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Retinal Structure in Pre-Clinical Age-Related Macular Degeneration (AMD)

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Abstract

Purpose: To determine if there are identifiable retinal structural changes associated with genetic risk for age-related macular degeneration (AMD).

Materials and Methods: Seventy-three subjects (range 51.5 to 68.9 years) participated in this prospective study. Subjects were recruited based on the presence of a family history of AMD in one or both parents. All participants underwent a complete ophthalmic exam and imagery for staging of disease severity and genetic testing to assess genetic risk for AMD development. Optical coherence tomography (OCT) imaging was performed on all participants. Semi-automated retinal layer segmentation was performed to assess retinal structural changes.

Results: Of 73 subjects, 47 subjects had normal appearing retina with no evidence of drusen or other changes consistent with AMD, 16 subjects were classified as early AMD, and 13 were designated as intermediate AMD. Retinal volume measures of total retina, outer retina, outer nuclear layer and the retinal pigment epithelium, were not related to AMD classification, genetic risk scores, or age. The thickness of the outer retina showed statistically significant thickening in the foveal region in only the intermediate AMD group and a statistically significant thickening of the RPE in early and intermediate AMD groups in the central retina.

Conclusion: No consistent changes were observed in retinal structure at multiple locations that are associated with pre-clinical AMD, based on AMD genetic risk or with aging within the age range of our cohort.

Keywords

age-related macular degeneration; AMD; OCT; retina; structure

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Declaration of Interest: There are no conflicts of interest for any of the authors.

Introduction

Molecular genetic studies of age-related macular degeneration (AMD) have yielded common and rare variants in at least 34 genetic loci that contribute to an individual's risk of developing advanced AMD (1). However, there are individuals across the spectrum of AMD risk who will develop AMD despite relatively low genetic risk and others who will not progress to advanced disease, even with multiple high-risk variants. As therapies are developed to prevent or slow disease progression, there is a need for preclinical biomarkers and phenotypes, that when combined with genetic risk, can provide greater sensitivity and specificity as to who will develop AMD and be appropriately selected for preventive treatment. These clinical biomarkers could also serve to measure disease progression and to monitor the effectiveness of these therapies prior to the development of advanced disease.

In an effort to identify pre-clinical biomarkers that might be useful in predicting who might develop AMD, we recently studied a highly select cohort of adult individuals between the ages of 50 and 70, with positive AMD family histories but without classic signs of early AMD, and asked whether we could detect regional functional abnormalities that would segregate with their AMD genetic risk scores (2). Genetic risk scores were determined on the basis of 17 key SNPs identified by the International AMD genetic consortium (Fritsche, Chen (3)). We found that AMD risk scores correlated with the detection of subclinical drusen (as detected by high resolution OCT) and early AMD classification, supporting the validity of our use of the genetic risk model as an AMD endophenotype (2). We also tested the association between AMD risk profiles in our pre-clinical cohort (those without drusen) and dark-adapted rod- and cone- mediated threshold sensitivities obtained with two-color dark-adapted perimetry. Though scotopic thresholds have been proposed for the detection of early AMD based on abnormalities seen in relatively normal areas of retina in AMD patients (4–7), we established that this retinal phenotype is not a useful discriminator in this pre-clinical high-risk population and did not contribute to our ability to refine the risk models that we currently have available (2).

In this study we employed segmentation analysis of high resolution OCT macular imaging to determine if there are structural changes in the retina that precede the emergence of drusen that would be associated with the AMD genetic risk. In the prior publication, we studied retinal structure in only a few patients. We now have a significantly larger cohort and appropriate analytic tools to provide a more detailed analysis of retinal structure in pre-clinical AMD.

Materials and Methods.

Subjects and Diagnostic Studies: Seventy-three subjects (mean age = 60.0 ± 4.3 years, range 51.5 to 68.9 years) participated in this prospective study. Subjects were recruited based on the presence of a family history of AMD in one or both parents. In nearly all cases, we had reliable clinical information that confirmed the diagnosis of AMD in the parents. All subjects underwent a complete ophthalmological exam by a retina specialist (MBG).

Retinal structure for all participants was measured with spectral-domain optical coherence tomography (sdOCT) (Heidelberg Spectralis HRA+OCT, Heidelberg Engineering, Germany). All images were recorded by the same technician who was certified to perform OCTs. Signal strength was evaluated during acquisition using the Heidelberg quality indicator with images repeated if signal strength fell below 30 (maximum of 40 indicating excellent image quality).

We first performed a volume scan consisting of 61 full-width (30°) scan lines, each consisting of the average of 9–15 high resolution (HR) scans. These images were carefully examined to determine whether drusen, ~~the hallmark of AMD~~, was present in either eye and was used to classify the subjects as having a normal, early AMD or intermediate AMD retina. AMD classification was based primarily on the presence of drusen, their size, and whether pigmentary changes were present (8). Individuals with no visible drusen or pigmentary changes were classified as normal. Individuals with medium size drusen (≥ 63 - < 125μm) and no pigmentary changes were classified as having early AMD. Finally, individuals with large drusen (> 125 μm) with or without pigmentary abnormalities, were classified as having intermediate AMD. These measurements were made for both eyes with the findings from the more involved eye determining the AMD classification for that subject. Short-wavelength and near-infrared autofluorescence images (30 and 50 degrees) were also examined to assess alterations that might be characteristic of AMD. The study was carried out with approval of the UCLA Institutional Review Board (IRB), informed consent was obtained from all subjects prior to participation, and the study was conducted in accordance with regulations of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Retinal function for many of these patients was published previously (2).

