only *ARMS2* was associated with drusen volume, although the *CFH* SNPs in our risk score and drusen area were not evaluated.[51]

The value in obtaining and following these OCT drusen measurements over time has been suggested. Folgar et al.[13] showed that greater baseline OCT drusen volume was associated with increased risk of progression to NV over 2 years. Sleiman et al.[52] demonstrated that OCT based drusen measurements were associated with appearance of geographic atrophy on color photographs over 4 years. Schmidt-Erfurth et al.[15] found that drusen area was an important quantitative feature for progression. Higher drusen volume was related to advanced AMD in a retrospective review by Lei et al.,[22] but other OCT parameters were more strongly related in their cases. A combination of drusen parameters along with other OCT-derived parameters such as hyper-reflective foci and retinal thickness may be the most informative and predictive of progression.[14,15,22]

The association between advanced AMD and the *CFH* Y402H variant, as well as the intronic SNP in this gene, *CFH* rs1410996, has been well documented.[5,19,37,41,53–55] The biologic mechanisms underlying their effect on drusen accumulation, however, have not been fully explored. In our previous analyses of these variants regarding progression, the effect of rs1410996 was stronger and the effect of Y402H was reduced when both were in the same model, suggesting that this variant may be more strongly associated with AMD.[4–6,55] *CFH* negatively regulates the alternate complement pathway
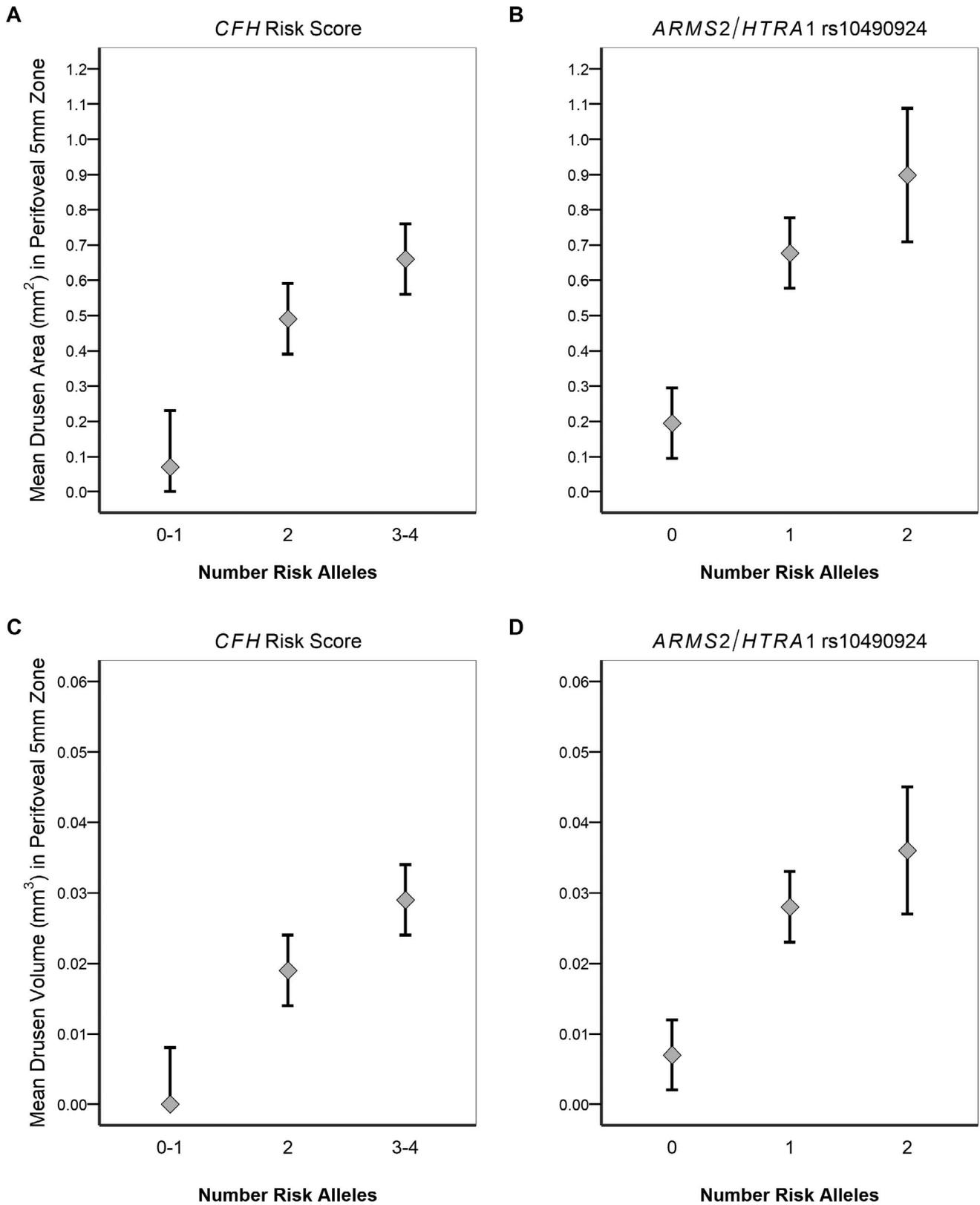
FIGURE 3. Independent associations between OCT-derived perifoveal drusen measurements and number of risk alleles for drusen area for (**A**) *CFH* score and (**B**) *ARMS2/HTRA1* rs10490924 (*P* trend = 0.004 and <0.001, respectively) and drusen volume for (**C**) *CFH* score and (**D**) *ARMS2/HTRA1* rs10490924 (*P* trend = 0.002 and <0.001, respectively). The *diamond* represents the adjusted mean controlling for age, sex, education, smoking, BMI, and AMD grade. The *vertical line* represents ±SE of the adjusted mean.

and dampens the excessive C3 convertase activated by either immune complex deposition or C3 convertase activation from pathogens or damaged cell surfaces. *CFH* risk variants are functionally less efficient at dampening down this response, leading to heightened complement activity, which can lead to AMD-related pathology.

