Heritability and Risk Factors of Incident Small and Large Drusen in the Copenhagen Twin Cohort Eye Study: A 20-Year Follow-Up

Mohamed Belmouhand\textsuperscript{a, b}, Simon Paul Rothenbuehler\textsuperscript{a, c}, Jakob Bjerager\textsuperscript{a}, Sami Dabbah\textsuperscript{a, d}, Jacob B. Hjelmborg\textsuperscript{e, f}, Inger Christine Munch\textsuperscript{g}, Christine Dalgård\textsuperscript{f, h}, Michael Larsen\textsuperscript{a, b}

\textsuperscript{a}Department of Ophthalmology, Rigshospitalet, Glostrup, Denmark; \textsuperscript{b}Department of Clinical Medicine, Faculty of Health and Medical Science, University of Copenhagen, Copenhagen, Denmark; \textsuperscript{c}Department of Ophthalmology, University Hospital Basel, Basel, Switzerland; \textsuperscript{d}Department of Ophthalmology, Odense University Hospital, Odense, Denmark; \textsuperscript{e}Epidemiology, Biostatistics and Biodynamics, Department of Public Health, University of Southern Denmark, Odense, Denmark; \textsuperscript{f}Danish Twin Research Center, University of Southern Denmark, Odense, Denmark; \textsuperscript{g}Centre for Clinical Research and Prevention, Bispebjerg and Frederiksberg Hospital, Frederiksberg, Denmark; \textsuperscript{h}Clinical Pharmacology, Pharmacy and Environmental Medicine, Department of Public Health, University of Southern Denmark, Odense, Denmark

\textbf{Keywords}
Drusen · Small hard drusen · Retina · Follow-up · Heritability · Twin study · Copenhagen Twin Cohort Eye Study

\textbf{Abstract}

\textbf{Introduction:} The transition from a normal fundus to one with early drusen (≥20 small hard drusen) to age-related macular degeneration (AMD) in the form of drusen ≥63 μm in diameter is of interest, because small hard drusen may be precursors of large drusen. Study of AMD precursor lesions may provide valuable insight into factors that initiate AMD. Here, the progression of drusen was studied over an interval of 20 years in a population-based twin cohort. \textbf{Methods:} Single-center, 20-year follow-up of 138 twins include biometry, fundus optical coherence tomography, and fundus photography. Macular characteristics were hierarchically classified as (per eye) (1) <20 small hard drusen, (2) ≥20 small hard drusen, (3) drusen ≥63 μm, or (4) ≥20 small hard drusen combined with drusen ≥63 μm. Additive and dominant genetic effects as well as shared and nonshared environmental effects were analyzed in a bivariate biprobit model with a classic liability-threshold approach and polygenic modeling with random effects. \textbf{Results:} Median participant age was 59 (range 41–66) years. Of 25 (18%) cases of incident macular drusen, 7 had ≥20 small hard drusen, and 18 had drusen ≥63 μm at follow-up, whereas no participant had developed both traits simultaneously. Smoking was associated with incident ≥20 small hard drusen (\(p = 0.04\)) and incident drusen ≥63 μm (\(p = 0.003\)). Having ≥20 small hard drusen at baseline was associated with incident drusen ≥63 μm at follow-up (\(p = 0.02\)). Development of drusen ≥63 μm was attributable to 49% genetic effects and 51% environmental effects. \textbf{Conclusion:} The risk of progressing from 0 to 19 small hard macular drusen per eye to having ≥20 small hard drusen or drusen ≥63 μm at follow-up was associated with smoking and genetic predisposition. Having ≥20 small hard drusen in the absence of drusen ≥63 μm at baseline was associated with incident drusen ≥63 μm when examined 20 years later. The study confirms that small hard macular drusen is a forewarning of AMD and that progression to AMD may be hindered by avoidance of smoking.

© 2022 The Author(s). Published by S. Karger AG, Basel

This is an Open Access article licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC) (http://www.karger.com/Services/OpenAccessLicense), applicable to the online version of the article only. Usage and distribution for commercial purposes requires written permission.

