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Title:

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Date:

2020-12-01

Citation:

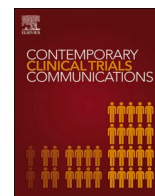
Robman, L. D., Phuong Thao, L. T., Guymer, R. H., Wolfe, R., Woods, R. L., Hodgson, L. A., Phung, J., Makeyeva, G. A., Le-Pham, Y. A., Orchard, S. G., Suleiman, J., Maguire, E., Trevaks, R. E., Ward, S. A., Riaz, M., Lacaze, P., Storey, E., Abhayaratna, W. P., Nelson, M. R., ... McNeil, J. J. (2020). Baseline characteristics and age-related macular degeneration in participants of the "ASpirin in Reducing Events in the Elderly" (ASPREE)-AMD trial. Contemporary Clinical Trials Communications, 20, <https://doi.org/10.1016/j.conctc.2020.100667>.

Persistent Link:

<https://hdl.handle.net/11343/272181>

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Research paper

Baseline characteristics and age-related macular degeneration in participants of the “ASpirin in Reducing Events in the Elderly” (ASPREE)-AMD trial

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ARTICLE INFO

Keywords:

Age-related macular degeneration
AMD
Aspirin
Randomized controlled trial
Elderly
Baseline characteristics

ABSTRACT

Purpose: To describe the baseline participant characteristics in the ASPREE-AMD study, investigating the effect of aspirin on AMD incidence and progression.

Methods: Australian participants from the ASPIrin in Reducing Events in the Elderly (ASPREE) trial, randomized to 100 mg aspirin daily or placebo, had non-mydratic, digital color fundus images graded according to the Beckman AMD classification. Associations with AMD were determined for baseline characteristics and genetic risk variants.

Results: ASPREE-AMD sub-study enrolled 4993 participants with gradable macular images. Median age was 73.4 years (IQR, 71.5, 76.6), 52% were female, 10% had diabetes mellitus, 73% had hypertension, and 44% were former/current smokers. Early, intermediate and late AMD (detected in 20.6%, 16.1%, 1.1%, respectively), significantly associated with age, were also associated with increasing HDL levels: OR = 1.52 (95%CI, 1.26, 1.84), OR = 1.43 (1.17, 1.77) and OR = 1.96 (1.02, 3.76), respectively. Female sex was associated with early [OR = 1.37 (1.16, 1.62)], and intermediate [OR = 1.35 (1.12, 1.63)] AMD, as was previous regular use of aspirin, with OR = 1.46 (1.11, 1.92) and OR = 1.37 (1.01, 1.85), respectively. Current smoking had increased odds for

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<https://doi.org/10.1016/j.conctc.2020.100667>

Received 18 May 2020; Received in revised form 22 August 2020; Accepted 3 October 2020

Available online 11 October 2020

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late AMD, OR = 4.02 (1.42, 11.36). Genetic risk variant rs3750846 (*ARMS2/HTRA1*) was associated with each AMD stage ($p < 0.001$), risk variants rs570618 and rs10922109 (*CFH*) with intermediate and late AMD ($p < 0.001$), and rare variant rs147859257 (*C3*) with late AMD ($p < 0.001$). The randomized groups were well balanced for all analyzed AMD risk factors.

Conclusions: Observed associations are typical of AMD. The ASPREE-AMD clinical trial provides a unique opportunity to determine the risks and benefits of low-dose aspirin for AMD incidence and progression in elderly population.

Trial registration: Australian New Zealand Clinical Trial Registry: ACTRN 12613000755730.

1. Introduction

The prevalence and incidence of age-related macular degeneration (AMD) are increasing as a result of the increasing proportion of elderly individuals in the population and this has a substantial impact on quality of life and the costs of care [1–7]. The absence of specific treatments that prevent progression from early to late stages of AMD (which threaten central vision), necessitates a search for measures that may delay the onset of this chronic disease or slow its progression.

The detailed rationale for the ASPREE-AMD study has been described previously [8]. In brief, aspirin has been considered of potential benefit in AMD because of its anti-inflammatory properties that could reduce low-grade inflammation in the earlier stages of AMD [9–15]. However, aspirin could also exacerbate retinal hemorrhages in late AMD [16–21]. Consistent with this paradox, previous observational and experimental studies in AMD have reported differing results related to risks and benefits of aspirin [14–19,21–34]. Meta-analyses have similarly not yielded definitive conclusions [20,35–37]. Therefore, given the widespread use of aspirin by older persons, a well-designed randomized controlled clinical trial of sufficient size and duration is needed [38–42].

The Aspirin in Reducing Events in the Elderly (ASPREE) primary prevention randomized controlled trial (2010–2017) provided the opportunity to undertake the ASPREE-AMD sub-study [43,44]. ASPREE investigated the risks and benefits of daily low-dose aspirin on a primary composite endpoint of disability- and dementia-free survival [45], and also mortality [46], cardiovascular events, major bleeding [47] and selected other conditions in 19,114 older participants [48–53]. The aspirin intervention was terminated after a median of 4.7 (interquartile range, 3.6 to 5.7) years of follow up in June 2017, for lack of benefit for the primary endpoint, disability-free and dementia-free survival, and higher rates of major hemorrhages and all-cause mortality [45–47]. Nonetheless, the ASPREE-AMD sub-study continued collecting the 5-year follow up retinal images (2016–2020) in order to investigate any possible delayed effect of long-term low-dose aspirin. Those measurements made after June 2017 have been flagged and sensitivity analyses will be undertaken with and without including these ‘post-intervention’ measures.

After completion of the primary ASPREE study in June 2017, its cohort is followed up in observational mode until 2024 as the ASPREE-XT (ASPREE-extension) study. Funded by the NHMRC and NIH/NIA, this study provides a substantial basis for the ASPREE-AMD sub-study by maintaining the infrastructure and database, providing scientific and administrative support, tracking the location of participants, and continuing uninterrupted collection of the data on adverse events, change of medications, morbidity, and mortality.

Here, we describe the baseline characteristics of ASPREE-AMD participants and associations between demographics, lifestyle, clinical and genetic risk factors and AMD stages in this cohort.

2. Materials and methods

The detailed methodologies of the principal ASPREE study [43,54] and the ASPREE-AMD sub-study [8] have been published previously. In brief, ASPREE was a multi-centre, randomized, double-blinded, placebo-controlled trial of daily 100 mg enteric-coated aspirin in 19,114 older

adults in both Australia and the USA [43]. In Australia, participants were healthy individuals who were 70 years and older, free of documented evidence of cardiovascular disease, cognitive impairment or physical disability and without a contraindication for aspirin use.

