



Heritability of retinal drusen in the Copenhagen Twin Cohort Eye Study

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ABSTRACT.

Purpose: To study age- and sex-adjusted heritability of small hard drusen and early age-related macular degeneration (AMD) in a population-based twin cohort.

Methods: This was a single-centre, cross-sectional, classical twin study with ophthalmic examination including refraction, biometry, best-corrected visual acuity assessment, colour and autofluorescence fundus photography, and fundus optical coherence tomography. Grading and categorization of drusen was by diameter and location.

Results: The study enrolled 176 same-sex pairs of twins of mean (SD) age 58.6 (9.9) years. The prevalence of the four phenotypes ≥ 20 small hard *macular* drusen (largest diameter $< 63 \mu\text{m}$), ≥ 20 small hard *extramacular* drusen, intermediate drusen (63–125 μm) anywhere, and large drusen ($> 125 \mu\text{m}$) anywhere was 12.4%, 36.4%, 5.8%, and 8.4%, respectively, and the respective heritabilities, adjusted for age and sex, were 78.2% [73.5–82.9], 69.1% [62.3–75.9], 30.1% [4.1–56.1], and 65.6% [26.4–100]. Age trajectory analysis supported a gradual transition to larger numbers of small hard drusen with increasing age. The heritability of ≥ 20 small hard drusen was markedly lower than the 99% found in the 40% overlapping twin cohort that was seen 20 years earlier.

Conclusion: Numerous (≥ 20) small hard drusen and larger drusen that fit the definition of dry AMD were highly heritable. Small hard drusen counts increased with age. Decreasing heritability with increasing age suggests that the impact of behavioural and environmental factors on the development of small hard drusen increases with age.

Key words: Copenhagen Twin Cohort Eye Study – drusen – heritability – small hard drusen – twins

ML has consulted, spoken or been a trial investigator for Novartis, Chiesi, Allergan, Bayer, Alcon, AbbVie, Biogen, Novo Nordisk, Eli Lilly, Spark Therapeutics, Biogen, Sanofi and Roche.

Introduction

Small hard drusen are a common fundus feature (Hammond et al. 2002; Munch et al. 2007) that is associated, when found in large numbers, with an increased risk of incident age-related macular degeneration (AMD) (van Leeuwen et al. 2003; Wang et al. 2003; Klein et al. 2015). The heritability of small hard drusen is high, amounting to 99% for the phenotype ≥ 20 small hard drusen per eye in a study of 114 twin pairs of both sexes aged 20–49 years (Munch et al. 2007) and 81% in an older cohort of 506 female-only twin pairs aged 49–79 years (Hammond et al. 2002). With mean participant ages of 35 years and 62 years, the two studies emphasized the pre-AMD age spectrum and the early AMD age spectrum, respectively. Overall heritability of any AMD is 45% (Hammond et al. 2002), but if numerous small hard drusen are indeed an AMD precursor, its high heritability and early detectability make it an attractive target for phenotype-genotype association studies, potentially having a narrower range of associations and more fundamental mechanistic effects than the pleomorphic spectrum of AMD abnormalities.

This study, carried out in 2019, follows up on the 1999 twin examination from our group with the aim of

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identifying any change in heritability regarding ≥ 20 small hard drusen over a 20-year period in a study population that is young enough to present the transition from pre-AMD to early AMD, including a comparison with a similar cross-sectional study (Hammond et al. 2002), and verify the findings by Hammond et al. in a sex-balanced cohort to exclude any sex biases.

The purpose of the present report is to assess the heritability of the phenotype ≥ 20 small hard drusen in twins of both sexes, at an age older than in the 1999 study, and the heritability of early AMD phenotypes, with reference to current drusen subclassification practice.

Methods

This observational study enrolled a zygosity-balanced cohort of same-sex pairs of twins from the Danish Twin Registry between March 2019 and June 2020. Half of the participants had previously participated in an eye examination in 1999, which recruited from the prospective GEMINAKAR study of volunteers without diabetes and cardiovascular disease (Schousboe et al. 2003; Munch et al. 2007). The other half of the study population, which was included to achieve statistical target power, was matched on zygosity and sex, whereas no selection was made for diabetes or cardiovascular characteristics. The target age range of the latter group, 30–80 years, was broader than the 44–66 years of the first round participants, because it was deemed value for the assessment of ageing effects. Zygosity was verified using forensic genetic testing. Oral and written informed consent was acquired from all participants. The study was approved by the regional Health Research Ethics Committee (registration number H-18052822) and the Danish Data Protection Agency (registration number VD-2018-434). The study complied with the tenets of the Declaration of Helsinki.