Correspondence to:
Mohamed Belmouhand, mohamed.belmouhand@regionh.dk
Introduction

Age-related macular degeneration (AMD) is a leading cause of blindness among older people in industrialized countries [1]. The earliest change associated with AMD is seen when the common age-related thickening of Bruch’s membrane, known from histology studies as basal laminar deposits, is supplemented by basal linear deposits, which can develop into the ophthalmoscopically visible focal aggregates called drusen [2, 3]. In vivo observations with a hypothetical link to degradation of retinal pigment epithelium can be seen from childhood as small bright well-circumscribed spots at the level of retinal pigment epithelium, called small hard drusen, because they are smaller than 63 μm in diameter that by convention defines AMD drusen [4]. Small hard drusen are not considered part of the AMD lesion spectrum. However, when they present in large numbers (≥20 per eye), they are associated with an increased risk of incident early AMD over periods of observations of 5–15 years [5, 6]. Once drusen ≥63 μm have formed, they tend to progress in size and number [7, 8]. Thus, a high density of small hard macular drusen adds to the empirical risk of AMD, together with age, genetics [9], drusen size [10, 11], certain dietary and smoking habits [9], and choriocapillaris flow deficits seen on optical coherence tomography (OCT) angiography [12].

Cross-sectional twin studies have shown that small hard drusen and AMD drusen are highly heritable [13–15]. The characteristics of AMD get more complex with progression to the later stages of the disease. Therefore, the targeted study of factors associated with AMD precursors such as small hard drusen may hypothetically identify a narrower set of risk factors responsible for the earlier stages of the disease and may form more attractive targets for intervention. Indeed, the dominating AMD-associated genotypes have shown to be associated with a higher degree with late AMD than early AMD [16].

Histological studies suggest a gradual transition from small hard drusen to AMD drusen [17, 18], but only non-invasive in vivo study with modern imaging methods can ultimately determine if AMD drusen are formed in situ, as a direct transformation of small hard drusen. This prospective study of early drusen phenotypes examined patterns and heritability of progression from baseline examination in mid-adulthood to a follow-up examination 20 years later.

Materials and Methods

This was a 20-year single-center, prospective cohort study. Twins who participated in a baseline examination in 1999 were invited to attend a follow-up visit in 2019/2020. Recruitment was through the Danish Twin Registry [15], a prospectively maintained registry of all twins born in Denmark [19]. Zygosity was verified by forensic genetic testing. Participants were enrolled between March 2019 and June 2020. Predefined inclusion criteria included being same-sex twins and being willing and able to provide written informed consent. Inclusion required participation of both twins. All examinations were made at the Department of Ophthalmology, Righospitalet, Copenhagen, Denmark.

The study followed the tenets of the Declaration of Helsinki. Relevant approvals were obtained before study inclusion (Regional Health Research Ethics Committee [No. H-18052822] and the Danish Data Protection Authority [No. VD-2018-434]).

A modified version of the questionnaire from 1999 was used to interview participants to obtain a detailed medical history, including current and previous diseases, current medication, family history, contact lens use, allergies, ethnicity, height and weight, smoking, alcohol use, and demographic data (shown in Fig. 1). Smoking was defined as active or history of smoking ≥1 pack-year (1 pack-year = 20 cigarettes daily for 365 consecutive days). Alcohol consumption was categorized as being either above or below 7 units per week for women and 14 units per week for men, inspired by a minimum-risk threshold promoted by the Danish National Board of Health.

Blood pressure was measured three times during the initial part of the examination, and the lowest measurement was used. Each participant provided a blood sample for estimation of triglycerides, low-density lipoprotein, high-density protein, very-low-density protein, and total cholesterol by means of a colorimetric slide test (VITROS 5600; Ortho Clinical Diagnostics, USA). Glycated hemoglobin (HbA1c) was examined using high-performance liquid chromatography (GLC; Tosoh Co., Japan). Risk factors, i.e., body mass index, smoking, mean arterial blood pressure, and blood samples, were assessed in 1999 and 2019; however, only accumulated/cross-sectional numbers from 2019 were used in the 20-year risk analysis.

