



Age-related macular degeneration in a randomized controlled trial of low-dose aspirin: Rationale and study design of the ASPREE-AMD study



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ABSTRACT

Purpose: Although aspirin therapy is used widely in older adults for prevention of cardiovascular disease, its impact on the incidence, progression and severity of age-related macular degeneration (AMD) is uncertain. The effect of low-dose aspirin on the course of AMD will be evaluated in this clinical trial.

Design: A sub-study of the 'ASpirin in Reducing Events in the Elderly' (ASPREE) trial, ASPREE-AMD is a 5-year follow-up double-blind, placebo-controlled, randomized trial of the effect of 100 mg daily aspirin on the course of AMD in 5000 subjects aged 70 years or older, with normal cognitive function and without cardiovascular disease at baseline. Non-mydratic fundus photography will be performed at baseline, 3-year and 5-year follow-up to determine AMD status.

Primary outcome measures: The incidence and progression of AMD. Exploratory analyses will determine whether aspirin affects the risk of retinal hemorrhage in late AMD, and whether other factors, such as genotype, systemic disease, inflammatory biomarkers, influence the effect of aspirin on AMD.

Conclusion: The study findings will be of significant clinical and public interest due to a potential to identify a possible low cost therapy for preventing AMD worldwide and to determine risk/benefit balance of the aspirin usage by the AMD-affected elderly. The ASPREE-AMD study provides a unique opportunity to determine the effect of aspirin on AMD incidence and progression, by adding retinal imaging to an ongoing, large-scale primary prevention randomized clinical trial.

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¹ The full list of the ASPREE Investigator Group is provided in the ASPREE Methodology paper, ref [44].

1. Introduction

Age-related macular degeneration (AMD) is a major cause of visual impairment and legal blindness amongst the elderly in developed countries [1–4]. Visual impairment from AMD leads to a loss of quality of life, with increased rates of depression, injury, social isolation and institutionalization. AMD is strongly associated with age, to the extent that more than 10% of adults aged over 80 are living with advanced AMD. Increasing life expectancy was estimated to double the number of people with this late-onset disease over the next two decades, with a substantial impact on quality of life and the costs of care [5–7]. The late forms of AMD lead to central vision loss as a result of neovascular (nAMD) complications or atrophic changes (geographic atrophy) in the retina. Anti-vascular endothelial growth factor (anti-VEGF) therapy has significantly improved the outcomes for nAMD, but there remains no proven treatment to specifically slow progression of geographic atrophy (GA). There is also no specific treatment that prevents progression from early or intermediate AMD to late AMD. Current recommendations, which are of limited efficacy, are centred upon the use of supplements, lifestyle and dietary advice [8–11]. As populations age, there is an imperative to delay the onset and progression of disability and chronic disease. Identifying an effective preventive agent for AMD, or one that can slow its progression, would have significant beneficial effects on quality of life as well as healthcare costs.

2. Rationale for the ASPREE-AMD study: a potential for aspirin to prevent or slow the AMD process

Inflammatory processes have been implicated in the pathogenesis of AMD and its progression and AMD is considered by many to be a chronic, systemic inflammatory disease [12–16]. As such it is plausible that aspirin, via its anti-inflammatory actions, may play a role in both the prevention and slowing of progression to vision loss through low grade inflammatory process. This was the rationale for two previous sub-studies in large primary prevention trials of low dose aspirin that evaluated self-reported AMD incidence as a secondary outcome. In these trials, alternate-daily aspirin versus placebo was administered in a population of 22,071 US physicians aged 40–84 years, with 5 years of follow-up [17] and in a population of 39,876 women aged over 45 years, with 10 years of follow-up [18]. Both studies reported a similar effect size of low dose aspirin in reducing the relative risk of visually significant AMD by more than 20%. The studies used self-reported diagnoses, confirmed with medical record data, in order to reduce random misclassification. While both studies are suggestive of a beneficial effect of aspirin with respect to AMD, the reliance on self-reported diagnosis and the low significance of the results due to the low number of incident cases in the relatively young study populations limit the weight that can be given to this evidence.

Results from observational studies with respect to aspirin's influence on AMD incidence and prevalence have been inconsistent, ranging from the generally positive ('harmful') associations with AMD prevalence or incidence, with emphasis on the increased frequency of sub-retinal or vitreous hemorrhages [19–24], to no association [25–32], to negative ('protective') association [33,34] (Table 1). Thus, the overall risk/benefit balances of aspirin in relation to incidence and prevalence of AMD are yet to be fully explored. In addition, if aspirin were to increase the risk of retinal or vitreous hemorrhage, this finding would have important implications when considering aspirin for widespread use in primary prevention. A growing need for a sufficiently powered randomized controlled trial to resolve the relationship between aspirin use and AMD was highlighted in several recent reviews and meta-analyses

[23,35–43]. The NIH-funded large randomized controlled trial - ASpirin in Reducing Events in the Elderly (ASPREE) - designed to address the role of aspirin in primary prevention on disability- and dementia-free survival in older adults, provides the opportunity for a sizable sub-study to address this need [44]. Taking into account the likelihood that a number of actions of aspirin (anti-inflammatory, anti-platelet, etc.) are likely to be seen with 100 mg daily in ASPREE, its effect could be multifaceted. Thus, along with the tested possible preventive effect by suppressing the inflammatory process at earlier stages of AMD, aspirin's antiplatelet property might appear to be exacerbating the late AMD processes. This phenomenon will be closely looked into. Adding fundus photography to examine a sub-set of ASPREE participants, the ASPREE-AMD sub-study will determine the effect of low-dose aspirin on the course of AMD.

3. Material and methods

3.1. Study design

This study is a 5-year follow-up, double-blind, randomized placebo-controlled trial of daily 100 mg aspirin versus placebo on the incidence and progression of AMD, in a population of healthy Australians aged 70 years or older. ASPREE-AMD is a sub-study of the principal ASPREE trial.

3.1.1. Ethics statement

The ASPREE-AMD study has been approved by the Human Research and Ethics Committee of Monash University, undertaken according to the Helsinki Declaration for research on humans and registered with the Australian New Zealand Clinical Trial Registry: ACTRN 12613000755730. The principal ASPREE study is registered with the International Standardized Randomized Controlled Trials Register, ASpirin in Reducing Events in the Elderly, Number: ISRCTN83772183 and clinicaltrials.gov, Number NCT01038583.

ASPREE participants consented separately to retinal photography. Participants in two other sub-studies of ASPREE, which together include more than 900 ASPREE participants with retinal photographs taken with the same cameras at baseline and after 3 years of treatment with study medication, will also be included in the ASPREE-AMD sub-study. These two sub-studies are:

- (1) ENVISION (ASpirin for the prevention of cognitive decline in the Elderly: a Neuro-Vascular Imaging Study - a two-centre, randomized, double-blind, placebo controlled trial of the effects of daily 100 mg enteric-coated aspirin on the rate of increase of magnetic resonance imaging (MRI)-based white matter hyperintensities (WMH) and silent brain infarctions (SBI), ACTRN12609000613202) [45];
- (2) SNORE-ASA (Study of Neurocognitive Outcomes, Radiological and Retinal Effects of ASpirin in Sleep Apnoea - a multi-centre, randomized, double-blind, placebo controlled trial of the effects of daily 100 mg enteric-coated aspirin on cognitive outcomes in the setting of sleep apnoea, in healthy older adults aged 70 and over, ACTRN 12612000891820).

The year 5 follow-up photography for these participants will be conducted as part of the ASPREE-AMD study.