Estimation of tobacco use, height, and weight was self-reported at the study visit. Active or previous tobacco users were considered as ‘smokers’ if ≥ 1 pack-year (≥ 20 cigarettes daily for 365 consecutive days). Blood pressure was measured at the beginning of the study visit, and mean arterial pressure was

used to evaluate blood pressure. A blood sample was taken from each participant to measure triglycerides, low-density lipoprotein, high-density lipoprotein and total cholesterol—the blood sample was stored in a tube with lithium heparin and analysed with a colorimetric test (VITROS, 5600, Ortho Clinical Diagnostics, USA).

Examinations included measurement of refraction and best-corrected visual acuity (BCVA) using an Early Treatment Diabetic Retinopathy Study (ETDRS) chart (4-meter original series, Precision-Vision, La Salle, IL, USA), pupil dilation using tropicamide 1%, slit-lamp biomicroscopy, mydriatic fundus photography, optical coherence tomography (OCT [Spectralis HRA + OCT2, Heidelberg Engineering, Heidelberg, Germany]) and four-field 50-degree digital colour fundus photography (TRC-50DX, Topcon Corp., Tokyo, Japan) centred on the foveal, the optic disc, the upper vascular arcade and the lower vascular arcade, and fovea-centred 30-degree blue-light fundus autofluorescence (Spectralis HRA + OCT2). Optical coherence tomography included a dense macular volume scan (20 × 20 degrees, 97 B-scans, high resolution, 20-scan averaging). Axial length was measured using the IOLMaster 700 (Carl Zeiss Meditec AG, Jena, Germany).

Colour- and red-free fundus images were visually inspected for drusen and other elements and annotated using ImageJ software (Fiji ImageJ v. 1.49 software). Drusen were categorized based on the largest diameter size: small hard drusen $< 63 \mu\text{m}$, intermediate drusen $63\text{--}125 \mu\text{m}$ and large drusen $> 125 \mu\text{m}$. The size of drusen $\geq 63 \mu\text{m}$ was based on the largest drusen diameter as measured by OCT; small hard drusen were rarely visible on OCT. Small hard drusen were subcategorized according to the number of drusen per eye (average of the two eyes): $n = 0\text{--}19$ small hard drusen per eye or ≥ 20 small hard drusen per eye. The cut-off value of 20 small hard drusen is arbitrarily defined by previous large epidemiological studies (Bressler et al. 1989) and used by comparable twin studies (Hammond et al. 2002; Munch et al. 2007). Therefore, we found this cut-off point suitable for the present study. One examiner (MB) only, masked to age, sex and zygosity,

counted and recounted the number of small hard drusen and drusen $\geq 63 \mu\text{m}$ for each eye in each participant. Every single drusen in each eye was marked and compared with the recount, and the recount did not result in a different small hard drusen phenotype grading (< 20 versus ≥ 20) in any participants. Due to the low variability between meticulous drusen counts and the limited capacity, an additional grader was not considered necessary. Drusen associated with a choroidal nevus were excluded ($n = 1$). Fundus images were graded twice. A second observer (ML) was consulted if arbitration was needed.

Data analysis included both small hard drusen within and outside the macula (Fig. 1), which was delineated by a fovea-centred circle with a radius extending to the temporal rim of the optic nerve head. Drusen within the circle were categorized as *macular* drusen. Otherwise, they were classified as *extramacular* drusen. All drusen were encircled, except when participants had a diffusely stippled peripheral retina that rendered counting impractical because the number of extramacular bright dots exceeded 500 per eye. In such case, the individual was considered to have ≥ 20 small hard *extramacular* drusen. Participants with eye disorders that significantly reduced retinal imaging quality were excluded from analysis.