Five twin pairs were not examined together: the median interval being 16 days (range 2–262 days). The 1999 and 2019 examinations included similar mydriatic and nonmydriatic examinations, although additional and newer imaging devices were used in 2019. The nonmydriatic examination included assessment of refraction and best-corrected visual acuity (Early Treatment Diabetic Retinopathy Study charts at 4 m; Precision Vision, La Salle, IL, USA), and intraocular pressure by rebound tonometry (iCare TA01i; Icare Oy, Helsinki, Finland). Structural mydriatic examination consisted of slit-lamp biomicroscopy and fundus photography, four-field 50-degree digital red-free fundus images centered on the fovea, upper vessel arc, lower vessel arc, and optic disk, respectively, and a 50-degree foveal-centered digital color fundus image (1999: Wratten 54 filter; Eastman Kodak, Inc., Rochester; NY/2019: TRC-50DX; Topcon Corp., Tokyo, Japan). The 2019 examination included in addition to slit-lamp biomicroscopy and fundus photography, OCT (Spectralis HRA+OCT2; Heidelberg Engineering, Heidelberg, Germany) in the form of a macula volume scan (20 × 20°, 97 B-scans, high resolution, and 20-frame averaging) and axial length measurement using the IOLMaster 700 (Carl Zeiss Meditec AG, Jena, Germany).
Fundus photographs were analyzed using digital software (Fiji Image v. 1.49 software) [20]. Every druse, independent of size, was graded according to its largest diameter and counted as small hard drusen (<63 μm) or intermediate/large drusen (≥63 μm). Furthermore, drusen ≥63 μm were subclassified into soft drusen, cuticular drusen, subretinal drusenoid deposits, or pachydrusen. Pachydrusen were defined as drusen that appear alone or in small clusters, often in the periphery of the macula and typically in an eye with a thick choroid [21]. The other three drusen types were defined with reference to classical AMD study practice [22]. Drusen located over choroidal nevi were omitted from analysis (n = 1). Drusen diameter was assessed by visual grading of fundus photographs and supplemented, as per grader discretion for 2019 examinations, by inspection of corresponding OCT scans and measurements using the manufacturer’s software calipers. Drusen that had a diameter less than half the width of a large arcade vein near the optic disk on fundus photographs and unidentifiable on OCT were classified as small hard drusen. Small hard drusen were classified as being macular, as defined by a fovea-centered circle touching the temporal edge of the optic disk. Only macular drusen were analyzed in follow-up analyses.

Analysis of change in drusen characteristics was based on the following hierarchical phenotype grading: (1) <20 small hard drusen, (2) ≥20 small hard drusen, (3) drusen ≥63 μm, or (4) drusen ≥63 μm combined with ≥20 small hard drusen, where the classification of the eye followed the highest grade of lesion present. The tabulated number of small hard drusen per eye was the average of a participant’s two eyes. Phenotype 3, drusen ≥63 μm, was assigned to a participant if one or more drusen ≥63 μm were observed in one of the participants’ two eyes. A single grader (M.B.) masked to age, sex, and zygosity graded every image twice. When discrepancies occurred, a recount was made. Ambiguous findings were settled by a second grader (M.L.).

Statistical Analysis
Data analysis was made using RStudio (RStudio: Integrated Development Environment for R, Inc., Boston, MA, URL: http://www.rstudio.com). The aims were to (1) identify factors associated
with incident ≥20 small hard drusen and incident drusen ≥63 μm and (2) assess if the risks of progression were attributable to genetic effects.

Descriptive characteristics were reported as medians with interquartile ranges. Counts are given as total numbers with corresponding percentages (Table 1). A violin plot was used to depict the distribution of small hard drusen. Risk factors associated with incident ≥20 small hard drusen and incident drusen ≥63 μm were analyzed using a logistic regression model and a conditional logistic regression model for a matched co-twin multivariable analysis, with estimates tabulated as odds ratio (OR) and 95% confidence interval (CI 95 ). Only risk factor data from the follow-up examination were included in the analysis. A Hosmer-Lemeshow goodness of fit test showed that the conditional logistic model was well calibrated (p = 0.75). Two-sided p values <0.05 were considered statistically significant.

The methodological essence of heritability studies in twins is that a higher intra-pair concordance for a given phenotype in monozygotic (MZ) twins than in dizygotic (DZ) twins, if assuming MZ twins to have an identical genotype, is attributable to genetic effects, whereas intra-pair discordance is attributable to environmental effects. The pair-wise concordance for the outcome incidence ≥20 small har d drusen and incident drusen ≥63 μm was estimated as the conditional risk of drusen in one twin if the other twin also developed drusen. Case-wise concordance was calculated using a liability-threshold model (R package “Mets”) [23, 24].

