Early middle age cholesterol levels and the association with age-related macular degeneration

Fabian Kananen,[1,2] iD Timo Strandberg,[1,3] Sirpa Loukovaara[1,4] iD and Ilkka Immonen[1,4]

[1]University of Helsinki, Helsinki, Finland
[2]Department of Ophthalmology, Örebro University Hospital, Örebro, Sweden
[3]Department of Geriatrics, Helsinki University Hospital, Helsinki, Finland
[4]Department of Ophthalmology, Helsinki University Hospital, Helsinki, Finland

ABSTRACT.

Purpose: To examine whether serum cholesterol in early middle age is associated with age-related macular degeneration (AMD) later in life.

Methods: A group of Helsinki Businessmen Study (HBS) participants (n = 209) were recruited for the study. Total cholesterol (TC), triglyceride and body mass index (BMI) were measured at the HBS baseline visit in 1964–1973. Lipid subfractions, BMI, smoking status and statin use were recorded in 2011 and fundus photographs graded for AMD in 2005–2012. The subjects were genotyped for the main AMD risk single nucleotide polymorphisms (SNPs).

Results: TC measured at baseline 1964–1973 was significantly higher in subjects later developing intermediate or late AMD (6.67 mmol/l versus 6.20 mmol/l, p = 0.024) or with drusen size of ≥125 μm (6.68 mmol/l versus 6.21 mmol/l, p = 0.030) compared with the rest of the study population. TC, LDL and TG values at follow-up 2011 were lower in subjects with AMD compared to those without, whereas HDL levels showed no difference. In multivariate analysis, baseline TC associated with intermediate or late AMD (OR 1.59, p = 0.004) and drusen size ≥ 125 μm (OR 1.57, p = 0.006) when corrected for age, BMI, AMD risk SNPs and smoking. Lipid values measured 2011 had no associations after correction.

Conclusions: High systemic total cholesterol in early middle age may have a role in the initial development of AMD, especially in patients later developing large drusen.

Key words: age-related macular degeneration – cardiovascular risk factors – drusen – genetics – lipids

Introduction

Age-related macular degeneration (AMD) is an important cause of visual impairment in high-income countries (Flaxman et al. 2017). The pathogenesis of AMD is multifactorial with genetic and environmental factors playing a role. Among environmental factors, the most consistently implied ones are age, smoking and nutrition (Age-Related Eye Disease Study Research Group, 2000; Smith et al. 2001). In addition, a large number of genes have been implicated in the development of AMD (Fritsche et al. 2016). Phenotypically early AMD is characterized by the formation of drusen with or without pigmentary abnormalities. Later stages involve atrophy of the retinal pigment epithelium (RPE) or development of choroidal neovascularization (CNV; Lim et al. 2012).

Lipid processing has a role in the development of AMD. Cholesterol accumulates in Bruch´s membrane with age at sites where basal linear deposits and drusen later form (Pauleikhoff et al. 1990; Ruberti et al. 2003). In addition, esterified and unesterified cholesterol constitute at least 40% of the volume of hard drusen (Wang et al. 2010). Several lipid pathway genes have been found to associate with AMD in genome-wide association studies (GWAS): Apolipoprotein E gene (APOE), cholesterol ester transferase gene (CETP), hepatic lipase gene (LIPC; Fritsche et al. 2013), ATP-binding cassette transporter A-1 (ABCA1), ABCA-7, apolipoprotein C2 (APOC2), APOC4 and phospho-lipid transfer protein (PLTP; Fritsche et al. 2016). In addition, candidate gene studies have suggested other lipid metabolism genes such as adiponectin receptor 1 (ADIPOR1; Kaarniranta et al. 2012), lipoprotein lipase (LPL; Wang et al. 2015), RAR related orphan

receptor A (RORA), roundabout guidance receptor 1 (ROBO1; Schaumberg et al. 2010; Jun et al. 2011), fatty acid desaturase 1–3 (FADS1–3; Fauser et al. 2011), LDL receptor related protein 5 (LRP5; Kloeckener-Gruissem et al. 2011), LRP6 and very low-density lipoprotein receptor (VLDLR; Haines et al. 2006), to be associated with AMD.