Genetic Analysis.

Our cohort of “at-risk” participants was screened for variants in genes commonly associated with AMD, including *ARMS2* and *HTRA1*, two genes in strong linkage disequilibrium on chromosome 10q26, as well as genes of the complement system. A complete description of the protocol is described elsewhere (2, 9, 10). Briefly, genotyping was done with an iPLEX panel of 88 SNPs that have been selected for known genes associated AMD and with the complement activation cascade, including CFH and CFH-related genes, CFI, C2/BF, and C3. The raw data was run through dbVor, a database system developed by Baron and Weeks for importing, editing and exporting genotype data (see http://watson.hgen.pitt.edu/register/docs/dbVORtutorial.html), to generate the data in Mega2 format (9). Mega2 was used to convert the data to PLINK (10). SNP’s with genotyping success rates <0.9 were removed (18 SNPs) and individuals with genotyping success rates of <0.9 were also excluded. The genetic loci for the 17 of the 19 risk SNPs that were included in the computation of the genetic risk score are described elsewhere (3). The two markers, rs2230199 and rs5749482, were removed because of excessive genotyping failure rates, 28.94% and 10.36%, respectively. All of the markers used in the AMD genetic risk score calculation showed no evidence of violation of the Hardy Weinberg Equilibrium. The list of SNPs used to calculate the AMD genetic risk score and the genotyping success rate are described elsewhere (2).

Segmentation analysis of Optical Coherence Tomography (OCT).

Semi-automated retinal layer segmentation was performed using software provided by the manufacturer (Heidelberg Engineering, Germany).. For the purposes of this study we report thickness measures for the total retina (inner limiting membrane to basal Bruch's membrane), the outer nuclear layer (outer plexiform layer to the external limiting membrane (ELM)), the photoreceptor layer (ELM to Bruch's membrane), and the retinal pigment epithelium (RPE) (RPE and Bruch's membrane). Segmentation lines were corrected manually as needed for each of the image scan lines to more accurately reflect local structural features. These corrections were required infrequently in eyes without evidence of drusen and classified as normal appearing. However, manual corrections were more frequent in patients with drusen and more significant structural changes. The volume HR scan was divided into nine sectors as shown in Figure. 1. Volume measurements for each sector are shown for each layer of interest.

Statistical Analyses.

For each layer of interest, the mean difference across nine sectors was compared using repeated measures ANOVA within and between AMD classification groups. Such comparison was first performed by combining subjects from all three subgroups (normal, early and intermediate AMD) together, then performed within each subgroup separately. The mean difference of each sector across the three AMD subgroups was compared using the Kruskal-Wallis test as the measurement from each subject was assumed to be independent. Finally, in subsequent analysis, the association of global and regional volumetric measurements with age and genetic risk was evaluated by Pearson's correlation coefficient and by linear regression.

Results.

AMD Classification.

Of the 73 subjects, 47 subjects (mean age = 59.5 ± 4.2 years) were considered to have normal appearing retina with no evidence of drusen or other changes consistent with AMD, 16 subjects (mean age = 59.9 ± 3.5 years) were classified as early AMD, and 13 (mean age = 62.1 ± 4.9 years) were designated as intermediate AMD. Because these subjects were recruited based on a family history of AMD, but without visual symptoms and/or a clinical diagnosis of AMD, the majority of the participants had a normal fundus appearance. No subject was considered to have advanced AMD.

Genetic Risk Scores are Correlated with AMD classification.

Genetic risk profiles for AMD based on 17 key SNPs were determined for each participant as described above. Individuals, whose DNA yielded low quality genotyping scores or more than two missing genotypes, were excluded from these analyses. Genetic risk scores ranged from 0.61 to 1.62. As shown in Figure 2, there was a clear association between AMD risk score and AMD classification (no t-test comparing the risk scores among the three AMD categories had p-values > 0.018). Since AMD classification was based on the presence of drusen and their size, the figure also implies that genetic risk is correlated with drusen size,

with generally higher risk scores associated with larger drusen. Preliminary findings were reported previously and establish the validity of the genetic model (2).

Retinal Volume Measures are not Related to AMD Classification.

Volume measures across the entire macular region were obtained for each retinal layer investigated. Except for a slight thickening of the retinal pigment epithelium and outer retina in in early and intermediate AMD (see Table 1), there were no significant layer thickness changes across the three classification groups. The small change in outer retinal thickness can be largely attributed to the change in RPE thickness, roughly representing a change of approximately 10% from the group with no drusen in either eye. The similarity in thickness volume measures is not a surprising finding in the context of the narrow definition used to segregate participants into the three AMD groups. For example, a participant with just one large localized druse (>125.0μ) in one eye without evidence of pigmentary changes was considered to have intermediate AMD (8). However, because changes in retinal thickness were localized, they tended to have little impact on overall retinal volume measures that were computed over wider expanses of the retina. Thus, one would expect that there would be considerable overlap in the distribution of the volume measures across the three groups.