Biometric modeling was used to model the effects of genetic and environmental factors such as additive genetic effects (A), dominant nonadditive genetic effects (D), common shared environmental effects (C), and unique environmental effects (E) [23]. Thus, analyses were made using the bivariate biprobit model by means of the classic liability-threshold approach, using polygenic modeling with random effects to assess the contribution from A + D and C + E. Broad-sense heritability was the proportion of genetic effects (A + D) relative to the total variance of all effects (A + D + C + E). Heritability was calculated by comparing concordance rates in MZ and DZ twins. Biometric modeling was achieved with the R package “Mets” using the function “twins.” The best-fit model was chosen among ACE versus ADE versus AE versus CE versus E based on the lowest Akaike information criterion (AIC) value.

### Results

One pair was excluded due to ungradable fundus images, thus leaving 138 twins from 69 pairs for analysis (Table 1, shown in Fig. 1). Median follow-up was 21 years (range 20–21 years), and the median age at follow-up was 59 years (range 41–66 years). All participants were Caucasian and born in Denmark, and all but one twin resided in Denmark at the time of examination.

Weighted kappa statistics for intra-rater reliability of total drusen counts per eye were 0.67 for right eyes and 0.70 for left eyes. One or more small hard drusen were found at baseline in 1999 in at least one eye in 107 (77.5%) participants, a rate that had increased to 123 (89.1%) participants at the 20-year follow-up (Table 1). Twenty or more small hard drusen were observed in 11 (8.0%) participants at baseline and 14 (10.1%) participants at follow-up. Drusen ≥63 μm were observed in 19 (13.8%) participants at follow-up, compared to only one (0.7%) participant at baseline. Variation in the number of small hard drusen per eye increased with age (shown in Fig. 2). The absolute small hard drusen count had increased in 88

| Variables                      | 1999       | 2019       |
|-------------------------------|------------|------------|
| Age, years                    | 38 (33–42)| 59 (54–63) |
| Sex                           | 78 women/60 men | 35 MZ pairs/34 DZ pairs |
| Body mass index, kg/m²        | 23.6 (21.9–25.1) | 24.5 (22.7–26.0) |
| Smoking, n (%)                | 56 (40.6) | 56 (40.6) |
| Pack-years, smokers only      | 7 (3–11) | 10 (7–20) |
| Mean arterial blood pressure, mm Hg | 85.3 (79.1–90.9) | 101.7 (95.0–110.8) |
| Cholesterol, mmol/L           | 5.5 (4.8–6.0) | 5.5 (4.9–6.2) |
| Low-density lipoprotein, mmol/L | 3.3 (2.7–3.9) | 3.2 (2.6–3.6) |
| High-density lipoprotein, mmol/L | 1.5 (1.3–1.7) | 1.6 (1.3–2.0) |
| Triglycerides, mmol/L         | 1.1 (0.8–1.5) | 1.3 (0.9–1.8) |
| Macular drusen, n (%)         |            |            |
| n < 20 small hard drusen      | 126 (91.3) | 105 (76.1) |
| n ≥ 20 small hard drusen      | 11 (8.0)  | 14 (10.1)  |
| Drusen ≥63 μm                 | 1 (0.7)   | 19 (13.8)  |

Table 1. Demographic characteristics of 138 twins at baseline (1999) and follow-up (2019)
The 20-Year Early Age-Related Macular Degeneration Incidence

(63.8%) participants, decreased in 33 (23.9%) participants, and remained unchanged in 17 (12.3%) participants. No participant developed choroidal neovascularization or geographic atrophy.

The 20-year incidence of having developed ≥20 small hard drusen was 5.6% (7 cases out of 126 participants without ≥20 small hard drusen at baseline), and the 20-year incidence of having developed drusen ≥63 μm was 13.1% (18 cases out of 137 participants) (Table 2). No participant presented with the combination of having developed both ≥20 small hard drusen and drusen ≥63 μm between baseline and follow-up.