Statistical analysis

RStudio statistical software (RStudio: Integrated Development Environment for R, Inc., Boston, MA) was used for

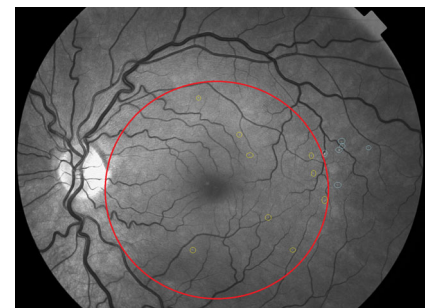


Fig. 1. Red-free fundus photograph of a 57-year-old female with 9 macular (yellow circles) and 6 extramacular (turquoise circles) small hard drusen $< 63 \mu\text{m}$. The red circle delineates the macula. No other small hard extramacular drusen were identified in other images displaying a larger field of the periphery.

data analysis. A classical twin study is set upon the comparison of phenotypic discrepancies in monozygotic (MZ) and dizygotic (DZ) twins. It is stipulated that MZ twins share identical genotypes, whereas DZ twins share approximately half of the genotypes. A higher similarity for a given phenotypic presentation among MZ twins indicates a genetic component for the development of that specific phenotype.

A descriptive comparison of MZ and DZ twins was reported as mean (standard deviation) unless otherwise specified—inference was adjusted for within-pair dependence. A Wald test for continuous variables or a Chi-square test for categorical variables was used to calculate p-values. Likelihood ratio testing showed no significant difference between a saturated model (assuming equal inter-twin variance and correlation) and an unsaturated model (assuming unequal inter-twin variance and correlation); therefore, we used a saturated model. Age trajectory of the proportions of various drusen categories based on the number of small hard drusen was estimated using the proportional odds model for modelling the dependence of ordinal variables. Regarding exposure to risk factors, the group of participants who only participated in 1999 was not significantly different from the group that participated in both 1999 and 2019 when analysed using a linear mixed regression model with fixed effects to adjust for dependencies. Weighted kappa statistics were used to assess the variability of the two drusen counts.

Cumulative incidence and casewise concordance

The risk of having ≥ 20 small hard drusen before a given age was estimated using the non-parametric cumulative incidence function of age as described elsewhere (Scheike et al. 2014). The casewise concordance of a specific drusen phenotype is the conditional risk of a given phenotype presentation in one twin before a certain age given that the other twin developed a similar phenotype before a certain age. A higher concordance rate between MZ twins than DZ twins is attributable to higher gene sharing and higher heritability of a given phenotype. The casewise concordances

were estimated using a liability threshold model (R-package ‘mets’) to test for the genetic component and quantify this effect (Scheike et al. 2014; Holst et al. 2016). The calculations in the present study assume identical environmental effects among twins.

Biometric modelling

Genetic and environmental effects can be estimated by twin modelling assessing the difference in MZ and DZ pair covariance to the total variance in phenotypic risks. A bivariate probit model, the classic liability threshold approach (Holst et al. 2016), using polygenic modelling with random effects was used to estimate these effects for a dichotomous outcome. From this model, the variance components of the polygenic biometric model from quantitative genetics (ACDE model) were calculated. Variance components are additive genetic effects (A), dominant (non-additive) genetic effects (D), common shared environmental effects (C) and unique environmental effects (E). The model with the lowest Akaike information criterion (AIC) value was considered the best fitting according to the principle of parsimony and is highlighted in bold in the presented tables. Genetic pleiotropic bivariate heritability modelling, adjusted for age and sex, was used to estimate broad-sense heritability according to the ratio of genetic effects (A + D) compared with the total variance of all effects (A + D + C + E). Genetic pleiotropy of left- and right eye variation in drusen outcomes was assessed by the joint (full Cholesky) biometric model allowing for AE components as above for each eye and thereby obtaining correlation between additive genetic effects (A of left eye with A of right eye) and similarly correlation of unique environmental effects (between E's of each eye). We chose this model to estimate true genetic effects more accurately; however, we believe that any discrepancies in genetic effects between the two eyes are results of stochastic drusen counts. Further, this allows for estimating the bivariate heritability of mutual drusen outcomes in eyes (the proportion of the phenotypic variation between the two eyes explained by genetic effects). A p-value < 0.05 , two-sided, was considered statistically significant.

Results

We invited 429 same-sex twin pairs, of whom 195 pairs responded. Of all who responded, 360 twins from 182 pairs showed up for examination and completed the study protocol, and 176 were available for analysis. Two pairs were excluded due to the inability to dilate pupils in one twin from each pair, preventing mydriatic fundus photography (narrow iridocorneal angle with high intraocular pressure and pregnancy), and the remaining four pairs were excluded due to no-show of a twin from each of the four pair. The study included 91 MZ and 85 DZ same-sex twin pairs, of whom 90 MZ pairs and 83 DZ pairs had gradable scans and were included in data analysis. The mean age of the participants was 58.5 (13.9) years (range, 30–80 years). The MZ and DZ groups were comparable (Table 1), with the exception that the DZ group had more small hard *macular* drusen and higher cumulative tobacco consumption. There was no association between tobacco consumption and the number of small hard *macular* drusen among DZ twins (data not shown). All participants were Danish-born and, except for one, resided in Denmark at the time of examination. There was broad agreement between the two counts of drusen number (weighted kappa statistics of 0.70 was found).