However, epidemiological studies have shown inconsistent results on the association of AMD and systemic lipids. Most smaller studies have found no or weak associations of AMD with total cholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C) and triglyceride (TG) levels in serum, often with conflicting results between the studies (van Leeuwen et al. 2018). Recent pooled data from the large European Eye Epidemiology (E3) Consortium showed a positive correlation between high-density cholesterol (HDL-C) levels and early, late or any AMD. A negative correlation was found with serum triglyceride (TG) and early or any AMD. LDL-C associated significantly only with early AMD whereas TC showed no associations (Colijn et al. 2019).

The current concept is that majority of lipid in drusen and around the RPE in AMD is of retinal origin with lipoproteins being assembled from multiple sources (Curcio 2018). Whether serum lipids accessing the outer Bruch's membrane from the choroidal circulation participate in AMD pathogenesis is still unknown.

The studies to date have examined systemic lipid levels in subjects in late middle age or later when clinical signs of AMD often already have developed. Such an approach does not necessarily address a longer-term lipid exposure or early development of AMD lesions. In this study, we examine the association between serum lipid values measured in early middle age and the presence of AMD in senior age in a homogenous cohort of male Finnish businessmen.

Methods

The Helsinki Businessmen Study (HBS) is a cohort study of Caucasian male executives and businessmen (n = 3490) born in 1919–1934. The subjects participated in a baseline examination and follow-health check-ups during the 1960s and early 1970s. Initially, the study had a cardiovascular disease (CVD) focus but with the years that has shifted to geriatric medicine. Current cohort follow-up time spans up to 55 years, making it one of the longest studies of its kind (Strandberg et al. 2016).

For the current study, a random subgroup of surviving, home-dwelling men (n = 209) from the HBS cohort were recruited for a study visit including colour fundus photography and a structured interview (including medications) in 2005–2012. TC, TG, blood glucose levels (including 1 hr oral glucose tolerance test, impaired tolerance defined as >10 mmol/l), body mass index (BMI) and blood pressure were acquired from the HBS baseline visit data in 1964–1973. In addition, TG levels were analysed in participants with the baseline visit occurring after 1969 (n = 70).

Follow-up blood pressure, TC, HDL-C, LDL-C, TG and BMI values were acquired from an HBS re-examination in 2011. The subjects were genotyped for the AMD risk single nucleotide polymorphisms (SNPs) of CFH/ARMS 1 rs1061170, LOC387715/ARMS 2 rs10490924, C3 rs2230199 as well as APOE rs429358 and rs7412. Information regarding smoking status and reported statin use in 2011 were collected from the HBS data.

Mydriatic colour fundus images were taken using either 30-degree stereoscopic film photography or 35-degree digital non-stereoscopic images. For film-based analysis, we used the AREDS template. Corresponding measurements were made in the digital images using the IMAGENET software (Topcon BV, Capelle aan den IJssel, The Netherlands).

The pictures were classified by two ophthalmologists into four AMD levels as suggested by the Age-Related Eye Disease Study Report Number 6 (Age-Related Eye Disease Study Research Group 2001). Level one (AREDS 1) was defined as no or normal age-related macular changes with a largest drusen size less than a circle with 63 μm diameter (C-0) and total area of less than a 125 μm diameter circle (C-1). Level two (AREDS 2), early AMD: medium size drusen (largest drusen size ≥63 μm but less than 125 μm) and/or retinal pigment abnormalities consistent with AMD. Level three (AREDS 3), intermediate AMD: large drusen (≥125 μm in diameter) as the largest drusen or intermediate drusen as largest drusen (≥63 μm in diameter) and a total drusen area of more than 0.241 disc diameter (I-2) if soft indistinct and 0.439 disc diameter (O-2) if soft distinct and/or geographic atrophy not affecting the centre of macula. Level 4 (AREDS 4), advanced AMD was defined as geographic atrophy involving the centre of macula and/or evidence of neovascular AMD (fibrovascular/serous pigment epithelial detachment (PED), serous or haemorrhagic sensory retinal detachment, subretinal haemorrhage, subretinal fibrous tissue or photocoagulation scars from AMD treatment). All patients who had received anti-vascular endothelial growth factor (VEGF) injections for AMD were classified as advanced AMD.