**Table 2.** The 20-year incidence of ≥20 small hard drusen and drusen ≥63 μm according to baseline phenotype

| Follow-up (2019) | participants, n | small hard drusen, n < 20 | small hard drusen, n ≥ 20 | drusen ≥63 μm |
|------------------|----------------|---------------------------|---------------------------|--------------|
| Baseline (1999)  | Participants, n | 105                       | 14                        | 19           |
|                   | Small hard drusen, n < 20 | 104 (82.5%) | 7 (5.6%) | 15 (11.9%) |
|                   | Small hard drusen, n ≥ 20 | 1 (9.1%) | 7 (63.6%) | 3 (27.3%) |
|                   | Drusen ≥63 μm | 1 | 0 | 1 (100%) |

Participants were categorized according to their highest drusen severity category. No participant developed a combination of two phenotypes or late AMD (choroidal neovascularization or geographic atrophy). Only macular drusen were included. Row-wise percentages are indicated for each baseline category. 25 individuals developed incident numerous drusen and large drusen (bold).

**Fig. 2.** Violin plot showing the distribution of small hard drusen per eye at baseline and follow-up. Individuals with >30 small drusen per eye were omitted from the graph (1999, n = 6; 2019, n = 7). Red dots represent the median number of small hard drusen per eye (1999 = 1, 2019 = 2.5).
Table 3. Factor associated with the 20-year ≥20 small hard drusen incidence and drusen ≥63 μm incidence

| Variables                                | Incident drusen ≥63 μm | Incident ≥20 small hard drusen | OR [CI95] | p value | OR [CI95] | p value |
|------------------------------------------|----------------------|--------------------------------|----------|---------|----------|---------|
| Age (years)                              |                      |                                | 1.04 [0.91–1.19] | 0.53    | 1.11 [0.99–1.25] | 0.07    |
| Male sex                                 |                      |                                | 0.58 [0.14–2.38] | 0.45    | 2.86 [0.38–21.65] | 0.31    |
| Body mass index (kg/m²)                  |                      |                                | 1.09 [0.94–1.27] | 0.24    | 0.84 [0.50–1.40] | 0.51    |
| Smoking, "yes"                           |                      |                                | 4.75 [1.73–13.08] | 0.003   | 8.75 [1.14–67.22] | 0.04    |
| Pack-years (smokers only)                |                      |                                | 0.97 [0.90–1.05] | 0.43    | 0.94 [0.88–1.02] | 0.13    |
| HbA1c (mmol/mol)                         |                      |                                | 1.07 [0.98–1.17] | 0.12    | 1.02 [0.89–1.16] | 0.80    |
| Excessive alcohol intake*                |                      |                                | 0.84 [0.20–3.64] | 0.82    | 0.41 [0.02–7.04] | 0.54    |
| Mean arterial blood pressure (mm Hg)     |                      |                                | 0.95 [0.90–1.01] | 0.08    | 0.98 [0.91–1.05] | 0.57    |
| Spherical equivalent, right eye (D)      |                      |                                | 1.10 [0.83–1.44] | 0.51    | 0.93 [0.45–1.92] | 0.84    |
| Axial length, right eye (mm)             |                      |                                | 1.29 [0.51–3.27] | 0.59    | 0.65 [0.13–3.32] | 0.61    |
| Subfoveal choroidal thickness, right eye, per 50 μm increase | 1.08 [0.74–1.57] | 1.22 [0.82–1.82] | 0.69 | 0.32 | – |

ORs are estimated per one unit increase unless otherwise indicated. A conditional logistic model was used for a matched co-twin design. An unconditional model did not yield other significant results. Only macular drusen were included. CI95, 95% confidence interval. Only accumulated/cross-sectional numbers from 2019 were used in the analysis. * Smoking was defined as active or history of smoking of ≥1 pack-year (1 pack-year = 20 cigarettes for 365 consecutive days). Excessive alcohol intake was defined as greater than 7/14 units per week for women/men, respectively.

Table 4. Case-wise concordance for the phenotypes 20-year incident ≥20 small hard drusen and incident drusen ≥63 μm

| Zygosity | Concordant pairs, n | Discordant pairs, n | Case-wise concordance [CI95] |
|----------|---------------------|---------------------|-----------------------------|
| Incident ≥20 small hard drusen | MZ | 0 | 1 | – |
| DZ | 1 | 3 | 0.22 [0.09–0.42] |
| Incident drusen ≥63 μm | MZ | 4 | 4 | 0.58 [0.25–0.85] |
| DZ | 0 | 6 | – |

Incident ≥20 small hard drusen was defined as advancement at the follow-up examination to at least 20 small hard drusen per eye (average of both eyes). In contrast, incident drusen ≥63 μm had only to appear in either or both eyes. Only macular drusen were included. CI95, 95% confidence interval; DZ, dizygotic; MZ, monozygotic.