In the macula, one or more small hard drusen was found in at least one eye in 305 (87.9%) participants. In the extramacular region, small hard drusen were found in at least one eye in 342 (98.6%) participants. The phenotype ≥ 20 small hard *macular* drusen was found in either eye in 59 (17.0%) participants and 136 (39.2%) when examining the extramacular region only. Figure 2 shows the cumulative frequency of ≥ 20 small hard *macular* drusen from 30 to 80 years of age. Figure 3 shows an age trajectory of the proportions for various small hard *macular* drusen categories, with shifts that reflect a general increase in lesion density with age. In the present study, eyes with ≥ 20 small hard *macular* drusen were typically not seen in participants younger than 50 years of age (Fig. 2)—the currently adopted cut-off age for an AMD diagnosis. Two-thirds of the study participants were in the early years (50–65 years) of the AMD

Table 1. Demographics, per participant.

	Total	Monozygotic	Dizygotic	p-value
No.	346	180	166	
Age, years	58.6 (9.9)	57.4 (10.1)	59.8 (9.5)	0.12
Women, No. (%)*	190 (54.8%)	98 (54.1%)	92 (55.4%)	0.90
Body mass index, kg/m ²	25.3 (4.3)	25.3 (3.9)	25.4 (4.4)	0.95
Smoking, No. (%)*	154 (44.4%)	72 (39.6%)	83 (48.8%)	0.09
Pack years, smokers only, median (interquartile range) [†]	12.0 (5.0–21.5)	10.8 (5.0–18.5)	14.0 (5.1–25.0)	0.03
Mean arterial blood pressure, mmHg	104.0 (12.8)	104.4 (13.7)	103.6 (11.8)	0.61
Spherical equivalent refraction, right eye, diopter	−0.20 (2.4)	−0.36 (2.6)	−0.02 (2.2)	0.28
LogMAR, right eye [Snellen equivalent]	−0.04 (0.20) [1.10]	−0.03 (0.24) [1.07]	−0.05 (0.15) (1.12)	0.65
Intra ocular pressure, right eye, mmHg	14.2 (3.5)	14.0 (3.5)	14.4 (3.4)	0.33
Axial length, right eye, mm	23.8 (1.2)	23.9 (1.2)	23.7 (1.1)	0.27
Cholesterol mmol/L	5.4 (1.0)	5.3 (1.0)	5.4 (1.0)	0.58
Low-density lipoprotein mmol/L	3.1 (0.9)	3.1 (0.8)	3.1 (0.9)	0.85
High-density lipoprotein mmol/L	1.6 (0.5)	1.6 (0.5)	1.7 (0.5)	0.38
Triglycerides mmol/L	1.5 (0.8)	1.5 (0.8)	1.5 (0.8)	0.87
Small hard macular drusen, <i>n</i> < 20 per eye, No. (%)*	303 (87.6%)	169 (93.9%)	134 (80.7%)	<0.001
Small hard macular drusen, <i>n</i> ≥ 20 per eye, No. (%)*	43 (12.4%)	11 (6.1%)	32 (19.3%)	
Small hard extramacular drusen, <i>n</i> ≥ 20 per eye, No. (%)*	126 (36.4%)	59 (32.6%)	67 (40.4%)	0.16
Intermediate drusen, 63–125 μm, No. (%)*	20 (5.8%)	11 (6.1%)	9 (5.4%)	0.98
Large drusen, >125 μm, No. (%)*	29 (8.4%)	14 (7.7%)	15 (9.0%)	0.81

Estimates are mean (standard deviation) unless otherwise indicated. Inference was adjusted for within-pair dependence. A Wald test for continuous variables or a Chi-square test for categorical variables was used to calculate p-values (monozygotic versus dizygotic) for a univariate outcome. Participants who were not part of the initial 1999 study were not significantly different in any of the above-listed parameters when analysed with a linear mixed regression model with fixed effects to adjust for dependencies (not shown).

* Number of participants and percentage.

[†] Number of pack years were not normally distributed; hence, estimates are median (interquartile range).