Retinal Volume Measures are not Correlated with the Genetic Risk Score

We then asked whether retinal volume measures for each layer of interest were associated with genetic risk score. The results are shown in Figure 3 (A-D). A significant association between genetic risk score and retinal volume was not apparent for either eye for any retinal layer. Genetic risk scores in the pre-clinical no drusen cohort were not predictive of retinal volume measures (black open and closed circles), however, when the analysis included early (red data points) and intermediate AMD (green data points), there was a small increase in volume of the outer retina (Figure 3C) perhaps due to the thickening of the RPE (Figure 3D) in intermediate AMD. However, none of the associations reached statistical significance.

Retinal Layer Volume is weakly Correlated with Age.

The relationship between age and retinal volume is shown in Figure 4A-D (total retina, ONL, photoreceptors, RPE, respectively) for each AMD classification. Overall, there was no strong association between age and global volumetric measures (Figure 4). To evaluate the association with age, we determined the slope of a linear regression relating the specific measure with age. There was a small but significant decrease in overall retinal volume in the “no AMD” group (slope = −0.028 + 0.013, p = 0.044), but for other retinal layers, the slope was not significantly different from zero (the most significant p of 0.18 was associated with thinning of the ONL). In the early AMD group, none of the slopes relating volume to age departed significantly from zero, although there was a trend in the direction of thickening for the total retina and the ONL, and a thinning of the photoreceptor and RPE layers. Finally, in the intermediate AMD group, none of the slopes relating volume to age departed significantly from zero, although there was a trend in the direction of thinning for the total retina, photoreceptors and RPE, and a thickening ONL. Overall, the trend with age for each retinal layer was relatively flat for both of the AMD groups. These observations are limited to the range of ages within our cohort and cannot necessarily be extrapolated to younger or older aged individuals.

Retinal Layer Thickness by Retinal Location, AMD Classification and Eye.

Since global measures of retinal volume were not significantly different across AMD categories, we asked whether we could identify consistent localized changes in retinal thickness measures that could be informative in the absence of drusen. Toward this goal, the OCT scans were analyzed separately in nine regions as shown in Figure 1. Average thickness for each sector were determined for each layer of interest and disease classification. All subsequent analyses are performed using this sector analysis.

There were no statistically significant interocular differences in most sectors, except for a few sectors, such as N1/N2 or T1/T2 in some layers and/or disease subgroups. In those instances where there were statistically significant differences, those differences were small. For example, in those participants without evidence of drusen, there was a significant interocular difference in total retina only in the nasal sector N2 (309 vs 311 μm for the right and left eye, respectively, $p < 0.0001$). All other sectors and retinal layers were not significantly different between eyes. Because of the non-specific and small differences between eyes and the relatively large numbers of comparisons, these differences most likely reflect chance variations, and the measurements from both eyes were averaged and used for subsequent analysis.

Retinal Layer Thickness Varies by Retinal Location.

As expected, for all groups, the total retina was thinnest in the foveal region (C0) and thickest in parafoveal regions N1, S1, T1, and I1, gradually decreasing toward more peripheral retinal locations for all groups ($p < 0.001$). (See Table 2.) In addition, there is a suggestion that the nasal retina is thicker than corresponding regions in the temporal retina. In contrast, both the ONL and the photoreceptor layers were thickest in the foveal region (C0) decreasing in thickness toward the peripheral retinal locations. The thickness of the RPE remained relatively constant across all regions.

Is there a difference in thickness measures for each of the 4 layers by retinal location and disease classification?

AMD causes non-homogenous changes in the retina leading to areas with differing degrees of drusen and retinal dysfunction. Thus we looked at variations in retinal thickness within different layers and locations among our cohort eyes. Total retina thickness by retinal region were not significantly different across the three disease classification groups (no comparison for a particular retinal location had a p value <0.15) (Table 2). The thickness of the outer retina (photoreceptors) showed statistically significant thickening in the foveal region (CO) in only the intermediate AMD group ($p = 0.05$). The thickness difference between intermediate AMD CO thickness and the normal group was 2.0 μm, or an approximate 2.4% change. There were no statistically significant differences among groups in other regions for the photoreceptor layer.

The ONL was thickest in CO and thinnest in the inferior retina, region I2 (Table 2). Although there was a trend in the direction of thinning of the ONL in CO in the intermediate group, this trend did not reach statistical significance. There were no statistically significant

differences in ONL thickness in other regions across the three groups (no comparison had a p value <0.20).

Finally, the greatest change in retinal thickness was in the RPE/Bruch's membrane complex. The RPE thickness varied across the retina as shown in Table 2. Overall, there was a statistically significant thickening of the RPE in early and intermediate AMD groups in the central retina. These changes reflect the impact of drusen in these eyes as well as basal laminar deposits that would thicken the RPE layer and which might not be qualitatively appreciated in a clinical setting.

Discussion.