3.1.2. The principal ASPREE trial

ASPREE is a multi-centre, randomized, double-blinded, placebo-controlled trial of daily 100 mg enteric-coated aspirin in 19,000+ healthy community dwelling older adults in Australia and the US. Age eligibility is 65 years and over for African Americans and Hispanics in the USA, and 70 years and over for all others,

Table 1
Studies of the association between regular aspirin intake and the course of age-related macular degeneration (AMD).

Reference	Details of study	Conclusion
Case studies		
el Baba, F. 1986 [19]	15 AMD cases of massive subretinal or vitreous hemorrhages. Of them, 4 on Warfarin, 1 on Aspirin, 1 on Clinoril and 1 on Ibuprofen. Doses of medications not provided.	Combining the author's cases with cases published before, 16/83(19%) of patients were on either warfarin (n = 14) or aspirin (n = 2) .
Case-control studies and retrospective studies		
Wilson, H.L. 2004 [33]	Retrospective consecutive case series of diagnosed AMD patients: 104 CNV, 18 GA and 204 intermediate AMD. Doses not provided.	Regular aspirin use [more than 6 months (LR assumption)] was associated with the decreased rates of CNV in the Multipredictor Model (hazard ratio = 0.63, 95% CI = 0.40–0.98, p = 0.04).
Kiernan, D. F. 2010 [24]	Retrospective cross-sectional study. Incident CNV: n = 195 eyes of 195 patients examined over 5 years. 80 (41%) used daily aspirin during a median follow-up of 27.0 months (range, 1–73 months). Doses not provided.	Incident intraocular hemorrhages (subretinal or vitreous) in patients with neovascular AMD were associated with daily antiplatelet or anticoagulant (AP/AC) medication usage (aspirin, clopidogrel, and warfarin). Aspirin was independently associated with hemorrhages, with OR 3.75 (95% CI 1.88–7.48). Aspirin was not associated with massive hemorrhages in AMD, whilst warfarin was associated.
Tilanus, M. A. 2000 [25]	Retrospective case-control study: 50 cases with massive haemorrhages (15 on Warfarin and 8 on aspirin) vs 50 cases with small AMD-related hemorrhages (2 on warfarin and 6 on aspirin) Aspirin doses varied from 80 to 100 mg daily.	Aspirin was not associated with massive hemorrhages in AMD, whilst warfarin was associated.
Jonczyk-Skorka, K. 2015 [26]	Case-control study: n = 292; 80 with drusen, 25 with GA, 52 with CNV and 135 controls. 27.1% of study population used aspirin. Doses not provided.	Aspirin was not associated with any form of AMD
DeAngelis M.M. 2004 [27]	Matched case-control study: 73 sibling pairs; one sibling with CNV, the other with no AMD. Aspirin was defined as regularly used if taken at least twice per week for at least 6 months before AMD diagnosis. Doses not provided.	In this study population of the extremely discordant sibling pairs, aspirin use was not associated with neovascular AMD (p = 0.48)
Cross-sectional studies		
de Jong, P.T.V.M., 2012 [20]	Population-based cross-sectional European Eye Study; n = 4691, age 65+ 36.4% early AMD and 3.3% late AMD Frequency of aspirin analysed: at least once monthly (n = 1786), weekly (n = 326) and daily (n = 839). Doses not provided.	For daily aspirin users (17.3% of population), aspirin was associated with prevalent AMD. AMD grade 1: OR 1.26 (95% CI, 1.08–1.46); AMD grade 2: OR 1.42 (95% CI, 1.18–1.70), and CNV: OR 2.22 (95% CI, 1.61–3.05). For a trend across aspirin intake frequency p < 0.001.
Cheung, N. 2013 [28]	Population-based cross-sectional Singapore Indian Eye Study; n = 3207. Aspirin use: yes/no answer to a question "currently taking any aspirin (solprin, cardiprin, disprin, ecotrin, but not panadol or dymadon?)". Doses not provided.	Overall aspirin use was not associated with prevalent early AMD. Aspirin was associated with early AMD in participants with a history of cardiovascular disease (OR 2.64, 95% CI 1.31–5.36) but not without it (OR 0.73; 95% CI 0.36–1.51 (interaction term, p = 0.011).
Chew, E. 2012 [34]	Cross-sectional analysis: 2046/4188 (48.8%) AREDS2 participants were taking aspirin; 661 controls (AREDS Simple Scale Score of 0, 1, and 2), 692 with bilateral large drusen and pigmentary change in one eye (Score 3), 1369 with bilateral large drusen and bilateral pigmentary changes (Score 4); 1466 had advanced AMD in one eye and bilateral large drusen in the other eye (Score 5). 1304 of the latter group had CNV and 162 had GA. Aspirin usage: < 5 times per week; > 5 times per week (<2 per day); or > 5 times per week (≥2 per day).	In adjusted multivariate analyses, an inverse relationship between the various stages of AMD with aspirin use at least 5 times a week was found, with OR (95% CI): for Score 3: 0.82 (0.65–1.02); for Score 4: 0.86 (0.70–1.05); for Score 5: 0.62 (0.50–0.76), for CNV 0.61 (0.49–0.75) and for GA 0.62 (0.42–0.94).
Rudnicka, A. R. 2010 [29]	Case-control study on association between markers of arterial thrombosis and late AMD: 81 late AMD cases and 77 controls.	Aspirin was weakly associated with a lower risk of AMD: Adjusted for age, sex, and smoking, aspirin reduced the risk of late AMD by 53% (OR 0.47; 95% CI 0.20–1.08); adjusted additionally for BMI, BP and total serum cholesterol, the OR was attenuated towards the null (OR 0.61; 95% CI 0.23–1.57).
Cohort studies		
Klein, B. E. 2012 [21]	Beaver Dam Eye Study – population-based Cohort: n = 4926 aged 43–86 at baseline; 5-yearly exams, average 14.8-years of follow-up. Aspirin usage primary measure (yes/no): at least twice a week for > 3months, asked at every 5-yr exam. (Doses not provided). At the 3rd, 4th and 5th exams, additional info on frequency (<1 aspirin every second day, 1 every second day, 1 daily, 2 daily, 3 to 7 daily, or ≥8 daily) and current dosage was obtained, to calculate an estimated average daily dose.	Regular aspirin intake was not associated with incidence of early AMD. Aspirin use 10 years prior to retinal examination was associated with late AMD (HR = 1.63 [95% CI, 1.01–2.63]), specifically with neovascular AMD (HR = 2.20 [95% CI, 1.20–4.15]).
Liew, G. 2013 [22]	Blue Mountains Eye Study – population-based cohort: n = 2389 Aged 49 years or older; 15 years of follow-up. Aspirin regular usage: ≥ 1 a week in the past year, confirmed with a list of medications taken for at least 1 month before examination and the medications brought in. Doses not provided.	Regular aspirin use was associated with developing neovascular AMD (OR 2.46; 95% CI 1.25–4.83) and was not associated with the incidence of geographic atrophy.

