Clinical Trials

Small, Hard Macular Drusen and Peripheral Drusen: Associations with AMD Genotypes in the Inter99 Eye Study

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Purpose. To study associations of small, hard macular drusen and peripheral drusen with genotypes associated with age-related macular degeneration (AMD).

Methods. Digital grayscale fundus photographs recorded in red-free illumination were graded for the presence of drusen in 1107 subjects aged 30 to 66 years. Participants were genotyped for AMD-related polymorphisms in complement factor H (*CFH*), in *LOC387715*, and in complement factor B (*CFB*).

Results. The prevalence of 20 or more small, hard macular drusen per eye was 14%, with no association to the investigated polymorphisms. Peripheral drusen were associated with *CFHY402H* (odds ratio [OR], 4.3; 95% confidence interval [95% CI], 1.4–13, for CC versus TT genotypes) as was macular drusen >63 μm (OR, 1.9; 95% CI, 1.1–3.1, for CC versus TT genotypes). Macular drusen >63 μm were associated with the presence of 20 or more small, hard macular drusen (OR, 1.7; 95% CI, 1.1–2.6) and with peripheral drusen (OR, 2.5; 95% CI,1.2–5.4)

Conclusions. In this study, the presence of 20 or more small, hard macular drusen per eye was not associated with known AMD-related polymorphisms, whereas the study confirmed an association of peripheral drusen with *CFHY402H.* (ClinicalTrials.gov number, NCT00289237.) (*Invest Ophthalmol Vis Sci.* 2010;51:2317–2321) DOI:10.1167/iovs.09-4482

Small, hard drusen in the retinal pigment epithelium are common in humans.[1] The prevalence was 93.6% in the Beaver Dam Eye study of subjects aged 43 to 86 years.[2] A large number of small, hard drusen in the macula is associated with increased risk of developing large, soft drusen and fundus pigment abnormalities.[3–7] Specifically, the Beaver Dam Eye Study found a more than threefold increased age-adjusted incidence of soft, indistinct drusen over 15 years in eyes with eight or more small, hard drusen at baseline, compared with eyes with one or two small, hard drusen per eye at baseline.[5]

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We found a heritability of 99% of the characteristic of more than 20 small, hard drusen in twins aged 20 to 46 years.[8] Comparable results have been found in a study of women aged 49 to 79 years,[9] in which 11.9% of participants had 20 or more small, hard macular drusen.

The prominent heritability of small, hard drusen and their association with age-related macular degeneration (AMD) has prompted us to examine small, hard drusen in relation to genetic polymorphisms known to be associated with AMD.[10–15]

Methods

Study Population

The Inter99 Study is a population-based study of cardiovascular and metabolic characteristics and lifestyle.[16] An age- and sex-stratified random sample of 13,016 subjects from seven birth cohorts (years of birth 1939–40, 1944–45, 1949–50, 1954–55, 1959–60, 1964–65, and 1969–70) from suburban Copenhagen were invited to participate, of which 6,906 volunteered. From the main study, 122 individuals were excluded because of alcoholism, drug abuse, or limited cooperation, leaving 6784 (52.5%) for analysis. The Inter99 Eye Study[17] was designed to investigate retinal characteristics in relation to risk factors for cardiovascular disease such as diabetes, elevated blood pressure, and high body mass index (BMI). The Inter99 Eye Study examined 1107 subjects from the Inter99 Study, comprising 567 subjects representative of the background population and a skewed sample of 540 subjects with cardiovascular high-risk profile. Parameters used to calculate the risk of cardiovascular disease were oral glucose tolerance test data, systolic blood pressure, total cholesterol and high density lipoprotein level, BMI, history of diabetes, history of cardiovascular disease, and family history of cardiovascular disease. Exclusions made before data analysis comprised 6 subjects with diabetic retinopathy that precluded reliable drusen counting, 86 with photographs of one or both maculae missing or of inferior quality, 1 with macular dystrophy, and 7 with clerical data errors. Inter99 Eye Study baseline examinations were conducted from 1999 to 2001 and from 2004 to 2006. All participants gave their informed consent. Ethics committee approval was obtained, and the research adhered to the tenets of the Declaration of Helsinki.

