Age-related Macular Degeneration in Older Populations: Long-Term Incidence, Progression and Associations

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PREFACE

This thesis describes the candidate's 2.5 year full-time (2011-2013) and 2.5 years part-time (2013-2015) work on age-related macular degeneration (AMD) incidence and progression, based on the Blue Mountains Eye Study (BMES) cohort data.

The BMES is a population based cohort study of vision and eye disease in an older Australian population residing in the Blue Mountains region, west of Sydney, Australia. The baseline examination was conducted from 1992 to 1994 (BMES I). The 5-, 10- and 15-year follow-up examinations were conducted during 1997-1999 (BMES II), 2002-2004 (BMES III) and 2007-2010 (BMES IV), respectively. Professor Jie Jin Wang (candidate's supervisor) was an associate investigator for BMES II and joint chief investigator for BMES III and IV. Professor Paul Mitchell (candidate's co-supervisor) was the principal investigator for BMES I, II, III and IV.

The findings presented in this thesis are primarily drawn from the Blue Mountains Eye Study (BMES) cohort. One comparative study included data from the BMES and Singapore Epidemiology of Eye Diseases (SEED) Study. A pooled data analysis included three population-based cohorts: the BMES, the Beaver Dam Eye Study (BDES) and the Rotterdam Study (RS), as a project in the Three Continent AMD Consortium (3CC).

This thesis examines the long-term incidence, progression and associated risk factors of age-related macular degeneration (AMD) and its component lesions including geographic atrophy, reticular drusen and medium drusen over 15 years in the BMES cohort, in Chapter 3. Comparison of two AMD classification scales, the Age-Related Eye Diseases Study Simplified Severity Scale and the newly developed Basic Clinical Classification Scale using

BMES data is shown in Chapter 4. The frequency and patterns of early AMD lesion distribution in Singaporean Asians compared to white Australians are documented in Chapter 5, and the progression from unilateral to bilateral AMD is examined in three Caucasian populations in Chapter 6.

ABSTRACT

Purpose

To assess the long-term (>10-year) incidence and progression of age-related macular degeneration (AMD) and associated risk factors in an older Australian population cohort, and conduct pooled data analysis of three Caucasian populations to assess progression from unilateral to bilateral AMD. In addition, to compare the pattern of early AMD lesion distribution between Singaporean Asian and Australian Caucasian samples.

Methods

The Blue Mountains Eye Study (BMES) is a population-based cohort study of persons aged 49 years and older, residing in the Blue Mountains region, west of Sydney, Australia. Baseline examinations were conducted from 1992 to 1994 and recruited 3654 participants (82.4% of those eligible). Surviving participants were invited to attend each follow-up examination thereafter. Of the surviving baseline participants, 2334 (75.8% of survivors), 1952 (76.7% of survivors) and 1149 (56.1% of survivors) participants attended the 5-year (1997-1999), 10-year (2002-2004) and 15-year (2007-2009) follow-up examinations respectively.

The SEED Study includes three population-based cohorts of Malays, Indians and Chinese aged 40-80 years old residing in Singapore. The baseline examinations were conducted consecutively between 2004 and 2011 starting with the Malay population, and recruited 3280 Malays (78.7 of those eligible), 3400 Indians (75.6% of those eligible) and 3353 Chinese (72.8% of those eligible).

The BDES recruited 4926 participants (83.2% of those eligible) aged 43-86 years old at the baseline examinations between 1988 and 1990. Of these, 3684, 2764, 2119 and 1913 participants attended the 5-year, 10-year, 15-year and 20-year examinations respectively. The RS recruited 7983 participants (77.7% participation rate) aged 55 years and older at the baseline examination (1990-1993), of which 6419 had retinal photography performed. Of these participants, 3637 were re-examined from 1997-1999 (6-year follow-up) and 2674 were re-examined from 2002-2004 (11-year follow-up).). The BMES, BDES and RS have been collaborating in the two decades, leading to the establishment of the 3CC in recent decade.

The presence of AMD lesions and the size and location of individual AMD lesions were assessed from colour retinal fundus photographs at each examination, in each study. The Wisconsin Age-Related Maculopathy Grading System was the primary grading protocol used in assessing AMD and AMD lesions. Side-by-side grading of baseline and follow-up retinal photographs was performed to confirm incident AMD and AMD lesions.

Results

Long-term incidence of AMD and AMD lesions in the BMES

The 15-year incidence before and after adjusting for competing risks were 22.7% and 15.1% for early AMD and 6.8% and 4.1% for late AMD, respectively. Age and risk alleles of *complement factor H* (*CFH-rs1061170*) or a*ge-related maculopathy susceptibility 2* (*ARMS2-rs10490924*) were independently associated with early AMD incidence. Current smoking and ≥1 risk allele of *CFH-rs1061170* or *ARMS2-rs10490924* were associated with late AMD incidence. At least one serving of fish per week was associated with 50% reduced incidence of late AMD. Severity of early AMD lesion characteristics was a strong predictor of progression to late AMD.

After excluding participants with GA or neovascular AMD at baseline, the 15-year incidence of GA was 3.6%. Current smoking and genetic risk from of *CFH-rs1061170* or *ARMS2-rs10490924* were independently associated with 15-year incident GA. Baseline early AMD lesion characteristics (drusen type, location closer to the fovea, larger drusen area and presence of retinal pigment epithelium (RPE) abnormalities) were strong predictors of 15-year incident GA. Fast progression of GA, defined as GA lesion growth of $\geq 2mm^2$/year, was more frequently observed among baseline current smokers, persons with the *CFH* or *ARMS2* risk genotypes and in pseudophakic eyes.

The 15-year cumulative incidence of reticular drusen was 4.0% after controlling for the competing risk of death. Increasing age, female sex, current smoking and risk alleles of *CFH-rs1061170* or *ARMS2-rs10490924* were independently associated with incident reticular drusen. A substantial proportion (33.9%) of eyes with reticular drusen progressed to late AMD in 5 years, and it was 4-fold progression to late AMD of the rate from eyes with other early AMD lesions but without reticular drusen. A significantly low risk of progression from reticular drusen to late AMD was found among those with increased dietary intake of lutein-zeaxanthin. There was no significant association between other AMD risk factors and progression from reticular drusen to late AMD.

The 15-year cumulative incidence of medium drusen (defined as drusen with a diameter ≥63μm and <125μm) was 13.9% after controlling for the competing risk of death. Increasing age and the presence of ≥3 risk alleles of the combined *CFH-rs1061170* and *ARMS2-rs10490924* genes were associated with an increased incidence. There was no association between past or current smoking and the development of medium drusen. The proportion of

eyes that progressed from medium drusen to late AMD was 5.0% over 15years, however, when medium drusen was co-present with RPE abnormalities, the progression rate to late AMD increased to 23.0%. Greater total area involved by, and central location of, medium drusen were associated with a greater likelihood of progression to worse stages of AMD.

Comparison of Two Classification Scales for AMD

The Age-Related Eye Disease Study (AREDS) Simplified Severity Scale calculates a risk score based on the number of early AMD risk factors (large drusen and pigment abnormalities) in both eyes, while the Basic Clinical Classification Scale that defines large drusen and pigment abnormalities as 'intermediate AMD' regardless of bilaterality and is based on the worse eye. The AREDS Scale classified similar proportions of participants who developed incident late AMD in 5 year across all AMD risk levels between population-based BMES and clinic-based AREDS cohorts. In comparison, the Basic Clinical Classification Scale consistently classified 50% to 75% lower late AMD incident rates across all risk levels in the BMES compared to the AREDS cohorts.

Comparison of Frequencies of Early AMD Lesions between Caucasians and Asians

After age-standardization to the BMES population, the prevalence of distinct soft drusen was significantly higher in Singaporean Asians compared to Australians (23.9%, 95% CI 22.9-25.0 versus 6.2%, 95% CI 5.3-7.0); adjusted OR 4.6 (95% CI 3.4-6.0). In contrast, the prevalence of indistinct soft or reticular drusen was non-significantly lower in Singaporeans compared to Australians (6.5%, 95% CI 5.9-7.1 versus 8.3%, 95% CI 7.4-9.3); adjusted OR 1.2, (95% CI 0.8-1.7). Soft drusen of any type were frequently present at the inner and outer macula (within a zone ≥500μm to <3000μm radius from the foveal centre) among

Singaporeans, while among Australians soft drusen were more frequently present at the central macula (<500μm radius).

Progression from Unilateral to Bilateral AMD in Three Caucasian Populations

In pooled data analysis of the BMES, BDES and RS cohorts, the 5-year progression rates from unilateral to bilateral any (early or late) AMD and bilateral late AMD ranged from 19% to 28% and 27% to 68%, respectively. Increasing age, the presence of 1 or ≥2 risk alleles from the *CFH* and *ARMS2* genes, past and current smoking status were significantly associated with increased risk of progression from unilateral to bilateral any AMD, after multivariable adjustment. The presence of ≥2 risk alleles from the *CFH* and *ARMS2* genes combined was significantly associated with increased progression from unilateral to bilateral late AMD.

Conclusions

Age and genetic risk from the *CFH* and *ARMS2* were associated with increased long-term incidence of early and late AMD, GA, reticular drusen, medium drusen, and also associated with an increased risk of progression from unilateral any AMD to bilateral within 5 years. Large macular area involved by, central location and co-presence of early AMD lesions are indicators of high risk of progression to late AMD. Of the modifiable risk factors, smoking is an important risk factor associated with the progression from early to late AMD, while weekly fish consumption and high dietary intake of lutein-zeaxanthin are associated with a reduced risk of late AMD. The knowledge of these modifiable factors should be advocated by physicians to patients with early AMD or with high risk of AMD. With Australia's ageing population, it is important to prevent adverse consequences of AMD by promoting healthy lifestyle behaviours to minimize the economic burden of AMD. Understanding mechanisms

explaining the differences in AMD presentation severities between Asians and Caucasians may help to reduce the burden of AMD among Caucasians.

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First and foremost, I sincerely thank my primary supervisor Professor Jie Jin Wang for her unwavering guidance, support and patience, for the many generous hours spent reviewing and improving my manuscripts and for the invaluable advice provided me in all aspects of this thesis. Her passion for research, intellectual rigor and discipline has been a huge inspiration for me throughout, and will continue to be in the future. I also sincerely thank my co-supervisor Professor Paul Mitchell for the opportunity to come back and work on the Blue Mountains Eye Study data and for his guidance and support. His research vigour, clarity of thought and countless research achievements have also been a great inspiration.

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PUBLICATIONS RELATING TO THIS THESIS

1. **Joachim N**, Mitchell P, Kifley A, Rochtchina E, Hong T, Wang JJ. Incidence and Progression of Geographic Atrophy: Observations from a Population-based Cohort. *Ophthalmology* 2013; 120: 2042-2050

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3. **Joachim N**, Mitchell P, Younan C, Burlutsky G, Cheng CY, Cheung CMG, Zheng Y, Moffitt M, Wong TY, Wang JJ. Ethnic variation in early age-related macular degeneration lesions between White Australians and Singaporean Asians. *Investigative Ophthalmology and Visual Science* 2014; 55 (7): 4421-4429

4. **Joachim N**, Mitchell P, Kifley A, Wang JJ. Incidence, Progression and Associated Risk Factors of Medium Drusen in Age-related Macular Degeneration: Findings from an Older Australian Cohort. *JAMA Ophthalmology* 2015; 133(6): 698-705

5. **Joachim N**, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. *Ophthalmology* 2015; 122 (12): 2482-2489

6. **Joachim N**, Colijn JM, Kifley A, Lee KE, Buitendijk GHS, Klein BEK, Myers C, Meuer SM, Tan AG, Holliday EG, Attia J, Liew G, Iyengar SK, de Jong PTVM,

Hofman A, Vingerling JR, *Mitchell P, *Klaver CCW, *Klein R, *Wang JJ. Five-year Progression from Unilateral to Bilateral Age-related Macular Degeneration: The Three Continent AMD Consortium Report. Submitted to *American Journal of Ophthalmology* (May 2016) (*co-senior authors)

7. Liew G, **Joachim N**, Burlutsky G, Mitchell P, Wang JJ. Validating the AREDS Simplified Severity Scale of Age-related Macular Degeneration with 5- and 10-Year Incident Data in Population Based Sample. Article in press: *Ophthalmology* (May 2016)

PRESENTATIONS RELATING TO THIS THESIS

Oral Presentations:

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2. **Joachim ND**, Mitchell P, Rochtchina E, Tan AG, Wang JJ. The Incidence and Progression of Reticular Drusen: Findings from an Older Australian Cohort. *Investigative Ophthalmology and Visual Science* (Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting 2013), 2012 54:367.
3. **Joachim ND**, Mitchell P, Rochtchina E, Tan AG, Wang JJ. Incidence and Progression of Reticular Drusen: Findings from the Blue Mountains Eye Study Cohort. The Westmead Association Hospital Week, 2013, Westmead Hospital, Sydney. Abstract 18.
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2. Wang JJ, **Joachim N**, Kifley A, Mitchell P. Long-term Incidence and Progression of Intermediate Drusen in Age-related Macular Degeneration. *Investigative Ophthalmology and Visual Science* (Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting 2015; presented by Wang JJ), 2015 56:3793.
3. Liew G, **Joachim N**, Burlutsky G, Mitchell P, Wang JJ. Validating Two Age-Related Macular Degeneration Classification Scales in a Population-Based Cohort. *Investigative Ophthalmology and Visual Science* (Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting 2015; presented by Liew G), 2015 56:3795.
4. **Joachim N**, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The 15-year Incidence and Progression of Age-Related Macular Degeneration: Findings from the Blue Mountains Eye Study. The Westmead Association Hospital Week, 2015, Sydney. Abstract 67.

CANDIDATE'S CONTRIBUTION

With the help of Professor Jie Jin Wang and guidance from Professor Paul Mitchell, the candidate developed the scientific questions and wrote all first-authored manuscripts included in this thesis.

The candidate developed grading forms and conducted side-by-side grading of the GA and reticular drusen lesions from the four examinations of the BMES after being trained in AMD grading by Professor Jie Jin Wang and Ms Mireille Moffitt. The candidate also performed computer planimetry and analysis of the GA lesions in the BMES. The candidate has additionally collected data for a project that was conducted by the International AMD Gene Consortium, and conducted grading tasks for studies outside the scope of the thesis.

The candidate was awarded scholarships to undertake two statistics subjects at the School of Public Health, The University of Sydney. However, as large data sets (the BMES, SEED, BDES and RS) were used in this thesis, all major analyses were conducted by statisticians, with review performed by the candidate. The candidate performed statistical analyses for a smaller data set which was outside the scope of this thesis.

The candidate also performed interviews and was trained and conducted subjective refraction on patients recruited into clinical trials of treatments for AMD.

The candidate gave oral presentations at the Association for Research in Vision and Ophthalmology (ARVO) annual meeting in 2012 and 2013, the Westmead Associations' Hospital Week in 2013 and at the Postgraduate Research Seminars (Westmead Hospital) in 2014. The candidate presented posters at the Westmead Associations' Hospital Week in 2012

and 2015 and contributed substantially to the posters presented at the ARVO annual meeting in 2015, where the candidate was second author.

The candidate drafted all the initial manuscripts and revision responses to journal reviewers for articles where the candidate was first author, and further improved the manuscripts to meet the journal submission standards with assistance from Professor Jie Jin Wang. This included performing literature searches, summarizing findings, reviewing and tabulating results from statistical outputs and creating composite images for the projects described in this thesis. The candidate provided substantial review on the paper in press entitled "Validating the AREDS Simplified Severity Scale of Age-Related Macular Degeneration with 5 and 10-year Incident Data in a Population Based Sample" where the candidate was second author.

ABBREVIATIONS

3CC	Three-Continent AMD Consortium
AMD	age-related macular degeneration
AREDS	Age-Related Eye Diseases Study
ARM	age-related maculopathy
ARMS2	age-related maculopathy susceptibility 2
AUC	area under the curve
BCVA	best-corrected visual acuity
BDES	Beaver Dam Eye Study
BMES	Blue Mountains Eye Study
BMI	body mass index
BP	blood pressure
CFH	complement factor H
CI	confidence interval
CRP	C-reactive protein
DA	disc area
DHA	docohexaenoic acid
EPA	eicosapentaenoic acid
ETDRS	Early Treatment Diabetic Retinopathy Study
FFQ	food frequency questionnaire
GA	geographic atrophy
GEE	generalized estimating equation
GWAS	genome-wide association scan
HDL	high-density lipoprotein
HPFS	Health Professionals Follow-up Study
HR	hazard ratio
IAMDGC	International AMD Gene Consortium
LALES	Los Angeles Latino Eye Study
LZ	lutein-zeaxanthin
MESA	Multi-Ethnic Study of Atherosclerosis
MVIP	Melbourne Visual Impairment Project
NAT 2	Nutritional AMD Treatment 2
NHMRC	National Health and Medical Research Council
NHS	Nurses' Health Study

OR	odds ratio
ROC	receiver operating characteristic
RPE	retinal pigment epithelium
RR	relative risk
RS	Rotterdam Study
SAS	Statistical Analysis System
SCES	Singapore Chinese Eye Study
SD	standard deviation
SEED	Singapore Epidemiology of Eye Diseases
SiMES	Singapore Malay Eye Study
SINDI	Singapore Indian Eye Study
SNP	single nucleotide polymorphism
WARMGS	Wisconsin Age-Related Maculopathy Grading System
WBCC	white blood cell count
WCC	white cell count
ω-3	omega-3
ω-6	omega-6

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Chapter 1

Incidence, Progression and Associated Risk Factors of Age-related Macular Degeneration: A Brief Literature Review

Age related macular degeneration (AMD) is the leading cause of irreversible severe visual impairment and blindness in the Western world, despite advances in treatment in recent decades[1-11]. A 2002 estimate by the World Health Organization revealed that 8.7% of the world's blindness was due to AMD, with 14 million people suffering blindness or severe visual impairment as a result of the disease[2].

1.1 Age-related macular degeneration disease classification and pathogenesis

Age-related macular degeneration is a complex, progressive degenerative disorder of the retina and commonly affects persons aged 50 years and older[1,3,7,12-14].

The sensory retina consists of nine cellular layers that are separated from the underlying choroidal vessels by a layer of retinal pigment epithelium (RPE) and a thin collagenous structure, the Bruch's membrane. Histopathologic findings indicate that AMD originates as deposits of extracellular material within or internal to the Bruch's membrane caused by the failure of the RPE cells to process photoreceptor cell debris[15-17]. These depositions are known as basal linear or basal laminar deposits[5,18-20]. Larger deposits termed drusen lying between the RPE and Bruch's membrane[5,19] are early clinical signs of AMD[20-25]. Other signs of AMD include RPE abnormalities (increased pigmentation in the sensory retina and/or decreased pigmentation of the RPE), geographic atrophy (GA) and varying manifestations of neovascular AMD that are visible on ophthalmoscopic examination and can be documented with retinal fundus photography. These degenerative changes of the retina in the macula and particularly the fovea (central macula) have a severe consequence of destroying central vision.

Clinical classification of AMD

In the past four decades, a number of classification systems have been developed for determining the presence and severity of AMD based on colour fundus photographs[13,26-32].

The Wisconsin Age-Related Maculopathy Grading System (WARMGS) was developed in the early 1990's by Klein and associates[26], which provided comprehensive definitions and a grading protocol that included assessment of lesion size and location within the macular area. The International Classification and Grading System for Age-Related Maculopathy (ARM) and AMD was later developed based on the WARMGS, a collaborative efforts by the leading ophthalmologists in this field around the world, in order to standardize definitions and terminologies for AMD and AMD lesions[13]. The aim was to reduce inconsistencies in definitions used by various studies and thus permit comparisons between studies.

Several AMD classification systems were proposed thereafter, by different researchers, to classify AMD into a scale with steps reflecting severity stages of the disease and inherent risk of progression to Late AMD at each specific step[27,28,30-32]. Examples include the Age-Related Eye Disease Study (AREDS) severity scales[27,28], the Beaver Dam Eye Study (BDES) scale[33], the Rotterdam Study (RS) scale[30], the harmonized Three-Continent AMD Consortium (3CC) scale[32] and the Basic Clinical Classification Scale (Beckman classification)[31]. Of these, the AREDS scale has been used and validated in different population-based cohort studies[34,35]. In the AREDS scale, AMD severity steps were determined by the presence of AMD lesions in one or both eyes, whereas the RS, BDES, 3CC and the Basic Clinical Classification scales stratify AMD severity stages based on lesions in the worse eye (**Table 1.1-1**).

Generally speaking, AMD has two broad disease stages; Early and Late AMD stages. The early stage was previously termed ARM while the late stage was termed AMD. However, the current convention defines both stages as AMD with differentiation imparted using the terms 'Early' and 'Late'. The Basic Clinical Classification Scale, the most recent AMD classification scale, also includes an 'intermediate' AMD stage in addition to early and late stages[31]. In this review and the thesis, only two stages, Early and Late AMD, are used. Early and Late AMD are denoted using initial capitals in this review chapter to facilitate reading.

Table 1.1-1: Summary of Classification Scales for AMD.

Classification Scale	Step or Level	Definition
Age-Related Eye Diseases Study (AREDS) simplified severity scale[28*]	**0**	No retinal pigment changes with none or small hard drusen in 1 or both eyes or intermediate (but not large) drusen in 1 eye only
	1	Pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only; No pigment changes in either eye but intermediate drusen in both eyes or large drusen in one eye
	2	Pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only; Pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye; No pigment changes in either eye but large drusen in both eyes.
	3	Pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye; Pigment changes in 1 eye with large drusen in both eyes.
	4	Pigment changes in both eyes with large drusen in both eyes
Beaver Dam Eye Study (BDES) six-level severity scale[33†]	**10**	Hard drusen or small soft drusen (<125µm in diameter) only, regardless of area of involvement, *and* no pigmentary abnormality present.
	20	Hard drusen or small soft drusen (<125µm in diameter), regardless of area of involvement, with increased retinal pigment present but no RPE depigmentation present; Soft drusen (≥125µm in diameter) with drusen area <196,350µm^2 (equivalent to a circle with a diameter of 500µm) and no pigmentary abnormalities present.
	30	Soft drusen (≥125µm in diameter) with drusen area <196,350µm^2 and RPE depigmentation present; Soft drusen (≥125µm in diameter) with drusen area ≥196,350µm^2 with or without increased retinal pigment but no RPE depigmentation present.
	40	Soft drusen (≥125 in diameter) with drusen area ≥196,350µm^2 involvement and RPE depigmentation present with or without increased retinal pigment.
	50	Pure geographic atrophy in absence of exudative macular degeneration.
	60	Exudative macular degeneration with or without geographic atrophy present.
Rotterdam Study (RS) stages of severity scale[30†]	**No ARM**	No ARM features or only drusen ≤63µm
	1a	Soft distinct drusen
	1b	Pigmentary irregularities
	2a	Soft indistinct or reticular drusen
	2b	Soft distinct drusen with pigmentary irregularities
	3	Soft indistinct or reticular drusen with pigmentary irregularities
	4	Atrophic or neovascular macular degeneration (AMD)
Three Continent AMD Consortium harmonized severity scale[32†]	**10**	***No AMD:*** No, questionable, small, or intermediate sized drusen (<125µm in diameter) only, regardless of area of involvement, and no pigmentary abnormalities (defined as increased retinal pigment or RPE depigmentation present); No definite drusen with any pigmentary abnormality.
	20	***Mild Early AMD:*** Small to intermediate sized drusen (<125µm in diameter), regardless of area of involvement, with any pigmentary abnormality; Large drusen (≥125µm in diameter) with drusen area <331,820µm^2 (equivalent to O-2 circle, defined as a circle with diameter of 650µm) and no pigmentary abnormalities.

	30	***Moderate Early AMD:*** Large drusen (≥125µm in diameter) with drusen area ≥331,820µm^2 and any pigmentary abnormality; Large drusen (≥125µm in diameter) with drusen area ≥331,820µm^2, with or without increased retinal pigment but no RPE depigmentation.
	40	***Severe Early AMD:*** Large drusen (≥125µm in diameter) with drusen area ≥331,820µm^2 and RPE depigmentation present, with or without increased retinal pigment.
	50	***Late AMD:*** Pure geographic atrophy in the absence of exudative macular degeneration; Exudative macular degeneration with or without geographic atrophy present.
Basic Clinical Classification Scale[31†]	***No apparent aging changes:*** No drusen and no AMD pigmentary abnormalities ***Normal aging changes:*** Only drupelets (small drusen ≤63µm) and no AMD pigmentary abnormalities ***Early AMD:*** Medium drusen >63µm and ≤125µm and no AMD pigmentary abnormalities. ***Intermediate AMD:*** Large drusen >125µm and/or any AMD pigmentary abnormalities. ***Late AMD:*** Neovascular AMD and/or any geographic atrophy	

[*]Scale refers to lesion changes in both eyes (person-specific)

[†]Scale refers to lesion changes one eye (eye-specific)

Early AMD

Early AMD is characterized by the presence of drusen and/or RPE abnormalities within the anatomic macular area. The features described forthwith refer to observations from retinal colour fundus photography and not other modes of retinal imaging such as optical coherence tomography. Drusen are identified as discrete white-yellow spots and vary in size and appearance, including small distinct and indistinct hard drusen, intermediate drusen, large distinct and indistinct soft drusen and reticular drusen (**Figure 1.1-1A**). The appearance of drusen edge: sharp or fuzzy, are descriptors for distinct or indistinct type, respectively; while size of drusen, denoted by the longest diameter of a single druse, is the separator for different types of drusen; hard drusen are <63μm, intermediate ≥63μm but <125μm, and soft drusen ≥125μm in diameter[13,26]. Reticular drusen are confluent and appear as ill-defined networks of interlacing ribbons[13]. Hard drusen are considered aging changes and not early AMD lesions.

Retinal pigment epithelium abnormalities include hyperpigmentation and RPE depigmentation (hypopigmentation). Hyperpigmentation refers to the presence of clumps of gray or black pigment granules within the sensory retina. RPE depigmentation refers to a sharply delineated area with apparent absence of the RPE but without visible choroidal vessels, in contrast to the appearance of GA where choroidal vessels are visible[13,26] (**Figure 1.1-1B**).

There are varying definitions of Early AMD used among different studies. In majority of population-based studies[12,14,36,37] including the BDES and BMES, Early AMD is defined as the presence of either (1) large (>125μm diameter) indistinct soft or reticular drusen or (2) the co-presence of distinct soft drusen and RPE abnormalities within the macula in the absence of Late AMD. This definition (termed BDES Early AMD definition in this chapter) does not

include the presence of intermediate drusen alone, distinct soft drusen alone or retinal pigmentary abnormalities alone into the Early AMD stage category. Other studies have defined Early AMD as two or more steps in a severity scale[30,38-40], or have included any drusen ≥63μm in diameter with or without the presence of retinal pigmentary abnormalities[41-43], which lead to variations in estimates of Early AMD prevalence and incidence.

Late AMD

The two late AMD lesions are geographic atrophy (dry form) and neovascular or exudative AMD (wet form)[13,26]. Geographic atrophy refers to a sharply defined area of RPE and sensory retinal disappearance with a diameter ≥175μm, exposing the choroidal vessels (**Figure 1.1-1C**). Neovascular AMD refers to various manifestations of exudative AMD including serous or hemorrhagic RPE/ neurosensory retinal detachment, retinal or subretinal haemhorrages, subretinal fibrosis (disciform scars) (**Figure 1.1 -1D**). Hard exudates may be present due to leakage of new vessels. The presence of photocoagulation scars, indicative of previous laser treatment of neovascularization, is also included in the definition of neovascular AMD (wet AMD)[35,36,44,45].

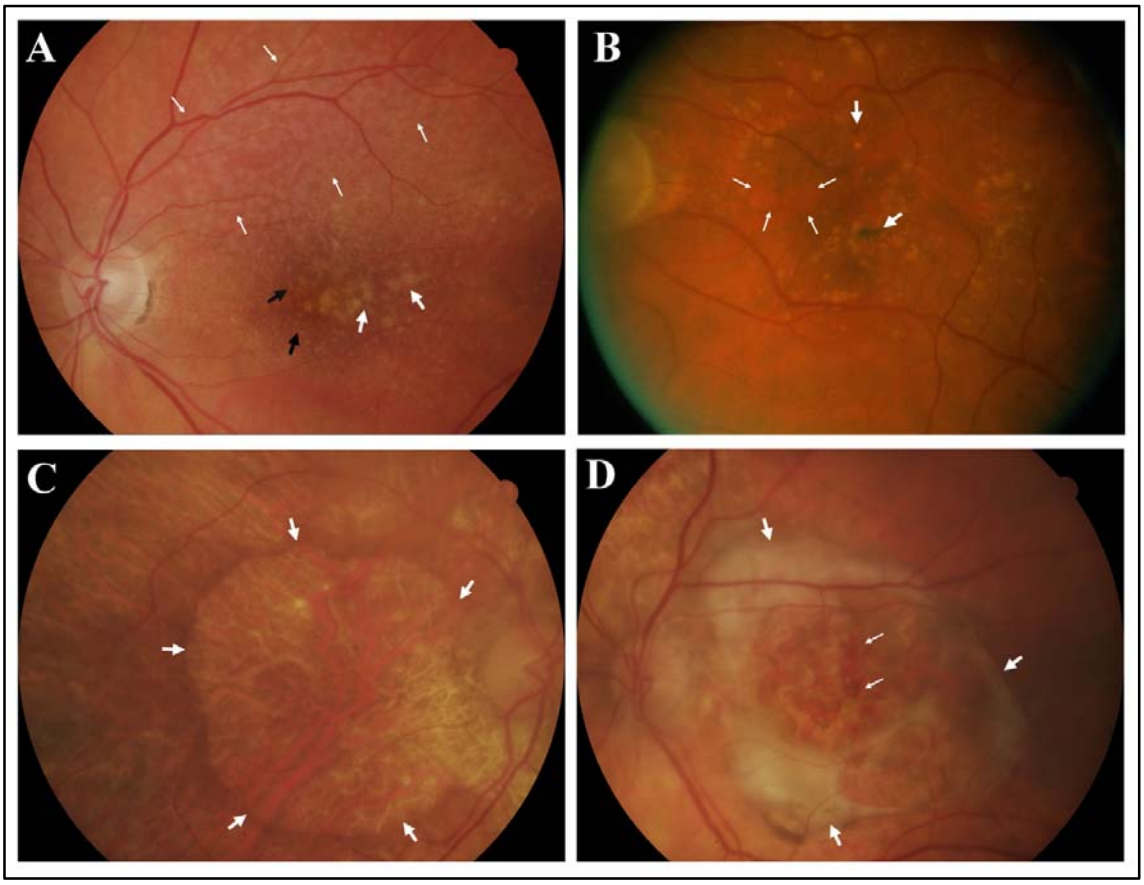

Figure 1.1-1 Early and Late AMD lesions visible on retinal fundus photographs. (A) Example of various drusen types including: reticular drusen present in the upper arcade (thin arrows) and predominant large indistinct soft drusen (thick white arrows), with scattered intermediate drusen (black arrows) in the central macular. (B) Example of hyperpigmentation (thick arrows) and retinal pigment epithelium (RPE) depigmentation (thin arrows). (C) Large area of geographic atrophy involving the entire macular area (wide arrows surrounding GA perimeter). (D) Presence of haemorrhages (thin arrows), sensory retinal detachment and subretinal fibrosis (thick arrows), a scaring stage of neovascular AMD.

1.2 Incidence and progression of age-related macular degeneration

Over the past four decades the prevalence, incidence and progression of AMD have been documented in a number of population-based studies around the world. While reports on AMD prevalence and short-term incidence were common, fewer reports were on the long-term (≥10-year) incidence and progression of AMD.

Relatively prominent population-based studies undertaken in the past 30 years and comprising AMD incident data from older populations are summarized in this chapter.

1.2.1 Methods and Definitions

The assessment of AMD incidence from retinal photographs is straightforward, however, estimation of incidence rates using various statistical methods, such as the Kaplan-Meier life table method, with or without consideration of competing risk of death, resulted in variations in estimates of incident rates.

Incidence of Early AMD is defined as the presence of Early AMD at follow-up where no Early and Late AMD was present at baseline or a previous visit. The incidence of individual AMD lesions is defined similarly. Incidence of Late AMD is defined as the presence of Late AMD at follow-up where no Late AMD was present at baseline or a previous visit but Early AMD might be present. Person-specific incidence refers to the incidence of AMD in the worse or the first eye, that were used in many studies included in this review [29,33-35,38,39,44,46-50], unless otherwise specified. Second eye (the fellow eye of persons with AMD in the first eye) incidence, or the incidence of bilateral AMD, was also investigated in some studies[29,33-35,38,39,45,47].

Various definitions of progression have been used across different studies. The two main approaches to assess progression included: (1) proportions of eyes that had an increased area involved by AMD lesions, such as an increase in the number of subfields involved by the same types of lesions, designated by the WARMGS grid that consists of 3 concentric circles divided into 9 subfields, and demarcates the anatomic macular area[29,33,34,44] (refer to **Figure 1.2.1-1**) and (2) the progression to Late AMD stage from different stages of Early AMD, according to early AMD lesion characteristics (type of drusen, total areas involved by, and location of drusen and retinal pigmentary abnormalities)[33,35]. Progression to Late AMD based on steps of AREDS AMD severity scale[27,28] and BDES severity scale[33,51] have also been reported[33,35]. Regression and disappearance of AMD lesions were assessed in some studies[29,34,48], however, this phenomenon is likely due to the development of more advanced stage lesions on the severity scale of AMD which should not be considered as true regression[4,52,53], and is not described in this review.

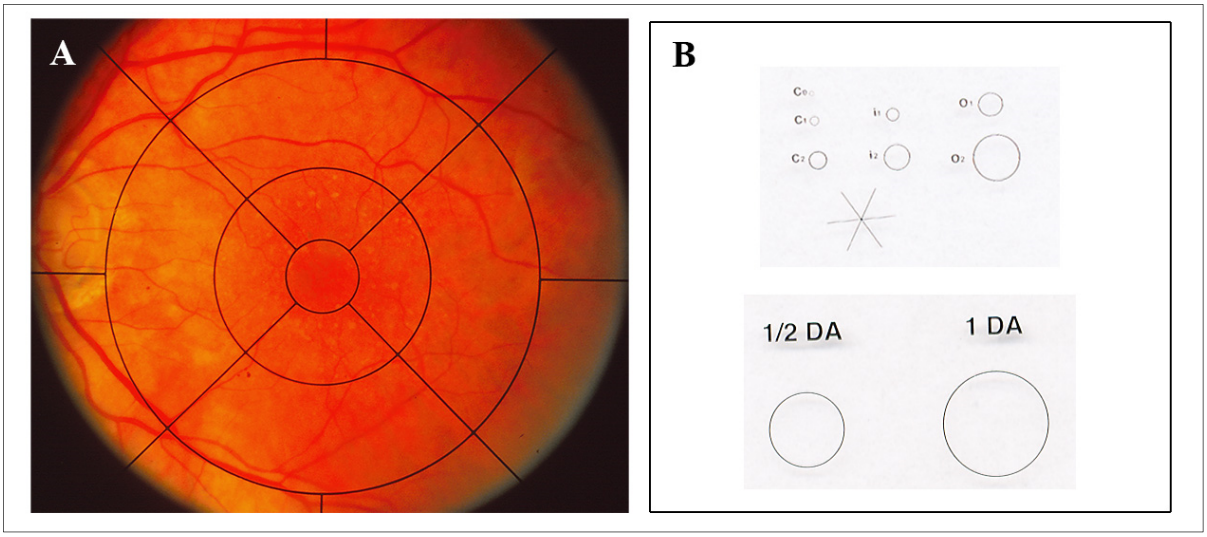

Figure 1.2.1-1 Wisconsin Age-Related Maculopathy Grading System (WARMGS) grid and measurement circles. (A) Placement of the WARMGS grid over a macula photograph with central subfield directly over the fovea; (B) Measurement circles used for estimating drusen size and AMD lesion area.

1.2.2 Australian studies

The ***Blue Mountains Eye Study (BMES)*** is a landmark study of vision and common eye disease in a representative sample of older Australians residing in the Blue Mountains region, west of Sydney[36]. Of 4433 eligible residents aged 49+ years, identified using a door-to-door census, 3654 (82.4% of those eligible) attended the baseline examinations between 1992 and 1994[36]. Of these participants, 2335 (75.1% of survivors) returned for the 5-year follow-up examinations (1997-1999)[44], 1952 (76.5% survivors) attended the 10-year follow-up examinations (2002-2004)[35] and 1149 (56.1% of survivors) attended the15-year follow-up examinations (2007-2009)[54]. Incidence of Early and Late AMD were calculated using Kaplan-Meier product limit survival estimates.

Late AMD was defined as described in the International ARM Classification[13]. The overall person-specific incidence of Late AMD was 1.1% and 3.7% at 5 and 10 years, respectively[35,44]. The incidence of neovascular AMD was 1.0% and 2.2%, and the incidence of GA 0.8% and 1.7% at 5 and 10 years, respectively. Of participants with unilateral AMD, 55.6% became bilateral at 5 years, and 100% bilateral at 10 years[35].

Early AMD was defined following the BDES Early AMD definitions[35,36,44]. The corresponding overall person-specific incidence of Early AMD was 8.7% and 14.1% at 5 and 10 years, respectively[35,44]. The 5-year incidence of individual early AMD lesions including distinct soft, indistinct soft, and combined indistinct soft and reticular drusen were 2.4%, 4.2% and 4.9%, respectively; and the 5-year incidence of retinal pigmentary abnormality, hyperpigmentation and RPE depigmentation were 7.0%, 7.0% and 3.8%, respectively[44]. The 10-year incidence of individual early AMD lesions were not reported, however, the incidence

of indistinct soft and reticular drusen combined was 11.7% and the incidence of retinal pigmentary abnormalities was 23.3%[35].

The progression of AMD was investigated (by eye) in the BMES. Progression of Late AMD was defined as an extension of GA and neovascular AMD lesions into the fovea, an increase in area involved by neovascular AMD lesions by 2 or more subfields on the WARMGS grid, or the appearance of more advanced stage lesions of neovascular AMD[44]. The 5-year progression of GA and neovascular AMD occurred in 42.9% and 71.4% of eyes, respectively[44].

The 10-year risk of progression to Late AMD was higher in eyes with larger individual drusen, greater total area involved by drusen and drusen location close to the fovea[35]. Progression rate to Late AMD over 10-years was also higher for persons with more advanced steps of AMD on the AREDS simplified severity scale[35].

The ***Melbourne Visual Impairment Project (MVIP)*** is a population-based survey of eye disease in Melbourne residents aged 40+ years in the Melbourne metropolitan region. The baseline study was conducted in 1992 to 1994 and examined 3271 participants (83% of eligible residents)[43]. Of these, 2594 participants (85% of survivors) returned at the 5-year follow-up visit during 1997-1999[46].

The overall 5-year incidence of Late AMD was 0.49%. The incidence of GA and neovascular AMD were not reported separately[46].

Early AMD was defined as the presence of distinct soft, indistinct soft or reticular drusen or the presence of retinal pigmentary abnormalities in the absence of Late AMD. Early AMD was also defined following the BDES Early AMD definitions. Using the latter definition, the overall 5-year incidence of Early AMD in the first eye was 5.4%[46]. The 5-year incidence of distinct soft drusen, indistinct soft drusen, hyperpigmentation and RPE depigmentation were 6.1%, 4.3%, 10.9% and 13.0%, respectively. The differences in the incident rates of Early AMD and early AMD lesions between the MVIP and the BMES may be due to age differences of the two populations: in the MVIP sample only 2% were aged 80+ years whereas in the BMES sample it was 4.8%. Differences in lesion classifications by different graders of these two studies, and different graders between the baseline and follow-up visits of the MVIP, may also have contributed to the differences[43,46].

Progression of AMD was assessed by 5 stages of AMD which ranged from 'no AMD' to either dry or wet form of Late AMD, where progression of Early AMD was defined as an increase in 1 or more stages, based on the worse eye[46]. Progression over 5 years occurred in 16.8% of participants with Early AMD at baseline. The 5-year risk of Late AMD for eyes present with indistinct soft or reticular drusen together and retinal pigmentary abnormalities (4^{th} stage) was 9-fold the risk for eyes present with soft drusen alone or retinal pigmentary abnormalities alone (2^{nd} stage)[46].

1.2.3 International studies

Caucasian Eye Studies

The ***Beaver Dam Eye Study (BDES)*** is a landmark population-based eye study of older Caucasian residents in the township of Beaver Dam, Wisconsin, USA. The study was one of the first comprehensive studies that collected demographic and lifestyle data and documented

the presence and development of retinal eye disease in a large sample of white population. It is also the only eye study of the general population with a follow-up period of 20 years. Baseline examinations were conducted in 1988-1990 and examined 4926 participants aged 43+ years (83.2% of those eligible)[14]. Of these participants, 3684 attended the 5-year follow-up examinations in 1993-1995[29], 2764 attended the 10-year follow-up in 1998-2000[34], 2119 attended the 15-year follow-up in 2003-2005[33] and 1913 attended the 20-year follow-up examinations in 2008-2010[6,40]. Incidence rates of Early and Late AMD, prior to the 15-year report, were assessed using Kaplan-Meier methods, and the 15-year incidence rates were reported after accounting for the competing risk of death[33]. The 20-year incidence of Late AMD, calculated directly using proportions, was reported in an article that addresses the long-term risk of AMD associated with smoking[40]. In a different report, the 20-year incidence of Early AMD was estimated after adjusting for competing risk of death[55].

Incidence of Late AMD in the first eye were 0.9%, 2.1%, 3.1% and 4.5% at 5, 10, 15 and 20 years, respectively[29,33,34,40]. The incidence of GA was 0.3% to 0.8% and 1.3%and the incidence of neovascular AMD was 0.6% to 1.4% and 2.0% at 5, 10 and 15 years, respectively. The progression from unilateral to bilateral Late AMD occurred in 22.2% of participants over 5 years[29] and 38.5% of participants over 10 years[34]. The 15-year cumulative incidence of bilateral Late AMD was 38.7%[33] among participants with unilateral AMD at baseline. There is no 20-year incidence of GA, neovascular AMD or second eye incidence of Late AMD reported from the BDES to date.

Early AMD was defined using the BDES Early AMD definitions: large (>125µm diameter) indistinct soft or reticular drusen or the co-presence of distinct soft drusen and retinal pigmentary abnormalities within the macula in the absence of Late AMD[29,33,34,40]. The

person-specific incidence rates of Early AMD were 8.2%, 12.1%, 14.3% and 24.4% at 5, 10, 15 and 20 years, respectively[29,33,34,40]. The 20-year cumulative incidence of Early AMD after adjusting for the competing risk of death was 23.0%[55]. The incidence rates of individual early AMD lesions in 5, 10 and 15 years were: 6.5%, 10.2% and 13.4%, respectively, for distinct soft drusen; 7.0%, 10.3% and 13.4% respectively for indistinct soft drusen; 4.9%, 8.5% and 9.8%, respectively, for hyperpigmentation; and 3.1%, 6.3% and 7.5%, respectively, for RPE depigmentation[29,33,34].

Progression of AMD lesions (in right eyes only) were defined as presence of the lesions in at least 1 subfield at baseline that had increased by 2 or more subfields at follow-up compared to baseline, in the absence of developing more severe lesions[29,34]. Progression of GA occurred in 50.0% and 55.6%, and neovascular AMD in 36.4% and 33.3% of eyes at 5 and 10 years, respectively[29,34]. The frequency of progression to GA and neovascular AMD at 5- and 10-year visits was highest in eyes with greater macular area involved by more advanced stage drusen (indistinct soft) or larger drusen (≥125μm diameter) compared to that involved by less advanced, smaller drusen (<63μm)[29,34]. The progression of AMD over 15 years was assessed along the BDES AMD severity scale as an increase by ≥2 steps from level 2 or 3, or by 1 step from level 4, and in the AREDS AMD severity scale as a ≥2-step increase in severity (**Table 1.1-1)**. The 15-year cumulative progression along the BDES scale, in the worse eye, was 12.2% and along the AREDS scale was 25.0%[33].

The ***Rotterdam Study (RS)*** is a population-based prospective cohort study conducted in Rotterdam, the Netherlands. Baseline examinations were conducted in 1990-1993, and 7983 participants aged 55 to 98 years (78% of those eligible) were recruited in the initial phase of the study. A subsample of 7983 participants (n=6872) underwent eye examinations, and of

the 6872, 6418 had gradable fundus photographs[30]. Of the 6418 participants, 4974, 3636 and 2674 were re-examined at the second (1993-1995)[30], third (1997-1999)[38] and fourth (2002-2004)[56,57] visits, respectively.

The 2-year cumulative incidence of Late AMD was 0.24%[30]. The overall 6-year incidence of Late AMD was 1.8 per 1000 person-years, with the incidence of GA and neovascular AMD 0.7 and 1.1 per 1000 person-years, respectively[38]. The cumulative incidence of Late AMD at 11 years was 2.1% [58]. The 2-year and 6-year cumulative incidence of bilateral Late AMD was 28.9% and 38.7%, respectively[30,38].

Early AMD was defined by mutually exclusive severity stages, developed by the RS investigators[30] (Table 1.2-1). Stages 2 and 3 correspond to Early AMD. The 6-year incidence of Early AMD (defined using the BDES Early AMD definitions) was 16.4 per 1000 person-years[38]. The cumulative incidence of Early AMD at 11 years was 12.2%44990].

Among participants with Early AMD, the 2-year cumulative progression to a more severe stage was 21.5%[30]. The 5-year risk of Late AMD was 0.0%, 0.9%, 7.8%, 28.0% for persons with stages 0, 1, 2 and 3 of the RS severity scale at baseline, respectively[38].

The ***Los Angeles Latino Eye Study (LALES)*** is a population-based cohort study of eye disease in self-identified Latinos aged 40+ years residing in the city of La Puente, Los Angeles County, California[59]. Latinos are a heterogeneous group of people that include Hispanics, Hispanic Americans and Latino Americans descended from a Spanish-speaking community, with a majority from Mexican ancestry (66%). Baseline examinations were performed on 6357 participants (82% of eligible participants) during 2000-2003[41]. Of these,

4658 participants (76.4% of survivors) were examined at the 4-year follow-up examinations[39].

The overall 4-year incidence of Late AMD was 0.2%, with the same incidence rate reported for pure GA (0.1%) and neovascular AMD (0.1%)[39]. No incidence of Late AMD in the second eye was reported.

The LALES defined Early AMD as any indistinct soft or reticular drusen; or retinal pigmentary abnormalities together with any type of drusen, in the absence of Late AMD, in the first eye. The 4-year incidence of Early AMD was 7.5%, and incident rates of distinct soft drusen, indistinct soft drusen, hyperpigmentation and RPE depigmentation were 10.5%, 2.7%, 3.1% and 1.9%, respectively[39]. The incidence of Early AMD in the second eye was 11.2% among persons with Early AMD in the first eye at baseline[39].

The progression of AMD was defined as a ≥2 step increase in the concatenated 11-step AREDS severity scale scores[27] at the follow-up visit. The overall 4-year progression of any (Early and Late) AMD by the AREDS scale was 9.2%[39]. GA progression, defined as an increase in size by ≥2 disc areas or an extension of GA to the macular centre, occurred in 33.3% of eyes with GA at baseline [39]. The progression of neovascular AMD was not reported.

The ***Copenhagen City Eye Study*** was one of the earliest population-based eye studies that examined 946 residents (96.9% of eligible participants) aged 60 to 80 years in the city of Copenhagen, between 1986 and 1988[60]. Follow-up examinations were conducted 14 years

later from 2000-2002, and examined 359 persons (97.3% of survivors, and 37.9% of the original 946 participants)[47].

The overall 14-year incidence of Late AMD was 16.9%, while pure GA and neovascular AMD incidence rates were 4.9% and 12.0%, in the first eye[47]. Of 46 participants with unilateral Late AMD at baseline, only 2 were re-examined at follow-up, and Late AMD developed in the second eye of both participants (100%) in 14 years[47].

Early AMD was defined using the same definitions as that used in the LALES. The 14-year incidence of Early AMD in the first eye was 37.8%. The incidence of distinct soft drusen, indistinct soft drusen, pigmentary abnormalities, hyperpigmentation and RPE depigmentation were 30.6%, 34.7%, 32.6%, 30.0% and 23.8%, respectively[47].

The risk of progression to Late AMD increased with larger drusen present at baseline[47]. Greater total macular area involved by drusen at baseline did not significantly affect the progression to Late AMD after adjustment for drusen type[47].

The ***Reykjavic Eye Study*** was conducted in Reykjavic, Iceland in 1996 and included 1045 participants aged 50 years and older (75.8% response rate)[61]. Of these, 846 participants (88.2% of survivors) attended the 5-year follow-up examination in 2001[45]. Incidence rates were calculated directly by proportions.

The 5-year incidence of Late AMD was 0.0%, 0.4%, 4.4% and 5.9% in the 50-59, 60-69, 70-79 and 80+ age groups, respectively[45]. The cumulative incidence of GA at 5 years was 4.6% and the incidence of neovascular AMD was 0%[45]. Bilateral development of GA occurred in 3

of 3 participants (100%) with unilateral GA at baseline. There were no participants who developed bilateral neovascular AMD in 5 years[45].

Early AMD included the presence of any soft drusen or reticular drusen, and/or retinal pigmentary abnormalities, within the macula. The 5-year incidence of Early AMD was 14.8%, 17.6%, 43.9% and 50.0% in those aged 50-59, 60-69, 70-79 and 80+ years, respectively[45].

Progression was not reported in the Reykjavic Eye Study.

Asian Eye Studies

The ***Beijing Eye Study*** was carried out in urban and rural communities in the north of central Beijing and south of Beijing, respectively. Baseline examinations were conducted in 2001 and recruited 4439 participants (83.4% response rate) aged 40-82 years[37]. Of these 3251 participants (73.2% of survivors) were re-examined in 2006[48].

The overall 5-year incidence of Late AMD was 0.1%[48]. The eye-specific 5-year incidence of GA and neovascular AMD were 0.02% and 0.1%, respectively[48]. Second eye incidence of Late AMD was not reported.

Early AMD was defined following the BDES Early AMD definitions[14]. The person-specific 5-year incidence of Early AMD was 4.2%[48]. The eye-specific 5-year incidence rates for distinct soft drusen, indistinct soft drusen, hyperpigmentaion and RPE depigmentation was 1.9%, 1.7%, 3.3% and 2.7%, respectively[48].

Progression of AMD was defined if the AMD lesion extended into the fovea, involved ≥2 more subfields of the WARMGS grid compared to baseline[26], or developed more advanced stage lesions of neovascular AMD (e.g. advancing from serous or hemorrhagic retinal detachment to subretinal scaring), by the time the 5-year follow-up visit was conducted. GA progression occurred in 16.7% and neovascular AMD progression in 20.0% of eyes with the corresponding lesions at baseline[48].

The ***Hisayama Study*** is a prospective population study conducted in Hisayama, southern Japan[42]. The baseline examination took place in 1998, and 1775 participants aged 50+ years (58.1% of those eligible) were recruited[42]. Of these, 961 (% of survivors not reported) participants attended the 5-year follow-up examinations in 2003[49], and 1401 (% of survivors not reported) attended the 9-year follow-up examination in 2007[50]. The 9-year incidence of Late AMD, Early AMD and its component lesions were reported after age-standardization to the 1998 World Health Organization standard population

The 5-year incidence of Late AMD was 0.8%, and incidence rates for GA and neovascular AMD were 0.3% and 0.5%, respectively[49]. The age-standardized 9-year incidence of Late AMD was 1.4% and incidence of GA and neovascular AMD were 0.04% and 1.4%, respectively[50].

Early AMD was defined as the presence of soft drusen (distinct and indistinct) or pigmentary abnormalities, in the first eye. The 5-year incidence of Early AMD was 8.5%, and incidence of soft drusen (combined distinct and indistinct types), and pigmentary abnormalities was 6.8% and 1.6%, respectively[49]. The 9-year incidence of Early AMD was 10.0% and incidence of soft drusen and pigmentary abnormality was 8.0% and 2.0%, respectively[50].

Progression of AMD was not reported from the 5-year follow-up visit[49], however, progression to Late AMD from Early AMD or specific early AMD lesions at baseline was reported from the 9-year follow-up visit[50]. The age-standardized 9-year progression to Late AMD from eyes with Early AMD was 4.4%, compared to only a 0.7% of eyes with no Early AMD at baseline[50]. The 9-year progression rates to Late AMD from soft drusen and pigmentary abnormality was 5.2% and 2.2%, respectively[50].

The incident rates of Early, Late AMD, and individual AMD lesions reported by population-based studies described above are summarized in **Tables 1.2-1, 1.2-2** and **1.2-3.**

In summary, the overall 5-year incidence of Late AMD was under 2% in all populations, and it increased with increasing length of follow-up period. The overall 5-year incidence of Early AMD varied from 5% to 15%, and it also increased with increasing length of follow-up period in a majority of the population-based studies included in this review. Some studies also reported incidence rates by age groups and sex[29,33-35,44].

An age-related increased incidence of AMD is evident in every study. In addition to age, many other systemic and lifestyle risk factors are also found to be associated with the incidence and progression of AMD.

Table 1.2-1: Summary of the incidence of early and late age-related macular degeneration among prominent Australian and International population-based cohort studies.

Name/Location of Study	Year of Baseline Examination	Sample Size at Baseline	Age at Baseline (years)	Years of Follow-up	Early AMD Incidence % (by years of follow-up period)			Late AMD Incidence % (by years of follow-up period)		
					≤5	>5 to ≤10	>10 to ≤15	≤5	>5 to ≤10	>10 to ≤15
Australian										
BMES[35,44]	1992-1994	3654	49-97	10	8.7	14.1	-	1.1	3.7	-
MVIP[46]	1992-1994	3271	40+	5	5.4	-	-	0.5	-	-
International										
Caucasian										
BDES[29,33,34]	1988-1990	4926	43-84	20	8.2	12.1	14.3	0.9	2.1	3.1
Rotterdam[38*]	1990-1993	6418	55-98	11	16.4	-	-	1.8	-	-
LALES[39]	2000-2003	6357	40+	4	7.5	-	-	0.2	-	-
Copenhagen[47]	1986-1988	946	60-80	14	-	-	37.8	-	-	16.9
Reykjavik[45†]	1996	1045	50+	5	14.8-50.0	-	-	0.0-5.9	-	-
Asian										
Beijing[48]	2001	4439	40-82	5	4.2	-	-	0.1	-	-
Hisayama[49,50]	1998	1482	50+	9	8.5	10.0‡	-	0.8	1.4‡	-

AMD=age-related macular degeneration, BMES=Blue Mountains Eye Study, MVIP=Melbourne Visual Impairment Project, BDES=Beaver Dam Eye Study, LALES=Los Angeles Latino Eye Study

*Incidence expressed as: incidence per 1000 person-years

†Overall incidence not reported; first value in range indicates incidence from 50-59 year age group and last value indicates incidence from 80+ year age group.

‡Incidence age-standardized to the 1998 World Health Organization standard population

Table 1.2-2: Summary of the incidence of individual early age-related macular degeneration lesions among prominent Australian and International population-based cohort studies.

Name/Location of Study	Drusen Incidence (% by years of follow-up period)						Pigmentary Abnormalities Incidence (% by years of follow-up period)								
	Soft distinct			Soft indistinct			Any			Hyperpigmentation			RPE depigmentation		
	≤5	>5 to ≤10	>10 to ≤15	≤5	>5 to ≤10	>10 to ≤15	≤5	>5 to ≤10	>10 to ≤15	≤5	>5 to ≤10	>10 to ≤15	≤5	>5 to ≤10	>10 to ≤15
Australian															
BMES[35,44]	2.4	-	-	4.9†	11.7†	-	7.0	23.3	-	7.0	-	-	3.8	-	-
MVIP[46]	6.1	-	-	4.3	-	-	-	-	-	10.9	-	-	13.0	-	-
International															
Caucasian															
BDES[29,33,34]	6.5	10.2	13.4	7.0	10.3	13.4	-	-	9.8	4.9	8.5	9.8	3.1	6.3	7.5
Rotterdam[38]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LALES[39]	10.5	-	-	2.7	-	-	-	-	-	3.1	-	-	1.9	-	-
Copenhagen[47]	-	-	30.6	-	-	34.7	-	-	32.6	-	-	30.0	-	-	23.8
Reykjavik[45*]	1.1-6.7	-	-	1.1-28.6	-	-	-	-	-	10.7-23.5	-	-	1.9-17.7	-	-
Asian															
Beijing[48]	1.9	-	-	1.7	-	-	-	-	-	3.3	-	-	2.7	-	-
Hisayama[49,50]	-	-	-	6.8‡	8.0‡§	-	1.6	2.0§	-	-	-	-	-	-	-

RPE=retinal pigment epithelium, BMES=Blue Mountains Eye Study, MVIP=Melbourne Visual Impairment Project, BDES=Beaver Dam Eye Sudy, LALES=Los Angeles Latino Eye Study

*Overall incidence not reported; first value in range indicates incidence from 50-59 year age group and last value indicates incidence from 80+ year age group.