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Table 1 (continued)

Reference	Details of study	Conclusion
Sen H. 2007 [31]	Age-Related Eye Disease Study (AREDS): 799 of the 1134 subjects in AREDS group 3 and 330 of the 499 subjects in AREDS groups 4 & 5; 11 years of follow-up. NSAIDs or aspirin usage defined as ≥ 5 days of the week for ≥ 3 months. Doses not provided.	Regular systemic use of NSAIDs or aspirin was not associated with developing advanced AMD
Ying 2016 [30]	A Cohort within the Comparison of Age-Related Macular Degeneration Treatments Trials (CATT): n = 1165 patients with CNV; 724 (62.1%) patients had retinal or subretinal hemorrhage at baseline; 514 (44.1%) used antiplatelets, 77 (6.6%) used anticoagulants, and 17 (1.5%) used both. Aspirin doses: none (0 mg), ≤ 81 mg and ≥ 82 mg. Warfarin doses: none, 1–4 mg and ≥ 5 mg. Duration of use: none (0 mg), < 5 yrs, ≥ 5 yrs and < 10 yrs, ≥ 10 yrs.	Hemorrhage was present in 64.5% of antiplatelet or anticoagulant users and in 59.6% of nonusers, OR 1.18; 95% CI 0.91–1.51. Baseline hemorrhages were not associated with the type, dose, or duration of antiplatelet or anticoagulant use. Incident hemorrhages 44/1078 (4.1%) were not associated with antiplatelet or anticoagulant use at baseline (p = 0.28) or during follow-up (P = 0.64). Among the participants with hypertension (n = 807), antiplatelet drug use was associated with a higher rate of hemorrhages at baseline OR 1.43; 95% CI 1.06–1.92.
Aronow, M.E [32].	Observational 6-year AMD progression data from the AREDS2. 957 aspirin users and 957 non-aspirin users (n = 1914) matched on the propensity score. Aspirin regular usage: at least 5 times a week. Doses not provided.	Aspirin propensity score adjusted for age, was not associated with AMD progression (OR 0.80, 95% CI 0.41–1.56). Analysed individually, neither geographic atrophy (OR 1.31, 95% CI 0.52–3.32) nor CNV (OR 0.60, 95% CI 0.23–1.58) was associated with the aspirin propensity score.
Randomized controlled trials		
Christen, W. G. 2001 [17]	5-year RCT of low-dose aspirin (325 mg every other day) and beta carotene (50 mg every other day) among US physicians; n = 22,071	Aspirin intake was not associated with self-reported AMD, RR 0.77; 95% CI 0.54–1.11. Additional testing for a potential beneficial effect in randomized trials of adequate size and duration is required; due to relatively young age of population, the detection of 23% risk reduction was not statistically significant
Christen, W. G. 2009 [18]	10-year RCT of low-dose aspirin (100 mg every other day), US female health professionals; n = 39,876	Aspirin intake was not associated with self-reported AMD, Hazard ratio 0.82; 95% CI, 0.64–1.06.
ASPREE Investigator Group 2013 [44]	Australian-American RCT "ASpirin in Reducing Events in the Elderly (ASPREE). n = 19,000, aged 65 years or above ("US minorities") and 70 years or above (non-"US minorities") 100 mg of aspirin daily.	Design: In contrast to other aspirin trials that have largely focused on one disease/group of diseases, ASPREE has a unique composite primary endpoint 'disability free survival', to capture the overall risk and benefit of aspirin, aiming to extend healthy independent lifespan. Pending results.
Meta-analyses		
Kahawita, S. K. 2014 [41]	2 cross-sectional, 1 population-based incidence and 1 cohort study; n = 10,292	High heterogeneity was emphasized. Aspirin use was associated with early ARMD. Pooled OR 1.43 (95%CI 1.09–1.95)
Ye, J. 2014 [40]	2 RCTs, 3 cohorts and 4 case-control studies n = 177,683	Aspirin use in total was not associated with AMD in total. Neovascular AMD was associated with aspirin use: RR 1.59; 95% CI 1.09–2.31
Zhu, W 2013 [35]	2 RCTs, 4 case-control and 4 cohort studies n = 171,729	Aspirin use was not associated with increased risk for Any ARMD (pooled RR 1.09, 95%CI 0.96–1.28), and it was so for either early or late AMD, in RCT case-control or cohort studies.
Li, L. 2015 [23]	Ten studies. N = 180 834. 2 Cohorts, 2 RCTs, 2 cross-sectional, 4 case-controls	Weakly, but statistically significantly, aspirin use was associated with AMD , with the pooled RR of 1.137 (95% CI, 1.003–1.289; I(2), 68.4%). The pooled RR was 1.19 (95% CI, 0.92–1.53; I(2) 82.6%) for early AMD, 1.95 (95% CI, 1.40–2.72; I(2), 27%) for CNV and 0.84 (95% CI, 0.62–1.15; I(2), 0%) for GA.
Reviews		
Sobrin, L. 2013 [37]	Analysis of findings and limitations of two RCTs, two population-based cross-sectional studies, one cohort and one case-control study.	Current data on the association between aspirin use and AMD do not fulfil criteria to declare that a causal relationship exists . The criteria that are not met include consistency, sufficient strength of association and specificity.
Wu, Y 2013 [36]	Aspirin and Age Related Macular Degeneration; the Possible Relationship. Review of multiple studies.	Evidence from the epidemiological studies has been contradictory and no persuasive conclusions have been made. "The current results should be challenged and acknowledged by well-designed, large-scale and long term follow-up studies"
Christen, W.G. 2014 [42]	Aspirin and risk of Neovascular AMD. Review of multiple studies.	The inherent limitations of observational studies preclude an interpretation of causality. Well-designed randomized trials of sufficient size and duration are needed to establish risk/benefit ratio of aspirin use by individuals at low-to-moderate risk of cardiovascular disease.
Chong, E.W. 2014 [39]	Aspirin and risk of AMD. Review-Commentary	Discussed the controversy surrounding aspirin use and highlighted the ongoing randomized controlled trial ASPREE .

Abbreviations: AMD, age-related macular degeneration; RCT, randomized controlled trial; GA, geographic atrophy; CNV, choroidal neovascularisation; OR, odds ratio; RR, relative risk; CI, confidence interval.

including all in the ASPREE-AMD, ENVISION and SNORE-ASA trials [44]. ASPREE will determine whether 100 mg aspirin daily extends disability- and dementia-free survival in the elderly, and it is a primary prevention study. The ASPREE study methods have been described in detail elsewhere [44]. In brief, the majority of ASPREE

Australian participants have been recruited through partnerships with general practitioner co-investigators. A minority has been recruited directly from the community.

The primary endpoint of ASPREE is a composite of death or dementia (adjudicated according to the DSM-IV criteria) or

persistent loss of one of the basic activities of daily living. Pre-specified secondary endpoints include death, cardiovascular and cerebrovascular disease, cancer, cognitive impairment, depression, physical disability and clinically significant bleeding. Clinical endpoints are adjudicated by independent committees provided with de-identified clinical information about the event [44]. **Inclusion criteria** include men and women who were able to give informed consent and able to attend a study visit for an estimated period of five years. **Exclusion criteria** include a past history of cardiovascular event or established cardiovascular disease (including stroke, transient ischemic attack, myocardial infarction, unstable angina, coronary artery reperfusion procedures and bypass grafting, abdominal aortic aneurysm, cardiac failure), atrial fibrillation, dementia or score of <78 on Modified Mini-Mental State (3MS) examination, disability as defined by severe difficulty or inability to perform any of the 6 Katz Activities of Daily Living (ADLs) [46], a condition with a high current or recurrent risk of bleeding, anaemia, a condition likely to cause death within 5 years, current use of other antiplatelet or antithrombotic medication, current use of aspirin for secondary prevention, and uncontrolled hypertension.

Participants meeting initial ASPREE eligibility at a screening study visit underwent a four-week placebo run-in phase and those with compliance equal or greater than 80% were randomized. Randomization of study drug followed a block randomization procedure and was stratified by site and age (65–79 and ≥80 years). An independent statistician generated the randomization list using the STATA ‘ralloc’ procedure. Participants were randomized to receive either 100 mg of enteric-coated aspirin or enteric-coated placebo, which are identical in appearance, in a ratio of 1:1. A 12-month supply of study medication was dispensed at trial entry and thereafter at each annual visit. Study participants, investigators and general practitioner co-investigators remain masked to treatment allocation.

All ASPREE participants have face-to-face study visits annually, with quarterly telephone contact in between visits. The 6-month phone call ascertains additional information relevant to study endpoints, including persistence of functional impairment.