Phenotyping

All subjects underwent a general ophthalmic examination including fundus photography with seven-field monochromatic 60° digital fundus photography (TRC-50X camera; Topcon Corp., Tokyo, Japan, with 1024 × 1024 pixel CV-1000 back-piece, AngioVision 1000; MediVision, Yokneam Elit, Israel) in red-free illumination (Wratten 54 filter; Eastman Kodak, Inc., Rochester, NY) and color diapositive photography (Ektachrome Elite 100; Eastman Kodak, Rochester, NY).

Fundus characteristics were assessed by a single ophthalmologist (ICM) masked to sex, age, systemic parameters, and genotypes. Digital images were examined on a computer screen and diapositive photo-

Table 1. Characteristics of the Study Population

Characteristic	Included (n = 1007)	Not Included (n = 5777)	P*
Age, y mean (SD)	48.4 (7.95)	45.7 (8.0)	<0.0001*
BMI, kg/m^2 mean (SD)	28.0 (5.3)	26.1 (4.5)	<0.0001*
Systolic blood pressure, mm Hg mean (SD)	133 (17.8)	128 (15.6)	<0.0001*
Diastolic blood pressure, mm Hg mean (SD)	84.1 (11.1)	80.8 (10.5)	<0.0001*
Sex, n (%)			0.42†
Males	502 (49.8)	2800 (48.5)	
Females	505 (50.2)	2977 (51.5)	
Smoking, n (%)	398 (39.8)	2233 (39.0)	0.61†
Diabetes, n (%)	307 (30.5)	181 (3.13)	<0.0001†
CFHY402H:rs1061170, TT/TC/CC (%)	355/436/152 (38/46/16)	2085/2587/783 (38/47/14)	0.36†
Number with genotype information	943	5455	
LOC387715A69S:rs10490924, CC/CT/TT (%)	610/286/45 (65/30/5)	3443/1783/255 (63/33/5)	0.43†
Number with genotype information	941	5481	
HtrA1:rs11200638, GG/GA/AA (%)	589/279/44 (65/31/5)	3348/1706/247 (63/32/5)	0.63†
Number with genotype information	912	5301	
CFBR32Q:rs641153, GG/GA/AA (%)	824/127/3 (86/13/0.31)	4742/737/26 (86/13/0.47)	0.79†
Number with genotype information	954	5505	
CFBL9H:rs4151667, TT/TA/AA (%)	874/78/4 (91/8.2/0.42)	5051/446/13 (92/8.1/0.24)	0.59†
Number with genotype information	956	5510	

Subjects included constitute a subpopulation of participants in the larger Inter99 Study.
* Student's t-test.
† χ^2 test.

graphs by using a hand-held pair of 15-D lenses. Histogram stretching was allowed during the evaluation of the digital photographs. Small, hard drusen were defined as any bright element with a diameter equal to or smaller than 63 μm, the shape, color or proximity to adjacent disease of which did not suggest that it could be hard exudate, subretinal precipitate, or focal loss of retinal pigment epithelium without drusen formation. This definition excluded drusen associated with nevi. When lesions on digital images were deemed questionable or other retinal disease was present, color diapositives were inspected, and a second ophthalmologist was consulted.

