Heredity of Small Hard Drusen in Twins Aged 20–46 Years

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Purpose. To examine the prevalence and heredity of small hard drusen in 220 healthy twins aged 20–46 years.

Methods. Grayscale digital fundus photography, four-field 50° nonstereoscopic, in red-free illumination was performed in 58 pairs of monozygotic (MZ) twins and 52 pairs of dizygotic (DZ) twins as part of a detailed biometric characterization. Small hard drusen (diameters, <63 μm) were counted and graded by distribution type.

Results. Small hard drusen were present in 212 of the 220 subjects. Five or more drusen per eye were found in 89 subjects, in three patterns of distribution: scattered drusen (66 subjects), macular drusen (18 subjects), and stippled, innumerable drusen (5 subjects). When analyzed as a continuous trait, the heritability of small hard drusen was 63% (95% confidence interval [CI], 43% to 77%). More than 20 drusen per eye were found in 26 subjects, and the heritability of this phenotype was 99% (95% CI, 82% to 100%).

Conclusions. Hard drusen are prevalent in young adults, and having more than 20 drusen per eye is a highly hereditary feature. Additional research is needed to determine whether the presence of small hard drusen correlates with the development of age-related macular degeneration later in life and to explore the relation to AMD genotypes. (*Invest Ophthalmol Vis Sci.* 2007;48:833–838) DOI:10.1167/iovs.06-0529

Small hard drusen are a common finding in healthy young and middle-aged subjects.[1,2] It is uncertain whether their presence predicts the development of symptomatic eye disease later in life and whether they offer an opportunity to monitor the effect of early intervention. It has been shown that large numbers of hard drusen predict the incidence of soft drusen and fundus pigmentation abnormalities, which are associated with an increased risk for geographic atrophy and exudative maculopathy.[3,4] Development of age-related maculopathy (ARM) and age-related macular degeneration (AMD) is highly dependent on genetic factors. Notably, a study of 840 elderly male twins demonstrated a heritability of 46% for overall AMD grade and 71% for advanced AMD.[5] If small hard drusen are indeed precursors for AMD, a genetic effect should be detectable in the distribution of small hard drusen in twins. A heritability of 81% of the phenotype ≥20 hard drusen per eye has previously been found in 506 female twin pairs (age range, 49–79 years).[6] In the present study, we examined the prevalence, systemic associations, concordance, and heritability of small hard drusen through the use of red-free fundus photography in twins of both sexes (age range, 20–46 years).

Methods

Subjects and Protocol

We examined 59 monozygotic (MZ) and 55 dizygotic (DZ) same-sex twin pairs 20 to 46 years of age. The subjects were recruited from the population-based Danish Twin Registry,[7] which includes more than 65,000 twin pairs in birth cohorts from 1870 to 1996. The ascertainment rate was 90% up to 1968 and 100% after 1968, when a fully comprehensive computerized national population database was introduced (Danish Civil Registry). Zygosity was determined through nine polymorphic DNA-based microsatellite markers (AmpFISTR Profiler Plus Kit; Perkin Elmer Applied Biosystems, Foster City, CA). This principle has an error probability of 0.003% or lower.[8] The twins participated in a larger study of diabetes-related metabolism,[9] for which participants were invited by way of a mailed questionnaire. Exclusion criteria were pregnancy, breastfeeding, known diabetes or cardiovascular disease, and conditions preventing the completion of an ergometric bicycle test. Of 2099 invited pairs, 764 pairs were eligible and willing to participate. Randomized exclusions were made in specific age groups to achieve a uniform age distribution, reducing the participant number to 621. Of these, twin pairs in which both twins lived on the island of Zeeland (Sjælland) were invited to participate in a separate ophthalmic examination, for which 114 pairs volunteered. Previously published data include lens autofluorescence,[10] retinal nerve fiber layer thickness,[11] the presence of cilioretinal arteries,[12] and retinal vessel diameters.[13] The present study excluded one MZ twin pair and three DZ twin pairs because fundus photography was unavailable on the day of examination. Thus, the study included 58 MZ and 52 DZ twin pairs.

All participants gave their informed consent. The study was approved by the regional medical ethics committee and followed the tenets of the Helsinki Declaration.