Table 5. The genetic and environmental effects of incident drusen ≥63 μm in 138 healthy twins

| Model | Genetic components | Environmental components | Fit statistics | p value |
|-------|--------------------|--------------------------|---------------|---------|
|       | A [CI95] | D [CI95] | C [CI95] | E [CI95] | AIC |       |
| ACE   | 0.49 [0.26–0.71] | – | 0 | 0.51 [0.29–0.74] | 86.4 | – |
| ADE   | 0 | 0.51 [0.29–0.72] | – | 0.49 [0.28–0.70] | 86.1 | – |
| AE    | 0.49 [0.26–0.71] | – | – | 0.51 [0.29–0.74] | 85.4 | 0.26 |
| CE    | – | – | 0.36 [0.15–0.57] | 0.64 [0.43–0.85] | 89.3 | <0.01 |
| E     | – | – | 1 | – | 96.2 | <0.01 |

Estimates are total variation proportion attributable to A, D, C, or E. p values are based on a likelihood ratio test (ref. ACE model). An AE model was the best-fit to explain effects (bold). Only macular drusen were included. A, additive genetic effects; D, dominant genetic effects; C, common environment effects; E, unique environment effects; AIC, Akaike information criterion; CI95, 95% confidence interval.
Of the 18 participants with incident drusen ≥63 μm, 10 had AMD drusen and 8 had pachydrusen. Only 1 participant regressed in drusen severity over 20 years, from ≥20 small hard drusen at baseline to <20 small hard drusen at follow-up. The participant, a never smoker, had 24 small hard drusen per eye at baseline but only three per eye at follow-up, and the change was comparable in the two eyes.

Smoking was significantly associated with incident ≥20 small hard drusen or incident drusen ≥63 μm (Table 3). Additionally, the incidence of drusen ≥63 μm was significantly associated with having ≥20 small hard drusen at baseline. An unconditional logistic regression model did not diverge from the conditional model, indicating robustness toward confounding factors.

Incident cases with ≥20 small hard drusen were concordant in 1 DZ twin pair and discordant in 1 MZ pair and in 3 DZ pairs (Table 4). Incident cases with drusen ≥63 μm were concordant in 4 MZ twin pairs but discordant in 4 MZ twin pairs and in 6 DZ twin pairs.

The case-wise concordance rates showed a higher numerical effect of genetics for drusen ≥63 μm than for ≥20 small hard drusen (Table 4). The higher number of informative cases enabled analysis of the relative contributions of genetic and environmental factors for the incidence of drusen ≥63 μm (Table 5). The polygenic model for quantitative genetic effects identified an AE model as the best-fitting model (lowest AIC value). Thus, additive genetic effects (A) accounted for 49% [CI95 26–71] and unique environmental effects for 51% [CI95 29–74] of the incidence of drusen ≥63 μm. The number of observations did not permit analysis of the contribution of smoking to the environmental effects. Assuming that broad-sense heritability is the sum of additive and nonadditive genetic effects, the heritability estimate for incident drusen ≥63 μm was $h^2 = 48.5\%$ [CI95 26.3–70.7]. There were too few observations of incident ≥20 small hard drusen to compute a heritability estimate for this phenotype.

The development of drusen ≥63 μm at a location that small hard drusen had occupied at baseline was seen in one or more locations in 3 participants (shown in Fig. 3). In all 3 cases, the incident drusen ≥63 μm were pachydrusen.

**Discussion**

This prospective population-based study of the development of macular drusen over 2 decades found that transition to higher small hard drusen counts or drusen ≥63 μm was strongly associated with smoking. Furthermore, a concordance analysis found that unshared environmental effects, e.g., smoking, dietary habits, lifestyle, and physical activity, explained one-half of the incidence of drusen ≥63 μm. In contrast, the other half was attributable to additive genetic effects, i.e., the influence of more than a single gene.
The present study used OCT in addition to fundus photography, which adds to purely fundus photographic studies by enabling better distinction between AMD drusen and pachydrusen [6–8, 21]. Fundus autofluorescence was of limited use, as most small hard drusen were neither hypo- nor hyperfluorescent. There was no case with reticular drusen, cuticular drusen, choroidal neovascularization, or geographic atrophy. Thus, the study was one of small hard drusen and early drusen of a size that is compatible, by convention, with the diagnosis of AMD.