2.1. ASPREE-AMD sub-study

The Australian ASPREE participants were the recruitment source for the ASPREE-AMD study. The ASPREE-AMD sub-study involved acquisition of non-mydratic digital retinal images of both eyes from the ASPREE participants at baseline and after 3 and 5 years of follow up. All ASPREE study inclusion and exclusion criteria applied to this sub-study, with the additional inclusion criterion of having gradable images of the macular area in at least one eye.

Study enrolment retinal photography was conducted between March 2010 and January 2015. The approved time frame for image collection from an individual participant was from 6 months before to 3 months after their randomization date. Bilateral digital 45° color fundus photographs, centered on the macula and optic disc, were captured using Canon Non-Mydratic fundus cameras (Canon Inc., Tokyo, Japan) with Digital Health Care software (UK). Digital images were viewed immediately and repeated if necessary.

All images were graded by two senior graders from the Centre for Eye Research Australia (CERA), according to the Beckman classification system, which is based upon the stage of disease in the worse affected eye [55]. The following Beckman risk categories of AMD will be used in the analysis:

- No apparent aging change: no drusen and no AMD pigmentation abnormalities
- Normal aging changes: only drupelets (small drusen $< 63 \mu\text{m}$) and no AMD pigmentation abnormalities
- Early AMD: Medium drusen ($\geq 63 \mu\text{m} - < 125 \mu\text{m}$) with no AMD pigmentation abnormalities
- Intermediate AMD: Medium drusen ($\geq 63 \mu\text{m} - < 125 \mu\text{m}$) with AMD pigmentation abnormalities or large drusen ($\geq 125 \mu\text{m}$)
- Advanced AMD: neovascular AMD (nAMD) or geographic atrophy

This severity scale will be used to define primary outcomes, which are the incidence and progression of AMD per person [8]. In addition, exploratory outcomes will be the rate of retinal hemorrhages and whether other factors, such as genotype, systemic disease, inflammatory biomarkers, or comorbidity influence the effect of aspirin on the course of AMD.

All cases of late AMD were adjudicated by clinical specialists. The graders had previously reported good intra- and inter-grader agreement in AMD grading, with kappa statistics ranging from 0.60 to 1.00 [56]. Graders showed high intra-grader repeatability for the separate AMD features, which varied from the direct agreement of 89% (kappa = 0.81) and 82% (kappa = 0.69) for intermediate and large drusen, respectively, to 99% (kappa = 0.97) for the most advanced form of late AMD – choroidal neovascularization (CNV). Inter-grader repeatability varied from 86% agreement (kappa = 0.72) for intermediate drusen to 97% (kappa = 0.65 or 0.68) for late AMD features. In testing for a temporal drift in grading, the overall person-based determination of AMD stages,

according to the Beckman classification, was in direct agreement in 89% ($\kappa = 0.845$). The measures of agreement were obtained before adjudication was provided.

2.2. Data collection and ethics approval

This study is collaboration between the School of Public Health and Preventive Medicine at Monash University, which coordinated the ASPREE study, including recruitment of participants and collection of demographic, anthropometric, lifestyle and clinical data, as well as blood samples and retinal images, and CERA, which graded macular photos for AMD and other pathology, and validated ophthalmic information. All participants signed a written informed consent form after explanation of the ASPREE-AMD study.

The ASPREE-AMD study protocol was approved by the Human Research and Ethics Committee of Monash University (MUHREC), undertaken according to the Helsinki Declaration for research on humans and registered with the Australian New Zealand Clinical Trial Registry: ACTRN 12613000755730. The ASPREE study was also registered with the International Standardized Randomized Controlled Trials Register, ASPirin in Reducing Events in the Elderly, Number: ISRCTN83772183 and clinicaltrials.gov, Number NCT01038583. Three additional sub-studies of ASPREE, ENVISION (Aspirin for the prevention of cognitive decline in the Elderly: a Neuro-Vascular Imaging Study, ACTRN12609000613202) [57], SNORE-ASA (Study of Neurocognitive Outcomes, Radiological and retinal Effects of Aspirin in Sleep Apnea, ANZCTRN12612000891820) [52], and the ASPREE Healthy Ageing Biobank, also contributed images to the ASPREE-AMD study. These sub-studies used the same methodology for fundus imaging as the ASPREE-AMD study. Written informed consents for retinal imaging were taken for all four contributing sub-studies of ASPREE. In addition, MUHREC approved the use of quality color images taken with the same method and provided by optometrists for those ASPREE participants who were willing to participate but for whom retinal photography by the study staff was not possible. Genetic analysis of stored blood samples from the ASPREE Healthy Ageing Biobank was approved by the Alfred Hospital Human Research Ethics Committee.

2.3. Genetic data

Genotyping was undertaken using the Axiom™ Precision Medicine Diversity Array (Thermo Fisher) following standard protocols at the Ramaciotti Centre for Genomics. Imputation of genetic variants was performed on European-ancestry samples using the haplotype reference consortium (HRC) panel on the Michigan imputation server [58]. In post imputation quality control step all single nucleotide polymorphisms (SNPs) < 0.3 imputation quality scores were excluded from further downstream analysis. Of the 52 SNPs previously associated with AMD [59], 40 imputed genetic variants were available for further analysis. The remaining 12 variants were not present in the ASPREE imputed genotype data because they were not included in the microarray of the whole ASPREE cohort.

2.4. Statistical analysis

Descriptive statistics included median $\pm$ interquartile range for continuous variables, or number (frequency %) for categorical variables. Multinomial logistic regression was used to study the association between AMD stages at baseline and selected factors reflecting demographics, general health and lifestyle. In this statistical model, AMD grade was considered as a nominal outcome variable with 4 categories: None/Normal ageing changes (No AMD), Early AMD, Intermediate AMD and Late AMD. All analyses were performed using R version 3.6.1 [60].

The variables of potential relevance to the risk of AMD included age and sex, lifestyle and general health characteristics: smoking, alcohol use, body-mass index (BMI), educational levels, non-fasting glucose,

high-density lipoprotein (HDL), low-density lipoprotein (LDL) and total cholesterol levels, history of diabetes mellitus or hypertension and previous regular use (irrespective of dose, frequency or longevity of use) of aspirin or statin, the latter defined as any medication whose ATC code places it in the statin group of medications, as statins may also have the potential to influence development of AMD [61].

Variable definitions: Diabetes mellitus is defined as self-report of diabetes or fasting glucose ≥ 126 mg/dL or on treatment for diabetes; Hypertension is defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg or on treatment for high blood pressure. Body-mass index (weight in kilograms divided by the square of the height in meters) categories: < 20 (underweight), 20–24 (normal weight), 25 to 29 (overweight), or ≥ 30 (obese); Smoking History is defined as smoking (Current), having smoked (Former) or never having smoked (Never) on average equal or more than one cigarette/cigar/pipe a day; Alcohol use is defined as having (Current), having had (Former) or never having had (Never) at least one alcoholic drink per week; non-fasting glucose, HDL, LDL and total cholesterol levels are continuous variables.