Subjects responded to a detailed questionnaire including information about lifelong smoking habits. Study examinations that have been described in detail[9–12] included oral glucose tolerance testing, blood pressure measurement, blood sampling, and measurement of height and weight. The ophthalmic examination included refractioning, determination of Snellen visual acuity, pupil dilation, slit lamp biomicroscopy, nonstereoscopic digital grayscale fundus photography on a

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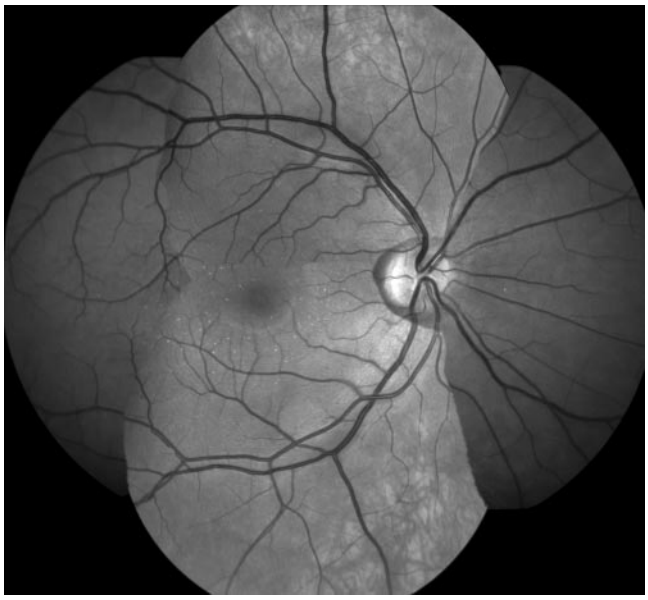

FIGURE 1. Montage of red-free fundus photographs representing macular distribution type of small hard drusen. In this eye, 75 small hard drusen were counted with the aid of digital contrast enhancement.

1024 × 1024-pixel image sensor, four 50° fields per eye, a single 20° optic disk–centered photograph in red-free illumination (Wratten 54 filter; Eastman Kodak, Inc., Rochester, NY), and color diapositive photography (four 50° fields per eye).

Fundus Grading

Fundus characteristics were visually assessed by examination of digital images on a computer screen and by diapositive inspection using a handheld 15-D lens. Histogram stretching was allowed during evaluation of the digital photographs. Small hard drusen were defined as any bright element smaller than 63 μm in diameter whose shape, color, or proximity to adjacent abnormality could not suggest that it was hard exudate. Specific attention was also given to the differential diagnosis of small hard drusen versus subretinal precipitate and multifocal retinal pigment epithelium depigmentation secondary to central serous chorioretinopathy.[14] Drusen associated with nevi were not included. A single observer (ICM), masked to the zygosity and relatedness of the subjects, examined all grayscale photographs in random order. Each drusen was counted and marked, regardless of location in the fundus, except in subjects with stippling, in whom the drusen were too numerous and too difficult to distinguish from the background. When lesions were deemed questionable or another retinal abnormality was present, color diapositives were evaluated and a second observer (ML) was consulted. After the first round of examination, the observer was able to identify three patterns of distribution of drusen. The photographs were reevaluated twice. Subjects with five or more drusen per eye (mean of the subject's two eyes) could consistently be classified into one of three categories: diffusely scattered drusen (not shown), macular drusen (Fig. 1), and stippled fundus with innumerable drusen (Fig. 2). The latter category was characterized by subtle stippling that appeared to represent minute drusen, ranging from definitely visible small hard drusen to smaller elements of the same type visible only after stretching of the luminosity range of the photograph. Manual counting of selected fundus areas and subsequent extrapolation to the entire combination of photographic field yielded estimates in excess of 1000 elements per eye. This phenotype has been described previously.[15]

Statistical Procedures

Classical twin data analysis is based on the assumption that MZ twins have identical genotypes; for this reason, all observed differences between twins in a pair are attributable to environmental factors. On average, however, DZ twins share 50% of their genes. The extent to which MZ twins are more alike than DZ twins is therefore assumed to reflect additional genetic sharing. Heritability is defined as the proportion of the total phenotypic variance attributable to genetic variance[16] and is calculated by means of structural equation modeling. Structural equation modeling quantifies sources of individual variation by decomposing the observed phenotypic variance into genetic and environmental variance. The genetic contribution can be further divided into an additive (A) genetic variance component, representing the influence of alleles at multiple loci acting in an additive manner, and a nonadditive (D) genetic variance component, representing intralocus interaction (dominance) and interlocus interaction (epistasis) of alleles. The environmental component can be subdivided into a common (C) environmental variance component, representing environmental factors affecting both twins in a pair, a source of similarity, and a random (E) environmental variance component, representing environmental factors not shared by twins, a source of dissimilarity that includes random factors and measurement errors.[17]