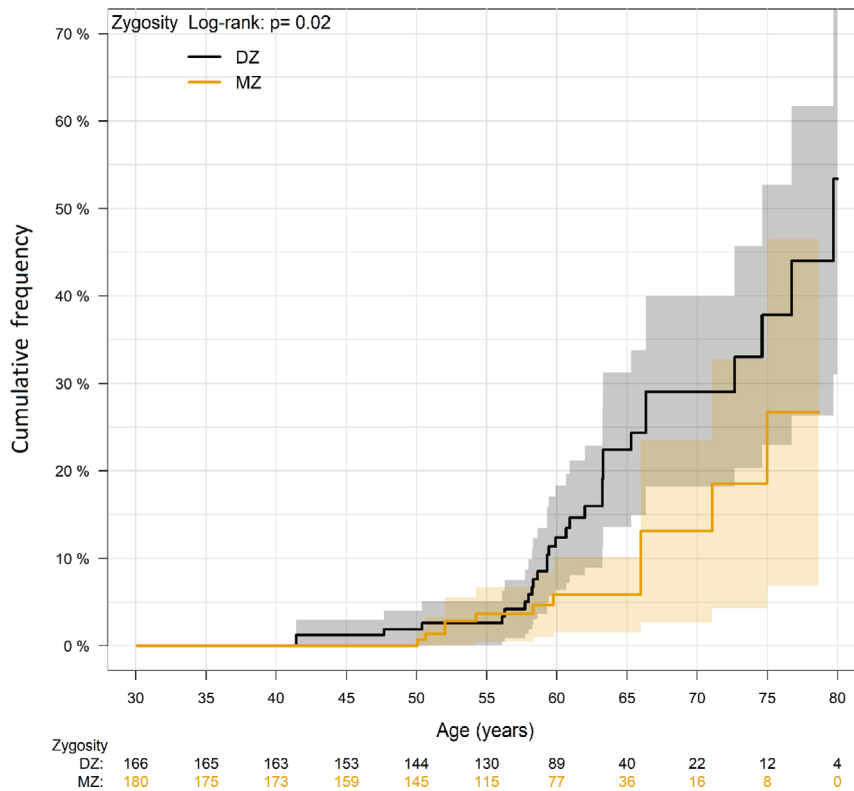


Fig. 2. Cumulative frequency (risk) of having ≥20 small hard macular drusen as a function of age and zygosity. Monozygotic twins appeared to have a lower cumulative incidence than dizygotic twins.