Annual ASPREE data collected and assessments conducted include: demographics, cognitive function, physical function, quality of life, blood pressure, cardiovascular biomarkers, health behaviours and lifestyle (Table 2). Compliance with study medication is checked by annual pill count. Clinical endpoints of the study are being adjudicated and confirmed. The ASPREE study began in

2010 and completed recruitment in December 2014, with 16,703 participants in Australia and 2411 in the USA, and will conclude in 2017/2018.

3.1.3. The ASPREE-AMD trial

The ASPREE-AMD trial involves the acquisition of digital retinal images of both eyes at baseline, 3 and 5 years in ASPREE participants after randomization to aspirin or placebo.

All Inclusion criteria for the parent ASPREE study applied to this project. All consecutive randomized ASPREE participants at each centre who also gave informed consent for retinal photography and were able to attend a retinal photography visit were deemed eligible for entry into the ASPREE-AMD sub-study.

All Exclusion criteria for the parent ASPREE study also applied, with the additional criterion of the examiner being unable to view the macula without pharmaceutical dilation to take a retinal image (mainly due to either ocular media opacity or small and rigid pupil).

Participants with bilateral late AMD at baseline were still enrolled, but will be excluded from the analysis of AMD incidence or progression. These participants will be followed up for assessment of the potential worsening of the condition due to possible new or recurrent hemorrhage.

3.1.4. Retinal photography

Two digital, 45°, non-stereoscopic, colour retinal photographs of each eye, with one image centred on the fovea and one on the optic disc, are taken on one of nine non-mydratic fundus cameras (Canon Inc., Tokyo, Japan), using Digital Health Care software (UK). Non-mydratic digital retinal imaging has been proven to be a reliable method of AMD detection [47–49]. These fundus cameras are either (1) located permanently at four ASPREE study stationary centers in three Australian states, or (2) installed permanently in the specifically designed three high-roofed clinic vehicles (Mercedes vans) operated from the Melbourne site to engage participants from remote regional and rural areas in the study, or (3) shipped on the regular basis in the flight cases from Melbourne to the most distant areas, with trained research staff travelling to and assembling the cameras at the pre-organized sites. The use of several mobile photographic units allowed involvement of rural and regional Australian population in this research.

Photographs are taken without pharmacological pupil dilation. The right eye is photographed first, with sufficient time (up to 5 min) allowed between the following photo shots for the pupils to

Table 2
Schedule of the ASPREE-AMD study and relevant components of the ASPREE trial.

Timeline	Baseline 2010–14	3-yr follow-up 2013–17	5-yr follow-up 2015–20
ASPIRIN and AMD			
Retinal photography	X	X	X
Screening for medically significant pathology	X	X	X
Grading digital images for AMD	X	X	X
Analysis of grading results		X	X
Medicare request on anti-VEGF intravitreal injections			X
Genotyping for AMD-related genes (further research)			X
Analysis and Reporting			X (2020–21)
ASPREE TRIAL			
Recruitment, Screening and Baseline enrolment	X		
Demographics, Cognitive & Physical Measurements ^a	X	X	
Blood Pressure and Cardiovascular Biomarkers ^b	X	X	
Health Behaviours and Lifestyle Measurements ^c	X	X	
Reporting			X (2018–2019)

^a First language, education, height, weight, abdominal circumference, family history and co-morbidity, cognitive and physical function measurements, depression measure.

^b Total cholesterol, LDL-C, HDL-C, triglycerides, Hb, fasting glucose & creatinine, UACR.

^c Physical ability, smoking history, alcohol use; SF-12, IADL, ADL.

recover from flash-induced constriction. Staff members were trained to assess image quality and re-capture if images are unsatisfactory. The images and participant identifiers are backed up on the portable hard discs and bulk-exported from each camera on a monthly basis. Prior to uploading the images to the ASPREE database, the batches of the exported images are converted into JPEG format and labelled (each image) with the participants' data entered into the computers linked to the fundus cameras, using for both procedures a custom-written script for automated bulk processing. Four pre-specified identifiers (participant ID, acrostic that consists of the combination of the shortened last and first names, date of imaging and DHC code) are used to match images with the ASPREE database records of the participants during bulk uploading of the images. The batches of images are initially screened for signs of clinically significant pathology requiring medical attention and if needed, the notification letters, automatically generated via the ASPREE database, are sent to the participants and their general practitioner. De-identified images are transferred to the ASPREE-AMD retinal image database housed on a secure server for detailed grading. Images are graded for AMD according to the Beckman classification by two independent, masked experienced graders [50]. Grading process closely follows the timing of image acquisition, aiming to complete the grading soon after completion of image collection. During side by side grading, the temporal sequence of photos will not be masked. In this study, the image labels include the date of photography as one of the identifiers important for data validation. Deleting the dates and relabelling the images would increase risk of errors, mostly due to the large scale of the study. However, the graders will at all times be independent of each other and masked to the allocation of study medications.

The graders assess quality of the image (for focus and field placement), as well as the presence, size and location of the AMD-related lesions within a 6000 μm circular grid calibrated for size on the optic disc and centred on the fovea [47]. Grading is checked periodically for inter-grader agreement and intra-grader repeatability on random selections of images.

Incident pathology or cases of progression will be confirmed via side-by-side comparison of baseline and follow-up images from the same participant and adjudicated by an ophthalmologist (RG) when required. After assessment of the baseline and follow-up retinal images for incidence, progression and severity of AMD, the effect of aspirin on the course of AMD will be analysed.

3.1.5. Adverse events

All adverse events and serious adverse events are reported according to good clinical practice guidelines and handled by the principal ASPREE study. An independent Data and Safety Monitoring Board (DSMB), established by the National Institute on Aging, monitors all ASPREE activities on a 6 monthly basis. Clinically significant retinal pathology at baseline or follow-up is reported back to the participant's primary care physician and to the participant. Participants with any bleeding disorder, including retinal hemorrhage, may be taken off study medication.

3.1.6. Acquisition of additional information on adverse events and anti-VEGF treatment

In the last decade, intravitreal anti-VEGF therapy for choroidal nAMD has been implemented in Australia. Once treated with anti-VEGF, signs of nAMD may not be visible on colour fundus images. Therefore, to improve the accuracy of diagnosis and correctly identify those who are receiving treatment with intravitreal ranibizumab and aflibercept between ASPREE-AMD baseline and follow-up time points, additional information will be obtained from two sources to ensure the development of nAMD is captured:

participant self-reported adverse events validated through medical records and the National Medicare data on intravitreal ranibizumab and aflibercept prescriptions being approved for nAMD treatment during the study period. These data will not capture those participants who are being treated with bevacizumab, an off label drug used for nAMD. As ranibizumab and aflibercept are fully subsidized through the pharmaceutical benefits scheme (PBS) in Australia for treatment of subfoveal nAMD, treatment with bevacizumab is likely to be uncommon. As anti-VEGF treatment via the PBS is used now for other retinal conditions as well, the diagnosis of AMD will be validated via medical records and the collected data on adverse events.

The data on anti-VEGF medications for nAMD will be collected for all ASPREE participants (not just those in ASPREE-AMD), as consent for their retrospective information to be obtained from Medicare is included in the ASPREE protocol, and this will provide an additional opportunity to conduct analysis on the effect of aspirin on the incidence of nAMD on a sample which will be considerably larger than the sample photographed as part of ASPREE-AMD.

3.1.7. Definitions of AMD

The following Beckman risk categories of AMD will be used in the analysis [50]:

- 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

3.1.8. Primary outcome measures

1) Incident AMD. Any case that progresses from bilateral 'normal' or 'normal aging change' to any grade of AMD in at least one eye will be classified as incident AMD.