To evaluate the relative importance of genetic and environmental influences on the number of hard drusen, structural equation modeling was used to fit different models to the observed data under a number of standard assumptions—no gene-environment interaction, random mating, and equal intrapair environments in MZ and DZ twin pairs. Stippled fundus was considered a separate phenotype, as opposed to the quasi-continuum found in the rest of the population; hence, five subjects with stippled fundi and their twins (eight subjects altogether, from 4 DZ twin pairs) were excluded from the heritability analysis. Thus, 116 MZ and 96 DZ twins remained. To deal with the highly discrete, skewed, and nonnormal distribution of the total number of drusen, a liability-threshold model was used.[18] For this purpose, the number of drusen per subject was grouped as follows: <1, 1–5, 6–10, 11–20, 21–40, >40. The liability-threshold model is based on the

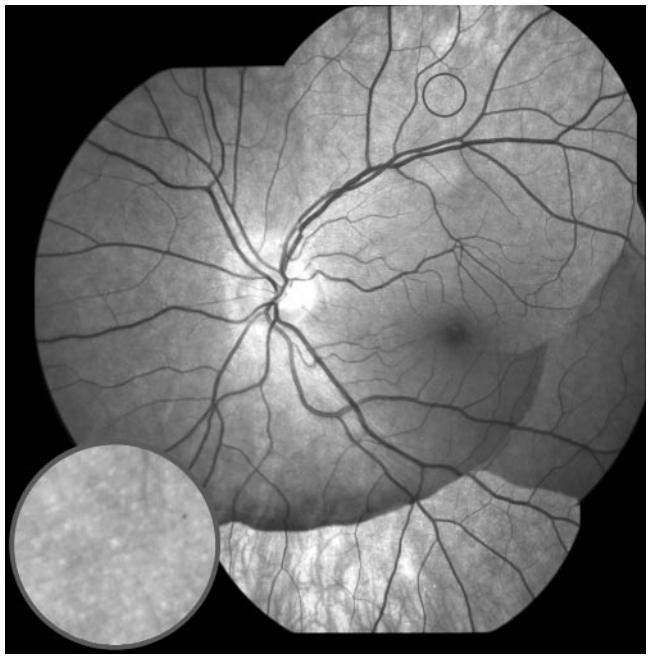

FIGURE 2. Montage of red-free fundus photographs representing small hard drusen of stippled, innumerable distribution type, with hundreds of drusen or drusen-like elements that ranged from those apparent at standard magnification and unaltered contrast to those distinguishable only after magnification and contrast enhancement (*inset*).

TABLE 1. Clinical Characteristics of Healthy Monozygotic and Dizygotic Twins*

Characteristic	MZ Twins	DZ Twins	*P*†
Pairs	58	48	—
Subjects	116	96	—
Male/female subjects	54/62	42/54	—
Smokers/nonsmokers	45/71	44/52	—
Pack years (smokers only)	8.04 (6.07)	10.04 (7.81)	0.23
Age (years)	34.9 (7.53)	34.6 (7.46)	0.82
Drusen per eye	6.01 (10.2)	8.52 (13.5)	0.22
Mean arterial blood pressure (mm Hg)	85.2 (10.1)	86.0 (8.78)	0.62
Body mass index (kg/m^2)	23.6 (3.53)	23.8 (3.77)	0.66
Fasting blood glucose‡ (mM)	4.88 (0.48)	4.86 (0.41)	0.79
2-h oral glucose tolerance test‡ (mM)	6.08 (1.26)	6.03 (1.20)	0.81
Total cholesterol (mM)	5.24 (1.00)	5.60 (1.08)	0.045
High-density lipoprotein (mM)	1.52 (0.35)	1.55 (0.48)	0.56
Low-density lipoprotein (mM)	3.18 (0.92)	3.39 (1.0)	0.21
Very-low-density lipoprotein (mM)	0.509 (0.19)	0.610 (0.27)	0.0093
Triglyceride (mM)	1.19 (0.74)	1.40 (0.70)	0.072

All values represent mean (SD) except pairs, subjects, sex, and numbers of smokers, which are given in actual numbers.