The number of small, hard drusen ≤63 μm was counted within a circle centered on the foveola, with a radius stretching to the temporal rim of the optic disc. Within this circle, the presence of drusen >63 μm was also noted. Both eyes were evaluated. Only subjects with gradable photographs from both eyes were included. Stippling of the retinal pigment epithelium was defined as diffusely scattered, minute, drusenlike elements resulting in a granular appearance of the fundus.[18] Peripheral drusen were defined as drusen >63 μm or confluent small, hard drusen located outside the temporal vascular arcades or nasal to the optic disc. Cuticular and reticular drusen were not graded separately, because they were included in the definition of peripheral drusen. Images of the peripheral retina were obtained only in red-free illumination, which precludes visualization of pigmentation features. Drusen anterior to the equator were not graded because this part of the fundus was covered only partly by the photography protocol.

The diagnosis of diabetes mellitus was based on oral glucose tolerance testing according to the WHO1999 criteria.[19] Blood pressure was measured twice with the patient supine and having rested for at least 5 minutes, by using standard mercury sphygmomanometry with an appropriately sized cuff. Smoking information was assessed by questionnaire.

Genotyping

DNA from whole blood was analyzed for five single-nucleotide polymorphisms (SNPs) associated with AMD: (1) complement factor H (*CFH)*, variant Y402H (rs1061170), nucleotide change T→C; (2) *LOC387715* (age-related macular degeneration susceptibility, *ARMS2*), variant A69S (rs10490924), nucleotide change C→T; (3) high temperature requirement factor A1(*HtrA1*), variant in the promoter region (rs11200638), nucleotide change G→A; (4) complement factor B (*CFB*), variant R32Q (rs641153), nucleotide change G→A; and (5) *CFB*, variant L9H (rs4151667), nucleotide change T→A. Genotyping of the five SNPs was performed by allelic discrimination (*Taq*Man; KBiosciences, Herts, UK). All genotyping success rates were above 95% (95.2%–98.8%). For each variant, 469 samples were genotyped in duplicate, with a mismatch rate below 0.7%. The distribution of genotypes for all variants was in Hardy-Weinberg equilibrium.

Statistical Analyses

In all analyses (SAS ver. 9.1; SAS Institute, Inc., Cary, NC), age was treated categorically in the following groups: 30 to 31, 35 to 36, 40 to 41, 45 to 46, 50 to 51, 55 to 56, 60 to 61, and 65 to 66 years (some individuals had their first examination in 2004–2006). Smoking history was coded into two groups: having smoked and never having smoked. Logistic regression (PROC LOGISTIC) was used to estimate odds ratio (OR) and its 95% confidence interval (95% CI). Test for trend was performed for categorical variables at several levels by considering the variable as a continuous ordered factor in logistic regression models. Test for multiplicative interaction between *CFHY402H* and recruitment group was calculated by inserting a cross-product term in the model. Power estimation was performed using the analyst application in SAS. The number of small, hard drusen was given as the mean of the subject's eyes. In all analyses, the individual subject was the unit of analysis.

Results

Genotype distributions in the eye study participants were comparable to those of the overall population of 6784 Inter 99 Study participants (Table 1). In agreement with the prespecified subgroup composition, the eye study participants were slightly older (48.4 vs. 45.7 years), had a higher BMI (28.0 vs. 26.1 kg/m^2), higher systolic (133 vs. 128 mm Hg) and diastolic (84.1 vs. 80.8 mm Hg) blood pressures, and a higher prevalence of diabetes (30.5% vs. 3.13%; Table 1).

Five or more small, hard drusen per eye (mean of two eyes) were found in 39% of the subjects, 10 or more in 26%, 20 or more in 14%, and 50 or more in 4.1% of the subjects (data not presented). The frequency of having 20 or more small, hard macular drusen increased with age, from 5.8% in subjects 30 to 36 years old, >13% in subjects aged 40 to 46 years, and 14% in subjects aged 50 to 56 years, to 19% in subjects aged 60 to 67 years (Table 2). The presence of peripheral drusen was signif-