†Soft indistinct and reticular drusen combined

‡Combined soft distinct and indistinct drusen

§Incidence age-standardized to the 1998 World Health Organization standard population

Table 1.2-3: Summary of the incidence of individual late age-related macular degeneration lesions among prominent Australian and International population-based cohort studies.

Name/Location of Study	Late AMD Incidence (% by years of follow-up period)					
	Geographic Atrophy			Neovascular AMD		
	≤5	>5 to ≤10	>10 to ≤15	≤5	>5 to ≤10	>10 to ≤15
Australian						
BMES[35,44]	0.8	1.7	-	1.0	2.2	-
MVIP[46]	-	-	-	-	-	-
International						
Caucasian						
BDES[29,33,34]	0.3	0.8	1.3	0.6	1.4	2.0
Rotterdam[38]	0.7	-	-	1.1	-	-
LALES[39]	0.1	-	-	0.1	-	-
Copenhagen[47]	-	-	4.9	-	-	7.6
Reykjavik[45]	4.6	-	-	-	-	-
Asian						
Beijing[48]	0.0	-	-	0.1	-	-
Hisayama[49,50]	0.3	0.04*	-	0.5	1.4*	-

AMD=age-related macular degeneration, BMES=Blue Mountains Eye Study, MVIP=Melbourne Visual Impairment Project, BDES=Beaver Dam Eye Sudy, LALES=Los Angeles Latino Eye Study

*Incidence age-standardized to the 1998 World Health Organization standard population

1.3 Risk factors associated with the incidence and progression of age-related macular degeneration

1.3.1 Systemic risk factors

Age

The association between increasing age and the incidence and progression of AMD are consistent across all populations[29,34,35,39,44,47], including clinic-based patient cohorts such as the AREDS[62]. In the BMES, the 5-year incidence of Late AMD increased with age from 0.0% in persons aged <60 years to 5.4% in those aged 80+ years and older. The corresponding incidence of Early AMD increased from 3.2% in persons aged <60 years to 18.3% in those aged 70-79 years, and 14.8% in those aged 80+ years[44]. Increased incidence of Early and Late AMD with increasing age was also found at the 10-year follow-up visit of the BMES[35], the 5- and 10-year follow-up visits in the BDES[29,34], the14 year visit in the Copenhagen Eye Study[47] and a number of other studies that assessed incidence of Early and Late AMD between intervals of 5 to 10 years[48-50,63]. Slightly decreased incident rates of both Early and Late AMD in the oldest old age group were also observed in the BDES 15-year follow-up visit, regardless of considering the competing risk of death[33].

Sex

While incident Early and Late AMD is consistently associated with age, the association with sex varies among different studies[29,34,35,38,44,47,48]. In the BMES, the 5- and 10-year incidence of AMD (Early and Late) was slightly higher in women than in men, and the incidence of neovascular AMD in women doubled that in men[35,44], although the differences were not statistically significant. Similar to the BMES, the BDES also reported a higher overall 5- and 10-year incidence of Early and Late AMD in women (9.0% and 1.3%, respectively at 5-year visit; 13.0 and 2.4% at 10-year visit) compared to men (7.1% and 0.5%, respectively at 5-year

visit; 10.9% and 1.7%, respectively at 10-year visit). These gender differences could not be explained by selective mortality, according to the authors' opinion[29,34]. The Rotterdam study did not find a significant gender difference in 5-year incident Late or Early AMD[38]. Likewise the incidence of any (Early and Late) AMD was also not significantly different between the two genders in the Copenhagen Study[47] and the Beijing Eye Study[48].

Similarly, in some Asian studies no significant difference in the incidence of AMD was documented between men and women[64,65], while in others, incident Late AMD was found to be greater in men compared to women[66-68], likely due to a substantially higher proportion of smokers among Asian men compared to Asian women. In the Hisayama Study population, 74.8% of men were current smokers compared to only 7.1% of women who were, and the 5-year incidence of Late AMD in this study population was 1.9% in men compared to 0.2% in women[49]. Similar findings were also reported from the 9-year follow-up of this cohort[50].

Overall there has been no strong and consistent evidence to support that women have a higher risk of AMD than men.

Genetic Risk of AMD

Genetic influences on AMD were initially documented in family and twin studies[69-73]. Studies using genome wide association scanning (GWAS) have revealed many gene loci related to AMD[74-78]. One with the largest sample size, the International AMD Gene Consortium (IAMDGC)[79], reported 19 gene loci associated with Late AMD. Of these loci, the *complement factor H (CFH)* and *age-related maculopathy susceptibility (ARMS2)* are the two showing the strongest risk magnitude. The association of the *CFH* with AMD has been

consistently documented in European studies and the association of *ARMS2* with AMD was relatively more prominent in Asian populations[57,79-86].

The *CFH* is a regulator gene in Chromosome 1 for the alternative pathway of the complement activation: the CFH protein binds to C-reactive protein induced by damaged tissues[87-89], a regulatory action to eliminate ongoing activation of complement system.

The *CFH*-rs1061170 (Y402H) polymorphism is relatively common in Caucasian populations, with this minor allele frequency ranging from 28% to 35%[90-95]. Each risk alleles of *CFH* increased the risk of incident Early or Late AMD in the order of 1.6 to 2.9 after age- and sex-adjustment, compared to persons with no risk allele of *CFH* [82,96,97]. Risk of progression from less to more advanced stage of Early AMD and from Early to Late AMD also increased with increased number of risk alleles of the *CFH*, after adjustment[81,97].

The *ARMS2* gene in Chromosome 10 is more common in Asians compared to Caucasians, and has been shown to be associated with an increased risk of AMD[84-86,98,99], independent of the *CFH* gene[100,101]. There have been several single nucleotide polymorphisms (SNPs; rs10490924, rs10490923, rs3750847,) identified; the first-reported SNP, rs10490924, is also known as the LOC387715. Many findings on the association between *ARMS2* and AMD have originated from case-control studies and limited data exist on the association between *ARMS2* and the incidence and progression of AMD[99,102].

The risk magnitudes for incident Late AMD were in the range of 2.7 to 4.1 with the presence of one or two risk alleles of *ARMS2*, compared to those with no risk allele of *ARMS2*[82,103].

The risk magnitude for incident Early AMD was 2.0 with both risk alleles of *ARMS2* compared to none, reported from a clinic-based study[82].

Prediction of AMD risk using risk scores generated based on the presence of risk alleles of AMD-related genes and known AMD risk factors (environmental exposures such as smoking, and the presence early AMD lesions) has been examined[104,105]. The risk of developing Late AMD was increased substantially in those with high risk score compared to low risk score in a model accounting for age, sex, 26 SNPs of known AMD-related genes, smoking, body mass index (BMI) and baseline early AMD phenotypes, reported from the 3CC[105]. In another study, the incidence of Late and Early AMD were increased in those with a higher genetic risk score compared to those with a lower genetic risk score, after adjusting for age[104]. Using area under the Receiver Operating Characteristic (ROC) curve to assess performance of the prediction models, findings consistently showed incremental gains after including known AMD environmental risk exposures such smoking, hypertension, history of physical activity, BMI, education, antioxidant and multivitamin use in age-adjusted prediction models containing genetic scores only[91,104-108]. Gene and environment risk models provided better performance in AMD risk prediction than models containing genes alone or environmental factors alone. Incorporating genetic factors into prediction models containing early AMD phenotypes provided a small improvement to the risk prediction performance[104,107].

Knowledge gained from GWAS studies of AMD genetic risk supports current understanding of complex disease that genetic influence contributes only a small proportion of the disease risk. Influence from environmental exposures, given that most of the exposures are modifiable, should be a main focus of research attention and public health interventions.

1.3.2 Lifestyle risk factors

Smoking

Cigarette smoke is known to increase oxidative stress levels, increase the number of inflammatory mediators and reduce antioxidant levels, all relevant to the proposed inflammation pathway, one of the proposed AMD pathogenesis theories[109-113]. Smoking is consistently identified to be associated with an increased risk of AMD in many studies[50,63,114-117]. Age-adjusted risk ratios for smokers compared to non-smokers in terms risk of Late and Early AMD are presented in **Table 1.3.2-1**.

The BMES and the Hisayama Study reported a significant association between current smoking and incident Late AMD, with overall risk ratios for current smokers compared to non-smokers ranging from 2.5 to 6.3, after adjusting for age [50,114,115]. One study found an increased risk of incident Early AMD among current smokers[116]. Other studies including BDES 5- and 10-year reports, the LALES and the Copenhagen Eye Study did not find a significant association between smoking and incident AMD[51,63,118,119].

Among past smokers, the Rotterdam Study reported a 3-fold risk of incident Late AMD compared to non-smokers, after adjusting for age[117]. In other studies, the risk ratios for Early and Late AMD among past smokers were comparable to that of non-smokers[114,115]. Any smoking (past or current) was significantly associated with incident Late AMD in the Hisayama Study[50].

Selective survival and small numbers of participants with incident Late AMD cases could have limited the power to detect an association between smoking and incident Late AMD[51,118,119]. Cessation of smoking after AMD development in some older participants, an

indication bias, could have also biased the association towards the null in cross-sectional studies[66,120-124], but not in longitudinal studies where smoking status is defined prior to AMD development.

Current smoking was also significantly associated with progression of AMD, defined by an increase by ≥2 steps in the BDES severity scale (**Table 1.1-1**) over 4 years in the LALES, after adjusting for age, sex, pulse pressure and history of diabetes[63]. Progression rate of AMD in the BDES, defined as an increase by ≥1 step in the BDES severity scale (**Table 1.1-1**), was also higher among current smokers compared to non-smokers, after adjusting for age, sex and baseline AMD severity level[116].

Table 1.3.2-1: Age-adjusted risk ratios for incident Late and Early age-related macular degeneration (AMD) among current and past smokers compared to non-smokers, summarized from Australian and International population-based cohort studies.

Name/Location of Study	Year (Incidence)	Age-adjusted Relative Risk/Odds Ratio (95% Confidence Intervals)			
		Current Smoker		Past Smoker	
		Late AMD	Early AMD	Late AMD	Early AMD
BMES	5	**2.5 (1.0-6.2)**	1.2 (0.8-1.9)	0.8 (0.4-1.8)	0.9 (0.7-1.2)
	10[*]	**4.9 (2.5-9.7)**	1.2 (0.8-1.9)	1.4 (0.8-2.5)	1.0 (0.8-1.4)
BDES	5 [men]	0.0	1.69 (0.96-2.98)	1.72 (0.23-13.05)	1.04 (0.64-1.68)
	5 [women]	0.44 (0.07-2.68)	0.80 (0.48-1.33)	0.85 (0.32-2.26)	1.11 (0.79-1.57)
	10	0.51 (0.18-1.46)	1.37 (0.98-1.94)	0.61 (0.33-1.13)	1.12 (0.85-1.47)
	15[†]	0.69 (0.27-1.76)[‡]	**1.47 (1.08-1.99)**	1.12 (0.62-2.01)[‡]	1.16 (0.91-1.48)
		0.18 (0.02-1.40)[§]		0.88 (0.41-1.88)[§]	
Rotterdam	5	**6.32 (2.10-19.1)**	-	**3.29 (1.17-9.22)**	-
LALES	4[l]	-	non-significant	-	non-significant
Copenhagen	14	1.6 (0.6-3.9)	1.2 (0.6-2.6)	1.3 (0.6-3.1)	1.1 (0.5-2.2)
Hisayama	5	**2.22 (1.14-4.33)**[¶]			
	9	Late AMD: **4.59 (1.86-11.3)**[#] / Early AMD: 1.07 (0.73-1.55)[#]			

BMES=Blue Mountains Eye Study, BDES=Beaver Dam Eye Study, LALES=Los Angeles Latino Eye Study

[*]Adjusted for age and sex

[†]Adjusted for age, sex and baseline AMD severity level

[‡]Neovascular AMD

[§]Geographic Atrophy

[l]OR not provided as association is non-significant for incidence of early or any AMD

[¶]OR for incidence of any (either Late or Early) AMD among past or current smokers combined

[#]OR for incidence of Late and Early AMD separately among past or current smokers combined

Fish Consumption

Omega-3 (ω-3), and omega-6 (ω-6) fatty acids, are found in high concentrations in the retina[125] and are important structural components of photoreceptor outer segments and the retinal vasculature[126,127].

Fish intake, and specifically intake of ω-3 fatty acids (including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)), have been found associated with a reduced risk of AMD in population-based cohorts[128-133]. In the BMES, fish consumption of ≥3 servings per week was associated with reduced 5-year incident rate of Late AMD [130]. The joint effect of low fish consumption (<1 serving per month) and current smoking status significantly increased the risk of 10-year Late AMD incidence in the BMES[115]. The Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS), and the Progression of Age-Related Macular Degeneration Study found inverse associations between fish consumption and incidence and progression of Late AMD[128,129].

In the BMES, the incidence of Early AMD at the 5- and 10-year follow-up visits was reduced by 30% to 40% among participants who consumed fish at least once per week[130,131]. The risk of incident Early AMD was reduced further among those who had a low dietary intake of linoleic acid (a type of ω-6 fatty acid) if they also consumed fish at least once per week, compared to those who consumed fish less than once a week, in the BMES and the Progression of Age-Related Macular Degeneration Study[129,131]. In contrast, the Reykjavic Eye Study and the Melbourne Collaborative Cohort Study found no significant association of fish consumption with the incidence of Early AMD or early AMD lesions[134,135]. Fish

consumption of 1-2 servings per week was not associated with a reduced risk of progression from bilateral drusen to Late AMD in the AREDS[132].

Associations between dietary intake of ω-3, ω-6 and incident AMD was also examined[130-133,135,136]. In the BMES, there was no significant association between high ω-3 or ω-6 intake and incident Late AMD in 5 or 10 years[130,131]. Similarly, no association of these fatty acids with incident Late AMD was observed in the Melbourne Collaborative Cohort Study[135]. In comparison, incident Early AMD was significantly reduced among participants with higher intake of ω-3 in the BMES and Melbourne Collaborative Cohort Study[130,131,135]. Further, higher intake of ω-3 long chain fatty acids (EPA + DHA) were shown associated with a reduced risk of developing visually impaired consequence (defined as best-corrected visual acuity 20/30 or worse) that was attributed to AMD in the Women's Health Study[136]. Higher intake of EPA + DHA was also associated with a reduced risk of progression to late AMD in the AREDS clinical trial[132,133].

Though a beneficial role of ω-3 fatty acids (EPA + DHA) in reducing AMD risk is likely, inconsistencies in findings in this regard suggest that more prospective studies and randomized controlled trials are needed to provide further evidence, in order to guide clinical recommendation[137,138].

Antioxidants

Antioxidants are known to have anti-inflammatory properties and have been investigated for their association with AMD[137,139-147]. Lutein and zeaxanthin (LZ) are of particular interest as they are found high in concentrations in the macula. The associations between LZ intake or other antioxidant intake and incident Late or Early AMD are summarized in **Table 1.3.2-2**.

There was a reduced likelihood of 10-year incidence of neovascular AMD associated with high baseline dietary intake of LZ in the BMES[139], whereas no association was found in the RS at 11 years[140], or in the Prospective Cohorts of the NHS and the HPFS populations, at 18 years[141]. However, by the time of 24-26 year follow-up visits of the same two prospective cohorts, a 40% reduced risk of incident Late AMD was observed among participants with higher plasma LZ levels at baseline[142].

An increased risk of incident neovascular AMD was found associated with high β-carotene intake from diet and supplements in the BMES, but not associated with dietary intake of β-carotene alone[139]. In comparison, a reduced risk or no association was found between combined diet and supplement intake of β-carotene and incident Late AMD in the NHS and HPFS, and the RS [140-142]. The AREDS investigators reported a decreased likelihood of incident Late AMD in participants with increased supplement intake of β-carotene[143], and a reduced risk of Late AMD in those who received antioxidants or antioxidants plus zinc supplements (which contained β-carotene) from the original AREDS formula[144]. However, in the AREDS2, a new trial focusing on LZ supplements in the AREDS population, found that elimination of β-carotene showed no effect on progression to Late AMD. The AREDS 2 also found that addition of either LZ, DHA + EPA or both to the original AREDS formula did not further reduce the risk of progression to Late AMD in the whole study sample[137], a generally well-nourished population. However, in a subgroup analysis, among participants in the lowest quintile of dietary LZ intake, those receiving LZ supplements had a significantly reduced risk of progression to late AMD compared to those who received the placebo[137,145]. Therefore, supplementary LZ was recommended as an appropriate substitute for β-carotene, as β-

carotene supplements may also potentially increase the incidence of lung cancer among former smokers[137,145].

High dietary intake of LZ was reported to be associated with a reduced risk of developing indistinct soft or reticular drusen at the 10-year visit of the BMES[139]. No significant associations were found between dietary LZ intake and incident Early AMD or early AMD lesions in the BMES and BDES, at 5 years[146,147]. Among other antioxidants, α-carotene was found associated with a reduced risk of incident Early AMD in the BDES 5-year follow-up visit[147].

Although only a limited number of studies have reported the risk of AMD in relation to dietary intake of antioxidants, many clinic-based studies and clinic trials including the AREDS have reported a protective effect of LZ supplements on AMD[137,145]. The AREDS2 investigators recommends the use of LZ supplements for patients with advanced stage of Early AMD who are at high risk of Late AMD[145].

Table 1.3.2-2: Summary of population-based cohort studies reporting risk of Late and Early age-related macular degeneration (AMD) associated with intake of lutein-zeaxanthin and other antioxidants.

Name/Location of Study	Type of Study	Method of analyses	Adjustment	Lutein-Zeaxanthin (dietary intake)	Other antioxidants (diet and/or supplement intake)
BMES[139,146]	Population-based cohort	Highest compared to lowest tertile	Age, sex, smoking and other factors	***Late AMD:*** RR 0.35 (95% CI 0.13-0.92) for 10-year incident neovascular AMD.	***Late AMD:*** RR 2.68 (95% CI 1.03-6.96) for 10-year incident neovascular AMD from baseline β-carotene intake (diet and supplements).
				Early AMD: No significant association for 5-year incident early AMD. RR 0.66 (95% CI 0.48-0.92) for 10-year incident indistinct soft or reticular drusen	***Early AMD:*** No significant associations.
BDES[147]	Population-based cohort	Highest compared to lowest quintile	Age, sex, smoking and other factors	***Early AMD:*** No significant association for 5-year incident early AMD, or early AMD lesions including large drusen and pigmentary abnormalitites.	***Early AMD:*** OR 0.52 (95% CI 0.3-1.0) for 5 year incident large drusen from previous intake of α-carotene (diet only).
Rotterdam[140]	Population-based cohort	Per 1-SD increase	Age, sex, smoking and other factors	***Any AMD:*** No significant association at 11 years.	***Any AMD:*** No significant association at 11 years.
NHS and HPFS[141,142]*	Prospective cohort	Highest compared to lowest quintile	Age, smoking and other factors	***Late AMD:*** No significant association between noeovascular AMD and LZ intake at 18 years. RR 0.59 (95% CI 0.48-0.73) for incident late AMD at 26 years. ***Early AMD:*** No significant association at 18 years.	***Late AMD*** (26-year follow-up): β-cryptoxanthin RR 0.73 (95% CI 0.60-0.89); α-carotene RR 0.69 (95% CI 0.56-0.84); β-carotene RR 0.82 (95% CI 0.67-1.01) (diet and supplements). ***Early AMD:*** No significant associations found at 26 years for any early AMD lesions.

BMES=Blue Mountains Eye Study, BDES=Beaver Dam eye Study, NHS=Nurses Health Study, HPFS=Health Professionals Follow-Up Study, SD=standard deviation, RR=relative risk, OR=odds ratio, CI=confidence interval

*Self-reported AMD with diagnosis confirmed by participants eye care professional

Vitamins and Zinc

The relationship between dietary intake of vitamins, vitamin supplement use, dietary or supplementary zinc and the incidence of AMD have also been investigated, with inconsistent findings [57,139,140,146,147]. The associations between vitamin or zinc intake and incident AMD in three population-based studies are summarized in **Table 1.3.2-3**.

After adjusting for age, sex, smoking and other factors, vitamin E intake from diet and supplements was associated with an increased risk of Late AMD at the 10-year follow-up visit of the BMES[139]; whereas, there was a reduced likelihood of incident any AMD associated with higher intake of vitamin E in the RS[140]. Increased vitamin E intake from diet alone was also associated with a reduced 5-year incidence of large drusen in the BDES[147] **(Table 1.3.2-3)**.

After adjusting for age, sex, smoking and family history of AMD, vitamin C intake from diet and supplements was associated with a 2-fold risk of Early AMD in 5 years, reported by the BMES team[146] **(Table 1.3.2-3)**. No other significant associations between vitamin C and incident AMD were reported from other studies.

After adjusting for age, sex, smoking and other factors, dietary zinc intake was associated with reduced incidence of any (Early or Late) AMD in both the BMES and RS[139,140]. Higher dietary zinc intake was found associated with reduced risk of Early AMD and early AMD lesions in the BMES[57,139] **(Table 1.3.2-3)**.

Dietary intake of combined vitamins (vitamin C, E and zinc) and antioxidant (β-carotene) was found associated with a 35% reduced risk of any (Early or Late) AMD at11 years in the RS, after adjusting for age, sex, smoking status and other factors[140].

The AREDS found zinc supplements to be beneficial to reduced progression to Late AMD among patients with advanced stage of Early AMD (AREDS categories 2 and 3) [144]. Thus zinc is an essential component of supplement formula recommended to patients with Early AMD[145].

Table 1.3.2-3: Summary of findings from population-based studies regarding the risk of Late AMD in relation to dietary and supplementary intake of vitamins and zinc.

Name/Location of Study	Type of Study	Method of analyses	Adjustment	Vitamins (diet and/or supplements)	Zinc (dietary intake)
BMES[139,146]	Population-based cohort	Highest compared to lowest tertile	Age, sex, smoking and other factors	***Late AMD:*** vitamin E intake (diet and supplements): RR 2.55 (95% CI 1.14-5.70) for 10-year incident late AMD.	***Any AMD:*** RR 0.56 (95% CI 0.32-0.97) for incident any AMD (top decile compared to rest of population)
				Early AMD: vitamin C intake (diet and supplements): OR 2.2 (95% CI 1.3-3.9) for 5-year incident early AMD.	***Early AMD:*** RR 0.54 (95% CI 0.30-0.97) for incident early AMD (NB. For top decile compared to rest of population)
BDES[147]	Population-based cohort	Highest compared to lowest quintile	Age, sex, smoking and other factors	***Early AMD:*** vitamin E intake (diet only): OR 0.4 (95% CI 0.2-0.9) for 5-year incident large drusen.	***Early AMD:*** No significant association for 5-year incident early AMD or early AMD lesions.
Rotterdam[140]	Population-based cohort	Per 1-SD increase	Age, sex, smoking and other factors	***Any AMD:*** vitamin E intake (diet and supplements): HR 0.92 (95% CI 0.84-1.00) for 11-year incident any AMD.	***Any AMD:*** HR 0.91 (0.83-0.98) for 11-year incident any AMD.

BMES=Blue Mountains Eye study, BDES=Beaver Dam Eye Study, AMD=age-related macular degeneration, SD=standard deviation, RR= relative risk, OR=odds ratio, HR=hazard ratio, CI=confidence interval

1.3.3 Other Risk Factors

Systemic risk factors found to be inconsistently associated incident AMD include blood pressure (BP), white blood cell count (WBCC), and serum lipids, in particular total cholesterol and high-density lipoprotein (HDL) cholesterol. A number of population-based cohort studies have reported the associations between these possible risk factors and prevalent AMD[148-153], however limited data exist on the associations with incidence or progression of AMD. Among dietary risk factors, alcohol consumption has also been investigated for its role in the incidence of AMD.

Blood Pressure

The relationship between increased BP or the presence of hypertension and incident AMD were assessed in majority of the studies mentioned in section 1.2. Overall, there were no significant associations between baseline BP and incident Early or Late AMD, after adjustment for age and sex in most cohorts[50,63,117,119,154,155], except for the 10-year follow-up report of the BDES where increase in systolic BP was found independently associated with the 10-year incidence of neovascular AMD[156]. Similarly, higher baseline pulse pressure was associated with the 10-year incidence of Early and Late AMD in the BDES[156] and the 4-year incidence of any AMD in the LALES[63], but not in any other population-based cohort.

White Blood Cell Count

The association between WBCC and incident AMD is inconsistent across different studies. Initial reports of this association were derived from observations of pathology specimens where leukocytes were present in neovascular membranes of eyes with neovascular AMD[157-159]. These findings suggested a link between inflammation and late

AMD, prompting the investigation of WBCC as a risk factor for AMD. C-reactive protein (CRP) is another inflammatory marker, found in drusen that may be considered as a risk factor for AMD.

There was no significant association between WBCC and incident Late AMD in most cohorts [117], except in the Hisayama study where higher circulating WBCC was associated with 9-year incident Late AMD after multivariable adjustment[50]. In the BMES, when stratified by sex and smoking status, there was an increased risk of 10-year incident Late AMD in men and in former smokers who had the highest compared to lowest tertile of baseline WBCC levels[160]. In the RS, increased high sensitivity CRP levels were associated with an increased risk of incident Late AMD at 11 years[161].

Of the two cohort studies that reported associations between WBCC and incident Early AMD, no significant associations were found in the BDES over 10 and 20 years[55,162], however, elevated baseline WBCC was associated with 10-year incidence of Early AMD in the BMES[160]. In an analysis of combined effects there was a >2-fold increased risk of 10-year incident Early AMD among those who had both WBCC in the highest tertile and ≥1 risk allele of *CFH*, compared to persons who had neither, in the BMES[96]. Combined effects for the presence of ≥1 risk allele of *ARMS2* together with a number of inflammatory markers on the incidence of Early AMD were demonstrated at 10-year follow-up of the BMES[102].

Lipids

The relationship between lipids, particularly total serum cholesterol and HDL cholesterol, and incident AMD have been widely examined, partly because cholesterol

was an important constituent of drusen[163-165]. Associations between cholesterol and incident AMD have been reported in a number of population-based cohorts[117,154-156].

Total cholesterol was associated with an increased risk of incident GA in a pooled data analyses of the BMES, BDES and RS[117], but was not associated with 5-year incidence of Late AMD in the Reykjavic Study[155].

High HDL cholesterol was associated with increased incidence of GA in the BDES[156], and also associated with increased incidence of GA and Late AMD in the Reykjavic Study[155]. In comparison, there was a reduced risk of GA and Late AMD over 5 and 10 years associated with increased baseline HDL cholesterol levels in the BMES[117,154].

Increased HDL cholesterol is known to be protective for cardiovascular disease risk[166-168], however, evidence to date about the associations between lipids and the long-term incidence and progression of AMD is largely inconsistent[169].

Alcohol

Alcohol is a modifiable dietary risk factor shown to increase oxidative stress, which can cause tissue damage in various organs including the retina[170,171]. Previous cross-sectional studies have shown that moderate alcohol consumption may provide a protective effect against AMD prevalence, with a J-shaped association[172,173]. Risk of developing AMD associated with low, moderate and high level consumption of alcohol also followed a similar pattern in magnitude of odds ratios in cohort studies, although the association was non-significant[122,174-176].

Heavy drinking or increased alcohol intake ≥4 drinks per day, compared to abstainers, was significantly associated with a 5-fold risk of developing Late AMD and a 9-fold risk of developing GA in the BDES, after adjusting for age, sex and other factors[118,177]. The effect was based on limited number of heavy drinkers, however, and therefore this observation could have been a chance finding[118,177].

Increased alcohol intake was significantly associated with increased incidence of Early AMD, observed in a number of studies [119,134,151], and marginally associated with 5-year incidence of Early AMD in men only after adjusting for age and sex in the BDES[178].

There were no significant associations between increased alcohol intake and incident Early or Late AMD in the Hisayama Study, Rotterdam Study, Physicians' Health Study and the NHS and HPFS over the follow-up periods of 8-13 years[50,174-176]. Lack of a uniform definition for heavy and moderate drinking across most population-based studies makes it difficult to compare findings about the associations between pattern of alcohol consumption and incident AMD.

1.3.4 Combined Risk Factors

Combinations of two or more risk factors have also been assessed for associations with AMD in a number of studies and in pooled cohort data of two or more study populations[56,57,115]. In the BMES, the 10-year incidence of Late AMD was increased among participants with joint exposure to smoking and low fish consumption compared to those with no exposure to either factor[115]. In the 3CC, pooled data analyses showed that the risk of Early AMD or any (Early and Late) AMD was significantly reduced among persons with high genetic risk (≥2 risk alleles of *CFH* and/or *ARMS2*) if they

consumed foods rich in LZ , compared to those with the same genetic risk but low dietary intake of LZ[57]. In the RS a similarly reduced risk of Early AMD was documented among persons with high genetic risk if they had high intake of zinc or zinc plus ω-3 fatty acids from diet or supplements, compared to those with low intake of the same nutrients[56].

Each risk factor may contribute a small proportion of risk for AMD development and progression. When two factors co-present, the risk difference between the exposed group (to both factors) and the non-exposed group (to either factor) would be stronger and easily detected.

In summary, age and genetic risk from the *CFH* and *ARMS2* genes are the most consistent risk factors for Early and Late AMD development[29,33-35,39,44,47-50,62,63,81,82,82,96,97,103,105]. A modifiable factor, smoking, is also consistently found to contribute an increased risk of Late AMD[50,63,114-117]. Of the other modifiable factors, diet rich in ω-3 fatty acids or regular fish consumption, intake of zinc and consumption of food rich in LZ, are relatively consistent protective factors associated with reduced incidence and progression of AMD, according to a majority of population-based and clinic-based studies[57,128-133,139,139,140,142,144]. Differentiated effect of these nutrients depending on the genetic susceptibility to AMD of individuals has been suggested[56,57,96,102], however, confirmation of these observations in different samples is needed.

Justification of the projects in the thesis

Many population-based cohort studies have described the incidence and progression of Early and Late AMD among older people of different ethnicities over the short- and mid-terms (<10 years in follow-up duration), and reported risk factors associated with the incidence and progression [29,30,38,39,44-46,48-50,50,63,96,114,117,122,130,134,140,146,147,155,175,178]. However, there is limited knowledge on the long-term (15+ years) incidence and progression of AMD and individual AMD lesions. There is also limited assessment of risk factors associated with the long-term incidence and progression of AMD. Projects included in this PhD thesis will fill in the gap of knowledge in this regard.

Chapter 2

Methods

2.1 Population-based cohorts

The findings presented in this thesis have been primarily drawn from the Blue Mountains Eye Study (BMES). In addition, comparisons between the BMES and the Singapore Epidemiology of Eye Diseases (SEED) study were made in one manuscript, and data from other cohorts within the Three Continent AMD Consortium (3CC) are also used in two manuscripts.

2.1.1 The Blue Mountains Eye Study (BMES)

Study Population

The BMES is a population-based study of vision and eye disease in older persons residing in the Blue Mountains region, west of Sydney Australia. The population was chosen from two postcode areas within the Blue Mountains region covering the suburbs of Katoomba, Leura and Medlow Bath (postcode 2780) and Wentworth Falls (postcode 2782).

Briefly, the two postcode areas were considered suitable for the study, with the population relatively stable and homogenous, largely European descents[36]. The population had an older age distribution compared with that of NSW and a slightly higher socioeconomic status compared to that of the Australian population[179,180].

At baseline, the BMES recruited 3654 participants (82.4% of those eligible) aged 49 years and older in 1992-1994 (BMES I). Surviving participants were invited to attend the next examination. Of the surviving baseline participants, 2334 (75.8% of survivors; 575 deceased) attended the 5-year follow up examinations in 1997-1999 (BMES II). The 10-year examinations were attended by 1952 participants (76.7% of survivors; a

further 535 deceased) in 2002-2004 (BMES III), and the final 15-year follow up examinations were attended by 1149 participants (56.1% of survivors; a further 496 deceased) in 2007-2009 (BMES IV). A flow chart of participation in the BMES is presented in **Figure 2.1.1-1**.

The mean (median, minimum and maximum) follow-up period for the 2334 BMES II participants was 5.1 years (4.9, 3.4 and 7.8 years); for the 1952 BMES III participants it was 10.5 years (10.4, 8.9 and 12.9 years) and for the 1149 BMES IV participants it was 15.6 years (15.5, 13.6 and 17.7 years). All 4 examinations were approved by Human Research Ethics Committees of the University of Sydney and Western Sydney Area Health Service, and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants at each visit.

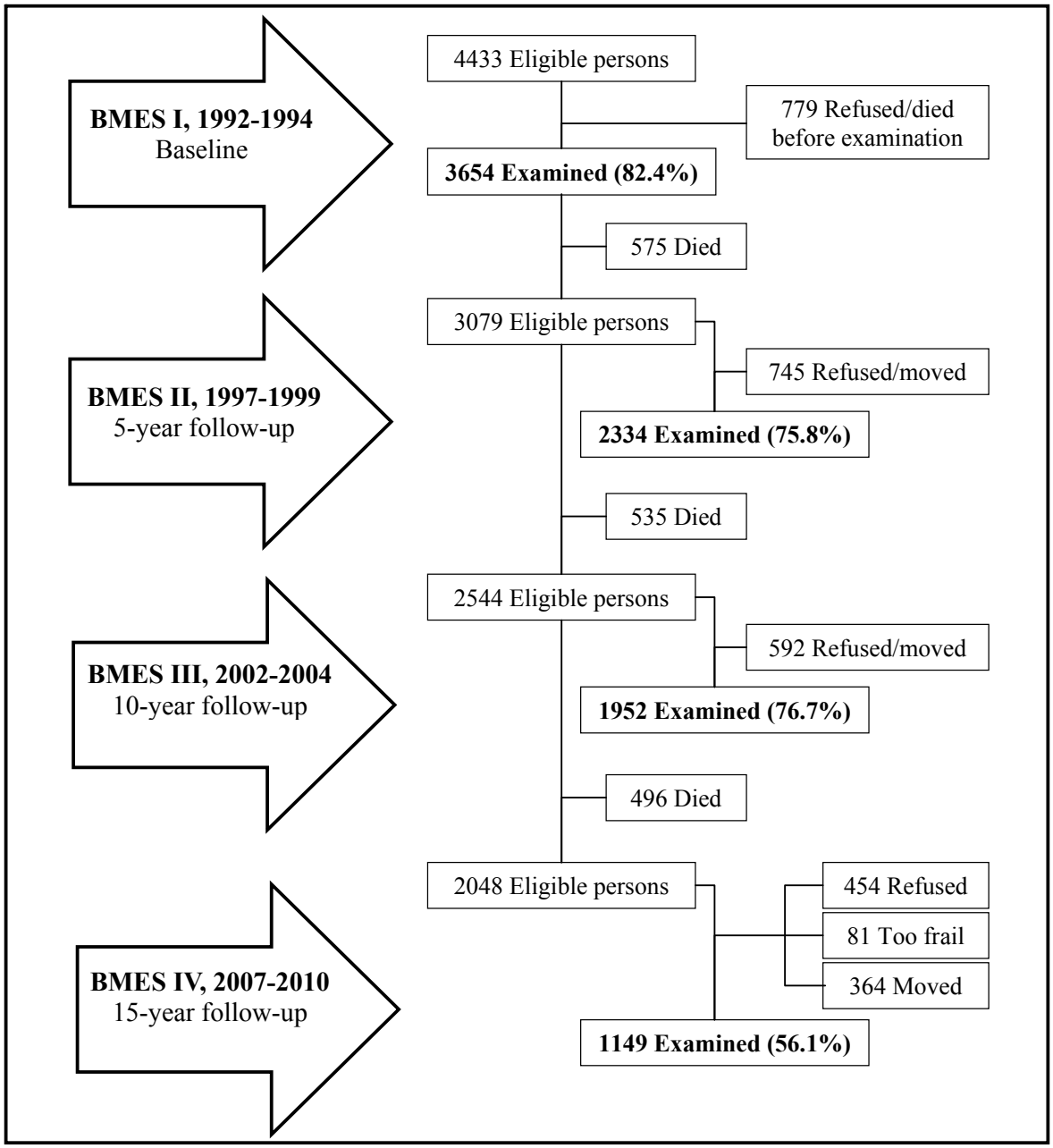

Figure 2.1.1-1 Flow chart of 15-year participation in the Blue Mountains Eye Study cohort, indicating eligible participants, refusals and examined participants at each time point.

Procedures

A comprehensive questionnaire was administered and eye examinations were performed at each visit, as described previously[35,36,44].

Trained interviewers completed a face-to-face interview with each participant using questionnaires that composed of comprehensive demographic and medical history information, including a detailed list of past and current medications, family histories of some systemic and ocular conditions, past medical diagnoses and surgeries, self-rated health and vision, smoking status and alcohol consumption (Appendix A). These questionnaires were completed prior to eye examinations.

Eye examinations included measurement of vision and subjective refraction using the BDES modification of the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol with logMAR chart[181], slit lamp examination of the lens and retinal fundus photography after pupil dilation using 1.0% tropicamide and 10% phenylephrine. Stereoscopic 30° retinal fundus photographs of the optic disc (ETDRS standard field 1) and macula (ETDRS standard field 2)[182] were obtained from both eyes. Non-stereoscopic photographs of the upper and lower arcades, fields temporal and nasal to the optic disc and temporal to the fovea were also taken (in total seven fields from each eye following the ETDRS protocol [182,183]. A Zeiss FF3 fundus camera (Carl Zeiss, Oberkochon, Germany) and Kodachrome 25 slide film (Kodak) were used for photography at the BMES I, II and III examinations. At the BMES IV examination, because of the unavailability of Kodachrome, 40° degree digital retinal images were obtained using a Canon CF-60 DSi fundus camera with DS Mark II body (Canon Inc., Tokyo, Japan).

Photographs were obtained from both eyes in 98%, 98%, 85% and 92% of the study populations at baseline, the 5-, 10- and 15-year examinations, and from at least 1 eye in 99%, 99%, 87% and 92% of the populations at baseline, the 5-, 10- and 15-year examinations, respectively.

Diet was assessed using a self-administered 145-item semi-quantitative food frequency questionnaire (FFQ) (Appendix B), which participants completed at home after attending their examination at baseline and each follow-up visit. Fasting blood samples were collected from participants who attended the BMES I, II and III examinations, for hematologic analysis and clinical biochemistry assessment. DNA extraction and genotyping were also performed in over 80% of BMES II and III participants.

2.1.2 The Singapore Epidemiology of Eye diseases Study (SEED)

Study Population

The SEED study includes three population-based study populations, namely the Singapore Malay Eye Study (SiMES), the Singapore Indian Eye Study (SINDI) and the Singapore Chinese Eye Study (SCES), representing the Malay, Indian and Chinese populations in Singapore. Baseline examinations of the SEED were conducted between 2004 and 2009.

Briefly, age-stratified random sampling was used to select 5600 Malay, 6350 Indian and 6752 Chinese names from the Ministry of Home Affairs[184-186]. Only residents aged 40-80 years were selected. After excluding participants who had moved from the residential address, had not lived at the address in the past 6 months, or were deceased

or terminally ill, the number of eligible participants included 4168 Malays, 4497 Indians and 4606 Chinese. Of these, a total of 3280 Malays (78.7% of those eligible) participated in the SiMES baseline examination between 2004-2006[187], 3400 Indians (75.6% of those eligible) participated in the SINDI baseline examination between 2007-2009, and 3353 Chinese (72.8% of those eligible) participated in the baseline examinations between 2009-2011[187]. All three studies were approved by the SingHealth Institutional Review Board and all examinations conducted at the Singapore Eye Research Institute in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to examinations.

Procedures

Each participant in the SEED studies underwent detailed interviewer-administered questionnaires and comprehensive eye examinations. The questionnaires contained sociodemographic, lifestyle data and medical histories, adapted from the BMES protocol and modified accordingly. The ocular examinations included assessment of visual acuity and subjective refraction, slit-lamp biomicroscopy, gonioscopy, intraocular pressure measurement using Goldmann applanation tonometry and ophthalmoscopy performed by ophthalmologists or ophthalmologist trainees. Pupils were dilated with 1% tropicamide and 2.5% phenylephrine hydrochloride, and 45° digital retinal photographs of the optic disc and macular fields were obtained using a Canon CR-DGi digital fundus camera, with10D SLR digital camera back (Canon, Tokyo, Japan). Retinal photographs of at least 1 eye were taken in 96.3% Malay, 97.9% Indian and 98.8% Chinese participants[187].

Non-fasting blood samples were collected for laboratory investigations including hematologic biochemistry tests and DNA extraction. Urine samples were also collected for biochemistry tests[188].

2.1.3 Cohorts of the Three Continent AMD Consortium (3CC)

The 3CC consists of four population-based cohort studies, including the BMES, BDES, RS and LALES. Only BDES and RS cohorts are briefly described in this sub-section.

The Beaver Dam Eye Study

Study Population

The BDES was conducted in the town of Beaver Dam, Wisconsin following a private census of the population from 1987-1988. Of 5924 eligible participants, 4926 (83.2%) aged between 43 and 86 years attended the baseline examination between 1988 and 1990. Of these participants, 3684 (81.1% of survivors) attended the 5-year follow-up examination from 1993-1995, 2764 (82.9% of survivors) attended the 10-year follow-up examination from 1998-2000, 2119 (85.4% of survivors) attended the 15-year follow-up examination from 2003-2005 and 1913 (over 80% of survivors) attended the 20-year follow-up examination from 2008-2010. Data were collected at each visit with institutional review board approval from the University of Wisconsin-Madison in conformity with all federal and state laws; the work was compliant with the Health Insurance Portability and Accountability Act and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants at each visit.

Procedures

At each visit, participants underwent a standardized face-to-face interview with questionnaires and examinations. Ocular examinations pertaining to AMD assessment included pharmacologic mydriasis, 30° stereoscopic color photographs of the optic disc and macula fields, and non-stereoscopic photographs of the other five retinal fields following the ETDRS protocol[182] were taken from both eyes, using a Zeiss FF4 fundus camera (Carl Zeiss, Oberkochon, Germany). Of the 2119 participants seen at the baseline, 5-, 10- and 15-year follow-up examinations, 96.4% had gradable retinal photographs in at least one eye at each visit. Casual blood specimens were also obtained at the time of each examination.

The Rotterdam Study

Study Population

The RS is a cohort study of persons aged 55+ years residing in Ommoord, a district of Rotterdam, the Netherlands. Names and addresses of residents were drawn from a municipal register. In random clusters all potential participants were invited to the study by letter and telephone call[189]. An interview was conducted at each participant's home that included questions of demographic characteristics, medical and ophthalmological histories, level of education obtained, ability in daily activities and a variety of other variables[190]. Physical examinations were later conducted at a screening centre. Residents living in nursing homes within the target area were also included, and examined in their homes[190].

At baseline (1990-1993), 7983 participants (77.7% participation rate) were examined in the RS, of whom 6419 had ophthalmic examinations and retinal photography

performed. Of these participants, 4977 (estimated 82.1% of survivors), 3637 (estimated 71.2% of survivors), and 2674 (number of survivors for this visit could not be estimated) were re-examined at the second (1993-1995), third (1997-1999) and fourth (2002-2004) visits, respectively. To correspond with the BMES and BDES 5-year follow-up interval, data from the second (2-year) follow-up visit (1993-1995) were excluded in analyses except for incident AMD cases that were included as the outcome event in the third (6-year) follow-up visit. All examinations were approved by the Medical Ethics Committee of the Erasmus Medical Centre and by the Ministry of Health, Welfare and Sport of the Netherlands, implementing the Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study). All examinations adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants at each visit.

Procedures

Prior to physical examinations, a 170-item validated semi-quantitative FFQ was administered during a face-to-face interview with a trained dietician, at the study site. Ocular examinations pertinent to AMD assessment included pharmacologic mydriasis and 35° stereoscopic colour retinal photographs of the macular field obtained using the Topcon TRV-50VT fundus camera (Topcon Optical Co., Tokyo, Japan) in the first 3 visits. At the fourth visit, 35° digital photographs were obtained using a Topcon TRC 50EX fundus camera with Sony DXC-950P digital camera (Topcon Optical Co.).

Casual blood samples were collected at baseline and fasting blood samples obtained at each of the follow-up examinations for clinical biochemical analyses and DNA extraction.

2.2 Assessing AMD

2.2.1 Photographic Grading

Photographic grading conducted for the BMES and SEED cohorts followed the Wisconsin Age-related Maculopathy Grading System (WARMGS) protocol[26], after simple modification by the BMES principal investigator (Paul Mitchell).

For each eye, the presence of AMD lesions, maximum individual drusen size, location and total macular areas involved by these lesions were assessed. The WARMGS grid was overlaid on the macular field of positive slides and a series of measurement circles were used to estimate drusen sizes and total areas involved by the lesions (**Figure 1.2.1-1)**. All retinal photographs taken from the same eye were placed into a clear plastic sheet to form the best stereo view of paired images for macular and optic fields. The sheet is then placed on a fluorescent viewing box (light box) with a Kelvin rating 6200°, and examined by graders using a Donaldson stereo-viewer with 5x magnification. For grading of digital images, a software program containing the digitized WARMGS grid and measurement circles (DH Client (Digital Healthcare: Image Management Systems, www.digital-healthcare.com, Cambridge, UK)) was used and applied in the same manner, overlying the macular photograph. Magnification was calculated and matched between the retinal images and WARMGS grid and measurement circles.

The WARMGS grid consists of 9 subfields demarcated by 3 concentric circles which are intersected by 4 radial lines (**Figure 1.2.1-1**). The radius of the inner circle corresponds to 500μm, the middle circle corresponds to 1500μm and the outer circle corresponds to 3000μm[26]. The subfields are designated as the following: the central subfield, the 'inner' superior, inferior, temporal and nasal subfields, and the 'outer'

superior, inferior, temporal and nasal subfields. An example of the WARMGS grid placement over the macular field, and the grading circles are shown in **Figure 1.2.1-1**.

The three sets of smaller measurement circles, C_0, C_1 and C_2, have diameters of 63µm, 125µm and 250µm, primarily used to estimated maximum drusen size[26,29]. The intermediate sized and large sized circles, I_1, I_2, O_1 and O_2, have diameters of 175µm, 350µm, 325µm and 650µm, respectively. These circles and additional two circles equivalent to a half a disc area (0.5 DA) and 1 disc area (1 DA) are primarily used to measure the collective area involved by various lesions including drusen, retinal pigment epithelium (RPE) abnormalities and sensory retinal or RPE detachment[29,36].

Retinal photographs taken at all visits of the BMES, and digital images of the SEED studies were graded in a masked manner by the same senior photographic graders in the BMES team. In the BDES and RS, retinal photographs were assessed by trained graders of each study team separately.

In the BMES, the presence, size and area of AMD lesions were recorded on a grading form (Appendix C). Inter- and intra-grader reliability showed good agreement for AMD grading, with quadratic weighted kappa values ranging from 0.64-0.93 and 0.54-0.94, respectively[36].

In the BMES and BDES, side-by-side grading was performed. For example, photographs of BMES I and II, and BMES I and III were graded side-by-side for participants with AMD lesions identified at any of the baseline and follow-up examinations[36,44]. Similar manner was also applied in the BDES.

2.2.2 Definitions of AMD and AMD Lesions

Late AMD Lesions

Neovascular AMD

Presence of either sub-retinal new vessels, serous or haemorrhagic detachment of the RPE or sensory retina, or sub-retinal fibrous scar in the macular area without other pathologic aetiology can be identified.

Geographic Atrophy (GA)

Sharply defined area of RPE and the sensory retinal loss with a diameter ≥175μm, exposing choroidal vessels.

Early AMD Lesions

RPE depigmentation

Sharply demarcated area with absence of the RPE but without visible choroidal vessels.

Hyperpigmentation

Granules or clumps of gray or black pigment in or beneath the retina.

Drusen

Discrete whitish-yellow material deposited between RPE cells and its basement membrane, the Bruch's membrane, which may be confluent, with sharp or fuzzy edges.

Types of Drusen

- Hard distinct drusen - drusen less than 63μm in diameter, with sharp edges but without a solid, thick, nodular appearance. This is not considered an AMD lesion.
- Intermediate soft drusen – maximum diameter of the drusen larger than 63μm but smaller than 125μm
- Soft distinct drusen - drusen greater than 125μm in diameter with uniform density, sharp edges and a solid thick appearance
- Soft indistinct drusen - drusen greater than 125μm in diameter with decreasing density from centre to periphery and fuzzy edges
- Reticular drusen – drusen that form ill-defined networks of broad interlacing ribbons.

Examples of neovascular AMD, GA, RPE depigmentation, hyperpigmentation and drusen were presented in **Figure 1.1-1** (Chapter 1).

AMD Stage

Late AMD

Late AMD was defined as the presence of any neovascular AMD lesions (RPE or sensory retinal detachment, disciform scars), photocoagulation scars with history of neovascular AMD, or GA[13].

Early AMD

Early AMD was defined as the presence of large (≥125μm in diameter) indistinct soft drusen, reticular drusen or the co-presence of large distinct soft drusen and retinal

pigmentary abnormalities (hyperpigmentation or RPE depigmentation) within the macula, in the absence of any late AMD lesions.

2.2.3 Definitions for AMD and AMD Lesion Incidence, Progression and Bilaterality

Incident AMD and incidence of individual AMD lesions; unilateral and bilateral AMD; and progression of AMD will be presented in chapters or sub-chapters that address these outcomes specifically.

Confirmation of AMD Incidence

All late AMD prevalent and incident cases in the BMES, BDES and RS were crosschecked by lead investigators of the 3CC study teams, and consensus was achieved on all late AMD cases that were used in research projects conducted by individual study teams or by the 3CC[32].

2.3 Risk Factor Assessment

Risk factors were assessed in relation to the incidence and progression of AMD and specific AMD lesions in the BMES. Common AMD risk factors assessed include age, sex and presence of genetic polymorphisms, as well as modifiable risk factors such as smoking and fish consumption.

Age, sex and smoking status were obtained from interviewer-administered examination questionnaires.

The Australian tables of food composition[191,192] and the United States Department of Agriculture carotenoid food composition database[193] were used to calculate the intake of nutrients from food and drink consumption specified in the FFQs.

Genetic polymorphisms were ascertained from genotyping. In the BMES, the *complement factor H (CFH)* single nucleotide polymorphism (SNP) *rs1061170* was genotyped using TaqMan assays (Applied Biosystems, Foster City, California)[96] and the *age-related maculopathy susceptibility 2 (ARMS2)* SNP *rs10490924* genotyped using restriction fragment length polymorphism analysis[102]. Additional genotyping was also performed with an Illumina Human 670-Quad custom array, version 1 (Illumina Inc., San Diego, CA) with stringent quality control testing using PLINK (Purcell S. PLINK version 1.07. Available at: http://pngu.mgh.harvard.edu/purcell/plink/ accessed July 12, 2013). After quality checking, genome-wide association scan (GWAS) data were imputed with the 1000 Genomes panel (Version 1 initially and Version 3 subsequently), using IMPUTE 2.0 (Department of Statistics, University of Oxford, Oxford, UK)[194]. The imputation r^2 was 0.968 for *CFH-rs1061170* and 0.996 for *ARMS2-rs104900924*.

2.4 Data Handling and Common Statistical Analyses

In the BMES, data recorded from the interviewer-administered questionnaires, the FFQ, blood test results and AMD grading sheets were entered into Microsoft Access databases by two independent data entry staff. Data entry errors were checked and any discrepancy between the two entries were verified and corrected.

The Statistical Analysis System, SAS[195], version 9.3 (SAS Institute Inc., Cary NC) was used for all statistical analyses.

Incident rates of AMD and AMD lesions were calculated using the Kaplan-Meier product-limit survival estimate and alternatively after adjusting for the competing risk of death. The BMES population was also directly age-standardized to the BDES population for comparison[33].

Frequency tables and the χ^2 statistic or Mantel-Haenszel procedure were used for comparing proportions. Logistic regression models were used to analyse associations between risk factors and the incidence or progression of AMD after adjusting for age, sex and smoking, and additional potential confounding variables. Generalized estimating equation (GEE) models using the GENMOD procedure in SAS[196] were applied to eye-specific data when assessing early AMD lesions characteristics in relation to AMD progression.

All means are presented with standard deviations (SD) and medians presented with maximum and minimum values. All association estimates are presented as odds ratios (ORs) with 95% confidence intervals (CIs).

Chapter 3

Long-Term Incidence of Age-related Macular Degeneration (AMD) and AMD Lesions in the Blue Mountains Eye Study

3.1 The 15-year incidence, progression and risk factors associated with age-related macular degeneration

Publication relating to this section of Chapter 3:

Joachim N, Mitchell P, Burlutsky G, Kifley A, Wang JJ. The Incidence and Progression of Age-Related Macular Degeneration over 15 Years: The Blue Mountains Eye Study. *Ophthalmology* 2015; 122 (12): 2482-2489

ABSTRACT

Purpose: To assess the 15-year incidence and progression of age-related macular degeneration (AMD) in an older Australian population.

Methods: Blue Mountains Eye Study (BMES) participants (n=3654), aged 49+ years were examined during 1992-1994. Of these 2334 participants (75.8% of survivors) were re-examined after 5 years (1997-1999), 1952 (76.7% of survivors) after 10 years (2002-2004) and 1149 (56.1% of survivors) after 15 years (2007-2010). Color retinal photographs were taken and comprehensive questionnaires administered at each visit. Blood samples were collected and DNA genotyped. Retinal photographic grading was performed by the same graders at each examination following the Wisconsin AMD grading protocol. Side-by-side comparisons were used to confirm newly-developed AMD lesions. Incidence was estimated using Kaplan-Meier estimates. Associations of AMD incidence with age, sex, smoking status, presence of the *complement factor H* (*CFH*)-*rs1061170* and *age-related maculopathy susceptibility 2* (*ARMS2*)-*rs10490924* polymorphisms and fish consumption were analyzed using discrete logistic regression models. Generalized estimation equation models were used to assess the risk of incident late AMD associated with baseline AMD lesion characteristics.

Results: The 15-year incidence was 22.7% for early AMD and 6.8% for late AMD. After adjusting for competing risks, early and late AMD incidence was 15.1% and 4.1%, respectively. Age was strongly associated with early and late AMD incidence (both $p<0.0001$). After age-standardization to the Beaver Dam Eye Study (BDES) population, early and late AMD incidence in the BMES was 13.1% and 3.3%, respectively. Female sex and the presence of both risk alleles of *CFH-rs1061170* or

ARMS2-rs10490924 were independently associated with early AMD incidence, whereas current smoking and presence of ≥1 risk allele of *CFH-rs1061170* or *ARMS2-rs10490924* were associated with late AMD incidence. Fish consumption was inversely associated with late but not early AMD incidence. Severity of early AMD lesion characteristics was a strong predictor of progression to late AMD.

Conclusion: We documented the 15-year incidence of early and late AMD in an older Australian population that were comparable to BDES observations. Risk of progression to late was strongly associated with severity of early AMD lesions.

BACKGROUND

Age-related macular degeneration continues to be one of the leading causes of blindness and visual impairment in older populations despite recent advances in treatments[6,45,54,197]. The incidence and progression of early and late-stage AMD over 5 and 10 years has been reported in a number of large population-based studies in the United States, Europe, Asia and Australia over the past two decades[29,34,35,38,44,45,48]. Greater severity of early AMD lesions including increased drusen area, presence of pigmentary abnormalities and location of lesions close to the fovea, was shown to be associated with greater risk of progression to late AMD[33-35,38]. The relationship between demographic and lifestyle risk factors including older age, sex and smoking status with the incidence and progression of AMD was also shown in some of these populations[117].

However, data on the incidence of AMD over the long-term (>10 years) are limited. The Copenhagen City Eye Study and the Beaver Dam Eye Study (BDES) are the only population-based studies thus far, to report 14- and 15-year AMD incidence, respectively[33,47]. In this report, we aimed to build on the previous 5- and 10-year AMD incidence findings to describe the 15-year incidence of early and late AMD and its component lesions in an older Australian population (the Blue Mountains Eye Study (BMES)); and to assess risk factors and baseline early AMD lesions characteristics associated with the risk of progression to late AMD over the longer term.

METHODS:

Study Population, Procedures and Photographic Grading

Details of the BMES population and the general procedures conducted at each examination have been described in Methods (Chapter 2). Retinal fundus photographs

were obtained and comprehensive demographic and lifestyle questionnaires administered at the baseline, 5-year, 10-year and 15-year examinations. Photographs were obtained for both eyes in 98%, 98%, 85% and 92% at the baseline, 5-, 10- and 15-year examinations, and for at least 1 eye in 99%, 99%, 87% and 92% at the baseline, 5-, 10- and 15-year examinations, respectively. Diet was assessed from a self-administered food frequency questionnaire completed by participants at each examination.