* After exclusion of pairs in which one subject or both subjects demonstrated the stippled fundus phenotype.

† Robust *t* test (adjusted for clustering).

‡ Capillary blood samples.

notion of an underlying bivariate normal liability variable related to the category of the number of drusen by means of a set of thresholds. For example, if the liability of a twin is between the first and second thresholds, the total number of drusen for this person is between 1 and 5. The liability variable is then decomposed into genetic and environmental components according to the methods of quantitative genetics. Respective proportions of variance (including heritability of liability) are estimated according to the maximum likelihood method, as implemented in a specialized software package (MX Statistical Modeling, Medical College of Virginia, VA). To adjust for the effects of age and sex, a linear relationship between the mean liability and the two covariates was assumed, resulting in two additional regression coefficients to be estimated.

Casewise concordance describes the risk for a certain phenotype given that the twin partner has this phenotype, and it is calculated from the formula $2C/(2C + D)$, where C is the number of concordant pairs and D is the number of discordant pairs. A confidence interval for the observed value can be calculated as previously described.[19]

The traditional *t* test used for comparison of means in a two-sample case is based on the assumptions of normality and independent observations. However, not all variables in the present dataset are normally distributed. Moreover, there is potential interdependence between the observations because they consist of members of twin pairs ("clustering"). To resolve this problem, we applied linear regression analysis using the so-called robust covariance matrix estimation, which takes clustering into account[20,21] as implemented in the statistical software (Stata 9; StataCorp, College Station, TX). Thus, the *P* values reported in Table 1 regarding comparisons of MZ and DZ twins are robust with respect to deviations from normality and presence of intrapair correlation.

RESULTS

Examination of the study population of 58 MZ and 52 DZ same-sex twin pairs of both sexes aged 20–46 years demonstrated that the MZ and DZ twin groups were comparable with regard to age, sex distribution, drusen number, smoking, and other study variables, except that the DZ twins had slightly higher plasma lipid concentrations (Table 1). Epiretinal fibrosis was found unilaterally in two subjects, a nevus with overlying drusen was found unilaterally in two subjects, scarring from multifocal choroiditis was found bilaterally in one subject, and a single drusen measuring between 63 μm and 125 μm in diameter was found unilaterally in two subjects. All these findings were made in only one member of a twin pair. None of these subjects were excluded from the study.

The presence of one or more small hard drusen in at least one eye was detected in 212 of the 220 (96%) subjects (Fig. 3). The mean number of small hard drusen was 6.0 in MZ twins and 8.5 in DZ twins ($P = 0.22$; Table 1). Masked recounting of 22 randomly selected subjects demonstrated a weighted kappa statistic of 0.67, indicating substantial agreement in drusen number used in the threshold-liability model. Five or more drusen per eye (the mean of both a subject's eyes) were found in 89 of 220 (40%) subjects. In five (2.2%) subjects, innumerable drusen—several hundreds or thousands per eye—created a stippled, granular fundus appearance in both eyes. The other subjects had fewer than 200 drusen per eye. The five subjects with stippled fundi were from 4 DZ female twin pairs. Their systemic characteristics were comparable to the remaining study population (data not shown).

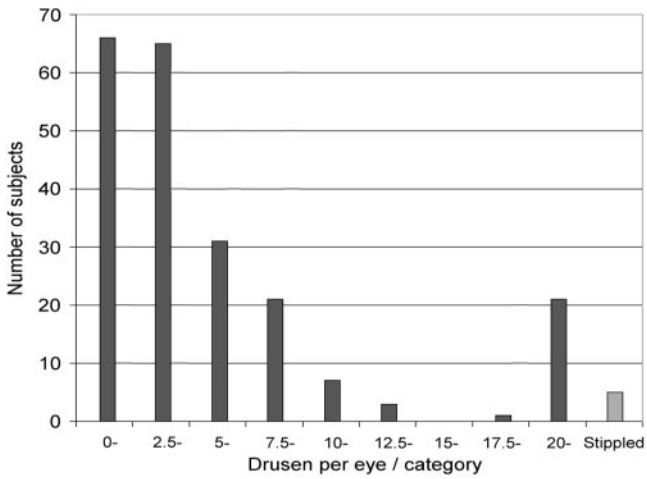

FIGURE 3. Frequency distribution of small hard drusen in 116 MZ and 104 DZ healthy twins 20–46 years of age. The stippled fundus phenotype is illustrated in Figure 2.