The AMD level and the size of the largest drusen in the worse eye were used for the analyses. Outcome variables were defined as follows: largest drusen diameter of ≥63 μm and ≥125 μm, any AMD (AREDS 2–4), intermediate or late AMD (AREDS 3–4) and late AMD (AREDS 4).

The study was performed with the approval of the local Medical Ethics Committee. Signed informed consent form was obtained from all participants in accordance with the Declaration of Helsinki.

Statistical analyses

Means are presented with standard deviation (SD). Univariate associations between lipid values, AMD risk SNPs, APOE, smoking status, statin use, AMD level and drusen size were tested with Student's t-test, chi-square test and Fischer's exact test when appropriate. Multivariate analysis was performed with binary logistic regression. All statistical analyses were done in SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0.; IBM Corp., Armonk, NY, USA).

Results

The main characteristics of our study population are presented in Table 1.

Fifteen subjects out of 209 (7.2%) had ungradable fundus images in both eyes due to media opacity or technical issues. At least one gradable fundus image was available in 194 subjects and

Table 1. Main parameters of the study cohort.

	Mean ± SD or n (%)	Range
Age at baseline (years)	38.9 ± 3.7	32.2–50.2
Age follow-up visit 2011 (years)	82.1 ± 3.3	76.2–91.6
Baseline examination 1963–1974		
Total cholesterol (mmol/l)	6.37 ± 1.28	3.30–10.91
Triglyceride (mmol/l), 70 subjects	1.44 ± 0.61	0.60–3.70
BMI (kg/m^2)	25.1 ± 2.5	17.9–33.4
Systolic blood pressure (mmHg)	131 ± 13	100–190
Diastolic blood pressure (mmHg)	84 ± 9	65–120
1-hr glucose stress test (mmol/l)	5.89 ± 1.77	2.50–13.90
Follow-up visit 2011		
Total cholesterol (mmol/l)	4.86 ± 0.90	2.60–7.40
HDL cholesterol (mmol/l)	1.55 ± 0.38	0.87–2.96
LDL cholesterol (mmol/l)	2.78 ± 0.75	1.26–4.92
Triglyceride (mmol/l)	1.16 ± 0.49	0.49–3.49
BMI (kg/m^2)	24.7 ± 2.8	18.1–33.4
Systolic blood pressure (mmHg)	149 ± 22	84–206
Diastolic blood pressure (mmHg)	82 ± 11	42–108
Largest drusen size		
No or <63 μm	126 (64.9%)	
≥63, <125 μm	24 (12.4%)	
≥125, <250 μm	26 (13.4%)	
≥250 μm	18 (9.2%)	
AMD status		
No AMD	84 (43.3%)	
Early AMD	61 (31.4%)	
Intermediate AMD	43 (22.2%)	
Late AMD	6 (3.1%)	
Smoking status 2011		
Never	96 (49.5%)	
Former	90 (46.6%)	
Current	8 (4.1%)	
Statin use 2011		
No	109 (56.2%)	
Yes	85 (43.8%)	

was used for the analysis. Figure 1 presents loss to follow-up from the HBS cohort and subjects included in our analysis.