The participant's awareness of having AMD prior to study entry was also recorded.

Extraction of genetic data and independent logistic regression analysis for each AMD stage (early, intermediate and late) versus controls (no AMD) was undertaken using the plink software (version 1.09) [62, 63]. We corrected for multiple testing using Bonferroni correction, and considered p values < 0.001 to be statistically significant.

It was estimated that a sample of 5000 participants, with gradable images at baseline and 4% per annum loss to follow-up, would provide 80% power with two-sided alpha of 0.05 to detect: (1) 24% reduction of early/intermediate AMD incidence, (2) 20% reduction of progression from early to intermediate stage of disease and (3) 53% reduction of progression from early or intermediate stage to late stage [8].

3. Results

3.1. Participants

A total of 14,247 of 16,703 participants from the ASPREE study in Australia consented to retinal photography. Given that ASPREE recruitment occurred throughout an extensive area of south-eastern Australia, facilities, geographic and scheduling limitations allowed collection of retinal images from only 5422 ASPREE participants. Of these, 4993 participants with gradable images in at least one eye were included for longitudinal follow up, and 4387/4993 (88%) of these participants had gradable images from both eyes. In some participants, small pupil size, along with increased nuclear density, made it impossible to take retinal images of gradable quality. Images with either poor focus or field or with a confounding lesion or artefact obscuring the macula were defined as ungradable. In 82/4993 (1.6%) cases the gradable images were provided by the participants' local eye care providers.

Fig. 1 outlines ASPREE-AMD recruitment, from the total number of Australians recruited to ASPREE to the sample of participants with gradable retinal images included in the ASPREE-AMD study.

3.2. Baseline characteristics

At the time of randomization, 69% of the photographed participants lived in capital cities and 31% lived in regional centers or rural areas of five south-eastern states and territories of Australia.

The median age of the 4993 enrolled participants was 73.4 years (IQR 71.5, 76.6) and 52% were women, 10% had diabetes mellitus, 73% had hypertension, 44% were former/current smokers, 46% were overweight, 29% were obese and 44% had less than 12 years of education. 7% of participants reported regular use of aspirin prior to entering the study, which they stopped in order to commence the study medication, and 31% were on prescribed statin medications. Only 4% reported

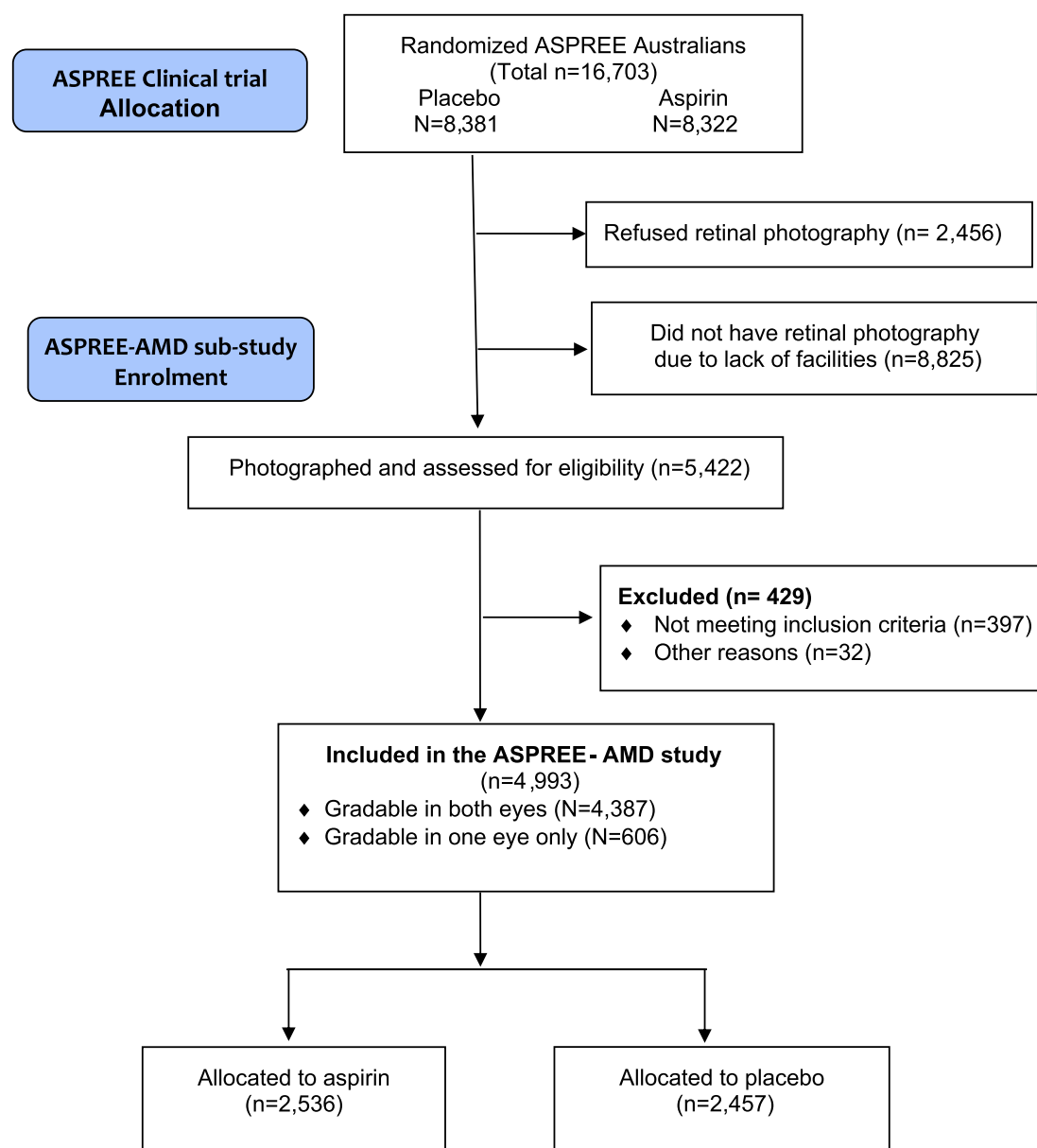


Fig. 1. ASPREE-AMD study CONSORT Flow Diagram.

having been told by a doctor of having AMD. The overall prevalence of early, intermediate and late AMD at baseline was 20.6%, 16.1% and 1.1%, respectively, with no significant difference in distribution of stages between the groups allocated to placebo and aspirin ($p < 0.53$).

Of the 11,710 Australian ASPREE participants not participating in the ASPREE-AMD study, their median age was one year older (74.3 vs 73.4), more frequently they were females (56% vs 52%); greater proportions had less than 12 years of education (53% vs 44%), were underweight (2% vs 1%), had hypertension (76% vs 73%), never consumed alcohol (17% vs 14%) and were aware of having AMD (9% vs 4%) than the 4993 who were included in the ASPREE-AMD study. Only those characteristics that had statistically significant differences have been cited.