TABLE 2. Classification of Small Hard Drusen in Healthy Twins with Five or More Drusen in Each Eye

	Zygosity	Cases in Concordant Pairs	Cases in Disconcordant Pairs	Casewise Concordance (95% CI)
Scattered drusen	MZ	22	16	0.58 (0.37–0.76)
	DZ	8	20	0.29 (0.09–0.54)
Macular drusen	MZ	2	3	0.40 (0.01–0.89)
	DZ	4	9	0.31 (0.04–0.68)
Stippled fundus	MZ	0	0	—
	DZ	2	3	0.40 (0.01–0.89)
≥20 drusen per eye*	MZ	6	1	0.86 (0.33–1.00)
	DZ	6	13	0.32 (0.08–0.63)
≥20 drusen per eye†	MZ	6	1	0.86 (0.33–1.00)
	DZ	4	10	0.29 (0.04–0.65)

$n = 89$.

* Including stippled fundus phenotype.

† Excluding stippled fundus phenotype.

In the 89 subjects with more than five drusen per eye who did not demonstrate the stippled phenotype, the distribution of subjects by drusen pattern was as follows: scattered drusen, 66 subjects; macular drusen, 18 subjects; stippled fundus, five subjects (Table 2). In the 26 subjects with more than 20 drusen per eye, 16 had predominately macular drusen, five had scattered drusen, and five had the stippled fundus phenotype. The pattern was the same in both eyes of all subjects.

Structural equation modeling yielded the superior fit with an AE model, regardless of whether it was started with an ADE or an ACE model (A, additive genetic factors; C, shared environment; D, nonadditive genetic factors; E, unshared environment; Table 3). Age was the only covariate that reached statistical significance because the number of drusen increased with age (Fig. 4). Estimated effects of age and sex (0 for males, 1 for females) on mean liability were −0.04 (95% confidence interval [CI]: −0.07 to −0.02), and −0.34 (95% CI: −0.69 to 0.01), respectively. No statistically significant association was found between drusen number and sex, smoking, serum lipids, fasting blood glucose, or blood pressure.

After adjustment for age and sex, the heritability of the number of small hard drusen was 63% (95% CI, 43% to 77%), and the effect of a random, nonshared environment was 37% (95% CI: 23% to 57%). The latter fraction includes the effect of random errors of measurement.

Casewise concordance was higher in MZ twins than in DZ twins for scattered drusen and macular drusen. Stippling of the fundus was not seen among MZ twins. For the phenotype (≥20 or more drusen per eye), the casewise concordance was 0.86 (95% CI: 0.33 to 1.0) in MZ twins and 0.29 (95% CI: 0.04 to 0.65) in DZ twins (Table 2). When applying structural equation modeling on the data with a threshold of 20 or more drusen per eye, the best fit calculated by the use of the Akaike Information Criterion (AIC) was a DE model with a heritability of 99% (95% CI: 85% to 100%) if the pairs with stippled fundus type were included and 99% (95% CI: 82% to 100%) if the pairs with stippled fundus type were excluded. In a DCE model, heritability of liability was 91% (95% CI: 36% to 100%) and 88% (85% CI: 29% to 100%), respectively.

DISCUSSION

In elderly persons, large numbers of hard drusen have been shown to predict the development of soft drusen,[3,4] but the significance of small hard drusen in younger subjects with normal visual function remains unknown.[22] Small drusen do not appear to have a composition that is fundamentally different from that of soft drusen. If they are clearly circumscribed, they are hard drusen; if they are diffusely circumscribed, they are soft drusen.[23] Small drusen are not associated with specific histologic characteristics, but they tend to be associated with deflection of overlying photoreceptors that are otherwise of normal appearance, whereas large drusen are associated with photoreceptor degeneration.[24]

In a previous study, one or more drusen of any size within the macula in at least one eye was found in 95.5% of a population of 4926 patients between 43 and 86 years of age.[2] We found at least one small hard drusen per subject in 96% of subjects 20 to 46 years of age. The drusen counted in this study