The mean TC levels decreased from 6.37 ± 1.28 mmol/l at baseline 1964–1973 to 4.86 ± 0.90 mmol/l in 2011 (p < 0.001). There was no significant change in mean TG or BMI between baseline and 2011. Subjects with statin treatment 2011 had significantly higher TC levels at baseline 6.82 ± 1.29 versus 6.03 ± 1.17 (p < 0.001) and significantly lower 2011 TC levels, 4.53 ± 0.88 versus 5.13 ± 0.82 (p < 0.001) compared to subjects without treatment. The TC levels in 2011 were significantly lower in both subject with and without statins (p < 0.001) compared to baseline.

Mean systolic blood pressure without correction for anti-hypertensive medication increased from baseline 131 ± 13 mmHg to 149 ± 22 mmHg (p < 0.001) 2011. There was no significant change in mean diastolic blood pressure between baseline and 2011.

Impaired glucose tolerance was present in 4/194 (2.1%) of subjects at baseline.

Compared with the rest of the original HBS cohort (nonsurvivors and survivors), our study subjects were younger (38.9 ± 3.7 years versus 41.9 ± 4.5 years, p < 0.001), had lower TC (6.37 ± 1.28 mmol/l versus 6.63 ± 1.17 mmol/l, p = 0.002), lower BMI (25.1 ± 2.5 kg/m^2 versus 25.9 ± 2.9 kg/m^2, p < 0.001), lower systolic blood pressure (131 ± 13 mmHg versus 136 ± 16 mmHg, p < 0.001), lower diastolic blood pressure (84 ± 9 mmHg versus 87 ± 11 mmHg, p < 0.001) and lower 1h oral glucose tolerance test (5.89 ± 1.77 mmol/l versus 6.37 ± 1.95 mmol/l, p = 0.002) at the HBS baseline visit in 1963–1974. There was no significant difference in mean TG levels between the groups (1.44 ± 0.61 mmol/l versus 1.56 ± 0.86 mmol/l, p = 0.25).

The HBS cohort participants (n = 1345) who died before 2005 and thus were not possible candidates for

our study were older (42.7 ± 4.41 years versus 41.0 ± 4.42 years, p < 0.001), had higher TC (6.76 ± 1.22 mmol/l versus 6.51 ± 1.14 mmol/l, p < 0.001), higher TG (1.64 ± 0.91 mmol/l versus 1.50 ± 0.81 mmol/l, p = 0.003) and higher BMI (26.3 ± 3.0 kg/m^2 versus 25.62 ± 2.7 kg/m^2, p < 0.001) at the HBS baseline visit in 1964–1973 compared with the rest of the HBS cohort.

In the 194 subjects with gradable retinal images, any AMD in the worse eye was detected in 110/194 (56.7%), with 61/194 (31.4%) having early AMD, 43/194 (22.2%) intermediate and 6/194 (3.1%) late AMD. 3/194 (1.5%) had neovascular AMD, and 2/194 (1.0%) had received anti-VEGF treatment.

In univariate analysis, TC measured at baseline 1964–1973 was higher in subjects with intermediate or late AMD and largest drusen size ≥125 μm, compared with the rest of the study population. At the follow-up in 2011, TC, LDL and TG, respectively, were lower in subjects with any AMD, compared to those with no AMD in univariate analysis (Table S1).

Univariate analyses disclosed significant associations of the CFH rs1061170 and C3 rs2230199 SNPs with largest drusen size ≥63 μm. The LOC387715/ARMS 2 rs10490924 SNP associated with all the outcome variables – the presence of any AMD, intermediate or late AMD, late AMD and drusen sizes of ≥63 and ≥125 μm. Smoking status and statin use 2011 did not show significant correlations (Table S2).

In multivariate logistic regression models corrected for age, AMD risk SNPs, APOE and smoking, the adjusted odds ratio (OR) for TC at baseline remained significant for both outcomes: no or early versus intermediate or late AMD (AREDS 1–2 versus 3–4; OR: 1.589, 95% CI 1.159–2.178, p = 0.004) and largest drusen size ≥ 125 μm (OR: 1.570, 95% CI 1.139–2.165, p = 0.006; Table 2).

