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, <65 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 ergonomic 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 Zealand (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 detail10–12 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
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 optic disk–centered photograph in red-free illumination (Wratten 54)

**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. 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.

**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 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.

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. 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
Thus, the multifocal choroiditis was found bilaterally in one subject, and drusen was found unilaterally in two subjects, scarring from was found unilaterally in two subjects, a nevus with overlying higher plasma lipid concentrations (Table 1). Epiretinal fibrosis other study variables, except that the DZ twins had slightly 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).

## 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 respect to deviations from normality and presence of intrapair correlation.

### Table 1. Clinical Characteristics of Healthy Monozygotic and Dizygotic Twins

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MZ Twins</th>
<th>DZ Twins</th>
<th>$P$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pairs</td>
<td>58</td>
<td>48</td>
<td>—</td>
</tr>
<tr>
<td>Subjects</td>
<td>116</td>
<td>96</td>
<td>—</td>
</tr>
<tr>
<td>Male/female subjects</td>
<td>54/62</td>
<td>42/54</td>
<td>—</td>
</tr>
<tr>
<td>Smokers/nonsmokers</td>
<td>45/71</td>
<td>44/52</td>
<td>—</td>
</tr>
<tr>
<td>Pack years (smokers only)</td>
<td>8.04 (6.07)</td>
<td>10.04 (7.81)</td>
<td>0.23</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.9 (7.53)</td>
<td>34.6 (7.46)</td>
<td>0.82</td>
</tr>
<tr>
<td>Drusen per eye</td>
<td>6.01 (10.2)</td>
<td>8.52 (13.5)</td>
<td>0.22</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>85.2 (10.1)</td>
<td>86.0 (8.78)</td>
<td>0.62</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.6 (5.53)</td>
<td>23.8 (3.77)</td>
<td>0.66</td>
</tr>
<tr>
<td>Fasting blood glucose† (mM)</td>
<td>4.88 (0.48)</td>
<td>4.86 (0.41)</td>
<td>0.79</td>
</tr>
<tr>
<td>2-h oral glucose tolerance test‡ (mM)</td>
<td>6.08 (1.26)</td>
<td>6.03 (1.20)</td>
<td>0.81</td>
</tr>
<tr>
<td>Total cholesterol (mM)</td>
<td>5.24 (1.00)</td>
<td>5.60 (1.08)</td>
<td>0.045</td>
</tr>
<tr>
<td>High-density lipoprotein (mM)</td>
<td>1.52 (0.35)</td>
<td>1.55 (0.48)</td>
<td>0.56</td>
</tr>
<tr>
<td>Low-density lipoprotein (mM)</td>
<td>3.18 (0.92)</td>
<td>3.39 (1.0)</td>
<td>0.21</td>
</tr>
<tr>
<td>Very-low-density lipoprotein (mM)</td>
<td>0.509 (0.19)</td>
<td>0.610 (0.27)</td>
<td>0.0093</td>
</tr>
<tr>
<td>Triglyceride (mM)</td>
<td>1.19 (0.74)</td>
<td>1.40 (0.70)</td>
<td>0.072</td>
</tr>
</tbody>
</table>

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.

The traditional 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 $\frac{2C}{2C + D}$, 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.79

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 account20,21 as implemented in the statistical software (Stata; 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 respect to deviations from normality and presence of intrapair correlation.
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 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 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 were 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 AIC and the 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 normal appearance, whereas large drusen are associated with photoreceptor degeneration.24

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have features in common with the Wisconsin AMD grading system categories hard distinct and hard indistinct drusen. \textsuperscript{3,4} Nevertheless, a number of fundamental differences exist between the method of fundus photography and the Wisconsin AMD grading system and the International Classification and Grading System of ARM and AMD.\textsuperscript{5-7} 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 \( \geq 3 \text{ \mu m} \) 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 (\( \geq 20 \text{ drusen} \) vs. \(< 20 \text{ drusen} \) 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., \textsuperscript{6} who observed that the heritability of scattered drusen (<20 per eye) was 19% compared with 81% for the phenotype (\( \geq 20 \text{ drusen per eye} \)) 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,\textsuperscript{26-28} elevated arterial blood pressure, and atherosclerosis.\textsuperscript{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.\textsuperscript{2} Large numbers of small hard drusen (>8) are associated with an increase in the incidence of soft drusen and pigment abnormalities,\textsuperscript{3} suggesting that large drusen may develop from small drusen.

Recent studies have implicated genes of the complement system in the pathogenesis of AMD,\textsuperscript{30-34} along with a gene of unknown function.\textsuperscript{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.\textsuperscript{3} 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