TABLE 3. Distribution Modeling of Small Hard Drusen in Healthy Twins

	Genetic Components		Environmental Components		Fit Statistics		
Model	**A***	**D***	**C***	**E***	**−2lnL**	**AIC**	***P***
ACE	0.60 (0.00–0.77)	—	0.03 (0.00–0.53)	0.37 (0.23–0.59)	603.765	0	
ADE	0.63 (0.00–0.77)	0.00 (0.00–0.76)	—	0.37 (0.23–0.57)	603.778	0.013	—
AE	**0.63 (0.43–0.77)**	—	—	**0.37 (0.23–0.57)**	**603.778**	**−1.987**	**0.909**
CE	—	—	0.48 (0.30–0.63)	0.52 (0.37–0.70)	607.354	1.589	0.058
E	—	—	—	1.00	629.589	21.824	<0.001

Boldface type indicates best-fitting model for liability to develop a total number of drusen per subject in a given range (categories: <1, 1–5, 6–10, 11–20, 21–40, >40) after correction for the effects of age and sex. Twin pairs with subjects exhibiting stippled fundus were excluded from the analysis.

* Proportion of total variation attributable to model component (95% CI). −2lnL minus 2 times log-likelihood of the data. AIC, Akaike information criterion. *P* value corresponds to the likelihood ratio test compared with the ACE model. Both the ACE and the ADE models were simplifiable to an AE model. A, additive genetic factors; C, shared environment; D, nonadditive genetic factors; E, unshared environment.

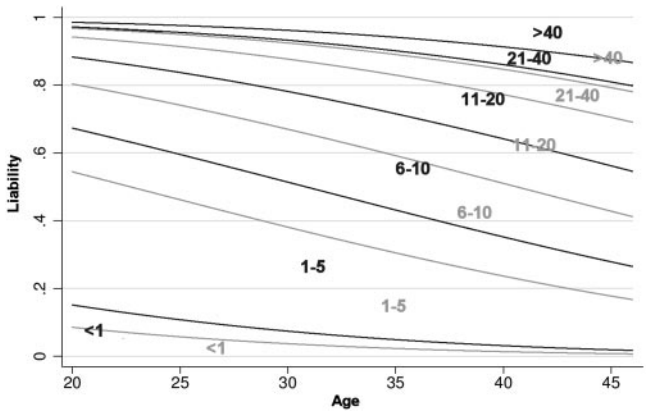

FIGURE 4. The age-trajectory of the proportions $p_i(x)$ for different drusen number categories ($I = 1,\ldots,6$ for <1, 1–5, 6–10, 11–20, 21–40, and >40 drusen, respectively), as predicted by the liability model. *Black lines*: males. *Gray lines*: females. Line graphs correspond to the function $f(x) = \Phi(t_i + \beta_{age}x + \beta_{sex}sex)$, where β_{age} and β_{sex} are the regression coefficients describing the effects of age and sex, with estimated values of −0.04 (95% CI: −0.07—−0.02) and −0.34 (95% CI: −0.69–0.01), respectively, t_i is the liability threshold, and Φ is the cumulative distribution function of the standard normal distribution. Thus, the drusen category proportions are given as the vertical distances between the curves, corresponding to $p_i(x) = \Phi(t_i + \beta_{age}x + \beta_{sex}sex) - \Phi(t_{i-1} + \beta_{age}x + \beta_{sex}sex)$.

have features in common with the Wisconsin AMD grading system categories *hard distinct* and *hard indistinct* drusen.[15] Nevertheless, a number of fundamental differences exist between the method of fundus photography and fundus grading used in the present study versus the Wisconsin AMD grading,[15] and the International Classification and Grading System of ARM and AMD.[25] We studied subjects younger than 50, whereas subjects younger than 50 were excluded, by convention, from the aforementioned AMD protocols. ARM is not conventionally diagnosed unless soft drusen 63 μm or larger in diameter, or more advanced lesions are present. This would have caused all but two of our participants to be excluded from having the diagnosis ARM/AMD made. Fundus photography in red-free illumination depicts small hard drusen at considerably higher contrast than the color diapositive fundus photography used in previous studies. Digital photography provided immediate feedback control of image focus and luminosity, enabling interactive adjustment of exposure settings. The grader was allowed to use digital adjustment of the image so as to facilitate the identification of drusen. Additionally we used total lesion count rather than subjective summation of total drusen area to quantify the amount of drusen. This approach was facilitated by the small hard drusen seen in the present study being of apparently uniform size (ARM and AMD often presents with drusen of widely differing dimensions). Most of our subjects showed little evidence of a predominantly macular location of small hard drusen; only 18 subjects demonstrated a distinct macular drusen pattern, whereas 66 subjects showed the scattered drusen pattern among subjects with five or more drusen per eye (Table 2). Limitations of the study included variation in the area of fundus covered and variation in drusen count (the latter resulted from difficulty in defining a lower limit of visibility of the drusen).