Of the 327 excluded participants whose non-mydratic fundus images were of insufficient quality in both eyes, the median age was two year older (75.3 vs. 73.4), and proportion of males was greater (55% vs 48%) compare to the study participants with gradable images at baseline. In other characteristics they were not significantly different from the analyzed sample.

The baseline characteristics are summarized by the stages of AMD in Table 1. Women were more likely to have AMD than men (43% vs 33%, respectively). Prevalence of late AMD was higher in current smokers (3%) and underweight participants (4%), compared with the rest of the sample (1% for both factors). The previous regular users of aspirin had a higher prevalence of early and intermediate AMD than non-users (26% and 19% versus 20% and 16%, respectively). There were almost equal proportions of the AMD stages amongst users and non-users of statin. The data on the awareness of having AMD was available from only 57% of the sample. Awareness of having AMD increased with increasing severity of the disease. Thus, 17/577 (3%) of participants with early AMD, 45/460 (10%) of participants with intermediate AMD and 34/42 (81%) of participants with late AMD were aware of having signs of AMD. Of interest, 31/1749 (1.8%) of participants without signs of AMD on their fundus images believed they had been told they had AMD. There were no obvious differences in distributions of other risk factors or comorbidities across AMD stages.

Table 1

Baseline characteristics of the ASPREE-AMD sample.
Number (%) or Median (IQR). *N* = 4993.

Characteristic	Total	No AMD*	Early AMD	Intermediate AMD	Late AMD
Age at randomization (years)	73.4 (71.5, 76.6) <i>N</i> = 4993	73.1 (71.4, 76.0) <i>N</i> = 3099	73.6 (71.5, 77.0) <i>N</i> = 1031	74.5 (71.9, 78.5) <i>N</i> = 806	77.3 (74.5, 82.2) <i>N</i> = 57
Age groups at randomization (years)	70–74	3220 (64)	2116 (66)	639 (20)	449 (14)
	75–79	1197 (24)	687 (57)	271 (23)	219 (18)
	80–84	446 (9)	234 (52)	100 (22)	97 (22)
	85+	130 (3)	62 (48)	21 (16)	41 (32)
Sex	Male	2412 (48)	1616 (67)	428 (18)	345 (14)
	Female	2581 (52)	1483 (57)	603 (23)	461 (18)
Education (years)	<12	2199 (44)	1344 (61)	462 (21)	365 (17)
	≥ 12	2794 (56)	1755 (63)	569 (20)	441 (16)
BMI groups	Underweight	69 (1)	40 (58)	20 (29)	6 (9)
	Normal	1146 (23)	688 (60)	236 (21)	209 (18)
	Overweight	2297 (46)	1426 (62)	489 (21)	359 (16)
	Obese	1462 (29)	932 (64)	282 (19)	230 (16)
Diabetes	No	4497 (90)	2777 (62)	937 (21)	732 (16)
	Yes	496 (10)	322 (65)	94 (19)	74 (15)
Hypertension	No	1335 (27)	822 (62)	288 (22)	209 (16)
	Yes	3658 (73)	2277 (62)	743 (20)	597 (16)
Smoking History	Current	149 (3)	91 (61)	24 (16)	29 (19)
	Former	2030 (41)	1278 (63)	383 (19)	348 (17)
	Never	2814 (56)	1730 (61)	624 (22)	429 (15)
Alcohol use	Current	4054 (81)	2520 (62)	837 (21)	654 (16)
	Former	240 (5)	161 (67)	43 (18)	31 (13)
	Never	699 (14)	418 (60)	151 (22)	121 (17)
Glucose (mmol/L)	Median (IQR)	5.3 (5.0, 5.8) <i>N</i> = 4894	5.4 (5.0, 5.8) <i>N</i> = 3034	5.3 (5.0, 5.8) <i>N</i> = 1017	5.3 (5.0, 5.8) <i>N</i> = 789
HDL (mmol/L)	Median (IQR)	1.5 (1.2, 1.8) <i>N</i> = 4858	1.5 (1.2, 1.8) <i>N</i> = 3013	1.6 (1.3, 1.9) <i>N</i> = 1004	1.6 (1.3, 1.9) <i>N</i> = 786
LDL (mmol/L)	Median (IQR)	3.0 (2.4, 3.6) <i>N</i> = 4842	3.0 (2.4, 3.6) <i>N</i> = 3000	3.0 (2.5, 3.6) <i>N</i> = 1002	3.0 (2.4, 3.6) <i>N</i> = 785
Cholesterol total (mmol/L)	Median (IQR)	5.2 (4.5, 5.8) <i>N</i> = 4938	5.1 (4.5, 5.8) <i>N</i> = 3063	5.3 (4.6, 5.8) <i>N</i> = 1020	5.1 (4.6, 5.9) <i>N</i> = 799
Previous regular use of aspirin	No	4649 (93)	2912 (63)	943 (20)	740 (16)
	Yes	344 (7)	187 (54)	88 (26)	66 (19)
Use of any statin	No	3461 (69)	2136 (62)	726 (21)	559 (16)
	Yes	1532 (31)	963 (63)	305 (20)	247 (16)
Awareness of having AMD	No	2701 (94)	1718 (64)	560 (21)	415 (15)
	Yes	127 (4)	31 (24)	17 (13)	45 (35)
	Unsure	40 (1)	23 (57.5)	3 (7.5)	14 (35)

Percentages are presented in column for Totals and by rows for the breakdown of AMD status. In some lines the total does not add up to 100% because of rounding. * - 'No AMD' category combines the cases from the Beckman categories None and Normal Ageing Changes.

3.3. Associations between the baseline characteristics and AMD stages

The results of univariable and multivariable multinomial logistic regression analysis of the associations between the baseline characteristics and AMD stages are presented in Table 2 and Table 3. Each stage of AMD was significantly associated with increasing age, with OR = 1.03, OR = 1.08 and OR = 1.23 for early, intermediate and late AMD, respectively. Females were more likely than males to have early and intermediate AMD, OR = 1.37 and OR = 1.35, respectively. Higher HDL levels were associated with all stages of AMD (OR = 1.52, OR = 1.43 and OR = 1.96, respectively), whereas LDL and total cholesterol levels were not associated with AMD. Current smokers were more likely to have late AMD (OR = 4.02) compared to non-smokers, whilst current or former alcohol use, hypertension or diabetes were not associated with any stage of AMD. Those participants who had taken aspirin on a regular basis prior to enrolment were more likely to have early or intermediate AMD (OR = 1.46 and OR = 1.37, respectively) than those who were not taking aspirin. The use of statin medications was not associated with any stage of AMD. Underweight participants were more likely to have late AMD in the univariable analysis, with OR = 3.97, but after adjusting for other variables in multivariable analysis, the association between low body weight and late AMD was no longer statistically significant. All other characteristics showed no statistically significant association with AMD.