2) Progression of AMD. An increase in the AMD severity status from early or intermediate AMD in either eye will be classified as AMD progression. Regression of AMD stage will also be documented.

3.1.9. Future genetic and inflammatory biomarker analyses

Another sub-study of the principal ASPREE study, the ASPREE Healthy Ageing Biobank (www.aspree.org), in parallel with ASPREE-AMD, collects, processes and stores components of blood and urine samples at baseline and at year 3 in the trial, with serum, EDTA plasma, sodium citrate plasma, red blood cells and buffy coat aliquots stored for future biomarker and genotyping analysis. Association studies will be conducted in relation to potential biomarker variables (inflammatory markers, AMD-related genetic polymorphisms) and AMD incidence and progression, as well as their possible influence on the effect of aspirin on the primary outcomes.

3.2. Statistical analysis

3.2.1. Sample size and study power

No single population-based study provided all relevant information that we required for sample size calculations, hence we used data from several studies.

Prevalence estimates: we used the results from the cross-sectional European Eye Study, conducted on participants of similar ethnic origin and similar age (65 years or older), and also used digital images of the retina [51]. The study found that approximately half of the participants had no AMD, about one third had medium drusen (early AMD) and about 15% had large drusen (intermediate AMD).

From an expected sample of 5000 ASPREE-AMD participants with gradable images at baseline, excluding an estimated 2% with existing 'late AMD' at baseline and allowing for a 4% per annum attrition, the cohort will have 3995 participants at 5-year follow-up.

Incidence estimates: Based on the age-specific data (70–79 and 80+ years) from the population-based longitudinal Melbourne Visual Impairment Project (VIP), also conducted in Victoria, Australia, among an estimated 1998 participants with no AMD at baseline (estimated to be 50% of 3995 followed-up participants), the expected 5-year incidence of early and intermediate AMD (combined) is approximately 20% [52].

Progression estimates:

- (i) Among an estimated 1398 participants with early AMD (medium size drusen) at baseline (35% of 3995 participants), we expect 35% to have 5-year progression to intermediate AMD. For this estimate we used the only available data - the AREDS study finding that medium sized drusen (63–125 μm) progress in five years to large drusen ($\geq 125 \mu\text{m}$) at the rate of 20% if drusen are in one eye only and at the rate of 50% if drusen are in both eyes [50]. As there is no data on the proportion of unilateral and bilateral drusen in a population aged 70 years or older, we took an average of 35%.
- (ii) Among an estimated 1998 participants with either early AMD or intermediate AMD at baseline, we expect at least 4% to progress to late AMD, based on the published late AMD incidence rates amongst people 70 years or older in two longitudinal studies, the Melbourne VIP and Blue Mountains Eye Study (BMES), both conducted in the Australian population [52,53].

Based on these estimates, our study will 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.

The detectable 5-year changes between the placebo and aspirin treatment groups in incidence and progression of AMD are provided in Table 3. Competing risks of death and debilitating diseases that might cause differential survivorship in study arms will be considered in the analysis [54,55].

Our power calculation describes percent reductions that can be detected with 80% power based on the observed effects, which are innately weaker than the "true" effect that could exist if everyone remained on their randomized treatment. Therefore, a naturally occurring incomplete compliance has been incorporated in the estimates. The reasons for non-compliance include the development of a non-fatal, non-disabling cardiovascular or cerebrovascular event necessitating aspirin therapy. Thus, the ASPREE protocol specifies an expectation that 5% per annum of placebo-group participants will initiate aspirin use and similarly that 5% of aspirin-group participants will cease taking study medication and not commence open-label aspirin use.

3.2.2. Statistical analysis plan

The primary analyses will be conducted using the intention-to-treat principle, *i.e.* according to the group to which participants were randomized and without reference to their actual compliance with assigned treatment. We will use the grading data from the participants with baseline and 5-year images and apply log-binomial regression models to directly compare event rates between treatment groups, to assess the effect of aspirin on the outcomes: AMD incidence and progression. Each model will be applied to the relevant eligible participant subset (see Table 3 for expected numbers) and the models will include a binary covariate to indicate randomization to aspirin or placebo; the parameter for this covariate can be translated as the estimated rate ratio for aspirin.

Secondary analyses will apply the same models but with adjustment for age, sex and smoking status at baseline, and further analyses will also adjust for any variables predictive of AMD progression and found to be different between the two groups at baseline.

An exploratory analysis will be undertaken to determine whether aspirin is associated with increased risk of retinal hemorrhages.

Given the large sample size, we anticipate that randomization will adequately balance baseline characteristics of participants between the two treatment groups. However, the use of aspirin may affect survivorship itself, which plays a major role in AMD statistics. Therefore, if the follow-up loss of retinal data due to death and disability is found to be unbalanced between the study arms, it will be included in the statistical model as a competing risk [55].

To assess sensitivity to participant dropout, the analyses will be repeated within a multiple imputation process, in which the imputation model will include 3-year image information, baseline characteristics and 5-year outcomes. Additionally, an analysis will be undertaken using baseline, 3-year and 5-year information from each participant in mixed effect models that are extensions of the

Table 3

Detectable risk reduction (%) in 3995 participants with gradable images free of late AMD at baseline in cumulative 5-year incidence or progression of AMD.

Outcome	Patient subset at risk (sample size)	5 years follow-up		
		Incidence rate in placebo group (%)	Detectable rate ratio ^b (aspirin rate/placebo rate)	
			Power 80%	Power 90%
Incidence of Early or Intermediate AMD	No AMD at baseline N = 1998 ^a	20	0.76	0.73
Progression from early to Intermediate AMD	Early AMD at baseline N = 1398 ^a	35	0.80	0.77
Progression from early or intermediate to Late AMD	Early/ Intermediate AMD at baseline N = 1998 ^a	4	0.47	0.40

^a Estimated from the sample size calculation.

^b Based on 1:1 allocation of aspirin and placebo, power 80% or 90%, two-sided alpha of 0.05.

log-binomial regression models with the inclusion of a random effect for participant to allow for intra-person correlation in outcome over time.

A 'per protocol' analysis will also be conducted for each outcome using the recorded data on study drug compliance and commencement of open-label aspirin use during follow-up with the aim of estimating complier-averaged causal effects of aspirin. The results of both Intention-To-Treat and "per protocol" analyses will be reported.

3.2.3. Pre-specified sub-group analyses

Subgroup analyses will use interaction terms involving the randomization covariate to examine whether variations in systemic diseases and inflammatory biomarker status influence the effect of aspirin on AMD. Pre-specified subgroup analyses will be undertaken by age and smoking status:

- a) Age below and above study median: The balance of the AMD-related risks and benefits due to aspirin may differ between age groups as a result of different rates of mortality, cardiovascular risk, cognitive decline, other disability and risk of adverse effects.
- b) Smoking: Current versus Never or Former smokers. Smoking is a major well-proven external risk factor for AMD. The effect of aspirin may be different in smokers and non-smokers.

Reporting of aspirin effects will be stratified by any covariate found to hold an interaction with the randomization variable.

We will also look in the future at common genetic variants associated with AMD and determine if the effect of aspirin was influenced by the genotype.

4. Discussion

In addition to its anti-inflammatory, antipyretic and analgesic qualities, aspirin, as a drug proven for the secondary prevention of occlusive cardiovascular events, has become the world's most widely used pharmaceutical drug, particularly by older adults. AMD is the most common cause of vision impairment in people over the age of 50 years in our community and it has profound effects on vision and quality of life. The ASPREE-AMD randomized clinical trial provides a unique opportunity to examine whether long-term low-dose aspirin influences the incidence or progression of AMD.