In a subset a of subjects with TG measurements at baseline (n = 70), TC associated positively (OR: 4.572, 95% CI 1.327–15.751, p = 0.016) and TG negatively (OR: 0.027, 95% CI 0.001–0.738, p = 0.032) to largest drusen size ≥125 μm after correction. Adjusted OR for no or early versus intermediate or

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│        HBS baseline cohort, n=3490 (100%)         │
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                    │        │ - Cumulative dead before 2005    │
                    │        │ n=1345 (38.5%)                   │
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│   Subjects alive at study start, n=2145 (61.5%)  │
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│      Enrolled in the study, n=209 (6.0%)          │
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                    │        │ - Ungradable fundus photographs  │
                    │        │ n=15 (0.4%)                      │
                    │        │ - No other exclusions            │
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│      Included in analysis, n=194 (5.6%)           │
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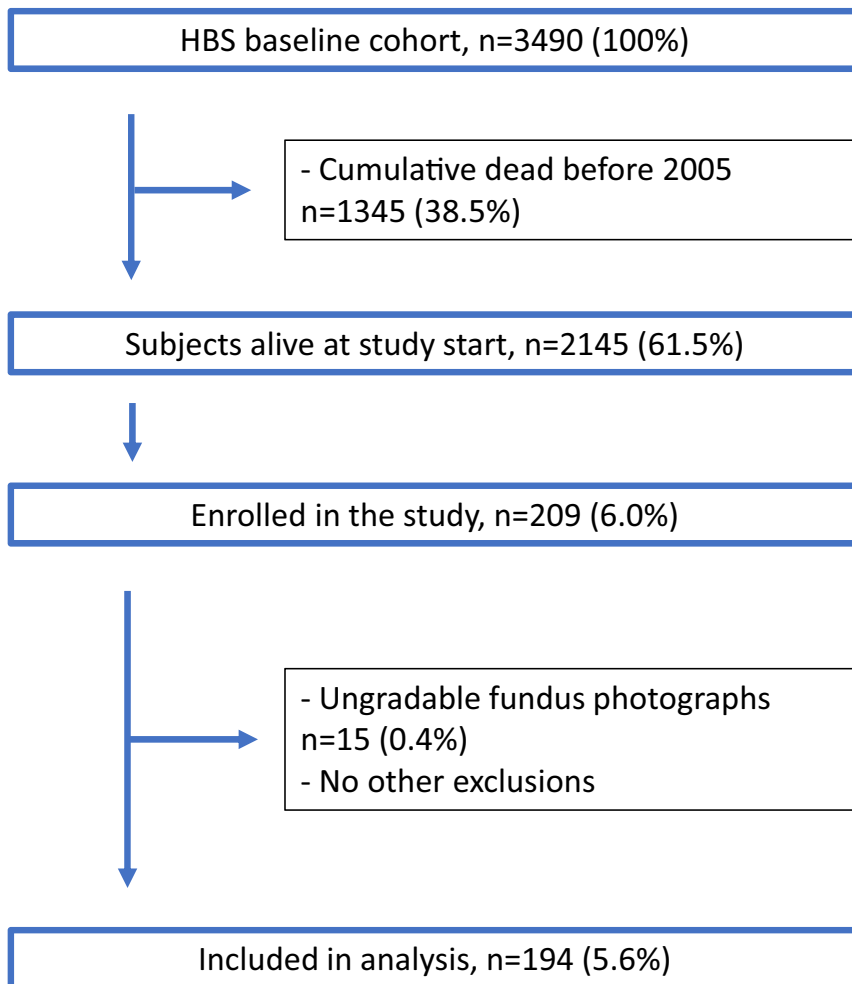
Fig. 1. Subjects included in the analysis, total number (*n*) and percentage of Helsinki Businessmen Study baseline cohort.

late AMD (AREDS 1–2 versus 3–4) was only significant for TC after correction, though TG showed a similar negative trend (Table S3).