3.4. Genetic analysis

Of the 52 SNPs previously associated with AMD [59], we were able to generate post-QC genotyping on 40 variants in 4391 ASPREE-AMD

samples. The overall genotyping rate was greater than 95% for the 40 AMD risk variants genotyped across 2735 'No AMD' (62%), 903 early (21%), 705 intermediate (16%) and 48 late (1%) AMD samples (Supplementary Table). Four SNPs were associated with one or more stages of AMD after correction for multiple testing ($p \leq 0.001$) (Table 4). These included rs3750846 at the *ARMS2/HTRA1* loci, in high Linkage Disequilibrium (LD) with rs10490924 ($r^2 = 1$) showing associations with all stages of AMD, and strength of association increased with increasing severity of AMD. Two common variants in the *CFH* gene, rs10922109 and rs570618 (in high LD with rs1061170, $r^2 = 1$), were associated with intermediate and late AMD ($p < 0.001$) but not early AMD. One rare variant in the *C3* gene (rs147859257) showed association only with late AMD ($p < 0.001$). All other SNPs analyzed showed no statistically significant association with AMD (Table 4).

3.5. Characteristics by treatment arm

Baseline characteristics by randomized treatment groups are presented in Table 5. The groups were well balanced for recognized risk factors for AMD, such as age, smoking and sex, the frequencies of the at-risk alleles in three known AMD-related genes and for all other characteristics included in the analysis.

4. Discussion

AMD complex pathogenesis includes low-grade inflammatory process in drusen at the early stages, and hemorrhages, scarring and atrophy of the central retina at the late stage of the disease. Anti-

Table 2

Univariable multinomial logistic regression analysis of the associations between baseline characteristics and AMD stages.

Characteristic		Early AMD	Intermediate AMD	Late AMD
Age at randomization (years)		1.03 (1.01, 1.05)	1.08 (1.06, 1.10)	1.21 (1.16, 1.27)
Age groups at randomization	70–74	ref	ref	ref
	75–79	1.31 (1.11, 1.54)	1.50 (1.25, 1.80)	3.85 (1.98, 7.47)
	80–84	1.42 (1.10, 1.82)	1.95 (1.51, 2.53)	8.48 (4.14, 17.37)
	85+	1.12 (0.68, 1.85)	3.12 (2.07, 4.68)	12.80 (4.84, 33.82)
Sex	Female	1.54 (1.33, 1.77)	1.46 (1.25, 1.70)	1.61 (0.94, 2.74)
Education (years)	≥ 12	0.94 (0.82, 1.09)	0.93 (0.79, 1.08)	0.80 (0.47, 1.34)
BMI groups	Underweight	1.46 (0.84, 2.54)	0.49 (0.21, 1.18)	3.97 (1.09, 14.49)
	Normal	ref	ref	ref
	Overweight	1.0 (0.83, 1.20)	0.83 (0.68, 1.01)	0.85 (0.43, 1.70)
	Obese	0.88 (0.72, 1.08)	0.81 (0.66, 1.00)	1.02 (0.50, 2.10)
Diabetes		0.87 (0.68, 1.10)	0.87 (0.67, 1.14)	1.01 (0.43, 2.38)
Hypertension		0.93 (0.80, 1.09)	1.03 (0.86, 1.23)	0.92 (0.51, 1.64)
Smoking History	Never	ref	ref	ref
	Former	0.83 (0.72, 0.96)	1.10 (0.94, 1.29)	0.92 (0.52, 1.60)
	Current	0.73 (0.46, 1.16)	1.29 (0.84, 1.98)	3.07 (1.16, 8.07)
Alcohol use	Never	ref	ref	ref
	Former	0.74 (0.50, 1.09)	0.67 (0.43, 1.03)	1.44 (0.48, 4.38)
	Current	0.92 (0.75, 1.13)	0.90 (0.72, 1.12)	0.79 (0.38, 1.64)
Glucose (mmol/L)		0.99 (0.92, 1.06)	0.95 (0.88, 1.04)	1.13 (0.91, 1.39)
HDL (mmol/L)		1.64 (1.40, 1.92)	1.57 (1.32, 1.86)	2.05 (1.20, 3.50)
LDL (mmol/L)		0.98 (0.90, 1.06)	0.99 (0.91, 1.09)	0.94 (0.69, 1.29)
Cholesterol total (mmol/L)		1.06 (0.98, 1.14)	1.05 (0.97, 1.14)	1.03 (0.78, 1.35)
Previous regular use of aspirin		1.45 (1.12, 1.89)	1.39 (1.04, 1.86)	0.87 (0.27, 2.79)
Use of any statin		0.93 (0.80, 1.09)	0.98 (0.83, 1.16)	1.11 (0.64, 1.93)
Awareness of having AMD		1.68 (0.92, 3.06)	6.01 (3.76, 9.61)	N/A

Odds Ratio (95% Confidence interval). ‘No AMD’ was the reference group for all stages of AMD. $N = 4993$.**Table 3**

Multivariable multinomial logistic regression analysis of the associations between baseline characteristics and AMD stages.

Baseline Characteristics		Early AMD	Intermediate AMD	Late AMD
Age at randomization (years)		1.03 (1.01, 1.05)	1.08 (1.06, 1.10)	1.23 (1.17, 1.30)
Sex	Female	1.37 (1.16, 1.62)	1.35 (1.12, 1.63)	1.32 (0.69, 2.54)
Education	≥ 12 years	0.95 (0.82, 1.10)	0.98 (0.83, 1.16)	0.87 (0.50, 1.53)
BMI groups	Normal	ref	ref	ref
	Underweight	1.23 (0.70, 2.18)	0.41 (0.17, 1.00)	2.19 (0.45, 10.08)
	Overweight	1.13 (0.93, 1.36)	0.91 (0.74, 1.12)	0.99 (0.48, 2.04)
	Obese	1.02 (0.82, 1.27)	0.93 (0.73, 1.17)	1.42 (0.64, 3.16)
Diabetes		0.87 (0.63, 1.20)	0.86 (0.60, 1.22)	0.57 (0.18, 1.83)
Hypertension		0.95 (0.80, 1.12)	0.99 (0.82, 1.19)	0.69 (0.37, 1.29)
Smoking History	Never	ref	ref	ref
	Former	0.88 (0.76, 1.04)	1.27 (1.07, 1.51)	1.22 (0.65, 2.28)
	Current	0.76 (0.47, 1.22)	1.41 (0.90, 2.22)	4.02 (1.42, 11.36)
Alcohol use	Never	ref	ref	ref
	Former	0.89 (0.59, 1.33)	0.69 (0.43, 1.09)	1.86 (0.57, 6.04)
	Current	1.06 (0.85, 1.32)	0.96 (0.76, 1.22)	0.87 (0.40, 1.92)
Glucose (mmol/L)		1.07 (0.98, 1.18)	1.02 (0.91, 1.14)	1.30 (1.00, 1.70)
Cholesterol total (mmol/L)		0.93 (0.85, 1.01)	0.95 (0.86, 1.05)	0.92 (0.65, 1.30)
HDL (mmol/L)		1.52 (1.26, 1.84)	1.43 (1.17, 1.77)	1.96 (1.02, 3.76)
Previous regular use of aspirin		1.46 (1.11, 1.92)	1.37 (1.01, 1.85)	0.92 (0.28, 3.04)
Use of any statin		0.87 (0.73, 1.05)	0.94 (0.77, 1.15)	1.11 (0.57, 2.16)