TABLE 2. Distribution of Drusen

Age Group (y)	*n*	Males *n* (%)	Macular Drusen >63 μm *n* (%)	Peripheral Drusen *n* (%)	Stippling *n* (%)	≥20 Small, Hard Macular Drusen *n* (%)
30–36	87	26 (29)	4 (4.6)	0	1 (1.2)	5 (5.8)
40–46	356	174 (49)	35 (9.8)	4 (1.1)	13 (3.7)	47(13)
50–56	437	225 (51)	72 (16)	15 (3.4)	16 (3.7)	61 (14)
60–66	127	77 (61)	38 (30)	14 (11)	5 (3.9)	24 (19)
Total	1007	502 (50)	149 (15)	33 (3.3)	35 (3.5)	137 (14)
Sex adjusted for age group, OR (95% CI)						
Male			1	1	1	1
Female			1.4 (0.96–2.0)	2.6 (1.2–5.6)	1.3 (0.64–2.5)	1.4 (0.96–2.0)
			$P = 0.087$	$P = 0.012$	$P = 0.51$	$P = 0.081$
Age group adjusted for sex, OR (95% CI)						
30–36			0.42 (0.14–1.2)	—	0.29 (0.038–2.3)	0.38 (0.15–0.98)
40–46			1	1	1	1
50–56			1.8 (1.2–2.8))	3.2 (1.1–9.8)	1.0 (0.48–2.1)	1.1 (0.71–1.6)
60–66			4.1 (2.4–6.9)	12 (4.0–39)	1.1 (0.30–3.2)	1.6 (0.93–2.7)
			$P < 0.0001$*	$P < 0.0001$*	$P = 0.68$*	$P = 0.0084$*

* *P* for trend.

icantly more frequent in women than in men (OR, 2.6; 95% CI, 1.2–5.6; Table 2).

One or more macular drusen >63 μm in either eye was found in 15% of subjects. The prevalence increased with age, from 4.6% in subjects aged 30 to 36 years to 30% in subjects aged 60 to 66 years (Table 2). Peripheral drusen were present in 33 subjects. The prevalence increased with age from 0 in subjects aged 30 to 36 year to 11% in subjects aged 60 to 66 years (Table 2). Of the 33 participants with peripheral drusen, 17 had no drusen in the macula, 3 had 20 or more small, hard drusen in the absence of other graded features, 10 had macular drusen >63 μm, and 3 had both macular drusen >63 μm and 20 or more small, hard drusen (data not presented). Stippling was observed in 3.5% of subjects, without any discernible effect of age or sex (Table 2).

The presence of macular drusen >63 μm was associated with the presence of 20 or more small, hard macular drusen (OR, 1.7; 95% CI, 1.1–2.6; $P = 0.029$, adjusted for age and sex; Table 3) and remained so after further adjustment for *CFHY402H* (OR, 1.8; 95% CI, 1.4–3.0; $P = 0.012$). Macular drusen >63 μm were also associated with peripheral drusen (OR, 2.5; 95% CI, 1.1–5.4; $P = 0.015$; Table 3).

Peripheral drusen were associated with *CFHY402H* with an OR of 4.3 (95% CI, 1.4–13) for genotype CC versus genotype TT and an OR of 2.4 (95% CI, 0.87–6.7) for the heterozygous genotype CT versus the homozygous genotype TT (ORs adjusted for the presence of macular drusen >63 μm). Likewise, an association was found between *CFHY402H* and the presence of macular drusen >63 μm, with an OR of 1.8 (95% CI, 1.1–3.0), for CC versus TT, and an OR of 1.2 (95% CI, 0.79–1.8), for CT versus TT (Table 4). In the subgroup analysis, the association between *CFHY402H* and macular drusen >63 μm was present only in the group of persons recruited because of cardiovascular high-risk profile, and there was significant interaction between recruitment group and *CFHY402H* (Table 4). For the presence of 20 or more small, hard macular drusen, there was no significant interaction between *CFHY402H* and recruitment group. Subgroup analysis on peripheral drusen and stippling was not performed because of the small sample.

