Received: 11 March 2023 | Accepted: 15 January 2024

DOI: 10.1111/aos.16645

Acta Ophthalmologica

ORIGINAL ARTICLE

Associations of drusen location with risk factors and incidence of late age-related macular degeneration in the Alienor study

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Funding information
Caisse nationale de solidarité pour l'autonomie; Agence Nationale de la Recherche; Université de Bordeaux; Laboratoires Théa; Fondation Voir et Entendre

Abstract

Purpose: To test the hypothesis that central drusen location is strongly linked with known Age-related Macular Degeneration (AMD) risk factors and risk of incident late AMD.

Methods: The Alienor study is a prospective population-based cohort study of residents of Bordeaux, France, followed from 2009 to 2017. On retinal photographs, we defined central drusen as at least one soft drusen (>63 μm) within 500 μm from fovea and pericentral drusen as at least one drusen 500–3000 μm from fovea, in the absence of any central drusen. Late AMD (atrophic and/or neovascular) was diagnosed using multimodal imaging. In total, 481 eyes were included in the analysis: 160 central and 321 pericentral. We investigated associations with systemic (age, sex, smoking, medical prescriptions, plasma concentrations of lipids and nutrients, UV exposure, blood pressure), ocular (retinal thickness, cataract extraction) and genetic risk scores (GRS).

Results: In multivariate logistic regression central drusen were associated with smoking (OR, 2.95 for smoking more than 20 pack-years, $p=0.02$), HDL-cholesterol (OR, 1.57 for 1 standard deviation (SD) increase, $p=0.0048$), pulse pressure (OR, 0.77 for 1 SD increase, $p=0.04$), Age-Related Maculopathy Susceptibility 2 (ARMS2) GRS (OR, 1.42; 95% CI, 1.11–1.83) and complement GRS (OR, 1.55; 95% CI, 1.15–2.10). In Cox modelling, the central location of drusen (at baseline or during the follow-up) was associated with a 4.41-fold increased risk (95% CI,1.98–9.81) for an incident late AMD.

Conclusion: Central drusen were strongly associated with AMD risk factors and incident late AMD, suggesting that it represents a key marker for AMD progression.

KEYWORDS
age-related macular degeneration, age-related macular degeneration/classification, epidemiologic studies, retinal drusen, retinal drusen/location

1 | INTRODUCTION

Age-related macular degeneration (AMD) remains the second largest non-avoidable cause of blindness worldwide in adults aged 50 years and older (GBD, 2019 Blindness and Vision Impairment Collaborators & Vision Loss Expert Group of the Global Burden of Disease Study 2021) and the first cause in Europe (Flaxman et al., 2017).

Relatively few population-based prospective studies of AMD have been conducted worldwide (Joachim et al., 2015; Klein et al., 2007; Leske et al., 2004; Varma et al., 2010; Yasuda et al., 2009; You et al., 2012) and even fewer in the European continent (Buch et al., 2005; Delcourt et al., 2005; Jonasson et al., 2005; van Leeuwen, Ikram, et al., 2003; van Leeuwen, Klaver, et al., 2003). These studies have helped identifying early retinal abnormalities (soft distinct and

indistinct drusen, reticular pseudodrusen, or pigmentary abnormalities) that precede and predict advanced forms of the disease (ie late AMD, ether atrophic or neovascular).

Several classifications have been proposed for early AMD, with varying definitions and stages (Davis et al., 2005; Ferris et al., 2013; Klein et al., 2014; Thee et al., 2020). But all of them define early AMD as the presence of drusen and pigmentary abnormalities in the ETDRS grid (3000 µm from the foveal centre), regardless of the location. Very few epidemiologic studies were interested in the drusen location (Delcourt et al., 2011; Joachim et al., 2015; Saunier et al., 2018). Only one study has specifically studied AMD according to drusen location (Knudtson et al., 2004), showing that lesions associated with early AMD were more likely to develop in specific locations in the macular area, and persons with lesions closer to the fovea may be associated with a higher risk of development of late AMD.

Early AMD represents a heterogeneous group of subjects, some of whom bear a very low risk of developing late AMD. This may be one of the reasons why associations of risk factors are often much weaker with early AMD than with late AMD in epidemiologic studies (Joachim et al., 2015, Saunier et al., 2018).

In these populations, the major risk factors identified for AMD are systemic (older age, sex, smoking status, plasma lipid and nutrient levels, blood pressure, ultraviolet exposure), ocular (retinal thickness and cataract extraction) and genetic (in particular, single-nucleotide polymorphisms in the complement factor H (CFH) gene and the age-related maculopathy susceptibility 2 (ARMS2) gene) (Chakravarthy et al., 2010; Colijn et al., 2021; Cougnard-Grégoire et al., 2013, 2014; Delcourt et al., 2014; Joachim et al., 2015; Lim et al., 2012; Merle et al., 2013, 2021).