Odds Ratio (95% Confidence interval). ‘No AMD’ was the reference group for all stages of AMD. $N = 4993$.

inflammatory property of aspirin could reduce low-grade inflammation in the earlier stages of AMD [9–15], thereby possibly preventing its progression to late AMD, whilst its inhibitory effect on platelet aggregation could exacerbate retinal hemorrhages in late AMD [16–21]. To date the evidence on the effect of aspirin on the course of AMD is mixed, with inconsistent results related to risks and benefits of aspirin in AMD from observational and experimental studies [14–19,21–34] and no definitive conclusions in meta-analyses [20,35–37], which highlighted the need for a well-designed randomized controlled clinical trial of sufficient size and duration [38–42].

The opportunity to conduct such a trial materialized by conducting assessment of the retina status in participants from the major international ASPirin in Reducing Events in the Elderly (ASPREE) clinical trial,

which investigated the effect of aspirin on the health of older people. As retinal photography and AMD grading required substantial additional effort and resources, they were funded separately as the ASPREE-AMD sub-study, a randomized, placebo controlled trial of almost five thousand participants, to examine associations between the incidence and progression of AMD and the use of low-dose aspirin. Three landmark ASPREE papers reported that aspirin use did not prolong disability-free survival in the elderly, but led to a higher rate of major hemorrhages and higher all-cause mortality than in the placebo group [45–47], therefore the intervention phase of the trial was ceased after a median 4.7 (IQR, 3.6, 5.7) years of follow-up. The observation of the participants’ health conditions continued.

Analysis of the ASPREE-AMD study cohort of 4993 people, majority

Table 4

Frequencies of 40 known genetic risk variants of AMD in the ASPREE-AMD Study participants and their association with AMD stages.

SNPs	Locus/Gene	Minor/ Major Allele (A1/A2)	Minor Allele Frequency by AMD stages				Associations with AMD Stages					
							Early AMD		Intermediate AMD		Late AMD	
			No AMD* N = 2735	Early AMD N = 903	Intermediate AMD N = 705	Late AMD N = 48	OR	p-value	OR	p-value	OR	p-value
rs3750846	ARMS2/ HTRA1	C/T	0.195	0.242	0.289	0.448	1.33	1.6E⁻⁰⁵	1.7	2.5E⁻¹⁴	3.64	3.9E⁻⁰⁹
rs570618	CFH	T/G	0.359	0.388	0.438	0.635	1.13	0.027	1.39	8.1E⁻⁰⁸	3.19	9.69E⁻⁰⁸
rs10922109	CFH	A/C	0.423	0.401	0.357	0.229	0.91	0.098	0.76	9.0E⁻⁰⁶	0.4	0.0002
rs147859257	C3	G/T	0.004	0.005	0.006	0.031	1.19	0.664	1.35	0.463	7.86	0.001
rs2230199	C3	C/G	0.199	0.213	0.235	0.292	1.08	0.246	1.23	0.004	1.63	0.029
rs6565597	NPLOC4/ TSPAN10	T/C	0.34	0.358	0.346	0.437	1.08	0.157	1.03	0.667	1.51	0.047
rs11080055	TMEM97/ VTN	A/C	0.471	0.488	0.496	0.573	1.07	0.206	1.11	0.083	1.51	0.048
rs3138141	RDH5/CD63	A/C	0.223	0.242	0.252	0.302	1.11	0.093	1.18	0.02	1.52	0.066
rs62247658	ADAMTS9- AS2	C/T	0.413	0.410	0.414	0.333	0.99	0.85	1.01	0.924	0.71	0.117
rs11884770	COL4A3	T/C	0.265	0.277	0.247	0.198	1.06	0.325	0.91	0.16	0.69	0.143
rs35292876	CFH	T/C	0.007	0.009	0.016	0.021	1.22	0.513	2.17	0.004	2.93	0.146
rs429358	APOE	C/T	0.133	0.136	0.104	0.083	1.03	0.732	0.75	0.003	0.59	0.153
rs2740488	ABCA1	C/A	0.269	0.293	0.282	0.333	1.13	0.045	1.07	0.348	1.36	0.162
rs7803454	PILRB/PILRA	T/C	0.179	0.192	0.209	0.125	1.09	0.235	1.22	0.009	0.65	0.169
rs12019136	RAD51B	A/G	0.045	0.044	0.040	0.021	0.97	0.801	0.89	0.442	0.45	0.266
rs140647181	COL8A1	C/T	0.018	0.017	0.021	0.031	0.96	0.832	1.2	0.401	1.79	0.334
rs943080	VEGFA	C/T	0.497	0.506	0.501	0.448	1.04	0.493	1.02	0.798	0.82	0.341
rs1626340	TGFBR1	A/G	0.223	0.209	0.212	0.187	0.92	0.211	0.94	0.384	0.81	0.41
rs72802342	CTRB2/ CTRB1	A/C	0.080	0.074	0.073	0.062	0.92	0.404	0.9	0.361	0.76	0.518
rs73036519	APOE	C/G	0.294	0.306	0.298	0.323	1.06	0.362	1.02	0.795	1.14	0.545
rs5754227	SYN3/TIMP3	C/T	0.1355	0.139	0.1121	0.1146	1.03	0.708	0.81	0.022	0.83	0.559
rs2842339	RAD51B	G/A	0.0885	0.0941	0.0844	0.1042	1.07	0.461	0.95	0.626	1.2	0.589
rs114092250	PRLR/SPEF2	A/G	0.0294	0.0288	0.0228	0.0208	0.98	0.888	0.74	0.13	0.7	0.621
rs10033900	CFI	T/C	0.4879	0.4751	0.4872	0.5104	0.95	0.339	1	0.962	1.1	0.657
rs9564692	B3GALT1	T/C	0.2868	0.2957	0.2865	0.2708	1.04	0.472	1	0.982	0.92	0.732
rs61941274	ACAD10	A/G	0.0165	0.0188	0.0213	0.0208	1.14	0.506	1.29	0.222	1.26	0.743
rs8135665	SLC16A8	T/C	0.2	0.1977	0.1936	0.2083	0.99	0.832	0.96	0.597	1.05	0.842
rs10781182	MIR6130/ RORB	T/G	0.3044	0.2918	0.344	0.3125	0.94	0.309	1.2	0.004	1.04	0.864
rs12357257	ARHGAP21	A/G	0.2245	0.2287	0.1965	0.2188	1.02	0.711	0.84	0.023	0.97	0.893
rs148553336	CFH	C/T	0.0093	0.0127	0.0121	0.0104	1.36	0.216	1.29	0.361	1.12	0.913
rs55975637	COL8A1	A/G	0.1122	0.1312	0.1241	0.1146	1.2	0.029	1.12	0.212	1.02	0.943
rs1142	KMT2E/ SRPK2	T/C	0.3523	0.361	0.383	0.3542	1.04	0.507	1.14	0.035	1.01	0.97
rs2043085	LIPC	T/C	0.3943	0.3715	0.3801	0.3958	0.91	0.084	0.94	0.331	1.01	0.976
rs2070895	LIPC	A/G	0.2199	0.2248	0.2113	0.2188	1.03	0.6667	0.95	0.487	0.99	0.978
rs17231506	CETP	T/C	0.3221	0.3389	0.3227	0.3229	1.08	0.191	1	0.968	1	0.987
rs62358361	C9	T/G	0.0101	0.0072	0.0128	0	0.72	0.281	1.26	0.387	NA	NA
rs141853578	CFI	T/C	0.0007	0.0011	0.0014	0	1.52	0.631	1.94	0.444	NA	NA
rs191281603	CFH	G/C	0.0048	0.0083	0.0057	0	1.71	0.092	1.18	0.669	NA	NA
rs181705462	C2/CFB/ SKIV2L	T/G	0.0095	0.0089	0.0085	0	0.93	0.806	0.89	0.727	NA	NA
rs121913059	CFH	T/C	0.0002	0.0006	0	0	3.03	0.433	NA	NA	NA	NA