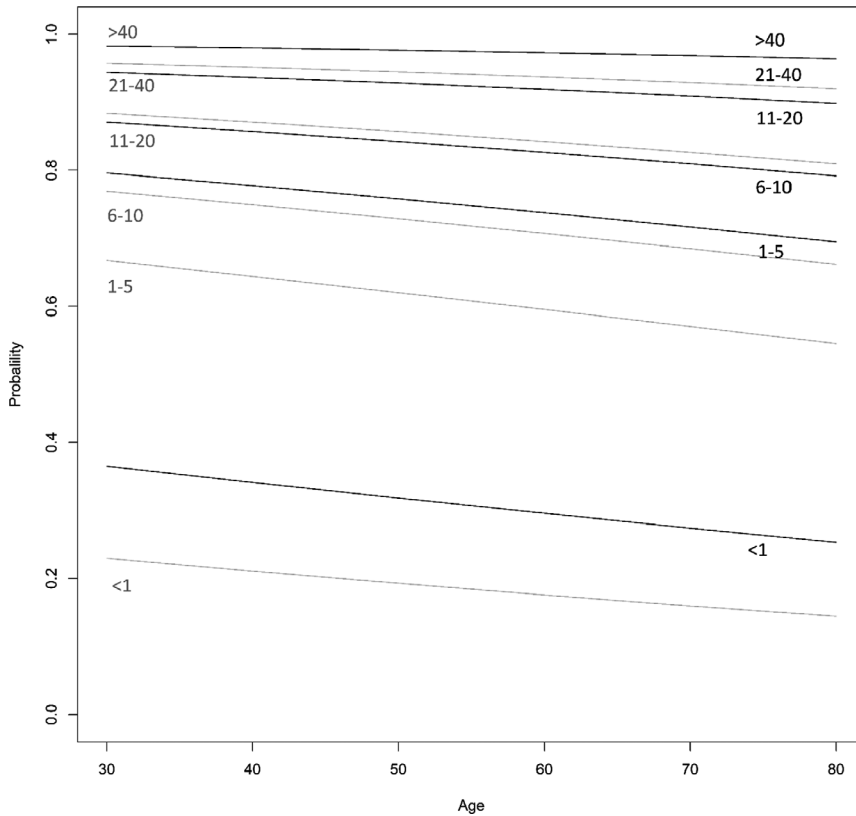


Fig. 3. Age trajectory of the proportions of various drusen categories as calculated by a probability model based on the number of small hard macular drusen (<1, 1–5, 6–10, 11–20, 21–40 and >40). Black lines represent males, and grey lines represent females. The curves are shown by means of the cumulative distribution function adjusted for age and sex. The vertical distance between the curves represents the proportional distribution of drusen.

age spectrum. Only two individuals (61 and 80 years old, respectively) had late-stage AMD.

The concordance of drusen phenotypes was generally higher for MZ twins than for DZ twins with high heritabilities for ≥ 20 small hard macular drusen, ≥ 20 extramacular drusen and drusen $>125 \mu\text{m}$ (Table 2). The concordance of the phenotype <20

small hard macular drusen per eye was similar between MZ and DZ twins.

A polygenetic model for quantitative genetics for different drusen phenotypes found that the best-fitted model (lowest AIC value) that explains the most amount of variation using fewest variables, although not significant, was an AE model when adjusting for age and sex (Table 3). The genetic effects

for ≥ 20 small hard macular drusen were attributable to additive effects ($A = 0.79\%$ [95% CI, 0.53%–1.00%]). The ACE and CE models suggest that shared and non-shared environmental factors could explain some of the effects. However, the confidence intervals were rather large. For ≥ 20 small hard extramacular drusen, the genetic effects were similarly attributable to additive effects ($A = 0.86\%$ [95% CI, 0.73%–0.99%]). Low numbers of affected individuals resulted in wide confidence intervals.

Genetic pleiotropic bivariate heritability modelling adjusted for age and sex found that an AE model best explains the data distribution (Table 4). The heritability of ≥ 20 small hard macular drusen was 78.2% (95% CI, 73.5%–82.9%) for right eyes and 68.4% (95% CI, 63.4%–73.4%) for left eyes; ≥ 20 small hard extramacular drusen showed similar but slightly lower estimates. Having soft drusen $>125 \mu\text{m}$ was also a heritable trait, 65.6% (95% CI, 26.4%–100%) for right eyes and 58.8% (95% CI, 23.0%–94.6%) for left eyes. Intermediate soft drusen 63–125 μm was the least heritable phenotype. Inter-eye genetic correlation, the genetic influence that is shared between the two eyes, was not 100% for all phenotypes; the random variation between the two eyes was ascribed to stochastic estimates of drusen counts. Stochastic effects would normally fall into the category of unique environmental effects (E).

Discussion

This study of a cohort of twins with a mean age of 58.6 years found high

Table 2. Casewise concordance of drusen phenotypes ($n_{\text{MZ pairs}} = 90$; $n_{\text{DZ pairs}} = 83$).

Phenotype	Zygoty	Concordant pairs (n)	Discordant pairs (n)	Prevalence	Casewise concordance [CI95]
≥ 20 small hard macular drusen per eye	MZ	2	7	0.03	0.36 [0.27–0.82]
	DZ	7	18	0.10	0.37 [0.20–0.58]
≥ 20 small hard extramacular drusen per eye	MZ	23	13	0.17	0.78 [0.67–0.88]
	DZ	20	26	0.20	0.61 [0.44–0.71]
<20 small hard macular drusen per eye	MZ	81	7	0.49	0.96 [0.88–0.97]
	DZ	57	18	0.41	0.86 [0.86–0.94]
Intermediate drusen 63–125 μm	MZ	2	7	0.02	0.36 [0.01–0.82]
	DZ	0	9	0	0
Large drusen $>125 \mu\text{m}$	MZ	5	4	0.04	0.71 [0.43–0.90]
	DZ	1	13	0.04	0.13 [0.02–0.52]

The phenotypic presentation was based on the average of the two eyes. Drusen $\geq 63 \mu\text{m}$ were considered present/not present if observed in any of a participant's two eyes.

CI₉₅ = 95% confidence interval, DZ = dizygotic, MZ = monozygotic.

Table 3. Polygenetic modelling for quantitative genetics of ≥ 20 small hard drusen per eye.