When the follow-up parameters, measured in 2011, were analysed for an association with no versus any AMD (AREDS 1 versus 2–4) or drusen size >125 μm in multivariate logistic regression models corrected for age, statin use, AMD risk SNPs, APOE and smoking, the associations between TC and TG seen in univariate analysis did not remain significant (Table S4). LDL-C levels measured 2011 showed multicollinearity with TC and were excluded from the analysis.

Discussion

In this study, we found an association between early middle age serum TC and later intermediate or late AMD, and largest drusen size of ≥125 μm. A

subset analysis showed a correlation between lower TG levels in early middle age and the presence of drusen ≥125 μm at older age.

Of serum lipids measured at older age, TC, LDL and TG showed a negative association to any AMD in univariate analysis that did not remain significant after correction for age, statin use, AMD risk SNPs and smoking. In our study, TC values had a high collinearity with LDL and were not tested separately.

We are not aware of previous published studies on the role of early lipid values on AMD development.

Although our material was not large compared to many recent studies, we feel that this is at least partly compensated by the homogeneity of the cohort: all were Caucasian Finnish men with similar socioeconomic status, ethnicity and cultural background. Together with the long cohort history,

this may have disclosed connections otherwise hidden by variability in the study material. On the other hand, this may limit the applicability of these results to more diverse populations. As the HBS cohort is all male, our results are gender biased and possible conclusions are thus not generalizable to the population as a whole.

Although lipid material in the Bruch's membrane and around the RPE has been considered important for the development of AMD, epidemiological studies have been inconclusive on the relationship between serum lipid levels and AMD. In contrast to carrying a lower risk to CVD, HDL-C has been reported to correlate with an increased risk of AMD (Colijn et al. 2019).

Our results suggest that serum lipids measured at senior age, when AMD lesions may already have developed, do not necessarily reflect the lifelong lipid exposure.

When considering our results, the limitations of this type of study must be kept in mind. The long time period between baseline and study visits carry a risk of bias. Selection bias may occur as our sample size is only 9.7% of the current HBS cohort and due to preferential survival (discussed further down). We do not have information on diet or dietary changes during the follow-up. Other lipid lowering medications than statins were not analysed, and the duration of lipid lowering medication was not registered. Therefore, the lipid exposure during the follow-up cannot be individually quantified. However, since the widespread use of statins started in early nineties, our subjects did have a statin-free exposure to serum lipid for at least 50–70 years. Correspondingly, the estimated maximum time of statin exposure at the time of photography is 15–22 years.

Age-related macular degeneration diagnosis was based on fundus photography alone, introducing a uncertainty around our results especially concerning neovascular AMD. Only two patients reported anti-VEGF injections and were classified as late AMD. All together the challenges inherent in an extremely long follow-up time must be kept in mind at interpreting these results.

In the late nineteen-sixties and early seventies, when the HSB cohort was first examined, quantitative analysis of

Table 2. Multivariate logistic regression model analysis on the association of baseline parameters, age-related macular degeneration (AMD) risk single nucleotide polymorphisms, apolipoprotein E gene (APOE) and smoking using three cut-off levels of AMD (1.) and two drusen cut-off levels (2.).