Comparison of the location of incident drusen ≥63 μm with the location of small hard drusen at baseline found 3 participants with pachydrusen out of 8 cases with pachydrusen that occurred where small hard drusen had been present 20 years earlier; no such observations were made for any other drusen ≥63 μm type. Lack of fundus photographs from the intervening years means that the detailed mode of transition cannot be described and that instances of transformation may have escaped documentation. Pachydrusen are characterized by being relatively large (>125 μm in diameter) and by being unevenly distributed in the posterior pole, occurring alone or in small clusters, and having well-defined borders [21]. Although pachydrusen are associated with a separate retinopathy called pachychoroid pigment epitheliopathy [25], and possibly more so than AMD, we chose to bundle drusen ≥63 μm into a single class due to limitations in methodology. Our observations indicate that studies of early drusen and drusen-like lesions of the retina may help refine the description of AMD and retinopathies with AMD-like characteristics.

The development of AMD has mainly been examined in large study populations aged 50 years and older, with a typical mean age of 65 years, and almost exclusively based on fundus photography [7–9, 26, 27]. Few of these studies have included OCT, which has mainly been used in smaller studies [10, 11]. Reported risk factors for the progression of AMD address notably the late stages of AMD including certain genotype variants [9, 26–29], age [9], the size and the area covered by drusen [9–11, 29], reduced choriocapillaris density and choriocapillaris flow impairment [30, 31], certain dietary habits [9], and smoking [9]. We examined a younger population suited for studying the transition from precursors of AMD to early AMD without choroidal neovascularization or geographic atrophy. We found a narrower range of risk factors than studies on older subjects. Although studies differ in design, there is now support for the assumption that the study of early AMD may narrow the search for risk factors to those that initiate AMD. The use of a population of twins allowed the assessment of the relative impact of genetic and environmental factors. Our main findings are that smoking and environmental factors increase the risk of developing AMD, at a magnitude comparable with that of genetics. The emergence of smoking as an identifiable risk factor between the baseline and follow-up visits in our cohort supports that AMD risk factors include exposures that are present late in life [15]. This agrees with the observation that the heritability of small hard drusen decreases with increasing age [32].

With an OR of 4.57 for as little as ten pack-years in a cohort with a median age of 59 years, the smoking effect is the highest recorded for smoking and early AMD [33]. Five prior large prospective studies with follow-up periods of 6.3–12 years examined the association between current smoking and AMD. Smoking was associated with incident soft large drusen in the Beaver Dam Eye Study (including a dose-response effect for the number of pack-years), with incident late AMD in the Blue Mountain Eye Study and the AREDS cohort [34–36], and with any incident AMD in both the Physicians’ Health Study (men-only) and the Nurses’ Health Study (female-only) [37, 38]. Although past smokers also had an increased risk of incident late AMD, the risk was only significant in the Blue Mountain Eye Study and the Nurses’ Health Study. However, the risk appeared to diminish after 20 years of smoking cessation [39]. Cross-sectional studies showed a stronger relationship between AMD and smoking (2- to 4-fold increase in risk for any AMD) as opposed to prospective studies (2-fold increase in primarily late AMD risk) among current smokers compared to nonsmokers. There is reason to suspect that this phenomenon may be explained by survival bias since smoking is associated with both increased risk of AMD and reduced survival [40]. After adjusting for increased mortality among smokers, the overall risk of developing late AMD among current smokers is 3.42 [CI95 1.57–5.15] compared to never smokers [40]. The risk is more prominent for people with a high-risk genetic profile [41].

The present study confirms prior studies that found evidence of a high number of small hard drusen per eye being associated with AMD development [5, 6]. Twin studies have also reproduced other findings from studies of unrelated participants, including the effect of genetic predisposition [13] and the heritability of both small hard drusen and advanced AMD [13–15].