We explored the hypothesis that drusen location is strongly linked with known AMD risk factors and incident late AMDs so that it could allow a better stratification of early AMD and constitute a powerful and easy biomarker for future epidemiologic studies.

In this report, we assessed the associations of drusen location (central or pericentral) with known AMD risk factors (systemic, ocular and genetic factors) and with the incidence of late AMD in a population-based study of elderly French individuals, to identify more specific early abnormalities associated with an increased risk of late AMD.

2 | METHODS

2.1 | Study sample

The study cohort for the ALIENOR (Antioxydants, Lipides Essentiels, Nutrition et Maladies Oculaires) Study was derived from the ongoing Three-City (3C) Study, a population-based investigation focused on vascular risk factors for dementia (3C Study Group, 2003). It consists of comprehensive eye examinations which are offered to 3C participants from Bordeaux approximatively every 2 years since 2006 (Delcourt et al., 2010). In 2006–2008, 963 (66.4%) participated in the first Alienor eye examination (Alienor 0), out of 1450 alive participants (Figure 1). Since then, 4 follow-up eye examinations have been conducted in 2009–2011 (Alienor 1), 2011–2012 (Alienor 2), 2013–2015 (Alienor 3) and 2015–2017 (Alienor 4).

2.2 | Eye examination

The eye examinations took place in the Department of Ophthalmology of the University Hospital of Bordeaux. A comprehensive assessment was undertaken, encompassing the recording of ophthalmic history, measures of visual acuity and refraction. Two

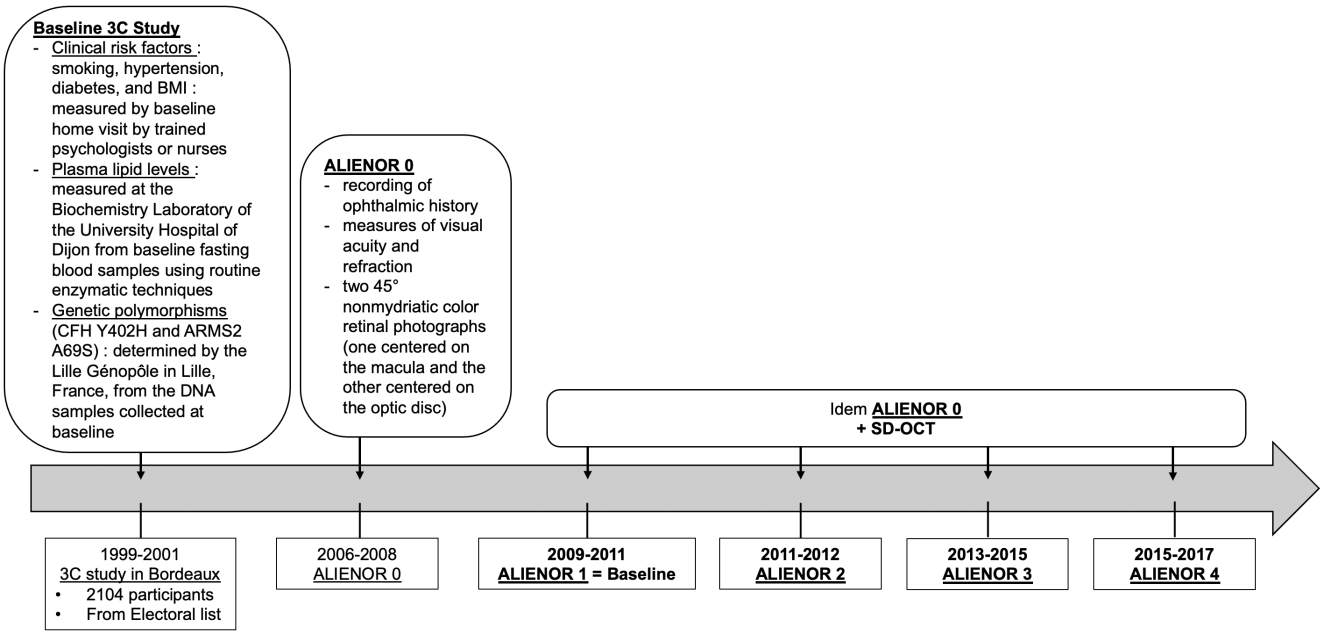

FIGURE 1 Timeline of alienor study.

45° nonmydriatic colour retinal photographs were obtained, with one focused on the macula and the other centred on the optic disc.

Retinal photographs were performed using a high-resolution digital nonmydriatic retinograph (TRC NW6S; Topcon).

In addition to retinal photographs, as of 2009, a spectral-domain optical coherence tomography (SD-OCT) examination of the macula was performed using Spectralis, version 5.4.7.0 (Heidelberg Engineering). All SD-OCT assessments were performed by the same experienced technician. For macular cube acquisition, conditions used for acquisition were the following: resolution mode, high speed; scan angle, 20°; size X, 1024 pixels (5.7 mm); size Z, 496 pixels (1.9 mm); scaling X, 5.54 µm/pixel; scaling Z, 3.87 µm/pixel; number of B-scans, 19; pattern size, 20 × 15° and distance between B-scans, 236 µm. A single horizontal and vertical B-scan images (1536 A-scans) centred on the fovea were also performed.