1.	No versus any AMD		No or early versus intermediate or late AMD		No, early or intermediate versus late AMD	
	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
Age fundus photo visit	0.031	0.924 (0.860–0.993)	0.624	1.021 (0.939–1.110)	0.297	0.558 (0.186–1.670)
Total cholesterol level 1964–1973	0.549	1.081 (0.838–1.395)	0.004	1.589 (1.159–2.178)	0.722	1.325 (0.281–6.247)
BMI 1964–1973	0.105	1.114 (0.978–1.269)	0.945	1.006 (0.858–1.179)	0.177	15.663 (0.287–853.579)
CFH genotype						
TT	[Ref.]		[Ref.]		[Ref.]	
CT	0.181	1.629 (0.797–3.333)	0.350	1.547 (0.619–3.864)	0.268	0.009 (<0.001–38.710)
CC	0.214	1.780 (0.716–4.420)	0.423	1.579 (0.517–4.823)	0.190	0.001 (<0.001–28.978)
LOC387715 genotype						
GG	[Ref.]		[Ref.]		[Ref.]	
TG	0.107	1.721 (0.890–3.331)	0.005	3.181 (1.423–7.111)	0.987	1.36×10^{14} (<0.001–∞)
TT	0.999	1.40×10^{9} (<0.001–∞)	<0.001	77.045 (8.105–732.414)	0.985	7.7×10^{15} (<0.001–∞)
C3 genotype						
CC	[Ref.]		[Ref.]		[Ref.]	
CG	0.175	1.664 (0.797–3.476)	0.465	1.388 (0.576–3.343)	0.900	1.334 (0.015–118.274)
GG	0.111	3.358 (0.758–14.876)	0.369	2.169 (0.401–11.729)	1.000	0.021 (<0.001–∞)
APOE type						
ε3	[Ref.]		[Ref.]		[Ref.]	
ε2	0.848	1.128 (0.327–3.889)	0.884	1.124 (0.234–5.400)	0.999	<0.001 (<0.001–∞)
ε4	0.103	1.928 (0.876–4.243)	0.962	0.977 (0.382–2.498)	0.996	<0.001 (<0.001–∞)
Smoking status						
Never smoker	[Ref.]		[Ref.]		[Ref.]	
Ever smoker	0.291	1.430 (0.737–2.775)	0.373	1.446 (0.642–3.255)	0.214	12.414 (0.233–660.671)
Current smoker	0.632	1.497 (0.287–7.804)	0.007	12.444 (1.982–78.117)	1.000	1.46×10^{4} (<0.001–∞)

2.	Largest drusen size ≥ 63 μm		Largest drusen size ≥ 125 μm	
	p	OR (95% CI)	p	OR (95% CI)
Age fundus photo visit	0.613	1.019 (0.947–1.096)	0.328	1.044 (0.958–1.138)
Total cholesterol level 1964–1973	0.697	1.054 (0.810–1.371)	0.006	1.570 (1.139–2.165)
BMI 1964–1973	0.135	1.108 (0.968–1.269)	0.982	1.002 (0.849–1.182)
CFH genotype				
TT	[Ref.]		[Ref.]	
CT	0.831	1.086 (0.508–2.324)	0.714	1.187 (0.475–2.970)
CC	0.061	2.409 (0.960–6.050)	0.743	1.206 (0.394–3.691)
LOC387715 genotype				
GG	[Ref.]		[Ref.]	
TG	0.055	1.954 (0.987–3.869)	0.001	4.041 (1.758–9.291)
TT	0.014	8.253 (1.525–44.682)	<0.001	41.288 (6.855–248.684)
C3 genotype				
CC	[Ref.]		[Ref.]	
CG	0.106	1.838 (0.878–3.847)	0.434	1.432 (0.583–3.515)
GG	0.077	3.502 (0.872–14.068)	0.220	2.815 (0.539–14.691)
APOE type				
ε3	[Ref.]		[Ref.]	
ε2	0.715	1.265 (0.359–4.460)	0.723	1.328 (0.277–6.358)
ε4	0.483	1.325 (0.604–2.904)	0.841	1.102 (0.428–2.832)
Smoking status				
Never smoker	[Ref.]		[Ref.]	
Ever smoker	0.559	1.227 (0.617–2.439)	0.478	1.348 (0.592–3.070)
Current smoker	0.902	1.124 (0.174–7.267)	0.274	3.049 (0.414–22.435)

cholesterol subclasses was not routine and their significance in CVD was not recognized. Thus, we only have TC and TG values available from that time and analysing the role of early lipoprotein subfraction levels for later development of AMD was not possible. However, the subjects in any AMD group did not have conspicuously high HDL levels and TC values showed high collinearity with LDL levels in the follow-up examination. Thus, it is unlikely that the higher baseline TC values in future AMD subjects would have been caused by exceptionally high HDL at baseline.