Genotypes that predispose to early AMD include complement factor H [42] and the immune-inflammatory pathway (ARMS2) [26] with AMD, but the associations are weaker for...
early AMD than for late AMD [16, 26, 28]. That may explain why we in a previous study did not find a relation between having ≥20 small hard drusen and the AMD-associated complement factor H and ARMS2 risk variants [43].

A strength of the present study includes the population-based twin cohort design and the random selection among all Danish-born twins aged 30–80 years who resided in the Capital Region or Region Zealand. This reduced selection bias, although women were overrepresented. Our follow-up time (20–21 years) is appreciably longer than any prior study in the field. Limitations include the small sample size of 138 twins, with only 25 having developed numerous small or large drusen, followed by a common bias for cohort study volunteers to be healthier than the background population. Thus, the study may under- or overestimate the effects of some risk factors (smoking, obesity, and blood pressure). The addition of OCT at the follow-up visit may have influenced the grading of drusen. Finally, the statistical model used in this study assumes identical environments among twins and thus a possible overestimation of the total heritability.

Conclusion

In conclusion, our study showed that smoking and genetic factors were significantly associated with incident ≥20 small hard drusen and incident drusen ≥63 μm in unselected adult twins. Having ≥20 small hard drusen at baseline increased the risk of developing drusen ≥63 μm after 20 years. Our observations support that further studies of early AMD may help identify targets for prevention and intervention against AMD at a stage where the remedies may be simpler and potentially benefit greater than later in life. Specifically, avoidance of smoking is recommended.

Statement of Ethics

This study protocol was reviewed and approved by the Danish Regional Health Research Ethics Committee, approval number [H-18052822]. All participants gave their written informed consent to participate in the present study.

Conflict of Interest Statement

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

Funding Sources

The study was supported by the Rigshospitalet (grant E-23334-02), P. Carl Petersens Fond (grant 19102), Helsefonden (grant 19-B-0063), Aase og Ejnar Danielsen's Fond (grant 18-10-0698), Beckett Fondens (grant 19-2-3490), Einar Willumsen Fondens (grant 500028), and Horizon 2020, the European Union’s Framework Programme for Research and Innovation, under grant agreement No. 780989 (MERLIN). JB was supported by the VELUX Foundation (grant 00028975). SPR was supported by the OPUS Foundation and the Alfred-Vogt Foundation. The funding organizations had no role in the design or conduct of this research.

Author Contributions

Concept and design: Mohamed Belmouhand, Michael Larsen, Simon Paul Rothenbuehler, Inger Christine Munch, and Christine Dalgård. Acquisition, analysis, or interpretation of data: Mohamed Belmouhand, Jacob B. Hjelmborg, Michael Larsen, Sami Dabbah, Jakob Bjerg, Simon Paul Rothenbuehler, and Inger Christine Munch. Drafting of the manuscript: Mohamed Belmouhand and Michael Larsen. Critical revisions of the manuscript for important intellectual content: Simon Paul Rothenbuehler, Inger Christine Munch, Jakob Bjerg, Sami Dabbah, Christine Dalgård, and Jacob B. Hjelmborg. Final approval of the manuscript: all authors.

Data Availability Statement

For previewing the dataset, please use the following URL: https://osf.io/fym6j. Further inquiries can be directed to the corresponding author.

References

1 Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, et al. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health. 2017 Dec;5(12):e1221–34.
2 Sarks SH. Ageing and degeneration in the macular region: a clinico-pathological study. Br J Ophthalmol. 1976 May;60(5):324–41.
3 Sarks JP, Sarks SH, Killingsworth MC. Evolution of soft drusen in age-related macular degeneration. Eye. 1994;8(Pt 3):269–83.
4 Munch IC, Li QX, Ahmad SSM, Olsen EM, Skovgaard AM, Larsen M. Small hard macular drusen and associations in 11- to 12-year-old children in the Copenhagen Child Cohort 2000 Eye Study. Invest Ophthalmol Vis Sci. 2019 Apr;60(5):1454–60.
5 Bressler NM, Munoz B, Maguire MG, Vitale SE, Schein OD, Taylor HR, et al. Five-year incidence and disappearance of drusen and retinal pigment epithelial abnormalities. Waterman study. Arch Ophthalmol. 1995 Mar;113(3):301–8.
Belmouhand et al. Ophthalmologica 2022;245:421–430
DOI: 10.1159/000525652