Most medical therapies are associated with some risks and costs in addition to their clinical benefit. While all of the approved treatments for AMD are focused on managing the end-stage complications of AMD (predominantly exudative AMD), newer therapies are being developed to address the underlying causes of AMD and will hopefully be used to prevent or greatly delay vision loss. For treatment to be optimally effective one needs to know whom to treat, when to treat, and whether or not the treatment is having a desired effect without having to wait for an end-stage outcome. Toward this goal, there is a critical need to find clinical markers that, when combined with the specificity of genetic testing, will identify individuals at the earliest stages of AMD who would benefit from preventive therapies. These clinical markers can greatly improve the diagnostic value of molecular genetic testing as well as serve as monitors for therapeutic response.

There are numerous studies that have demonstrated decreases in rod-mediated sensitivity and prolonged dark-adaptation kinetics even in *early* AMD (11–19). It is tempting to select structural and/or functional measures of retinal status that have been shown to be abnormal in patients with AMD as possible early biomarkers. However the value of these measures must be established in individuals who do not necessarily have clinical evidence of AMD and we have previously shown that one can use the AMD genetic risk profile as an endophenotype of AMD to test these hypothetical cases (2). In a recent study, we tested the association between AMD genetic risk profiles in a *pre-clinical* cohort with dark-adapted rod- and cone- mediated threshold sensitivities. We demonstrated that this functional phenotype failed to discriminate between individuals with high and low genetic risk profiles in a pre-clinical at-risk population. However, Owsley et al (2016) recently demonstrated that delayed rod-mediated dark-adaptation kinetics in older adults with normal macular health is associated with incident early AMD three years later (20). We are in the process of developing the means for testing this psychophysical response at multiple retinal loci (given the nature of AMD-related retinal changes) with our preclinical cohort to test for an association with their genetic risk profiles.

In this study, we asked whether there were any pre-clinical structural changes that are associated with the AMD genetic risk scores and which may enhance our predictive abilities for the development of AMD. While this study was focused on identifying structural changes that might precede the development of drusen, we also included patients within our cohort who had been diagnosed with early and intermediate AMD and thus had evidence of

one or more drusen. We did not find a consistent structural change in the volumes of the retinal layers at multiple locations that were associated with pre-clinical AMD, based on AMD genetic risk or with aging within the age range of our cohort. We did observe a modest thinning of the total retina in early and intermediate AMD in foveal and parafoveal regions, including the nasal and superior parafoveal retina as has been previously reported (21, 22); however, we also observed a gradual thickening of the outer retina (Fig 3C) presumably driven by changes within the RPE layer in early and intermediate AMD. Variable thinning and thickening of the RPE has been reported previously in early and intermediate AMD (23). Overall, however, most differences were too small to reach statistical significance and/or be of any predictive value.

Although we did not find any structural changes in pre-clinical AMD that are associated with genetic risk, which was the main focus of this study, that finding does not preclude the possibility of functional deficits in these structurally normal retinas that might be correlated with genetic risk and predictive of disease emergence (24). That work is currently in progress.

Conclusions.

Considerable effort has focused on understanding the structural and functional changes that occur in early and later stages of AMD. However, these studies were carried out using individuals with an AMD diagnosis characterized by the presence of one or more drusen of appropriate size. However, there is a paucity of data of data identifying changes, either structural or functional, in pre-clinical at-risk AMD individuals that would be predictive of the development of disease before the classic signs of AMD emerge. Genetic risk models alone are useful in comparing those who are at higher risk for the development of AMD with those with lower risk profiles and can be used to test for the associations with other clinical biomarkers, but these genetic risk scores are not sufficiently predictive of the development of AMD to be clinically useful on an individual basis. These current genetics-based models, even when they incorporate dietary, age and smoking histories, have not been validated for individuals who have no evidence of AMD and who might wish to know if they should take steps to lower their risk of developing AMD. While a number of abnormalities of retinal structure and function have been implicated as comorbidities of AMD, we have shown that some features, including final dark-adapted visual thresholds and the regional thicknesses of retinal layers are not discriminative for preclinical AMD and do not contribute to our ability to refine the current AMD risk models (2). The search for a pre-clinical biomarker with respect to retinal function with sufficient sensitivity and specificity to identify AMD at-risk individuals will continue. In addition, serum biomarkers associated with lipid metabolism (e.g., apolipoprotein E), retinoid uptake and transport, carotenoid transport, inflammatory markers including C-reactive protein, circulating complement factors and regulatory proteins, may be useful biomarkers but have yet to be investigated in an appropriate preclinical AMD cohort such as we have described.

Finally, epigenetics may play a major role in the pathogenesis of AMD but this has yet to be clearly established. The important consideration is that epigenetic changes in DNA are both age and tissue-specific. We are using the genetic risk score as an endophenotype of AMD to

allow for testing the hypothesis that other changes (eg structural changes in this study) may reflect preclinical or early changes in AMD. One can certainly use this approach to test the hypothesis that specific epigenetic factors may precede the onset of other findings of AMD, but that was beyond the scope of our study.