Retinal photographic grading was performed by 2 senior graders and closely followed the Wisconsin Age-Related Maculopathy Grading System (WARMGS) protocol[26]. Adjudication was provided by a senior retinal specialist (Paul Mitchell) if needed. Consensus on BMES incident late AMD cases was provided by lead investigators of the Three Continent AMD Consortium[32].

Late AMD was defined as the presence of neovascular AMD, indicated by retinal pigment epithelial or neurosensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars with history of neovascular AMD; or the presence of pure geographic atrophy (GA) within the macula, as described in the International Age-Related Maculopathy Classification[13]. Early AMD was defined as the presence of large (≥125μm in diameter) indistinct soft drusen, reticular drusen or the co-presence of large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or depigmentation of retinal pigment epithelial (RPE) cells), within the macula, in the absence of any late AMD lesions. The maximal diameter of individual drusen and collective macular areas involved by drusen and pigmentary abnormalities within the eye was estimated using the WARMGS measurement circles [26].

Definition of AMD Incidence

Incident late AMD in the first eye was defined as the appearance of neovascular AMD or GA in either eye at any follow-up examination when the lesion was not present in either eye at baseline. For participants at risk of incident neovascular AMD, cases with this lesion at baseline were excluded but cases with GA at baseline were not excluded. Participants with GA or neovascular AMD at baseline and with neovascular AMD at follow-up were excluded from those at risk of incident GA. If GA was secondary to neovascular AMD or laser treatment of neovascular AMD it was not considered as incident GA. The BMES participants who developed late AMD during the follow-up period were all seen by the principal investigator of the BMES (Paul Mitchell) for confirmation, and were treated and followed at the Eye Clinic, Westmead Hospital. These participants also were labelled using BMES identification numbers in their patient records and were included as incident late AMD cases.

Incident early AMD in the first eye was defined as the appearance of either indistinct soft or reticular drusen, or the co-presence of distinct soft drusen and retinal pigmentary abnormalities in either eye, at any follow-up examination in whom no late or early AMD was present in either eye at baseline. Participants with either distinct soft drusen or retinal pigmentary abnormalities alone at baseline who later developed complementary lesions that comprised a diagnosis of early AMD were included as incident early AMD cases. Incidence of indistinct soft or reticular drusen was defined as the appearance of these lesions in either eye at follow-up visits, where none were present at baseline, and excluding late AMD, regardless of the presence of retinal pigmentary abnormalities. Incidence of retinal pigmentary abnormalities was defined as

the appearance of these abnormalities in either eye at follow-up visits in participants with no pigment abnormalities at baseline, and no late AMD at any follow-up visits.

The incidence of early and late AMD in the second eye of participants with unilateral early or late AMD at baseline, and the progression from early AMD to late AMD in at least one eye over 15 years, were assessed among persons with AMD in one or both eyes at baseline.

Risk Factors for Incident AMD

Lifestyle, genetic and dietary data were obtained at each examination as described in Methods (Chapter 2). Briefly, for smoking history, participants were classified as non-smokers if they answered 'no' to the question whether they smoke regularly. Past smoking was defined if participants had smoked regularly but quit smoking more than 1 year prior to the examination. Current smoking was defined if participants were current smokers or had stopped smoking <1 year before the examination. Regular fish consumption was defined as consuming ≥1 serving of fish per week.

Statistical Analyses

The 15-year person-specific incidence of early and late AMD and their component lesions were estimated using Kaplan-Meier product limit survival estimates, and alternatively, using competing risk analyses to control for the risk of death. The BMES population was also directly age-standardized to the BDES population[33], to compare 15-year AMD incident rates between the two populations. Further, the probabilities of late AMD development from different severity levels of AMD over 15 years were reported according to steps on the AREDS simplified severity scale[28].

The associations between known AMD risk factors (age, sex, smoking, fish consumption, the complement factor H (*CFH*)-*rs1061170* and age-related maculopathy susceptibility 2 (*ARMS2*)-*rs10490924* risk alleles) and the 15-year incidence of early and late AMD were assessed using age-sex-adjusted, and multivariable-adjusted discrete logistic regression models. Generalized estimating equation models were applied to eye-specific data to assess the associations between incidence of late AMD and early AMD lesion characteristics (area and location of drusen and retinal pigmentary abnormality). For comparison, the association between 15-year incident late AMD and steps on the AREDS simplified severity scale[28] were also assessed using person-specific data, with time intervals included in the model. Association estimates are presented as age-sex-smoking-adjusted or multivariable-adjusted (age, sex, smoking, fish consumption, the *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles) odds ratios (ORs) and 95% confidence intervals (CIs).

RESULTS

We included participants who were censored up to either the 5-, 10- or 15-year follow-up examination to estimate incidence. Of 3654 baseline participants, 854 (23.4%) died with no follow-up information available and 326 (8.9%) were lost to follow-up and had no retinal photographs available at all 3 time points, leaving 2474 (67.7%) with gradable retinal photographs who were examined at either the 5-, 10- or 15-year examinations, or 2 or all 3 examinations. Of these 2474 participants, 574 (23.2%), 75 (3.0%) and 7 (0.3%) were seen only at 5-, 10- or 15-year examination, respectively, 789 (31.9%) were seen at two of the examinations (5- and 10-year, 5- and 15-year or 10- and 15-year examinations) and 1029 (41.6%) were seen at all three examinations.

Table 3.1-1 compares the baseline characteristics between participants examined at either or all of the 5-, 10- or 15-year examination (n=2474) and those who were alive but not examined at any follow-up examination (n=326) or those who died (n=854) without re-examination in the BMES cohort. Compared to those who were followed, those who were lost to follow-up were more likely to have been younger at baseline (mean age: 60.6 versus 64.3 years), to have a lower socioeconomic status (defined by homeownership and trade or higher qualification), to be current smokers (22.5% versus 13.1%) but less likely to have a history of heart disease (7.7% versus 14.4%). Histories of stroke, cancer, diabetes, hypertension and self-ranked health were not significantly different among those examined versus those not examined. Participants who died without attending any follow-up were on average 10 years older, more likely to be living alone, to have walking disabilities and systemic diseases and to use community services at baseline (**Table 3.1-1**).

Table 3.1-1: Comparison of baseline characteristics between participants examined and not examined at 15 years in the Blue Mountains Eye Study Cohort.

Baseline Characteristics	Examined (n=2474)	Not examined or no photographs (n=326)	P Value*	Died (n=854)
Mean age (years) (95% CI)	64.3 (64.0-64.7)	60.6 (59.8-61.5)	<0.0001	73.7 (73.1-74.4)
Age group:	**%**	**%**		**%**
<60	31.6	50.3	<0.0001	8.7
60-69	40.7	35.0	0.05	22.1
70-79	22.9	13.5	0.0001	41.0
≥80	4.8	1.2	0.003	28.2
Women	57.6	63.5	0.04	51.5
Currently married	66.1	62.0	0.1	53.0
Home owner	91.1	82.4	<0.0001	83.8
Low job prestige	36.3	37.4	0.7	42.5
Trade or higher qualification	60.6	55.3	0.08	51.2
Living alone	25.5	24.2	0.6	34.6
Walking disability	3.3	3.4	1.0	20.4
Regular use community services	3.7	4.6	0.4	15.8
Self-ranked health:				
Excellent	21.8	20.9	0.7	13.8
Good	57.1	56.7	0.9	47.4
Fair	18.9	18.4	0.8	29.8
Poor	2.2	4.1	0.04	9.0
History of Stroke	3.3	3.1	0.8	12.1
History of heart disease	14.4	7.7	0.0009	24.3
History of cancer	7.4	6.1	0.4	12.5
History of diabetes	6.3	8.6	0.1	11.6
Presence of hypertension	70.0	66.6	0.2	78.2
Smoking status:				
Never	85.6	75.9	<0.0001	80.6
Past	1.3	1.6	0.6	1.3
Current	13.1	22.5	<0.0001	18.1
Fish Consumption (≥1 serving/wk)	59.4	62.0	0.4	60.2
CFH-rs1061170				
TT	39.3	25.0		20.0
CT	46.7	75.0	0.5†	60.0
CC	14.0	0.0		20.0
ARMS2-rs10490924				
GG	61.8	25.0		50.0
GT	34.0	75.0	0.2†	50.0
TT	4.3	0.0		0.0

n=sample size; CI=confidence interval; CFH=complement factor H (C risk allele); ARMS2=age-related maculopathy susceptibility gene 2 (T risk allele)

*P-value for difference between participants examined and not examined (or had no photographs), excluding those who had died.

†Unadjusted tests for heterogeneity used to calculate P values

The 15-year incidence of early and late stage AMD lesions by age and sex is presented in **Table 3.1-2**. There was an increased 15-year incidence of late AMD associated with older age. Although a similar increase was observed for early AMD including up to 80 years of age, the 15-year incidence of early AMD, particularly incidence of indistinct or reticular drusen, decreased in those aged ≥80 years old at baseline. The 15-year incidence of both early AMD and neovascular AMD was higher in women compared to men. After adjusting for gender, age was strongly associated with incidence of early and late AMD lesions (both $P<0.0001$).

Table 3.1-2: Fifteen-year incidence of late and early AMD lesions by age and sex.

Incident AMD Lesions	Age at Baseline (years)										Women	Men
	<60		60-69		70-79		≥80		All Ages			
	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	Incidence (%)	Incidence (%)
Geographic atrophy	774	0.4	960	2.1	519	9.5	102	19.3	2355	2.6	2.5	2.8
Neovascular AMD	778	0.7	988	4.8	547	12.9	108	13.8	2421	4.4	5.2	3.3
Any late AMD	778	1.1	988	6.8	547	20.2	108	27.3	2421	6.8	7.5	6.0
Early AMD	737	8.7	855	26.9	388	51.4	56	29.3	2036	22.7	25.7	18.4
Indistinct/ reticular drusen	755	7.1	918	21.0	440	40.3	72	19.2	2185	18.1	21.5	13.5
Pigmentary abnormality	729	21.4	871	32.4	440	48.7	83	42.6	2124	31.1	32.1	29.6

*Using Kaplan-Meier estimates incorporating persons censored up to the 5-, 10- and 15-year examinations.

The 15-year incidence of early and late AMD in the BMES population, after adjusting for the competing risk of death, was 15.1% and 4.1%, respectively. Using the competing risk method, the BMES early and late AMD incidence was age-standardized to that of the BDES population. We found similar age-standardized incident rates of early AMD (13.1%, 95% CI 11.7% -14.6%, versus 14.3%, estimated 95% CI 13.1% - 15.5%) and late AMD (3.3%, 95% CI 2.6%-4.0%, versus 3.1%, estimated 95% CI 2.6%-3.6%) over 15 years in the BMES when compared to the corresponding incidence rates in the BDES population[33].

The AREDS simplified severity scale was applied to the BMES baseline AMD status, and the probabilities of developing late AMD over 15 years from various levels in the scale are shown in **Table 3.1-3**. The 15-year incidence of late AMD among persons at Step 0 at baseline ranged from 1.1% to 4.5%; at Step 1, 2 and 3, the 15-year incidence of late AMD ranged from 4.5% to 12.0%, 21.1% to 41.7% and from 0.0% to 33.3%, respectively. Corresponding incidence among persons at Step 4 (baseline) was 76.5% (**Table 3.1-3**).

Table 3.1-3: Number and proportion of participants who developed late AMD over 15 years by levels of the Age-Related Eye Disease Study (AREDS) simplified severity scale for AMD at baseline

Drusen size and number of eyes	Pigment Abnormality					
	None (C1)		One eye (C2)		Two eyes (C3)	
	No. of events/ No. of subjects	%*	No. of events/ No. of subjects	%*	No. of events/ No. of subjects	%*
None or small, only one or both eyes (R1)	19/1573	1.2 (0.6)	3/67	4.5 (1.5)	4/19	21.1 (21.1)
Intermediate, one eye (no large) (R2)	10/224	4.5 (2.7)	3/25	12.0 (4.0)	5/12	41.7 (36.4)
Intermediate, both eyes (no large) (R3)	6/55	10.9 (7.4)	3/10	30.0 (20.0)	0/4	0.0 (0.0)
Large, one eye (R4)	7/61	11.5 (8.1)	4/14	28.6 (28.6)	5/26	19.2 (11.5)
Large, both eyes (R5)	5/18	27.8 (27.8)	3/9	33.3 (33.3)	13/17	76.5 (70.6)

*Blue Mountains Eye Study 10-year incidence rates in parentheses.

AREDS Step 0 = R1C1 and R2C1; No retinal pigment changes with none or small hard drusen in 1 or both eyes or intermediate (but not large) drusen in 1 eye only.

AREDS Step 1 = R1C2, R2C2, R3C1 and R4C1; Pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/no pigment changes in either eye but intermediate drusen in both eyes or large drusen in one eye.

AREDS Step 2 = R1C3, R2C3, R3C2, R4C2 and R5C1; Pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye/no pigment changes in either eye but large drusen in both eyes.

AREDS Step 3 = R3C3, R4C3, R5C2; Pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye/ pigment changes in 1 eye with large drusen in both eyes.

AREDS Step 4 = R5C3; Pigment changes in both eyes with large drusen in both eyes.

After controlling for the competing risk of death, the cumulative incidence of early AMD in the second eye of persons with early AMD in the first eye was 67.0% over the follow-up period. The corresponding incidence of late AMD in the second eye of persons with late AMD in the first eye was 35.4%, and of those with early AMD in the first eye, was 24.6%.

Known AMD risk factors associated with 15-year incident early and late AMD are listed in **Table 3.1-4**. In the age-sex adjusted model, 2 risk alleles of *CFH-rs1061170* (OR 2.8) and either 1 or 2 risk alleles of *ARMS2-rs10490924* (ORs 1.6-2.6) were associated with greater risk of 15-year incident early AMD. There was no significant association between smoking or fish consumption and 15-year early AMD incidence. Current smoking at baseline and the presence of at least 1 risk allele of *CFH-rs1061170* (ORs 1.9-3.8) or *ARMS2-rs10490924* (ORs 1.8-4.9) were significantly associated with an increased risk of 15-year incident late AMD. Conversely, the incidence of late AMD was significantly reduced among persons who consumed ≥1serving of fish per week (OR 0.5) compared to those who consumed fish less than weekly. These associations remained significant after multivariable adjustment as shown in **Table 3.1-4**.

Table 3.1-4: Common AMD risk factors associated with 15-year incidence of early and late stage AMD

Risk Factor	15-year Incidence of Age-related Macular Degeneration			
	Early AMD		Late AMD	
	Age-sex adjusted OR (95% CI)	Multivariable-adjusted OR (95% CI)*	Age-sex adjusted OR (95% CI)	Multivariable-adjusted OR (95% CI)*
Age, per 10 years	1.12 (1.10-1.14)	1.11 (1.09-1.14)	1.17 (1.13-1.20)	1.20 (1.16-1.25)
Sex (male)	0.70 (0.55-0.90)	0.66 (0.48-0.90)	0.77 (0.50-1.16)	0.80 (0.48-1.32)
Smoking (current)	1.14 (0.74-1.73)	1.45 (0.90-2.36)	3.96 (2.31-6.80)	3.63 (1.86-7.06)
CFH-rs1061170				
TT	1.00	1.00	1.00	1.00
CT	1.18 (0.88-1.59)	1.08 (0.78-1.51)	1.91 (1.12-3.29)	2.25 (1.22-4.15)
CC	2.81 (1.97-4.00)	2.56 (1.71-3.84)	3.77 (2.03-6.99)	4.45 (2.19-9.03)
ARMS2-rs10490924				
GG	1.00	1.00	1.00	1.00
GT	1.63 (1.24-2.14)	1.53 (1.12-2.08)	1.79 (1.14-2.80)	2.59 (1.56-4.31)
TT	2.58 (1.38-4.81)	2.16 (1.07-4.37)	4.88 (2.06-11.55)	5.81 (2.09-16.12)
Fish Consumption (≥1 servings/wk)	0.90 (0.69-1.17)	0.92 (0.68-1.24)	0.45 (0.29-0.71)	0.48 (0.29-0.79)

OR=odds ratio; CI=confidence interval; CFH=complement factor H (C risk allele); ARMS2= age-related maculopathy susceptibility gene 2 (T risk allele)

*Mulivariable-adjusted logistic regression model including age, sex, smoking, CFH and ARMS2 polymorphisms and fish consumption.

Table 3.1-5 presents the eye-specific associations between baseline early AMD lesion characteristics and the 15-year incidence of late AMD. After adjusting for age, sex and smoking, the presence of large drusen (OR 7.0), indistinct soft drusen (OR 19.3), drusen location closer to the foveal centre (OR 7.3-21.0) and larger drusen area (OR 7.2-30.9) were highly predictive of the late AMD development. Likewise, the presence of retinal pigmentary abnormalities (OR 6.6) was associated with increased risk of late AMD as was each step increment in the AREDS 5-Step scale (ORs 4.8-169.4). These associations remained after further adjustment for fish consumption and *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms.

Table 3.1-5: The 15-year risk of late AMD by baseline early AMD characteristics.

Baseline Early AMD Lesion Characteristics[*]	Crude Late AMD Incidence (%)			Age, Sex and Smoking Adjusted OR (95% CI)	Multivariable Adjusted OR (95% CI)[†]
	5 Year	10 Year	15 Year		
	Eye-Specific				
Maximum Drusen size					
None or <125µm	0.5	3.1	3.0	1.0	1.0
≥125µm	13.0	39.7	14.3	7.0 (4.0-12.2)	7.4 (4.0-14.0)
Drusen type					
None or small drusen <125µm	0.5	2.9	3.0	1.0	1.0
Distinct soft drusen	1.3	11.5	3.7	1.3 (0.5-3.9)	1.6 (0.5-5.1)
Indistinct soft drusen	22.6	62.5	50.0	19.3 (9.7-38.6)	21.4 (9.4-48.5)
Drusen location					
None or <63µm with an area <250µm in diameter	0.3	2.0	2.5	1.0	1.0
1500-3000µm from foveal centre	1.0	2.6	5.9	1.0 (0.3-2.8)	1.4 (0.5-4.3)
500-1500µm from foveal centre	9.3	39.3	21.4	7.3 (3.7-14.5)	8.2 (3.7-18.2)
within 500µm radius of foveal centre	17.8	51.2	50.0	21.0 (10.7-41.0)	18.5 (8.7-39.2)
Drusen area					
None or <375µm in diameter	0.5	2.9	3.0	1.0	1.0
≥375µm in diameter to <0.5 disc area	7.0	34.8	16.7	7.2 (3.2-16.3)	7.9 (3.2-19.7)
≥0.5 disc area	30.0	79.0	66.7	30.9 (13.8-69.2)	33.0 (12.3-88.4)
Retinal pigmentary abnormality					
Absent	0.4	3.4	2.8	1.0	1.0
Present	10.9	34.6	13.1	6.6 (3.9-11.1)	7.2 (4.1-12.8)
	Person-Specific				
AREDS simplified AMD severity scale					
Step 0[‡]	0.2	1.8	1.9	1.0	1.0
Step 1[§]	2.4	9.0	13.3	4.8 (2.5-9.3)	7.8 (3.6-17.0)
Step 2[ǁ]	11.0	50.0	10.5	12.6 (6.2-25.6)	15.2 (6.8-34.0)
Step 3[¶]	12.8	9.1	22.2	10.1 (3.5-28.8)	13.1 (3.3-52.1)
Step 4[#]	47.1	80.0	100.0	169.4 (55.2-519.9)	119.4 (24.6-580.5)

OR = odds ratio, CI = confidence interval, AREDS = Age-Related Eye Disease Study

[*]Assessed within the anatomical macular area (within 3000µm radius of the foveal centre)

[†]Multivariable ORs adjusted for age, sex, smoking status, *CFH-rs1061170*, *ARMS2-rs10490924* and fish consumption

[‡]No retinal pigment changes with none or small hard drusen in 1 or both eyes or intermediate (but not large) drusen in 1 eye only.

[§]Pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/no pigment changes in either eye but intermediate drusen in both eyes or large drusen in one eye.

[ǁ]Pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye/no pigment changes in either eye but large drusen in both eyes.

[¶]Pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye/ pigment changes in 1 eye with large drusen in both eyes.

[#]Pigment changes in both eyes with large drusen in both eyes.

DISCUSSION

We found an overall 15-year incidence of 22.7% for early AMD and 6.8% for late AMD, in persons aged 49+ years in this older Australian cohort. After adjusting for the competing risk of death, the incidence of early and late AMD was 15.1% and 4.1%, respectively. The 15-year incidence of late AMD or late AMD lesions increased with increasing age, however, the incidence of early AMD decreased in persons aged 80+ years at baseline. Women had a higher 15-year incidence of any AMD compared to men.

Our study is one of very few studies to report the long-term (>10 years) incidence of AMD from an older population-based cohort. The Copenhagen City Eye Study[47] reported the 14-year incidence of early and late AMD as 31.5% and 14.8%, respectively, considerably higher than our incidence estimates observed in the BMES. The older age range (60-80 years) of the Copenhagen study sample and the low 14-year follow-up rate of 38%[47] could explain the relatively high incidence found in this study. In the BDES, the 15-year early AMD incidence was 14.3%, while late AMD was 3.1%[33], comparable to our 15-year incidence estimates in the BMES, after age standardization to the BDES population.

We found that the overall 15-year incidence of both early and late AMD was substantially higher, compared to the 10-year incident rates in the BMES cohort. However, the 15-year incidence of early AMD was relatively lower among participants aged 80 years or older, dissimilar to previous findings of 10-year early AMD incidence in the same cohort[35]. The lower incidence in this oldest old age group may be due to

high mortality and the low number of subjects who were at risk of early AMD at this age.

The AREDS simplified severity scale was previously validated using the 10-year incidence of late AMD data from the BMES[35]. We found that the probabilities of developing late AMD by baseline AREDS scale Steps 0 and 1 over 15 years were twice as high as the 10-year late AMD incidence from the same steps. Whereas the probabilities of developing late AMD by baseline AREDS scale Steps 2, 3 or 4 (when both eyes had large soft drusen and/or retinal pigmentary changes) over 15 years were somewhat similar to the 10-year late AMD incident rates[35]. This observation could suggest that the time needed for progression from severe early AMD to late AMD may likely be within 10 years or less, while the duration of 15 years is more applicable to those with less severe stages of early AMD at baseline.

Second eye incidence of late AMD has been reported in a number of clinic-based studies[198-201]. In the BMES, we found substantially higher incidence of early or late AMD in the second eye of persons with either early or late AMD in the first eye at baseline. This is comparable to previous observations in the BDES which reported a 39% second eye incidence of late AMD in those with unilateral late AMD at baseline[33].

We previously demonstrated that incidence of specific early AMD lesions and late AMD was greater in women compared with men[35,44]. Although we found a similar pattern in this report, the association between female sex and 15-year incident AMD was only significant for early but not for late AMD. We also found that current smoking at baseline was significantly associated with incident late but not incident early AMD

over 15 years. This is in keeping with previous findings from the BMES and other studies of white populations including the Rotterdam study[115,117,123,150]. Weekly fish consumption of at least one or more servings was associated with a reduced risk of late but not early AMD over the 15-year follow-up period. Similar findings were reported in a meta-analysis of pooled data from nine studies, in that fish intake at least twice a week was associated with reduced risks of early and late AMD[202]. Consistent with genetic knowledge of AMD, 2 risk alleles of *CFH* and either 1 or 2 risk alleles of *ARMS2* were significantly associated with increased risk of early and late AMD over the longer term, with a greater risk magnitude for late AMD[78].

Increasing severity of baseline early AMD lesions, including larger drusen size (≥125μm), drusen location closer to the fovea, larger area involved by drusen and the presence of RPE abnormalities, have been well-recognized to predict the incidence of late AMD[33-35,38]. We also previously reported high risk of developing GA from eyes with indistinct soft and reticular drusen, central location and larger area involved by drusen over 15 years[203]. These associations were generally consistent across the 10- and 15-year AMD incidence although with different risk magnitude: the risk of late AMD associated with baseline early AMD lesion characteristics was higher for the 10-year than the 15-year data, and particularly so for central location of soft drusen. This observation may suggest that early AMD characteristics are indicative of a more severe stage likely leading to late AMD within 10 years rather than 15 years.

We were able to follow about 75% of survivors of this older cohort at the 5- and 10-year visits. However, follow-up rate reduced to only 56% by the time the 15-year examinations were performed. This could have introduced selection bias due to

selective follow-up and survival. Persons who died were older and more likely to have chronic systemic conditions and disabilities. Participants who were lost to follow up were likely to have been younger (mean age, 61, versus 64) and more likely to smoke (23% versus 13%) at baseline. It is thus likely that our estimates of the 15-year incidence of AMD could have been underestimated, although the "true incidence" would not be more useful than the current estimate, as only those who survived demand eye health and aged care services.

Strengths of this study include the relatively long-term follow-up of a population-based cohort, the use of retinal photographs to document macular conditions and a validated AMD grading system to assess size and location of AMD lesions. A major limitation of our study is the substantial number of participants lost to follow-up at the 15-year visit, as mentioned above. A further limitation includes lack of high-resolution imaging (e.g. spectral-domain optical coherence tomography), unavailable at the time of the BMES examinations, that might have increased the ability to detect some AMD lesions such as early stage GA.

SUMMARY

This report shows the 15-year incidence of early and late AMD in an older Australian cohort, particularly among persons with no lesions or only early-stage lesions at baseline. The incidence rates for early and late AMD in this cohort were comparable to those reported in the BDES population over the same follow-up period. Current smoking at baseline was a stronger risk factor for 15-year incidence of late AMD than early AMD.

3.2 Long-Term Incidence and Natural History of Geographic Atrophy Secondary to Age-related Macular Degeneration

Publication relating to this section of Chapter 3:

Joachim N, Mitchell P, Kifley A, Rochtchina E, Hong T, Wang JJ. Incidence and Progression of Geographic Atrophy: Observations from a Population-Based Cohort. *Ophthalmology* 2013; 120: 2042-2050

ABSTRACT

Purpose: To examine early AMD lesion characteristics and risk factors associated with the long-term development and progression of geographic atrophy (GA).

Methods: Of 3654 participants aged 49+ years in the Blue Mountains Eye Study, 75.8%, 76.7% and 56.1% of survivors attended the 5-, 10- and 15-year follow-up examinations, respectively. Retinal photographs were taken at each visit. Incident GA was confirmed using a side-by-side grading method. Computer planimetry was used to measure the area involved by GA. Fast and slow/normal progression rates were defined as GA area enlargement by ≥2 and <2 mm^2/year, respectively. Incident GA was estimated using the Kaplan-Meier product-limit method. Early AMD lesion characteristics were assessed for association with GA incidence, using eye-specific data and generalized estimating equation models adjusting for age, current smoking and presence of risk alleles of the *complement factor H (CFH)* or *age-related maculopathy susceptibility 2 (ARMS2)* genes, genotyped or imputed using genome-wide scan data.

Results: By excluding 41 subjects with GA at baseline, of 2503 participants at risk of GA, incident pure GA (without co-existing neovascular AMD lesions) was confirmed in 57 participants, a 15-year incidence of 3.6%. Baseline early AMD lesion characteristics associated with GA incidence included drusen type (soft indistinct: OR 59.0, 95% CI 20.4-171.0, and reticular drusen: OR 13.9, 95% CI 4.0-47.6); drusen location within 500μm radius of the fovea (OR 15.1, 95% CI 7.4-30.8); drusen area greater than 375μm in diameter (OR 10.1, 95% CI 4.0-25.6), presence of retinal pigment epithelial depigmentation (OR 9.0, 95% CI 4.1-19.8) or hyperpigmentation (OR 12.0, 95% CI 6.1-23.5), referenced to subjects with no or hard drusen only. Fast progression was more

frequent among baseline current smokers at baseline, subjects with the *CFH* or *ARMS2* risk genotypes and pseudophakic eyes.

Conclusions: Early AMD lesion characteristics (type, location, area involved) were strongly associated with higher long-term risk of developing GA, independent of age, smoking and AMD genetic susceptibility from the *CFH* or *ARMS2* genes. Known AMD risk factors were also more frequently present among fast progressing GA cases.

BACKGROUND

Geographic atrophy (GA) is 1 of 2 types of late AMD and is characterized by a sharply defined area of RPE degeneration in which choroidal blood vessels are visible[26]. It accounts for approximately 35% to 40% of late stage AMD cases[148]. There is currently no effective treatment for GA, and once the foveal centre is involved, affected patients are deprived of central vision and may develop legal blindness[204-206].

Although the short term (e.g., 2 to 5 years) incidence of GA is available from population- based studies[30,45,63,207], there are limited data available on long-term incidence (over 10 to 15 years). Two large population-based studies with long-term follow-up, the Beaver Dam Eye Study (BDES) and the Blue Mountains Eye Study (BMES), reported that the 10-year incidence of pure GA was 0.8%[34] and 1.7%[35], respectively. The 15-year incidence of pure GA in the BDES was 1.3%[33]. In both studies, the presence of large drusen and retinal pigmentary abnormalities were found to be associated with increased long-term incidence of late AMD, including GA and neovascular AMD[29,34,35,208]. However, population-based data for the associations of early AMD lesions with long-term incidence of pure GA are limited.

The natural history and progression of GA have also been assessed in both the BDES and BMES populations[44,205] and clinic-based cohort studies[209,210]. Larger atrophy areas at baseline were found to be associated with fast progression of GA lesions[204,209], and the shape of the atrophic area, termed 'GA configuration' (classic, multifocal and merged), also appears to have different progression rates[205]. In a previous BMES report, 5-year progression of GA occurred in 43% of eyes with GA at baseline, where GA progression was defined as an increase in the atrophic area by ≥2 subfields of the

Wisconsin grading grid or by extension of atrophy into the foveal center without quantitative measures[44].

We aimed in this report to examine the 15-year incidence of pure GA, and associations of early AMD lesion characteristics with GA incidence in the BMES cohort. We also assess the 5-year GA progression rate, indicated by quantitative measures of the size of atrophic areas and GA involvement with respect to the fovea, and explore factors associated with fast versus slow progression of prevalent and incident cases of pure GA.

METHODS

Procedures and Photographic Grading

At each BMES examination, in addition to retinal fundus photography, visual acuity was assessed following the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol[211]. Demographic and lifestyle questionnaires were also administered and blood collected for genotyping, as described previously (Methods, Chapter 2).

Geographic atrophy refers to a sharply defined area of RPE and the sensory retinal loss with a diameter ≥175μm, exposing choroidal vessels.For this report, side-by-side grading of retinal photographs of each subject taken at BMES I, II, III and IV was performed to confirm incident pure GA cases. Incident pure GA was defined as the first appearance of GA in either eye of subjects who had no sign of GA at the previous visit and no neovascular AMD (including pigment epithelial or sensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars) at all previous and current examinations. All

incident late AMD cases including incident GA cases were also confirmed by BDES and Rotterdam Eye Study principal investigators.

Early AMD and early AMD lesion characteristics were defined as in Methods (Chapter 2)

Computer Planimetry

GA progression was assessed in both prevalent (from baseline examination, BMES I) and incident pure GA cases (detected at follow-up visits, either BMES II or BMES III) that had retinal photographs available at subsequent visits. Progression was measured as increasing size of the atrophic areas over 5 years from one BMES examination to the next, and converted to progression rate in mm^2 per year. The retinal photographs from BMES I, II and III, on 35mm film, were scanned using a CanoScan 5600F scanner at 2400 dots per inch. The digital format of the Wisconsin Age-Related Maculopathy Grading System (WARMGS) grid was enlarged according to the resolution of the scanned photographs using Adobe Photoshop CS4 (Adobe Systems Incorporated, USA) so that the measurements could be performed digitally. The resolution of the grid was amended according to the resolution of BMES IV digital photographs when used for images taken at BMES IV. A random sample of retinal photographs without pathology from each BMES examination were used to obtain the scaling factor (microns per pixel) using Photoshop (Adobe Systems Incorporated, USA). This was calculated by dividing 4500μm, taken as the constant distance between the center of the optic disc to the center of the fovea, by the same distance measured in pixels, to obtain the number of microns per pixel. This scale factor was then set into Image J (National Institutes of Health,

NIH)[212] to allow measurements to be read in microns. Tracings were made along the margin of GA and area measurements obtained using 'region of interest' manager.

Definition of GA Progression

The average progression of GA was calculated using data from the BMES, BDES[205] and the Age Related Eye Disease Study (AREDS)[213] and was found to be an area between 1 and2mm^2 per year. We defined this as the normal progression rate. If the GA progression rate was greater than 2mm^2/year this was defined as fast progression, and if less than 1mm^2/year, was defined as slow progression. **Figure 3.2-1** shows examples of cases with different progression rates.

The configuration of GA has been used and described by previous studies[205,214]. The 'classic' configuration is defined as a single round atrophic area, 'multifocal' as two or more areas of atrophy within the macula region and the 'merged' configuration as two or more initially separate areas (multifocal) which subsequently amalgamated into a large irregular atrophic area. We added a 'mixed' configuration in our report to include eyes with two or more configuration types described above.

GA progression was assessed using two indicators: enlargement of the atrophic area and progression to involve the fovea associated with worsened best-corrected visual acuity (BCVA), in eyes that had not yet developed GA at the fovea when it was first detected.

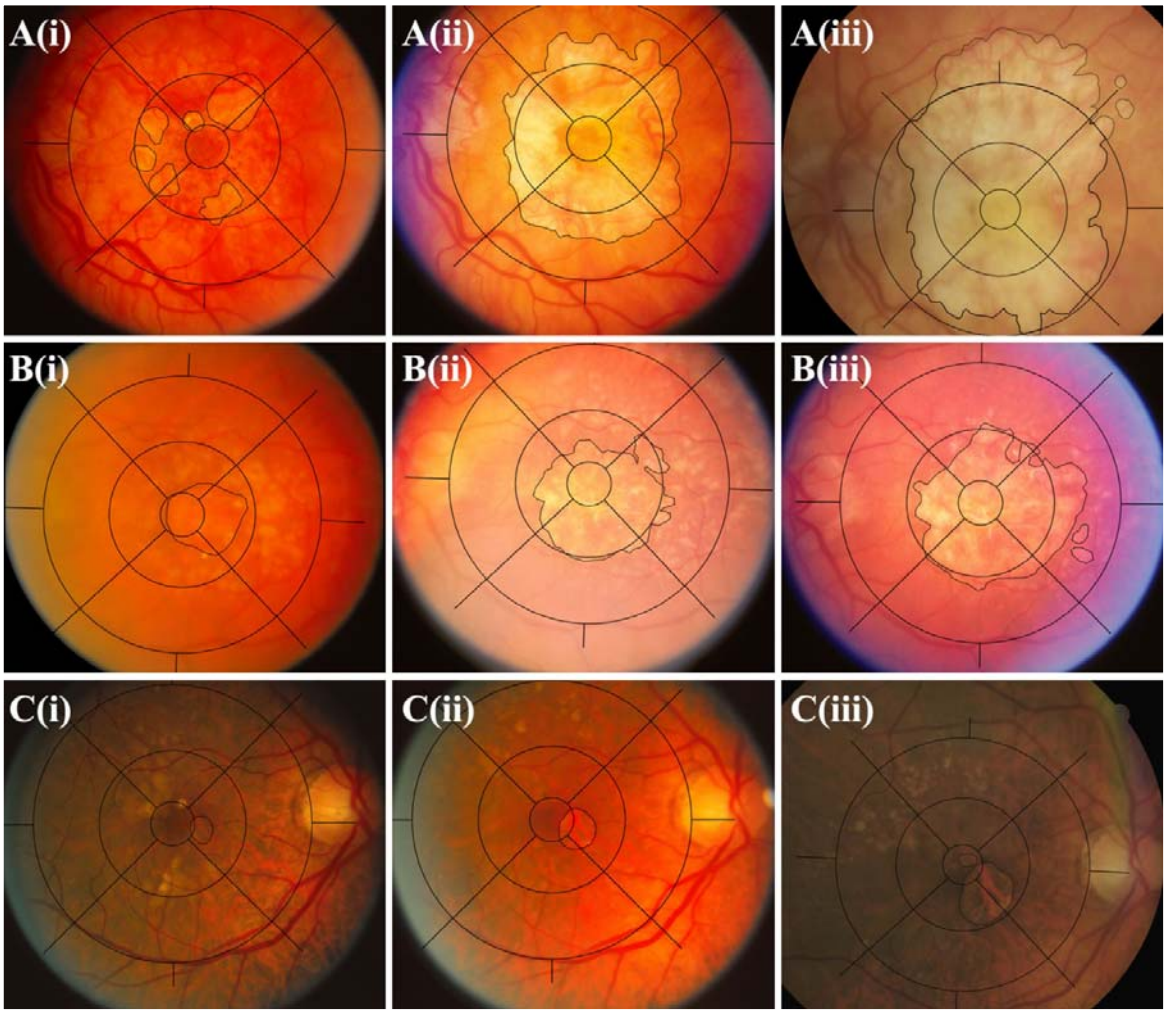

Figure 3.2-1: Examples of the fast (A), normal (B) and slow (C) progression of geographic atrophy (GA) by enlargement of atrophic areas. Tracings around the GA perimeter show the increase in area from the first detection of GA (i), to 5 years (ii), and to 10 years (iii) after first detection of GA.

Genotyping

The complement factor H (*CFH*) single nucleotide polymorphism (SNP) *rs1061170* and the age-related maculopathy susceptibility gene 2 (*ARMS2*) SNP *rs10490924* were genotyped in 1874 and 593 participants, respectively, and imputation performed in the remaining subjects, as described in Methods (Chapter 2). In our study sample, 1501 and 509 participants had both typed and imputed *rs1061170* and *rs10490924*, respectively, and the concordance rate between typed and imputed SNPs were 99.6% for *rs1061170* and 99.2% for *rs10490924*. Of 2503 participants used in our analyses, 151 participants had *rs1061170* imputed and 1287 had *rs10490924* imputed.

Definition of Baseline Variables Associated with GA

For smoking history, if participants answered 'no' to smoking regularly they were classified as non-smokers. If participants answered 'yes' and had given up smoking ≥1 year prior to the baseline examination, they were classified as past smokers. Current smokers were defined as participants who currently smoked or stopped smoking <1 year before baseline examinations. Regular fish consumption was defined as 1 or more servings per week, compared to infrequent consumption (<1 serving per week).

Statistical Methods

Fifteen-year person-specific GA incidence was assessed using Kaplan-Meier product limit survival estimates, including overall and incident rates by the AREDS simplified severity scale (5 steps)[28] according to baseline AMD levels. Estimates were then re-calculated after adjusting for the competing risk of death.

Associations between 15-year incidence of GA and common AMD risk factors (age, current smoking status, sex, presence of risk alleles of *CFH-rs1061170* or *ARMS2-rs10490924*, and regular fish consumption) were assessed using age-adjusted and multivariable adjusted discrete logistic regression models, and expressed as ORs. *CFH-rs1061170* and *ARMS2-rs10490924* were assessed categorically, in a general model, using homozygous wild genotype (no risk alleles) as the referent and heterozygous (one risk allele) and homozygous risk genotypes (two risk alleles) as separate categories. These SNPs were also assessed in an additive model where genotype was treated as a continuous variable. Eye-specific data were used to assess the association between early AMD risk characteristics (type, area and location of early lesions) and the 15-year incidence of GA. To handle the correlation between the two eyes of the same individual and multiple measures of the same eyes (observations from the same individual are in a cluster), the GENMOD procedure in SAS was used to perform generalized estimation equation modeling with an exchangeable working correlation matrix, using subject identification as a cluster indicator[196]. Due to the small number of cases available for assessment of GA progression, only descriptive data are provided.

RESULTS

Of 3654 baseline participants, 2572 had been followed-up at least once since the baseline examination. Of these, 68 participants developed late AMD at baseline or neovascular AMD at first follow-up after baseline (and were thus not at risk of developing GA), and 1 participant had ungradable photographs at all visits. This left 2503 participants included in the assessment of GA incidence. **Table 3.2-1** presents baseline characteristics of the BMES participants with incident GA and without any late AMD. Subjects with incident GA were significantly older and had significantly worse

BCVA in both their better and worse eyes, compared to those without late AMD. Participants with incident GA were also more likely to have two risk alleles of the *CFH* gene compared to those without any late AMD. The frequency of one or two *ARMS2* gene risk alleles was slightly higher in subjects with incident GA, but the differences were not statistically significant (**Table 3.2-1**).

Table 3.2-1: Comparison of baseline characteristics between participants with and without incident GA, examined up to 15 years in the Blue Mountains Eye Study cohort

Characteristic	% Participants*		P-value†
	No Late AMD (n=2446)	Incident GA (n=57)	
Mean age, years (SD)	63.9 (8.5)	72.3 (6.7)	<0.0001
Mean BCVA, no. of letters read (SD)			
Better eye	56.2 (4.8)	52.8 (5.5)	<0.0001
Worse eye	51.5 (10.9)	46.5 (10.6)	0.0006
Sex (male)	42.6	36.8	0.4
Smoking			
Current	12.9	19.6	0.1
Past	35.7	30.4	0.4
Fish consumption (≥1 serving/week)	60.4	43.5	0.02
CFH-rs1061170			
TT	40.3	24.0	0.0
CT	46.5	46.0	0.9
CC	13.3	30.0	0.0007
ARMS2-rs10490924			
GG	62.5	54.0	0.2
GT	33.6	40.0	0.3
TT	4.0	6.0	0.5

AMD = age-related macular degeneration; SD = standard deviation; BCVA = best corrected visual acuity; *CFH* = complement factor H gene (C risk allele); *ARMS2* = age-related maculopathy susceptibility gene 2 (T risk allele).

*% where appropriate.

†Mantel-Haenszel Chi-Square.

Incidence of pure geographic atrophy

Incident pure GA was identified in 57 participants (82 eyes) of 2503 subjects at risk, with an overall 15-year incidence of 3.6% (95% confidence interval (CI) 2.7-4.7). After accounting for the competing risk of death, the 15-year incidence of GA reduced to 2.2% (95% CI 1.6-2.8). After age-standardization to the Australian census 2011 population aged 50+ years, the estimated 15-year incidence became 1.8% (95% CI 1.2-2.4). Bilateral involvement occurred in 27 of the 57 participants (47.4%). Of the bilateral cases, 21 were bilateral at the same follow-up visit, 4 became bilateral at the subsequent follow-up visit and 2 cases that had unilateral GA at baseline developed incident GA in the fellow eye during the follow-up period.

Table 3.2-2 presents the incidence of pure GA at the 5-, 10- and 15-year follow-up examinations by different stages of AMD at baseline according to the AREDS 5-step severity scale[28]. Age was significantly associated with advancing severity level (p for trend < 0.0001). At each of the follow-up visits, incidence of pure GA increased with increasing baseline AREDS severity step from 0 to 4, except for cases with AREDS scale step 3 (**Table 3.2-2**).

Table 3.2-2: Incidence of pure GA at the 5-, 10- and 15-year follow-up visits of the Blue Mountains Eye Study population by the Age-Related Eye Disease Study (AREDS) 5-step severity scale[28].

AREDS category at baseline	Number of participants (n=2503)	Mean age in years ±SD)	Mean BCVA in worse eye, no. of letters read ±SD	Incidence GA (%)		
				5 year	10 year	15 year
Step 0[*]	1873	62.6±7.9	53.4±6.8	0.0	0.2	1.0
Step 1[†]	207	67.4±8.4	50.0±10.8	0.5	4.7	9.6
Step2[‡]	72	71.3±9.0	50.6±7.4	6.9	23.9	38.4
Step 3[§]	36	71.1±8.5	49.5±7.8	8.3	8.3	19.8
Step 4[I]	13	70.5±7.0	48.1±9.7	30.8	60.4	100.0

n = sample size; SD = standard deviation; BCVA = best-corrected visual acuity

[*]no retinal pigment changes with none or small hard drusen in one or both eyes or intermediate (but not large) drusen in one eye only.

[†]pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only; no pigment changes in either eye but intermediate drusen in both eyes or large drusen in 1 eye.

[§]pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only; pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye; no pigment changes in either eye but large drusen in both eyes.

[d]pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye; pigment changes in 1 eye with large drusen in both eyes.

[I]pigment changes in both eyes with large drusen in both eyes.

Age-related macular degeneration risk factors assessed for association with the 15-year incidence of GA are presented in **Table 3.2-3**. Increasing age was significantly associated with the development of GA within 15 years (p<0.0001). In an age-adjusted model, current smoking (p=0.0001) and homozygous risk genotypes of *CFH-rs1061170* (p=0.002) and *ARMS2-rs10490924* (p=0.04) were associated with an increased risk of incident GA. There was no significant sex difference found in the long-term risk of GA. Regular fish consumption had a significant protective effect against the development of GA (p=0.02). In a model simultaneously adjusting for the above mentioned risk factors, the significant associations of age, current smoking, and genetic risks from the *CFH* and *ARMS2* with GA incidence remained, and the association of weekly fish consumption with incident GA became non-significant (**Table 3.2-3**).

Table 3.2-3: Common age-related macular degeneration risk factors associated with 15-year incidence of GA

Risk factor	15-year incidence of GA			
	Age-adjusted (where appropriate)		Multivariate adjusted*	
	OR	95% CI	OR	95% CI
Age, per year	1.18	1.14-1.22	1.22	1.16-1.27
Sex (male)	0.84	0.49-1.47	0.77	0.39-1.49
Smoking (current)	4.11	2.01-8.38	4.20	1.76-10.02
CFH-rs1061170†	1.92	1.28-2.88	1.99	1.26-3.15
TT	1.00		-	-
CT	1.66	0.82-3.38	-	-
CC	3.65	1.66-8.03	-	-
ARMS2-rs10490924†	1.66	1.02-2.70	2.38	1.41-4.02
GG	1.00		-	-
GT	1.54	0.85-2.79	-	-
TT	3.32	0.96-11.53	-	-
Fish Consumption (≥ 1 servings/wk)	0.48	0.27-0.88	0.54	0.28-1.03

OR = odds ratio; CI = confidence interval; *CFH* = complement factor H gene (C risk allele); *ARMS2* = age-related maculopathy susceptibility gene 2 (T risk allele).
*multivariate model adjusted for age, sex, smoking, *CFH* and *ARMS2* and regular fish consumption.
†computed using additive model

Table 3.2-4 demonstrates the relationship between early AMD lesion characteristics and 15-year incidence of GA using eye-specific data. Drusen characteristics that were strongly associated with increased risk of developing GA over the 15-year period included soft indistinct (OR 28.58) and reticular drusen (OR 14.39), drusen within 500μm radius of the foveal center (OR 9.97) and a collective drusen area greater than 375μm in diameter (ORs 7.62-33.36). The baseline presence of RPE depigmentation (OR 6.99) and hyperpigmentation (OR 11.27) were significantly associated with higher risk of developing pure GA over the same period, as was location of pigmentary abnormalities within 1500μm radius of the foveal center. These associations remained significant after adjusting for age, sex, smoking, regular fish consumption, and presence of the *CFH* and *ARMS2* risk alleles (**Table 3.2-4**).

Table 3.2-4: Relationship between baseline drusen and retinal pigmentary abnormalities and the 15-year incidence of geographic atrophy (GA), analyzed by eye, and presented as odds ratios (OR) with 95% confidence intervals (CI).

Early AMD characteristics	No. that developed GA	No. at risk	15-year incidence of GA			
			Age-adjusted		Multivariate adjusted*	
			OR	95% CI	OR	95% CI
Drusen Type:						
None or hard drusen only	27	4094	1.00		1.00	
Intermediate	24	488	3.92	2.36-6.52	5.30	2.67-10.49
Soft distinct	2	100	1.61	0.41-6.26	3.02	0.70-13.08
Soft indistinct	24	100	28.58	12.66-64.53	59.02	20.38-170.95
Reticular	15	44	14.39	5.56-37.22	13.85	4.03-47.64
Drusen Location:						
None or ≥ 3000µm from foveal centre	27	4094	1.00		1.00	
1500-3000µm from foveal centre	1	42	1.93	0.38-9.92	3.40	0.49-23.73
500-1500µm from foveal centre	4	188	1.83	0.61-5.50	3.65	1.21-10.99
Within 500µm radius of foveal centre	48	451	9.97	5.88-16.90	15.08	7.38-30.82
Drusen Area:						
None or <375µm in diameter	39	4535	1.00		1.00	
≥ 375 to <0.5 disc area	16	156	7.62	3.76-15.4	10.11	4.00-25.57
≥ 0.5 disc area	25	87	33.36	13.65-81.56	61.12	18.37-203.34
RPE Depigmentation:						
Present vs absent	20	144	6.99	3.87-12.32	9.02	4.10-19.80
Location						
None or ≥1500µm from foveal centre	62	4881	1.00		1.00	
500-1500µm from foveal centre	8	72	5.24	2.31-11.90	8.04	2.84-22.80
Within 500µm radius of foveal centre	12	67	10.35	4.74-22.67	11.20	3.61-34.75
Area						
None to <375µm in diameter	70	4932	1.00		1.00	
>375µm to <2 disc areas	12	88	8.40	4.04-17.46	5.62	2.07-15.28
≥ 2 disc areas	-	-	-		-	
Hyperpigmentation:						
Present vs absent	41	317	11.27	6.70-18.96	12.02	6.14-23.53
Location						
None or ≥1500µm from foveal centre	41	4723	1.00		1.00	
500-1500µm from foveal centre	16	98	13.17	6.33-27.38	14.91	6.47-34.37
Within 500µm radius of foveal centre	25	146	16.37	8.55-31.33	14.97	5.95-37.63
Area						
None or <64µm in diameter	45	4727	1.00		1.00	
≥ 64µm in diameter	37	240	12.21	7.08-21.05	11.18	5.65-22.10

AMD = age-related macular degeneration

*multivariate model adjusted for age, sex, smoking, regular fish consumption, the *CFH* and *ARMS2* risk alleles.

Progression of pure geographic atrophy

To investigate the progression of pure GA, both prevalent (n=7) and incident GA cases (n=12) with gradable follow-up retinal photographs were included. There were 41 baseline participants with GA but only 7 were pure GA cases (10 eyes) and had follow-up retinal photographs available for this analysis. There were 16 participants (23 eyes) with incident GA and follow-up retinal photographs, and 4 persons (5 eyes) were excluded due to subsequent development of neovascular AMD, leaving a total of 19 participants (28 eyes) that met the criteria for analysis of progression rate.

The average size of the GA lesions at first observation among eyes with baseline pure GA and incident pure GA was 5.0 mm^2 (standard deviation (SD) 7.0) and 4.6 mm^2 (SD 4.5), respectively. Five years after the first detection of GA, average size of baseline pure GA lesions had increased to 13.0 mm^2 (SD 8.8) and incident GA lesions increased to 15.9 mm^2 (SD 8.1). The average GA progression rate for baseline and incident eyes combined (n=28) was 1.95mm^2/year. **Table 3.2-5** presents the three indicators of GA progression (enlargement of atrophic area, progression of atrophy into the fovea and worsening in BCVA). Of participants with baseline GA, none survived to the 15-year follow-up. These participants were significantly older than participants without GA at baseline (mean age 82.6 years versus mean age 65.9 years, respectively). The multifocal configuration of GA had the fastest progression rate from the first detection to the next examination 5 years later, while the slowest progression rate was observed in eyes presenting with the classic configuration. Central GA (involving the fovea) was present in 57% (n=16) of 28 eyes with GA at the first detection. Of the remainder, 92% of 12 eyes with GA had progressed into the fovea within the next 5 years. The mean BCVA deteriorated by an average 18 letters over the same period.

Table 3.2-5: Progression in atrophic area, foveal involvement and best-corrected visual acuity over 5 and 10 years in eyes with pure geographic atrophy (GA).

Characteristics of progression	GA progression					
	First observation - 5 years		Between 5 - 10 years		First observation - 10 years	
	n	mean mm^2 (SD)	n	mean mm^2 (SD)	n	mean mm^2 (SD)
Change in mean GA area						
Prevalent (baseline) GA eyes (n=10)	9	7.7 (5.2)	3	4.5 (2.9)	4	7.5 (4.4)
Incident GA eyes (n=18)	18	11.2 (6.0)	3	8.6 (6.3)	3	20.3 (16.2)
All GA eyes (n=28)	27	10.1 (5.9)	6	6.6 (4.9)	7	13.0 (12.0)
Change in mean GA area (by GA configuration)*						
Classic	7	5.5 (5.3)	4	4.7 (4.6)	5	9.3 (10.3)
Merged	7	10.3 (6.0)	0	-	0	-
Multifocal	6	13.8 (6.2)	3	13.7 (1.0)	3	31.1 (1.9)
Mixed	7	11.2 (3.8)	1	-	1	-
Change in foveal involvement*	n	%	n	%	n	%
	11	92	0	0	2	67
Change in BCVA*	n	Difference in the mean no. of letters read correctly (SD)	n	Difference in the mean no. of letters read correctly (SD)	n	Difference in the mean no. of letters read correctly (SD)
(later visit minus previous visit)	27	-18.6 (17.9)	8	1.25 (6.6)	8	-9.5 (21.6)

N=number of eyes; SD=standard deviation; BCVA=best-corrected visual acuity.

*prevalent and incident GA eyes were combined.

NB. For foveal involvement n: number of eyes which had GA that progressed of the number of eyes with GA that were at risk of progression into the fovea (eyes which had already developed GA in the fovea were not included).

Table 3.2-6 presents the frequencies of risk factors in fast or slow/normal progression groups. Fast, slow and normal progression were observed in 10, 7 and 2 participants respectively, according to the worse eye if the cases were bilateral (n=2). A larger proportion of participants with fast progressing GA were current smokers (30% versus 0% in the slow/normal progression group), had one or both eyes pseudophakic prior to or at first detection of GA in the same eye (30% versus 11%), and had the homozygous risk genotype of either the *CFH* (50% versus 22%) or the *ARMS2* (10% versus 0%), compared to subjects who had slow/normal progress. Participants that regularly consumed fish were more frequently represented in the slow/normal progress groups compared to the fast progress group (57% versus 38%).

Table 3.2-6: Proportion of participants presenting with the selected risk factors by fast and slow progression of pure geographic atrophy (GA).

Risk characteristics	**% Participants with GA progression**		
	Fast (≥2mm^2/year) (n=10)	**Slow (<1mm^2/year) (n=7)**	**Slow/Normal (<2mm^2/year) (n=9)**
Mean baseline age (years)	71.9	72.4	75.2
Sex (male)	30.0	28.6	33.3
Smoking			
Past	40.0	28.6	33.3
Current	30.0	0.0	0.0
Fish consumption (≥1 serving/wk)	37.5	50.0	57.1
Pseudophakic[*]	30.0	14.3	11.1
CFH-rs1061170			
TT	10.0	71.4	55.6
CT	40.0	14.3	22.2
CC	50.0	14.3	22.2
ARMS2-rs10490924			
GG	50.0	57.1	55.6
GT	40.0	42.9	44.4
TT	10.0	0.0	0.0

n = sample size; *CFH* = complement factor H gene; *ARMS2* = age-related maculopathy susceptibility gene 2.

[*]pseudophakia detected prior to or at the same examination as GA detected.

DISCUSSION

In this older Australian cohort we found an overall 3.6% incidence of pure GA over 15 years. Common AMD risk factors including increasing age, history of smoking, genetic risk from the *CFH* and *ARMS2* alleles and regular fish consumption were associated with long-term incidence of pure GA. In addition, early AMD lesion characteristics strongly predicted risk of GA independent of the known risk factors mentioned above. We also found that fast progression of GA was more likely to occur in affected individuals who were current smokers, or possessed at least one risk allele of the *CFH* gene or both risk alleles of the *ARMS2* gene, and in pseudophakic eyes, while slow progression of GA was more likely to occur in those who consumed one or more servings of fish weekly.

The BMES and the BDES[33] are the only two studies to have reported GA incidence over a 15-year follow-up period in population-based older samples. Both study protocols and methods used including examination procedures and retinal photographic grading of AMD signs were similar, while the BDES population had a slightly younger age limit (43+ years) than the BMES (49+ years). The BMES found an incidence of 3.6% that was double the 1.3% rate observed in the BDES[33] over the 15-year period. The 10-year cumulative incidence of GA was also double in the BMES (1.7%)[35] than in the BDES (0.8%)[34]. After accounting for the competing risk of death and age-standardization of the BMES population to the general Australian population, the 15-year incidence of pure GA was 1.8%, however, the 95% CI 1.2-2.4 is consistent with the finding of a 1.3% 15-year incidence reported from the BDES. Apart from the different age ranges of the two study samples, there are likely different environmental exposures (smoking, sunlight exposure) that could explain the difference in GA

incidence between the two studies. The association between smoking and the incidence of late AMD was somewhat dissimilar in the BMES compared to the BDES, as previously reported[115,117,118]. There was a higher frequency of baseline current smokers in the BDES (men 21%; women 18%), compared to the BMES (men 15%; women 11%). However, current smokers had a 4-fold greater risk of incident late AMD compared to non-smokers in the BMES[115], while no similar association was found between past or current smoking and incident late AMD (or GA), in the BDES[116,118].