Aspirin is established for the management of acute cardiovascular diseases (CVD) and their secondary prevention [56–59]. The role of aspirin in primary prevention of CVD is less defined and is currently under further investigation [44,54,60–64]. Nevertheless, it is widely used by older adults, with 20%–50% of older persons in the USA, without cardiovascular disease, being regular users of aspirin [65,66]. The role of low dose aspirin in cancer prevention and management is also under investigation [65,67–70].

A number of studies have been undertaken to establish the association of aspirin with the incidence and progression of AMD, but the results have been inconsistent. Self-reported data from two large randomized controlled trials suggest a beneficial effect of aspirin on AMD [17,18]. Recently, considerable publicity was given to the results of cross-sectional and cohort studies, which reported that aspirin exacerbated the AMD process and contributed to blindness [20–22]. In the latest report from the Blue Mountains Eye Study (BMES), the 15-year cumulative nAMD incidence was 9.3% in aspirin users and 3.7% in nonusers. Users were defined as those who took aspirin (doses were not recorded) once or more per week in the year before the baseline. Adjusted for age, sex, smoking, history of cardiovascular disease, systolic blood pressure, and body mass index, nAMD was associated with regular use of aspirin (OR

2.46; 95% CI, 1.25–4.83). In this study, aspirin use was not updated at follow-up examinations and the systemic survival bias could have affected the results, as 46% participants from the original BMES cohort were not available for the 15-year follow-up. Aspirin users may have had prolonged survival rates allowing them to develop AMD. Competing risks of death were not adjusted for in the BMES study. Thus, the risk/benefit profile of aspirin use with respect to AMD remains unanswered. These issues are important given that aspirin is already the world's most widely used therapeutic agent, being taken regularly by more than 100 million individuals [71].

The principal ASPREE trial will be informative on the use of aspirin in primary prevention of death, physical and cognitive disability in an elderly population, whilst ASPREE-AMD will clarify whether aspirin is efficacious as a primary prevention for AMD and useful in slowing its progression. Additionally, it will allow the question regarding a possible increased risk of retinal hemorrhage associated with nAMD to be addressed. The ASPREE-AMD study differs from other studies in that it is a randomized controlled trial, with photo-documented detailed AMD assessment and a large sample size in the at-risk, relatively healthy, participants aged 70 years or older. The strong detailed records of the exposure data, which include checking the routine of pill taking at every 3-monthly phone calls and counting untaken pills in the returned containers, add weight to the study merit.

As part of ASPREE, other health outcomes will be captured, which can be used when interpreting the ASPREE-AMD results.

A weakness of this study is the reliance upon only non-stereoscopic colour fundus photography to diagnose AMD, without the added benefit of multimodal imaging, such as optical coherence tomography, auto-fluorescence and infrared imaging that would allow for more thorough phenotyping. In particular, our ability to detect reticular pseudo-drusen, a risk factor for progression to late AMD, is limited.[72–74] However, the randomization should ensure equal distribution of this limitation across both groups. Another weakness of reliance upon colour imaging is that nAMD may not be detectable on colour images due to the use of anti-VEGF treatment, which can lead to underestimation of the number of nAMD cases. This will be mitigated by including information collected through registered adverse events in ASPREE, validated through medical records, and also from linking the ASPREE-AMD data to Australian Federal Government Medicare electronic records on the use of the anti-VEGF intravitreal injections.

5. Conclusion

The results of the study are likely to be of substantial clinical significance and public health importance due to a potential for the ASPREE-AMD sub-study to identify a possible low cost therapy for the prevention or slowing progression of AMD worldwide. The large size of the trial will also provide information that may indicate sub-groups of people who could benefit more, or less, than the overall population. Similarly, the trial will establish how the presence of various common co-morbidities influences the risk/benefit of aspirin.