In this study, the second eye examination (Alienor1) conducted between 2009 and 2011 was designated as the baseline (Figure 1). This decision was motivated by the systematic implementation of SD-OCT imaging from this point onward, providing a consistent and comprehensive dataset. Subsequent follow-up examinations were conducted in intervals spanning from 2011 to 2012 (Alienor 2), from 2013 to 2015 (Alienor 3) and from 2015 to 2017 (Alienor 4). Participation in the study required both a baseline examination and at least one gradable follow-up examination.

2.3 | Classification of age-related macular degeneration

Procedures for the classification of AMD have been described in detail previously (Delcourt et al., 2011; Saunier et al., 2018).

In summary, retinal photographs were independently interpreted by two specially trained technicians according to international classification (Bird et al., 1995) and to a modification of the grading scheme used in the Multi-Ethnic Study of Atherosclerosis (Klein et al., 2006) for drusen size, location and area. Inconsistencies between the interpretations were resolved by a senior grader (C.D.) for the classification of AMD and other retinal diseases.

Late AMD was defined by the presence of neovascular AMD or geographic atrophy within the ETDRS grid (3000 µm from the foveal centre). This determination was based on a comprehensive evaluation, including retinal photographs, OCT scans, ophthalmologic history and treatment records. All cases of late AMD were reviewed and confirmed by specialists (J.F.K., M.N.D., or M.B.R.). Incident late AMD referred to the onset of late AMD during the follow-up period, observed in eyes without late AMD at the baseline. The date of late AMD occurrence was calculated as the midpoint between the last visit without late AMD and the first visit with late AMD.

Early AMD was classified into two groups (in the absence of late AMD): early AMD1 (soft distinct drusen without pigmentary abnormalities or pigmentary abnormalities without large drusen [125 µm]); and early AMD2 (soft indistinct drusen and/or reticular drusen and/or soft distinct drusen associated with pigmentary abnormalities [hyperpigmentation or hypopigmentation]).

In addition, detailed characteristics of early AMD abnormalities were studied, including intermediate drusen (63–125 µm in diameter), large soft distinct and indistinct drusen (>125 µm in diameter), large area of drusen (>500 µm in diameter), hyperpigmentation and hypopigmentation. For each item, there were four exclusive categories: absent, questionable, present in pericentral area (500–3000 µm from fovea), present in central area (within 500 µm from fovea) (Delcourt et al., 2011).

Drusen were divided into two groups: 'central drusen' defined as at least one intermediate or large soft drusen in the central area, and 'pericentral drusen' defined as at least one drusen outside the pericentral area in the absence of any drusen within the central area.

Although our analysis relied exclusively on retinal photographs, information regarding retinal thickness was incorporated into the analysis starting from the ALIENOR 1 visit, utilizing data from SD-OCT.

2.4 | Assessment of risk factors

During the period from 1999 to 2001, comprehensive data were obtained through face-to-face interviews employing a standardized questionnaire administered by trained psychologists or nurses. Demographic and lifestyle variables, including age, sex and smoking history in pack-years, were recorded. Lifetime exposure to ambient ultraviolet (UV) radiation was estimated from residential history and Eurosun satellite-based estimations of ground UVR, as described previously (Delcourt et al., 2014). Medical variables, collected at ALIENOR 1 (2009–2011), encompassed diastolic and systolic blood pressure, retinal thickness on OCT, history of cataract surgery and an inventory of all medications used in the preceding month, with special emphasis on lipid-lowering treatments. Medical prescriptions and, when feasible, the medications themselves were reviewed by the interviewer.

Biological data, drawn from a fasting blood sample collected during the baseline examination of the 3C study (1999–2001), included lipid concentrations (total, HDL, LDL-cholesterol), nutrients (Omega3 fatty acids, Zeaxanthin, Lutein, vitamin D), as detailed previously (Cougnard-Grégoire et al., 2014, 2015; Merle et al., 2013, 2021). Genotyping was performed on DNA extracted from leukocytes at 3C study baseline (1999–2001) and kept frozen at −80°C. Genome-wide scan analysis was conducted at Lille Génopôle (Lille, France) from DNA samples collected during the baseline of the 3C study (1999–2001) (Delcourt et al., 2011, 2012).