Stippling and the presence of more than 20 small, hard macular drusen were not associated with *CFHY402H* (Table 4) or any other of the polymorphisms assessed in the study. The power to detect a twofold increase in the proportion of having 20 or more small, hard macular drusen in subjects with one or two copies of *CFHY402H* was 0.93 at a significance level of 0.05. No significant association was found between any investigated phenotype and the polymorphisms *LOC387715A69S*, *HtrA1:*rs11200638, *CFBR32Q*, and *CFBL9H* (data not presented).

TABLE 3. Association of Macular Drusen ≤63 μm and >63 μm with the Presence of Other Drusen

	All Macular Drusen ≤63 μm *n* (%)	Macular Drusen >63 μm *n* (%)
≥20 Small, hard macular drusen OR (95% CI)*	106/858 (12) 1	31/149 (21) 1.7 (1.1–2.6) $P = 0.029$
Peripheral drusen OR (95% CI)*	20/858 (2.3) 1	13/149 (8.7) 2.5 (1.2–5.4) $P = 0.015$
Stippling OR (95% CI)*	29/858 (3.4) 1	6/149 (4) 1.1 (0.44–2.8) $P = 0.83$

* Adjusted for age and sex.

DISCUSSION

This study provided a unique opportunity to examine small, hard drusen and early AMD-related retinal lesions in a population of relatively young subjects aged 30 to 66 years, of which few had AMD, but many are destined to develop the disease. The prevalence of numerous small, hard drusen in the macula increased with age. Despite being a highly heritable condition[8,9] associated with future development of AMD,[3,5–7,20] the presence of numerous small, hard drusen lacked a detectable association with known AMD-related genotypes. Specifically, no association was found between the presence of more than 20 small, hard macular drusen and the polymorphisms *CFHY402H*, *LOC387715A69S*, *HtrA1*:rs11200638, *CFBR32Q*, and *CFBL9H*.

Previous studies of AMD genetics have emphasized advanced stages of AMD[21,22] and applied a threshold definition of AMD of one soft, distinct drusen >63 μm[23–25] or >125 μm.[25,26] Consequently, subjects with only small, hard drusen have systematically been assigned to control groups. Other studies have been confined entirely to subjects with AMD.[27,28] Such study designs cannot yield information about the genetic background of small, hard macular drusen.

TABLE 4. Drusen Phenotypes in Relation to *CFHY402H* by Recruitment Subgroup

	CFHY402H:rs1061170		
	TT	**TC**	**CC**
Macular Drusen >63 μm			
All participants			
n (%)	44/355 (12)	63/436 (14)	31/152 (20)
OR (95% CI)	1	1.2 (0.79–1.8)	1.8 (1.1–3.0)
P (trend)			0.029
Stratified by recruitment group, *P*-value for interaction = 0.0045			
Cardiovascular disease high-risk characteristics			
n (%)	17/167 (10)	36/210 (17)	21/72 (29)
OR (95% CI)	1	1.8 (0.99–3.4)	3.6 (1.8–7.4)
P (trend)			0.0004
Population-matched			
n (%)	27/188 (14)	27/226 (12)	10/80 (13)
OR (95% CI)	1	0.81 (0.46–1.4)	0.85 (0.39–1.9)
P (trend)			0.57
≥20 Small Hard Drusen*			
All participants			
n (%)	43/355 (12)	72/436 (17)	14/152 (9)
OR (95% CI)	1	1.4 (0.94–2.1)	0.69 (0.36–1.3)
P (trend)			0.70
Stratified by recruitment group *P*-value for interaction = 0.24			
Cardiovascular disease high-risk characteristics			
n (%)	24/167 (14)	37/210 (18)	6/72 (8.3)
OR (95% CI)	1	1.2 (0.70–2.2)	0.49 (0.19–1.3)
P (trend)			0.36
Population-matched			
n (%)	19/188 (10)	35/226 (15)	8/80 (10)
OR (95% CI)	1	1.7 (0.93–3.11)	1.0 (0.42–2.4)
P (trend)			0.56
Stippling*			
All participants			
n (%)	11/355 (3.1)	16/436 (3.7)	7/152 (4.6)
OR (95% CI)	1	1.2 (0.55–2.6)	1.5 (0.57–4.0)
P (trend)			0.41
Peripheral Drusen*			
All participants			
n (%)	5/355 (1.4)	15/436 (3.4)	10/152 (6.6)
OR (95 % CI)	1	2.4 (0.87–6.7)	4.3 (1.4–13)
P (trend)			0.0073