The TC values in 2011 were lower in both subjects with statin treatment and without compared to baseline. Statin

treated subjects had higher TC levels at baseline and lower 2011 TC. This fact highlights the difficulty of analysing a possible correlation between AMD and lipid values based on current data when AMD is already present. The TC values measured at baseline were higher than those measured in Finnish men of the same age group today. On a population basis, the decline of TC values over time is explained by dietary causes (Borodulin et al. 2015). Thus, the role of today's early middle age TC levels on future development of AMD is unanswered by this study. Anyhow, the current decline in TC values may have a bearing on the age-cohort-related decline of AMD prevalence found by some of the recent studies (Klein et al. 2011; Colijn et al. 2017; Cruickshanks et al. 2017).

Compared with the rest of the original HBS cohort, our study population were younger and had significantly lower CVD risk factors (TC, BMI, systolic and diastolic blood pressure at baseline) except triglyceride. The difference is likely to be explained at least partly by preferential survival of individuals with lower CVD risk as previous HBS studies have shown long-term mortality to be predicted by midlife TC, blood pressure, BMI and 1-hr postload glucose (Strandberg et al. 2016). The role of this selection on our results is undisclosed.

In the whole study cohort, the prevalence of late AMD (3.1%) was comparable to other studies of northern Europeans (Reykjavik (Jonasson et al. 2003), Oslo (Bjornsson et al. 2006) and Tromsø studies (Erke et al. 2012)). Differences in grading protocols and age distribution limit the comparison of early AMD prevalence between studies. The prevalence of early AMD (53.6%) in our study cohort is in the same range as Tromsø (59.0%) and Oslo (43.1%) studies but higher than in the Reykjavik study (17.9%).

The biological mechanisms of the observed associations between higher TC in early middle age and AMD in the senior age are not disclosed by this study. However, taking into account the accumulation of lipids and lipoproteins in Bruch's membrane throughout adulthood and results suggesting diet as the major fatty acid source, either directly or recycled from the RPE and photoreceptors (Curcio 2018), it could be hypothesized that early long-term exposure to high levels of serum lipids contribute to an excess of cholesterol and fatty acids in and around the RPE. Drusen would form later due to impeded debris processing and transport through the aged Bruch's membrane.

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Correspondence:
Fabian Kananen, MD
Department of Ophthalmology
Örebro University Hospital
701 85 Örebro
Sweden
Tel: +46736391676
Fax +46196022561
Email: fabian.kananen@regionorebrolan.se

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Serum lipids and BMI stratified according to AMD grade (three cut-off levels) and drusen size (two cut-off levels) at baseline 1964–73 and at the follow-up examination in 2011.

Table S2. Associations of AMD risk SNPs, APOE, statin use 2011 and smoking status 2011 with AMD (three cut-off levels) and drusen size (two cut-off levels) without correction for confounding factors.

Table S3. Multivariate logistic regression model analysis on the association of baseline parameters, AMD risk SNPs, APOE and smoking using three cut-off levels of AMD (1.) and two drusen cut-off levels (2.) in a subset of patients ($N = 70$) with TG values measured at baseline.

Table S4. The follow-up parameters measured in 2011, analyzed for associations with three cut-off levels of AMD (1.) and two drusen cut-off levels (2.) with multivariate logistic regression models corrected for age, statin use, AMD risk SNPs, APOE and smoking. LDL measured in 2011 showed multicollinearity with TC and was excluded from the analysis.