A Danish study of 946 subjects aged 60+ years, which used a similar grading system to detect AMD from color fundus photographs, reported a 14-year incidence of GA of 4.9%[47], substantially higher than the 15-year incidence found in the BDES and BMES. The difference could be due to an older baseline age (60+ years) of the Danish study sample compared to the baseline ages of the BDES or BMES. Participants aged under 60 years at baseline comprised 32.5% of the BMES population compared to none in the same age group in the Danish Study, which could have accounted for the differences in the incidence rates between the two studies. We do not have directly comparable data from the Danish study, so that direct age-standardization between the two study samples is not possible. Due to relatively high proportions of study participants who had died or were lost to follow-up (37.9%) in the Danish study[47] and in the 15-year follow-up examinations of our study cohort, survival bias could have also affected differently the incidence estimates of the two studies.

Previous studies have documented that eyes with early AMD lesions had a substantially higher risk of progression to late AMD including GA and neovascular AMD[29,44,215].

Therefore the strong association between early AMD lesion characteristics and risk of developing GA, found in our study, is expected.

We found that average progression of GA, measured as enlargement in GA area, was between 1.95mm^2/year, which is similar to the average progression found in other population-based studies (1.2-1.8 mm^2/year)[205,213] but lower than the progression rate found in a clinic-based sample (2.6 mm^2/year)[209]. In defining the progression rate in terms of enlargement in area, we have assumed a steady, similar enlargement rate in GA area across all stages of GA progression. Whether such an assumption holds is unclear, as indicated by Klein et al[205]. We also found a faster progression rate among cases with multifocal compared to classic GA configuration, consistent with the BDES finding in pure GA cases[205]. However, multifocal GA configuration usually indicates a more advanced stage of GA, and therefore the different progression rates observed may be due to differing stages of GA rather than the morphological differences of GA configurations per se. Nevertheless, these findings were based on a very small number of GA cases and should be interpreted with caution.

Older age and history of smoking have been consistently identified in population-based studies as risk factors for late AMD[63,123,216,217]. The association between smoking and 15-year incident GA, documented in this study, is in keeping with previous observations[115,117]. Past smokers in the BMES were shown to have a 3-fold higher risk of incident GA after adjusting for age, sex and other factors as previously reported[115]. We found that similar risk factors that were associated with high risk of developing GA were also associated with fast progression of GA, including genetic influence from the *CFH* and *ARMS2* genes, current smoking and less frequent consumption of fish. The

higher proportion of participants who consumed fish at least weekly in the slow/normal progression group compared to the fast progression group accords with evidence suggesting beneficial effect of omega 3 fatty acids on the development and progression to either form of late AMD[218-220]. The higher proportion of pseudophakic participants in the fast than slow/normal progression groups lends support to the hypothesis that sunlight exposure could also be a risk factor for GA[221,222].

Strengths of our study include its long-term follow-up of an older predominantly Caucasian cohort that is comparable with the BDES cohort. The low follow-up rate at the 15-year examinations, however, could have led to either an over- or underestimation of GA incidence. Our findings from all GA cases including both clinical and subclinical cases without apparent visual symptoms reflect the natural history of the condition. We were able to assess GA progression and risk characteristics associated with the progression by comparing fast to slow/normal progression groups. However, we cannot exclude the possibility that GA progresses differently at different stages of the disease and that fast progress cases may occur in a more advanced stage. Limitations of this study include the lack of optical coherence tomography (OCT) measures, due to timing (early 1990s when OCT was not available), and lack of fundus autofluorescence images, which may have allowed for better detection of the borders and extent of GA. We also could not statistically validate the association between certain risk factors and progression of GA lesions due to the small number of cases available to analyze progression. Concordance studies have shown differences in progression between bilateral GA cases and unilateral GA cases with early AMD in the fellow eye[223], however, as above, we were unable to assess this due to very small number of GA cases in these subgroups.

SUMMARY

This report shows the 15-year incidence of GA to be 3.6% in this older Australian cohort, and confirmed strong associations between baseline early AMD lesion characteristics with higher risk of developing GA over the long-term, independent of age, smoking and genetic susceptibility from the two major AMD genes (*CFH* and *ARMS2*). Similar AMD risk factors were also found to be associated with fast progression of GA over 5 to 10 years.

3.3 The incidence and progression of reticular drusen in age-related macular degeneration

Publication relating to this section of Chapter 3:

Joachim N, Mitchell P, Rochtchina E, Tan AG, Wang JJ. Incidence and Progression of Reticular Drusen in Age-related Macular Degeneration: Findings from an Older Australian Cohort. *Ophthalmology* 2014; 121: 917-925

ABSTRACT

Purpose: To assess the 15-year incidence and progression of reticular drusen and associations of this lesion with age-related macular degeneration (AMD) risk factors.

Methods: Of 3654 Blue Mountains Eye Study (BMES) participants aged 49+ years, examined at baseline, 75.8%, 76.7% and 56.1% of survivors attended 5-, 10- and 15-year follow-up examinations, respectively. Color retinal photographs were obtained and comprehensive questionnaires administered at each visit, and DNA samples were genotyped. Fundus autofluorescence images were not available. Reticular drusen identified from photographs were confirmed with side-by-side grading, using the Wisconsin AMD grading protocol. Incidence was assessed using Kaplan-Meier product limit survival methods, controlling for competing risk of death. Associations between smoking, fish consumption, serum lipids, systemic and dietary factors, the complement factor H (*CFH-rs1061170*) and age-related maculopathy susceptibility 2 (*ARMS2-rs10490924*) genes and the 15-year incidence of reticular drusen were analyzed in discrete logistic regression models. Generalized estimating equation models were used to analyze eye-specific relationships between these risk factors and 5-year progression from reticular drusen to late AMD.

Results: The 15-year cumulative incidence of reticular drusen was 4.0% (n=95). Increasing age (per decade increase; odds ratio, OR 3.4, 95% confidence interval, CI, 2.6-4.4), female sex (OR 2.0, 95% CI 1.3-3.2) and presence of risk alleles of *CFH-rs1061170* (OR 1.8, 95% CI 1.3-2.4) or *ARMS2-rs10490924* (OR 3.0, 95% CI 2.1-4.4) were associated with higher reticular drusen incidence. Current smoking at baseline predicted higher reticular drusen incidence (OR 2.1, 95% CI 1.0-4.5) after adjusting for

age, sex, *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms. Of 118 eyes with reticular drusen, 40 (33.9%) developed late AMD over 5 years. A higher proportion of eyes with reticular drusen located outside versus within the macular area progressed to late AMD (50.0% versus 37.8%). Dietary lutein-zeaxanthin intake was associated with decreased likelihood of progression from reticular drusen to late AMD (adjusted OR 0.5, 95% CI 0.3-1.0).

Conclusions: Known AMD risk factors were associated with greater long-term risk of reticular drusen. Neither total area nor central location of reticular drusen predicted 5-year progression to late AMD. Increased consumption of lutein-zeaxanthin predicted a lower risk of progression.

BACKGROUND

Described as soft, confluent drusen forming ill-defined networks of interlacing ribbons[26], reticular drusen have been relatively under-researched and their incidence, prognosis and risk factors have not been well characterized. Reticular drusen are also termed 'reticular pseudodrusen' and 'subretinal drusenoid deposits', as a result of observations using different imaging modalities[224-226]. For the purpose of this report, we retain the term reticular drusen designated by Klein et al for reticular drusen lesions detected using only color fundus photographic grading[26].

In most previous studies, reticular drusen were grouped together with large drusen into the category of soft indistinct drusen, with only a few studies reporting the prevalence and incidence of reticular drusen as a separate lesion. The Beaver Dam Eye Study (BDES) reported a reticular drusen prevalence of 0.7% and an overall 15-year incidence of 3.0% in a population aged 43 to 86 years at the baseline examination[227]. We previously reported a 2.0% 5-year incidence of reticular drusen in the Blue Mountains Eye Study (BMES) population[44]. In a report of BMES 10-year AMD incidence, we included both reticular drusen and soft indistinct drusen in the incidence of 11.7% [35].

Findings from clinic-based samples that used multimodal imaging methods have shown strong associations between reticular drusen and late AMD[224,228-230]. Some studies reported that reticular drusen were detected commonly co-existing with geographic atrophy (GA)[225,231], whereas others suggested that reticular drusen commonly accompanied neovascular AMD[229]. Lack of longitudinal data in these studies, however, precludes important prognostic information that ophthalmologists need in providing

advice to patients found to have signs of reticular drusen, for example about the timeframe of progression from reticular drusen to late AMD.

In this report we aimed to assess the prevalence, and 15-year incidence and progression of reticular drusen as a standalone lesion, in a well-characterized older Australian population sample. We also aimed to assess associations of common AMD risk factors and other early AMD lesion characteristics with the incidence of reticular drusen, as well as its progression to late AMD, over a 15-year period.

METHODS

Procedures

Retinal photography was conducted at each BMES examination as described in Methods, Chapter 2.

Reticular drusen was defined as confluent drusen forming an interlacing ribbon-like network, with individual lesions usually >125μm in diameter[26] **(Figure 3.3-1)**.

Systolic and diastolic blood pressure (BP) measurements were recorded from the first and fifth Korotkoff sounds using a mercury sphygmomanometer after participants were seated for at least 5 minutes[232]. Diet was assessed from a self-administered food frequency questionnaire completed by participants at each examination, which included questions on dietary supplement use[139]. The Australian tables of food composition[191,192] and the United States Department of Agriculture carotenoid food composition database[193] were used to calculate or estimate, respectively, the intake of nutrients, including lutein and zeaxanthin in μg.

Fasting blood samples were collected from 3222 participants at BMES I to assess white cell count (WCC) and cholesterol levels, as previously described[154,160,233]. Briefly, WCC ($x10^9$/L) was determined using Coulter Counter methods (Beckman Coulter, Inc, Fullerton, CA)[160] or an Advir 120 autoanalyzer (Bayer, Leverkusen, Germany)[102], and total cholesterol, high density lipoprotein (HDL) and triglyceride concentrations (mmol/L) were measured on a Reflotron reflectance photometric analyser (Roche Diagnostics, Germany)[233]. Blood samples collected from 2272 participants during the BMES II and III examinations were additionally used for genotyping.

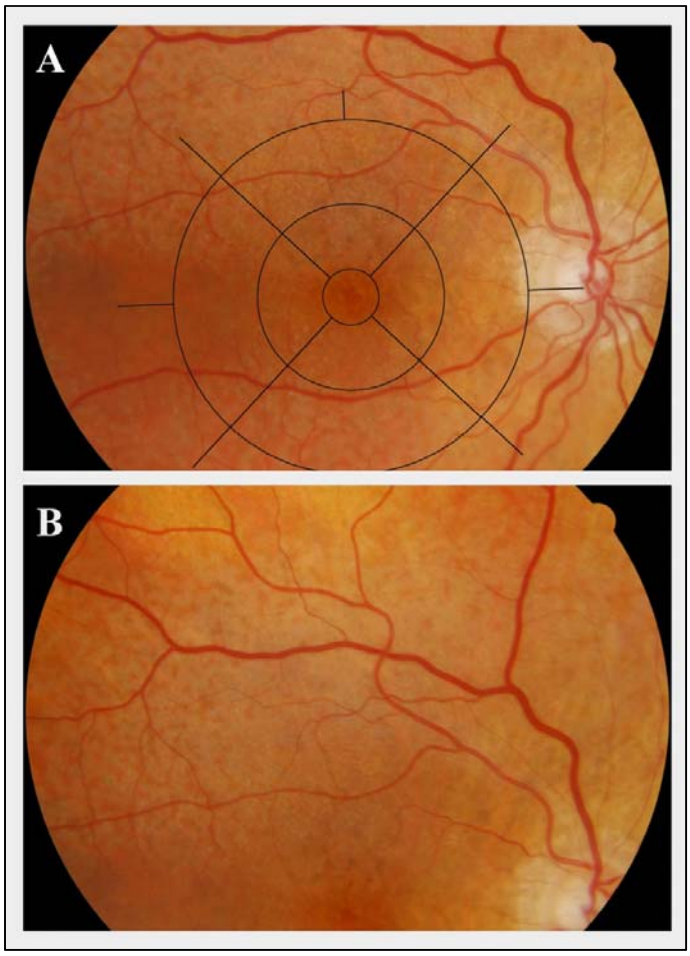

Figure 3.3-1: (**A)** Example of reticular drusen (distinguished as soft confluent drusen that form broad ribbon-like networks) in the upper and lower arcades of the macula with the Wisconsin Age-Related Maculopathy Grading System (WARMGS) grid centered on the fovea; **(B)** Reticular drusen visible in the upper arcade of the same eye in Figure 1(A).

Photographic Grading

Retinal photographic grading was conducted by two senior graders using the Wisconsin Age-Related Maculopathy Grading System (WARMGS) protocol, as described previously[36]. The presence and location of reticular drusen within or outside the WARMGS grid was recorded.

The author (Nichole Joachim) re-graded cases with confirmed or questionable reticular drusen in a side-by-side manner using all available retinal photographs from baseline and follow-up visits. Location of reticular drusen was recorded as presence in each subfield of the WARMGS grid. Reticular drusen area was measured for three concentric circles, within a 500μm, 1000μm and 3000μm radius of the foveal center. Difficult cases, where the presence of reticular drusen was questionable or where discrepancies existed between the original and subsequent side-by-side grading, were adjudicated and verified by a senior researcher with grading experience (Jie Jin Wang) and a retinal specialist (Paul Mitchell).

Late and early AMD were defined in Methods, Chapter 2. Early AMD lesion type, area and location were defined using the WARMGS protocol[26]. Briefly, individual lesion size and collective area of drusen and pigmentary abnormalities were estimated using the WARMGS measurement circles. Location of lesions was defined as within 500μm, between 500 and 1500μm and between 1500 and 3000μm radius of the foveal center, in the WARMGS grid[26].

Definition of Reticular Drusen Incidence and Progression

Incident reticular drusen was defined as the first occurrence of reticular drusen at the 5-, 10- or 15-year follow-up in eyes without reticular drusen or any late AMD, at previous visits. For cases where reticular drusen and late AMD were both present for the first time in a follow-up visit, we assumed that reticular drusen developed first, before the occurrence of late AMD during the 5-year interval between the two visits.

Progression from reticular drusen to late AMD was defined as the development of any late AMD (GA or neovascular AMD) that followed the development of reticular drusen. Progression was assessed for baseline and incident reticular drusen cases where reticular drusen progress could be assessed for at least 5 years (with 5 or more years follow-up information). Location of reticular drusen was defined by subfields of the WARMGS grid, and the area of reticular drusen defined as small if <2DA and large if ≥2 DA, within the confines of the WARMGS grid[26].

Definition of Risk Factors

Lifestyle, genetic and dietary data was obtained as described in Methods, Chapter 2. Smoking status was ascertained from an interviewer-administered questionnaire and participants were classified as non-smokers if they answered 'no' to smoking regularly. If participants had quit smoking more than 1 year prior to the examination they were classed as past smokers. Current smokers were defined as participants who currently smoked or had stopped smoking <1 year before the examination. Regular fish consumption was defined as consuming ≥1 serving of fish per week. Alcohol consumption (including beer, wine or spirits) was categorized as none, >0 to ≤2 standard drinks and >2 standard drinks, per day. These categories were formulated

based on Australian National Health and Medical Research Council (NHMRC) recommendations of up to 2 standard drinks per day[234].

Statistical Analyses

SAS (version 9.1, SAS Institute Inc, Cary, NC) was used. The 15-year incidence of reticular drusen was estimated using the Kaplan-Meier product limit survival method controlling for competing risk of death. Age was treated categorically (49-54, 55-64, 65-74 and 75+ years) and the 15-year incidence of reticular drusen is presented by sex within each age-group. Age- and sex-adjusted discrete logistic regression models were used to assess associations between well known AMD risk factors (age, sex, smoking, presence of risk alleles for *CFH-rs1061170* and *ARMS2-rs10490924*, BP, WCC, total cholesterol, HDL, triglycerides, fish consumption, alcohol consumption, vitamin supplementation and lutein-zeaxanthin dietary intake) and the 15-year development of reticular drusen using person-specific data. If risk factors were significantly associated with the 15-year incidence of reticular drusen in age-and sex-adjusted models, they were further adjusted for in a multivariable-adjusted model, and expressed as odds ratios (ORs). The two SNPs (*CFH-rs1061170* and *ARMS2-rs10490924*) were assessed additively, where genotype (none, one or two risk alleles) was treated as a continuous variable.

Generalized estimating equation models, using the GENMOD procedure in SAS[196] was used to analyze eye-specific data for associations between other early AMD lesion characteristics and the 15-year incidence of reticular drusen. This method was also used to assess the relationship between the common AMD risk factors and 5-year progression of reticular drusen to late AMD using eye-specific data. Each risk factor

was defined using information collected at the time when reticular drusen was detected. Estimates are expressed as age- and sex-adjusted or multivariable-adjusted (age, sex, smoking, *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms) OR and 95% confidence intervals (CI).

RESULTS

Prevalence and Incidence of Reticular Drusen

Reticular drusen were present in 1.95% of the baseline BMES population sample. Of the 65 participants with reticular drusen, in 38 (58.5%) this sign was present in both eyes (bilateral). The co-presence of reticular drusen with late AMD occurred in 7 of the total 103 eyes (6.8%) with prevalent reticular drusen.

Incident reticular drusen were identified in 95 (152 eyes) of 2738 participants at risk, with an overall 15-year incidence of 4.0% (95% CI 3.2-4.8) after controlling for the competing risk of death. Of the 95 cases, 57 (60.0%) were bilateral. Baseline characteristics of participants with and without incident reticular drusen are presented in **Table 3.3-1**. Participants who developed reticular drusen were more likely to be female, older at baseline, to have at least one risk allele of *CFH-rs1061170* or *ARMS2-rs10490924*, and to have higher mean HDL and lower mean triglyceride levels than participants who did not develop reticular drusen.

Table 3.3-1: Comparison of baseline characteristics between participants with and without incident reticular drusen.

Characteristic	% Participants		P-value
	No reticular drusen (n=2135)	Incident reticular drusen (n=95)	
Mean Age, years (SD)	63.4 (8.2)	69.5 (6.4)	<0.0001
Sex (female)	56.6	73.7	0.001
Smoking			
Non-smoker	51.8	57.5	
Ex-smoker	35.3	30.9	0.6*
Current smoker	13.0	11.7	
CFH-rs1061170			
TT	40.3	22.0	
CT	46.2	53.7	0.0008*
CC	13.5	24.4	
ARMS2-rs10490924			
GG	62.9	37.0	
GT	33.2	51.9	<0.0001*
TT	3.8	11.1	
Mean Blood Pressure (mmHg; SD)			
Systolic	144.6 (20.4)	147.7 (20.4)	0.1
Diastolic	83.5 (9.9)	83.2 (8.4)	0.7
Mean White Cell Count (x10^9cells/L; SD)	6.4 (1.7)	6.5 (1.5)	0.3
Mean Total Cholesterol (mmol/L; SD)	6.0 (1.0)	6.2 (1.1)	0.3
Mean High Density Lipoprotein (mmol/L; SD)	1.4 (0.4)	1.5 (0.4)	0.01
Mean Triglycerides (mmol/L; SD)	1.8 (1.1)	1.6 (0.8)	0.02
Fish Consumption (≥1 servings/week)	60.1	52.0	0.2
Daily Alcohol Consumption			
None	20.0	18.2	
>0 to ≤2 standard drinks	59.7	64.9	0.6*
>2 standard drinks	20.2	16.9	
Any Current Vitamin Supplement Intake	39.6	40.3	0.9
Mean Dietary Lutein and Zeaxanthin Intake (μg; SD)	829.0 (480.2)	907.5 (691.4)	0.3

SD = standard deviation; CFH = complement factor H (C risk allele); ARMS2 = age-related maculopathy susceptibility gene 2 (T risk allele)

*Unadjusted tests for heterogeneity used to calculate p-values

The 15-year cumulative incidence of reticular drusen by age and sex is presented in **Table 3.3-2**. It was significantly associated with increasing age (P for trend <0.0001) in both men and women. After adjusting for age, the 15-year cumulative incidence of reticular drusen was twice as likely in women than in men (5.6%, 95% CI, 5.59-5.61 versus 2.2%, 95% CI, 2.19-2.21; **Table 3.3-2**).

Table 3.3-2: Fifteen-year cumulative incidence of reticular drusen by age and sex

Age (years)	15-year Incidence of Reticular Drusen					
	Women		Men		Both	
	No. of cases/ no. at risk	% (95% CI)	No. of cases/ no. at risk	% (95% CI)	No. of cases/ no. at risk	% (95% CI)
49 to 54	0/205	0	1/162	0.8 (-0.8-2.4)	1/367	0.4 (-0.4-1.2)
55 to 64	13/519	3.1 (1.4-4.8)	4/420	1.1 (0.0-2.2)	17/939	2.2 (1.2-3.2)
65 to 74	42/530	10.3 (7.4-13.2)	12/414	3.2 (1.4-5.0)	54/944	7.0 (5.2-8.8)
75+	15/259	6.2 (3.3-9.1)	8/229	3.5 (1.2-5.8)	23/488	4.9 (3.0-6.8)
***P* trend**	< . 0001		< . 0001		< . 0001	
Total	70/1513	5.6 (4.3-6.9)*	25/1225	2.2 (1.3-3.1)*	95/2738	4.0 (3.2-4.8)

CI = confidence interval

*Age-adjusted difference between sex, P = 0.0032

In an age- and sex-adjusted model (**Table 3.3-3**), each additional risk allele of *CFH-rs1061170* and *ARMS2-rs10490924* was also associated with a greater 15-year risk of incident reticular drusen ($p=0.0004$ and $p<0.0001$, respectively). Increasing WCC was positively associated with reticular drusen incidence, but this association was marginally non-significant ($p=0.07$). Current smoking at baseline was not associated with the 15-year development of reticular drusen in the age- and sex-adjusted model, but became so after adjusting for age, sex and presence of the *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms. In the multivariable-adjusted model, increasing age, female sex and presence of risk alleles for *CFH-rs1061170* and *ARMS2-rs10490924* remained significantly associated with an increased risk of incident reticular drusen, in addition to current smoking status (**Table 3.3-3**).

Table 3.3-3: Associations between well-known age-related macular degeneration risk factors and the 15-year incidence of reticular drusen.

Risk Factor	15-year Incidence of Reticular Drusen			
	Age and Sex Adjusted (where appropriate)		Multivariable Adjusted*	
	OR	95% CI	OR	95% CI
Age, per 10 years	3.4	2.6-4.4	4.3	3.1-5.9
Female Sex	2.0	1.3-3.2	2.2	1.3-3.8
Smoking				
Ex-smoker	1.0	0.6-1.7	1.3	0.7-2.1
Current smoker	1.7	0.9-3.4	2.1	1.0-4.5
CFH allele - rs1061170†	1.8	1.3-2.4	1.8	1.3-2.6
ARMS2 allele - rs10490924†	3.0	2.1-4.4	3.2	2.2-4.6
Blood Pressure, per 10mmHg				
Systolic	1.0	0.9-1.1	-	-
Diastolic	1.0	0.8-1.3	-	-
White Cell Count‡	1.2	1.0-1.5	-	-
Total Cholesterol‡	1.0	0.8-1.3	-	-
High Density Lipoprotein‡	1.2	0.9-1.4	-	-
Triglycerides‡	0.8	0.6-1.1	-	-
Fish Consumption (≥1 servings/wk)	0.8	0.5-1.2	-	-
Daily Liquor Consumption				
None	0.7	0.4-1.2	-	-
>0 to ≤2 standard drinks (referent)	1.0			
>2 standard drinks	1.0	0.5-1.9	-	-
Vitamin Supplementation (current baseline intake)	1.0	0.6-1.5	-	-
Lutein-Zeaxanthin Intake‡	1.1	0.9-1.4	-	-

OR = odds ratio; CI = confidence interval; CFH = complement factor H; ARMS2 = age-related maculopathy susceptibility gene 2

*Multivariable ORs adjusted for age, sex, past and current smoking, the *CFH* and *ARMS2* risk alleles as additive variables

†Analyzed using additive models; estimates represent risk associated with each additional single nucleotide polymorphism of the minor variants.

‡Per standard deviation increase in each risk factor

The association between early AMD lesion characteristics and the 15-year incidence of reticular drusen is shown in **Table 3.3-4**. After adjusting for age and sex, the presence of soft indistinct drusen (excluding reticular drusen) (OR 4.3), location of any soft drusen within 500μm radius of the foveola (OR 2.6) and a collective area of any soft drusen ≥375μm (ORs 3.1 – 6.3) were all predictive of the development of reticular drusen. After further adjusting for smoking and the *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles, only the location of any soft drusen at close proximity to the fovea (OR 2.2) and an area of soft drusen ≥375μm but <0.5DA in diameter (OR 3.2) were significantly associated with a greater risk of reticular drusen (**Table 3.3-4**).

Table 3.3-4: Relationship between baseline early age-related macular degeneration (AMD) lesion characteristics and the 15-year incidence of reticular drusen, analyzed by eye.

Early AMD Characteristics	No. eyes that progressed/ no. eyes at risk of progression to reticular drusen	15-year Incidence of Reticular Drusen			
		Age and Sex Adjusted		Multivariable Adjusted*	
		OR	95% CI	OR	95% CI
Drusen Type					
None or hard distinct	99/3823	1.0		1.0	
Intermediate	21/439	1.1	0.7, 1.8	1.1	0.6, 1.8
Soft distinct	9/94	2.0	0.9, 4.3	1.8	0.7, 4.4
Soft indistinct	12/73	4.3	1.7, 10.8	2.1	0.6, 7.7
Drusen Location					
None or ≥500µm radius of foveal centre	101/4034	1.0		1.0	
<500µm radius of foveal centre	40/385	2.6	1.7, 4.1	2.2	1.3, 3.7
Drusen Area					
None or <375µm in diameter	111/4238	1.0		1.0	
≥375µm to <0.5 disc area in diameter	21/143	3.1	1.8, 5.4	3.2	1.7, 5.8
≥ 0.5 disc area in diameter	8/40	6.3	2.2, 18.1	2.3	0.4, 12.2
RPE Depigmentation					
Absent	141/4402	1.0		1.0	
Present	9/131	1.3	0.6, 2.7	1.5	0.6, 3.4
Location					
None or ≥1500µm radius of foveal centre	141/4405	1.0		1.0	
≥500µm to <1500µm radius of foveal centre	4/67	1.4	0.6, 3.1	1.8	0.7, 4.5
<500µm radius of foveal centre	5/61	1.3	0.4, 4.5	1.2	0.3, 5.6
Area					
None or <375µm in diameter	146/4451	1.0		1.0	
≥375µm in diameter	4/82	1.2	0.4, 3.7	1.3	0.4, 4.7
Hyperpigmentation					
Absent	135/4260	1.0		1.0	
Present	15/274	1.2	0.6, 2.2	1.2	0.6, 2.7
Location					
None or ≥1500µm radius of foveal centre	135/4278	1.0		1.0	
≥500µm to <1500µm radius of foveal centre	4/86	1.1	0.5, 2.5	1.4	0.6, 3.3
<500µm radius of foveal centre	10/124	1.4	0.5, 3.7	1.3	0.4, 4.4
Area					
None or <64µm in diameter	137/4280	1.0		1.0	
≥64µm in diameter	12/208	1.1	0.5, 2.3	1.1	0.5, 2.6

OR = odds ratio; CI = confidence interval; RPE = retinal pigment epithelial

*Multivariable ORs adjusted for age, sex, current smoking, the *CFH* and *ARMS2* risk alleles

Progression of Reticular Drusen

Of the 65 participants with prevalent reticular drusen, 37 had retinal photographs available from at least one follow-up visit. Of these, 18 participants (48.6%) progressed to late AMD within 15 years, in at least one eye. Five-year progression from reticular drusen to late AMD occurred in 14 of the 37 (37.8%) participants (18 of 59 eyes; 30.5%).

Of the 95 participants (152 eyes) with incident reticular drusen detected at either of the 5-, 10- or 15-year follow-up visits, only 40 participants (59 eyes) had follow-up information. Overall, 18 of 40 participants (26 of 59 eyes) progressed to late AMD within 5 to 10 years. Of the 26 eyes with reticular drusen that progressed, 22 eyes progressed to late AMD in 5 years. This included eyes in which both reticular drusen and late AMD were detected at the same visit and in which reticular drusen was assumed to have developed prior to the late AMD.

Of a total 118 eyes with either prevalent or incident reticular drusen that were available for assessment of progression, 33.9% (40 eyes; 18 with prevalent and 22 with incident reticular drusen) progressed to late AMD over 5 years. Of these, 23 (57.5%) progressed to GA, 12 (30.0%) progressed to neovascular AMD and 5 (12.5%) developed both GA and neovascular AMD in the same eye. By comparison, of 722 eyes with other early AMD lesions (excluding reticular drusen), that were available for assessment of progression, 62 (7.9%) eyes progressed to late AMD over 5 years, including 27 (43.6% of the 62 eyes) that developed GA, 30 (48.4%) that developed neovascular AMD and 5 (8.1%) that developed both late AMD lesions. Further, of 4176 eyes with no prior early

AMD lesions, 6 (0.1%) progressed to late AMD over 5 years, all of which developed neovascular AMD.

A higher proportion of eyes with reticular drusen outside the WARGMS grid progressed to late AMD compared to eyes with reticular drusen in the central, inner or outer circles (50.0% versus 23.5-37.8%). Of eyes with reticular drusen present within the grid, there was no difference in proportion of eyes that progressed to late AMD between eyes with small (28.6%) or large (37.9%) areas of reticular drusen.

No significant associations were found between the 5-year progression from reticular drusen to late AMD and most known AMD risk factors assessed (age, female sex, smoking status, genetic risk from *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms, BP, WCC, total cholesterol, HDL, triglycerides, fish consumption, alcohol consumption and vitamin supplementation; data not shown). However, a significantly lower risk of progression to late AMD was evident in the eyes of subjects with increased intake of dietary lutein-zeaxanthin (OR 0.5, 95% CI 0.3-1.0, p=0.046) after adjusting for age, sex, current smoking, and presence of the *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms.

DISCUSSION

We found an overall 15-year incidence of reticular drusen of 4.0% in this older Australian cohort, with over 50% of incident cases developing bilaterally. The incidence of reticular drusen rose with increasing age and was significantly higher in women than in men. Current smoking and presence of the *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles were also independently associated with higher 15-year incidence of

reticular drusen. A substantially higher proportion (34%) of eyes with reticular drusen (with or without early AMD lesions) compared to eyes without reticular drusen but with other early AMD lesions (8%) progressed to late stage AMD within 5 years. Increased dietary lutein-zeaxanthin intake was associated with a lower risk of progression from reticular drusen to late AMD over 5 years.

The BDES and the BMES are the only two longitudinal population-based studies to have reported 15-year incidence of reticular drusen in older cohorts to date. Although the prevalence of reticular drusen in the BMES (1.95%) was more than double the prevalence reported in the BDES (0.7%), the 15-year incidence of reticular drusen in the BMES, after accounting for competing risk of death (4.0%) was similar to the incidence in the BDES (3.0%), given that the BMES population was slightly older than the BDES population at baseline (43 to 86 years for the BDES; and 49 to 97 years for the BMES). In addition to age, differences in environmental exposures (smoking, diet, sunlight exposure) could also account for the differences in prevalence and incidence of reticular drusen between these two studies.

We confirmed that older age and female sex are associated with a greater risk of reticular drusen, consistent with the BDES[227] and previous cross-sectional studies that reported a female preponderance for reticular drusen[225,228]. Smoking was independently associated with incident reticular drusen, as it was with late AMD in the BMES[115]. The risk of incident reticular drusen for current smokers was 2.1-fold the risk for non-smokers in our cohort, similar to that found in the BDES (current smokers had 1.9 times the risk of reticular drusen compared to non-current smokers)[227], though no similar association was found between smoking and incident late AMD in the BDES[34,116,117].

The BDES previously reported a higher prevalence of reticular drusen in those with than without the *CFH-rs1061170* polymorphism[227], however, the increased risk of reticular drusen associated with the presence of either or both the *CFH-rs1061170* and *ARMS2-rs10490924* minor alleles, found in our study, has not been demonstrated previously [227]. An inflammatory basis has been hypothesized for the development of reticular drusen[228,235], however, we did not observe a significant relationship between WCC levels and 15-year incident reticular drusen.

Of other early AMD lesions and early AMD lesion characteristics, we found that soft drusen location close to the fovea and an intermediate area of soft drusen was associated with 15-year incident reticular drusen. This finding is inconsistent with the BDES finding that presence of more severe drusen type but not drusen location or area predicted 15-year development of reticular drusen[227].

About one in three eyes with reticular drusen progressed to late AMD in 5 years in our study, compared to >50% of eyes that progressed to late AMD in a cohort of the Nutritional AMD Treatment 2 (NAT 2) study[236]. The NAT 2 study, however, assessed participants who had unilateral neovascular AMD already present, which may explain the higher frequency of eyes that progressed from reticular drusen to late AMD[35]. We found that progression from reticular drusen to pure GA was slightly more frequent than the progression to neovascular AMD, a finding that appears to conflict with other reports, although the previous findings were based on associations between reticular drusen and late AMD detected at the same visit[224,229,231].

In the Geographic Atrophy Progression study population [237], the average growth rate of reticular drusen area was found to be 4.4mm^2/year over a period of 18 months; although this observation was obtained from 16 eyes only[237]. We did not conduct quantitative measures of areas of reticular drusen, as the majority of reticular drusen were located outside the WARMGS grid and therefore the photographic fields taken might not include all of the reticular drusen in the eye.

We found that the proportion of eyes with reticular drusen that progressed to late AMD in 5 years was 4-fold the corresponding proportion of eyes without reticular drusen but with other early AMD lesions. This finding is consistent with clinicians' experience as well as observation from other studies. Klein et al[227] demonstrated that eyes with reticular drusen conferred a 6-fold risk of late AMD compared to eyes with soft indistinct drusen but no reticular drusen. We also observed that reticular drusen progressed to RPE depigmentation followed by GA. Fading of reticular drusen as neovascular AMD developed has also been documented in previous studies[236].

Although we observed a decreased risk of 5-year progression from reticular drusen to late AMD associated with increased dietary intake of lutein-zeaxanthin, this observation was based on a relatively small sample of eyes of participants who had dietary intake information available. Nevertheless, our observation is in keeping with findings from the Age-Related Eye Disease Study (AREDS) that showed a protective effect of dietary lutein-zeaxanthin intake on the development of late AMD[143] and a similar protective effect for treatment with lutein-zeaxanthin supplements in participants with low dietary intake of lutein-zeaxanthin (AREDS2)[137].

Strengths of this study include long-term follow-up of participants in an older Australian cohort, and side-by-side grading of retinal photographs taken over the follow-up period of the same participants. The low follow-up rate in the 15-year visits, however, could have led to an over- or under-estimation of reticular drusen incidence. Limitations include that only color retinal fundus photographs were used to detect reticular drusen, resulting in substantial under-estimation of reticular drusen prevalence and incidence, compared to multimodal imaging methods (fundus autofluorescence, infrared reflectance, blue light photography and spectral domain optical coherence tomography), as used in other studies [225,231,238,239], therefore caution must be exercised when interpreting these findings. The distinction between soft indistinct drusen and reticular drusen can be differentiated well with monochromatic blue light as well as red free images, and therefore misclassification of drusen was unlikely in our study.

SUMMARY

This report shows the 15-year incidence of reticular drusen was 4.0% in this older Australian cohort, and was associated with well-known AMD risk factors. Reticular drusen was, however, documented only from standard color photography, which could have substantially underestimated the prevalence and incidence compared to that found using multimodal imaging methods. The proportion of eyes that progressed from reticular drusen to late AMD over 5 years was four-fold that in eyes without reticular drusen, suggesting that this sign portends a higher risk. High dietary intake of lutein-zeaxanthin was associated with a lower risk of progression from reticular drusen to late AMD over 5 years, though confirmation of this finding in future studies will be important.

3.4 The incidence and progression of medium drusen over 15 years

Publication relating to this section of Chapter 3:

Joachim N, Mitchell P, Kifley A, Wang JJ. Incidence, Progression and Associated Risk Factors of Medium Drusen in Age-related Macular Degeneration: Findings from an Older Australian Cohort. *JAMA Ophthalmology* 2015; 133(6): 698-705

ABSTRACT

Purpose: To assess the 15-year incidence and progression of medium drusen and associated risk factors.

Methods: Of 3654 participants, aged 49+ years who attended baseline examinations of the Blue Mountains Eye Study (1992-1994), 75.8%, 76.7% and 56.1% of survivors attended 5-, 10- and 15-year follow-up examinations, respectively. Colour retinal fundus photographs were taken at each examination. Incidence and progression of medium drusen (maximum diameter ≥63μm-<125μm) were assessed using Kaplan-Meier product limit survival methods controlling for the competing risk of death. Factors associated with the 15-year incidence of medium drusen were assessed using discrete logistic regression models after adjusting for age, sex, smoking, serum lipids, systemic and dietary factors, *complement factor H (CFH-rs1061170)* and *age-related maculopathy susceptibility 2 (ARMS2-rs10490924)* polymorphisms. Associations between lesion characteristics and progression to late AMD were assessed using generalized estimating equation models and eye-specific data.

Results: Of 1317 participants at risk the 15-year cumulative incidence of medium drusen was 13.9% (n=281). Increasing age (per decade older, odds ratio, OR 1.4; 95% confidence interval (CI) 1.2-1.8) and the presence of ≥3 risk alleles of the *CFH-rs1061170* or *ARMS2-rs10490924* genes (OR 2.1, 95% CI 1.1-4.1) were associated with a higher incidence. There was no association between past or current smoking and development of medium drusen (OR's 0.8, 95% CI 0.6-1.1 and 0.6, 95% CI 0.4-1.1, respectively). The progression rate to late AMD in eyes that had both medium drusen and retinal pigmentary abnormalities was 4-fold higher than that of eyes with medium

drusen alone. Larger total area and central location of medium drusen were associated with a greater likelihood of progression to worse stages of AMD.

Conclusion: Older age and presence of *CFH* and *ARMS2* risk alleles are two main risk factors associated with medium drusen development. The co-presence of medium drusen and retinal pigmentary abnormalities signals a greater risk of progression to late AMD than the presence of medium drusen alone.

BACKGROUND

Early age-related macular degeneration (AMD) is characterized by the presence of drusen and retinal pigmentary abnormalities[13,28]. Drusen vary in size (with diameters ranging from ≤63μm to ≥250μm) and type (hard, soft, distinct and indistinct). Pigment abnormalities include clusters of pigment granules within the sensory retina (increased pigmentation) and sharply demarcated areas of retinal pigment epithelial (RPE) depigmentation.

The International Classification and Grading System for AMD termed medium drusen as 'intermediate' soft drusen, defined as drusen with maximum diameter ≥63μm but <125μm, larger than the maximum diameter of hard drusen (<63μm) but smaller than the minimum diameter of large soft drusen (≥125μm)[13]. A similar definition of this drusen type was used by Age-Related Eye Disease Study (AREDS)[28] and the Basic Clinical Classification System[31], and termed 'medium' drusen. Similarly the Wisconsin Age-Related Maculopathy Grading System (WARMGS) defines medium drusen by maximum diameter, although the term medium drusen is not used[26]. In this report, we use the term 'medium' drusen for this type of drusen.

Although medium drusen have attracted some attention recently and was included in AMD incidence reports[61,62], knowledge of the associated risk factors and progression of medium drusen is limited. It has been relatively under researched compared to large, soft drusen and pigmentary lesions[29,31,240,241]. In this report, we aimed to assess the 15-year incidence and progression of medium drusen in an older Australian cohort and associations between common AMD risk factors and the development and progression of medium drusen.

METHODS

Procedures

The study population and procedures for retinal fundus photography acquisition and grading of AMD are described in Methods, Chapter 2.

Late and early AMD were also defined in Methods, Chapter 2. The maximal diameter of individual drusen and collective macular areas involved by drusen and retinal pigment epithelium (RPE) abnormalities within the eye were estimated using the WARMGS measurement circles[26].

Definition of Medium Drusen Incidence and Progression

Medium drusen is defined as soft drusen with a diameter larger than 63μm but smaller than 125μm. Incidence of medium drusen were defined as its presence at the 5-, 10- or 15-year follow-up in persons who had no drusen or hard drusen only in any eye at the baseline visits. Progression of medium drusen was defined as the progression to worse AMD lesions including large soft drusen, retinal pigmentary abnormalities or late AMD at follow-up visits, in eyes with medium drusen as the most severe lesion at baseline.

Genotyping

Genotyping was performed on 2761 participants with blood samples collected during the BMES II examinations as described in Methods (Chapter 2) After quality checking, 2534 subjects with genome-wide association scan (GWAS) data were imputed.

The *complement factor H (CFH)* single nucleotide polymorphism (SNP) *rs1061170* was also genotyped in 1874 participants and the *age-related maculopathy susceptibility 2 (ARMS2)* SNP *rs10490924* genotyped in 593 participants as described (Methods, Chapter 2). Imputed SNPs of these two genes were used for the remaining participants. Of the 1501 and 509 participants who had both typed and imputed *rs1061170* and *rs10490924*, respectively, the concordance rates between typed and imputed SNPs were 99.6% for *rs1061170* and 99.2% for *rs10490924*.

Other Risk Factors

Participants were classified as non-smokers if they answered 'no' to the question whether they smoke regularly. Past smoking was defined if participants had smoked regularly but quit smoking more than 1 year prior to the examination. Current smoking was defined if participants were current smokers or had stopped smoking <1 year before the examination. Alcohol consumption (including beer, wine or spirits) was categorized as none, >0 but ≤2 standard drinks and >2 standard drinks per day. These categories were based on Australian National Health and Medical Research Council (NHMRC) recommendations of up to 2 standard drinks per day[234].

Systolic and diastolic blood pressure (BP) measurements were recorded from the first and fifth Korotkoff sounds using a mercury sphygmomanometer after participants were seated for at least 5 minutes[232]. Dietary consumption and supplement use were extracted from the self-administered FFQ [139]. The Australian tables of food composition[191,192] and the United States Department of Agriculture carotenoid food composition database[193] were used to estimate, respectively, the intake of nutrients, including lutein and

zeaxanthin in μg. Regular fish consumption was defined as consuming ≥1 serving of fish per week.

Fasting blood samples were collected from 3222 baseline participants to assess white cell count (WCC) and cholesterol levels, as previously described[154,160,233]. Briefly, WCC ($x10^9$/L) was determined using Coulter Counter methods (Beckman Coulter, Inc, Fullerton, CA)[160], and total cholesterol, high density lipoprotein (HDL) and triglyceride concentrations (mmol/L) were measured on a Reflotron reflectance photometric analyser (Roche Diagnostics, Germany)[233].

Statistical Analyses

SAS software version 9.3 (SAS Institute, Inc., Cary NC) was used. The 15-year incidence of medium drusen was estimated using Kaplan-Meier product limit survival estimates and competing risk analyses that control for the competing risk of death among persons at risk of medium drusen, after excluding participants with any worse stage of early or late AMD lesions at baseline. The associations between common AMD risk factors (age, sex, smoking status, numbers of *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles, BP, WCC, total cholesterol, HDL, triglycerides, fish consumption, alcohol, antioxidant and zinc supplementation intake) and the 15-year incidence of medium drusen were assessed using age and sex-adjusted discrete logistic regression models. If these risk factors had a *P* value ≤0.09 in the age and sex-adjusted regression models, they were included in the multivariable-adjusted logistic regression model. The final multivariable-adjusted logistic regression model includes age, sex, past and current smoking, zinc supplementation and the combined *CFH-rs1061170* and

ARMS2-rs10490924 risk alleles as co-variables. The combined *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles were categorized as none, 1, 2 or ≥3.

The frequencies of progression from medium drusen alone, and from medium drusen plus RPE abnormalities to worsening stages of AMD were reported. Generalized estimating equation models, using the GENMOD procedure in SAS[196], were applied to eye-specific data to assess the associations between medium drusen area and location characteristics and progression to early or late AMD. Association estimates are presented as age- and sex-adjusted or multivariable-adjusted odds ratio (ORs) and 95% confidence intervals (CIs).

RESULTS

Prevalence of Medium Drusen

Of 3654 baseline participants, we excluded persons with late AMD (n=75), early AMD (n=185) and large soft distinct drusen (n=113), and included 3281 for the assessment of medium drusen. Medium drusen status can be clearly defined in 2959 participants, of which 534 (18.0%) had medium drusen, including, 445 (83.3%) present with medium drusen only and 89 (16.7%) co-present with retinal pigmentary abnormalities. Medium drusen was bilateral in 16.6% (74/ 445).

Incidence of Medium Drusen

Among 1317 persons without any AMD lesions at baseline who had been followed up, the 5-, 10- and 15-year cumulative incidence of medium drusen was 10.1% (95% CI 8.6 - 11.9), 17.7% (95% CI 15.6 - 20.1) and 30.8% (95% CI 27.7 - 34.3), respectively. After controlling for competing risk of death, the 5-, 10- and 15-year cumulative incidence of

medium drusen was 5.7% (95% CI 4.8 – 6.6), 9.2% (95% CI 9.1 – 9.3) and 13.9% (95% CI 12.5-15.3), respectively. The 15-year incidence of medium drusen by age and sex are presented in **Table 3.4-1**. The incidence rates across all age groups were comparable, except for those aged 75 years and older. Incidence of medium drusen was slightly lower in men compared with women.

Table 3.4-1: Fifteen-year incidence of medium drusen by age and sex.

Age (years)	15-year Kaplan-Meier Incidence of Medium Drusen*					
	Women		Men		Both	
	No of cases/ No. at risk	*% (95% CI)*	*No of cases/ No. at risk*	*% (95% CI)*	*No of cases/ No. at risk*	*% (95% CI)*
49-54	32/145	28.5 (20.8-38.3)	17/110	19.9 (12.7-30.3)	49/255	24.7 (19.1-31.6)
55-64	68/319	28.9 (23.3-35.4)	49/257	27.0 (20.8-34.6)	117/576	28.1 (23.8-32.9)
65-74	59/219	41.1 (31.3-52.7)	43/167	43.9 (32.8-57.0)	102/386	42.7 (35.1-51.3)
75+	Aug-51	15.7 (8.2-28.9)	May-49	15.8 (5.5-40.5)	13/100	16.2 (8.6-29.4)
Total	**167/734**	**32.2 (28.0-36.9)**	**114/583**	**29.0 (24.5-34.3)**	**281/1317**	**30.8 (27.7-34.3)**

Age (years)	15-year Cumulative Incidence of Medium Drusen after Controlling for Competing Risk of Death					
	Women		Men		Both	
	No. of deaths/ No. at risk	*% (95% CI)*	*No. of deaths/ No. at risk*	*% (95% CI)*	*No. of deaths/ No. at risk*	*% (95% CI)*
49-54	13/145	24.1 (17.0-31.2)	8/110	16.8 (9.7-23.9)	21/255	20.9 (15.8-25.9)
55-64	37/319	22.2 (17.6-26.7)	48/257	17.6 (13.3-21.9)	85/576	20.0 (16.9-23.2)
65-74	63/213	17.8 (13.9-21.8)	79/167	13.0 (9.7-16.4)	142/386	15.5 (13.0-18.1)
75+	40/51	2.5 (0.8-4.1)	39/49	1.8 (0.3-3.3)	79/100	2.2 (1.0-3.3)
Total	**153/734**	**15.8 (13.7-17.9)**	**174/583**	**11.8 (9.9-13.7)**	**327/1317**	**13.9 (12.5-15.3)**

CI = confidence interval

*Definition of medium drusen herein excludes larger soft drusen, pigment abnormalities and late age-related macular degeneration

Baseline characteristics of participants with and without incident medium drusen are presented in **Table 3.4-2**. There were no significant differences in the mean age or the frequency of females between participants with and without incident medium drusen. However, participants with incident medium drusen were marginally more likely to have ≥1 risk allele of *ARMS2-rs10490924*, lower mean WCC and were less likely to be past or current smokers or to take zinc supplements.

Table 3.4-2: Comparison of baseline characteristics of participants with versus without incident medium drusen.

Baseline Characteristic	Participants (%)		*P* value
	No Medium Drusen	Incident Medium Drusen	
Mean age (SD), yrs	62.1 (8.1)	62.3 (7.5)	0.7
Female sex	54.7	59.4	0.2
Smoking Status			
non-smoker	47.8	61.8	
past smoker	37.3	28.7	<.001*
current smoker	14.8	9.5	
CFH-rs1061170			
TT	42.3	38.2	
CT	47	49	0.4*
CC	10.6	12.9	
ARMS2-rs10490924			
GG	65.6	57.9	
GT	30.4	37.8	0.09*
GT	4	4.3	
***CFH* and *ARMS2* combined risk**			
No risk alleles	28.7	21.3	
1 risk allele	40.6	44	0.2*
2 risk alleles	25.5	27.6	
≥3 risk alleles	5.2	87.1	
Mean blood pressure (SD), mmHg			
Systolic	143.4 (19.9)	144.6 (21.1)	0.4
Diastolic	83.2 (10.0)	83.8 (9.1)	0.4
Mean white cell count (SD), x10^9 cells/l	6.4 (1.7)	6.2 (1.4)	0.06
Mean total cholesterol (SD), mmol/l	6.0 (1.1)	6.1 (1.1)	0.6
Mean high-density lipoprotein (SD), mmol/l	1.4 (0.5)	1.5 (0.4)	0.3
Mean triglycerides (SD), mmol/l	1.8 (1.1)	1.7 (1.1)	0.2
Fish consumption (≥1 serving/wk)	60.3	60.2	1
Alcohol consumption			
none	29.6	31.6	
>0 to ≤2 standard drinks/day	58.6	55.5	0.7*
>2 standard drinks/day	11.8	12.9	
Any antioxidant supplement	36.1	35.2	0.8
Any zinc supplementation	16.9	11.6	0.04
Mean dietary lutein-zeaxanthin intake (SD), µg	0.8 (0.5)	0.8 (0.4)	0.6

CFH = complement factor H, ARMS2 = age-related maculopathy susceptibility gene 2, SD = standard deviation

*Unadjusted tests for heterogeneity used to calculate *P* values.

Table 3.4-3 presents associations between known AMD risk factors and the incidence of medium drusen. Each decade increase in age was significantly associated with 15-year incident medium drusen after adjusting for sex. The presence of ≥3 risk alleles of *CFH-rs1061170* and *ARMS2-rs10490924* combined were significantly associated with an increased risk of developing medium drusen (OR 2.1, 95% CI 1.1-3.9), after age-sex adjustment. These associations remained similar after additional adjustment for past and current smoking, and zinc supplementation (**Table 3.4-3**). The 15-year incidence of medium drusen was inversely associated with higher intake of zinc, however, this association was marginally non-significant in both age-sex-adjusted and multivariable-adjusted models. Risk factors including smoking, blood pressure, WCC, serum lipids, fish and liquor consumption, antioxidant supplementation and lutein-zeaxanthin intake were not significantly associated with the 15-year incidence of medium drusen in the age-sex adjusted models (data not shown).

Table 3.4-3: Associations between known age-related macular degeneration risk factors and the 15-year incidence of medium drusen

Risk Factor	15-year Incidence of Medium Drusen	
	Age and Sex Adjusted (where appropriate) OR (95% CI)	**Multivariable Adjusted[*] OR (95%CI)**
Age, per 10 years	1.4 (1.2-1.7)	1.4 (1.2-1.8)
Male Sex	0.2 (0.6-1.0)	0.9 (0.6-1.2)
Smoking status		
Never Smoker	1.0 (reference)	1.0 (reference)
Past smoker	0.7 (0.5-0.9)	0.8 (0.6-1.1)
Current smoker	0.6 (0.4-1.0)	0.6 (0.4-1.1)
***CFH* and *ARMS2* combined risk[†]**		
No risk alleles	1.0 (reference)	1.0 (reference)
1 risk allele	1.4 (1.0-2.0)	1.6 (1.1-2.4)
2 risk alleles	1.4 (0.9-2.1)	1.5 (0.9-2.2)
≥3 risk alleles	2.1 (1.1-3.9)	2.1 (1.1-4.1)
Zinc supplementation	0.7 (0.5-1.1)	0.7 (0.4-1.0)

OR = odds ratio, CI = confidence interval, CFH = complement factor H, ARMS2 = age-related maculopathy susceptibility gene 2

[*]Adjusted for age, sex, past and current smoking, any zinc supplementation and the combined *CFH* and *ARMS2* risk alleles.

[†]Single nucleotide polymorphisms *CFH-rs1061170* and *ARMS2-rs10490924*; reference group = no risk alleles

Progression of Medium Drusen

The frequency of progression to large soft drusen, was similar in eyes with medium drusen only as in eyes with medium drusen and RPE abnormalities (41.8% and 50.0%, respectively, p=0.2). However, progression to late stage AMD or late AMD lesions (either geographic atrophy or neovascular AMD) was 4-fold greater in eyes co-present with medium drusen and RPE abnormalities, compared to eyes with medium drusen alone (23.0% versus 5.0%, respectively). An example of medium drusen progression is shown in **Figure 3.4-1**.

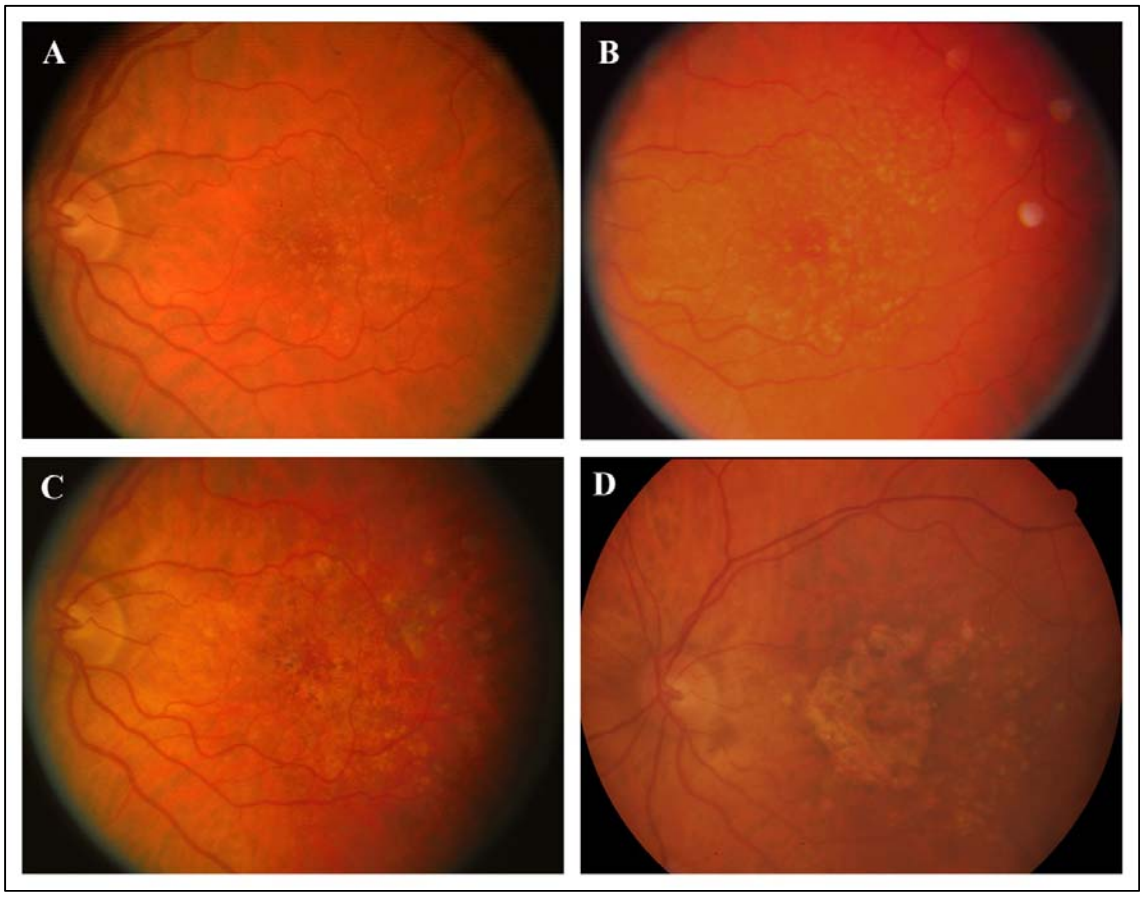

Figure 3.4-1: An example of the progression of medium drusen over 15 years. **(A)** Left eye with medium drusen and retinal pigment epithelium abnormalities at baseline; **(B)** Large soft drusen at 5 years; **(C)** More apparent retinal pigmentary abnormalities at 10 years; **(D)** Geographic atrophy at 15 years.