A Genetic Risk Score (GRS) for 49 AMD-associated risk variants was calculated as the sum of the βs of the risk variants from the genome-wide association study of the International AMD Genomics Consortium, as described previously (de Breuk et al., 2021; Fritsche

et al., 2016). In a European population-based cohort (Colijn et al., 2021), the mean (SD) score was 0.40 (1.24) and showed a normal distribution. In patients with late AMD, GRS was higher than control participants and was positive in >85%. As previously described (Colijn et al., 2021), pathway-specific GRSs were constructed using subsets of Single-nucleotide Polymorphisms (SNP): complement GRS (CFH, CFI, C9, C2, TMEM97/VTN, C3 genes); lipid GRS (ABCA1, LIPC, CETP and APOE genes); extracellular matrix (ECM) GRS (COL4A3, ADAMTS9AS2, COL8A1, VEGFA and SYN3/TIMP3 genes).

2.5 | Statistical analyses

Results are presented using mean values and standard deviations (mean (SD)) for continuous variables, and number and percentage for non-continuous variables (No. (%)).

First, associations of drusen location (central or pericentral) and potential risk factors were estimated using logistic Generalized Estimating Equation (GEE) models, adjusted for age and sex (Model 1) (Zeger et al., 1988). Second, a multivariate model was performed including all variables associated with drusen location in Model 1, with forced entry for age and sex (p-value<0.10). The associations are presented as odds ratios (ORs) and 95% confidence intervals (CIs). Eyes with pericentral drusen were the reference in all models.

Then, the association of drusen location with the incidence of late AMD were analysed using the Cox proportional hazards model, adjusted for age and sex (Lamarca et al., 1998). The individual eye was used as the unit of analysis by using PROC PHREG with the covariance aggregate option. This survival model allows the use of correlated data in eye-specific analyses and uses a robust estimate of the variance of estimated parameters to account for the correlation between right and left eyes (Glynn & Rosner, 2012). We considered the potential evolution of a pericentral drusen into a central drusen during follow-up using a time-dependent variable.

Statistical significance was set at $p<0.05$. Statistical analyses were performed between September 2021, and March 2022 using SAS, version 9.4 (SAS Institute, Inc, Cary, NC; procedure GENMOD for the GEE analysis).

Data were analysed from December 2021 to September 2022.

3 | RESULTS

3.1 | Study sample

Out of the initial 1248 eyes (624 participants) in the Alienor 1 examination (Figure 2), 82 eyes were excluded due to late AMD, 303 eyes due to ungradable images and 382 eyes due to the absence of central or pericentral drusen. Consequently, 481 eyes with central or pericentral drusen were included in the analysis, comprising 160 eyes with central drusen and 321 eyes with pericentral drusen. The mean (SD) follow-up period for these participants was 4.8 (2.0) years. Of these eyes, 65 (13.5%) did not participate in any follow-up examinations, and 24 (5.8%) had ungradable AMD status at all available follow-ups. Thus, 392 eyes were available for the analysis of incident late AMD (Cox analysis).

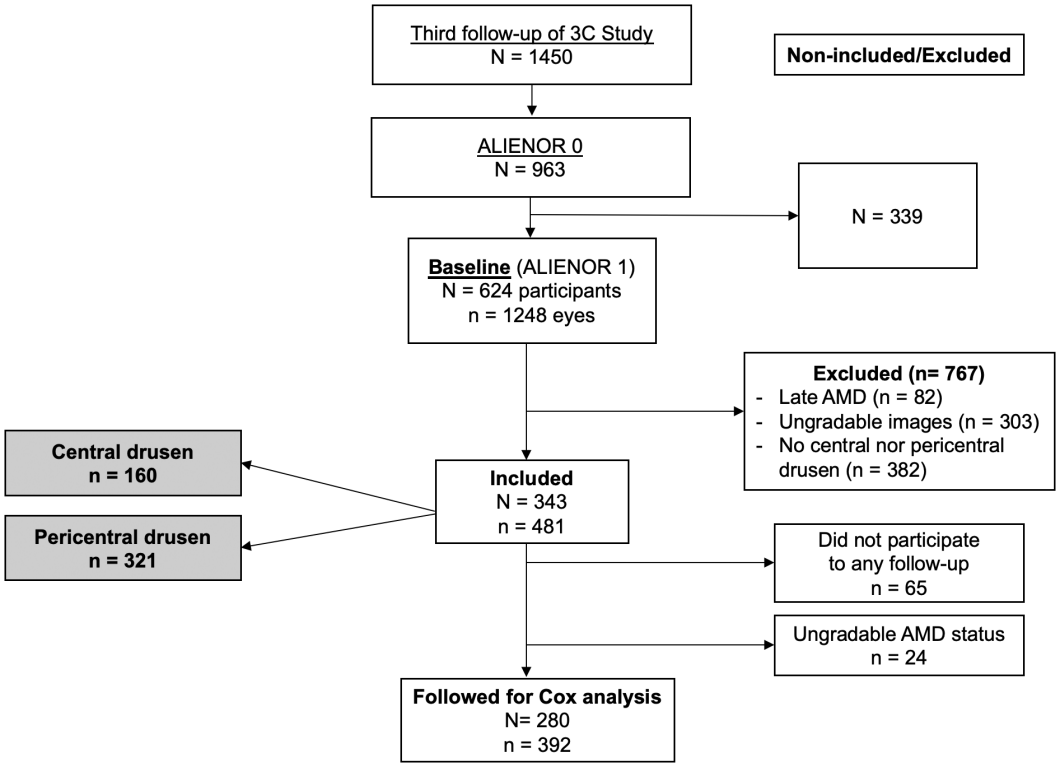

FIGURE 2 Flow chart showing selection of eyes. *N*, number of participants, *n*, number of eyes.