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References

- [1] WHO International Council of Ophthalmology. Causes of blindness and visual impairment. Priority eye diseases: Age-related macular degeneration 2016 [cited 07 Nov 2016]. Available from: <http://www.who.int/blindness/causes/priority/en/index7.html>.
- [2] D. Pascolini, S.P. Mariotti, Global estimates of visual impairment: 2010, *BJO 96* (5) (2012) 614–618.
- [3] K. Attebo, P. Mitchell, W. Smith, Visual acuity and the causes of visual loss in Australia. The Blue Mountains Eye Study, *Ophthalmology* 103 (1996) 357–364.
- [4] L.M. Weih, M.R. VanNewkirk, C.A. McCarty, H.R. Taylor, Age-specific causes of bilateral visual impairment, *Arch. Ophthalmol.* 118 (2000) 264–269.
- [5] Australian Bureau of Statistics, 3201.0 - Population by Age and Sex, Australian States and Territories, 2010.
- [6] Access Economics, Centrally Focussed "The Impact of Age-related Macular Degeneration" – a Dynamic Economic Model and Report, Eye Research Australia, Melbourne, 2006.
- [7] Deloitte Access Economics, Eyes on the Future. A Clear Outlook on Age-related Macular Degeneration, Macular Degeneration Foundation, 2011.
- [8] Age-Related Eye Disease Study Research Group, A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8, *Arch. Ophthalmol.* 119 (10) (2001) 1417–1436.
- [9] C.J. Chiu, R.C. Milton, R. Klein, G. Gensler, A. Taylor, Dietary carbohydrate and the progression of age-related macular degeneration: a prospective study from the age-related eye disease study, *Am. J. Clin. Nutr.* 86 (4) (2007) 1210–1218.
- [10] Age-Related Eye Disease Study 2 Research Group, Lutein + zeaxanthin and omega-3 fatty acids for age-related macular degeneration: the Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial, *JAMA* 309 (19) (2013) 2005–2015.
- [11] K.J. Meyers, Z. Liu, A.E. Millen, S.K. Iyengar, B.A. Blodi, E. Johnson, et al., Joint associations of diet, lifestyle, and genes with age-related macular degeneration, *Ophthalmology* 122 (11) (2015) 2286–2294.
- [12] D.H. Anderson, R.F. Mullins, G.S. Hageman, L.V. Johnson, A role for local inflammation in the formation of drusen in the aging eye, *Am. J. Ophthalmol.* 134 (3) (2002) 411–431.
- [13] J.M. Seddon, S. George, B. Rosner, N. Rifai, Progression of age-related macular degeneration: prospective assessment of C-reactive protein, interleukin 6, and other cardiovascular biomarkers, *Arch. Ophthalmol.* 123 (6) (2005) 774–782.
- [14] D.H. Anderson, M.J. Radeke, N.B. Gallo, E.A. Chapin, P.T. Johnson, C.R. Curletti, et al., The pivotal role of the complement system in aging and age-related macular degeneration: hypothesis re-visited, *Prog. Retin. Eye Res.* 29 (2010) 95–112.
- [15] M. Laine, H. Jarva, S. Seitsonen, K. Haapasalo, M.J. Lehtinen, N. Lindeman, et al., Y402H polymorphism of complement factor H affects binding affinity to C-reactive protein, *J. Immunol.* 178 (6) (2007) 3831–3836.
- [16] L. Robman, P.N. Baird, P.N. Dimitrov, A.J. Richardson, R.H. Guymer, C-reactive protein levels and complement factor H polymorphism interaction in age-related macular degeneration and its progression, *Ophthalmology* 117 (10) (2010) 1982–1988.
- [17] W.G. Christen, R.J. Glynn, U.A. Ajani, D.A. Schaumberg, E.Y. Chew, J.E. Buring, et al., Age-related maculopathy in a randomized trial of low-dose aspirin among US physicians, *Arch. Ophthalmol.* 119 (8) (2001) 1143–1149.
- [18] W.G. Christen, R.J. Glynn, E.Y. Chew, J.E. Buring, Low-dose aspirin and medical record-confirmed age-related macular degeneration in a randomized trial of women, *Ophthalmology* 116 (12) (2009) 2386–2392.
- [19] F. el Baba, W.H. Jarrett 2nd, T.S. Harbin Jr., S.L. Fine, R.G. Michels, A.P. Schachat, et al., Massive hemorrhage complicating age-related macular degeneration. Clinicopathologic correlation and role of anticoagulants, *Ophthalmology* 93 (12) (1986) 1581–1592.
- [20] P.T.V.M. de Jong, U. Chakravarthy, M. Rahu, J. Seland, G. Soubrane, F. Topouzis, et al., Associations between aspirin use and aging macula disorder: the European Eye Study, *Ophthalmology* 119 (2012) 112–118.
- [21] B.E. Klein, K.P. Howard, R.E. Gangnon, J.O. Dreyer, K.E. Lee, R. Klein, Long-term use of aspirin and age-related macular degeneration, *JAMA* 308 (23) (2012) 2469–2478.
- [22] G. Liew, P. Mitchell, T.Y. Wong, E. Rochtchina, J.J. Wang, The association of aspirin use with age-related macular degeneration, *JAMA Intern. Med.* 173 (4) (2013) 258–264.
- [23] L. Li, W. Li, C.Z. Chen, Z.H. Yi, Y.Y. Zhou, Is aspirin use associated with age-related macular degeneration? A meta-analysis, *J. Clin. Pharm. Ther.* 40 (2) (2015) 144–154.
- [24] D.F. Kiernan, S.M. Hariprasad, I.M. Rusu, S.V. Mehta, W.F. Mieler, R.D. Jager, Epidemiology of the association between anticoagulants and intraocular hemorrhage in patients with neovascular age-related macular degeneration, *Retina* 30 (10) (2010) 1573–1578.
- [25] M.A. Tilanus, W. Vaandrager, M.H. Cuyppers, A.M. Verbeek, C.B. Hoyng, Relationship between anticoagulant medication and massive intraocular hemorrhage in age-related macular degeneration, *Graefes Arch. Clin. Exp. Ophthalmol.* 238 (6) (2000) 482–485.
- [26] K. Jonczyk-Skorcka, D. Sliwczynska-Rodziewicz, A. Jarmak, J. Kowalski, Does acetylsalicylic acid and vitamin K antagonists are risk factors of macular degeneration related with age? *Pol. Merkur. Lek.* 38 (225) (2015) 144–149.
- [27] M.M. DeAngelis, A.M. Lane, C.P. Shah, J. Ott, T.P. Dryja, J.W. Miller, Extremely discordant sib-pair study design to determine risk factors for neovascular age-related macular degeneration, *Arch. Ophthalmol.* 122 (4) (2004) 575–580.
- [28] N. Cheung, W.T. Tay, G.C. Cheung, J.J. Wang, P. Mitchell, T.Y. Wong, Is aspirin intake associated with early age-related macular degeneration? The Singapore Indian Eye Study, *BJO 97* (6) (2013) 785–788.
- [29] A.R. Rudnicka, P.K. MacCallum, R. Whitlocke, T.W. Meade, Circulating markers of arterial thrombosis and late-stage age-related macular degeneration: a case-control study, *Eye* 24 (7) (2010) 1199–1206.
- [30] G. Ying, M. Maguire, E. Daniel, J. Grunwald, O. Ahmed, D. Martin, et al., Association between antiplatelet or anticoagulant drugs and retinal or subretinal hemorrhage in the comparison of age-related macular degeneration treatment trials, *Ophthalmology* 123 (2) (2016) 352–360.
- [31] H. Sen, E.Y. Chew, E. Agron, G.F. Reed, J. SanGiovanni, F.L. Ferris, Systemic NSAids and age-related macular degeneration: The Age-Related Eye Disease Study (AREDS), *Invest. Ophthalmol. Vis. Sci.* 48 (5) (2007) 2166.
- [32] M. Aronow, M. Klein, T.E. Clemons, F.L. Ferris, E. Agron, B.A. Blodi, et al., Effect of aspirin use on progression of age-related macular degeneration in the Age-Related Eye Disease Study 2 (AREDS2) participants, *Invest. Ophthalmol. Vis. Sci.* 55 (13) (2014) 2993.
- [33] H.L. Wilson, D.M. Schwartz, H.R. Bhatt, C.E. McCulloch, J.L. Duncan, Statin and aspirin therapy are associated with decreased rates of choroidal neovascularization among patients with age-related macular degeneration, *Am. J. Ophthalmol.* 137 (4) (2004) 615–624.
- [34] E.Y. Chew, T.E. Clemons, G. Gensler, Age-Related Eye Disease Study 2 (AREDS2) ResearchGroup, The association of aspirin use with age-related macular degeneration in AREDS2, *Invest. Ophthalmol. Vis. Sci.* 53 (14) (2012) 1325.
- [35] W. Zhu, Y. Wu, D. Xu, Y.H. Li, B. Jun, X.L. Zhang, et al., Aspirin use and risk of age-related macular degeneration: a meta-analysis, *PLoS One* 8 (3) (2013) e58821.
- [36] Y. Wu, W. Zhu, Y.H. Li, J. Yu, Aspirin and age related macular degeneration; the possible relationship, *Med. Hypothesis, Discov. Innov. Ophthalmol.* 2 (3) (2013) 59–68.
- [37] L. Sobrin, J.M. Seddon, Regular aspirin use and risk of age-related macular degeneration, *Am. J. Ophthalmol.* 156 (2) (2013) 213–217.
- [38] W.G. Christen, E. Chew, Long-term use of aspirin and age-related macular degeneration, *US Ophthalmic Rev.* 6 (2) (2013) 144–147.
- [39] E.W. Chong, R.H. Guymer, L.D. Robman, Does aspirin increase the risk of age-related macular degeneration? *Expert Opin. Drug Saf.* 13 (6) (2014) 691–693.
- [40] J. Ye, Y.F. Xu, J.J. He, L.X. Lou, Association between aspirin use and age-related macular degeneration: a meta-analysis, *Invest. Ophthalmol. Vis. Sci.* 55 (4) (2014) 2687–2696.
- [41] S.K. Kahawita, R.J. Casson, Aspirin use and early age-related macular degeneration: a meta-analysis, *Can. J. Ophthalmol.* 49 (1) (2014) 35–39.
- [42] W.G. Christen, E.Y. Chew, Does long-term aspirin use increase the risk of neovascular age-related macular degeneration? *Expert Opin. Drug Saf.* 13 (4) (2014) 421–429.
- [43] J.M. Guirguis-Blake, C.V. Evans, C.A. Senger, M.G. Rowland, E.A. O'Connor, E.P. Whitlock, Aspirin for the Primary Prevention of Cardiovascular Events: a Systematic Evidence Review for the U.S. Preventive Services Task Force, Evidence Syntheses, No. 131, Report No.: 13-05195-EF-1, Agency for Healthcare Research and Quality (US), Rockville (MD), 2015.
- [44] ASPREE Investigator Group, Study design of ASPirin in Reducing Events in the Elderly (ASPREE): a randomized, controlled trial, *Contemp. Clin. Trials* 36 (2) (2013) 555–564.
- [45] C.M. Reid, E. Storey, T.Y. Wong, R. Woods, A. Tonkin, J.J. Wang, et al., Aspirin for the prevention of cognitive decline in the elderly: rationale and design of a neuro-vascular imaging study (ENVIS-ion), *BMC Neurol.* 12 (2012) 3.
- [46] S. Katz, C.A. Akpom, A measure of primary sociobiological functions, *Int. J. Health Serv. Plan. Adm. Eval.* 6 (3) (1976) 493–508.
- [47] K.Z. Aung, L. Robman, E.W. Chong, D.R. English, G.G. Giles, R.H. Guymer, Non-