Table 3.4-4 presents the 15-year progression of medium drusen to early and late AMD in relation to total areas and locations of medium drusen. Eyes co-present with medium drusen and RPE abnormalities were excluded. After adjusting for age and sex, a large (≥375μm) compared to small total area (<375μm) of medium drusen, was significantly associated with high risk of developing early AMD (OR 2.9, 95% CI 1.5-5.4) and any (early or late) AMD (OR 3.0, 95% CI 1.6-5.5). However, it was not significantly associated with risk of late AMD (OR 2.3, 95% CI 0.8-6.8). After further adjusting for past and current smoking, fish consumption and the *CFH* and *ARMS2* risk alleles, these associations remained (**Table 3.4-4**). Similarly, the association between central location of medium drusen and the development of early AMD and any AMD was significant after adjusting for these co-variables (OR 2.6, 95% CI 1.4-4.8 and OR 2.4, 95% CI 1.3-4.5, respectively; **Table 3.4-4**).

Table 3.4-4: Fifteen-year progression of medium drusen to worse age-related macular degeneration stages by medium drusen are and location at baseline.

Meidum Drusen Characteristics	15-year Progression to Worse AMD Stage								
	Early AMD*			Any Late AMD			Any Early* or Late AMD		
	No. of cases/ No. at risk	Age-sex adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)†	No. of cases/ No. at risk	Age-sex adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)†	No. of cases/ No. at risk	Age-sex adjusted OR (95% CI)	Multivariable adjusted OR (95% CI)†
Area									
<375µm in diameter	73/308	1.0	1.0	13/327	1.0	1.0	76/311	1.0	1.0
≥375µm in diameter	41/68	2.9 (1.5-5.4)	3.2 (1.4-7.2)	9/71	2.3 (0.8-6.8)	1.6 (0.4-6.6)	44/71	3.0 (1.6-5.5)	3.3 (1.5-7.4)
P value‡	<0.0001	-	-	0.03	-	-	<0.0001	-	-
Location									
≥500µm radius of the foveal centre	30/150	1.0	1.0	5/160	1.0	1.0	32/152	1.0	1.0
<500µm radius of the foveal centre	82/222	2.6 (1.5-4.3)	2.6 (1.4-4.8)	17/235	3.1 (0.8-11.4)	3.5 (0.6-20.4)	86/226	2.5 (1.5-4.2)	2.4 (1.3-4.5)
P value‡	0.0002	-	-	0.06	-	-	0.0002	-	-

AMD = age-related macular degeneration, OR = odds ratio; CI = confidence interval

*Early AMD as defined in the BMES: large indistinct soft or reticular drusen or large distinct soft drusen with retinal pigmentary abnormalities.

†Odds ratios adjusted for age, sex, past and current smoking, fish consumption and increasing numbers of *complement factor H* and *age-related maculopathy susceptibility* gene risk alleles (0, 1 or 2).

‡*P* values for differences in number of cases between small versus large areas or between further versus closer locations of intermediate drusen by AMD stage.

DISCUSSION

We found an overall 13.9% of persons aged 49+ years developed medium drusen over 15 years. The incidence of medium drusen did not appear to be different across the three age groups from 49 to 74 years, but was slightly higher in women compared to men. The lower incidence of medium drusen observed in participants aged 75+ years at baseline is likely due to the fact that most persons in this age group had either died or passed this very early stage of AMD, and very few numbers of participants in this age group were considered at risk of medium drusen.

Per decade increase in age, and the presence of ≥3 risk alleles of the *CFH-rs1061170* and *ARMS2-rs10490924* combined were independently associated with increased risk of developing medium drusen. No other known AMD risk factors were found to be associated with the incidence.

There were limited numbers of population-based reports on the incidence of medium drusen available in the literature. The 5-, 10- and 15-year incidence rates of medium drusen in the BMES were 5.7%, 9.2%, and 13.9%, respectively. In the Reykjavik Eye Study, a population-based study of residents in Iceland, the 5-year incidence rate of medium drusen increased with increasing age, ranged from 5.0% in 50-59 year olds to 22.7% in those aged 70-79 years[45]. In comparison, the overall 5-year incidence of medium drusen was 10.1% in the BMES (Kaplan–Meier method), or 5.7% after accounting for competing risk of death. The Copenhagen Eye Study reported the 14-year incidence of medium drusen to be 27.4%, among persons aged 60-80 years at baseline[47]. The Beaver Dam Eye Study (BDES) found the 10- and 15-year cumulative incidence of medium drusen to be 14.0% and 23.9%, respectively, after adjusting for the

competing risk of death[33,34], higher than the 9.2% and 13.9% of incidence in 10amd 15 years in our cohort study. Sources of disparity between findings of our and the above-mentioned two studies likely include variations in defining medium drusen and different methods used to calculate incidence estimates, e.g., the competing risk approach was not used in both the Reykjavik and Copenhagen Eye Studies. While the BDES had a lower age limit (43 years at baseline) in the study sample, variations in other environmental exposures in addition to grading variations in defining medium drusen, could also explain the difference in incidence between the two studies.

The age-related increase in medium drusen incidence before adjusting for competing risk of death, found in our study, is consistent with other AMD stages and lesions[35,44,242]. Interestingly, past or current smoking was not associated with the 15-year incidence of medium drusen, a finding in keeping with previous BMES observations although current smoking was strongly associated with prevalence and incidence of late AMD, the association between smoking and early AMD was much weaker than that with late AMD[115,117]. Smoking may likely be a promoter playing a greater role in the progression from early to late AMD than in the initiation of early AMD.

We demonstrated that the risk of progression of medium drusen to late AMD was substantially higher if medium drusen was co-present with RPE abnormalities. This parallels previous findings that demonstrated the faster progression from early to late AMD in eyes with large drusen and RPE abnormalities compared to eyes with large drusen alone[30,35,243]. This observation supports severity scales that incorporate multiple lesion types to better classify risk of progression to late AMD [28,31,32].

We also found that eyes with medium drusen located closer to the fovea or eyes with large total macular areas involved by medium drusen were more likely to progress to early AMD. The non-significant association of these characteristics with the progression to late AMD was likely due to the relatively small numbers of incident late AMD cases in this cohort. These findings are consistent with the BDES that the 15-year incidence of both early and late AMD were higher in eyes with a large compared to small total area of medium drusen at baseline[33].

The strengths of this study include its long-term follow-up of an older Australian cohort, the use of retinal photographs and a validated AMD grading system to assess size and location of AMD lesions. Limitations include the relatively low follow-up rate at the 15-year examination that could have led to an over- or under-estimation of the incidence. As only colour fundus photographs, rather than high resolution imaging (e.g. spectral-domain optical coherence tomography) was available during the baseline and follow-up examinations, this may have led to an underestimation of the prevalence and incidence. However, we used colour photographs only throughout the BMES cohort to make sure that the comparisons are valid. Although the medium drusen category was included in the retinal photographic grading since the 5-year follow-up examinations, side-by-side grading of baseline and follow-up retinal images provided precise classification of this drusen type.

SUMMARY

In this report, we document the 15-year incidence of medium drusen as 13.9% in this older Australian cohort. The proportion of eyes that progressed to late AMD was

significantly higher when medium drusen co-presented with RPE abnormalities, compared to eyes with medium drusen alone. Large total area and close to foveal location of medium drusen were associated with a high likelihood of progression to more advanced AMD lesions. These findings are informative for the monitoring and management of patients at risk of early and late AMD.

Chapter 4

Comparison of Two Classification Scales for Age-related Macular Degeneration

Comparing the predictive ability of two severity scales for classification of age-related macular degeneration in population and clinic based samples

Publication relating to this Chapter:

Liew G, Joachim N, Mitchell P, Burlutsky G, Wang JJ. Validating the AREDS Simplified Severity Scale of Age-related Macular Degeneration with 5- and 10-Year Incident Data in a Population-Based Sample. (Article in Press, *Ophthalmology*: May 2016)

ABSTRACT

Purpose: A number of classification systems for age-related macular degeneration (AMD) have been developed from observations of patients in clinical trials. We aimed to validate two AMD classification systems using 5-year incident late AMD data from the population-based Blue Mountains Eye Study (BMES) cohort, and compare the published 5-year late AMD incident estimates from the Age-Related Eye Diseases Study (AREDS) clinical trial.

Methods: This comparative study included BMES participants aged 49-97 years at baseline (n=2134) and AREDS participants aged 55-80 years (n=3640). AMD lesions in the BMES were graded according to the Wisconsin AMD grading protocol from stereoscopic color photographs and classified according to: 1) the AREDS Simplified Severity Scale, and the 2) Basic Clinical Classification Scale. The AREDS Simplified Severity Scale calculates a risk score based on the number of early AMD risk factors (large drusen and pigment abnormalities) in both eyes. The Basic Clinical Classification Scale categorizes large drusen and pigment abnormalities as intermediate AMD regardless of bilaterality and is based on the worse eye. The main outcome measure was the 5-year incidence of late AMD.

Results: Over 5 years there were 32 participants who developed late AMD in either eye in the BMES, and 316 participants in the AREDS. The AREDS Simplified Severity Scale classified similar proportions of participants who developed incident late AMD in both the BMES and AREDS. The Basic Clinical Classification Scale consistently categorized 2-3 fold lower late AMD incidence rates across all risk levels in BMES compared to AREDS.

Conclusions: The AREDS Simplified Severity Scale classified late AMD risk levels similarly when applied to population-based and clinic-based samples, whereas the Basic Clinical Classification resulted in lower incidence rates in population-based samples. The choice of classification system may need to take into account the study population as actual observed incidence rates may differ from predicted rates.

BACKGROUND

There are several classification systems for age-related macular degeneration (AMD)[13,27,28,31,32,48,244]. Most are derived from observations of patients in clinical trials. Recently two widely used AMD severity scales have been updated and simplified for use in clinical practice. The Age-Related Eye Diseases Study (AREDS) Simplified Severity Scale was published in 2005[28], and is based on data from 3212 participants of the AREDS clinical trial population[27]. The scale uses a scoring system that assigns 1 risk factor for the presence of 1 or more large (≥125 μm) drusen and 1 risk factor for the presence of any retinal pigment abnormalities in an eye.(Table 1) Risk factors are summed across both eyes, forming a 5-step scale (steps 0 to step 4) for which the 5-year risk of developing advanced AMD in at least one eye increases as follows: 0 factors, 0.5%; 1 factor, 3%; 2 factors, 12%; 3 factors, 25%; and 4 factors, 50%[28].

The Basic Clinical Classification Scale was published in 2013[31] and is also based on data from the AREDS. This was an update on the International Classification System first published in 1995[13]. It is based on the same lesions as the AREDS Simplified Severity Scale but differs in that both large drusen and retinal pigment abnormalities are categorized into the same risk category, with both lesions assigned to the same risk regardless of lesion type and their bilaterality, while medium (or intermediate) drusen are given their own risk category (refer to **Table 1.1-1**). Five categories are defined based on the lesions in the worst eye, and the 5-year risk of developing late AMD for persons with none/normal ageing changes, early AMD, and intermediate AMD is 0.5%, 0.9% and 20.5 %, respectively, based on published data[13,31]. In both severity scales, advanced or late AMD is identical and defined as the presence of geographic atrophy or choroidal neovascularization.

Severity scales are useful to stratify risk categories and likely prognosis within a defined period, and facilitate communication and comparison across different samples. They are also useful endpoints for interventional therapeutic trials, where sample size and study power calculation are often based on risk estimates of incident late AMD. However, patients in clinical trials may not be representative of the general population and therefore risk estimates that were developed based on patient samples may differ when applied to general populations. This has implications for study design and power calculations and for projections of future burden of disease in populations.

We therefore aimed to examine the ability of two AMD classification systems to predict 5-year incident late AMD in a population-based cohort and compared the results with published estimates from a clinical trial.

METHODS

We used data from the population-based Blue Mountains Eye Study (BMES)(n=2134)[36,44], and compared this to incident data from the AREDS clinical trial (n=3640)[27,245].

Population-based cohort

Details of the BMES population, examination procedures and photographic grading were described in Methods, Chapter 2.

In this report, incident late AMD was defined as the appearance at 5-year follow up of either two characteristic late AMD lesions, geographic atrophy (GA) and neovascular

AMD, in either eye of persons in whom no late AMD lesion was present in both eyes at baseline.

Clinic-based cohort

The AREDS is a multicenter prospective cohort study of the clinical course, prognosis, and risk factors for AMD and cataract conducted between 1992 and 1998, when 11 retina clinics in the United States enrolled 4757 people aged 55 to 80 years[245]. The study included a randomized, placebo-controlled clinical trial of treatment with high-dose antioxidant vitamins and/or zinc on the incidence of late AMD and vision loss. Participants were examined at baseline, at the 2-year visit, and annually thereafter. This report uses published data from the baseline and 5-year visit.

Methods for taking and grading stereoscopic colour fundus photographs in AREDS have been described elsewhere[245,246]. In short, stereoscopic colour fundus photograph pairs of fields 1 (optic disc) and 2 (macula) and a single photograph of field 3 (temporal to the macula) were taken with 30° retinal fundus cameras.

In the AREDS, AMD and early AMD lesion characteristics such as drusen number, size and location was graded in a similar masked manner as in the BMES. Incident late AMD was also defined as that used in the BMES.

Statistical Analyses

We tabulated the 5-year incidence of late AMD in the BMES according to baseline severity levels of both classification systems, the AREDS Simplified Severity Scale[28], and the Basic Clinical Classification Scale[31]. These estimates were compared to the

published estimates from the AREDS. The 5-year incident late AMD rates from the AREDS for the AREDS Simplified Severity Scale were published in 2005[28] while the rates for the Basic Clinical Classification Scale are derived from Table 2 in the 2013 publication[31]. Patients with late AMD in either eye at baseline were excluded.

RESULTS

The baseline characteristics of participants differed between the BMES and AREDS populations, as the BMES is a population-based study and the AREDS, a clinical trial of patients with AMD. In the BMES, participants were slightly younger, (median age 65 years versus 69 years) and had a higher proportion of current smokers at baseline (13% versus 8%), compared to the AREDS[44,245]. In both studies, a similar proportion of participants were female (58% versus 56%)[44,245].

Table 1.1-1 (Chapter 1) summarizes the two classification systems and definitions of AMD lesions used in this report.

Figures 4-1 and **4-2** show the 5-year observed incidence of late AMD lesions in the BMES cohort compared to the predicted incidence by the two severity scales. There were 32 patients with incident late AMD in either eye in BMES, and 316 in AREDS. The AREDS Simplified Severity Scale resulted in similar proportions of patients developing incident late AMD when applied to both the BMES and AREDS samples (**Figure 4-1**). Categories 1, 2 and 4 had similar incidence rates (0.2 vs 0.5%, 2.4 vs 3.0%, 11.0 vs 12.0% and 47.1 vs 50.0% respectively), but in category 3 the incidence of late AMD in the BMES was half that in AREDS (12.8% vs 25.0%). The Basic Clinical Classification Scale showed major differences in the incidence of late AMD, with

consistently 2-3 fold lower rates at all categories in the BMES compared to AREDS, i.e., for patients with No or Normal ageing changes, Early AMD and Intermediate AMD, the rates were 0.1 vs 0.4%, 0.4 vs 0.9% and 7.0 vs 19.6% respectively (**Figure 4-2**).

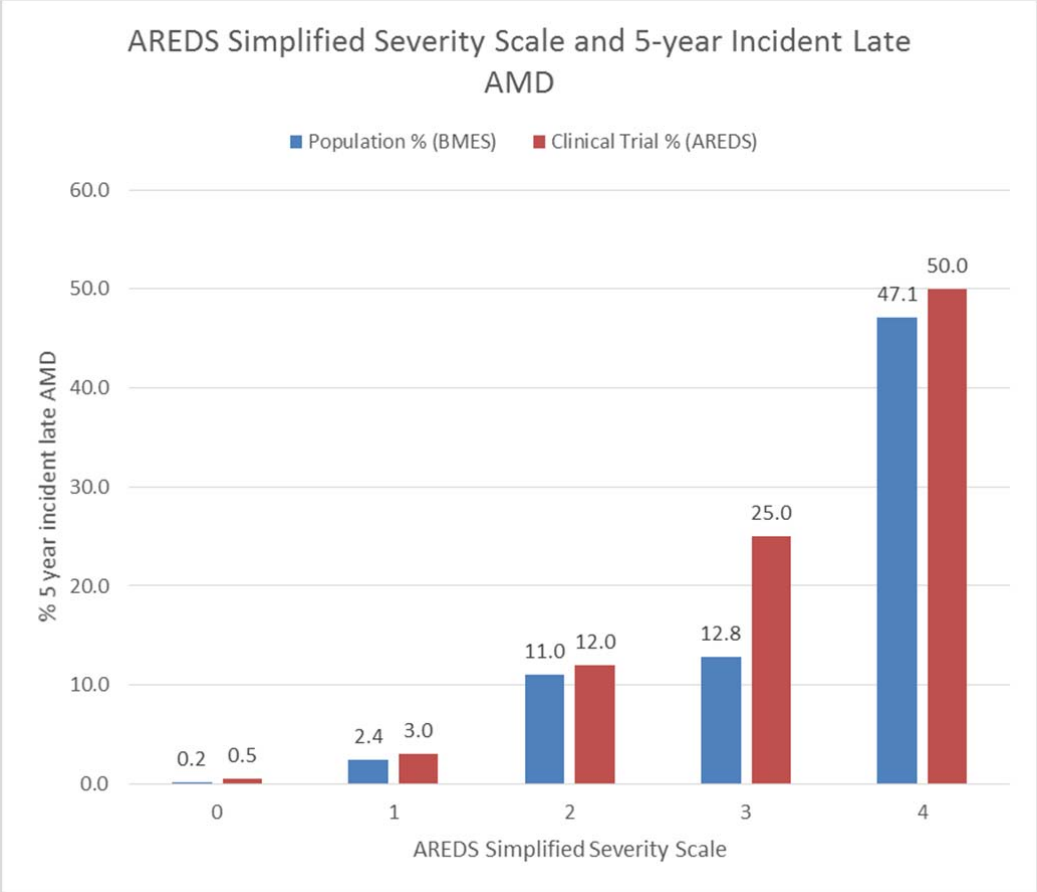

Figure 4-1 Observed 5-year incident late age-related macular degeneration (AMD) by categories on the AREDS Simplified Severity Scale. AREDS refers to the Age-Related Eye Diseases Study clinical trial. BMES refers to the population-based Blue Mountains Eye Study.

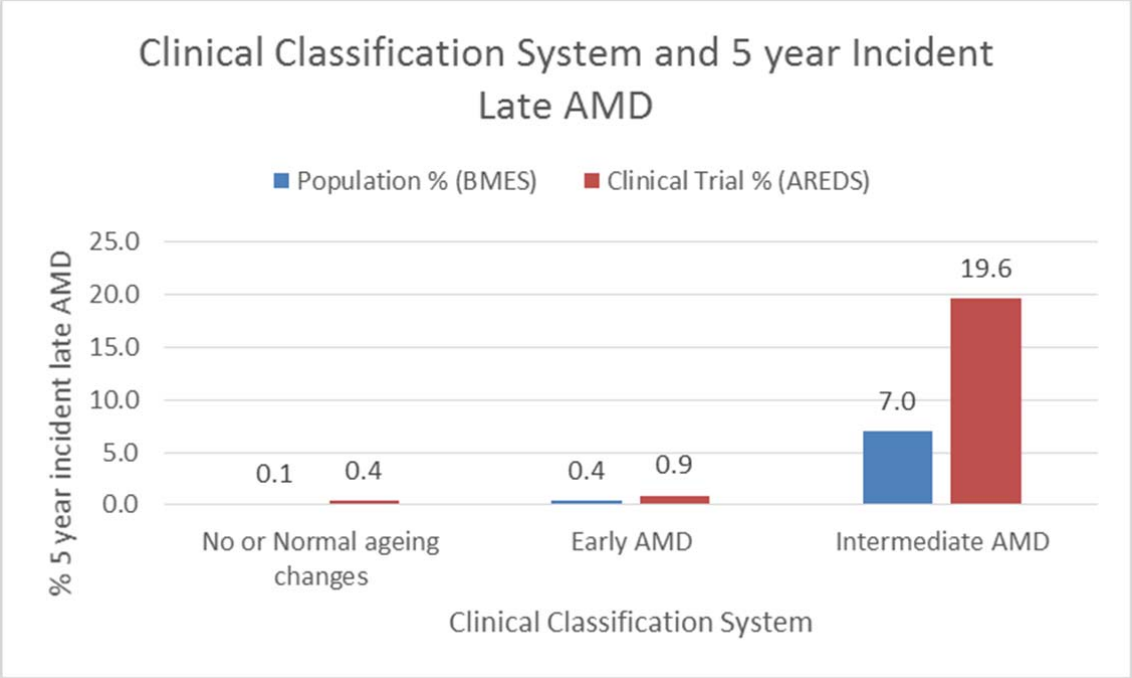

Figure 4-2 Observed 5-year incident late age-related macular degeneration (AMD) by categories on the Basic Clinical Classification. AREDS refers to the Age-Related Eye Diseases Study clinical trial. BMES refers to the population-based Blue Mountains Eye Study.

DISCUSSION

There is increasing interest in evaluating interventions that may slow progression from early AMD to vision-threatening late AMD. Severity scales are useful for documenting progression and comparing the effects of interventions and several have been proposed[13,27,28,31,32,48,244]. Most are based on data from clinical trials, in particular the AREDS trial, but have not been validated in general populations. We therefore sought to compare the predicted rates of 5-year incident late AMD to observed rates in the population based BMES. We report that the AREDS Simplified Severity Scale performed similarly when applied to population-based as well as clinic-based samples. The Basic Clinical Classification consistently resulted in lower rates in population-based compared to clinic-based samples. These results suggest that the AREDS Simplified Severity Scale may be more robust when applied to different populations.

It is worthwhile to bear in mind when interpreting the findings, that the AREDS Simplified Severity Scale calculates a risk score based on the number of risk factors (namely large drusen and pigment abnormalities) in both eyes, whereas, the Basic Clinical Classification Scale assigns patients with these lesions the same risk regardless of the lesion type and their bilaterality, and is driven by the lesions in the worse eye. The divergent results may be related to differences in the way the two scales are constructed, as mentioned above. The AREDS scale places greater emphasis on the presence of large drusen and pigment abnormalities, and acccounts for the presence of these lesions in both eyes[28]. Data from both eyes and from the more advanced and fellow eye are used in calculating risk categories. It has incorporated limited quantitative information (number of eyes involved) in the scale. Whereas the Basic Clinical Classification Scale categorizes large drusen and pigment abnormalities as the

same category, termed Intermediate AMD, and assigns patients with these lesions in the worse eye to the same risk level regardless of the type of lesion and their bilaterality[31]. It is therefore determined primarily by the type of lesions in the worse eye, but not the numbers of eyes with these lesions. The closer concordance between observed 5-year late AMD risk in clinic-based and population-based samples for the AREDS Simplified Severity Scale suggests that the number of large drusen, retinal pigment abnormalities, and bilaterality are important variables in predicting late AMD, and that concatenating these variables into a single risk category as in the Basic Clinical Classification Scale may lose important prognostic information.

A second likely reason for the difference in performance of the two scales is the difference in baseline prevalence of no AMD lesions and early AMD lesions (such as medium drusen or retinal pigmentary abnormalities). The BMES had only 7.2% of baseline participants with early AMD lesions and a large majority of participants without any drusen or other AMD signs[36]. In contrast, the large majority of the AREDS clinical trial population had early AMD at baseline[245]. There were thus far fewer patients in the early and intermediate AMD categories of the Basic Clinical Classification in the BMES than in the AREDS study, which may partially account for the lower incidence of late AMD in these categories observed in the BMES.

Our results suggest the choice of using AMD classification scale may have a major impact on late AMD risk estimates depending on the study samples. This has implications for power calculations for intervention trials of early AMD. If the study population to be recruited is similar in composition to the AREDS clinical trial population, our results suggest both the Simplified Severity Scale and Basic Clinical

Classification Scale will result in similar late AMD incidence estimates. However, if the study population is more similar to a general population, then the Basic Clinical Classification may overestimate the predicted incidence by 2-3 folds.

Limitations of the report are, firstly, the small number of incident late AMD cases in the BMES. Longer follow-up will result in a higher number of incident cases[247] but these data have not yet been published for the AREDS and we therefore cannot compare longer term incidence rates. Secondly, comparing data across different studies is difficult due to differences in methodology. In this context it is reassuring that similar incidence estimates were obtained from the BMES and AREDS using the Simplified Severity Scale, as it suggests there is a reasonable degree of similarity in defining AMD lesions and levels between the two studies. Finally, these risk estimates are derived from predominantly white populations. It remains unclear how the severity scales will perform when applied to other populations such as Asian or black populations where the incidence of AMD may be lower[48].

SUMMARY

In this report, we validated and compared the performance of two AMD classification systems in predicting incident late AMD. We found that the AREDS Simplified Severity Scale classified late AMD risk levels similarly when applied to population-based and clinic-based samples, whereas the Basic Clinical Classification resulted in 2-3 fold lower incidence rates in a population-based sample. The choice of classification system may need to take into account the study population as actual observed incidence rates may differ considerably from predicted rates.

Chapter 5

Comparison of Frequencies of Early AMD Lesions between Caucasians and Asians

Ethnic variation in early age-related macular degeneration lesions between White Australians and Singaporean Asians

Publication relating to this Chapter:

Joachim N, Mitchell P, Younan C, Burlutsky G, Cheng CY, Cheung CMG, Zheng Y, Moffitt M, Wong TY, Wang JJ. Ethnic Variation in Early Age-related Macular Degeneration Lesions between White Australians and Singaporean Asians. *Investigative Ophthalmology and Visual Science* 2014; 55 (7): 4421-4429

ABSTRACT

Purpose: To compare early age-related macular degeneration (AMD) lesion characteristics between white Australians and Singaporean Asians.

Methods: Participants of the Blue Mountains Eye Study (BMES; whites, n=3508) and the Singapore Epidemiology of Eye Disease Study (SEED, Malay, n=3280, Indian, n=3400 and Chinese, n=3353) underwent examinations including retinal photography. AMD lesions were assessed following the Wisconsin AMD grading protocol by the same photographic grader. Prevalence and characteristics of early AMD lesions were compared between the BMES and the SEED. The associations between ethnicity and early AMD lesion types were analyzed using logistic regression models adjusting for age, sex, smoking status, lipids and genetic polymorphisms associated with AMD.

Results: After age-standardization to the BMES population, the prevalence of distinct soft drusen was significantly higher in Singaporeans compared to Australians (23.9%, 95% confidence interval (CI) 22.9-25.0 versus 6.2%, 95% CI 5.3-7.0), with an adjusted odds ratio (OR) 4.6 (95% CI 3.4-6.0). In contrast, the prevalence of indistinct soft or reticular drusen was significantly lower in Singaporeans compared to Australians (6.5%, 95% CI 5.9-7.1 versus 8.3%, 95% CI 7.4-9.3), with non-significant adjusted OR 1.2, (95% CI 0.8-1.7). Soft drusen of any type were frequently present at the inner and outer macula (within a zone ≥500μm to <3000μm radius from the foveal centre) among Singaporeans, while among Australians soft drusen were more frequently present at the central macula (<500μm radius).

Conclusion: Singaporean Asians had a milder spectrum of early AMD lesions and lesion characteristics (predominantly distinct soft drusen and noncentral location) compared to white Australians.

BACKGROUND

Differences in the prevalence of early and late signs of age-related macular degeneration (AMD) and specific AMD lesions, between whites and blacks residing in the U.S. have long been observed and documented; these findings have been suggested to reflect underlying ethnic predisposition to AMD [248-250]. For example, larger drusen and retinal pigmentary abnormalities have been reported to be more frequently present, and the advanced forms of AMD more prevalent, in whites compared to blacks [248,250].

Emerging data on AMD in Asians are now available[251,252]. Although it was suggested that AMD was not common in Asians[251], the pooled prevalence of early (6.8%) and late AMD (0.56%) in four Asian populations in a meta-analysis was comparable to the prevalence of early (8.8%) and late AMD (0.59%) in white populations[68], challenging this previous assumption that AMD is less frequent in Asians than in whites[253]. However, these previous studies comparing differences in prevalence of AMD between white and Asian populations have only focused on the overall prevalence of any, early or late AMD. The prevalence of individual early AMD lesions in particular, may exhibit ethnic variability. Similar to white populations, the type and frequency of different early AMD lesions in Asians may indicate a certain risk of progression to late AMD, for example, eyes with indistinct soft drusen having a higher risk of developing late AMD compared to those with distinct soft drusen, in mainly Caucasian populations[28,254]. Few studies have assessed the differences in specific AMD lesion characteristics and distributions between different ethnic groups[255,256]. Such information may yield further insights into the early pathogenesis and presentation of AMD in diverse ethnic groups.

In this report we aim to directly compare the frequencies of different types of early AMD lesions, bilateral involvement and lesion location between population-based samples of white Australians and Asians living in Singapore

METHODS

Study Populations and Procedures

Details of the BMES study population are described in Methods, Chapter 2. At the time of the 5-year examinations an additional 1378 eligible permanent residents were identified following a second door-to-door census in 1999. This included residents who had moved into the study area or had reached 49 years of age during the intervening period. Of these newly eligible persons, 1174 participated in the study (85.2% of those eligible). Prevalence of AMD was derived from the entire BMES II survey sample (BMES II-a: returning participants of the BMES baseline cohort and BMES II-b: newly identified participants) with a total number of 3508 participants.

Details of the SEED study are described in Methods, Chapter 2. Each participant in the BMES and the SEED study cohort underwent a comprehensive ocular examination and retinal fundus photography as previously described (Methods, Chapter 2).

Blood samples were collected from 3222 BMES and 9670 SEED participants at examination. Total cholesterol and high density lipoprotein (HDL) concentrations (mmol/l) were measured on a Reflotron reflectance photometric analyser (Roche Diagnostics, Manheim, Germany) in the BMES, or were obtained from the biochemistry tests conducted.at the Singapore National University Hospital Reference Laboratory. Blood samples collected were also used for genotyping.

AMD Grading and Definitions

Retinal photographs taken from participants of both the BMES and SEED studies were graded by a single senior grader (Mireille Moffitt) in a masked manner at the Centre for Vision Research, University of Sydney, Australia, and adjudication provided by a senior researcher (Jie Jin Wang) or retinal specialist (Paul Mitchell). The presence and location of AMD lesions were graded using the WARMGS grid and measurement circles as described in Methods (Chapter 2).

Late AMD was defined as the presence of any sign of neovascular AMD (pigment epithelial or neuro-sensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars) as described in the International Age-Related Maculopathy classification[13] or geographic atrophy (GA) involving the fovea. Distinct soft drusen were distinguished as discrete whitish-yellow nodules >125μm in diameter with uniform density and sharp edges. Indistinct soft drusen were described as >125μm in diameter with decreasing density from centre outwards to the periphery and fuzzy edges. Confluent indistinct soft drusen with the appearance of broad interlacing ribbons were distinguished as reticular drusen. Retinal pigment epithelial (RPE) depigmentation was defined as faded but sharply demarcated areas of the RPE without visible choroidal vessels underneath. Clumps of granules of gray or black pigment beneath the retina were distinguished as hyperpigmentation[26]. Early AMD was defined as the presence of either large (>125μm diameter) indistinct soft or reticular drusen, or distinct soft drusen with retinal pigmentary abnormalities (RPE depigmentation and hyperpigmentation). In the following analyses we have included reticular drusen under the broader category of

indistinct soft drusen. Macular areas involved by early AMD lesions were categorized as small, intermediate and large areas for each lesion type; and location of early AMD lesions categorized as within the central macula, inner macula, outer macula or outside the macula area, as shown in **Table 5-1**.

Bilateral involvement of early or late AMD was defined among persons with early or late AMD respectively. Participants with early AMD in one eye and late AMD in the fellow eye were considered bilateral for any AMD but unilateral for early or late AMD. If maculopathy data were missing in one eye, the study subject was excluded from analysis of bilateral involvement.

Table 5-1 Definitions of the area and location of the early AMD lesions assessed.

Area and Location	Definitions
Drusen Area	
Small	None or <375μm in diameter
Intermediate	≥375μm to <0.5 disc area in diameter
Large	≥ 0.5 disc area in diameter
RPE Depigmentation Area	
Small	None or <375μm in diameter
Intermediate	≥375μm to <2 disc area in diameter
Large	≥2 disc area in diameter
Hyperpigmentation Area	
Small	None or <64μm in diameter
Intermediate	≥64μm to <660μm in diameter
Large	≥660μm in diameter
Location (All Lesions)	
Central macula	<500μm radius from the foveal centre
Inner macula zone	≥500μm to <1500μm radius from the foveal centre
Outer macula zone	≥1500μm to <3000μm radius from the foveal centre
Outside macula	≥3000μm radius from the foveal centre

RPE = retinal pigment epithelium

Genotyping

Genotyping procedures carried out in the BMES were described in Methods (Chapter 2). In the SEED, genotyping was performed using the Illumina Human 610-Quad array. Similar quality control procedures were applied in the SEED as in the BMES prior to analysis. Imputation was then performed using HapMap II penal and IMPUTE 5.0.

Definitions of Other Variables

For smoking status, participants categorized as non-smokers were those who answered "no" to smoking regularly. If participants answered "yes" but had stopped smoking ≥1 year prior to the examination they were categorized as past smokers. Participants who currently smoked or had stopped smoking <1 year prior to the examination were categorized as current smokers. In the BMES, hypertension was considered present if participants were taking anti-hypertensive medication at the time of examination, or blood pressure was ≥140mmHg or diastolic blood pressure was ≥90mmHg at examination. In the SEED, hypertension was considered present if systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg or with previous physician diagnosis reported by the participant. Body mass index (BMI) was calculated from weight and height measurements taken at examination.

Statistical Analyses

SAS (version 9.1, SAS Institute Inc. Cary, NC) was used for all analyses. We included participants aged 50 years or older only, with 3508 from the BMES, 2453 from the SiMES, 2427 from the SINDI and 2633 from the SCES. The worse eye prevalence of late and early AMD in the BMES were compared with worse eye prevalence estimates of the SiMES, SINDI and SCES, respectively after direct age-standardization to the

BMES population. Comparisons were also performed within subgroups stratified by smoking status after age-standardization to the BMES population. Differences in the frequencies of bilateral involvement of early AMD lesions between the ethnic groups were assessed using Fisher's exact test. Logistic regression models adjusted for age, sex, smoking, lipids, hypertension, BMI and the *CFH* and *ARMS2* polymorphisms were used to estimate association magnitudes between each Asian ethnicity and the presence of distinct soft drusen and indistinct soft drusen, with reference to the BMES.

Early AMD lesion characteristics, including the areas involved by the lesions and location of the lesions within the central, inner and outer zones were compared between the BMES and the Asian samples combined. Data of both eyes and generalized estimating equation (GEE) models were used in these analyses.

RESULTS

Table 5-2 shows the characteristics of participants without AMD and with early AMD in the BMES compared to the SiMES, SINDI, SCES or these Asian samples combined. Among participants with and without early AMD, both the mean age and frequency of women were lowere in the the three Asian populations combined, compared to the BMES ($p<0.0001$ for age and sex). There was a lower frequency of past smokers and higher frequency of current smokers in the combined Singaporean Asian sample compared to the BMES (both $p<0.0001$). The mean ages of participants with early AMD were higher than among participants without any AMD across all the study samples (**Table 5-2**). The frequency of participants with 1 or 2 risk alleles of *CFH* was significantly lower in the SiMES, SINDI and SCES compared to the BMES, whereas, there was a higher frequency of 2 risk alleles for ARMS2 across the Singaporean Asian

samples compared to the BMES sample (all $p<0.0001$). Mean cholesterol, high density lipoprotein and BMI were significantly lower in the Asian samples compared to the BMES in both subgroups with and without early AMD lesions (**Table 5-2**).

Table 5-2 Characteristics in participants without AMD and with early AMD in the Blue Mountains Eye Study compared to participants of the Singapore Malay Eye study, the Singapore Indian Eye Study, the Singapore Chinese Eye Study and the three Asian samples combined.

Characteristic	% participants with no AMD								
	BMES (n=2867)	**SiMES (n=1746)**	**P-value***	**SINDI (n=1806)**	**P-value***	**SCES (n=2046)**	**P-value***	**Combined Asian (n=5598)**	**P-value†**
Mean age (years, SD)	65.1 (8.7)	61.2 (8.1)	<0.0001	60.0 (7.8)	<0.0001	60.9 (8.2)	<0.0001	60.7 (8.0)	<0.0001
Sex (female)	56.5	51.7	0.002	48.3	<0.0001	52.2	0.003	50.8	<0.0001
Smoking status									
non-smoker	49.2	62.3		73.1		74.8		70.4	
past smoker	40.1	18.5	<0.0001‡	12.6	<0.0001‡	12.9	<0.0001‡	14.5	<0.0001‡
current smoker	10.8	19.2		14.3		12.4		15.1	
Hypertension (present)	75.5	75.3	0.9	62.7	<0.0001	61.9	<0.0001	66.3	<0.0001
CFH (rs1061170-BMES/rs1080155-SiMES, SINDI, SCES)									
no risk alleles	38.4	83.0		54.0		93.7		75.7	
1 risk allele	47.3	15.8	<0.0001‡	37.9	<0.0001‡	6.2	<0.0001‡	21.0	<0.0001‡
2 risk alleles	14.4	1.2		8.1		0.1		3.4	
ARMS2 (rs10490924-BMES/rs3750847-SiMES, SINDI, SCES)									
no risk alleles	62.6	37.8		40.8		31.5		37.1	
1 risk allele	32.7	47.4	<0.0001‡	47.7	<0.0001‡	49.3	<0.0001‡	48.0	<0.0001‡
2 risk alleles	4.8	14.8		11.5		19.2		14.9	
Mean total cholesterol (mmol/L; SD)	5.8 (1.1)	5.7 (1.2)	<0.0001	5.2 (1.1)	<0.0001	5.5 (1.1)	<0.0001	5.5 (1.1)	<0.0001
Mean HDL (mmol/L; SD)	1.5 (0.4)	1.4 (0.3)	<0.0001	1.1 (0.3)	<0.0001	1.3 (0.4)	<0.0001	1.3 (0.4)	<0.0001
Mean BMI (kg/m^2; SD)	27.8 (4.9)	26.4 (4.9)	<0.0001	26.3 (4.7)	<0.0001	23.7 (3.7)	<0.0001	25.4 (4.6)	<0.0001

	% participants with early AMD							
	n=284	**n=147**		**n=166**		**n=219**		**n=532**	
Mean age (years, SD)	75.1 (8.4)	67.6 (7.8)	<0.0001	67.0 (8.6)	<0.0001	66.9 (8.1)	<0.0001	67.1 (8.2)	<0.0001
Sex (female)	62.7	39.5	<0.0001	44.0	0.0001	39.3	<0.0001	40.8	<0.0001
Smoking status									
non-smoker	53.0	57.9		68.7		69.9		66.2	
past smoker	38.8	26.9	0.01‡	19.9	0.0002‡	18.7	<0.0001‡	21.3	<0.0001‡
current smoker	8.2	15.2		11.5		11.4		12.5	
Hypertension (present)	82.0	85.7	0.3	78.9	0.4	78.1	0.3	80.5	0.6
CFH (rs1061170-BMES/rs1080155-SiMES, SINDI, SCES)									
no risk alleles	26.6	80.5		52.0		90.3		74.6	
1 risk allele	50.0	19.5	<0.0001‡	35.0	<0.0001‡	9.0	<0.0001‡	20.8	<0.0001‡
2 risk alleles	23.4	0.0		13.0		0.8		4.6	
ARMS2 (rs10490924-BMES/rs3750847-SiMES, SINDI, SCES)									
no risk alleles	48.8	32.7		43.9		20.2		31.9	
1 risk allele	47.3	41.6	<0.0001‡	37.4	<0.0001‡	54.5	<0.0001‡	44.9	<0.0001‡
2 risk alleles	4.0	25.7		18.7		25.4		23.2	
Mean total cholesterol (mmol/L; SD)	5.9 (1.0)	5.6 (1.2)	0.01	4.8 (1.1)	<0.0001	5.3 (1.1)	<0.0001	5.2 (1.2)	<0.0001
Mean HDL (mmol/L; SD)	1.5 (0.4)	1.4 (0.4)	<0.0001	1.1 (0.3)	<0.0001	1.3 (0.4)	<0.0001	1.3 (0.4)	<0.0001
Mean BMI (kg/m^2; SD)	27.0 (4.5)	26.1 (5.5)	0.09	26.2 (4.8)	0.1	23.2 (3.7)	<0.0001	24.9 (4.8)	<0.0001

AMD = age-related macular degeneration, BMES = Blue Mountains Eye Study, SiMES = Singapore Malay eye study, SINDI = Singapore Indian eye study, SCES = Singapore Chinese eye study, Combined Asian = SiMES, SINDI and SCES combined, n = sample size, SD = standard deviation, CFH = complement factor H, ARMS2 = age-related maculopathy susceptibility gene 2, BMI = body mass index.

*P-value for comparison between participants of the BMES and SiMES or SINDI or SCES

†P-value for comparison between participants of the BMES and participants of the SiMES, SINDI and SCES combined

‡Unadjusted tests for heterogeneity used to calculate p-values

The crude prevalence of AMD in each study population is presented in **Table 5-3** and age-standardized prevalence presented in **Table 5-4**. After direct age-standardization, late AMD prevalence was non-significantly lower in the combined Asian sample compared to the BMES, whereas the prevalence of early AMD was significantly higher in the combined Asian samples compared to the BMES.

The prevalence of distinct soft drusen was substantially higher in each of the Asian samples or in the combined Asian samples, compared to the BMES. In contrast, the prevalence of indistinct soft drusen was significantly lower in SiMES and SCES, or in the combined Asian samples, compared to the BMES sample. A finding of note was that the prevalence of indistinct soft drusen in SINDI was similar to the BMES, after age-standardization to the BMES sample (**Table 5-4**). There was no difference in the prevalence of retinal pigmentary abnormalities in the combined Asian samples compared to the BMES sample.

Table 5-3 Comparison of the crude prevalence of age-related macular degeneration in the Blue Mountains Eye Study to the Singapore Malay, Singapore Indian and Singapore Chinese Eye Study samples and the three Asian samples combined.

Lesion	BMES	SiMES		SINDI		SCES		Combined Asian	
	Prevalence % (No. affected/ total no.)	Prevalence % (No. affected/ total no.)	P-value*	Prevalence % (No. affected/ total no.)	P-value*	Prevalence % (No. affected/ total no.)	P-value*	Prevalence % (No. affected/ total no.)	P-value*
Any late AMD	1.76 (60/3410)	0.97 (20/2060)	0.02	0.54 (13/2427)	<0.0001	0.95 (25/2633)	0.008	0.81 (58/7120)	<0.0001
Pure GA	0.67 (23/3410)	0.39 (8/2060)	0.2	0.00 (0/2427)	<0.0001	0.19 (5/2633)	0.006	0.18 (13/7120)	<0.0001
Neovascular AMD	1.09 (37/3409)	0.58 (12/2059)	0.06	0.54 (13/2427)	0.02	0.76 (20/2633)	0.2	0.63 (45/7119)	0.01
Any early AMD	9.01 (284/3152)	7.76 (147/1894)	0.1	8.42 (166/1972)	0.5	9.67 (219/2265)	0.4	8.68 (532/6132)	0.6
Any large drusen‡	13.06 (412/3154)	20.62 (398/1930)	<0.0001	21.11 (422/1999)	<0.0001	26.83 (620/2311)	<0.0001	23.07 (1440/6241)	<0.0001
Soft distinct drusen	6.00 (194/3235)	17.02 (326/1915)	<0.0001	17.54 (348/1984)	<0.0001	24.12 (556/2305)	<0.0001	19.82 (1230/6205)	<0.0001
Soft indistinct or reticular drusen	7.65 (241/3149)	4.35 (82/1886)	<0.0001	4.33 (85/1964)	<0.0001	3.63 (82/2258)	<0.0001	4.08 (249/6109)	<0.0001
Any pigment abnormality	15.08 (487/3229)	15.65 (323/2064)	0.6	14.98 (319/2129)	0.9	16.04 (385/2400)	0.3	15.57 (1027/6594)	0.5
RPE depigmentation	9.34 (301/3221)	11.49 (236/2054)	0.01	7.91 (169/2136)	0.07	7.71 (185/2398)	0.03	8.95 (590/6589)	0.5
Hyperpigmentation	14.33 (463/3230)	11.25 (231/2053)	0.001	12.71 (270/2124)	0.09	14.11 (338/2395)	0.8	12.76 (839/6573)	0.03

BMES = Blue Mountains Eye Study, SiMES = Singapore Malay Eye Study, SINDI = Singapore Indian Eye Study, SCES = Singapore Chinese Eye Study, Combined Asian = SiMES, SINDI and SCES combined, AMD = age-related macular degeneration, GA = geographic atrophy, RPE = retinal pigment epithelial

*P-value for crude prevalence comparison between participants of the BMES and the SiMES, SINDI, SCES or Combined Asian sample

†Unadjusted tests for heterogeneity used to calculate p-values

‡Includes soft distinct, soft indistinct and reticular drusen

Table 5-4 Prevalence of age-related macular degeneration lesions in the Singapore Malay, Singapore Indian, Singapore Chinese and combined Asian eye study samples age-standardized to the Blue Mountains Eye Study population.

Lesion	Age-standardized prevalence % (95% CI)				
	BMES	**SiMES**	**SINDI**	**SCES**	**Combined Asian**
Any late AMD	1.76 (1.32-2.20)	1.09 (0.64-1.54)	0.76 (0.42-1.11)	1.52 (1.05-1.99)	1.3 (1.04-1.56)
Pure GA	0.68 (0.40-0.95)	0.43 (0.15-0.72)	0.00 (0.00-0.00)	0.28 (0.08-0.48)	0.26 (0.15-0.38)
Neovascular AMD	1.09 (0.74-1.43)	0.66 (0.31-1.01)	0.76 (0.42-1.11)	1.24 (0.82-1.67)	1.03 (0.80-1.27)
Any early AMD	**9.72 (8.69-10.76)**	10.98 (9.57-12.38)	**13.53 (12.02-15.04)**	**13.01 (11.63-14.40)**	**12.51 (11.69-13.34)**
Any large drusen*	**13.88 (12.68-15.09)**	**26.72 (24.75-28.69)**	**28.35 (26.37-30.33)**	**32.36 (30.45-34.26)**	**28.84 (27.72-29.96)**
Soft distinct drusen	**6.15 (5.32-6.98)**	**22.73 (20.85-24.60)**	**21.75 (19.94-23.57)**	**28.76 (26.91-30.60)**	**23.93 (22.87-24.99)**
Soft indistinct or reticular drusen	**8.32 (7.35-9.28)**	**4.92 (3.94-5.90)**	8.36 (7.14-9.59)	**5.20 (4.28-6.11)**	**6.52 (5.91-7.14)**
Any pigment abnormality	**15.52 (14.27-16.77)**	15.76 (14.19-17.34)	18.02 (16.39-19.65)	**18.50 (16.95-20.06)**	17.62 (16.70-18.54)
RPE depigmentation	9.63 (8.61-10.65)	10.35 (9.03-11.66)	9.07 (7.85-10.28)	7.65 (6.59-8.72)	8.97 (8.28-9.66)
Hyperpigmentation	**14.78 (13.56-16.01)**	**12.03 (10.62-13.44)**	15.64 (14.10-17.19)	16.74 (15.24-18.23)	15.06 (14.20-15.93)

BMES = Blue Mountains Eye Study, SiMES = Singapore Malay Eye Study, SINDI = Singapore Indian Eye Study, SCES = Singapore Chinese Eye Study, Combined Asian = SiMES, SINDI and SCES combined, AMD = age-related macular degeneration, GA = geographic atrophy, RPE = retinal pigment epithelial, CI = confidence interval

*Includes soft distinct, soft indistinct and reticular drusen

Bold font indicates significant difference between the BMES and SiMES, SINDI, SCES or the Combined Asian samples.

Compared to the BMES, the combined Asian sample had a lower frequency of bilateral late AMD (17.24% versus 58.33%, $p<0.0001$) and bilateral early AMD (30.31% versus 48.13%, $p<0.0001$). Bilateral retinal pigment abnormalities were also less frequent in the SiMES, SINDI and SCES compared to the BMES (all $p<0.0001$). However, there was no difference in the frequencies of bilateral reticular drusen or other soft drusen between the Asian and the BMES samples (all $p>0.05$; data not shown).

After stratifying by smoking status, the age-standardized prevalence of early AMD was not significantly different between the combined Asian and the BMES sample in each category of non-smokers and current smokers. However, there was a significantly higher prevalence of early AMD in past smokers in the combined Asian sample compared to the BMES (**Table 5-5**). Distinct soft drusen prevalence remained significantly higher in all three Asian ethnicities compared to the BMES, regardless of smoking status, whereas indistinct soft drusen prevalence remained significantly lower in the combined Asian samples compared to the BMES in the non-smoking subgroup. In the two subgroups of past-smokers and current smokers, the differences in the prevalence of indistinct soft drusen between the combined Asian samples and the BMES were non-significant (**Table 5-5**).

Table 5-5 Comparison of the prevalence of early AMD by smoking status in the Blue Mountains Eye Study to the Singapore Malay, Singapore Indian, Singapore Chinese Eye Studies and the three Asian samples combined.

AMD Lesion	Age-standardized Prevalence (95% CI)				
	BMES	**SiMES**	**SINDI**	**SCES**	**Combined Asian**
			Non-smoker		
Any early AMD	10.41 (8.88-11.94)	10.49 (8.74-12.25)	13.41 (11.64-15.17)	12.77 (11.18-14.37)	12.20 (11.22-13.18)
Any large drusen	**14.72 (12.94-16.49)**	**25.03 (22.57-27.48)**	**28.42 (26.10-30.74)**	**32.48 (30.26-34.70)**	**28.86 (27.51-30.20)**
Soft distinct drusen	**6.71 (5.48-7.95)**	**19.64 (17.37-21.90)**	**22.54 (20.39-24.70)**	**29.03 (26.87-31.18)**	**24.18 (22.90-25.45)**
Soft indistinct or reticular drusen	**8.66 (7.25-10.07)**	6.40 (4.99-7.80)	7.69 (6.31-9.07)	**4.98 (3.94-6.02)**	**6.15 (5.43-6.87)**
Any pigment abnormality	15.74 (13.94-17.54)	13.49 (11.62-15.37)	18.96 (17.02-20.91)	16.51 (14.79-18.24)	16.31 (15.25-17.38)
RPE depigmentation	**9.33 (7.89-10.77)**	7.87 (6.39-9.35)	8.38 (7.00-9.75)	**5.94 (4.84-7.04)**	7.27 (6.52-8.02)
Hyperpigmentation	**14.74 (12.99-16.50)**	**10.77 (9.07-12.47)**	16.47 (14.63-18.32)	15.36 (13.68-17.04)	14.27 (13.26-15.28)
			Past Smoker		
Any early AMD	**9.44 (7.81-11.07)**	13.46 (9.94-16.98)	**15.53 (11.14-19.93)**	14.57 (10.61-18.54)	**14.30 (12.04-16.55)**
Any large drusen	**13.77 (11.85-15.68)**	**26.60 (22.09-31.11)**	**28.42 (26.10-30.74)**	**33.12 (27.94-38.30)**	**29.92 (27.01-32.83)**
Soft distinct drusen	**6.27 (4.94-7.61)**	**21.29 (17.09-25.50)**	**22.54 (20.39-24.70)**	**29.78 (24.74-34.82)**	**24.93 (22.17-27.69)**
Soft indistinct or reticular drusen	8.10 (6.58-9.62)	7.73 (4.97-10.50)	9.27 (5.74-12.80)	5.07 (2.61-7.54)	7.07 (5.42-8.73)
Any pigment abnormality	**14.44 (12.50-16.37)**	**22.07 (18.00-26.13)**	20.50 (15.76-25.23)	**22.58 (18.04-27.11)**	**21.74 (19.19-24.29)**
RPE depigmentation	**9.15 (7.56-10.74)**	**17.14 (13.43-20.85)**	12.80 (8.89-16.72)	12.29 (8.74-15.84)	**14.26 (12.10-16.42)**
Hyperpigmentation	14.12 (12.20-16.03)	16.30 (12.66-19.94)	17.86 (13.37-22.36)	19.27 (14.99-23.55)	17.63 (15.27-19.99)
			Current Smoker		
Any early AMD	7.66 (4.78-10.55)	7.85 (5.06-10.64)	7.86 (4.69-11.04)	10.66 (7.03-14.29)	8.82 (6.98-10.66)
Any large drusen	**10.68 (7.34-14.03)**	**22.77 (18.46-27.08)**	**20.85 (16.08-25.61)**	**30.67 (25.32-36.03)**	**24.82 (22.04-27.60)**
Soft distinct drusen	**3.60 (1.60-5.60)**	**17.11 (13.23-20.98)**	**17.44 (12.98-21.90)**	**27.03 (21.86-32.21)**	**20.32 (17.72-22.91)**
Soft indistinct or reticular drusen	7.37 (4.54-10.20)	5.63 (3.23-8.02)	4.24 (1.87-6.61)	5.24 (2.61-7.87)	5.21 (3.77-6.66)
Any pigment abnormality	18.99 (14.78-23.31)	19.45 (15.48-23.42)	16.37 (12.12-20.62)	21.74 (17.03-26.46)	19.42 (16.92-21.91)
RPE depigmentation	12.51 (8.96-16.07)	15.98 (12.30-19.65)	10.58 (7.06-14.10)	13.17 (9.30-17.03)	13.44 (11.29-15.59)
Hyperpigmentation	17.81 (13.70-21.92)	13.13 (9.73-16.53)	12.92 (9.07-16.77)	17.81 (13.41-22.20)	14.72 (12.48-16.96)

BMES = Blue Mountains Eye Study, SiMES = Singapore Malay Eye Study, SINDI = Singapore Indian Eye Study, SCES = Singapore Chinese Eye Study, Combined Asian Sample = SiMES, SINDI and SCES combined, CI = confidence interval, AMD = age-related macular degeneration, RPE = retinal pigment epithelial

Bold font indicates significant difference between the BMES and SiMES, SINDI, SCES or the Combined Asian samples.

Table 5-6 presents a comparison of the areas and location of early AMD lesions in right eyes, between the BMES and the combined Asian samples, shown with age-adjusted p values. Larger areas of soft drusen were more frequent in the SiMES, SINDI and SCES than in the BMES (age-adjusted $p<0.0001$). The frequencies of larger areas involved by RPE depigmentation were similar in both Australian and Singaporean Asian samples, while small areas involved by hyperpigmentation were more frequent in theAsian sample compared to the BMES (6.7% versus 9.6%; age-adjusted $p=0.01$). Singaporean Asians were more likely to have eyes with drusen located only at the inner and outer macula zones, but were significantly less likely to have eyes with drusen located at the central macula compared to the BMES sample (age-adjusted $p<0.0001$; **Table 5-6**, examples shown in **Figure 5-1**). A more central location for hyperpigmentation was also significantly less likely in the Singaporean Asian samples than in the BMES (age-adjusted $p<0.03$).

Table 5-6 Area and location of drusen and pigmentary abnormalities in the Blue Mountains Eye Study compared to the combined Asian samples (Singapore Malay, Singapore Indian and Singapore Chinese Eye Studies combined).

Early AMD lesion Characteristics	% Eyes		Age-adjusted P-value†	Odds Ratio (95% CI)‡
	BMES	Combined Asian Samples*		
AREA				
Drusen				
None or <375µm in diameter	92.4	91.8	<0.0001	1.0
≥375µm in diameter	7.7	8.2		1.8 (1.6-2.1)
RPE Depigmentation				
None or <375µm in diameter	96.3	96.7	0.9	1.0
≥375µm in diameter	3.7	3.3		1.0 (0.9-1.2)
Hyperpigmentation				
None or <64µm in diameter	90.5	93.3	0.01	1.0
≥64µm in diameter	9.6	6.7		0.9 (0.8-1.0)
LOCATION				
Drusen				
Central macula	87.5	59.3	<0.0001	1.0
Inner and outer macula zone	12.5	40.7		5.8 (2.7-12.3)
RPE Depigmentation				
Central macula	58.4	62.4	0.7	1.0
Inner and outer macula zone	41.6	37.6		0.9 (0.5-1.5)
Hyperpigmentation				
Central macula	80.8	63.6	0.03	1.0
Inner and outer macula zone	19.2	36.4		2.0 (1.1-4.0)

AMD= age-related macular degeneration, BMES = Blue Mountains Eye Study, RPE = retinal pigment epithelial

Location definitions: Central macula = <500µm radius from foveal centre; Inner macula zone = ≥500µm to <1500µm radius from the foveal centre; Outer macula zone = ≥1500µm to <3000µm radius from the foveal centre

* Singapore Malay, Indian and Chinese Eye Study samples combined

†Unadjusted tests for heterogeneity used to calculate p-values

‡Odds ratios estimated with BMES as reference group, using generalized estimating equation models and both eyes from each participant.