TABLE 1 Age- and sex-adjusted associations of drusen location per eye with potential systemic and ocular risk factors of AMD in the Alienor study.

	No	Central ($n=160$ eyes)	Pericentral ($n=321$ eyes)	OR	95% CI	p-value
Systemic factors						
Age	481	81.90 (4.33)	82.07 (4.14)	0.93[b]	0.74–1.17	0.53
Sex	481					0.12
Men		41 (25.6)	109 (34.0)	ref		
Women		119 (74.4)	212 (66.0)	1.48	0.91–2.41	
Smoking status	478					0.06
Nonsmoker		96 (60.4)	218 (68.3)	ref		
<20 pack-years[a]		35 (22.0)	53 (16.6)	1.88	1.04–3.38	
≥20 pack-years[a]		28 (17.6)	48 (15.1)	1.79	0.94–3.38	
Lipid-lowering treatment	476					0.34
No		101 (64.3)	184 (57.7)	ref		
Statin therapy		13 (8.3)	37 (11.6)	0.58	0.27–1.27	
Others		43 (27.4)	98 (30.7)	0.91	0.55–1.52	
Plasma lipid concentration, (mmol/L)						
Total cholesterol	455	5.82 (0.92)	5.80 (0.96)	1.01[b]	0.81–1.27	0.93
HDL-cholesterol	455	1.72 (0.41)	1.59 (0.37)	1.40[b]	1.11–1.77	0.0045
LDL-cholesterol	455	3.60 (0.81)	3.67 (0.86)	0.91[b]	0.72–1.13	0.39
Triglycerid	455	1.11 (0.54)	1.19 (0.56)	0.88[b]	0.68–1.13	0.31
Plasma Omega3 fatty acids (% of total fatty acids)	393	4.10 (1.37)	4.23 (1.39)	0.89[b]	0.70–1.14	0.37
Plasma Zeaxanthin (μg/L)	431	42.37 (24.48)	43.04 (26.89)	0.95[b]	0.74–1.20	0.65
Plasma Lutein (μg/L)	431	178.70 (89.91)	175.98 (88.26)	1.00[b]	0.79–1.26	0.99
Plasma Lutein+zeaxanthin (μg/L)	431	221.07 (110.13)	219.02 (110.02)	0.99[b]	0.78–1.24	0.90
Plasma Vitamin D (ng/mL)	436	13.63 (6.20)	14.83 (9.67)	0.94[b]	0.75–1.17	0.57
UV exposure (kJ/cm^2)	432	40.02 (2.06)	40.11 (1.82)	1.01[b]	0.80–1.28	0.91
Pulse pressure=systolic-diastolic (mmHg)	449	65.50 (14.24)	70.26 (14.51)	0.76[b]	0.60–0.97	0.03
Ocular factors						
Central retinal thickness (μm)	475	275.10 (29.35)	283.04 (37.05)	0.91[b]	0.74–1.12	0.38
Pericentral retinal thickness (mean of the 4 pericentral subfields) (μm)	475	322.95 (21.45)	328.10 (25.66)	0.86[b]	0.67–1.10	0.22
Cataract extraction	471					0.17
No		56 (36.4)	137 (43.2)	ref		
Yes		98 (63.6)	180 (56.8)	1.35	0.88–2.08	

Note: Results are presented using mean values and standard deviations (mean (SD)) for continuous variables, and number and percentage for non-continuous variables (No. (%)).

Abbreviations: CI, Confidence Interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; UV: Ultra-Violet.

[a]Pack-year calculated as number of smoking years × mean number of cigarettes per day/20.

[b]ORs were estimated for 1-SD increase.

3.2 | Characteristics of eyes with central drusen and association with risk factors

Tables 1 and 2, present the associations of central drusen with AMD risk factors, after adjustment for age and sex, using subjects with pericentral drusen as the reference group.

Regarding systemic and ocular risk factors, a positive association with central drusen was found for plasma HDL-cholesterol (OR, 1.40; $p=0.0045$) and pulse pressure (OR, 0.76; $p=0.03$). The association with smoking was of borderline significance ($p=0.06$). No significant associations were found between drusen location and age, lipid-lowering treatments, plasma lipids and nutrients, UV exposure, retinal thickness or cataract surgery.