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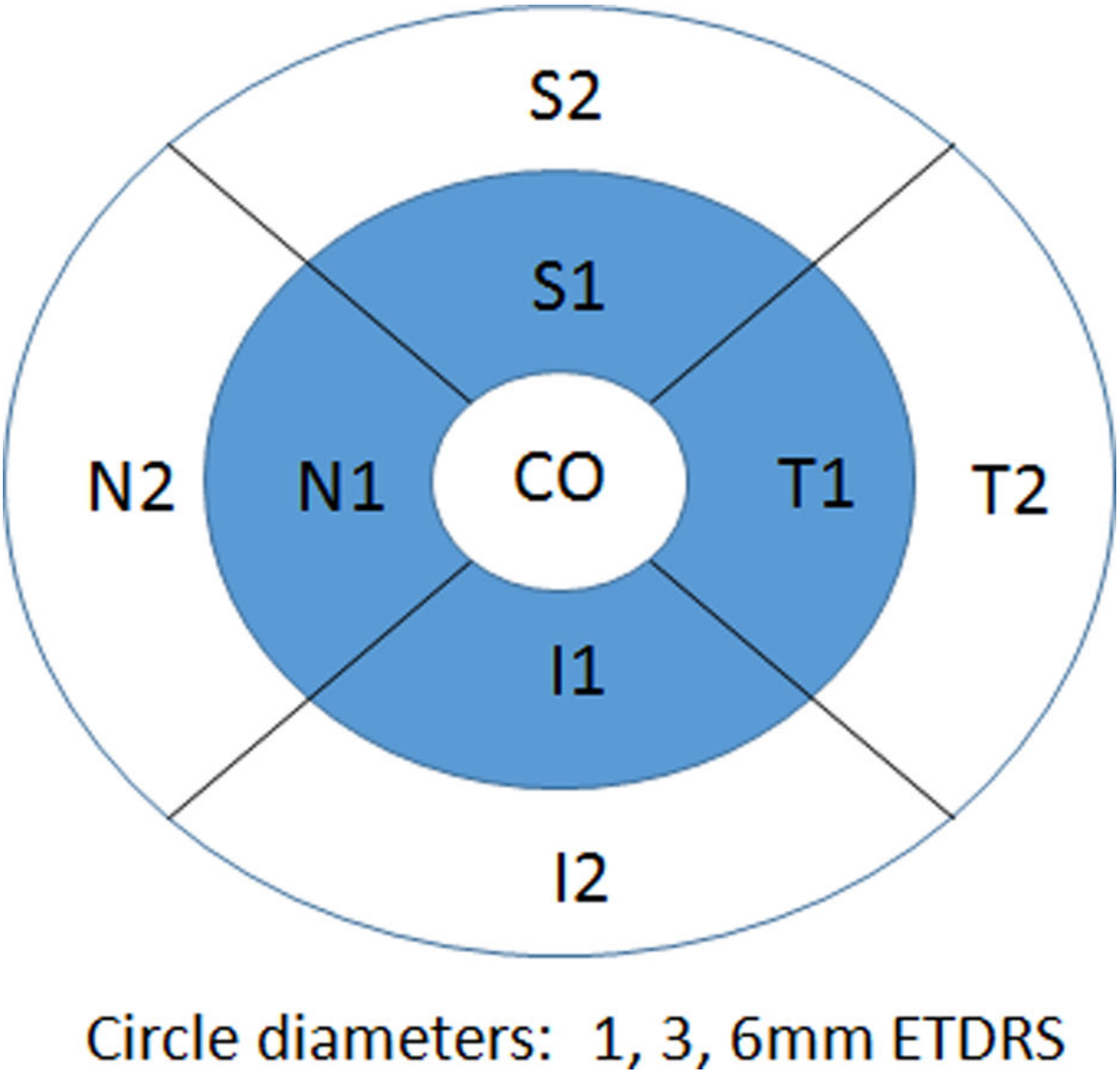

Figure 1.

Volume measurements for retinal layers of interest were determined for nine retinal regions. Measurements were mirror-reversed so that regions from both eyes were in correspondence for the purposes of averaging. C = central, N = nasal, T = temporal, S = superior and I = inferior.