Abbreviations: SNP: Single Nucleotide Polymorphism, A1: Minor Allele, A2: Major Allele, OR: odd ratio, P values: Logistic regression p value.

* - 'No AMD' category combines the cases from the Beckman categories None and Normal Ageing Changes. 'No AMD' was the reference group for all stages of AMD.

of whom (88%) had the baseline retinal images gradable for AMD in both eyes, revealed the presence of early, intermediate and late AMD in 20.6%, 16.1% and 1.1%, respectively. The prevalence of early-intermediate AMD stages in the ASPREE-AMD study participants per corresponding age group was comparable with three Australian population-based AMD studies: the Blue Mountain Eye Study (BMES), the Melbourne Visual Impairment Project (Melbourne VIP) and the Australian National Eye Health Survey. However, late AMD prevalence in ASPREE (1.1%) was lower than that seen in the other studies, which reported on prevalence of 4.8% (in participants aged 64 years or older) in BMES [64], of about 4% in the 70–79 age group (increasing to 23% in those aged 90+) in the Melbourne VIP [65], and of 2% in the 70+ age group (nonindigenous participants) in the Australian National Eye Health Survey [66]. Most likely, this lower prevalence was due to the ASPREE eligibility criteria that limited enrolment to generally healthy

older individuals, rather than being population-based, and not including nursing home residents, thus potentially excluding those who would be more likely to have late AMD.

In ASPREE-AMD, 7% of the participants used aspirin regularly prior to entry into the study. Amongst these individuals the prevalence of early and intermediate AMD was higher than in those with no regular prior use. There was no difference in rates of late AMD though. The strong potential for confounding reinforces the need to conduct the ongoing follow-up ASPREE-AMD study, which will provide more robust evidence to confirm or refute an association. Longitudinal follow up of this cohort has the potential to address important questions that remain.

In the cross-sectional analysis of the ASPREE-AMD cohort at study entry, the prevalence and severity of AMD increased with age. Associations were also found with female gender, higher HDL levels, current smokers, four already identified AMD-related genetic risk variants and

Table 5Baseline characteristics of the ASPREE-AMD sample by treatment arms. Number (%) or Median (IQR). $N = 4993$.

Characteristic	Total	Placebo	Aspirin
Age at randomization (years)	73.4 (71.5, 76.6) $N = 4993$	73.3 (71.5, 76.5) $N = 2457$	73.5 (71.5, 76.8) $N = 2536$
Age groups at randomization (years)			
70–74	3220 (64)	1602 (65)	1618 (64)
75–79	1197 (24)	577 (23)	620 (24)
80–84	446 (9)	215 (9)	231 (9)
85+	130 (3)	63 (3)	67 (3)
Sex			
Male	2412 (48)	1174 (48)	1238 (49)
Female	2581 (52)	1283 (52)	1298 (51)
Education (years)			
<12	2199 (44)	1102 (45)	1097 (43)
≥ 12	2794 (56)	1355 (55)	1439 (57)
BMI groups			
Underweight	69 (1)	34 (1)	35 (1)
Normal	1146 (23)	538 (22)	608 (24)
Overweight	2297 (46)	1130 (46)	1167 (46)
Obese	1462 (29)	744 (30)	718 (28)
Diabetes			
No	4497 (90)	2211 (90)	2286 (90)
Yes	496 (10)	246 (10)	250 (10)
Hypertension			
No	1335 (27)	647 (26)	688 (27)
Yes	3658 (73)	1810 (74)	1848 (73)
Smoking history			
Current	149 (3)	73 (3)	76 (3)
Former	2030 (41)	983 (40)	1047 (41)
Never	2814 (56)	1401 (57)	1413 (56)
Alcohol use			
Current	4054 (81)	1997 (81)	2057 (81)
Former	240 (5)	109 (4)	131 (5)
Never	699 (14)	351 (14)	348 (14)
Glucose (mmol/L)	5.3 (5.0, 5.8) $N = 4894$	5.3 (5.0, 5.8) $N = 2411$	5.3 (5.0, 5.8) $N = 2483$
HDL (mmol/L)	1.5 (1.2, 1.8) $N = 4858$	1.5 (1.2, 1.8) $N = 2387$	1.5 (1.2, 1.8) $N = 2471$
Cholesterol total (mmol/L)	5.2 (4.5, 5.8) $N = 4938$	5.2 (4.6, 5.8) $N = 2435$	5.1 (4.5, 5.8) $N = 2503$
Previous use of aspirin			
No	4649 (93)	2298 (94)	2351 (93)
Yes	344 (7)	159 (6)	185 (7)
Use of any statin			
No	3461 (69)	1739 (71)	1722 (68)
Yes	1532 (31)	718 (29)	814 (32)
AMD status			
None/Normal aging (No AMD)	3099 (62)	1536 (62)	1563 (61)
Early	1031 (21)	509 (21)	522 (21)
Intermediate	806 (16)	381 (16)	425 (17)
Late	57 (1)	31 (1)	26 (1)
ARMS2/HTRA1 rs3750846			
CC	210 (5)	109 (5.1)	101 (4.5)
CT	1534 (35)	754 (35)	780 (35)
TT	2647 (60)	1282 (60)	1365 (61)
CFH rs570618			
GG	1705 (39)	840 (39)	865 (39)
TG	2028 (46)	985 (46)	1043 (46)
TT	658 (15)	320 (15)	338 (15)
C3 rs147859257			
GT	43 (1.0)	19 (0.9)	24 (1.1)
TT	4348 (99)	2126 (99)	2222 (99)
Awareness of having AMD			
Unsure	40 (1)	15 (1)	25 (2)
No	2701 (94)	1326 (94)	1375 (94)
Yes	127 (4)	63 (4)	64 (4)

prior use of aspirin. The majority of these findings replicate results from previously published Australian studies [64–66].