Regarding genetic risk factors, significant associations were observed with Complement Factor H (CFH) (OR, 2.43; $p=0.04$), Age-Related Maculopathy Susceptibility (ARMS2) (OR, 4.86; $p=0.03$), the Genetic Risk Score (OR, 1.82; $p<0.0001$, 95% CI, 1.39–2.37), as well as the ARMS2 (OR, 1.35, $p=0.01$) and complement specific pathways (OR, 1.56, $p=0.0007$). By contrast, the extracellular matrix and lipid pathways were not significantly associated with central drusen. Figure 3 visually depicts the variations in the distribution of the Genetic Risk Score based on the location of drusen. While the

TABLE 2 Age- and sex-adjusted associations of drusen location per eye with potential genetic risk factors of AMD in the Alienor Study.

	n	Central (*n* = 160 eyes)	Pericentral (*n* = 321 eyes)	OR	95% CI	*p*-value
CFH Y402H rs1061170 genotype	443					0.04
TT (non-risk)		46 (30.9)	131 (44.6)	ref		
TC		77 (51.7)	132 (44.9)	1.56	0.94–2.57	
CC		26 (17.4)	31 (10.5)	2.43	1.18–4.97	
ARMS2 A69S rs10490924 genotype	397					0.03
GG (non-risk)		78 (58.6)	183 (69.3)	ref		
GT		44 (33.1)	74 (28.0)	1.47	0.87–2.48	
TT		11 (8.3)	7 (2.7)	4.86	1.62–14.63	
Genetic score[a]	398	0.88 (1.16)	0.16 (1.25)	1.82[b]	1.39–2.37	<0.0001
ARMS2 pathway		0.52 (0.70)	0.37 (0.57)	1.35[b]	1.07–1.70	0.01
ECM pathway		−0.05 (0.19)	−0.07 (0.21)	1.08[b]	0.85–1.36	0.54
Complement pathway		0.39 (0.88)	−0.06 (0.95)	1.56[b]	1.20–2.01	0.0007
Lipid pathway		−0.06 (0.25)	−0.11 (0.24)	1.22[b]	0.95–1.56	0.12

Note: Results are presented using mean values and standard deviations (mean (SD)) for continuous variables, and number and percentage for non-continuous variables (No. (%)).

Abbreviations: ARMS2, age-related maculopathy susceptibility 2; CFH, complement factor H; CI, Confidence Interval; ECM, Extra Cellular Matrix; OR, odds ratio.

[a]Based on 49 polymorphisms related to Age-related Macular Degeneration.

[b]ORs were estimated for 1-SD increase.

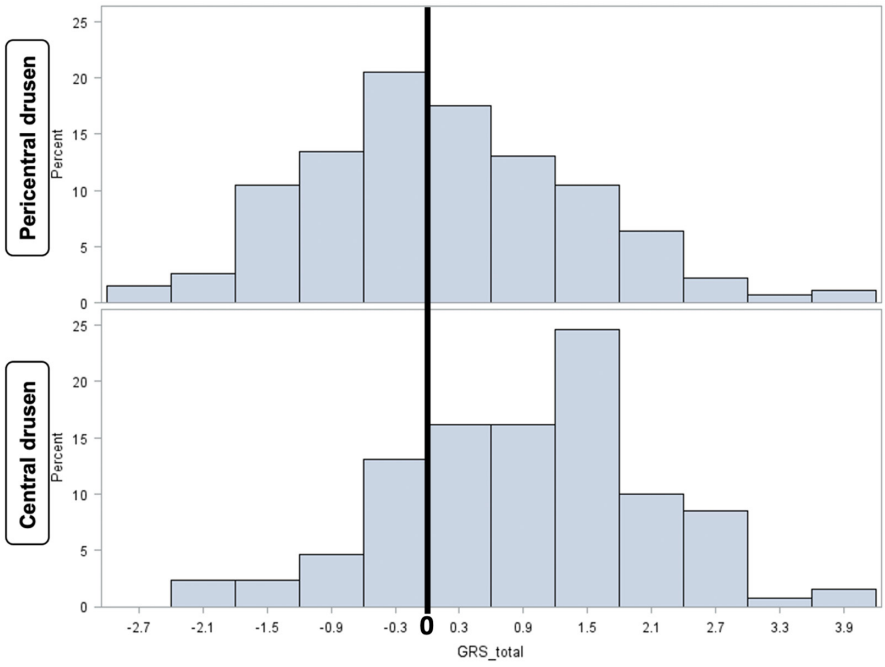

FIGURE 3 Bar graphs showing distributions of the total AMD genetic risk score (GRS) according to the drusen location (top panel) pericentral drusen, (bottom panel) central drusen.

distribution of pericentral drusen was centred on 0, 77.7% of individuals with central drusen had positive values. This highlights the higher genetic susceptibility of participants with central drusen.