	Genetic components		Environmental components		Fit Statistics AIC	p-value
	A [CI95]	D [CI95]	C [CI95]	E [CI95]		
≥ 20 small hard macular drusen per eye						
ACE	0.54 [0–1.00]	-	0.23 [0–0.98]	0.23 [0–0.56]	248.3	
ADE	0.79 [0.53–1.00]	0	-	0.21 [0–0.47]	248.1	-
AE	0.79 [0.53–1.00]	-	-	0.21 [0–0.47]	246.1	0.58
CE	-	-	0.57 [0.31–0.83]	0.43 [0.17–0.69]	246.8	0.32
≥ 20 small hard extramacular drusen per eye						
ACE	0.75 [0.10–1.00]	-	0.10 [0–0.70]	0.15 [0–0.29]	393.1	
ADE	0.86 [0.73–0.99]	0	-	0.14 [0.01–0.27]	393.4	-
AE	0.86 [0.73–0.99]	-	-	0.14 [0.01–0.27]	391.4	0.75
CE	-	-	0.68 [0.52–0.85]	0.32 [0.15–0.48]	396.8	0.02

The confidence intervals are age- and sex-adjusted.

A = additive genetic effects, AIC = Akaike’s information criterion, C = common environment effects, CI₉₅ = 95% confidence interval, D = dominant genetic effects, E = unique environmental effects.

Estimates are proportions attributable to each A, C, D and E factor [95% CI]. Best-fitted model was based on lowest AIC value and shown in bold. p-values are based on a likelihood ratio test (ref. ACE model).

Table 4. Estimates [CI₉₅] of broad-sense inter-eye heritability and environmental effects.

	h^2	Genetic correlation*	e^2	Environment correlation*
≥ 20 Small hard macular drusen per eye				
Right eye	78.2 [73.5–82.9]	88.5 [79.7–97.2]	21.8 [17.1–26.5]	12.8 [0–36.4]
Left eye	68.4 [63.4–73.4]		31.6 [26.6–36.6]	
≥ 20 Small hard extramacular drusen per eye				
Right eye	69.1 [62.3–75.9]	97.1 [91.9–1.00]	30.9 [24.1–37.7]	53.1 [49.0–57.2]
Left eye	61.3 [54.5–68.1]		38.7 [31.9–45.5]	
Soft drusen, 63–125 μm				
Right eye	30.1 [4.1–56.1]	98.3 [85.2–100]	69.9 [43.9–95.9]	71.9 [36.1–100]
Left eye	29.0 [3.7–54.3]		71.0 [45.7–96.3]	
Soft drusen, > 125 μm				
Right eye	65.6 [26.4–100]	100 [95.7–100]	34.4 [0–73.6]	51.6 [17.0–86.3]
Left eye	58.8 [23.0–94.6]		41.2 [5.4–77.0]	

e^2 = environmental effect, h^2 = broad-sense heritability.

Estimates are adjusted for age and sex.

* Nominal genetic and environment correlation (inter-eye correlation).

heritabilities of the phenotypes ≥ 20 small hard *macular* drusen per eye, ≥ 20 small hard *extramacular* drusen per eye, and large drusen >125 μm . Heritability was primarily explained by additive genetic effects (numerous genes exerting influence or modifying the phenotype). Intermediate drusen, of diameter 63–125 μm , were not significantly heritable, an outcome that may be attributable to this phenotype being a short-lived transient stage of AMD, where even a minor desynchronization within a pair of twins can result in phenotype discordance.

Comparison with a 40% overlapping cohort of twins with a mean age of 34.8 years (Munch et al. 2007) showed a decline in heritability, from 99% for ≥ 20 small hard drusen per eye to 68%–78% for ≥ 20 small hard drusen per eye in the macula at a mean age of 58.6 years. The latter is close to the

age of the cohorts studied by Meyers (1994) and Hammond et al. (2002) and in the age range near the age where early age-related AMD appears. The decrease is not in absolute heritability, but in genetic effects relative to environmental effects and may thus be affected by non-shared environmental factors (e.g. smoking, dietary habits, lifestyle, and physical activity) that have a cumulative character that grows in proportion to the effect of genetics. This assumption fits the observation that the risk of AMD is strongly related to smoking, fatty foods and a high body mass index, and the role of behaviour and choice in the exposure to such risk factors (Smith et al. 2001; Seddon et al. 2003a, 2003b).