- mydriatic digital macular photography: how good is the second eye photograph? *Ophthalmic epidemiol.* 16 (4) (2009) 254–261.
- [48] R. Klein, S.M. Meuer, S.E. Moss, B.E. Klein, Detection of drusen and early signs of age-related maculopathy using a nonmydriatic camera and a standard fundus camera, *Ophthalmology* 99 (1992) 1686–1692.
- [49] A. Muller, H.T. Vu, J.G. Ferraro, J.E. Keeffe, H.R. Taylor, Rapid and cost-effective method to assess vision disorders in a population. *Clin. Exp. Ophthalmol.* 34 (6) (2006) 521–525.
- [50] F.L. Ferris 3rd, C.P. Wilkinson, A. Bird, U. Chakravarthy, E. Chew, K. Csaky, et al., Clinical classification of age-related macular degeneration, *Ophthalmology* 120 (4) (2013) 844–851.
- [51] C.A. Augood, J.R. Vingerling, P.T. de Jong, U. Chakravarthy, J. Seland, G. Soubrane, et al., Prevalence of age-related maculopathy in older Europeans: the European Eye Study (EUREYE), *Arch. Ophthalmol.* 124 (4) (2006) 529–535.
- [52] B.N. Mukesh, P.N. Dimitrov, S. Leikin, J.J. Wang, P. Mitchell, C.A. McCarty, et al., Five-year incidence of age-related maculopathy: the Visual Impairment Project, *Ophthalmology* 111 (6) (2004) 1176–1182.
- [53] J.J. Wang, S. Foran, W. Smith, P. Mitchell, Risk of age-related macular degeneration in eyes with macular drusen or hyperpigmentation: the Blue Mountains Eye Study cohort, *Arch. Ophthalmol.* 121 (5) (2003) 658–663.
- [54] Antithrombotic Trialists Collaboration, C. Baigent, L. Blackwell, R. Collins, J. Emberson, J. Godwin, et al., Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials, *Lancet* 373 (9678) (2009) 1849–1860.
- [55] E.J. Tchetgen Tchetgen, K. Phiri, R. Shapiro, A simple regression-based approach to account for survival bias in birth outcomes research, *Epidemiology* 26 (4) (2015) 473–480.
- [56] Z.M. Chen, P. Sandercock, H.C. Pan, C. Counsell, R. Collins, L.S. Liu, et al., Indications for early aspirin use in acute ischemic stroke: a combined analysis of 40 000 randomized patients from the Chinese acute stroke trial and the international stroke trial. On behalf of the CAST and IST collaborative groups, *Stroke A J. Cereb. Circ.* 31 (6) (2000) 1240–1249.
- [57] Antithrombotic Trialists Collaboration, Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients, *BMJ* 324 (7329) (2002) 71–86.
- [58] J. Hung, for the Medical Issues Committee of the National Heart Foundation of Australia. Aspirin for primary prevention, *Med. J. Aust.* 179 (3) (2003) 147–152.
- [59] L. Robertson, M.A. Ghouri, F. Kovacs, Antiplatelet and anticoagulant drugs for prevention of restenosis/reocclusion following peripheral endovascular treatment, *Cochrane Database Syst. Rev.* 8 (2012) CD002071.
- [60] P.M. Ridker, M. Cushman, M.J. Stampfer, R.P. Tracy, C.H. Hennekens, Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men, *NEJM* 336 (14) (1997) 973–979.
- [61] M. Hayden, M. Pignone, C. Phillips, C. Mulrow, Aspirin for the primary prevention of cardiovascular events: a summary of the evidence for the U.S. Preventive Services Task Force, *Ann. Intern. Med.* 136 (2) (2002) 161–172.
- [62] P.S. Sanmuganathan, P. Ghahramani, P.R. Jackson, E.J. Wallis, L.E. Ramsay, Aspirin for primary prevention of coronary heart disease: safety and absolute benefit related to coronary risk derived from meta-analysis of randomised trials, *Heart* 85 (3) (2001) 265–271.
- [63] P.M. Ridker, N.R. Cook, I.M. Lee, D. Gordon, J.M. Gaziano, J.E. Manson, et al., A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women, *NEJM* 352 (13) (2005) 1293–1304.
- [64] G. De Berardis, M. Sacco, G.F. Strippoli, F. Pellegrini, G. Graziano, G. Tognoni, et al., Aspirin for primary prevention of cardiovascular events in people with diabetes: meta-analysis of randomised controlled trials, *BMJ* 339 (2009) b4531.
- [65] C.D. Williams, A.T. Chan, M.R. Elman, A.H. Kristensen, W.F. Miser, M.P. Pignone, et al., Aspirin use among adults in the U.S.: results of a national survey, *Am. J. Prev. Med.* 48 (5) (2015) 501–508.
- [66] J.J. VanWormer, A.W. Miller, S.H. Rezkalla, Aspirin overutilization for the primary prevention of cardiovascular disease, *Clin. Epidemiol.* 6 (2014) 433–440.
- [67] S. Chell, A. Kaidi, A.C. Williams, C. Paraskeva, Mediators of PGE2 synthesis and signalling downstream of COX-2 represent potential targets for the prevention/treatment of colorectal cancer, *Biochim. Biophys. Acta* 1766 (1) (2006) 104–119.
- [68] A.C. Vidal, S.J. Freedland, Aspirin and prostate cancer prevention, *Aging (Albany NY)* 7 (5) (2015) 292–293.
- [69] P.C. Elwood, G. Morgan, J.E. Pickering, J. Galante, A.L. Weightman, D. Morris, et al., Aspirin in the treatment of cancer: reductions in metastatic spread and in mortality: a systematic review and meta-analyses of published studies, *PLoS One* 11 (4) (2016) e0152402.
- [70] E. Whitlock, S. Williams, B. Burda, A. Feightner, T. Beil. Aspirin Use in Adults: Cancer, All-cause Mortality, and Harms. A Systematic Evidence Review for the U.S. Preventive Services Task Force. Rockville (MD): Agency for Healthcare Research and Quality (US). (Evidence Syntheses, No. 132. Report No.: 13-05193-EF-1.) (2015 Sep.) Available from: <https://www.ncbi.nlm.nih.gov/books/NBK321643/> (Accessed 6 March 2017).
- [71] R. Roy, The impact of synthetic chemistry on human health. Chapter 5, in: J. García-Martínez, E. Serrano-Torregrosa (Eds.), *The Chemical Element: Chemistry's Contribution to Our Global Future*, WILEY-VCH, 2011.
- [72] R.P. Finger, E. Chong, M.B. McGuinness, L.D. Robman, K.Z. Aung, G. Giles, et al., Reticular pseudodrusen and their association with age-related macular degeneration: the Melbourne Collaborative Cohort Study, *Ophthalmology* 123 (3) (2016) 599–608.
- [73] M.J. van Grinsven, G.H. Buitendijk, C. Brussee, B. van Ginneken, C.B. Hoyng, T. Theelen, et al., Automatic identification of reticular pseudodrusen using multimodal retinal image analysis, *Invest. Ophthalmol. Vis. Sci.* 56 (1) (2015) 633–639.
- [74] Z. Wu, L.N. Ayton, C.D. Luu, P.N. Baird, R.H. Guymer, Reticular pseudodrusen in intermediate age-related macular degeneration: prevalence, detection, clinical, environmental, and genetic associations, *Invest. Ophthalmol. Vis. Sci.* 57 (3) (2016) 1310–1316.