In a multivariate model including variables with P-value below 0.10 (smoking, HDL-cholesterol, pulse pressure, ARMS2 and complement pathways), there was a statistically significant association of central drusen with smoking ($p=0.02$), HDL-cholesterol (OR, 1.57; $p=0.0048$), pulse pressure (OR 0.77; $p=0.04$), ARMS2 (OR, 1.42; $p=0.01$) and complement pathway (OR, 1.55, $p=0.0029$) (Table 3). No association with age and sex was observed.

3.3 | Risk of incident late AMD according to drusen location

Among the 392 eyes with follow-up data, 57 eyes with pericentral drusen developed central drusen and 33 eyes

TABLE 3 Multivariate associations of drusen location with systemic and genetic risk factors assessed by multivariate linear mixed model.

	OR[b]	95% CI	*p*-value
Age	0.99[c]	0.73–1.32	0.92
Sex			0.08
Men	Ref		
Women	1.95	0.92–4.15	
Smoking status			0.02
Nonsmoker	Ref		
<20 pack-years[a]	2.21	1.06–4.63	
>=20 pack-years[a]	2.95	1.25–6.97	
Plasma HDL-cholesterol[c]	1.57[c]	1.18–2.08	0.0048
Pulse pressure[c] = systolic – diastolic	0.77[c]	0.60–0.99	0.04
ARMS2 pathway	1.42[c]	1.11–1.83	0.01
Complement pathway	1.55[c]	1.15–2.10	0.0029

Abbreviations: ARMS2, age-related maculopathy susceptibility 2; CI, Confidence Interval; HDL, high-density lipoprotein; OR, odds ratio.

[a]Pack-year calculated as number of smoking years × mean number of cigarettes per day/20.

[b]All variables with a *p*-value below 0.10 in the univariate model were forced into the model: smoking, HDL-cholesterol, Pulse pressure, Complement Factor H, ARMS2.

[c]ORs were estimated for a 1-SD increase.

(8.4%) developed incident late AMD, with 25 from the central drusen group and 8 from the pericentral group. Of these, 20 (61%) were atrophic AMD, and 13 (39%) were neovascular AMD.

A cox-model shows that central location of drusen (at baseline or during follow-up) was associated with a 4.41-fold increased risk (95% CI,1.98–9.81) for incident late AMD.

4 | DISCUSSION

In this large population-based longitudinal study, central drusen location was strongly associated with known AMD risk factors. The multivariate model highlights systemic (smoking status, HDL concentration and pulse pressure) and genetic (AMRS2 and complement pathways) associations. In addition, the longitudinal analysis found a strong association of central drusen location with the incidence of late AMD. Therefore, the central drusen location could constitute a powerful characterization of drusen for analysing associations with risk factors and predict evolution to late AMD.

Our observations are consistent with previous epidemiological studies, showing an increased risk of progression to late AMD in eye with central drusen. Indeed, in the Beaver Dam Eye Study (Knudtson et al., 2004), in an American population, individuals with drusen closer to the fovea faced an elevated risk of developing late AMD. Similarly, the Blue Mountains Eye Study, conducted in an Australian population (Joachim et al., 2015), demonstrated that the proximity of drusen to the foveal centre was a robust predictor of incident late AMD. This association persisted even after adjusting for age, sex and smoking, and remained significant following further adjustments for factors such as fish consumption, CFH- rs1061170 and ARMS2-rs10490924 polymorphisms.

Furthermore, in our study, the presence of central drusen was associated with a high genetic susceptibility to AMD. In the EYE-RISK database (Colijn et al., 2021), mean (SD) GRS was 0.26 (1.16) in control participants and 0.83 (1.33) in intermediate AMD patients. These scores are close to those found in our study for pericentral (mean, 0.16; SD, 1.25) and central drusen (0.88; SD, 1.16). Moreover, in the present study, central drusen were associated with ARMS2 and complement pathways, which are the strongest pathways associated with AMD (Colijn et al., 2021).

In the present study, central drusen were also associated with non-genetic AMD risk factors, in particular smoking, the best-characterized lifestyle risk factor for AMD (Thornton et al., 2005), and HDL-cholesterol. This finding aligns with existing research linking HDL-cholesterol and AMD (Colijn et al., 2019; Cougnard-Grégoire et al., 2014). However, surprisingly, we did not find an association between drusen location and the genetic lipid pathway. This discrepancy may be attributed to the diverse lipid genes included in the pathway, such as ABCA1, LIPC, CETP and APOE (Colijn et al., 2021). While lipid metabolism is clearly involved in drusen formation, the exact mechanisms remain elusive (Curcio et al., 2011).

By contrast, central drusen were not significantly associated with plasma concentrations of several nutrients (in particular omega3 fatty acids and lutein and zeaxanthin), which have been repeatedly associated with AMD in several studies, including the Alienor study (Delcourt et al., 2006; Merle et al., 2013, 2014, 2021; Souied et al., 2016). However, these nutrients were mostly associated with late AMD in previous studies, suggesting that they might be more associated with the progression to late AMD, rather than in the constitution of drusen.