It is of interest that this study also identified that higher HDL levels were associated with prevalent AMD, when higher HDL is considered a beneficial factor for cardiovascular disease. This finding has been reported in other studies and as yet no good rationale has been put forward [67–71]. Higher levels of HDL have been associated with a range of factors, including high levels of physical activity and intake of olive oil and fatty fish, all of which have previously been reported to be potentially protective for AMD [72–77]. Further work on this interesting, yet counter-intuitive association is needed, including studying behavioral changes in older age.

The ASPREE-AMD study did not find associations between AMD and alcohol intake, unlike other studies [78–81], however not all studies have reported such associations [82–84]. We were unable to detect any association between taking statins and AMD, where previous reports have been contradictory [61,85]. It must be remembered that in ASPREE the cohort was required to be healthy on entry and potential participants were excluded if they had some pre-existing chronic illnesses. This difference from other population-based or cohort studies is likely to have a significant impact on disease associations in cross-sectional mode.

Genetic data was available on the majority (88%) of participants, and we found significant associations with four of the previously

described AMD genetic risk loci *ARMS2/HTRA1*, *CFH* and *C3* [59]. However, beyond these variants, we were unable to replicate other AMD risk variants associated with the disease, likely due to the small number of samples in each AMD group and absence of the 12/52 AMD risk variants in the microarray of the whole ASPREE cohort.

4.1. Strengths and limitations

The strengths of this study include its randomized controlled design, the age brackets appropriate for AMD, recruitment in both urban and rural areas, comprehensive interviews and general examinations via the ASPREE study, high quality digital fundus photography, genotyping and a relatively large sample size. The proportion of the participants from regional centers and rural areas (31%) was similar that in the total Australian population (29%), which increases the external validity of ASPREE-AMD's findings.

The proportion of ungradable images in one eye (11.2%) or both eyes (7.4%) was relatively low, compared to other non-mydratic photography studies [66,86,87].

A limitation of the ASPREE-AMD study is the use of only non-mydratic color photography to document AMD with no multimodal imaging available. However, it was not logistically feasible to provide multimodal imaging equipment to the large number of geographically

distant sites and we believe that much can still be learnt from the high-quality color fundus images. Another limitation is the potential to miss the diagnosis of neovascular AMD given the widespread use of *anti*-VEGF drugs, which can mask the neovascular complications. To ensure we capture virtually all cases of neovascular AMD, we will obtain information on treatment with *anti*-VEGF medications via data linkage with the Australian Government's Pharmaceutical Benefit Scheme. Data on the use of *anti*-VEGF treatment will be integrated with extensive ASPREE study data for analysis.

The ENVISION and SNORE sub-studies that provided retinal images from 548 (11.0%) and 331 (6.6%) participants, respectively, had additional exclusion criteria related to MRI, such as the presence of metal implants or claustrophobia, which may have meant that their cohorts are not as representative of the wider population. We anticipate no significant influence of this difference, but this will be investigated when considering the results and sensitivity analyses will be undertaken with and without including these cases.

Due to the wealth of other high quality data collected in ASPREE and its 15 sub-studies, related to cardiovascular health, depression, dementia, obstructive sleep apnea, frailty, genetic predisposition, social activity, dietary specifics, and more, there will be opportunities to explore additional associations with the development and progression of AMD. The ASPREE-AMD study results, analyzed in the context of the primary outcomes of the ASPREE trial and its sub-studies focused on various conditions of old age might help to find an optimal balance between risks and benefits of low dose aspirin treatment. The results may further refine medical recommendations about the use of aspirin in cases where AMD is combined with other diseases/conditions.

5. Conclusion

The ASPREE-AMD study leveraged opportunities offered by the large-scale, randomized, double-blinded primary prevention ASPREE trial, to recruit 4993 participants for 5 years of follow up to investigate the effect of long-term low-dose aspirin on incidence and progression of AMD. Prevalence of the early/intermediate stages of AMD was similar to that in the corresponding age groups from the three Australian population-based studies, while prevalence of late AMD was lower. Associations between AMD and age, current smoking, higher HDL levels, female sex and previous regular aspirin use were similar to that expected in this population of otherwise healthy elderly participants. Strong genetic associations of AMD with variants of *ARMS2/HTRA1*, *CFH* and *C3* genes were consistent with previous associations. Importantly, the randomization into treatment groups resulted in well balanced study arms with respect to known AMD risk factors and as such will provide an excellent opportunity to study the associations of long-term aspirin with the incidence and/or progression of AMD.

Funding

The principal ASPREE study was supported by the National Institute on Aging and the National Cancer Institute at the National Institutes of Health (grant #U01AG029824); the National Health and Medical Research Council of Australia (grants #334047 and #1127060); Monash University (Australia); and the Victorian Cancer Agency (Australia). A. G. Bayer provided aspirin and matching placebo but played no other role in the trial. Bio platform Australia funded genotyping with a SNP array on the ASPREE cohort.

The ASPREE-AMD sub-study was supported by the National Health and Medical Research Council of Australia (research grant #APP1051625 and equipment grant) and the National Eye Institute at the National Institutes of Health (grant #R01EY026890), the Phyllis Connor Memorial Trust, Jack Brockhoff Foundation and Eric Ormond Baker Charitable Trust. CERA provided retinal cameras; Monash University funded two Bio bus clinical vehicles for photography in more remote regions. CERA receives Operational Infrastructure Support from

the Victorian Government.

MRN received travel and meeting payment for an advisory board of A. G. Bayer.

The funding organizations did not have any role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

All Co-authors declared 'No Commercial Relationship'.

Acknowledgements

The investigators acknowledge the work of all retinal photographers, graders and ASPREE field research staff members who conduct study visits and collect data from ASPREE participants. The investigators also acknowledge the valued contribution of the ASPREE participants and the support from their general practitioners and eye care providers.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.conctc.2020.100667>.

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