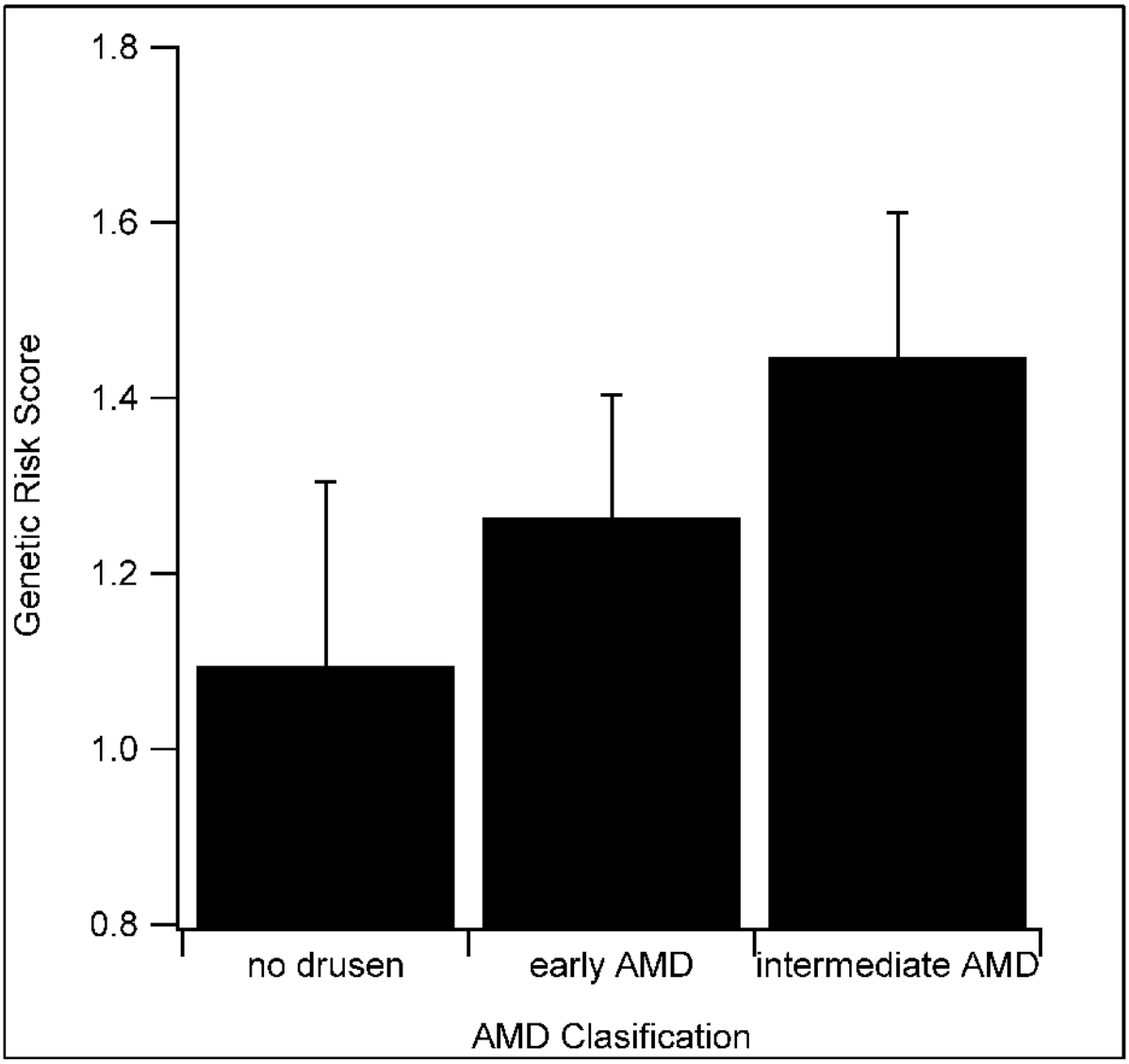

Figure 2.
The association of genetic risk scores and AMD classification.