When analyzed as a continuous trait, the heritability of small hard drusen was 63% (95% CI: 43% to 77%). When analyzed as a dichotomous trait (≥20 drusen vs. <20 drusen per eye), heritability was as high as 99% (95% CI: 82% to 100%). The lower heritability achieved when including eyes with small numbers of scattered drusen may be a result of the error of measurement being high for eyes with low drusen numbers. It seems plausible that a high number of drusen is a distinctly heritable phenotype. This is supported by the study of Hammond et al.,[6] who observed that the heritability of scattered drusen (<20 per eye) was 19% compared with 81% for the phenotype (≥20 drusen per eye) in elderly women.

The present study was small, and its power to differentiate different subgroups among the 26 subjects with more than 20 drusen per eye was limited. Most subjects (16/26) had predominately macular drusen, a small fraction (5/26) displayed the stippled fundus type, and another small fraction (5/26) was categorized as having scattered drusen predominantly outside the macula. Excluding subjects with the stippled fundus type did not change heritability. A fundamental methodological issue is whether small hard drusen in the macular fundus have a different relation to AMD than small hard drusen in the peripheral fundus. The present study does not enable evaluation of the potential long-term impact of small hard drusen by number and pattern on incident ARM and AMD.

Factors empirically associated with AMD include smoking,[26–28] elevated arterial blood pressure, and atherosclerosis.[29] The present study was not designed to detect disease associations. The absence of any detectable effect of these factors in the present study can be ascribed to three factors: subjects with known cardiovascular disease were excluded from the study, study size was small, and the study population was young.

In the present study, we were able to demonstrate that the number of small hard drusen increased with increasing age. Lesions compatible with AMD were nearly completely absent in the study population, presumably because it was too young for small hard drusen to have begun to be replaced by soft drusen. In elderly patients, the prevalence of small hard drusen has been shown to decrease with age, in concert with an increasing prevalence of large drusen.[2] Large numbers of small hard drusen (>8) are associated with an increase in the incidence of soft drusen and pigment abnormalities,[3] suggesting that large drusen may develop from small drusen.

Recent studies have implicated genes of the complement system in the pathogenesis of AMD,[30–34] along with a gene of unknown function.[35,36] Our study showed that having more than 20 drusen per eye in young adulthood is highly hereditary. Previous studies have shown that in older subjects, this feature is associated with AMD.[3,4] It is likely that the study of established genetic risk factors for AMD in relation to small hard drusen in subjects younger than 50 years of age can help determine whether small hard drusen are truly early markers of AMD.

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References

1. Bressler NM, Bressler SB, West SK, et al. The grading and prevalence of macular degeneration in Chesapeake Bay watermen. *Arch Ophthalmol.* 1989;107:847–852.
2. Klein R, Klein BE, Linton KL. Prevalence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology.* 1992; 99:933–943.
3. Klein R, Klein BE, Tomany SC, et al. Ten-year incidence and progression of age-related maculopathy: the Beaver Dam eye study. *Ophthalmology.* 2002;109:1767–1779.
4. Bressler NM, Munoz B, Maguire MG, et al. Five-year incidence and disappearance of drusen and retinal pigment epithelial abnormalities: Waterman study. *Arch Ophthalmol.* 1995;113:301–308.
5. Seddon JM, Cote J, Page WF, et al. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. *Arch Ophthalmol.* 2005;123:321–327.