Most people older than 40 years have small hard drusen, but having 5 or more small hard drusen in the

macula or a very large area with small hard drusen in the macula is associated with an elevated risk of developing AMD and large drusen (Bressler et al. 1995; van Leeuwen et al. 2003; Klein et al. 2015). Light and electron microscopy of drusen has shown that small hard and soft drusen share many characteristics, but small hard macular drusen are less homogeneous than peripheral small hard drusen and soft drusen (Sarks et al. 1999; Rudolf et al. 2008).

The study of heritability in AMD was pioneered by Meyers et al., who examined 111 twin pairs and found 100% concordance for AMD in 25 MZ twin pairs, compared to 42% in 11 DZ twin pairs (Meyers 1994). The only prior twin study was a case report by Melrose et al. (1985). In a later study of 506 female twin pairs, Hammond et al. (2002) found a concordance for

AMD of 59% and a total heritability of 45%. For the phenotype ≥ 20 small hard drusen per eye, a heritability of 81% was found. In a study of 391 male twin pairs, who were older and had a higher AMD prevalence, Seddon et al. (2005) observed a heritability for intermediate and late AMD of 67%. In this context, it is worth considering whether small variations in the time of transition between stages and in the time spent in a given stage give rise to large variations in estimated heritability. Seddon et al. studied the phenotype ≥ 15 small hard but did not report a specific heritability for this phenotype. There are other small twin studies that have found a high concordance rate for late AMD, but they did not include the phenotype numerous small hard drusen (Klein et al. 1994; Gottfredsdottir et al. 1999).

Whereas AMD has been shown repeatedly to be associated with specific risk alleles, notably in CFH (complement factor H) and ARMS2 (age-related maculopathy susceptibility 2) (Edwards et al. 2005; Haines et al. 2005; Klein et al. 2005; Rivera et al. 2005; Fisher et al. 2016), no such associations have been found for small hard drusen (Munch et al. 2010), despite CFH and ARMS2 having been specifically looked for (Klein et al. 2015; Munch et al. 2016). Assuming that this was not because of lack of statistical power, one may infer that the aforementioned genes are not involved in the hypothetical precursor stage of AMD where small hard drusen appear, but in the later stage of manifest AMD (Holliday et al. 2013; Klein et al. 2015). Likewise, the non-genetic risk factors for early AMD may also differ from those of late AMD. In this context, one must consider the possibility that small hard drusen may be precursors of specific subtypes of larger drusen. Of particular interest, besides soft drusen, is the most recently added class of drusen subtypes, pachydrusen, which are associated with central serous chorioretinopathy and a thicker choroid (Zhang & Sivaprasad 2021). People with pachydrusen have a lower frequency of AMD genotypes than people with soft drusen or reticular drusen (Fukuda et al. 2019) and no detectable association with AMD genotypes compared with a general background population (Takahashi et al. 2021).

Compared with our previous study of the heritability of small hard drusen heritability (Munch et al. 2007), the present study had a mean participant age that was 20 years older and near the typical age of onset of AMD. We used OCT and fundus autofluorescence to exclude confounding conditions. The recruitment was random and population-based, which should serve to minimize selection bias (MZ twins are known to be more likely to participate compared with DZ twins), and it included both sexes (Hammond et al. 2002). However, a higher cumulative frequency of ≥ 20 small hard macular drusen, per eye, was observed among DZ twins, indicating a possible drusen distribution skewness and thereof an under- or overestimation of heritability. Women were overrepresented. A diabetes prevalence lower than half of the national age-adjusted average and higher socioeconomic status suggests that our study participants may be healthier than the background population. A major limitation, compared with previous studies, is that the sample size is smaller. In line with this, the low heritability of intermediate drusen compared with other drusen phenotypes might be explained by sample size and a study cohort not comparable to the background population. The prevalence of numerous small hard drusen or large drusen was indeed relatively low. The clinical presentation of various phenotypes of interest, including a higher prevalence of numerous small hard *macular* drusen among DZ twins, may be explained by stochastic interactions or selection bias that cannot be attributed to either genetic or environmental factors. Potential analysis bias is inherent in the assumption of identical environments among twins, which may lead to overestimation of the heritability of a given phenotype. Furthermore, dietary habits and physical activity, two potential pivotal risk factors, were not systematically recorded.

In conclusion, ≥ 20 small hard *macular* and *extramacular* drusen, and large drusen were found to be highly heritable. Our results confirm that potential AMD precursors are genetically influenced and strengthen the need for genome-wide associations studies to identify likely associations with currently known AMD-related risk genotypes.

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