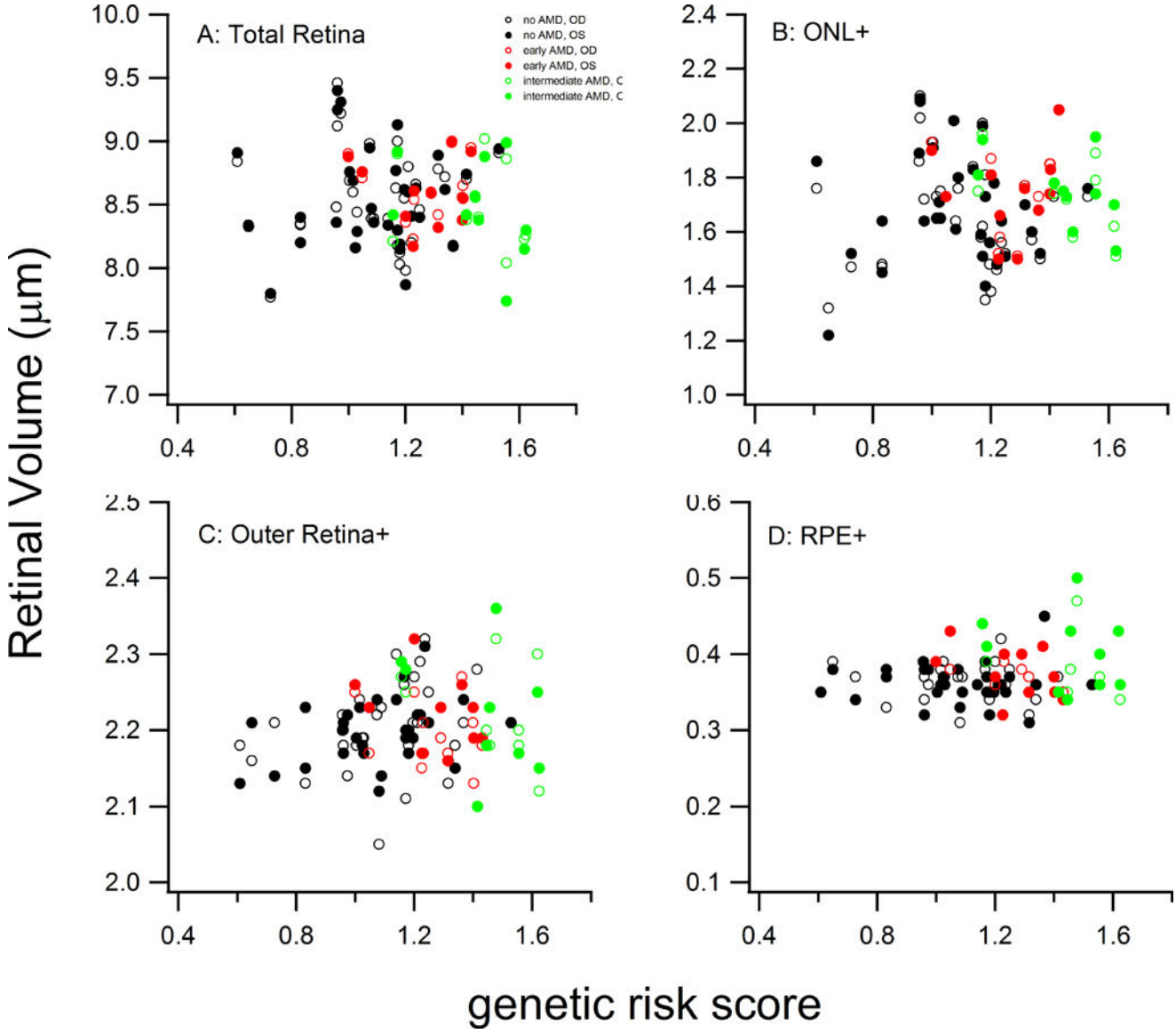

Figure 3.

The association of volumetric measurements with the calculated genetic risk score. Total retina (inner limiting membrane to basal Bruch's membrane), ONL+ (outer plexiform layer to the external limiting membrane (ELM)), outer retina+ (ELM to Bruch's membrane), and RPE+ (RPE and Bruch's membrane).

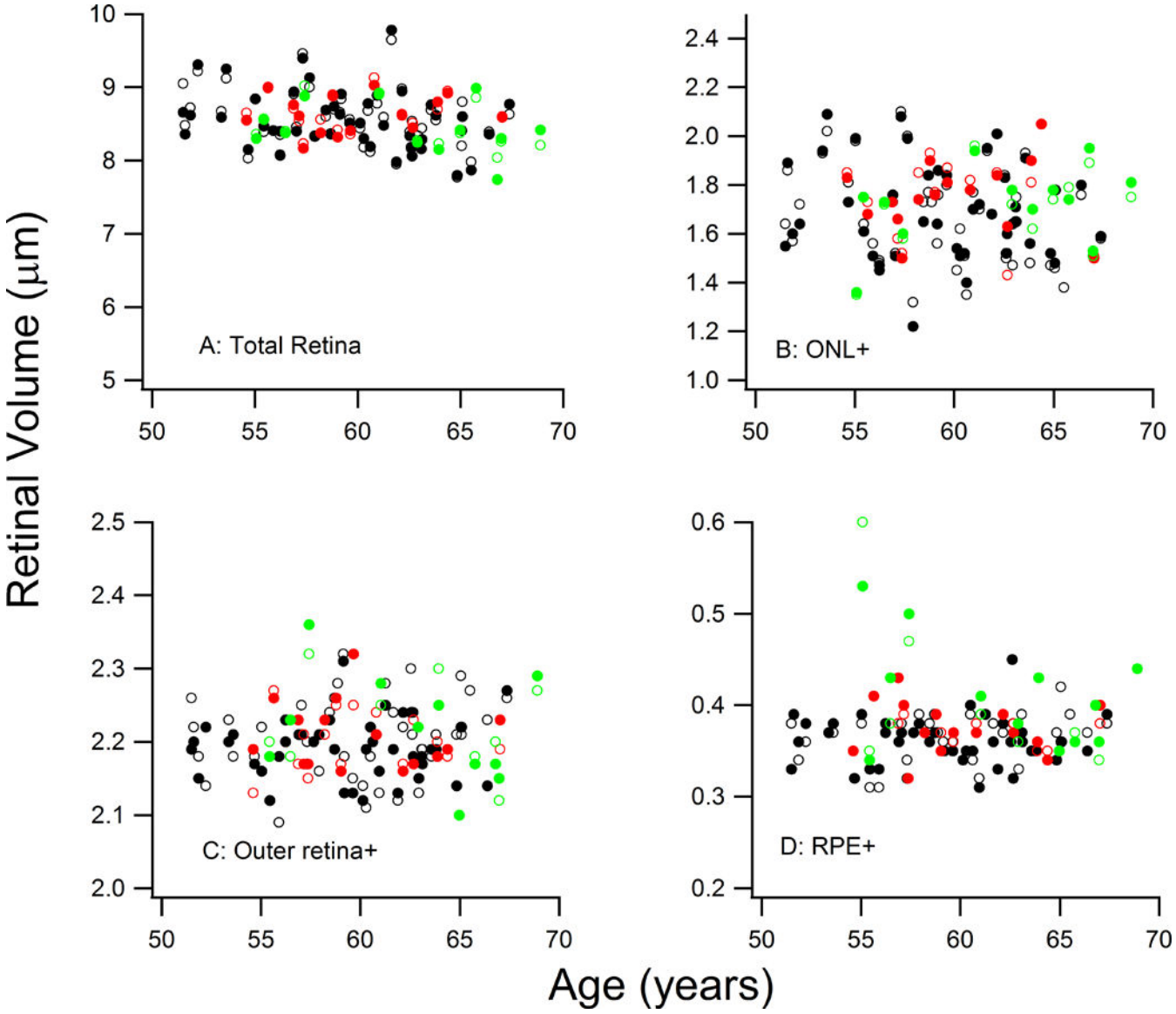

Figure 4.