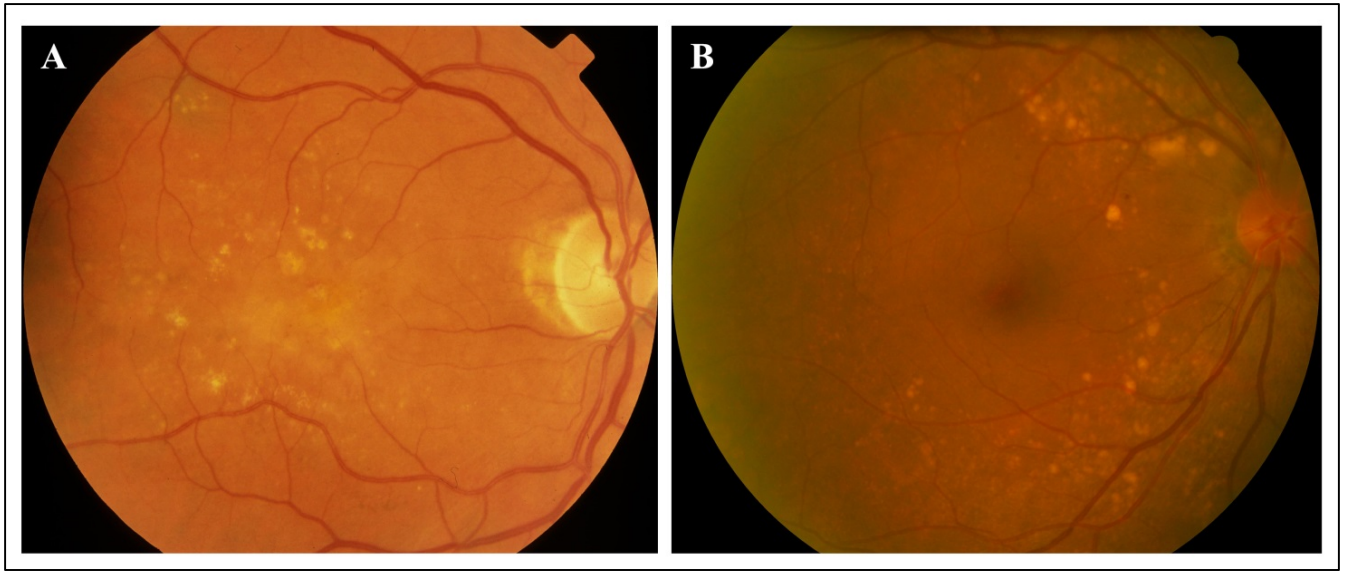

Figure 5-1 Examples of the different distribution of early age-related macular degeneration lesions in white Australians and Singaporean Asians: Central location of drusen in an Australian participant (A), compared to the more peripheral location of drusen in an Asian participant (B).

The associations between ethnicity and soft drusen types were further assessed after adjusting for age, sex, smoking status, total cholesterol, HDL, hypertension, BMI and the *CHF* SNP's *rs1061170* and *rs1080155* and *ARMS2* SNP's *rs10490924* and *rs3750847* (**Table 5-7**). With reference to Australians, there was a significantly higher likelihood of having distinct soft drusen in Malays, Indians or Chinese (ORs 4.0, 4.6 and 7.0, respectively). By comparison, there was a similar likelihood of having indistinct soft or reticular drusen among Malays, Indians and Chinese, compared to the Australian population (OR's 1.1, 1.1 and 1.3, respectively), though these associations were not significant (**Table 5-7**).

Table 5-7 The association between Asian ethnicity (Singapore Malay, Singapore Indian, and Singapore Chinese and combined Asian sample) and the prevalence of soft drusen, with reference to whites (the Blue Mountains Eye Study population), shown as odds ratios (OR) with 95% confidence intervals (95% CI).

Ethnic Group	Soft Drusen Type	
	Distinct Soft	**Indistinct Soft and Reticular**
	Multivariable adjusted OR* (95% CI)	**Multivariable adjusted OR* (95% CI)**
BMES (reference group)	1.0	1.0
SiMES	**4.0 (3.0, 5.4)**	1.1 (0.7, 1.7)
SINDI	**4.6 (3.4, 6.3)**	1.1 (0.7, 1.8)
SCES	**7.0 (5.1, 9.6)**	1.3 (0.7, 2.2)
Combined Asian Sample	**4.6 (3.4, 6.0)**	1.2 (0.8, 1.7)

OR = odds ratio, CI = confidence interval, BMES = Blue Mountains Eye Study, SiMES = Singapore Malay eye study, SINDI = Singapore Indian eye study, SCES = Singapore Chinese eye study, Combined Asian = SiMES, SINDI and SCES combined

*Adjusted for age, sex, smoking, cholesterol, high density lipoprotein, hypertension, body mass index and the complement factor H and age-related maculopathy susceptibility gene 2 risk alleles as categorical variables.

Bold font indicates significant differences.

DISCUSSION

In this study, we found a significantly lower prevalence of distinct soft drusen in Australians compared to Asians (Malays, Indians and Chinese). In contrast, we found a significantly higher prevalence of indistinct soft drusen in Australians compared to Malays and Chinese; while Indians had a similar prevalence of indistinct soft drusen to Australians. This similarity between Indians and whites could be explained by their relatively close genetic make-up, compared to less similarity in the genome between whites and other Asian ethnicities.

Similar to the previous report by Kawasaki et al. of a meta-analysis of findings from nine Asian population-based samples aged 40-79 years[68], we found a 1.76% prevalence of late AMD in the BMES population to be comparable to the 1.30% prevalence in the combined Asian samples of Malays, Indians and Chinese, all aged 50 years or older. The age-standardized prevalence of early AMD was slightly lower in Australians and Malays compared to that of Indians or Chinese (9.72% and 10.98% versus 13.53% and 13.01%, respectively).

The early AMD prevalence of 10.98% and 13.53% found in our study contrasts with previously published prevalence of 3.5% and 4.5% for the same Singaporean population of Malays[67] and Indians[257], respectively. These differences are likely due to the age of the populations under investigation: ≥50 years in our study versus 40 to 80 years in the previous study; and age-standardization to the BMES in our study versus age-standardization to the Singapore population at the 2000 Singapore census[67,257]. Our finding also contrasts with another study of a different multiethnic cohort of Singaporean Malays, Indians and Chinese aged 40+ years that reported similar

prevalence of any AMD among the three ethnic groups (5.7%-7.7%)[256], which was comparable to white populations around the world.

Although the overall prevalence of early AMD was found to be similar between Australians and Singaporean Asians in our study, the frequency of various lesions forming early AMD was different. Malays and Chinese predominantly presented with a milder spectrum of lesions such as distinct soft drusen and a lower frequency of advanced lesions including indistinct soft drusen, compared to Australians. Of a few studies that have specifically investigated the prevalence of the individual lesions of late and early AMD, our observation is consistent with previous findings from the Multi-Ethnic Study of Atherosclerosis (MESA) that found higher prevalence of distinct soft drusen among Chinese compared to black, white or Hispanic participants[249].

The BMES consists of participants with predominantly European ancestry and has found comparable early and late AMD prevalence and incidence to other population-based epidemiological studies including the Beaver Dam Eye Study and the Rotterdam Eye study[14,29,36,44,189]. Singaporeans consist of many different Asian ethnicities, however the three ethnic groups captured in the SEED study were the three predominant ethnic groups of Asians[184,188]. Similar estimates of early and late AMD prevalence were found between Singaporean Indians and Indians living in India[257].

The single nucleotide polymorphisms (SNP's) at the *CFH* and *ARMS2* loci are significant risk factors for early AMD, though they present a weaker risk for early than for late AMD, as shown in a recent genome wide association study (GWAS) meta-

analysis[78]. The differences in early AMD lesion prevalence between Australians and Asians could be partly explained by genetic differences[78,258-261].

The bilateral involvement of early, late and any AMD were shown to be comparable between the Malay and Australian populations in a previous report[255]. In the current analysis, we found that the frequency of bilateral late and early AMD was significantly lower in Malays, Indians and Chinese compared to Australians. The higher frequencies of bilateral involvement of late and early AMD in Australians compared to Singaporean Asians in our study could have been partly due to differences in age ranges of the samples, as age-standardization was not performed in the earlier comparison of bilateral involvement, due to small numbers. The lower frequency of bilateral early AMD in Asians may also be explained by the lower prevalence of the *CFH Y402H* polymorphism in Asian populations[262,263], which has been found to be associated with bilateral early AMD involvement[264,265].

In addition to increasing age, smoking is an established risk factor for AMD in many white populations[115,148,266]. Similar associations between smoking and an increased AMD risk has also been documented in Asians[64,120,267]. Among past and current smokers, we found no difference in the age-standardized prevalence of indistinct soft and reticular drusen between Australians and Singaporean Asians. This could have been due to reduced numbers of subjects in these smoking subgroups.

A higher risk of developing late AMD has been associated with a more central location of drusen and more advanced stages of early AMD lesions[35,208]. Our findings of a predominantly distinct soft drusen pattern among early AMD lesions and relatively high

prevalence of drusen and retinal pigmentary changes located away from the foveal center in Singaporean Asians compared to white Australians, reinforce the impression that Asians present a milder spectrum of early AMD lesions than whites, despite the overall lack of a substantive difference in the prevalence of early and late AMD between the Singaporean Asians and white Australians.

The strengths of this study include the large sample size of each ethnic group and the direct comparison of AMD lesion and lesion characteristics among the studies. All studies used the same standardized grading protocol to determine AMD lesions and lesion characteristics, with AMD grading performed by the same principle grader (Mireille Moffit), adjudicated by the same senior researcher (Jie Jin Wang) and ophthalmologist (Christine Younan), with all late AMD cases confirmed by the same retinal specialist (Paul Mitchell). Limitations of the study include the small sample size in some groups when bilaterality and area and location of early AMD lesions were assessed, where it was not possible to obtain an age-standardized frequency. There is also a difference in the examination time between the BMES II study and the three Asian studies conducted in succession of each other, and this may have influenced the comparison between the studies if there was a temporal change in the prevalence of AMD.[255] This does not seem likely given the relatively narrow time interval. The difference in types of fundus photographs taken between the BMES and the three Asian studies (35mm colour film versus colour digital images; 30° versus 45° photographs; and stereo versus non-stereo, respectively), however, could have had subtle effects on the quantitative measurements of lesion area and location but is unlikely to have affected the assessment of the prevalence of different AMD lesions.

SUMMARY

In this report, we found that overall, Asians (including the three major Asian ethnic groups, Malays, Indians and Chinese) living in Singapore, had a predominantly milder spectrum of early AMD lesions compared to whites living in Australia, after age standardization. Further studies of environmental and genetic risk factors and their associations with early AMD lesions and lesion characteristics may elucidate insights into the similarities and dissimilarities in mechanisms that lead to AMD occurring in either Asians or whites.

Chapter 6

Progression from Unilateral to Bilateral AMD among Three Caucasian Populations

Five-year progression from unilateral to bilateral age-related macular degeneration: The Three Continent AMD Consortium report

Submission relating to this Chapter:

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ABSTRACT

Purpose: To assess the 5-year progression from unilateral to bilateral age-related macular degeneration (AMD) and associated risk factors in populations.

Methods: Participants of the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES) and Rotterdam Study (RS) cohorts underwent similar examination procedures. Color retinal photographs were taken at each visit and comprehensive questionnaires administered. Blood samples were collected and DNA genotyped. Photographic grading was performed by trained graders following the modified Wisconsin AMD grading protocol. Five-year progression to bilateral any (early and late AMD) or late AMD was assessed in persons with unilateral any or late AMD in each study and in the pooled cohort. Associations of known AMD risk factors with progression to bilateral AMD were assessed using logistic regression models and expressed as odds ratios (ORs) and 95% confidence intervals (CIs).

Results: In any 5-year duration over the follow-up periods, progression rates ranged from 19% to 28% for bilateral any AMD and 27% to 68% for bilateral late AMD in persons with prior unilateral involvement. In the pooled cohort, progression to bilateral any AMD was associated with increasing age and increased number of risk alleles of the *CFH* and *ARMS2* genes (all $P_{trend}<0.0001$). After multivariable adjustment, the presence of 1 or ≥2 risk alleles from the *complement factor H (CFH)* and *age-related maculopathy susceptibility 2 (ARMS2)* genes was associated with increased progression to bilateral any AMD (OR 1.76, CI 1.17-2.64 or OR 3.34, CI 2.21-5.04), as was past (OR 1.64, CI 1.16-2.33) and current smoking status (OR 1.67, CI 1.10-2.55). In addition to age, the presence of ≥2 risk alleles form the *CFH* and *ARMS2* genes combined was

significantly associated with increased progression to bilateral late AMD (OR 12.46, CI 1.52-101.97). Increased cholesterol levels were inversely associated with progression to bilateral late AMD (OR 0.47, CI 0.26-0.84).

Conclusions: One in five to one in four persons with unilateral any AMD progressed to bilateral, and nearly one in two with unilateral late AMD progressed to bilateral in 5 years. Carrying risk alleles of *CFH* or *ARMS2* and smoking increased this progression risk.

BACKGROUND

Age-related macular degeneration (AMD) is a leading cause of blindness in western populations around the world[2,211]. While the presence of AMD in one eye can be debilitating, vision loss and eventual blindness in both eyes due to bilateral AMD will have severe consequences for the affected individuals [268,269].

Population-based studies with follow-up data and few clinic-based studies have reported the development of bilateral late AMD (fellow eye involved) to be between 20% to 50% over 5 to 10 years[29,33-35,38,44,270]. However, the progression from unilateral early AMD to bilateral early or any AMD has been less well described[271]. The relationship between known AMD risk factors and the progression from unilateral to bilateral AMD is also not well-documented.

We therefore aim to report the 5-year progression rates from unilateral to bilateral involvement by any or late AMD (termed progression to bilateral any or late AMD in this report). Also to investigate the progression in relation to age, genotypes of the *complement factor H* (*CFH*) and *age-related maculopathy susceptibility 2* (*ARMS2*) genes, smoking status and other known AMD risk factors, in the Three Continent AMD Consortium (3CC).

METHODS

We included three cohorts of non-Hispanic white, European-origin populations of the 3CC conducted in Australia, USA and the Netherlands respectively: the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES) and Rotterdam Study (RS); all had a follow-up period of 10 or more years[32,169]. Details of the three study populations are

described in Methods, Chapter 2. The total follow-up period in the BMES, BDES and RS taken into account in this study was 15 years, 20 years and 10 years, respectively, on average. To correspond with the BMES and BDES 5-year follow-up visit intervals, data from the second follow-up visit (1993-1995) of the RS were excluded.

Photographic Grading and Definitions of AMD

Retinal photographs of both eyes were initially graded by trained graders of each study following the Wisconsin Age-related Maculopathy Grading System[26]. All late AMD incident cases detected from each study were adjudicated and confirmed by the retinal specialists of the corresponding study team, followed by cross-checking among chief investigators of the BMES, BDES and RS[57].

A 5-step severity scale that was developed after phenotype harmonization of AMD by the Three Continent AMD Consortium[32], was used to define AMD severity stage (refer to **Table 1.1-1**). The scale defines AMD by levels 10, 20, 30, 40 and 50, corresponding to: no AMD, mild early, moderate early, severe early and late AMD. Early AMD was defined as levels 20-40 and late AMD was defined as level 50 that includes geographic atrophy and neovascular AMD. Unilateral any AMD was defined as the presence of either early or late AMD in one eye, without any AMD in the fellow eye. Unilateral late AMD was defined as the presence of late AMD in one eye without late AMD and with or without early AMD in the fellow eye. Bilateral any AMD was defined as presence of any AMD in both eyes, and the same held for bilateral late AMD.

Total drusen area, measured as a proportion of the Wisconsin Age-Related Maculopathy Grading System (WARMGS), and presence of any retinal pigmentary abnormality in

the unilaterally affected eye at 'baseline' were also assessed as prognostic factors for bilateral involvement. As the studies differed slightly in their method of calculation of total drusen area, we derived quintiles of drusen area within each study to obtain comparable measures. Next, drusen area was categorized as small, intermediate or large, representing participants who had the lowest 20%, the middle 60% and the highest 20% of drusen area in each specific population accordingly.

Genotyping

In the BMES genotyping was performed as described in Methods (Chapter 2).After quality-control checking, genotypes of 2534 participants (544, 802 single nucleotide polymorphisms (SNPs)) were imputed. Additionally, genotype data was previously obtained for *CFH* SNP rs1061170 in 1840 participants and *ARMS2* SNP rs10490924 in 615 participants who attended the 5-year follow-up[96], and these subjects had one or both of the SNPs typed and imputed. The concordance rates between typed and imputed SNPs were 99.6% for rs1061170 and 99.2% for rs10490924. The genotyped SNPs were used whenever available.

In the BDES, *CFH*-rs1061170 was genotyped using TaqMan assays (Applied Biosystems, Foster City, CA) in 3015 participants, and a custom Illumina array in 2940 participants with subsequent data imputation techniques performed with Markov chain haplotyping, version 1.0.32 (http://www.sph.umich.edu/csg/abecasis/MACH/)[104]. The concordance rate between typed and imputed data among 1476 samples was 99.8%. Similarly the *ARMS2*-rs10490924 SNP was genotyped using 2 platforms, including TaqMan assay and an iSelect array (Illumina, Inc.). The concordance rate between the 588 samples genotyped with both platforms was 99.7%[104].

In the RS, *CFH*-rs1061170 was successfully genotyped in 6345 participants and *ARMS2*-rs10490924 in 6411, using TaqMan assays (Applied Biosystems, Foster City, CA). Additionally, for participants without genotyped data, data was imputed from a genome-wide association scan dataset, genotyped using the Illumina Infinium II HumanHap550. Imputation was performed using Markov Chain Haplotyping software version 1.0.15 (http://www.sph.umich.edu/csg/abecasis/MACH/, accessed June 4, 2013) and HapMap CEU data (NCBI build 36, release 22, The International HapMap Project). There were 6478 participants with typed or imputed *CFH* and *ARMS2* SNPs.

Assessment of Other Risk Factors

In each study, smoking status was assessed using an interviewer-administered questionnaire. In the BMES, participants were classified as non-smokers if they answered 'no' to smoking regularly. If participants had quit smoking more than 1 year prior to the examination, they were classed as past smokers. Current smokers were defined as participants who currently smoked or had stopped smoking <1 year before the examination. In the BDES, participants were classified as non-smokers if they had smoked fewer than 100 cigarettes in their lifetime, as a past smoker if they smoked ≥100 cigarettes but had stopped smoking before the examination, or a current smoker if they had not stopped smoking[118]. In the RS, smoking status was defined as never, past or current according to participants responses 'no, never smoked', 'yes, stopped smoking' and 'yes, still smoking', respectively[123].

In each study, the mean systolic and diastolic blood pressures were taken from the average of 2 readings of systolic and diastolic blood pressure. Serum total cholesterol

levels, high density lipoprotein (HDL) levels and white blood cell count were measured at baseline from non-fasting blood samples in the BDES and RS and fasting blood samples in the BMES[117].

Statistical Analyses

All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary NC). Progression to bilateral AMD was assessed using discrete time survival analysis focusing solely on the first 5 year interval after detection of unilateral AMD. Participants were included in the cohort at first detection of unilateral AMD, whether at the baseline, 5-year, 10-year or 15-year visits, and were assessed for progression to bilateral involvement 5 years later.

The progression was assessed by age, the number of risk alleles of the *CFH* and *ARMS2* genes individually or in combination (categorized as combined genetic risk scores: 0 risk alleles, 1 risk allele and 2-4 risk alleles), and smoking status, by each study sample and next in pooled data of three cohorts, using Mantel-Haenzel χ^2 tests for linear association.

Associations between progression to bilateral involvement and known or potential AMD risk factors (age, sex, smoking status, numbers of the *CFH* and *ARMS2* risk alleles, blood pressure, white blood cell count, total cholesterol and HDL-cholesterol levels) were assessed in age-adjusted and multivariable-adjusted logistic regression models, both within each cohort and in pooled three cohorts. Age was defined as a time-dependent variable corresponding to the visit when unilateral AMD was first detected. All other co-variables were defined using data collected at baseline visits. Final models

included age, sex, smoking status and any other variables that remained statistically significant at $p<0.05$. In addition, indicators of study site were included in models using pooled data.

Total drusen area and the presence of retinal pigmentary abnormalities in the unilaterally affected eye at 'baseline' (i.e. the beginning of each 5-year interval) were also included in the final models to assess whether these early AMD lesion characteristics contribute to an increased progression risk to bilateral any AMD.

To exclude possible influence of different timing when unilateral AMD was detected on the associations, additional analyses were performed to include secular trends in final models, to accommodate potential cohort effect. This was to assess whether there were systematic differences between individuals who developed unilateral AMD at the beginning of the study period and those who developed unilateral AMD in later periods. Association estimates are presented as age-adjusted or multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs).

To assess how useful the final model might be in predicting progression to bilateral any AMD in 5 years, we also produced the receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) to gauge the prognostic value of the model. The AUC is a measure of discrimination and, in this case, indicates the probability that a person with progression will have a higher score in the model than a person without progression.

The association between the systemic risk factors and progression to bilateral late AMD could not be assessed in the BMES, BDES and RS separately due to small number of cases, and therefore was only examined in pooled three cohort data. The association between ocular factors (drusen area, presence of retinal pigmentary abnormalities) and progression to bilateral late AMD could not be assessed even in pooled data, due to the small number of cases. Therefore a ROC curve and AUC were not produced for this model.

RESULTS

Participants aged 51+ years from the BMES, aged 44+ years from the BDES and aged 55+ years from the RS who had unilateral any or late AMD were included in this report. Of 1490 participants (BMES n=335, BDES n=625 and RS n=530) who had unilateral any AMD detected at any visit except for the last visit, 94 (28%) progressed to bilateral in the BMES, 119 (19%) in the BDES and 126 (24%) in the RS. Of 96 participants (BMES n=25, BDES n=51 and RS n=20) who had unilateral late AMD detected at any visits except for the last visit, 17 (68%) progressed to bilateral in the BMES, 14 (27%) in the BDES and 11 (55%) in the RS.

Factors associated with progression to bilateral any AMD

Table 6-1 presents comparisons of baseline characteristics between those who did and did not progress in the separate and pooled three populations. Compared to participants who remained unilateral, those who progressed to bilateral were older, and more likely to have at least 1 risk allele of *CFH* or *ARMS2* or at least 2 risk alleles of combined *CFH* and *ARMS2*. There was no difference in smoking status among those who progressed compared to those who did not (**Table 6-1**).

Table 6-1 Comparison of baseline characteristics of participants who did and those who did not progress from unilateral to bilateral any age-related macular degeneration (AMD), or from unilateral to bilateral late AMD, in the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES), Rotterdam Study (RS) individually and combined three cohorts.

Characteristics		5-Year Progression											
		BMES			BDES			RS			Combined		
		No progression	Progression	P-value*	No progression	Progression	P-value*	No progression	Progression	P-value*	No progression	Progression	P-value*
		Unilateral Any AMD to Bilateral Any AMD											
Participants, n(%)		241	94 (28.1)		506	119 (19.0)		404	126 (23.8)		1151	339 (22.8)	
Mean Age (SD)		66.1 (7.5)	72.4 (7.12)	<0.0001	63.6 (9.4)	69.8 (9.0)	<0.0001	68.1 (7.3)	70.9 (7.8)	0.0002	65.7 (8.5)	70.9 (8.1)	<0.0001
Sex (male) %		41.5	46.8	0.4	49.2	42.9	0.2	44.1	40.5	0.5	45.8	43.1	0.4
Smoking Status %	Never	57.5	49.5	0.3	43.1	40.3	0.9	34.3	29.6	0.6	42.9	38.9	0.3
	Past	28.8	37.6		37.2	39.5		45.0	48.8		38.2	42.4	
	Current	13.7	12.9		19.8	20.2		20.8	21.6		18.9	18.7	
***CFH* (rs1061170) %**	*TT*	44.2	32.1	0.04	40.3	21.9	0.0003	45.9	34.5	0.02	43.0	29.2	<0.0001
	CT	40.9	41.7		47.8	57.1		42.0	44.5		44.5	48.5	
	CC	14.9	26.2		11.9	21.0		12.2	21.0		12.6	22.4	
***ARMS2* (rs10490924) %**	*GG*	66.7	46.8	0.003	62.5	47.1	0.003	65.7	53.8	0.004	64.4	49.5	<0.0001
	GT	31.8	46.8		33.0	42.9		32.2	38.7		32.5	42.3	
	TT	1.5	6.3		4.6	10.1		2.1	7.6		3.1	8.2	
Combined Genetic Risk Score‡ %	0 risk alleles	27.8	11.4	0.007	23.5	10.9	<0.0001	29.6	18.5	0.003	26.5	13.9	<0.001
	1 risk allele	43.3	45.6		46.3	35.3		43.1	38.7		44.6	39.1	
	2-4 risk alleles	28.9	43.0		30.2	53.8		27.3	42.9		28.9	47.0	
Mean Systolic BP (SD), mmHg		142.9 (19.4)	147.6 (21.5)	0.05	130.4 (19.9)	131.9 (18.7)	0.4	140.9 (21.4)	140.5 (20.6)	0.9	136.7 (21.1)	139.4 (21.1)	0.03
Mean Diastolic BP (SD), mmHg		84.6 (10.2)	83.2 (9.9)	0.3	78.4 (10.1)	76.3 (10.0)	0.04	75.6 (11.7)	71.3 (10.0)	0.0002	78.7 (11.1)	76.4 (11.0)	0.0006
Mean WBCC (SD), x10^9 cells/L		6.6 (1.8)	6.4 (1.6)	0.4	7.2 (1.9)	7.2 (1.9)	0.8	6.4 (1.8)	6.3 (1.6)	0.5	6.8 (1.9)	6.7 (1.8)	0.2
Mean Total Cholesterol (SD), mmol/L		6.1 (0.9)	6.3 (1.0)	0.09	6.0 (1.1)	6.1 (1.0)	0.7	6.4 (1.1)	6.2 (1.1)	0.09	6.2 (1.1)	6.2 (1.0)	0.9
Mean HDL Cholesterol (SD), mmol/L		1.4 (0.4)	1.5 (0.5)	0.2	1.4 (0.5)	1.3 (0.4)	0.4	1.4 (0.4)	1.4 (0.4)	0.6	1.4 (0.4)	1.4 (0.4)	0.6

		Unilateral Late AMD to Bilateral Late AMD											
Participants n (%)		8	17 (68.0)		37	14 (27.5)		9	11 (55.0)		54	42 (43.8)	
Mean Age (SD)		77.3 (6.2)	76.5 (6.2)	0.8	73.2 (8.0)	79.4 (6.8)	0.01	73.4 (6.8)	75.9 (5.3)	0.4	73.8 (7.6)	77.3 (6.2)	0.02
Sex (male) %		25.0	23.5	0.9	46.0	35.7	0.5	66.7	63.6	0.9	46.3	38.1	0.4
Smoking Status %	Never	37.5	47.1	0.6	56.8	50.0	0.3	11.1	9.1	0.6	46.3	38.1	0.5
	Past	50.0	29.4		32.4	50.0		66.7	45.5		40.7	40.5	
	Current	12.5	23.5		10.8	0.0		22.2	45.5		13.0	21.4	
***CFH* (rs1061170) %**	*TT*	12.5	31.3	0.6	16.2	14.3	0.9	33.3	9.1	0.3	18.5	19.5	0.6
	CT	50.0	43.8		67.6	64.3		55.6	54.6		63.0	53.7	
	CC	37.5	25.0		16.2	21.4		11.1	36.4		18.5	26.8	
***ARMS2* (rs10490924) %**	*GG*	62.5	31.3	0.2	29.7	21.4	0.03	33.3	27.3	0.6	35.2	26.8	0.2
	GT	37.5	50.0		40.5	78.6		66.7	63.6		44.4	63.4	
	TT	0.0	18.8		29.7	0.0		0.0	9.1		20.4	9.8	
Combined Genetic Risk Score[†] %	0 risk alleles	12.5	6.3	0.8	13.5	0.0	0.3	22.2	9.1	0.4	14.8	4.9	0.3
	1 risk allele	37.5	31.3		16.2	21.4		22.2	9.1		20.4	22.0	
	2-4 risk alleles	50.0	62.5		70.3	78.6		55.6	81.8		64.8	73.2	
Mean Systolic BP (SD), mmHg		146.4 (20.3)	147.5 (16.2)	0.9	136.4 (19.8)	136.6 (18.0)	0.97	137.6 (12.2)	144.3 (17.7)	0.4	138.1 (18.9)	143.0 (6.2)	0.2
Mean Diastolic BP (SD), mmHg		82.9 (8.5)	83.5 (8.2)	0.9	75.7 (9.3)	71.7 (11.2)	0.2	71.6 (10.2)	78.4 (11.2)	0.2	76.1 (9.7)	78.2 (11.0)	0.3
Mean WBCC (SD), x10^9 cells/L		6.9 (2.3)	6.8 (1.1)	0.8	7.2 (2.3)	7.1 (2.1)	0.9	6.6 (1.2)	6.9 (1.6)	0.6	7.0 (2.2)	6.9 (1.6)	0.8
Mean Total Cholesterol (SD), mmol/L		6.6 (1.3)	6.3 (0.9)	0.4	6.1 (1.2)	5.7 (0.8)	0.3	5.9 (1.0)	5.8 (0.9)	0.7	6.1 (1.2)	5.9 (0.9)	0.4
Mean HDL Cholesterol (SD), mmol/L		1.5 (0.3)	1.4 (0.5)	0.6	1.4 (0.5)	1.6 (0.4)	0.4	1.4 (0.3)	1.6 (0.8)	0.4	1.4 (0.4)	1.5 (0.5)	0.4

n=sample size, SD=standard deviation, *CFH*=complement factor H (risk allele C), *ARMS2*=age-related maculopathy susceptibility gene 2 (risk allele T), BP=blood pressure, WBCC=white blood cell count, HDL=high density lipoprotein

[*]P-value for association between baseline characteristics and progression of AMD from unilateral to bilateral (categorical factors) and P-value for difference in mean baseline level (continuous factors).

[†]Total number of risk alleles from *CFH* and *ARMS2* combined

Table 6-2 presents proportions of progression to bilateral any AMD by age, genotype and smoking status in separate and pooled populations. The progression was associated with increasing age and increasing numbers of risk alleles of the *CFH* and *ARMS2* genes. However, there was no significant crude association between smoking status and progression to bilateral any AMD (**Table 6-2**).

Table 6-2 Five-year progression from unilateral to bilateral any and late AMD, by age, genotype and smoking status in the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES), Rotterdam Study (RS) individually and combined three cohorts.

Factors	5-Year Progression from Unilateral to Bilateral AMD															
	BMES				BDES				RS				Combined			
	Any AMD*		Late AMD†		Any AMD*		Late AMD†		Any AMD*		Late AMD†		Any AMD*		Late AMD†	
	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%	No. of cases/ No. at risk	%
Age (years)																
40-49	-	-	-	-	3/44	6.8	0/0	0.0	-	-	-	-	3/44	6.8	0/0	0.0
50-59	4/47	8.5	0/0	0.0	11/137	8.0	0/1	0.0	11/58	19.0	0/0	0.0	26/242	10.7	0/1	0.0
60-69	24/136	17.7	2/3	66.7	42/241	17.4	1/11	9.1	40/233	17.2	1/3	33.3	106/610	17.4	4/17	23.5
70-79	51/127	40.2	10/14	71.4	46/163	28.2	8/27	29.6	59/197	30.0	7/12	58.3	156/487	32.0	25/53	47.2
80+	15/25	60.0	5/8	62.5	17/40	42.5	5/12	41.7	16/42	38.1	3/5	60.0	48/107	44.9	13/25	52.0
Total	**94/335**	**28.1**	**17/25**	**68.0**	**119/625**	**19.0**	**14/51**	**27.5**	**126/530**	**23.8**	**11/20**	**55.0**	**339/1490**	**22.8**	**42/96**	**43.8**
***P* trend‡**	**<0.0001**		**0.8**		**<0.0001**		**0.07**		**0.0005**		**0.5**		**<0.0001**		**0.06**	
Smoking Status																
Never	46/180	25.6	8/11	72.7	48/266	18.1	7/28	25.0	37/174	21.3	1/2	50.0	131/620	21.1	16/41	39.0
Past	35/102	34.3	5/9	55.6	47/235	20.0	7/19	36.8	61/241	25.3	5/11	45.5	143/578	24.7	17/39	43.6
Current	12/44	27.3	4/5	80.0	24/124	19.4	0/4	0.0	27/110	24.6	5/7	71.4	63/278	22.7	9/16	56.3
***P* trend‡**	**0.1**		**0.4**		**0.6**		**0.8**		**0.4**		**0.3**		**0.4**		**0.2**	
CFH (rs1061170)																
TT	27/119	22.7	5/6	83.3	26/230	11.3	2/8	25.0	41/218	18.8	1/4	25.0	94/567	16.6	8/18	44.4
CT	35/120	29.2	7/11	63.6	68/310	21.9	9/34	26.5	53/215	24.7	6/11	54.6	156/645	24.2	22/56	39.3
CC	22/53	41.5	4/7	57.1	25/85	29.4	3/9	33.3	25/72	34.7	4/5	80.0	72/210	34.3	11/21	52.4
***P* trend‡**	**0.01**		**0.3**		**<0.0001**		**0.6**		**0.006**		**0.1**		**<0.0001**		**0.5**	
ARMS2 (rs10490924)																
GG	37/169	21.9	5/10	50.0	56/372	15.1	3/14	21.4	64/317	20.2	3/6	50.0	157/858	18.3	11/30	36.7
GT	37/100	37.0	8/11	72.7	51/218	23.4	11/26	42.3	46/124	27.1	7/13	53.9	134/488	27.5	26/50	52.0
TT	5/8	62.5	3/3	100.0	12/35	34.3	0/11	0.0	9/17	52.9	1/1	100.0	26/60	43.3	4/15	26.7
***P* trend‡**	**0.0008**		**0.09**		**0.0006**		**0.3**		**0.003**		**0.5**		**<0.0001**		**0.8**	

Combined Genetic Risk Score[§]																
0 risk alleles	9/63	14.3	1/2	50.0	13/132	9.9	0/5	0.0	22/136	16.2	1/3	33.3	44/331	13.3	2/10	20.0
1 risk allele	36/120	30.0	5/8	62.5	42/276	15.2	3/9	33.3	46/212	21.7	1/3	33.3	124/608	20.4	9/20	45.0
2-4 risk alleles	34/90	37.8	10/14	71.4	64/217	29.5	11/37	29.7	51/156	32.7	9/14	64.3	149/463	32.2	30/65	46.2
***P* trend**[‡]	**0.002**		**0.5**		**<0.0001**		**0.3**		**0.0008**		**0.2**		**<0.0001**		**0.2**	

CFH=complement factor H (risk allele C), *ARMS2*=age-related maculopathy susceptibility 2 (risk allele T)

[*]Unilateral any AMD progression to bilateral any AMD

[†]Unilateral late AMD progression to bilateral late AMD

[‡]*P* trend calculated using Mantel-Haenszel χ^2 test for linear association

[§]Combined risk dichotomised as 0 or 1 risk allele of *CFH* or *ARMS2* or 2 to 4 risk alleles of *CFH* and/or *ARMS2*

Table 6-3 presents ORs of AMD risk factors associated with progression to bilateral any AMD by individual cohorts. After adjusting for sex, smoking, diastolic BP, total drusen area, presence of retinal pigmentary abnormalities and number of risk alleles of the *CFH* and *ARMS2* genes, age was associated with the risk of progression. Similarly, the presence of risk genotypes of the *CFH* or *ARMS2,* or the presence of ≥2 risk alleles from these two genes, was associated with an increased risk of progression. These associations were consistent across three cohorts while smoking was non-significantly associated with this progression. Large total drusen area (the highest quintile compared to the lowest) contributed significantly to the risk of progression in each cohort. Presence of any retinal pigmentary abnormality was associated with a significantly increased risk of progression in the BMES and BDES but not in the RS (**Table 6-3**).

Table 6-3 Associations of age-related macular degeneration (AMD) risk factors with 5-year progression from unilateral to bilateral any AMD in the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES) and Rotterdam Study (RS) populations.

Risk Factors		BMES		BDES		RS	
		Age-Adjusted OR (95% CI)	**Multivariable-Adjusted* OR (95% CI)**	**Age-Adjusted OR (95% CI)**	**Multivariable-Adjusted* OR (95% CI)**	**Age-Adjusted OR (95% CI)**	**Multivariable-Adjusted* OR (95% CI)**
Age per year		**1.12 (1.08-1.16)**	**1.15 (1.09-1.21)**	**1.08 (1.05-1.10)**	**1.07 (1.04-1.10)**	**1.05 (1.02-1.09)**	**1.04 (1.00-1.07)**
Sex (male)		1.54 (0.91-2.60)	1.02 (0.49-2.12)	0.90 (0.59-1.36)	0.76 (0.47-1.24)	0.96 (0.63-1.46)	0.92 (0.54-1.56)
Smoking Status	Never	1.00	1.00	1.00	1.00	1.00	1.00
	Past	1.74 (0.98-3.08)	1.43 (0.65-3.12)	1.33 (0.83-2.12)	1.57 (0.91-2.70)	1.51 (0.93-2.45)	1.79 (0.98-3.29)
	Current	1.61 (0.70-3.67)	2.10 (0.73-5.98)	1.48 (0.83-2.63)	1.47 (0.78-2.77)	1.71 (0.93-3.13)	1.69 (0.83-3.43)
***CFH* (rs1061170)**	*TT*	1.00	-	1.00	-	1.00	-
	CT	1.33 (0.70-2.51)	-	**2.37 (1.43-3.93)**	-	1.35 (0.84-2.15)	-
	CC	**3.55 (1.64-7.67)**	-	**3.53 (1.85-6.74)**	-	**2.42 (1.32-4.41)**	-
***ARMS2* (rs10490924)**	*GG*	1.00	-	1.00	-	1.00	-
	GT	**2.72 (1.48-4.99)**	-	**1.94 (1.24-3.02)**	-	1.39 (0.89-2.16)	-
	TT	**7.48 (1.57-35.6)**	-	**4.37 (1.96-9.74)**	-	**4.55 (1.67-12.4)**	-
Combined Genetic Risk Score†	0 risk alleles	1.00	1.00	1.00	1.00	1.00	1.00
	1 risk allele	**2.93 (1.22-7.05)**	**3.46 (1.33-9.02)**	1.71 (0.87-3.37)	1.57 (0.77-3.20)	1.42 (0.80-2.49)	1.52 (0.83-2.79)
	2-4 risk alleles	**5.36 (2.16-13.30)**	**5.19 (1.93-13.93)**	**4.75 (2.43-9.27)**	**4.25 (2.11-8.54)**	**2.49 (1.40-4.40)**	**2.51 (1.35-4.67)**
Blood Pressure (per 10mmHg)	Systolic	1.03 (0.91-1.17)	-	0.99 (0.90-1.11)	-	0.95 (0.86-1.05)	-
	Diastolic	0.90 (0.70-1.16)	0.96 (0.68-1.34)	0.97 (0.78-1.21)	0.93 (0.73-1.18)	**0.72 (0.59-0.86)**	**0.71 (0.57-0.88)**
WBCC (per SD increase)		0.93 (0.69-1.23)	-	1.12 (0.88-1.43)	-	0.96 (0.77-1.19)	-
Total Cholesterol (per SD increase)		1.25 (0.92-1.69)	-	0.95 (0.76-1.19)	-	0.88 (0.71-1.08)	-
HDL Cholesterol (per SD increase)		1.15 (0.86-1.54)	-	0.93 (0.75-1.15)	-	1.04 (0.85-1.27)	-
Drusen Area‡	Low	1.00	1.00	1.00	1.00	1.00	1.00
	Intermediate	1.32 (0.58-2.99)	1.83 (0.75-4.49)	1.79 (0.88-3.66)	**2.75 (1.26-5.99)**	**2.99 (1.52-5.90)**	**2.65 (1.32-5.31)**
	High	**9.62 (3.77-24.55)**	**15.26 (5.08-45.83)**	**7.93 (3.69-17.04)**	**12.32 (5.10-29.79)**	**9.11 (4.27-19.43)**	**9.62 (4.29-21.57)**
RPE Abnormality Presence		1.18 (0.65-2.15)	**2.61 (1.21-5.66)**	0.93 (0.61-1.42)	**1.73 (1.03-2.90)**	1.02 (0.67-1.56)	1.49 (0.91-2.43)

OR=odds ratio, CI=confidence interval, *CFH=complement factor H*, *ARMS2=age-related maculopathy susceptibility gene 2*, WBCC=white blood cell count, HDL=high density lipoprotein, SD=standard deviation, RPE=retinal pigment epithelium

*Adjusted for age, sex, smoking, combined genetic risk score, diastolic blood pressure, drusen area and RPE abnormalities.

†Total number of risk alleles from *CFH* and *ARMS2* combined (reference: 0 risk alleles)

‡Total drusen area categorised as low, intermediate and high representing the lowest 20% of drusen area, the central 60%, and highest 20%, respectively.

Bold values indicate significant odds ratios.

Table 6-4 presents ORs of AMD risk factors associated with progression to bilateral any AMD in pooled data after age-adjustment and multivariable adjustment including age, sex, smoking, diastolic blood pressure, total drusen area and the presence of retinal pigmentary abnormalities. This progression was more commonly observed in the BMES compared to the BDES. Older age was associated with progression while sex was not. Past and current smoking was significantly associated with increased risk of progression, compared to persons who never smoked. The presence of ≥1 risk allele of *CFH*, *ARMS2,* or in combination, also showed an increased risk of progression (**Table 6-4**). Large total drusen area and retinal pigmentary abnormalities substantially increased risk of the progression. The ROC curve of this final model is shown in **Figure 6-1**, and the AUC was 0.79, which indicates reasonable but not excellent discrimination for a clinical tool.

Table 6-4 Associations of age-related macular degeneration (AMD) risk factors with 5-year progression from unilateral to bilateral any AMD and late AMD in pooled data of the Blue Mountains Eye Study (BMES), Beaver Dam Eye Study (BDES) and Rotterdam Study (RS).

Risk Factors		BILATERAL ANY AMD		BILATERAL LATE AMD	
		Age-Adjusted OR (95% CI)	Multivariable-Adjusted* OR (95% CI)	Age-Adjusted OR (95% CI)	Multivariable-Adjusted† OR (95% CI)
Study Population (ref: BDES)	BMES	**1.42 (1.03-1.97)**	**1.71 (1.16-2.54)**	**5.45 (1.86-15.90)**	**7.30 (2.05-25.96)**
	RS	1.06 (0.79-1.42)	1.10 (0.79-1.53)	**3.54 (1.16-10.80)**	3.43 (0.82-14.31)
Age (per year)		**1.08 (1.06-1.09)**	**1.07 (1.05-1.09)**	**1.08 (1.01-1.15)**	**1.13 (1.05-1.23)**
Sex (male)		1.06 (0.82-1.37)	0.89 (0.65-1.22)	0.86 (0.33-2.23)	0.81 (0.28-2.34)
Smoking Status	Never	1.00	1.00	1.00	1.00
	Past	**1.51 (1.13-2.01)**	**1.64 (1.16-2.33)**	1.32 (0.46-3.77)	1.97 (0.59-6.55)
	Current	**1.65 (1.14-2.38)**	**1.67 (1.10-2.55)**	2.14 (0.47-9.76)	2.01 (0.38-10.57)
***CFH* (rs1061170)**	*TT*	1.00	-	1.00	-
	CT	**1.64 (1.22-2.21)**	-	1.24 (0.38-4.07)	-
	CC	**2.92 (2.00-4.25)**	-	2.06 (0.50-8.39)	-
***ARMS2* (rs10490924)**	*GG*	1.00	-	1.00	-
	GT	**1.77 (1.34-2.33)**	-	2.79 (0.97-8.03)	-
	TT	**4.64 (2.62-8.21)**	-	1.18 (0.25-5.51)	-
Combined Genetic Risk Score‡	0 risk alleles	1.00	1.00	1.00	1.00
	1 risk allele	**1.72 (1.17-2.54)**	**1.76 (1.17-2.64)**	3.61 (0.52-25.34)	4.91 (0.60-40.03)
	2-4 risk alleles	**3.56 (2.42-5.25)**	**3.34 (2.21-5.04)**	**6.39 (1.04-39.09)**	**12.46 (1.52-101.97)**
Blood Pressure (per 10mmHg)	Systolic	0.99 (0.93-1.05)	-	1.05 (0.81-1.36)	-
	Diastolic	**0.84 (0.74-0.95)**	**0.82 (0.71-0.95)**	1.07 (0.67-1.70)	-
WBCC (per SD increase)		1.01 (0.87-1.17)	-	1.04 (0.65-1.64)	-
Total Cholesterol (per SD increase)		0.98 (0.86-1.12)	-	0.62 (0.36-1.04)	**0.47 (0.26-0.84)**
HDL Cholesterol (per SD increase)		1.02 (0.88-1.17)	-	1.19 (0.77-1.83)	-
Drusen Area§	Low	1.00	1.00	-	-
	Intermediate	**2.04 (1.34-3.10)**	**2.32 (1.50-3.59)**	-	-
	High	**8.57 (5.42-13.56)**	**10.67 (6.45-17.67)**	-	-
RPE Abnormality Presence		0.99 (0.76-1.29)	**1.68 (1.23-2.29)**	-	-

OR=odds ratio, CI=confidence interval, *CFH=complement factor H*, *ARMS2=age-related maculopathy susceptibility gene 2*, WBCC=white blood cell count, HDL=high density lipoprotein, SD=standard deviation, RPE=retinal pigment epithelium

*Adjusted for study population, age, sex, smoking, combined genetic risk, diastolic blood pressure, drusen area and RPE abnormalities.

†Adjusted for study population, age, sex, smoking, combined genetic risk and total cholesterol.

‡Total number of risk alleles from *CFH* and *ARMS2* combined (reference: 0 risk alleles).

§Total drusen area categorised as low, intermediate and high representing the lowest 20% of drusen area, the central 60%, and highest 20%, respectively.

Bold values indicate significant odds ratios.

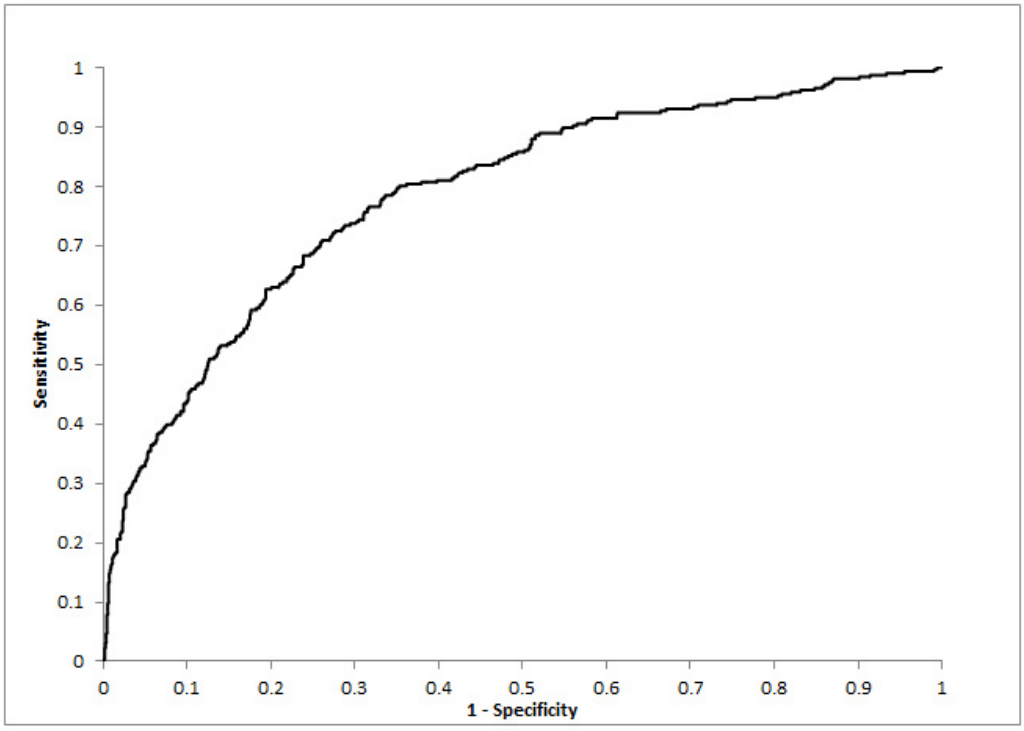

Figure 6-1 Receiver operating characteristic (ROC) curve indicating the prognostic performance of the model in predicting probabilities of 5-year progression from unilateral to bilateral involvement by any AMD.

A supplementary analysis using a recently developed comprehensive gene-environment risk score[105] found no additional improvement to the adjusted model for predicting progression to bilateral any AMD (data not shown). There was no meaningful difference in the association between these risk factors and progression to bilateral any AMD after inclusion of secular trend terms in the model (data not shown).

Factors associated with progression to bilateral late AMD

Compared to participants who stayed in the unilateral late AMD category over 5 years, those who progressed to bilateral late AMD were on average older (**Table 6-1**). However, there was no significant trend for age, smoking or genetic associations with this progression in either separate or pooled cohorts (**Table 6-2**).

Progression to bilateral late AMD was more commonly observed in the BMES compared to the BDES (**Table 6-4**). The presence of ≥2 risk alleles from the *CFH* and *ARMS2* genes combined presented a 12-fold risk of progression after multivariable adjustment. Increased serum total cholesterol was independently associated with decreased risk of progression. After inclusion of secular trend terms, progression to bilateral late AMD was also more common in the RS compared to the BDES. However, the association between AMD risk factors and the progression remained essentially the same as in the model without secular trends (data not shown).

DISCUSSION

This is one of few studies to assess progression to bilateral any or late AMD over a clinically relevant time (5-year) interval. On average, the progression rate to bilateral

any AMD was 19% to 28%, and to bilateral late AMD it was 27% to 68% in persons with unilateral involvement 5 years prior.

More severe levels of AMD in one eye were previously reported to be associated with increased incidence and progression of AMD in the fellow eye[271]. Using data from the 3CC we additionally examined risk factors associated with progression to bilateral involvement, and documented that three well known AMD risk factors (older age, genetic risk and smoking), as well as early AMD lesion characteristics, are associated with an increased risk of progression to bilateral involvement in pooled data of three cohorts.

The BDES population includes a younger age spectrum (age 43+ years) compared to the BMES (aged 51+ years) and the RS (aged 55+ years), which may, in part, explain why higher proportions of progression were present in the BMES and RS relative to the BDES (Tables 6-1 and 6-4).

One clinic-based study reported a 5-year incidence of late AMD in the fellow eye of 26% of unilateral late AMD patients enrolled in a randomized clinical trial[270]. Similarly in the BMES 29% and in the BDES 22% were progressed to late AMD in the fellow (second) eye in 5 years among participants with unilateral late AMD[29,44], while in the RS the 5-year cumulative incidence of late AMD in the fellow eye was greater at 39%[38]. These previous findings are comparatively lower than the 68% progression to bilateral late AMD found in the BMES, 28% in the BDES and 55% in the RS in this report. As we examined AMD progression within any 5-year period over the 10-20 year follow-up periods, rather than in a single 5-year interval, the mean age of participants included in

this report is considerably older than the mean ages of 64.5 years in the BMES, 61.7 years in the BDES and 68.9 years in the RS baseline samples only.

Factors associated with progression to bilateral any AMD

There are limited data available in the literature for comparison with our findings. In pooled data analyses of the 3CC, either past or current smoking was significantly associated with a 50% greater risk of progression to bilateral any AMD. The risk magnitude and 95% confidence intervals of the associations of past and current smoking with the progression were similar compared to never smoking. This finding could be due to the fact that some past smokers had only recently stopped smoking. The RS and BDES used definitions slightly different from those used in the BMES for current and past smoking. Despite heterogeneity in the smoking questions asked and in definitions of current and past smokers across three studies, the contribution of smoking to progression to bilateral AMD is evident in the increased sample size of pooled-data.

Consistent with a previous BMES finding[265], we also documented increased risk of progression among those with ≥1 risk allele of the *CFH* or *ARMS2* gene individually or in combination, in our pooled data. The inverse association between increased diastolic blood pressure and reduced risk of progression to bilateral any AMD is not readily explained.

As in previous studies, drusen area was found to be the strongest predictor of progression to bilateral any AMD[272]. An AUC of 0.79 for the final model suggests that the model has considerable value in distinguishing persons who progress to bilaterality from those who do not.

Factors associated with progression to bilateral late AMD

The relatively small numbers of participants with unilateral late AMD in each individual population and in pooled data is likely the reason for the lack of associations with smoking found in our analyses. Risk alleles of the *CFH* and *ARMS2* genes were not significantly associated individually with progression to bilateral late AMD in each study sample or in pooled data. However, the presence of ≥2 risk alleles of the *CFH* and *ARMS2* genes combined was associated with a significantly high risk of progression to bilateral involvement by late AMD, a blinding condition with significant impact on the affected individuals. Note that due to small numbers resulting in substantially wide confidence intervals (95% CI 1.5-102), this bilateral late AMD risk estimate (OR 12.5) is likely unstable.

The inverse association between increased cholesterol and risk of progression to bilateral late AMD is not readily explained. In a previous report on the pooled data analysis from the same 3CC, an increase in serum total cholesterol levels was also found to be associated with a reduced incidence of neovascular AMD[117]. Findings concerning the relationship between serum total cholesterol levels and AMD risk are largely inconsistent[169,266,273,274]. While HDL cholesterol is protective for cardiovascular disease risk[166-168], the association between HDL cholesterol and AMD risk has similarly been inconsistent[154,156,274]. In this study we found that HDL cholesterol levels were not associated with progression to bilateral any or late AMD.

Study strengths and limitations

Strengths of this study include the relatively large sample size for unilateral any AMD cases from the 3CC Caucasian cohorts. Care has been taken to harmonize AMD grading and to confirm all late AMD cases across three cohorts[57]. Additionally, the 5-step severity scale developed after harmonization of AMD phenotypes[32] in the 3CC was used to uniformly define any and late AMD. We were also able to document that secular trends had no impact on the association between risk factors and the progression to bilateral AMD.

Limitations of this study include the relatively small sample size within each cohort and in the pooled samples for unilateral late AMD cases, resulting in low study power to assess some risk factors. The cohort samples included in this study are almost Caucasians of Northern and Western European descent, and therefore the results presented may not be applicable to other ethnic populations.

SUMMARY

Our findings from this report show that in 5 years, one in five to one in four older persons with unilateral any AMD progressed to bilateral any AMD, and nearly one in two with unilateral late AMD progressed to bilateral late AMD. Findings from pooled data of three cohorts suggest that three known AMD risk factors (age, smoking and AMD genetic variants) and early AMD lesion characteristics are significantly associated with progression to bilateral involvement among unilateral AMD cases. Of these risk factors, only smoking is modifiable.

Chapter 7

Implications of the Findings from this Thesis

My work in this thesis documented the long-term incidence, progression and associated risk factors of age-related macular degeneration (AMD) and its component lesions in an older Australian population-based cohort, the Blue Mountains Eye Study.

The 15-year incidence rates of Early and Late AMD were 15.1% and 4.1%, respectively, in the BMES. The 15-year incidence of pure geographic atrophy (GA) was 2.2 %, and progression of GA measured as enlargement of the GA lesion was 1.95mm^2/year. The 15-year incidence of reticular drusen was 4.0%, and 34% of eyes with this lesion progressed to late AMD within 5 years. The 15-year incidence of medium drusen was 13.9%, and the progression to late AMD from this lesion was 5.0%, over 15 years. Progression to late AMD from medium drusen with co-presented pigmentary abnormalities was 4-fold (23.0%) of that for eyes with medium drusen alone. The prevalence of distinct soft drusen was 23.9% in Singaporean Asians compared to 6.2% in white Australians, and the prevalence of indistinct soft and reticular drusen was 6.5% in Singaporean Asians compared to 8.3% in white Australians. One in five to one in four persons with unilateral any AMD progressed to bilateral, and nearly one in two with unilateral late AMD progressed to bilateral in 5 years.

Age and known genetic risk from the *CFH* and *ARMS2* were significantly associated with the 15-year incidence of early AMD, late AMD, GA, reticular drusen, medium drusen and the progression from unilateral any AMD to bilateral. Current smoking was associated with the long-term incidence of late AMD, GA, reticular drusen and the progression from unilateral any AMD to bilateral. There was no longitudinal association between smoking and the incidence of early AMD or medium drusen. Weekly fish

consumption was associated with a reduced risk of 15-year incident late AMD, and the progression from reticular drusen to late AMD in 5 years was reduced in those with high dietary intake of lutein-zeaxanthin.