* Adjusted for macular drusen >63 μm.

Several studies have shown a decreasing number of small, hard drusen with age, concomitant with an increasing prevalence of larger drusen.[20,29,30] Our finding of an increasing prevalence of small, hard drusen with increasing age is not in conflict with these studies because nearly all our participants were sufficiently young not to have had their small, hard drusen replaced by larger drusen. Our result is comparable to a study of female twins aged 49 to 74 years, in which the prevalence of having 20 or more small, hard macular drusen was found to increase with age.[9]

The present study confirmed that the prevalence of macular drusen >63 μm, a trait that falls within the definition of AMD, increases with age and that these drusen were associated with *CFHY402H*. We observed a significant association between *CFHY402H* in patients who had a high-risk cardiovascular profile. This finding suggests that *CFHY402H* may exert its effect through cardiovascular risk factors, although previous studies of advanced AMD have failed to identify a significant interaction between cardiovascular risk factors and *CFHY402H*.[31,32] We observed no association between macular drusen >63 μm and the polymorphisms *LOC387715A69S*, *HtrA1*:rs11200638, *CFBL9H*, or *CFBR32Q*. This result is in agreement with a recent study of drusen ≥63 μm in very early AMD.[23]

In the present study, the prevalence of peripheral drusen, most of which were found immediately outside the temporal vascular arcades or on the nasal side of the optic disc, increased with age, and it was higher in the women than in the men. The presence of peripheral drusen was associated with *CFHY402H*, with a fourfold increase in the odds of peripheral drusen among subjects homozygotic for *CFHY402H* compared with those in subjects without this polymorphism. Previously, a threefold increase has been observed in a study of elderly subjects.[33] The coupling of peripheral drusen to *CFHY402H* may explain why peripheral drusen are significantly more common among AMD patients with affected family members than in isolated cases of AMD.[34] It is thus increasingly evident that peripheral drusen are part of the morphologic and genetic spectrum of AMD.

Fundus stippling, which appears like hundreds or thousands of minute drusen spread over the entire fundus or large parts of it, was found in 3.5% of subjects independent of age, sex, and AMD genotypes and without any detectable association with macular drusen >63 μm. This finding indicates that fundus stippling is a nondegenerative condition without any association with AMD.

The present study had the power to exclude major associations between small, hard drusen and known AMD genotypes,

whereas weak associations may have escaped detection. Another limitation is that the number of subjects with drusen was too small, in our opinion, to justify classification and analysis by subtype (e.g., intermediate, confluent, reticular, cuticular). Furthermore, subjects with increased cardiovascular risk were overrepresented in the study. Nevertheless, the prevalence of small, hard drusen and peripheral drusen did not differ from that in previous studies.[9,33]

In summary, we examined fundus lesions that precede, but are not included in the current definition of AMD, and the results confirmed the association of *CFHY402H* with macular drusen >63 μm and with peripheral drusen. On the contrary, we did not observe an association between *CFHY402H* and numerous small, hard macular drusen, despite such drusen predisposing for the development of larger drusen. Specifically, our data showed no association between having 20 or more small, hard macular drusen per eye and the AMD-related polymorphisms *CFHY402H* and *LOC387715A69S*. We conclude that there may be unknown genetic determinants of this hereditary AMD-precursor.

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