The association of volumetric measurements with age. Total retina (inner limiting membrane to basal Bruch's membrane), ONL+ (outer plexiform layer to the external limiting membrane (ELM)), outer retina+ (ELM to Bruch's membrane), and RPE+ (RPE and Bruch's membrane).

Table 1.

The association of global retinal volume measurements with AMD classification. Total retina (inner limiting membrane to basal Bruch's membrane), ONL+ (outer plexiform layer to the external limiting membrane (ELM)), outer retina+ (ELM to Bruch's membrane), and RPE+ (RPE and Bruch's membrane).

	No Drusen (n=46)		**Early AMD (n=15)**		**Intermediate AMD (n=12)**	
	Right Eye	**Left Eye**	**Right Eye**	**Left Eye**	**Right Eye**	**Left Eye**
RPE+	0.37 (0.03)	0.36 (0.03)	0.37 (0.02)	0.37 (0.09)	0.41 (0.07)	0.41 (0.06)
Outer Retina+	2.20 (0.06)	2.19 (0.04)	2.20 (0.04)	2.21 (0.05)	2.25 (0.14)	2.25 (0.14)
ONL+	1.68 (0.20)	1.69 (0.20)	1.75 (0.17)	1.75 (0.15)	1.72 (0.16)	1.72 (0.16)
Total	8.56 (0.39)	8.55 (0.41)	8.66 (0.25)	8.63 (0.26)	8.46 (0.31)	8.44 (0.36)

Table 2.

The association of regional volumetric measurements with AMD classification. Total retina (inner limiting membrane to basal Bruch's membrane), ONL+ (outer plexiform layer to the external limiting membrane (ELM)), outer retina+ (ELM to Bruch's membrane), and RPE+ (RPE and Bruch's membrane). CO = foveal region, N=nasal, T=temporal, I=inferior, and S=superior retina.

Total Retina		**CO**	**N1**	**N2**	**S1**	**S2**	**T1**	**T2**	**I1**	**I2**
No drusen		279.4 (3.3)	344.5 (2.3)	310.4 (2.5)	341.9 (2.4)	295.7 (2.1)	329.8 (2.3)	280.7 (1.8)	339.4 (2.3)	284.1 (2.2)
Early AMD		279.0 (2.9)	344.6 (1.7)	314.5 (1.9)	343.7 (1.8)	298.9 (1.3)	331.3 (1.6)	284.0 (1.2)	340.9 (1.7)	289.1 (1.5)
Intermediate AMD		276.5 (3.5)	336.2 (2.6)	306.4 (1.9)	331.3 (3.0)	292.5 (1.8)	322.8 (2.5)	279.2 (1.4)	332.7 (2.7)	282.9 (1.4)
Photoreceptors+										
No drusen		86.8 (0.61)	80.4 (0.3)	77.0 (0.25)	79.7 (0.29)	77.9 (0.27)	79.6 (0.27)	76.9 (0.26)	78.6 (0.29)	75.9 (0.27)
Early AMD		84.5 (0.45)	80.4 (0.28)	77.0 (0.25)	79.5 (0.23)	78.2 (0.21)	79.7 (0.23)	77.4 (0.25)	78.7 (0.25)	76.7 (0.22)
Intermediate AMD		88.8 (0.84)	82.0 (0.67)	78.2. (0.73)	81.5 (0.73)	79.8 (0.76)	82.7 (1.06)	78.7 (0.85)	81.6 (0.95)	77.5 (0.76)
ONL+										
No drusen		93.2 (1.39)	72.1 (1.68)	54.4 (1.21)	69.1 (1.34)	59.4 (1.03)	71.3 (1.33)	56.5 (1.0)	65.1 (1.83)	51.3 (0.98)
Early AMD		96.2 (0.88)	75.1 (0.67)	58.0 (1.12)	69.7 (1.19)	61.1 (0.91)	74.2 (0.95)	58.2 (0.88)	69.1 (1.16)	53.9 (0.88)
Intermediate AMD		62.1 (0.73)	91.9 (1.99)	75.2 (1.28)	57.0 (0.69)	65.1 (2.07)	58.5 (1.09)	70.9 (1.09)	56.7 (0.92)	69.4 (0.97)
RPE+										
No drusen		15.9 (0.2)	14.3 (0.17)	12.4 (0.18)	14.4 (0.15)	12.7 (0.14)	13.7 (0.13)	12.3 (0.14)	13.7 (0.19)	12.2 (0.16)
Early AMD		16.0 (0.21)	14.7 (0.28)	12.5 (0.16)	14.5 (0.18)	12.9 (0.14)	14.1 (0.14)	12.8 (0.15)	13.9 (0.18)	12.6 (0.13)
Intermediate AMD		20.3 (0.79)	15.8 (0.36)	13.3 (0.36)	15.8 (0.48)	14.2 (0.36)	17.2 (0.72)	14.0 (0.57)	15.9 (0.43)	13.1 (0.41)