Finally, central drusen was significantly associated with a lower pulse pressure. This is in contradiction with previous observations of a higher risk for advanced AMD in subjects with high pulse pressure, both in the Alienor study and several other epidemiological studies (Choudhury et al., 2011; Cougnard-Grégoire et al., 2013; Klein et al., 2003; van Leeuwen, Ikram, et al., 2003; van Leeuwen, Klaver, et al., 2003).

Overall, our results suggest that central drusen constitutes a high-risk feature of AMD. Further insights may be expected from progress in the processing of OCT imaging. Using a computer-assisted segmentation of drusen on OCT macular scans, Pollreiz et al (Pollreisz et al., 2021) showed that drusen density was about 20-fold higher in the central circle of the ETDRS (1 mm diameter) than in the outer ETDRS circle (3–6 mm in diameter). They suggested that drusen biogenesis may be related to the foveal singularity in the distribution of cells (in particular high density of cones and Müller cells). Moreover, in several clinical studies using automated OCT segmentation, drusen volume exceeding 0.03 mm^3 in the 3 mm central circle was associated with a high risk of developing late AMD (Abdelfattah et al., 2016; Hirabayashi et al., 2023; Wakatsuki et al., 2023). Future studies, documenting drusen volume also in the 1-mm

central circle might allow a more targeted identification of high-risk patients.

A potential limitation of our results is the questionable representativeness of the sample. Indeed, about two thirds of the participants in the 3C Study accepted the eye examination. In this subsample, subjects of younger age and higher socio-economic status were overrepresented among subjects participating in the 3C Study (Delcourt et al., 2010). However, for most parameters of interest in our study, in particular, smoking, nutritional and cardiovascular risk factors, subjects included in the ALIENOR study were not different from those who did not participate (Delcourt et al., 2010). The age-and sex-specific prevalence rates of AMD were also similar to those observed in other studies performed in Europe (Augood et al., 2006; Vingerling et al., 1995) and other industrialized countries (Friedman et al., 2004). Moreover, the individuals ($n=630$) still participating in the 3C Bordeaux cohort in 2009 (10-year follow-up) were representative of the inhabitants of Bordeaux 75 years or older ($n=19\,232$) (Tabue-Teguo et al., 2017). The frequencies of the genotypes of *CFH Y402H* (Despriet et al., 2006; Klein et al., 2008; Xing et al., 2008) and *ARMS2* (Thee et al., 2022) polymorphisms were also similar to those observed in previous studies in Caucasian subjects. Finally, such differences are unlikely to bias the estimation of the associations of drusen location with AMD risk factors and incident late AMD.

We obtained a good follow-up rate (81%), with a 6-year follow-up and 3-time points evaluations. In particular, most previous studies (Joachim et al., 2015; Jonasson et al., 2005; Klein et al., 2007; Leske et al., 2004; You et al., 2012) used 5 years intervals between follow-up examinations. This may have led to survival bias and decreased the incidence rates, as some participants may have developed AMD and died within this 5 years interval and therefore would not be counted as incident cases. Our much shorter intervals (1–2 years between examinations) probably have decreased much of this survival bias.

In conclusion, this study confirms the strong association of drusen location with AMD risk factors and incidence of late AMD. Characterization of the central location of drusen could modify the classification of early and intermediate AMD. A more accurate classification of the early stages of the disease will help identify AMD risk factors and predict evolution to the late stage of disease, providing a better identification of high-risk patients.

FUNDING INFORMATION

Laboratoires Théa (Clermont-Ferrand, France), Universite de Bordeaux (Bordeaux, France), Fondation Voir et Entendre (Paris, France), Agence Nationale de la Recherche (2010-PRSP-011), CFSR Recherche (Club Francophone des Spécialistes de la Rétine), the French Ministry of Health (PHRC, 2012, PHRC12_157 ECLAIR) and CNSA (Caisse Nationale pour la Solidarité et l'Autonomie) (Paris, France). Laboratoires Théa participated in the design of the study, but none of the sponsors participated in the collection, management, statistical analysis and interpretation of the data, or in the preparation, review or approval of the present manuscript.

CONFLICT OF INTEREST STATEMENT

None.

ETHICAL APPROVAL

The Alienor Study was conducted in compliance with the Declaration of Helsinki. The design of the Alienor Study was approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006, ensuring that all aspects of the study were conducted in accordance with ethical standards. All participants were informed about the study and provided their written informed consent.

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How to cite this article: Sénéclauze, A., Le Goff, M., Cougnard-Grégoire, A., Korobelnik, J.-F., Rougier, M.-B., Delyfer, M.-N. et al. (2024) Associations of drusen location with risk factors and incidence of late age-related macular degeneration in the Alienor study. *Acta Ophthalmologica*, 00, 1–10. Available from: https://doi.org/10.1111/aos.16645