While genetic screening is not conventional practice in ophthalmology clinics, being genetically susceptible to AMD is an important risk stratifier of AMD. Smoking may have a promoting role in progression from early to late AMD. Weekly fish consumption and dietary intake of food rich in lutein-zeaxanthin may help to prevent or slow the development of late AMD.

Importantly, early AMD characteristics including large macular areas involved by, and central location of early AMD lesions, the co-presence of different types of early AMD lesions and the presence of reticular drusen, are indicative of high risk of progressing to late AMD. The choice of AMD severity scales used in assessing risk of late AMD should be considered carefully as it may have different risk estimates depending on the type of study sample, i.e. population-based or clinic-based, and may have implication in study power calculations for intervention trials.

It is also important to understand the differences in the risk and severity of AMD lesions in different racial populations given the current demographic of Australia being ethnically diverse. In 2014 it was estimated that 28% of Australia's population was born overseas, and nearly half this number originated from Asia[275]. The relatively milder spectrum of early AMD lesions in Asian population implies that the risk of progression to late AMD in Asians likely differs from that of Caucasians. Further studies are needed to explore the interrelationship of environmental and genetic risk factors relating to

AMD risk, which may provide insight into mechanisms explaining ethnic differences in risk of AMD between Caucasians and other ethnic populations.

The findings presented in this thesis were all derived from population-based studies that used retinal fundus photography todocument and assess AMD and its component lesions. While this method of assessment has provided much knowledge on current understanding of the characteristics, development and progression of AMD, the advent of new technologies in retinal imaging in recent years have furthered this knowledge and will continue to do so in the future. Novel features of OCT are currently used to examine structural alternations associated with early and late stages of AMD including drusen thickness and volume, RPE and choroidal thickness, examining vitreoretinal interface abnormalities, the hyperfluorescent edge of GA during progression and the presence of fluid and other features of neovascular AMD[210,276-285]. However, while use of these technologies in clinical settings may be straightforward, their implementation in large scale population-based studies would be very challenging, particularly the logistics of time demanded for having multiple imaging examination methods and the willingness of participants to undergo such rigorous testing of their eyes during examinations. The use of multimodal imaging should nevertheless be employed, if possible, in new population-based studies as it allows better classification of certain AMD lesions and quantification of some AMD characteristics such as GA progression.

Given Australia's ageing population and that AMD is strongly age-related, estimates of the long-term incidence and progression, and examination of associated risk factors of AMD are essential to understanding the aetiology of AMD, developing strategies to prevent adverse consequence of AMD, and improving management and services for the

affected individuals. Knowledge about modifiable lifestyle behaviours that can either increase or reduce risk of late AMD should be advocated and included in education provided to patients with early AMD. Contribution from my work in this thesis should ultimately help maintain good quality of life for an ageing population and minimise the economic burden of AMD.

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Appendix A

Examination questionnaire from the BMES

Blue Mountains Eye Study

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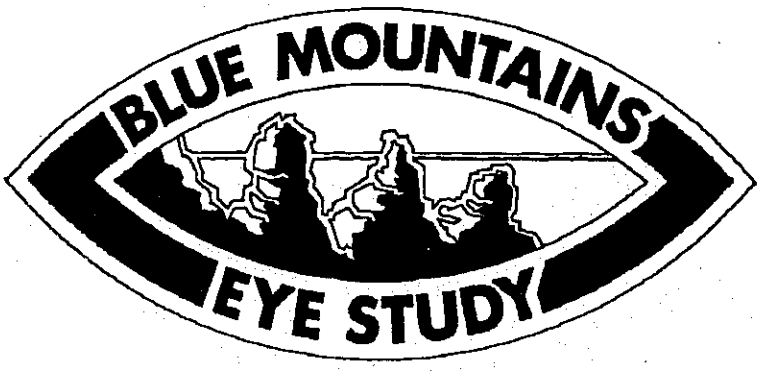

Questionnaire and study flow sheet

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BLUE MOUNTAINS EYE STUDY QUESTIONNAIRE AND STUDY FLOW SHEET

Page 1 *(detach from rest of questionnaire after completion)*

X1. *(IDnum)* _____ ID number X2. *(IDHosp)*_____ hosp number

---

X3. *(surname)* Surname _____

X4. *(name)* First Name(s) _____

X5. *(DOB)* What is your date of birth? ___/___/___ *(calculate age)*

X6. *(age)* So you are _____ years old now ?
if <49 years stop and explain inability to include.

X7. *(address)* Your address?_____

Postcode _____

X8. *(usuadrs)* Is this your usual address? yes 1
no 2
(more than 6 months of the year)

X9. *(phno)* Your phone number? _____

X10. *(WMHpt)* Have you ever been seen at Westmead Hospital as an outpatient or admitted there? yes 1
no 2

X11.*(WMHno)* _____ *(Hospital Number)*

X12. *(rels)* Do you have any relatives living in Katoomba, Leura or Medlow Bath who are aged 49 or older? yes 1
no 2

1. Name _____

Address _____

2. Name _____

Address _____

BLUE MOUNTAINS EYE STUDY QUESTIONNAIRE AND STUDY FLOW SHEET

PHASE 1: *for completion by Examiner 1.*

A1. *(IDnum)* _____ ID number A2. *(IDHosp)*_____hosp number

A3. *(DOB)* Date of birth ___/___/___

A4. *(examday)* Exam date ___/___/9__

A5. *(examloc)*
Blue Mts Hospital 1
Nursing Home ..... 2 specify_____ code_____
Other site ..... 3 specify_____ code_____

A6. *(sex)* Sex: Female 1
Male 2

A7. *(hrstart)* Time began _____ hrs A8. *(temp)* Temperature _____ °C

Thank you for taking part in our eye study. As a part of this study we will be asking you some general questions about you, your health and your eyes. All information you provide will be strictly confidential.

A9. *(yrsBM)* How long have you lived in the Blue Mountains? _____ yrs
don't know 888

A10. *(transprt)* How did you get to the eye study today?
walked .....1 friend/rel drove 5
bus .....2 hospital bus 6
taxi .....3 voluntary driver ... 7
drove yourself ..... 4 other_____ code_____

A11. *(GP)* Who is your GP?_____

A12. *(adrGP)* In which suburb is his/her surgery? _____

A13. *(whoeye1)* Who was the last person you saw for your eyes, for glasses or any eye treatment? _____

A14. *(wheneye)* When was that? _____

A15. *(optoph)* Was it an optometrist ?... 1
or an eyedoctor ?.....2
don't know 8

A16. *(adreye)* In which suburb are his/her rooms? _____

A17. *(longeye)* How long before that had you seen someone for your eyes? _____

A18. *(whoeye2)* Who did you see? _____

If not an ophthalmologist in A18or A13, then ask:

A19. *(seenoph)* Have you ever seen an eye doctor?
yes 1
no ..... 2 *goto A22.*
don't know..8 *goto A22.*

A20. *(whooph)* Who did you see?_____

A21. *(whenoph)* When did you see him/her? _____

A22. *(birthpl)* Where were you born? _____ | code _____
(write state or territory if in Australia, country if born overseas)

A23. *(ageAust)* *If not born in Australia,* how old were you when you came to Australia? _____ yrs

---

A24. *(mumborn)* Where was your mother born? _____ | code _____
(write state or territory if in Australia, country if born overseas)

A25. *(dadborn)* Where was your father born? _____ | code _____
(write state or territory if in Australia, country if born overseas)

A26. *(race)* *Racial group:* *examiner to fill in, ask only if unsure*

White .....1 *Aboriginal* ..... 2 *Indian* 7
Negroid ..... 3 *Hispanic* 4
Oceanian .....5 *Asian* 6

A27. *(speak)* At home, do you usually speak a language other than English?

yes..... 1
no..... 2 *goto A 29.*
don't know ..... 8 *goto A 29.*

A28. *(spoken)* What language do you speak? _____ | code _____

A29. *(qualif)* After leaving school, did you get any other qualification or learn a trade?

yes 1
no ..... 2 *goto A31.*
don't know ..... 8 *goto A31.*

A30. *(qualnm)* Qualification name _____ | code _____

A31. *(jobstat)* Are you retired or still employed?

houseduties..... 1 medical disability 4
retired ..... 2 unemployed 5
employed ..... 3 other 6

A32. *(retired)* *If retired,* how old were you when you retired? _____ yrs
don't know 88

A33. *(presjob)* *If employed,* what is your present occupation?
_____ | code _____

A34. *(mainjob)* In your working life what has been your main job?
_____ | code _____

A35. *(othjobs)* Could you list other jobs you have had? (≥5 yrs) what age?

_____ | code _____ _____
_____ | code _____ _____
_____ | code _____ _____
_____ | code _____ _____
_____ | code _____ _____

A36. *(marital)* What is your marital status? *(choose most appropriate option)*

never married .....1 divorced 4
now married .....2 widowed 5
separated but not divorced 3

A37. *(spoujob) If now married or widowed:*
What kind of work does/did your spouse do for most of his/her life?

_____ | code ____

A39. *(pension)* Do you receive a pension?

yes 1
no ..... 2 *goto A 39.*
don't know..... 8 *goto A 39.*

A39. *(sort)* What sort of a pension is it?

age pension ..... 1 blind pension 4
invalid pension .... 2 other 5
veteran's 3

A40. *(other$)* Are you receiving any other income or superannuation?

yes 1
no 2
refused 3

A41. *($detail)*

source 1_____ | code ____
source 2_____ | code ____

A42. *(abode)* What sort of a place do you live in?

own house ..... 1 boarding house 6
own flat/ILU..... 2 nursing home 7
rent house ..... 3 with relatives 8
rent flat ..... 4 caravan 9
hostel ..... 5 other _____ | code ____

A43. *(wholive1)* .....and who lives with you? friend 3

nobody ..... 1 daughter 4
spouse ..... 2 son 5

A44. *(wholive2)* other _____ | code ____

A45. Do you get regular help at home from the following?

(MOW) Meals on Wheels yes .....1 no ... 2
(nurse) community nurse yes .....1 no ... 2
(hmhelp) home help yes .....1 no ... 2

A46. *(othhelp)* other _____ | code ____

A47. *(cleanhs)* Who usually cleans your house?

you ..... 1 other relative 5
spouse ..... 2 friend 6
daughter ..... 3 home help 7
son ..... 4 other_____ | code ____

A48. *(whoshop)* Who usually does your shopping?

you ..... 1 other relative 5
spouse ..... 2 friend 6
daughter 3
son ..... 4 other_____ | code ____

	Are you able to go out alone?			How often do you go?
A49. ...to do the shopping *(goshop)*	yes ... 1	no 2	A50 *(noshop)*	_____
A51. ...to visit someone *(govisit)*	yes ... 1	no 2	A52 *(novisit)*	_____
A53. ...to go to 'town' *(gotown)*	yes ... 1	no 2	A54 *(notown)*	_____

Medications

Now I would like to ask you some questions about the tablets or vitamins you've taken in the last week. May I see the medications you are taking now?

Examine contents of plastic bag. List medications,write chemist's name from bottles

B1. *(Chemist)* Chemist's Name _____

	Name of drug	code	approx period
none ☐			
B2.*(drug1)*	1 _____	|	| _____
B3.*(drug2)*	2 _____	|	| _____
B4.*(drug3)*	3 _____	|	| _____
B5.*(drug4)*	4 _____	|	| _____
B6.*(drug5)*	5 _____	|	| _____
B7.*(drug6)*	6 _____	|	| _____
B8.*(drug7)*	7 _____	|	| _____
B9.*(drug8)*	8 _____	|	| _____
B10.*(drug9)*	9 _____	|	| _____
B11.*(drug10)*	10 _____	|	| _____
B12.*(drug11)*	11 _____	|	| _____

ASK "are there any other tablets, sprays or medications you take that you have not mentioned here?"

"Can you recall any other tablets you have taken for more than 3 months in the past?"

	Name of drug	code	approx period taken
none ☐			
B13.*(tab1)*	1 _____	|	| _____
B14.*(tab2)*	2 _____	|	| _____
B15.*(tab3)*	3 _____	|	| _____
B16.*(tab4)*	4 _____	|	| _____
B17-19*tab5-7)*	5 _____	|	| _____

B20. *(aspirin)* Over the past year, about how often have you taken an **aspirin** tablet? (Solprin, Disprin, Ecotrin or Cardiprin, but not Panadol, Dymadon or Digesic)

Was it: less than once a month ..... 1 *goto B22*
more than once a month but less than once a week ..... 2 *goto B22*
once a week or more 3

B21. *(aspnum)* How many aspirin tablets do you usually take each week? _____

B22. *(aspyrs)* For how many years have you been taking this number? _____ yrs

---

B23. *(asppast)* Is that: more than 1
the same ..... 2 or
less than..... 3
the number of aspirins you were taking 10 years ago ? don't know 8

B24. *(steroid)* Have you ever taken **steroid** tablets such as Prednisone, Cortisone, or Decadron for asthma, arthritis or another condition, for longer than one month altogether? (ring steroid taken)
yes 1
no 2
don't know 8

B25. *(steryrs)* *If yes*, about how long altogether were you taking these tablets?
_____weeks _____ months _____ years

B26. *(sterdos)* What dose were you taking? _____

B27. *(stertot)* (Try to estimate total dose_____)
(say 15mg/ day for 3 mos then 5 mg/ day for 3 months)

B28. *(sterdx)* What condition did you take them for?_____
(eg asthma, arthritis) I code _____

B29. *(Becot)* Have you ever used a **Becotide** puffer, a brown coloured puffer used in asthma and other chest problems?
yes 1
no 2
don't know 8

B30. *(Becdos)* *If yes*, how often do/did you need to take it? _____
(puffs/day or week or month etc)

B31. *(sedat)* Have you ever taken tablets for "nerves" or to help you sleep like tranquilizers, antidepressants, or antianxiety drugs ?
yes ..... 1 *add name here*
no ..... 2 _____ I code _____
don't know ..... 8 _____ I code _____

B32. *(calc)* Do you take or have you ever taken calcium tablets like Sandocal, Cal-Sup or Caltrate regularly? *(at least once a week for more than a month)?*
yes..... 1 *add name here*
no ..... 2 _____ I code _____
don't know 8

Female Medical History *females only, if male, goto page 7*

Now I would like to ask you a few questions about your menstrual history

W1. *(menarch)* How old were you when you started having periods? ___ yrs DK 88

W2. *(ifmenop)* Have you stopped having periods?
yes .....1 no ... 2 *goto W7*
don't know ..... 8 *goto W5*

W3. *(menopau)* How old were you when you stopped? _____ years DK.....88

W4. *(menowhy)* Did you stop naturally or because of a hysterectomy?
naturally ..... 1 hysterectomy ..... 2 *goto W6*
other ..... 3 don't know 8

W5. *(hystrec)* Have you had a hysterectomy, that is, an operation to remove the uterus?
yes ..... 1; what age?_____ yrs
no ..... 2 don't know..8 *goto W7*

W6. *(ovarect)* Were both ovaries removed? yes..... 1; same age?____yrs
no ..... 2 don't know..88

W7. *(hormTx)* Have you ever been on hormone replacement therapy, such as oestrogens and/or progesterones for menopausal symptoms or after the menopause?
yes..... 1 don't know ..... 8 *add name here*
no ..... 2 _____|code_____

W8. *(OCP)* Have you ever taken oral contraceptive pills for birth control or other medical reasons?
yes..... 1 don't know ..... 8 *add name here*
no ..... 2 _____|code_____

W9. *(pregnan)* Have you ever been pregnant?
yes ..... 1 E48*(pregnum)* _____ times
no ..... 2 *goto W11.*
don't know..... 8 *goto W11..*

W10.*(parity)* Of these pregnancies, how many children have you had? ____children

---

W11. *(Papkno)* Have you ever heard of a **Pap Smear Test?**
yes 1
no ..... 2 don't know.....8 *goto W14.*

A Pap Smear Test, sometimes called a Pap Test, is a routine test carried out by a doctor. It is recommended for all women to detect cancer of the cervix.

W12. *(Paphad)* Have you ever had a Pap Smear Test?
yes 1
no ..... 2 don't know.....8 *goto W14.*

W13. *(PapWhen)* When did you have your last Pap Smear Test?
less than one year ago ..... 1 5 or more years ago 4
1 year to less than 3 years ago .... 2 don't know 8
3 years to less than 5 years ago ... 3

W14. *(MamKno)* Have you ever heard of a **mammogram?**
yes 1
no ..... 2 don't know.....8 *goto C1.*

A mammogram is an x-ray taken of the breasts by a machine that presses against the breast while the picture is taken.

W15. *(Mamhad)* Have you ever had a mammogram?
yes 1
no ..... 2 don't know.....8 *goto C1.*

W16. *(Mamwhen)* When did you have your last mammogram?
less than one year ago ..... 1 5 or more years ago 4
1 year to less than 3 years ago .... 2 don't know 8
3 years to less than 5 years ago ... 3

For Interviewer	DID PARTICIPANT:	yes	no	DK
D1.*(hearimp)*	Have a hearing impairment?	☐	☐	☐
D2.*(walkdif)*	Have walking difficulties?	☐	☐	☐
D3.*(cane)*	Use a cane/crutches/walker?	☐	☐	☐
D4.*(wheelch)*	Use a wheelchair?	☐	☐	☐
D5.*(cough)*	Have shortness of breath,cough continually?	☐	☐	☐
D6. *(lanprob)*	Have a language problem?	☐	☐	☐
D7.*(speechp)*	Have a speech but not a language problem?	☐	☐	☐
D8.*(dement)*	Appear demented?	☐	☐	☐

Who mainly answered the questionnaire?

D9.*(answer)*

Participant 1
Spouse 2
Son, Daughter..... 3
Sibling 4
Other relative..... 5
Friend 6
Other (specify)_____ |code___

Attach any lists of medications or medical history

Glasses Can I now please check your glasses?

C1.*(typegls)* Current glasses:

unifocal	1
bifocal	2
multifocal	3
separate pairs	4
no glasses	5
glasses not brought	6

Lens Analyzer 1) current glasses: *2) separate readers, ifworn:*

C2.*(RglDS)*
C3.*(RglDC)*

C4.*(Rglax)*

C5.*(LglDS)*
C6.*(LglDC)*
C7.*(Lglax)*

C8.*(RglDS2)*
C9.*(RglDC2)*

C10.*(Rglax2)*

C11.*(LglDS2)*
C12.*(LglDC2)*
C13.*(Lglax2)*

Humphrey autorefractor: attach printout here:

C14.*(RARac1)* C15.*(RARODS)*
C16.*(RARODC)* C17.*(RAROax)*
C18.*(RARac2)* C19.*(RARSDS)*
C20.*(RARSDC)* C21.*(RARSax)*
C22.*(LARac1)* C23. *(LARODS)*
C24.*(LARODC)* C25.*(LAROax)*
C26. *(LARac2)* C27.*(LARSDS)*
C28. *(LARSDC)* C29.*(LARSax)*

PHASE 2: *for completion by Examiner 2.*

Visual Symptoms I am going to ask you some questions about your eyes and then test your vision.

E1.*(distgls)* Do you wear glasses to see clearly in the distance, or have you in the past, (that includes bifocals or multifocals)?
yes 1
no 2
don't know.. 8

E2.*(agegls)* How old were you when you first needed to wear glasses to see clearly in the distance?
_____ years old
don't know 88

E3.*(readgls)* Do you wear reading glasses or bifocals?
yes 1
no ..... 2 *goto E5.*
don't know.. 8

E4.*(presby)* How old were you when you first needed reading glasses, bifocals or multifocals?
_____ years old
don't know 88

E5.*(timegls)* How long have you had your current glasses? _____ years
don't know 88

Reading Vision
I am now going to test how well you can read with each eye using your current reading glasses.

E6,7,8.*(rdtype)* Smallest Logmar type read **R** _____ **L** _____ **both eyes** _____

---

		able	not able
E9.*(rdnewsp)*	Can you read this newspaper paragraph?	1	2
E10.*(rdbill)*	Can you read the amount owing on this electricity bill:	1	2
E11.*(rdprice)*	Can you read the price on this can?	1	2
E12. *rdpills)*	Can you read the directions on this bottle of pills ?	1	2

E13. *(qcat)* Have you ever heard of cataracts? Yes.... 1 No... 2
E14. *(dcat)* What do you think a cataract is?

E15. *(acat)* Answer: _____
_____ code _____

E16. *(qAMD)* Have you ever heard of macular degeneration? Yes.... 1 No.... 2
E17. *(dAMD)* What do you think macular degeneration is?

E18. *(aAMD)* Answer: _____
_____ code _____

E19. *(qglauc)* Have you ever heard of glaucoma? Yes.... 1 No... 2
E20. *(dglauc)* What do you think glaucoma is?

E21. *(aglauc)* Answer: _____
_____ code _____

I am now going to test your vision with your glassses, if you wear them.

Vision Examination: Logmar visual acuity score or E - equivalent

measure at 2.4 metres (8 ft) with best distance correction; if unable to see any letters, then try at one metre

What distance was chart read? R 2.4m ☐ 1m ☐ E22. *(Rrddist)* L 2.4m ☐ 1m ☐ E23. *(Lrddist)*

CURRENT DISTANCE GLASSES yes ..... 1 no 2

at 2.4 m	Right eye					no. correct	Left eye					no. correct	
6/60	H	V	Z	D	S	____	H	V	Z	D	S	____	5
6/48	N	C	V	K	D	____	N	C	V	K	D	____	10
6/38	C	Z	S	H	N	____	C	Z	S	H	N	____	15
6/30	O	N	V	S	R	____	O	N	V	S	R	____	20
6/24	K	D	N	R	O	____	K	D	N	R	O	____	25
6/19	Z	K	C	S	V	____	Z	K	C	S	V	____	30
6/15	D	V	O	H	C	____	D	V	O	H	C	____	35
6/12	O	H	V	C	K	____	O	H	V	C	K	____	40
6/9.5	H	Z	C	K	O	____	H	Z	C	K	O	____	45
6/7.5	N	C	K	H	D	____	N	C	K	H	D	____	50
6/6	Z	H	C	S	R	____	Z	H	C	S	R	____	55
6/4.8	S	Z	R	D	N	____	S	Z	R	D	N	____	60
6/3.8	H	C	D	R	O	____	H	C	D	R	O	____	65
6/3.0	R	D	O	S	N	____	R	D	O	S	N	____	70

E24.*(RmarVA)* Logmar VA R ____ E25.*(LmarVA)* Logmar VA L ____

E26. *(RPH)* Pinhole R ____ E27. *(LPH)* Pinhole L ____

If **vision < 6/60** E28.*(RpoorVA)* R _____ E29. *(LpoorVA)* L _____

Right CF.....1 HM....2 PL.....3 NPL...4 *Left* CF..... 1 HM....2 PL.....3 NPL...4

Logmar VA modified Sheridan-Gardiner

E30.*(RSheGar)* R _____ E31.*(LSheGar)* L _____

E32. *(amblyop)* *If one eye weaker (2 line difference) ask:*

Has your Right/Left eye always been weaker?

Right eye -yes 1
Left eye -yes 2
no 3
don't know 8

E33. *(visdis)* *If visual disability, eg field defect or severe visual loss (< 6/60) in both eyes, ask:*

Have you sought help from ;

Low vision clinic 1
Royal Blind Society..... 2
Guide Dogs 3
Other _____ code _____

E34.*(demenVA)* *Did mental disability or dementia prevent measurement of VA?*

yes 1
no 2
don't know 8

at **VISUAL ACUITY WITH BEST SUBJECTIVE REFRACTION**

2.4 m	Right eye					no. correct	Left eye					no. correct	
6/60	H	V	Z	D	S	____	H	V	Z	D	S	____	5
6/48	N	C	V	K	D	____	N	C	V	K	D	____	10
6/38	C	Z	S	H	N	____	C	Z	S	H	N	____	15
6/30	O	N	V	S	R	____	O	N	V	S	R	____	20
6/24	K	D	N	R	O	____	K	D	N	R	O	____	25
6/19	Z	K	C	S	V	____	Z	K	C	S	V	____	30
6/15	D	V	O	H	C	____	D	V	O	H	C	____	35
6/12	O	H	V	C	K	____	O	H	V	C	K	____	40
6/9.5	H	Z	C	K	O	____	H	Z	C	K	O	____	45
6/7.5	N	C	K	H	D	____	N	C	K	H	D	____	50
6/6	Z	H	C	S	R	____	Z	H	C	S	R	____	55
6/4.8	S	Z	R	D	N	____	S	Z	R	D	N	____	60
6/3.8	H	C	D	R	O	____	H	C	D	R	O	____	65
6/3.0	R	D	O	S	N	____	R	D	O	S	N	____	70

E35. *{RsubjVA}* Logmar VA **Right** ____ E36.*{LsubjVA}* Logmar VA **Left** ____

Best subjective refraction RIGHT **LEFT**

E37. *{RSrefDS}* sph _____ E41. *{LSrefDS}* sph _____

E38. *{RSrefDC}* cyl _____ E42. *{LSrefDC}* cyl _____

E39. *{RSrefax}* axis _____ E43. *{LSrefax}* axis _____

E40. *{RSadd}* reading add_____ E44.*{LSadd}* reading add: _____

E45,6,7.*{newtype}* Logmar reading chart **R** _____ **L** _____both eyes _____

Contrast Sensitivity Testing (Vectorvision CSV-1000 chart) / Glare Testing (BAT)

subject wearing best distance correction

right eye

T1.*{RCSrowA}*	Row A (3cpd)	1 t	2 b	3 t	4 t	5 b	6 b	7 b	8 t
T2.*{RCSrowB}*	Row B (6cpd)	1 b	2 b	3 t	4 b	5 t	6 t	7 b	8 b
T3.*{RCSrowC}*	Row C (12cpd)	1 b	2 t	3 b	4 t	5 b	6 b	7 t	8 t
T4.*{RCSrowD}*	Row D (18cpd)	1 t	2 t	3 b	4 t	5 b	6 t	7 b	8 t

left eye

T5.*{LCSrowA}*	Row A (3cpd)	1 t	2 b	3 t	4 t	5 b	6 b	7 b	8 t
T6.*{LCSrowB}*	Row B (6cpd)	1 b	2 b	3 t	4 b	5 t	6 t	7 b	8 b
T7.*{LCSrowC}*	Row C (12cpd)	1 b	2 t	3 b	4 t	5 b	6 b	7 t	8 t
T8.*{LCSrowD}*	Row D (18cpd)	1 t	2 t	3 b	4 t	5 b	6 t	7 b	8 t

right eye with BAT (setting No. 2)

T10.*{RBatB}*	Row B (6cpd)	1 b	2 b	3 t	4 b	5 t	6 t	7 b	8 b
T11.*{RBatC}*	Row C (12cpd)	1 b	2 t	3 b	4 t	5 b	6 b	7 t	8 t

left eye with BAT (setting No. 2)

T14.*{LBatB}*	Row B (6cpd)	1 b	2 b	3 t	4 b	5 t	6 t	7 b	8 b
T15.*{LBatC}*	Row C (12cpd)	1 b	2 t	3 b	4 t	5 b	6 b	7 t	8 t

PHASE 3: *for completion by Examiner 3.*

F1. *(height)* **Height** _____ metres
reliable .....1 not done 2
unreliable.... 3 other _____(eg amputee)

F2. *(weight)* **Weight** _____ kilograms
reliable .....1 not done 2
unreliable.... 3 other _____(eg amputee)

F3. *(wtmost)* What is the most you have ever weighed ? (not counting pregnancy)
_____ stones _____ kgs don't know..... 888

Strabismus

G1.*(strab)* Have you ever been told you had a turned or lazy eye, or had treatment for any eye muscle problems?
yes.....1
no .....2 don't know.....8

G2.*(strabage)* *If yes,* how old were you when this was first noticed?_____yrs

G3.*(patch)* Did you wear a patch on one eye?
yes.....1
no .....2 don't know.....8 *goto F39.*

G4.*(agepcth)* *If yes,* From what age_____yrs G5.*(lngptch)* For how long_____

G6. *(coverT)* Perform Cover Test 6m_____

G7. *(otherT)* Other _____

Exercise

H1.*(walkex)* In the past 2 weeks, did you walk for recreation or exercise?
yes.....1 H2.*(walkhrs)* How many times? _____
no .....2 don't know.....8

H3.*(vigact)* In the past 2 weeks, did you engage in vigorous activity or exercise, which made you breathe harder or puff and pant? (eg, carrying loads, heavy gardening, chopping wood, labouring - at home, during work or anywhere else)
yes.....1
no .....2 don't know.....8

H4.*(actimes)* How many sessions of vigorous activity did you have over the 2 week period?_____

H5.*(acthrs)* How much time was spent in vigorous activity over the past 2 wks?
_____ hrs _____ mins

Cigarette smoking

H6. *(smoking)* Have you ever smoked cigarettes, cigars or a pipe regularly?
yes..... 1
no ..... 2 *goto H12.*
don't know..... 8 *goto H12.*

H7. *(smokyrs)* At what age did you start smoking regularly? _____ yrs

H8. *(smokcurr)* Have you given up smoking?
yes 1
no ..... 2 *goto G6.*

H9. *(smokcurr)* When did you last give up smoking? _____ years ago

H10. *(pastcig)* How much did you usually smoke just before you stopped?

_____ manufactured cigs/day
_____ grams "hand-rolled"
_____ cigars/week
_____ grams pipe tobacco/wk

H11. *(smoknow)* *If currently smoking:* How much do you usually smoke?

_____ manufactured cigs/day
_____ grams "hand-rolled"
_____ cigars/week
_____ grams pipe tobacco/wk

H12. *(cigspou)* Does or did your husband/ wife smoke cigarettes?

yes.....1
no .....2 don't know.....8

Caffeine intake Do you drink tea?

H13. *(tea)*
yes .....1 H14.*(Tcups)* Cups per day_____
no 2
don't know 8

H15. *(Ttype)* How do you usually make your tea?
Teabags 1
Tealeaves..... 2
Both..... 3

H16. *(coffee)* Do you drink coffee?
yes .....1 H17.*(Cofups)* Cups per day_____
no ..... 2 (exclude decaffeinated)
don't know 8

H18.*(coftype)* How do you usually make your coffee?
Instant 1
Percolated 2
Both..... 3

H19,20. *(pastcaf)* About how many cups of coffee or tea did you drink per day 10 years ago?

coffee	tea
number of cups _____	number of cups _____

Alcohol I am now going to ask you some questions to see whether alcohol is related to eye problems or whether it has any benefits for the eyes.

H21. *(alcohol)* How many days a week would you usually have an alcoholic drink now?
never.....1 *goto H24.*
<once a week 2
1-2 days a week.....3
3-4 days a week.....4
5-6 days a week.....5
every day.....6

H22. *(alctype)* What do you mostly drink?
light beer 1
beer 2
wine 3
spirit 4
other _____ code_____

H23. *(alcnum)* On the days when you have a drink, how many drinks do you usually have ?
13 or more drinks 1
9-12 drinks 2
5-8 drinks 3
3-4 drinks 4
1-2 drinks 5

H24. *(alcmore)* Has there been a period in your life when you drank quite a bit more than you do now?
yes 1
no 2
don't know..8

H25. *(alcstop)* *If yes,* how many years ago was that? _____ yrs

---

Driving and Vision: Have you ever driven a car regularly?

V1. *(drive)*
yes, still driving ... 1 *goto V3.*
yes, stopped 2
never driven..... 3 *goto V9.*

V2. *(carlast)* *If no,* when did you last drive? _____

V3. *(carvis)* Did you stop because of problems with your vision?
yes 1
no..... 2
don't know..... 8

V4. *(acciden)* Have you had any car accidents in the last year?
yes ..... 1 V4A. *(accidVA)*
no..... 2 visual contribution yes..... 1
don't know..... 8 no..... 2

V5. *(accidet)* *If yes,* number of accidents _____ don't know..8

V6. *(carabil)* Do you think your driving abilility is as good now as it used to be?
yes 1
no..... 2 don't know..... 8

V7. *(abildet)* *If no,* do you think this might be related to your vision?
yes 1
no..... 2 why _____
don't know..... 8

V8. *(carnite)* Any problems driving at night?
yes 1
no..... 2 don't know..... 8

V9. *(seenite)* Do you think you have more difficulty seeing in the dark than others of your age?
yes 1
no..... 2 don't know..... 8

V10. *(glare)* Do you think you are more sensitive than others of your age to sunlight or glare?
yes 1
no..... 2 *goto V12.*
don't know..... 8 *goto V12.*

V11. *(glareyr)* For how long has this been a problem? _____

Are you able to:

V12. *(seefar)* recognise a friend across the street? yes 1
no.....2
don't know..8

V13. *(seenear)* recognise a friend close to you? yes 1
no.....2
don't know..8

V14. *(seeTV)* recognise detail on TV? yes 1
no.....2
don't know..8

V15. *(rdnewsp)* Can you read the ordinary print in the newspaper reasonably well, that is comfortably, with or without reading glasses?
yes ..... 1 *(if yes go to V18)*
no..... 2
don't know..... 8

V16. *(lastrd)* *If no,* when were you last able to do this? _____

V17. *(magnif)* Do you need to use a magnifier to read (eg. paper)?
yes 1
no..... 2
don't know..... 8

V18.*(Vworse)* Are you aware of a deterioration of vision in one or both eyes?
yes, R eye 1
yes, L eye 2
yes, both eyes 3
no 4
don't know 8

V19.*(Rworyes)* When did your **right eye** worsen?

_____ yrs ___ mths ____wks

V20.*(Lworyes)* When did your **left eye** worsen?

___ yrs ___ mths ____wks

Did you notice any:-		how long?		how long?
V21.*(Rdistor)* distortion of straight lines?	___	_____	V26.*(Ldistor)* ___	_____
V22.*(Rpatch)* a dark patch?	___	_____	V27.*(Lpatch)* ___	_____
V23.*(Rblur)* or just blurring of vision?	___	_____	V28.*(Lblur)* ___	_____
V24.*(Rchange)* any other changes?	___	_____	V29.*(Lchange)* ___	_____

V25. *(Rdetail)* R details_____

V30. *(Ldetail)* L details_____

Hearing Have you ever had a problem with your **hearing**?

H26. *(hearing)* yes 1
no..... 2 *goto H29.*
don't know..... 8 *goto H29.*

H27.*(hearsev)* Is it? mild..... 1
moderate..... 2
severe..... 3

H28. *(hearear)* Which ear? left..... 1
right..... 2
both ears.... 3

H28A. *(hearaid)* Do you wear a hearing aid? yes 1
no ... 2
sometimes ... 3

H29. *(hearcon)* Do you have trouble in hearing normal conversation with several other people? yes 1
no 2
don't know..... 8

H30. *(heartin)* Do you have any annoying noises (ringing, buzzing, hissing) in your ears?
yes 1
no 2
don't know..... 8

Blood pressure

I am now going to check your blood pressure. *(put cuff on right arm)*

F4. *(cuff)* **Cuff size:** small .....1 adult .....2 large 3

Blood pressure (Right arm)

F5.*(systBP)* SystolicBP _____ mmHg F6.*(diastBP)* Diastolic BP _____ mmHg

---

Humphrey Visual field testing

		Right F7. *(RHumph)*	Left F8. *(LHumph)*
76-point threshold-related suprathreshold program		**H**	**H**
or	Goldmann kinetic/ static perimetry	**G**	**G**
or	Bjerrum 1 metre tangent screen	**B**	**B**

Proceed to 30-2 program if specified field defects either eye, on second day.

F9. *(Rlens)* correction used R _____ F10. *(Llens)* L _____

F11. *(Rfield)* test done yes .....1 no .....2 F12. *(Lfield)* test done yes .....1 no 2

If no,
F13. *(RVFwhy)* reason _____ *If no,* F14. *(LVFwhy)* reason _____

Slit lamp examination

Iris Colour

F15. *(Riris)* **Right eye**	F16. *(Liris)* **Left eye**
< std #1 (blue) 1	<std #1 (blue) 1
< std #2 (hazel/green)..... 2	< std #2 (hazel/green) 2
< std #3 (tan/brown)..... 3	< std #3 (tan/brown) 3
> std #3 (dark brown) 4	> std #3 (dark brown)..... 4
cannot judge/ not done 5	cannot judge/ not done 5

F17. *(occlude)* **Is angle occludable?** yes ..... 1 questionable 3
no ..... 2 can't judge 4

Intraocular pressure

F18. *(RIOP)*	R _____ mmHg	F20. *(LIOP)*	L _____ mmHg
F19. *(RIOPrel)*	unreliable 1	F21. *(LIOPrel)*	unreliable 1
	unobtainable 2		unobtainable 2

Instill dilating drops: Tropicamide 1% and Phenylephrine 10%, twice.

(End of Phase 3)

PHASE 4: *for completion by Examiner 4.*

Past Medical History

I would like to ask some questions about your general health; to find whether this is related to eye diseases.

M1.*(health)* For someone of your age, how would you rate your overall health?

excellent ..... 1 poor 4
good ..... 2 don't know..... 8
fair 3

M2.*(hosadm)* Have you had any admissions to a hospital in the last 12 months?

_____times don't know..... 88

If admitted,

M3.*(hosdis)* What for? M4.*(hosnam)* Which hospital?

1 _____
2 _____
3 _____

Has a doctor ever said that you have any of the following conditions ?

		yes	no	DK	age/yrs 1st told	Rx yrs
M5,6,6A*(hypert)*	**high blood pressure**	☐	☐	☐	_____ yrs	____
M7,8.*(angina)*	**angina**..... (chest pain form your heart)	☐	☐	☐	_____ yrs	
M9,10.*(AMI)*	**heart attack** ..... (a coronary, myocardial infarct)	☐	☐	☐	_____ yrs	
M11,12.*(stroke)*	**stroke**	☐	☐	☐	_____ yrs	
M13,14.*(cholest)*	**high cholesterol**	☐	☐	☐	_____ yrs	
M15,16.*(diabet)*	**diabetes,**	☐	☐	☐	_____ yrs	
M17.*(diadiet)*	sugar in your urine	Treated with:			diet alone?_____	yrs
M18.*(diatab)*	or high blood sugar				tablets?_____	yrs
M19.*(diains)*					insulin?_____	yrs
M20,21.*(cancer)*	**cancer**	☐	☐	☐	_____ yrs	
M22.*(CAtype)*	What type of cancer? _____					
M23.*(CAtreat)*	Treated with: surgery ..... 1 radiation ..... 2 chemotherapy 3				no treatment ..... 4 don't know 5	
M24,25.*(skinCA)*	**sunspots or skin cancer,** ... treated by freezing or surgery	☐	☐	☐	_____ yrs	
M26,27.*(Thyroid)*	**thyroid condition**	☐	☐	☐	_____ yrs	
M28. *(ThyrRx)*	Treated with: radioactive iodine..1 surgery ..... 2 thyroxine tablets ... 3 _____ code:_____					
M28a.*(Asthma)*	**asthma**	☐	☐	☐	_____ yrs	
M29,30.*(Arthrit)*	**arthritis**	☐	☐	☐	_____ yrs	
M31. *(ArthTyp)*	What type: osteoarthritis ..... 1 rheumatoid ..... 2 other ..... 3 _____ code:_____					
M31a *(Gout)*	**gout**	☐	☐	☐	_____ yrs	

M32.*(arthtab)* Have you ever taken tablets for gout or arthritis such as Zyloprim, Indocid, Voltaren, Feldene, Naprosyn, Panamax or Dymadon?

yes 1
no 2
don't know 8

add name here

_____ | code ____
_____ | code ____
_____ | code ____

M33.*(clorquin)* Have you ever taken Chloroquine or Plaquenil for arthritis or malaria?

yes 1
no 2
don't know 8

add name here

_____ | code ____
_____ | code ____

M34.*(migrain)* Have you ever had migraine headaches ?

(severe headaches, usually on one side, may have changes in vision like zig-zag lines or blurring, often nausea or vomiting and you usually need to lie down with the lights off)

yes, typical 1
yes, but atypical ... 9
no 2
don't know 8

If yes,

M34a *(mig1st)*
age started _____ yrs
M34b *(migstop)*
age stopped_____ yrs

M35.*(falls)* During the past 12 months, have you had any falls where you have landed on the ground or floor? *number of falls* _____

If fallen,

M36.*(fallvis)* How many of these do you think were due to problems with your vision?
number of falls _____

M37. *(TetYrs)* Can you recall when you had your last tetanus shot? _____ yrs ago
M38. *(TetNo)* don't know.....98 never had one..... 99

Have you had any other serious or major illnesses or operations?

M39.*(illnes1)* _____ | code ____

M40.*(illnes2)* _____ | code ____

M41.*(illnes3)* _____ | code ____

M42.*(illnes4)* _____ | code ____

M43. *(CAT)* Have you ever had a CAT scan of the head or brain?
M44. *(CATYr)*

yes 1
no..... 2
don't know..... 8

years _____

M45. *(shock)* Have you ever been in hospital with a sharp drop in your blood pressure or severe loss of blood (shock)?

yes..... 1
no 2
possible..... 3
don't know.. 8

M46.*(shokdet)* details _____

Family History We want to know if eye disease runs in families.

Have your parents or your brothers or sisters or children had:

(tick)	glauc?	cataract?	macular degen?	blind?	other eg turned eye?
I1. *(FHxmo)* mother have	____	____	____	____	_____
I2. *(FHxfa)* father have	____	____	____	____	_____
I3. *(FHxbro)* brothers /of no.	____	____	____	____	_____
I4. *(FHxsist)* sisters / of no.	____	____	____	____	_____
I5. *(FHxchil)* natural children?	____	____	____	____	_____

I6. *(alivefa)* Is your father still alive? yes ..... 1 no ..... 2 don't know..... 8

I7. *(fathage)* approx age at death _____years (if young, why?) _____

I8. *(alivemo)* Is your mother still alive? yes ..... 1 no ..... 2 don't know..... 8

I9. *(mothage)* approx age at death _____years (if young, why?) _____

I10. *(broth)* How many full brothers have you had, that is alive or dead? ___

I11. *(sist)* How many full sisters have you had, that is alive or dead? ___

I12. *(FHxdiab)* Do you have a family history of diabetes?

none	1	children	5
mother	2	maternal aunts,uncles	6
father	3	maternal grandparents	7
brothers,sisters	4	pat aunts,uncles	8
		paternal grandparents	9

Sunlight Exposure I would now like to ask you some questions about your exposure to sunlight

J1. *(colour)* As a teenager,what was the natural colour of your hair? Was it:

blonde	1	black	4
red	2	other	5
brown	3	don't know	8

J2. *(burn)* In the past, when your skin was exposed to the summer sun, did it?

- always burn, never tan 1
- usually burn, tan with difficulty..... 2
- burn and tan about average 3
- rarely burn, tan above average 4
- don't know 8

J3. *(burn-no)* How many bad sunburns, that is with soreness lasting more than a day, would you estimate you have had during your life, including childhood?

none	1	>10	4
one	2	don't know	8
2-10	3		

J4.*(skincol)* *Non-exposed skin colour:* *examiner to estimate*

very fair	1	dark olive	4
fair	2	brown	5
light olive	3	black	6

J5.*(skindam)* *Degree of sun-induced skin damage:* *examiner to estimate*

none	1	moderate	3
mild	2	severe	4

Eye medications Now a few questions about your eyes.

K1. Are you using any eyedrops at present? yes ...1 no ... 2 don't know ... 8

list: how long have you been taking these?

K2. *(drop1)* 1. _____ | code _____ _____

K3. *(drop2)* 2. _____ | code _____ _____

K4. *(drop3)* 3. _____ | code _____ _____

K5. *(drop4)* 4. _____ | code _____ _____

K6. *(stedrop)* Any steroid eye drops? yes ... 1 no ... 2 don't know ... 8

add here _____ | code _____

Eye Diseases Now about some eye conditions

K7. *(catarac)* Have you ever been told you have cataracts?

yes 1

no ..... 2 don't know ..... 8 *goto K15.*

K8. *(catadet)* details _____

K9. *(cataage)* How many years ago were you first told? ____yrs _____age

K10. *(catarop)* *If yes,* have you ever had an operation for cataract?

yes 1

no ..... 2 don't know ..... 8 *goto K15.*

K11. *(catdet)* details _____

K12. *(catawho)* Who performed it? _____

K13. *(cataYAG)* Have you had YAG laser to improve your vision after cataract surgery?

yes 1

no ..... 2 don't know 8

K14. *(catar-YAG)* details _____

K15. *(AMD)* Have you ever been told you have macular degeneration, sometimes called 'hardening of the arteries at the back of the eye', or 'degeneration of the retina'?

yes 1

no ..... 2 don't know ..... 8 *goto K20.*

K16. *(AMDdet)* details _____

K17. *(AMDage)* How many years ago were you first told? ____yrs _____age

K18. *(AMDlase)* *If yes,* have you had laser treatment for macular degeneration?

yes 1

no ..... 2 don't know ..... 8 *goto K20.*

K19. *(lasedet)* details _____

K20. *(glaucom)* Have you ever beeen told that you have glaucoma or 'raised pressure in the eyes'?

yes 1

no ..... 2 don't know ..... 8 *goto K26.*

K21. *(glaudet)* *If yes,* details _____

K22. *(glauage)* How many years ago were you first told? ____yrs _____age

K23. *(glaucRx)* Have you ever used eyedrops or medications for glaucoma?

yes 1

no ..... 2 don't know ..... 8 *goto K26.*

K24. *(glaucop)* Have you had an operation or laser treatment for glaucoma?

yes 1

no ..... 2 don't know ..... 8 *goto K26.*

K25. *(opdet)* details _____

K26. *(retina)* Have you ever been told you have a problem in the retina or the 'back of the eye'? like retinal detachment or vessel blockage or bleeding ?

yes 1
no ..... 2 don't know ..... 8 *goto K29.*

K27. *(retidet)* details _____

K28. *(retinyr)* How many years ago were you first told? ____yrs _____age

---

K29. *(injury)* Have you ever had any serious eye injury requiring doctor's care?

yes 1
no ..... 2 don't know ..... 8 *goto K31.*

K30. *(inj-det)* details_____

Any other eye problems or eye surgery that I haven't asked you about?

K31. *(eyedet1)*_____ code_____

K32. *(eyedet2)*_____ code_____

Eye Examination: Slit lamp examination

	R eye	L eye
Corneal Arcus		
	L1.*(Rarcus)*	L2. *(Larcus)*
	none....1 quest ....2 ≤180°...3 >180° 4	none....1 quest ....2 ≤180°...3 >180° 4
Pingueculum		
	L3.*(Rpingue)*	L4. *(Lpingue)*
	absent...1 quest .....2 present....3	absent...1 quest .....2 present....3
Pterygium		
	L5. *(Rpteryg)*	L6. *(Lpteryg)*
	absent...1 quest .....2 present....3 pres, axis involved 4	absent...1 quest .....2 present....3 pres, axis involved 4
Pseudoexfoliation		
	L7. *(Rexfol)*	L9. *(Lexfol)*
	absent...1 quest .....2 present....3	absent...1 quest .....2 present....3
	L8. *(Rdegree)* R degree_____	L10. *(Ldegree)* L degree_____
Corneal opacities		
	L11. *(Ropac)*	L12. *(Lopac)*
	absent...1 quest .....2 present....3 pres, axis involved 4	absent...1 quest .....2 present....3 pres, axis involved 4

Other slit lamp abnormalities

L13*(Rabn1)*_____ L14*(Labn1)*_____

L15*(Rabn2)*_____ L16*(Labn2)*_____

L17*(Rabn3)*_____ L18*(Labn3)*_____

L19*(Rabn4)*_____ L20*(Labn4)*_____

L21*(Rabn5)*_____ L22*(Labn5)*_____

		right	*left*
L23,24*(Topcon)*	*Topcon Nuclear Lens PhotographTaken*	_____	_____
L25,26*(Neitz)*	*Neitz Cortical/PSC Lens Photograph Taken*	_____	_____

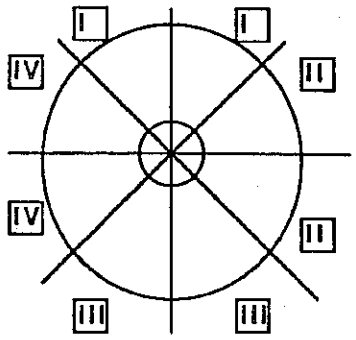
N1. *(Rphakia)* **Right Lens presence:**
phakic..... 1
aphakic, no lens ... 2
aphakic, AC IOL 3
aphakic, PC IOL 4
enucleated 5

Nuclear cataract:

N2. *(Rnuccat)* WISC	N46.*(RnucJH)* JH
≤std#1 1	 1
≤std#2 2	 2
≤std#3 3	 3
≤std#4 4	 4
>std#4 5	 5
mature..... 6	 6
can't grade... 7	 7

Cortical Cataract
N47.*(RcorJH)*
JH
none.....1
≤45°..... 2
≤90°..... 3
≤180°.....4
cortical dots. 6
can't grade....7

WISC



N23*(Rantopa)* are white anterior cortical opacities present?
absent...1 present...2 quest...3

Cortical and posterior subcapsular cataract:
record % of each lens quadrant involved for each type of opacity.

	PSC	Cortical		Vacuoles anterior	Vacuoles posterior
Quadrant I	N3_____	N4_____	N5	_____	N6 _____
Quadrant II	N7_____	N8_____	N9	_____	N10_____
Quadrant III	N11_____	N12_____	N13	_____	N14_____
Quadrant IV	N15_____	N16_____	N17	_____	N18_____
central circle	N19_____	N20_____	N21	_____	N22_____

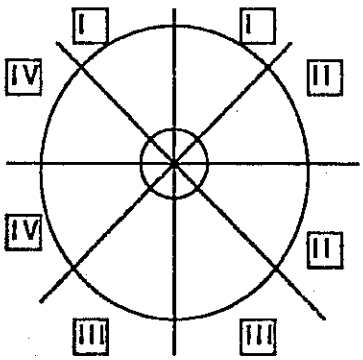
N24.*(Lphakia)* **Left Lens presence:**
phakic..... 1
aphakic, no lens ... 2
aphakic, AC IOL 3
aphakic, PC IOL 4
enucleated 5

Nuclear cataract:

N25.*(Lnuccat)* WISC	N48.*(LnucJH)* JH
≤std#1 1	 1
≤std#2 2	 2
≤std#3 3	 3
≤std#4 4	 4
>std#4 5	 5
mature..... 6	 6
can't grade...7	 7

Cortical Cataract
N49.*(LcorJH)*
JH
none.....1
≤45°..... 2
≤90°..... 3
≤180°.....4
cortical dots. 6
can't grade....7

WISC



N46.*(Lantopa)* are white anterior cortical opacities present?
absent...1 present...2 quest...3

Cortical and posterior subcapsular cataract:
record % of each lens quadrant involved for each type of opacity.

	PSC	Cortical		Vacuoles anterior	Vacuoles posterior
Quadrant I	N26_____	N27_____	N28	_____	N29 _____
Quadrant II	N30_____	N31_____	N32	_____	N33_____
Quadrant III	N34 _____	N35_____	N36	_____	N37 _____
Quadrant IV	N38_____	N39_____	N40	_____	N41 _____
central circle	N42 _____	N43_____	N44	_____	N45 _____

Fundus Examination right eye **left eye**

O1.*(RhardD)* **Hard macular drusen**
absent...1 quest .....2 present....3

O2.*(LhardD)* **Hard macular drusen**
absent...1 quest .....2 present....3

O3.*(RsoftD)* **Soft macular drusen**
absent...1 quest .....2 present....3

O4.*(LsoftD)* **Soft macular drusen**
absent...1 quest .....2 present....3

O5.*(Rpig)* **Visible Pigment**
absent...1 quest .....2 present....3

O6. *(Lpig)* **Visible Pigment**
absent...1 quest .....2 present....3

O7.*(Ratrophy)* **Geographic atrophy**
absent...1 quest .....2 present....3

O8.*(Latroph)* **Geographic atrophy**
absent...1 quest .....2 present....3

O9.*(Rdiscif)* **Disciform degeneration**
absent...1 quest .....2 present....3

O10.*(Ldiscif)* **Disciform degeneration**
absent...1 quest .....2 present....3

O11.*(Rretinop)* **Diabetic retinopathy**
absent...1 quest .....2 present....3

O12.*(Lretinop)* **Diabetic retinopathy**
absent...1 quest .....2 present....3

other retinal abnormalities
O13*(Rabnorm1)*_____
O15*(Rabnorm2)*_____
O17*(Rabnorm3)*_____
O19*(Rabnorm4)*_____

other retinal abnormalities
O14*(Labnorm1)*_____
O16*(Labnorm2)*_____
O18*(Labnorm3)*_____
O20*(Labnorm4)*_____

Estimated cause of visual loss **right eye** **left eye**
If vision in eyes is 6/12 or worse, estimate proportion caused by:

O21*(Renucl)*	enucleation	_____	O22*(Lenucl)*	enucleation	_____
O23*(Rambly)*	amblyopia	_____	O24*(Lambly)*	amblyopia	_____
O25*(Rcatara)*	cataract	_____	O26*(Lcatara)*	cataract	_____
O27*(RAMD)*	AMD	_____	O28*(LAMD)*	AMD	_____
O29*(Rretina)*	other retinal disease	____	O30*(Lretina)*	other retinal disease	_____
O31*(Rglauc)*	glaucoma	_____	O32*(Lglauc)*	glaucoma	_____
O33*(RopticN)*	other optic nerve dis	____	O34*(LopticN)*	other optic nerve dis	____
O35*(Rcornea)*	corneal disease	_____	O36*(Lcornea)*	corneal disease	_____
O37*(Rvitreo)*	vitreous media	_____	O38*(Lvitreo)*	vitreous media	_____
O39*(Runsure)*	unsure	_____	O40*(Lunsure)*	unsure	_____
O41*(Rdiseas)*	other,		O42*(Ldiseas)*	other,	
describe_____			describe_____		

O43*(colphot)* ***Colour fundus photographs taken?*** _____ _____

O44*(NFLphot)* ***Nerve Fibre Layer photographs taken?*** _____ _____

The examination is completed. Thank you very much for taking part.

O47. *(Pathday)* Date of blood test ___/___/9___
O45. *(PathT)* Take blood _____ time
O46. *(hrmeal)* How long ago did you last eat a meal? _____hours

Appendix B

Participant food frequency questionnaire from the BMES

BLUE MOUNTAINS EYE STUDY 2

Investigators: Paul Mitchell, Wayne Smith
Robert Cumming, Stephen Leeder

FOOD QUESTIONNAIRE

- This questionnaire is for all people who were examined in the Blue Mountains Eye Study during 1992- 1994.
- As part of the 5-year examinations, we would appreciate further information from you about your eating habits.
- This questionnaire will take you about an hour to fill in, but it is very important that you try to answer all questions as accurately as you can.
- We want you to fill this out yourself, but if you need help from your spouse or another member of your household please consult them as necessary.
- The results of your food questionnaire, are entirely confidential, that is:
  - only the researchers will see your answers
  - your results will be combined with others to summarise the overall picture of food habits in the community
  - no individuals can be identified in the analysis or in any reports.

If you have any queries about completing it, please telephone

Ms. Vicki Flood, Ph (02) 9845 6677
Dr. Karen Webb, Ph (02) 9845 6677
Ms. Michele Barbeau, Ph (047) 806 166

THANK YOU VERY MUCH FOR YOUR TIME IN COMPLETING THIS IMPORTANT QUESTIONNAIRE

Name:_____

ID._ _ _ _ _

Date completed:_____

This study is being carried out by the Departments of Ophthalmology, Public Health and Community Medicine, Westmead Hospital, and the National Centre for Epidemiology and Population Health, the Australian National University. The project is funded by the National Health and Medical Research Council.

WE WOULD LIKE TO ASK YOU WHICH FOODS YOU EAT, AND HOW MUCH YOU EAT OF EACH.

On the next page you will see a list of foods with an amount written next to each food. For each food we would like you to indicate with a tick **how often**, on average, you have eaten the **given amount over the last twelve months**. This may vary from never to four or more times as much as the given amount per day.

To help get you started, here are some examples of what we mean. If you can take a few minutes to work through these examples, you will quickly get the idea.

EXAMPLE 1: How often do you drink 250 ml (8oz) of whole milk?

If you drink a 250 ml glass of whole milk every day, on average (including milk you use on cereal, or in tea or coffee), you would place a tick in the 1 per day column, like this:

		Number of times used this amount over last 12 months								
		Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day
Whole Milk	250 ml (8oz.) glass							✓		

If you drink **twice** this amount, that is a total of about **two** 8oz. glasses of whole milk every day, you would place a tick in the 2-3 **per day** column, like this:

Whole Milk	250 ml (8oz.) glass								✓	

EXAMPLE 2: How often do you eat 1/2 cup of green beans?

If you eat 1/2 cup of green beans every 2 weeks, on average, you would place a tick in the **1-3 per month** column, like this:

		Number of times used this amount over last 12 months								
		Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day
Green Beans	1/2 cup			✓						

If you eat 1 cup of green beans a **week**, on average, this is the same as eating 1/2 cup of green beans 2 times a week, so you would place a tick in the **2-4 per week** column, like this:

Green Beans	1/2 cup					✓				

If there are any foods that you never eat, please place a tick in the **NEVER** column. Do not leave it blank.