It should be noted that SNPs in the genes *ARMS2* and *HTRA1* at the chromosome 10q26 locus are in very high linkage disequilibrium, and functional studies are needed to determine which gene products lead to AMD pathology.[43] The function of the ARMS2 protein in humans and the consequences of the A69S variant have been explored but have not been confirmed. One study found that ARMS2 was expressed in human monocytes and microglia cells and facilitated removal of cellular debris by local complement activation; the A69S variant resulted in ARMS2 deficiency, possibly impairing the removal of cellular debris at Bruch's membrane, leading to the development of drusen.[56] On the other hand, another study reported that ARMS2 mRNA and protein are expressed at extremely low levels in eye tissues and presented support for *HTRA1* as the risk factor for AMD.[57]

We found that the OCT algorithm yielded drusen measurements that were highly associated with the manual CARMS grading. This advanced RPE analysis algorithm or a similar method may be useful for ophthalmologists or reading centers to categorize the stage of AMD more efficiently, with higher precision and with the ability to quantify the measurements, compared with clinical observation or photography. Higher-resolution OCT may reduce variability in OCT grading and enhance the application of the RPE analysis algorithm. Future studies will continue to provide more information about the impact of genetic variants on drusen morphology.

Strengths

Strengths of this study include the assessment of plausible genetic pathways, the inclusion of high-quality scans, and use of a commercially available means of estimating drusen measurements that is incorporated into Cirrus SD-OCT instruments. Automated RPE analysis has been shown to be consistent and reproducible, especially between repeated scans.[35,58] We also conducted eye-specific analyses adjusting for correlation between eyes, analyzed both drusen area and volume, and controlled for demographic and behavioral risk factors associated with AMD.

Limitations

Some of the AMD-associated genes did not show an association with drusen area or volume in eyes with early and intermediate AMD. This may be due to lack of an association or insufficient power secondary to a limited number of eyes in the homozygous risk or protective genotype category in some of these genes. We did not find a significant association between OCT-derived drusen measurement and the HDL pathway genes (*ABCA1, LIPC,* and *CETP*), although effect estimates were in the direction previously seen in a prospective analysis.[19] Of note, the same genes (*CFH* and *ARMS2/HTRA1*) significantly related to the transition from early to intermediate AMD in Yu et al.[19] based on color photographs were also associated with larger OCT-derived drusen measurements in our analyses as seen in Tables 1 to 5 of the current paper.

We observed that some small drusen seen on color photographs were classified as having no measurable drusen by the RPE algorithm on OCT. This may due to the basis of the algorithm that has a threshold of 10-pixel elevation (19.5 μm) before the measurements can be calculated.[34] The developers of the algorithm used this to prevent a false-positive RPE elevation in the OCT due to noise. Drusen area and volume are estimates assuming an eye with 24.46 mm in length so there may be magnification differences between eyes. Furthermore, drusen outside the perifoveal zone were not measured because they were located outside the scanned or analyzed zone.[59] Because the same algorithm for measuring drusen parameters was applied to all data uniformly, the relative rankings of the area and volume measurements were internally valid.

CONCLUSIONS

In summary, this study determined that risk alleles in AMD-associated genes, *CFH* and *ARMS2/HTRA1*, were independently associated with greater drusen area and volume based on automated measurements of RPE using the Zeiss SD-OCT algorithm. The associations between these genes and drusen remained significant after controlling for all other genes, as well as the nongenetic covariates. The *C3* variant was associated with drusen area and volume only in univariate analyses. Results confirm some findings in the few previous studies in different populations but also contribute new information and analyses to support the involvement of both *CFH* and *ARMS2/HTRA1* genes in drusen development.

As our understanding of the genetic determinants of AMD becomes clearer, it will be important to understand the specific patho-biologic consequences of these genetic variants. SD-OCT can quantify variation in morphology in a way that is accurate and reproducible. Other AMD phenotypes detectable on OCT including subfoveal fluid, retinal and choroidal thickness, retinal hyper-reflective foci, and other drusen characteristics such as hyper-reflectivity and homogeneity, are opportunities for exploration in the context of subtyping AMD and assessing risk of progression,[14,15,22,52] as well as defining the associations with genetic variants. Understanding the relationship between disease severity, morphologic features, and genetic factors has the potential to enhance individualized patient management and treatment.

Genetic risk is associated with a higher drusen burden assessed by OCT in eyes with early and intermediate stages of AMD. Further characterization of drusen and retinal and choroidal morphology may lead to the identification of biomarkers that could serve as early anatomic end points for clinical trials and facilitate the development of new therapies for AMD.

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