6. Hammond CJ, Webster AR, Snieder H, et al. Genetic influence on early age-related maculopathy: a twin study. *Ophthalmology*. 2002;109:730–736.
7. Kyvik KO, Christensen K, Skytthe A, et al. The Danish Twin Register. *Dan Med Bull*. 1996;43:467–470.
8. Scheurlen W, von Stockhausen HB, Kreth HW. Diagnose der Monozygotie bei Mehrlingen durch DNA-Fingerprinting. *Monatsschr Kinderheilkd*. 1991;139:30–32.
9. Schousboe K, Visscher PM, Henriksen JE, et al. Twin study of genetic and environmental influences on glucose tolerance and indices of insulin sensitivity and secretion. *Diabetologia*. 2003;46:1276–1283.
10. Kessel L, Hougaard JL, Sander B, et al. Lens ageing as an indicator of tissue damage associated with smoking and non-enzymatic glycation—a twin study. *Diabetologia*. 2002;45:1457–1462.
11. Hougaard JL, Kessel L, Sander B, et al. Evaluation of heredity as a determinant of retinal nerve fiber layer thickness as measured by optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2003;44:3011–3016.
12. Taarnhoj NC, Munch IC, Kyvik KO, et al. Heritability of cilioretinal arteries: a twin study. *Invest Ophthalmol Vis Sci*. 2005;46:3850–3854.
13. Taarnhoj NCBB, Larsen M, Sander B, et al. Heritability of retinal vessel diameters and blood pressure: a twin study. *Invest Ophthalmol Vis Sci.* 2006;47:3539–3544.
14. Wang M, Sander B, la Cour M, Larsen M. Clinical characteristics of subretinal deposits in central serous chorioretinopathy. *Acta Ophthalmol Scand*. 2005;83:691–696.
15. Klein R, Davis MD, Magli YL, et al. The Wisconsin Age-Related Maculopathy Grading System. *Ophthalmology*. 1991;98:1128–1134.
16. Day INM, Humphries SE. *Genetics of Common Diseases*. Oxford: BIOS Scientific Publishers Ltd.; 1997:19–32.
17. Plomin R, DeFries JC, McClearn GE, Rutter M. *Behavioral Genetics*. 3rd ed. New York: W.H. Freeman and Company; 1997:305–310.
18. Neale MC, Cardon LR. *Methodology for Genetic Studies of Twins and Families*. Dordrecht, The Netherlands: Klüwer Academic Publishers; 1992.
19. Witte JS, Carlin JB, Hopper JL. Likelihood-based approach to estimating twin concordance for dichotomous traits. *Genet Epidemiol*. 1999;16:290–304.
20. Rogers WH. Regression standard error in clustered samples. *Stata Tech Bull*. 1993;13:19–23.
21. Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics*. 2000;56:645–646.
22. Sarks SH, Arnold JJ, Killingsworth MC, Sarks JP. Early drusen formation in the normal and aging eye and their relation to age related maculopathy: a clinicopathological study. *Br J Ophthalmol*. 1999;83:358–368.
23. Hageman GS, Mullins RF. Molecular composition of drusen as related to substructural phenotype. *Mol Vis*. 1999;5:28.
24. Johnson PT, Lewis GP, Talaga KC, et al. Drusen-associated degeneration in the retina. *Invest Ophthalmol Vis Sci*. 2003;44:4481–4488.
25. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration: the International ARM Epidemiological Study Group. *Surv Ophthalmol*. 1995;39:367–374.
26. Fraser-Bell S, Wu J, Klein R, et al. Smoking, alcohol intake, estrogen use, and age-related macular degeneration in Latinos: the Los Angeles Latino Eye Study. *Am J Ophthalmol*. 2006;141:79–87.
27. Thornton J, Edwards R, Mitchell P, et al. Smoking and age-related macular degeneration: a review of association. *Eye*. 2005;19:935–944.
28. Khan JC, Thurlby DA, Shahid H, et al. Smoking and age related macular degeneration: the number of pack years of cigarette smoking is a major determinant of risk for both geographic atrophy and choroidal neovascularisation. *Br J Ophthalmol*. 2006;90:75–80.
29. van Leeuwen R, Ikram MK, Vingerling JR, et al. Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci*. 2003;44:3771–3777.
30. Hagemann GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci USA*. 2005;102:7227–7232.
31. Edwards AO, Ritter R III, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science*. 2005;308:421–424.
32. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science*. 2005;308:419–421.
33. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science*. 2005;308:385–389.
34. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet*. 2006;38:458–462.
35. Schmidt S, Hauser MA, Scott WK, et al. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *Am J Hum Genet*. 2006;78:852–864.
36. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum Mol Genet*. 2005;14:3227–3236.