Q 1 **Now**, please look at the list of foods below. For **each** food listed indicate with a tick how often, on average, you have eaten this food, in the given amount, during the past year. Please try to think carefully about **each** food, and try not to leave any blank lines.

		Number of times used this amount over last 12 months									
DAIRY FOODS **Foods**	**Amount**	**Never**	**Less than 1 per month**	**1-3 per month**	**1 per week**	**2-4 per week**	**5-6 per week**	**1 per day**	**2-3 per day**	**4+ per day**	
Skim milk	**250 ml (8oz.) glass**										**6** _
Low fat milk	**250 ml (8oz.) glass**										_
Whole milk	**250 ml (8oz.) glass**										_
Cream e.g. thickened, pouring	**1 tblsp.**										_
Ice cream	**1/2 cup**										**10** _
Yoghurt, flav/plain	**1 small carton**										_
Yoghurt, low fat, flav/plain	**1 small carton**										_
Cottage or ricotta cheese	**1/2 cup**										_
Other cheese, e.g. Coon	**1 slice or 1 oz. serving**										_
Margarine, added to food or bread: Exclude use in cooking	**1 teasp.**										**15** _
Butter, added to food or bread: Exclude use in cooking	**1 teasp.**										_

Q 2 What form of margarine do you use most often for spreading on bread, adding to vegetables etc? (Exclude use in cooking) (Circle one)

1.	Cooking margarine	4.	Low fat margarine	
2.	Table margarine	5.	Do not use margarine	
3.	Polyunsaturated margarine	6.	Other, please specify_____	17 _

What brand do you use most often? _____ 18 _ _

Q 3 What form of butter do you use most often for spreading on bread, adding to vegetables etc? (Exclude use in cooking) (Circle one)

1.	Ordinary butter	4.	Dairy blend, reduced fat	
2.	Reduced fat butter	5.	Do not use butter	
3.	Dairy blend, regular			20 _

Q 4a. Do you usually add butter or margarine to your cooked vegetables before you eat them? (Circle one)

1.	Yes	2.	No	21 _

Q. 4b. What type of ice cream and other ice confection do you usually use? (Circle one)

1.	Regular ice cream	4.	Reduced fat frozen yoghurt
2.	Reduced fat ice cream	5.	Vitari, sorbet or other fruit ices
3.	Regular frozen yoghurt	6.	Other, please specify _____

Q. 4 c. What type of cheese do you usually have?

1. Cottage / ricotta
2. Traditional types (cheddar, tasty, processed, Camembert, etc.)
3. Fat modified/ reduced fat types
4. Don't know/ can't say

Q 5 SEASONAL FRUITS Please indicate how often on average you eat these fruits when they are in season. Foods — Amount	Number of times used this amount over last 12 months									
	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Fresh peaches, apricots, plums or nectarines — 1										22
Fresh grapes — small bunch (about 20)										
Fresh strawberries — 1/2 cup										
Other fresh berries — 1/2 cup										25
Fresh cantaloupe or rockmelon — 1/4 melon										
Fresh mangoes — 1										
Fresh paw-paw — 1 slice										
Fresh pineapple — 1 slice										
Watermelon — 1 slice										30
Avocado — 1/2 avocado										

Q 5		Number of times used this amount over last 12 months									
OTHER FRUITS		Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Fresh apple or pear	1										32
Fresh orange	1										
Fresh grapefruit	1/2										
Fresh banana	1										35
Prunes	1/2 cup										
Dried apricots	4 - 5 halves										
Dried peaches	4 - 5 halves										
Other dried fruits	1 tblsp.										
Canned apricots or peaches	1/2 cup										40
Other canned fruit	1/2 cup										

Q 6 SEASONAL VEGETABLES (Please indicate how often on average you eat these vegetables when they are in season.) Foods	Amount	Number of times used this amount over last 12 months Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Broccoli	1/2 cup										42 _
Cauliflower	1/2 cup										_
Spinach, Silverbeet, cooked	1/2 cup										_
Spring onions, shallots	1 medium										45 _

Q 6		Number of times used this amount over last 12 months									
OTHER VEGETABLES (fresh, frozen or canned) **Foods**	**Amount**	**Never**	**Less than 1 per month**	**1-3 per month**	**1 per week**	**2-4 per week**	**5-6 per week**	**1 per day**	**2-3 per day**	**4+ per day**	
Potato, boiled or mashed	**1 medium, 1/2 cup**										46
Potato, baked	**1 medium**										
Hot chips	**1 cup**										
Pumpkin, boiled or mashed	**1 med. piece, 1/2 cup**										
Pumpkin, baked	**1 medium piece**										50
Sweet potato	**1/2 cup**										
Peas	**1/2 cup**										
Green beans	**1/2 cup**										
Cabbage	**1/2 cup**										
Brussel sprouts	**3-5 fresh or frozen**										55
Carrots	**1 medium whole or 1/2 cup cooked**										

Q 6		Number of times used this amount over last 12 months								
OTHER VEGETABLES (fresh, frozen or canned) Continued Foods	Amount	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day
Sweet corn	1 cob or 1/2 cup frozen or canned									
Eggplant, zucchini or squash	1/2 cup									
Mushrooms	6-7 small									
Tomatoes	1									
Lettuce	2 medium leaves									
Coleslaw	1/2 cup									
Celery	10cm (4 inch) stick									
Bean sprouts	1/2 cup									
Baked beans	1/2 cup									
Soybeans	1/2 cup									
Other beans or lentils	1/2 cup									

Q 7 MEATS, FISH & EGGS Foods / Amount	Number of times used this amount over last 12 months Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Beef, pork or lamb as main dish e.g. steak, roast — 1 small t-bone or 3 slices										68 _
Beef, pork or lamb mixed dish e.g. stew, casserole — 1/2 cup										_
Ham, beef, pork or lamb in sandwich — 1 slice										70 _
Chicken with skin — 1 drumstick or 2 slices										_
Chicken without skin — 1 drumstick or 2 slices										_
Sausages — 2 thick or 3 thin										_
Hamburger patty or rissole — 1										_
Mince in tomato sauce e.g. spaghetti sauce — 1 cup										75 _
Other mince meat dishes — 1 cup										_
Bacon — 2 slices										_

Q 7		Number of times used this amount over last 12 months									
MEATS, FISH & EGGS (continued) Foods	Amount	Never	Less than 1 per month	1-3 per month	1 per week	2-3 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Liver	100 g (4 oz.)										
Meat pie	1										
Sausage roll	1										80
Processed meats e.g. Devon, Chicken roll	1 piece or slice										
Frankfurt, saveloy	1 large or 3 small										
Boiled or poached egg	1										
Fried egg	1										
Scrambled egg or omelette	1										85
Tuna canned in oil	1/2 cup										
Tuna, salmon canned in water	1/2 cup										
Sardines	1/2 cup										
Other fish (e.g. fried, baked)	1 small fillet										
Other seafood e.g. prawns, crabs scallops as a main dish	1/2 cup										90

Q 8 BREAD, CEREALS, STARCHES Foods / Amount	Number of times used this amount over last 12 months Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Cold breakfast cereal — 1 cup										91 _
Cooked oatmeal — 1 cup										_
White bread or toast — 1 slice										_
Wholemeal/mixed grain bread or toast — 1 slice										_
Scone, pikelet — 1 scone, 3 pikelets										95 _
Brown rice — 1 cup (cooked)										_
White rice — 1 cup (cooked)										_
Pasta e.g. spaghetti, noodles, etc. — 1 cup										_
Crispbread, cracker, etc. — 1										_

Q9 What kind of breakfast cereal do you use most often (e.g. Uncle Toby's Toasted Muesli, Kellogg's Corn Flakes) Please specify type(s) and brand(s):

1._____ 100 _ _

2._____ 102 _ _

Q 10a		Number of times used this amount over last 12 months									
BEVERAGES	Amount	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Orange juice	1 small glass										104 _
Pineapple juice	1 small glass										_
Grape juice	1 small glass										_
Tomato juice	1 small glass										_
Carrot juice	1 small glass										_
Other juice	1 small glass										_
Low calorie cola e.g. Diet Coke	1 can										110 _
Other low calorie soft drink e.g. Diet Solo	1 can										_
Coke, Pepsi or other cola	1 can										_
Other soft drink, e.g. Lemonade	1 can										_
Cordial	1 glass										_
Coffee	1 cup										115 _
Decaf Coffee	1 cup										_
Tea (not herbal teas)	1 cup										_
Herbal tea	1 cup										_

Q 10a		Number of times used this amount over last 12 months								
BEVERAGES WITH ALCOHOL	Amount	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day
Beer (ordinary or heavy)	1 stubbie, can									
Beer (low alcohol)	1 stubbie, can									
Red Wine	1 wine glass									
White Wine or Champagne	1 wine glass									
Sherry or Port	1/2 wine glass									
Spirits (e.g. whiskey, gin)	1 drink or nip									

119 _
_
_
_
_
_

Q.10 b. **What type(s) and brand(s) of fruit juice do you use?**

1. _____

2. _____

3. _____

Q 11 SWEETS, BAKED GOODS & SNACKS Foods / Amount	Number of times used this amount over last 12 months									
	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Custard 1/2 cup										125
Cake 1 slice										
Tart or pie 1 slice										
Pastry, Pavlova, Cheesecake, etc 1 slice										
Sweet roll, bun 1										
Plain sweet biscuits, commercial 1										130
Fancy biscuits, commercial, e.g. chocolate coated 1										
Chocolate 1										
Lollies 3-5										
Jam, marmalade, syrup or honey 1 tblsp.										
Peanut paste 1 tblsp.										135
Vegemite or Marmite 1 teasp.										
Nuts 1 tblsp.										
Potato chips (crisps), corn chips, twisties etc. 1 small bag										

Q 11		Number of times used this amount over last 12 months									
OTHER FOODS Foods	Amount	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Pizza	2 slices										139 _
Olives/gherkins/pickled vegs	1/3 cup										_
Cream soup	1 cup										_
Oil and vinegar dressing, e.g. French	1 tblsp.										_
Mayonnaise or other creamy salad dressing	1 tblsp.										_

Q 12 Are there any other foods not listed above that you usually eat at least once per week?

Other foods that you usually use at least once per week	Usual serving size	Average use per week	
(a)			144 _ _ _ _
(b)			148 _ _ _ _
(c)			152 _ _ _ _

Q 13 How many teaspoons of sugar altogether do you add to your food and drink each day? (Include sugar added to your tea, coffee, cereal, fruit etc.)

Total _____ teasp **156** _ _

Q 14 What do you do with the visible fat on your meat? (Circle one)

1. Eat most of it
2. Eat some of it
3. Eat as little as possible
4. Don't eat meat

158 _

Q 15 What type of cooking oil is used <u>most often</u> in your home? (e.g. Bertolli olive oil, Meadow Lea sunflower oil)

Please specify type and brand _____ 159 _

Q 16 What kind of fat is used most often in your home for frying or roasting meat or vegetables?

1.	Butter	5.	Table margarine	
2.	Lard	6.	Vegetable oil	
3.	Cooking margarine	7.	Other, please specify	
4.	Polyunsaturated margarine /polyunsat. table margarine	8.	None	**160 _**

Q 17 How often do you eat food that is fried at home? (Include any foods cooked in a pan or on a hot plate e.g. pan frying or dry frying) (Circle one)

1.	Less than once per week	4.	Daily	
2.	1 - 3 times per week	5.	2 or more times per day	
3.	4 - 6 times per week			161 _

Q 18 How often do you eat take-away that is fried food e.g. Chips, battered foods, chicken fried fish? (Circle one)

1.	Less than once a week	3.	4 - 6 times per week	
2.	1 - 3 times per week	4.	Daily	162 _

Q 19 Do you take vitamin pills (or liquid)? (Circle One)

1. Yes 2. No 157 _

If YES, do you regularly (in most weeks) take any of the vitamins listed below?

1. Yes 2. No 158 _

If YES, please look at the bottle to help answer the following:

Name of Vitamin	Brand Name	Used for how many years	No. of pills, capsules or teaspoons per day	Strength in mg or other units - see bottles	
Multi-vitamin					**159** _ _ _ _ _ _
Vitamin A retinol					**165** _ _ _ _ _ _ _ _ _ _
Beta - carotene					**175** _ _ _ _ _ _ _ _ _ _
Vitamin C					**185** _ _ _ _ _ _ _ _ _ _
Vitamin E					**195** _ _ _ _ _ _ _ _ _ _

Q 20 Do you take other dietary supplements or minerals? (Circle one)

1. Yes 2. No 205 _

If YES, please specify for each supplement, the type, number or amount taken and how often taken.

Name of Supplement or Mineral	Brand Name	Used for how many years	Amount taken per day	If applicable - strength in mg or other units	
(a)					206 _ _ _ _ _ _ _ _ _ _
(b)					216 _ _ _ _ _ _ _ _ _ _
(c)					226 _ _ _ _ _ _ _ _ _ _
(d)					236 _ _ _ _ _ _ _ _ _ _
(e)					246 _ _ _ _ _ _ _ _ _ _

Q. 21. These are more detailed questions about meat Foods / Amount	Number of times used this amount over last 12 months									
	Never	Less than 1 per month	1-3 per month	1 per week	2-4 per week	5-6 per week	1 per day	2-3 per day	4+ per day	
Beef or veal as main dish eg steak or roast — 1 small t-bone or 3 slices										250 _
Pork or ham as main dish eg chops or roast — 3 slices										_
Lamb as main dish eg chops or roast — 3 slices										252 _
Beef or veal mixed dish e.g. stew, casserole, stir fry — 1/2 cup										_
Pork or ham mixed dish e.g. stew, casserole, stir fry — 1/2 cup										254_
Lamb mixed dish e.g. stew, casserole, stir fry — 1/2 cup										_
Ham or pork in sandwich — 1 slice										256_
Beef or veal in sandwich — 1 slice										_
Lamb in sandwich — 1 slice										258_

Q.22 For each of the following types of food I would like you to tell me about how often you usually eat the food at this time of year. For example, over the last 3 months have you eaten a particular food, once a day, twice a week, three times a month - whatever is easier. Think about all the food you eat - both at home and away from home.

A. How often do you eat fried food with a batter or breadcrumb coating?

1. _____ per day
2. _____ per week
3. _____ per month
4. _____ rarely or never
5. _____ don't know / can't say

B. How often do you eat meat products such as sausages, frankfurters, belgium, devon, salami, meat pies, bacon or ham?

1. _____ per day
2. _____ per week
3. _____ per month
4. _____ rarely or never
5. _____ don't know / can't say

C. How often do you eat chips, french fries, wedges, fried potatoes or crisps?

1. _____ per day
2. _____ per week
3. _____ per month
4. _____ rarely or never
5. _____ don't know / can't say

D. How is your meat <u>usually cooked?</u>

1. fried
2. stewed/casserole
3. grilled/roasted with added fat or oil
4. grilled/roasted without added fat
5. Rarely or never eat meat
6. Don't know/can't say

E. What type of milk do you <u>usually have?</u>

1. regular milk (whole or full cream milk)
2. Life full cream
3. Lite white
4. Farmer's best
5. Life reduced fat
6. Shape
7. Skim milk
8. Other, please specify _____
9. Don't have milk
10. Don't know/can't say

F. Which <u>one</u> of the following <u>best</u> describes your usual way of eating?

1. no special way of eating
2. vegetarian
3. weight reduction diet
4. diabetic diet
5. fat modified diet to lower blood fat (cholesterol)
6. Other, please specify _____

Q. 23. **How many serves of vegetables do you <u>usually</u> eat each day?**
(a 'serve' = ½ cup of cooked vegetables or 1 cup of salad vegetables)

1. _____ serves per day (0,1,2,3, etc)
2. don't eat vegetables

Q.24 **How many serves of fruit do you <u>usually</u> eat each day?**
(a `serve' = 1 medium piece or 2 small pieces of fruit or 1 cup of diced pieces)

1. _____ serves per day (0,1,2,3, etc)
2. don't eat fruit

Q.25 **How many slices of bread do you <u>usually</u> eat each day?**
(A slice of bread is equal to 1 small bread roll or 1 bagel or ½ a large bread roll or ½ bread muffin or 1 scone or ½ a pita bread)

1. _____ slices per day (0,1,2,3, etc)
2. don't eat bread
3. don't know

Q.26 **How many cups of cooked pasta, rice, noodles, or other cooked cereals do you <u>usually</u> eat each <u>week</u>? (Not including cooked breakfast cereals). I am asking you about <u>per week</u> here!**

1. _____ cups per day (0,½ ,1,1½,2,2½,3, etc)
2. don't eat these foods
3. don't know

Q.27 **How many cups of breakfast cereal do you usually eat each day?**
(One cup is equal to 2 weetbix or ½ cup of cooked porridge or ⅛ of a cup of muesli or ½ cup of allbran)

1. _____ cups per day (0,½ ,1,1½,2,2½,3, etc)
2. don't eat breakfast cereals
3. don't know

Q.28 **How many serves of meat do you <u>usually</u> eat each day?**
(A 'serve' = 3 slices meat or one small t-bone)

1. _____ serves per day (O, 1, 2, 3, etc.)
2. don't eat meat

Q. 29 a. **In the last 5 years, have you changed your eating habits in any way?**

1. Yes 2. No

If **YES**, how? _____

_____

Q.29 b. **Over the past 5 years, would you say you have increased, decreased, or not changed the amount you eat of the following foods and nutrients. (Please tick)**

Food	Increased	Decreased	Not Changed	Don't Eat
Salt				
Starches (eg, cereals, pasta, rice, bread, grains)				
Fibre				
Fruit				
Vegetables				
Total fat				
Saturated fats (eg fat in meat, milk, cheese, butter)				
Polyunsaturated fats (eg vegetable oils, polyunsaturated margarine)				
Monounsaturated fats (eg olive oil, canola oil or canola margarine)				
Cholesterol				
Alcohol				
Energy (kilojoules or calories)				

Q. 29 c. **Do you think you will make any changes to your eating habits during the next five years?**

1. Yes 2. No

If **YES**, what changes? _____

_____

Q.30 How would you rate the amount you eat of each of these foods and nutrients? (Please tick)

Food	Too much	About right	Too little	Don't Eat
Salt				
Starches (eg, cereals, pasta, rice, bread, grains)				
Fibre				
Fruit				
Vegetables				
Total fat				
Saturated fats (eg fat in meat, milk, cheese, butter)				
Polyunsaturated fats (eg vegetable oils, polyunsaturated margarine)				
Monounsaturated fats (eg olive oil, canola oil or canola margarine)				
Cholesterol				
Alcohol				
Energy (kilojoules or calories)				

The next nine questions are about your body weight.

31. Do you consider yourself to be

1. Acceptable weight
2. Underweight
3. Overweight

32. How tall are you without shoes?

_____centimetres

OR

_____ feet _____inches

33. How much do you weigh without clothes or shoes?

_____kilograms

OR

_____stones _____pounds

34. Compared to the same time last year, has your weight:

1. Increased
2. Decreased
3. Stayed the same
4. Don't know

35. If your weight has changed, what do you think were the reasons for this weight change?

_____

_____

36. Have you tried to lose weight in the past 12 months?

1. Yes
2. No
3. Not sure

37. If you have tried to lose weight in the past 12 months, which weight loss methods have you used? (You can mark more than one response)

1. I dieted
2. I exercised
3. I used organised programs
4. I used vitamins
5. I used meal replacements
6. I used diet supplements
7. I used over-the-counter pharmaceutical products Eg. Diet pills or appetite suppressants
8. I had surgery, eg. Liposuction

If you circled 3, please specify program type

_____

If you circled 4, 5, 6 or 7, please specify type and brand:

_____

38. Which one of the following statements best describes you at the moment?

1. I am actively doing things to try to **gain** weight at the moment
2. I am actively doing things to **avoid gaining** weight at the moment
3. I am actively doing things to try to **lose** weight at the moment
4. I am **not doing anything** in particular for my weight at the moment

Q. 39 Here are a series of statements that people have made about their food situation. Please circle one response to each question, indicating whether the statement is often true, sometimes true or never true for your household or the individuals in your household.

A. I worry whether my food will run out before I get money to buy more.

1. often true 2. sometimes true 3. never true

B. I worry about whether the food that I can afford to buy for my household will be enough.

1. often true 2. sometimes true 3. never true

C. The food that I bought just didn't last, and I didn't have money to get more.

1. often true 2. sometimes true 3. never true

D. I ran out of the foods that I needed to put together a meal and I didn't have money to get more food.

1. often true 2. sometimes true 3. never true

E. We eat the same thing for several days in a row because we only have a few different kinds of food on hand and don't have money to buy more.

1. often true 2. sometimes true 3. never true

F. I am often hungry, but I don't eat because I can't afford enough food.

1. often true 2. sometimes true 3. never true

G. I eat less than I think I should because I don't have enough money for food.

1. often true 2. sometimes true 3. never true

H. I can't afford to eat properly.

1. often true 2. sometimes true 3. never true

I. Sometimes people lose weight because they don't have enough to eat. In the past year, did you lose weight because there wasn't enough food?

1. often true 2. sometimes true 3. never true

J. In the past year, have you had hunger pangs but couldn't eat because you couldn't afford food?

1. often true 2. sometimes true 3. never true

K. In the last 12 months, were there times that your household ran out of food and there wasn't money to buy any more food?

1. often true 2. sometimes true 3. never true

L. In the last 12 months, has anyone in your household eaten less than they should because you couldn't afford enough food?

1. often true 2. sometimes true 3. never true

Thank you for your co-operation

We really appreciate the time and effort you have put into completing this questionnaire

Appendix C

Summary grading form for AMD used in the BMES

I.D. # _ _ _ _ _ _ Eye ___

BMES Maculopathy Summary Grading Form Oct, 2003

Photo date: _____
Grader ___ ___
Date __ __/__ __/__ __
Entered _____
Verified _____

Fields	Fld 1	Fld 2	Fld 3	Nasal	F2 Qual	Focus	Field	Stereo
gradeable	0	0	0	0	good	1	1	1
ungradeable	1	1	1	1	fair	2	2	2
not present	2	2	2	2	poor	3	3	3

Maculopathy

None 0 Quest or Def 2 CG 8

1. **Detachments** None 0 Quest 1 Yes 2 Area within field 2 _____ DA
   Old/Atrophic Disciform None 0 Quest 1 Yes 2

	Field 2				Central + Inner Subfields			
	None	Quest	Yes	CG	None	Quest	Yes	CG
Drusenoid PED	0	1	2	8	0	1	2	8
Non-Drusenoid PED	0	1	2	8	0	1	2	8
Type (circle one)		1. Dome				2. Shallow		3. Irregular
SSR/Haem RD	0	1	2	8	0	1	2	8
Hard Exudate	0	1	2	8	0	1	2	8
SubRet/SubRPE Haem	0	1	2	8	0	1	2	8
Subretinal Fibrosis	0	1	2	8	0	1	2	8
Laser Rx AMD	0	1	2	8	0	1	2	8

2. **Geographic Atrophy**	None	Quest	< I 2	< O 2	<half DA	< 1 DA	< 2 DA	≥ 2DA	CG
Area Centre Point	0	1	2						8
Area Centre Subfield	0	1	2	3	4	5			8
Area Centre + Inners	0	1	2	3	4	5	6	7	8
Area within Grid	0	1	2	3	4	5	6	7	8

3. **End-Stage Maculopathy** None 0 Quest 1 Yes 2
   Type disciform/detach ("wet") 1 GS ("dry") 2

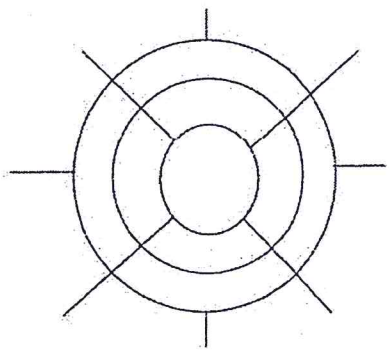
4. **RPE Depigmentation**	None	Quest	< I 2	< O 2	<half DA	< 1 DA	< 2 DA	≥ 2DA	CG
Area Centre Subfield	0	1	2	3	4	5			8
Area Centre + Inners	0	1	2	3	4	5	6	7	8
Area within Grid	0	1	2	3	4	5	6	7	8

5. **Increased Pigment**	None	Quest	< C 0	< C 1	< C 2	< O 2	≥ O 2	Other C	CG
Area Centre Subfield	0	1	2	3	4	5			8
Area Centre + Inners	0	1	2	3	4	5	6	7	8
Area within Grid	0	1	2	3	4	5	6	7	8

Drusen Grading

6. **Confounding Ocular Lesions**	None	Quest	Yes	CG
Prevent Grading Drusen	0	1	2	8

7. **Drusen Within Grid**	None	Quest or Def	CG
	0	2	8



8. **Max Drusen Size**

None	0
Quest	1
< C-0 (63)	2
< C-1 (125)	3
< C-2 (250)	4
≥ C-2	5
Can't Grade	8

9. **Drusen Number**

None	0
Quest/stippling	1
< 10	2
≥ 10	3
Can't Grade	8

10. **Soft Drusen**

None	0
Soft Distinct	1
Soft Indistinct	2
Can't Grade	8
Intermediate soft drusen (>C0, ≤C1)	3

11. **Drusen Area**	None/Q/< C 0	< C-1	< C-2	< I-2	< O-2	<half DA	< 1 DA	≥ 1 DA	CG
C/Sub Only	0	1	2	3	4	5	6	7	8
Centre + Inners	0	1	2	3	4	5	6	7	8
Within Grid	0	1	2	3	4	5	6	7	8

Out Grid + F1 + F3 None 0 < O-2 1 ≥ O 2 2 CG 8

	None	Quest	Outside Grid	Within and Outside Grid	CG
12. **Reticular Drusen**	0	1	2	3	8
13. **Calcified Drusen**	0	1	2	3	8

14. **Comments**

_____

_____

15. **Other Ocular Lesions**

	None		Yes	CG		
Quest/Def Present	0		2	8		
	No	Quest	Yes	CG	Lesion #	Description/Abbreviation
Lesion 1	0	1	2	8	_____	_____
Lesion 2	0	1	2	8	_____	_____
Lesion 3	0	1	2	8	_____	_____
Lesion 4	0	1	2	8	_____	_____
Lesion 5	0	1	2	8	_____	_____
Lesion 6	0	1	2	8	_____	_____
Lesion 7	0	1	2	8	_____	_____
Lesion 8	0	1	2	8	_____	_____

Abbreviations for Common Lesions

Retinop		Possible diabetic retinopathy, (add:Haem, MA, or H/MA)
Def Ret	C	Definite diabetic retinopathy
Chor Scr		Chorioretinal scar > 1500 microns from centre (various causes)
Mac Scr	C	Chorioretinal scar < 1500 microns from centre (various causes)
ToxoP	C?	Old chorioretinal scar typical of Toxoplasmosis
Mac Oed	C	Macular oedema
Mac Hole	C	Macular hole/cyst
Mac Oth	C	Macular other lesion, < 1500 microns from centre
SWR	C?	Surface wrinkling retinopathy (preretinal fibrosis), with folds, tension lines or a patch (confounding if ≥ 1 disc area in extent)
Cello R		Cellophane reflex only
Vit Det		Prominent posterior vitreous detachment
Laser		Photocoagulation scars, other (i.e. non-AMD)
Laser C	C	confounding if < 1500 microns from centre
P/V Haem	C	Preretinal or vitreous haemorrhage
Ret Det	C	Retinal detachment
Myop Ret	C	Myopic crescent, > half longest disc diameter
RAO	C	Retinal artery occlusion , central or branch
BRVO	C	Branch retinal vein occlusion
CRVO	C	Central retinal vein occlusion
Ret Emb		Retinal artery embolus (Hollenhorst plaque)
Naevus		Choroidal Naevus
Op Atr		Optic atrophy
Op Oed		Optic disc oedema
Op Dru		Optic disc drusen
Gl Rem		Glia remnant, optic disc
PP Atr		Peripapillary Atrophy
Ang Stk	C?	Angioid streaks
Ast Hyl		Asteroid Hyalosis
Lg Cup		Large opticcup, cup-disc ratio (add characteristics: undercutting, notching, rim eroded)
Cat	C	Cataracts preclude grading
	C	*Lesion confounding grading of drusen or other AMD lesions*

Comments, Other Lesions

_____

_____

_____

Appendix D

Published papers arising from this thesis

CrossMark

American Academy of Ophthalmology
The Eye M.D. Association

The Incidence and Progression of Age-Related Macular Degeneration over 15 Years

The Blue Mountains Eye Study

Nichole Joachim, BSc (Hons), Paul Mitchell, MD, PhD, George Burlutsky, MApplStat, Annette Kifley, MApplStat, PhD, Jie Jin Wang, MMed, PhD

Purpose: To assess the 15-year incidence and progression of age-related macular degeneration (AMD) in an older Australian population.

Design: Population-based cohort study.

Participants: Blue Mountains Eye Study (BMES) participants (n = 3654) aged 49+ years were examined during 1992−1994. Of these, 2334 (75.8% of survivors) were reexamined after 5 years (1997−1999), 1952 (76.7% of survivors) after 10 years (2002−2004), and 1149 (56.1% of survivors) after 15 years (2007−2010).

Methods: Color retinal photographs were taken, and comprehensive questionnaires were administered at each visit and DNA was genotyped. Retinal photographic grading was performed by the same graders following the Wisconsin AMD grading protocol. Side-by-side comparisons were used to confirm newly developed AMD lesions. Incidence was estimated using Kaplan−Meier estimates. Associations of AMD incidence with age, sex, smoking status, presence of the *complement factor H (CFH)-rs1061170* and *age-related maculopathy susceptibility 2 (ARMS2)-rs10490924* polymorphisms, and fish consumption were analyzed using discrete logistic regression models. Generalized estimation equation models were used to assess the risk of incident late AMD associated with baseline AMD lesion characteristics.

Main Outcome Measures: The 15-year incidence and progression of AMD, and associated factors.

Results: The 15-year incidence was 22.7% for early AMD and 6.8% for late AMD. After adjusting for competing risks, early and late AMD incidence were 15.1% and 4.1%, respectively. Age was strongly associated with early and late AMD incidence (both $P < 0.0001$). After age standardization to the Beaver Dam Eye Study (BDES) population, early and late AMD incidence in the BMES were 13.1% and 3.3%, respectively. Female sex and the presence of both risk alleles of *CFH-rs1061170* or *ARMS2-rs10490924* were independently associated with early AMD incidence, whereas current smoking and presence of ≥1 risk allele of *CFH-rs1061170* or *ARMS2-rs10490924* were associated with late AMD incidence. Fish consumption was inversely associated with late but not early AMD incidence. Severity of early AMD lesion characteristics was a strong predictor of progression to late AMD.

Conclusions: We documented the 15-year incidence of early and late AMD in an older Australian population that were comparable to BDES observations. Risk of progression to late AMD was strongly associated with severity of early AMD lesions. *Ophthalmology 2015;122:2482-2489 © 2015 by the American Academy of Ophthalmology.*

Age-related macular degeneration (AMD) continues to be one of the leading causes of blindness and visual impairment in older populations despite recent advances in treatments.[1−4] The incidence and progression of early and late-stage AMD over 5 and 10 years have been reported in a number of large population-based studies in the United States, Europe, Asia, and Australia over the past 2 decades.[2,5−10] Greater severity of early AMD lesions, including increased drusen area, presence of pigmentary abnormalities, and location of lesions close to the fovea, was shown to be associated with greater risk of progression to late AMD.[6,7,10,11] The relationship between demographic and lifestyle risk factors, including older age, sex, and smoking status, with the incidence and progression of AMD was also shown in some of these populations.[12]

However, data on the incidence of AMD over the long-term (>10 years) are limited. The Copenhagen City Eye Study and the Beaver Dam Eye Study (BDES) are the only population-based studies thus far to report 14- and 15-year AMD incidence, respectively.[11,13] In this report, we aimed to build on the previous 5- and 10-year AMD incidence findings to describe the 15-year incidence of early and late AMD and its component lesions in an older Australian population (the Blue Mountains Eye Study [BMES]) and to assess risk factors and baseline early AMD lesions characteristics associated with the risk of progression to late AMD over the longer term.

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Methods

Study Population

The BMES is a population-based study of vision and eye disease in persons aged 49 years and older residing in the Blue Mountains region, west of Sydney Australia. The study recruited 3654 participants (82.4% of those eligible) during baseline examinations (1992−1994, BMES I). Of these, 2334 participants (75.8% of survivors; 575 deceased) attended 5-year follow-up examinations (1997−1999, BMES II); 10-year examinations were attended by 1952 participants (76.7% of survivors; a further 535 died; 2002−2004, BMES III). The final 15-year follow up examinations were attended by 1149 participants (56.1% of survivors; a further 496 died; 2007−2009, BMES IV). The mean (median, minimum, and maximum) follow-up period was 5.1 years (4.9, 3.4, and 7.8, respectively) for the 2334 BMES II participants; 10.5 years (10.4, 8.9, and 12.9, respectively) for the 1952 BMES III participants; and 15.6 years (15.5, 13.6, and 17.7, respectively) for the 1149 BMES IV participants. All 4 examinations were approved by the University of Sydney and Western Sydney Area Health Service Human Research Ethics Committees and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants at each visit.

Procedures

A comprehensive questionnaire was administered, and eye examinations were performed at each visit, as previously described.[10,14] Briefly, 30° stereoscopic retinal fundus photographs of the macula and other retinal fields of both eyes were obtained using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany) and Kodachrome 25 slide film (Kodak) at BMES I, II, and III examinations. At the BMES IV examination, because of the unavailability of Kodachrome, 40° digital photographs were obtained with a Canon CF-60 DSi fundus camera with a DS Mark II body (Canon Inc., Tokyo, Japan). Photographs were obtained for both eyes in 98%, 98%, 85%, and 92% at the baseline, 5-, 10-, and 15-year examinations, respectively, and for at least 1 eye in 99%, 99%, 87%, and 92% at the baseline, 5-, 10-, and 15-year examinations, respectively. Diet was assessed from a self-administered food frequency questionnaire completed by participants at each examination. Blood samples were collected from participants at the BMES II and III examinations, and DNA extraction and genotyping were performed in >80% of these BMES participants.

Photographic Grading

Retinal photographic grading was performed by 2 senior graders and closely followed the Wisconsin Age-Related Maculopathy Grading System protocol.[15] As previously described, film fundus photographs were initially graded in a masked manner, and side-by-side grading between BMES I and II, and BMES I and III was performed subsequently for participants with AMD lesions identified after each follow-up examination.[9,14] Inter- and intra-grader reliability showed good agreement for AMD grading, with quadratic weighted kappa values ranging from 0.64−0.93 and 0.54−0.94, respectively.[14] Adjudication was provided by a senior retinal specialist (P.M.) if needed. The BMES IV digital retinal photographs were graded in the same masked manner using the grading software DH Client (Digital Healthcare: Image Management Systems, www.digital-healthcare.com, Cambridge, UK). Consensus on BMES incident late AMD cases was provided by lead investigators of the Three Continent AMD Consortium.[16]

Late AMD was defined as the presence of neovascular AMD, indicated by retinal pigment epithelial or neurosensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars with a history of neovascular AMD, or the presence of pure geographic atrophy (GA) within the macula, as described in the International Age-Related Maculopathy Classification.[17] Early AMD was defined as the presence of large (≥125 μm in diameter) indistinct soft drusen, reticular drusen, or the copresence of large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or depigmentation of retinal pigment epithelial cells), within the macula, in the absence of any late AMD lesions. The maximal diameter of individual drusen and collective macular areas involved by drusen and pigmentary abnormalities within the eye was estimated as specified in the Wisconsin Age-Related Maculopathy Grading System, using circles with diameters of 63 μm, 125 μm, 250 μm, 350 μm, and 644 μm, 0.5 or 1 disc area.[15]

Definition of Age-Related Macular Degeneration Incidence in the First Eye

Incident late AMD in the first eye was defined as the appearance of neovascular AMD or GA in either eye at any follow-up examination when the lesion was not present in either eye at baseline. For participants at risk of incident neovascular AMD, cases with this lesion at baseline were excluded but cases with GA at baseline were not excluded. Participants with GA or neovascular AMD at baseline and with neovascular AMD at follow-up were excluded from those at risk of incident GA. If GA was secondary to neovascular AMD or laser treatment of neovascular AMD, it was not considered as incident GA.

The BMES participants who developed late AMD during the follow-up period were all seen by the principal investigator of the BMES (PM) for confirmation and were treated and followed at the Eye Clinic, Westmead Hospital. These participants also were labeled using BMES identification numbers in their patient records and were included as incident late AMD cases.

Incident early AMD was defined as the appearance of indistinct soft or reticular drusen, or the co-presence of distinct soft drusen and retinal pigmentary abnormalities in either eye, at any follow-up examination where no late or early AMD was present in either eye at baseline. Participants with distinct soft drusen or retinal pigmentary abnormalities alone at baseline who later developed complementary lesions that comprised a diagnosis of early AMD were included as incident early AMD cases. Incidence of indistinct soft or reticular drusen was defined as the appearance of these lesions in either eye at follow-up visits, where none were present at baseline, and excluding late AMD, regardless of the presence of retinal pigmentary abnormalities. Incidence of retinal pigmentary abnormalities was defined as the appearance of these abnormalities in either eye at follow-up visits in participants with no pigment abnormalities at baseline and no late AMD at any follow-up visits.

Other Study Outcomes

The incidence of early and late AMD in the second eye of participants with unilateral early or late AMD at baseline and the progression from early AMD to late AMD in at least 1 eye over 15 years were assessed among persons with AMD in 1 or both eyes at baseline.

Genotyping

Genotyping was performed on the BMES cohort and the BMES Extension Survey (1999−2000) samples using an Illumina Human 670-Quad custom array version 1 (Illumina Inc., San Diego, CA) with stringent quality-control testing using PLINK (Purcell S. PLINK version 1.07. Available at: http://pngu.mgh.harvard.edu/purcell/plink/,

accessed July 12, 2013). After quality checking, genome-wide association scan data were imputed with the 1000 Genomes panel (Version 1), using IMPUTE 2.0 (Department of Statistics, University of Oxford, Oxford, UK).[18] The imputation r^2 was 0.968 for *complement factor H (CFH)-rs1061170* and 0.996 for *age-related maculopathy susceptibility 2 (ARMS2)-rs104900924*.

The *CFH* single nucleotide polymorphism (SNP) *rs1061170* was also genotyped in 1928 participants using TaqMan assays (Applied Biosystems, Foster City, CA)[19] and the *ARMS2* SNP *rs10490924* was genotyped in 638 participants using restriction fragment length polymorphism analysis.[20] In this report of the BMES cohort sample, we used typed SNPs when this information was available and imputed SNPs for the remaining participants.

Other Risk Factors

Participants were classified as nonsmokers if they answered "no" to the question whether they smoke regularly. Past smoking was defined if participants had smoked regularly but quit smoking more than 1 year before the examination. Current smoking was defined if participants were current smokers or had stopped smoking <1 year before the examination. Regular fish consumption was defined as consuming ≥1 serving of fish per week.

Statistical Analyses

SAS software version 9.3 (SAS Inc, Cary NC) was used for analyses. The 15-year person-specific incidence of early and late AMD and their component lesions were estimated using Kaplan−Meier product limit survival estimates and, alternatively, competing risk analyses to control for the risk of death. The BMES population was also directly age-standardized to the BDES population[11] to compare 15-year AMD incident rates between the 2 populations. Further, the probabilities of late AMD development from different severity levels of AMD over 15 years were reported according to steps on the AREDS simplified severity scale.[21]

The associations between known AMD risk factors (age, sex, smoking, fish consumption, the *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles) and the 15-year incidence of early and late AMD were assessed using age-, sex-, and multivariate-adjusted discrete logistic regression models. Generalized estimating equation models, using the GENMOD procedure in SAS,[22] were applied to eye-specific data to assess the associations between incidence of late AMD and early AMD lesion characteristics (area and location of drusen and retinal pigmentary abnormality). For comparison, the association between 15-year incident late AMD and steps on the AREDS simplified severity scale[21] was also assessed using the GENMOD procedure applied to person-specific data, with time intervals included in the model. Association estimates are presented as age-, sex-, and smoking-adjusted or multivariable-adjusted (age, sex, smoking, fish consumption, the *CFH-rs1061170* and *ARMS2-rs10490924* risk alleles) odds ratios (ORs) and 95% confidence intervals (CIs).

Results

We included participants who were censored up to the 5-, 10-, or 15-year follow-up examination to estimate incidence. Of 3654 baseline participants, 854 (23.4%) died with no follow-up information available and 326 (8.9%) were lost to follow-up and had no retinal photographs available at all 3 time points, leaving 2474 (67.7%) with gradable retinal photographs who were examined at the 5-, 10-, or 15-year examination, or 2 or all 3 examinations. Of these 2474 participants, 574 (23.2%), 75 (3.0%), and 7 (0.3%) were seen only at 5-, 10-, or 15-year examination, respectively; 789 (31.9%) were seen at 2 of the examinations (5- and 10-year, 5- and 15-year, or 10- and 15-year examinations); and 1029 (41.6%) were seen at all 3 examinations.

Table 1 compares the baseline characteristics between participants examined at either or all of the 5-, 10-, or 15-year examinations (n = 2474) and those who were alive but not examined at any follow-up examination (n = 326) or those who died (n=854) without reexamination in the BMES cohort. Compared with those who were followed, those who were lost to follow-up were more likely to have been younger at baseline (mean age, 60.6 vs. 64.3 years), to have a lower socioeconomic status (defined by homeownership and trade or higher qualification), and to be current smokers (22.5% vs. 13.1%), but less likely to have a history of heart disease (7.7% vs. 14.4%). Histories of stroke, cancer, diabetes, hypertension, and self-ranked health were not significantly different among those examined versus those not examined. Participants who died without attending any follow-up were on average 10 years older and more likely to be living alone, to have walking disabilities and systemic diseases, and to use community services at baseline (Table 1).

The 15-year incidence of early- and late-stage AMD lesions by age and sex is presented in Table 2. There was an increased 15-year incidence of late AMD associated with older age. Although a similar increase was observed for early AMD including up to 80 years of age, the 15-year incidence of early AMD, particularly incidence of indistinct or reticular drusen, decreased in those aged ≥80 years at baseline. The 15-year incidence of both early AMD and neovascular AMD was higher in women compared with men. After adjusting for gender, age was strongly associated with incidence of early and late AMD lesions (both $P < 0.0001$).

The 15-year incidence of early and late AMD in the BMES population, after adjusting for the competing risk of death, was 15.1% and 4.1%, respectively. By using the competing risk method, the BMES early and late AMD incidence rates were age standardized to that of the BDES population. We found similar age-standardized incident rates of early AMD (13.1%; 95% CI, 11.7−14.6, vs. 14.3%, estimated 95% CI, 13.1−15.5) and late AMD (3.3%, 95% CI, 2.6−4.0, vs. 3.1%, estimated 95% CI, 2.6−3.6) over 15 years in the BMES when compared with the corresponding incidence rates in the BDES population.[11]

The AREDS simplified severity scale was applied to the BMES baseline AMD status, and the probabilities of developing late AMD over 15 years from various levels in the scale are shown in Table 3. The 15-year incidence of late AMD among persons at Step 0 at baseline ranged from 1.2% to 4.5%; at Steps 1, 2, and 3, the 15-year incidence of late AMD ranged from 4.5% to 12.0%, 21.1% to 41.7%, and 0.0% to 33.3%, respectively. Corresponding incidence among persons at Step 4 (baseline) was 76.5% (Table 3).

After controlling for the competing risk of death, the cumulative incidence of early AMD in the second eye of persons with early AMD in the first eye was 67.0% over the follow-up period. The corresponding incidence of late AMD in the second eye of persons with late AMD in the first eye was 35.4% and of those with early AMD in the first eye was 24.6%.

Known AMD risk factors associated with 15-year incident early and late AMD are listed in Table 4. In the age-sex adjusted model, 2 risk alleles of *CFH-rs1061170* (OR, 2.8) and either 1 or 2 risk alleles of *ARMS2-rs10490924* (ORs, 1.6−2.6) were associated

Table 1. Comparison of Baseline Characteristics between Participants Examined and Not Examined at 15 Years in the Blue Mountains Eye Study Cohort

Baseline Characteristics	Examined (n = 2474)	Not Examined or No Photographs (n = 326)	*P* Value*	Died (n = 854)
Mean age (yrs) (95% CI)	64.3 (64.0–64.7)	60.6 (59.8–61.5)	<0.0001	73.7 (73.1–74.4)
Age group	%	%		%
<60 yrs	31.6	50.3	<0.0001	8.7
60–69 yrs	40.7	35.0	0.05	22.1
70–79 yrs	22.9	13.5	0.0001	41.0
≥80 yrs	4.8	1.2	0.003	28.2
Women	57.6	63.5	0.04	51.5
Currently married	66.1	62.0	0.1	53.0
Home owner	91.1	82.4	<0.0001	83.8
Low job prestige	36.3	37.4	0.7	42.5
Trade or higher qualification	60.6	55.3	0.08	51.2
Living alone	25.5	24.2	0.6	34.6
Walking disability	3.3	3.4	1.0	20.4
Regular use of community services	3.7	4.6	0.4	15.8
Self-ranked health				
Excellent	21.8	20.9	0.7	13.8
Good	57.1	56.7	0.9	47.4
Fair	18.9	18.4	0.8	29.8
Poor	2.2	4.1	0.04	9.0
History of stroke	3.3	3.1	0.8	12.1
History of heart disease	14.4	7.7	0.0009	24.3
History of cancer	7.4	6.1	0.4	12.5
History of diabetes	6.3	8.6	0.1	11.6
Presence of hypertension	70.0	66.6	0.2	78.2
Smoking status				
Never	85.6	75.9	<0.0001	80.6
Past	1.3	1.6	0.6	1.3
Current	13.1	22.5	<0.0001	18.1
Fish consumption (≥1 serving/wk)	59.4	62.0	0.4	60.2
CFH-rs1061170				
TT	39.3	25.0	0.5†	20.0
CT	46.7	75.0		60.0
CC	14.0	0.0		20.0
ARMS2-rs10490924				
GG	61.8	25.0	0.2†	50.0
GT	34.0	75.0		50.0
TT	4.3	0.0		0.0

ARMS2 = age-related maculopathy susceptibility gene 2 (T risk allele); *CFH = complement factor H* (C risk allele); CI = confidence interval.
**P* value for difference between participants examined and not examined (or had no photographs), excluding those who had died.
†Unadjusted tests for heterogeneity used to calculate *P* values.

Table 2. Fifteen-Year Incidence* of Late and Early Age-Related Macular Degeneration Lesions by Age and Sex

	Age at Baseline (Yrs)											
	<60		60–69		70–79		≥80		All Ages		Women	Men
Incident AMD Lesions	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	No. at Risk	Incidence (%)	Incidence (%)	Incidence (%)
GA	774	0.4	960	2.1	519	9.5	102	19.3	2355	2.6	2.5	2.8
Neovascular AMD	778	0.7	988	4.8	547	12.9	108	13.8	2421	4.4	5.2	3.3
Any late AMD	778	1.1	988	6.8	547	20.2	108	27.3	2421	6.8	7.5	6.0
Early AMD	737	8.7	855	26.9	388	51.4	56	29.3	2036	22.7	25.7	18.4
Indistinct/reticular drusen	755	7.1	918	21.0	440	40.3	72	19.2	2185	18.1	21.5	13.5
Pigmentary abnormality	729	21.4	871	32.4	440	48.7	83	42.6	2124	31.1	32.1	29.6

AMD = age-related macular degeneration; GA = geographic atrophy.
*Using Kaplan–Meier estimates incorporating persons censored up to the 5-, 10-, and 15-year examinations.

Table 3. Number and Proportion of Participants Who Developed Late Age-Related Macular Degeneration over 15 Years by Levels of the Age-Related Eye Disease Study Simplified Severity Scale for Age-Related Macular Degeneration at Baseline

	Pigment Abnormality					
	None (C1)		1 Eye (C2)		2 Eyes (C3)	
Drusen Size and No. of Eyes	No. of Events/ No. of Subjects	%*	No. of Events/ No. of Subjects	%*	No. of Events/No. of Subjects	%*
None or small, only 1 or both eyes (R1)	19/1573	1.2 (0.6)	3/67	4.5 (1.5)	4/19	21.1 (21.1)
Intermediate, 1 eye (no large) (R2)	10/224	4.5 (2.7)	3/25	12.0 (4.0)	5/12	41.7 (36.4)
Intermediate, both eyes (no large) (R3)	6/55	10.9 (7.4)	3/10	30.0 (20.0)	0/4	0.0 (0.0)
Large, 1 eye (R4)	7/61	11.5 (8.1)	4/14	28.6 (28.6)	5/26	19.2 (11.5)
Large, both eyes (R5)	5/18	27.8 (27.8)	3/9	33.3 (33.3)	13/17	76.5 (70.6)

AREDS Step 0 = R1C1 and R2C1; no retinal pigment changes with no or small hard drusen in 1 or both eyes or intermediate (but not large) drusen in 1 eye only.
AREDS Step 1 = R1C2, R2C2, R3C1, and R4C1; pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/no pigment changes in either eye but intermediate drusen in both eyes or large drusen in 1 eye.
AREDS Step 2 = R1C3, R2C3, R3C2, R4C2, and R5C1; pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye/no pigment changes in either eye but large drusen in both eyes.
AREDS Step 3 = R3C3, R4C3, R5C2; pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye/pigment changes in 1 eye with large drusen in both eyes.
AREDS Step 4 = R5C3; pigment changes in both eyes with large drusen in both eyes.
*The BMES 10-year incidence rates are in parentheses.

with a greater risk of 15-year incident early AMD. There was no significant association between smoking or fish consumption and 15-year early AMD incidence. Current smoking at baseline and the presence of at least 1 risk allele of *CFH-rs1061170* (ORs, 1.9–3.8) or *ARMS2-rs10490924* (ORs, 1.8–4.9) were significantly associated with an increased risk of 15-year incident late AMD. Conversely, the incidence of late AMD was significantly reduced among persons who consumed ≥1 serving of fish per week (OR, 0.5) compared with those who consumed fish less than weekly. These associations remained significant after multivariable adjustment as shown in Table 4.

Table 5 presents the eye-specific associations between the baseline early AMD lesion characteristics and the 15-year incidence of late AMD. After adjusting for age, sex, and smoking, the presence of large drusen (OR, 7.0), indistinct soft drusen (OR, 19.3), drusen location closer to the foveal center (OR, 7.3–21.0), and larger drusen area (OR, 7.2–30.9) were highly predictive of the late AMD development. Likewise, the presence of retinal pigmentary abnormalities (OR, 6.6) was associated with an increased risk of late AMD, as was each step increment in the AREDS 5-Step scale (ORs, 4.8–169.4). These associations remained after further adjustment for fish

Table 4. Common Age-Related Macular Degeneration Risk Factors Associated with 15-Year Incidence of Early and Late Stage Age-Related Macular Degeneration

	15-Year Incidence of Age-Related Macular Degeneration			
	Early AMD		Late AMD	
Risk Factor	Age- and Sex-Adjusted OR (95% CI)	Multivariable-Adjusted OR (95% CI)*	Age- and Sex-Adjusted OR (95% CI)	Multivariable-Adjusted OR (95% CI)*
Age, per 10 yrs	1.12 (1.10–1.14)	1.11 (1.09–1.14)	1.17 (1.13–1.20)	1.20 (1.16–1.25)
Sex (male)	0.70 (0.55–0.90)	0.66 (0.48–0.90)	0.77 (0.50–1.16)	0.80 (0.48–1.32)
Smoking (current)	1.14 (0.74–1.73)	1.45 (0.90–2.36)	3.96 (2.31–6.80)	3.63 (1.86–7.06)
CFH-rs1061170				
TT	1.00	1.00	1.00	1.00
CT	1.18 (0.88–1.59)	1.08 (0.78–1.51)	1.91 (1.12–3.29)	2.25 (1.22–4.15)
CC	2.81 (1.97–4.00)	2.56 (1.71–3.84)	3.77 (2.03–6.99)	4.45 (2.19–9.03)
ARMS2-rs10490924				
GG	1.00	1.00	1.00	1.00
GT	1.63 (1.24–2.14)	1.53 (1.12–2.08)	1.79 (1.14–2.80)	2.59 (1.56–4.31)
TT	2.58 (1.38–4.81)	2.16 (1.07–4.37)	4.88 (2.06–11.55)	5.81 (2.09–16.12)
Fish consumption (≥1 servings/wk)	0.90 (0.69–1.17)	0.92 (0.68–1.24)	0.45 (0.29–0.71)	0.48 (0.29–0.79)

ARMS2 = age-related maculopathy susceptibility gene 2 (T risk allele); *CFH = complement factor H* (C risk allele); CI = confidence interval; OR = odds ratio.
*Multivariable-adjusted logistic regression model including age, sex, smoking, *CFH*, *ARMS2* polymorphisms, and fish consumption.

Table 5. The 15-Year Risk of Late Age-Related Macular Degeneration by Baseline Early Age-Related Macular Degeneration Characteristics

Baseline Early AMD Lesion Characteristics*	Crude Late AMD Incidence (%)			Age-, Sex-, and Smoking-Adjusted OR (95% CI)	Multivariable-Adjusted OR (95% CI)†
	5 Years	*10 Years*	*15 Years*		
Eye-Specific					
Maximum drusen size					
None or <125 μm	0.5	3.1	3.0	1.0	1.0
≥125 μm	13.0	39.7	14.3	7.0 (4.0–12.2)	7.4 (4.0–14.0)
Drusen type					
None or small drusen <125 μm	0.5	2.9	3.0	1.0	1.0
Distinct soft drusen	1.3	11.5	3.7	1.3 (0.5–3.9)	1.6 (0.5–5.1)
Indistinct soft drusen	22.6	62.5	50.0	19.3 (9.7–38.6)	21.4 (9.4–48.5)
Drusen location					
None or <63 μm with an area <250 μm in diameter	0.3	2.0	2.5	1.0	1.0
1500–3000 μm from foveal center	1.0	2.6	5.9	1.0 (0.3–2.8)	1.4 (0.5–4.3)
500–1500 μm from foveal center	9.3	39.3	21.4	7.3 (3.7–14.5)	8.2 (3.7–18.2)
Within 500 μm radius of foveal center	17.8	51.2	50.0	21.0 (10.7–41.0)	18.5 (8.7–39.2)
Drusen area					
None or <375 μm in diameter	0.5	2.9	3.0	1.0	1.0
≥375 μm in diameter to <0.5 disc area	7.0	34.8	16.7	7.2 (3.2–16.3)	7.9 (3.2–19.7)
≥0.5 disc area	30.0	79.0	66.7	30.9 (13.8–69.2)	33.0 (12.3–88.4)
Retinal pigmentary abnormality					
Absent	0.4	3.4	2.8	1.0	1.0
Present	10.9	34.6	13.1	6.6 (3.9–11.1)	7.2 (4.1–12.8)
Person-Specific					
AREDS simplified AMD severity scale					
Step 0‡	0.2	1.8	1.9	1.0	1.0
Step 1§	2.4	9.0	13.3	4.8 (2.5–9.3)	7.8 (3.6–17.0)
Step 2‖	11.0	50.0	10.5	12.6 (6.2–25.6)	15.2 (6.8–34.0)
Step 3¶	12.8	9.1	22.2	10.1 (3.5–28.8)	13.1 (3.3–52.1)
Step 4#	47.1	80.0	100.0	169.4 (55.2–519.9)	119.4 (24.6–580.5)

AMD = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; CI = confidence interval; OR = odds ratio.
*Assessed within the anatomic macular area (within 3000 μm radius of the foveal center).
†Multivariable ORs adjusted for age, sex, smoking status, *CFH-rs1061170*, *ARMS2-rs10490924*, and fish consumption.
‡No retinal pigment changes with no or small hard drusen in 1 or both eyes or intermediate (but not large) drusen in 1 eye only.
§Pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/no pigment changes in either eye but intermediate drusen in both eyes or large drusen in 1 eye.
‖Pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye/no pigment changes in either eye but large drusen in both eyes.
¶Pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye/pigment changes in 1 eye with large drusen in both eyes.
#Pigment changes in both eyes with large drusen in both eyes.

consumption and *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms.

Discussion

We found an overall 15-year incidence of 22.7% for early AMD and 6.8% for late AMD in persons aged 49 years or more in this older Australian cohort. After adjusting for the competing risk of death, the incidence of early and late AMD was 15.1% and 4.1%, respectively. The 15-year incidence of late AMD or late AMD lesions increased with increasing age, but the incidence of early AMD decreased in persons 80 years of age or older at baseline. Women had a higher 15-year incidence of any AMD compared with men.

Our study is one of very few studies to report the long-term (>10 years) incidence of AMD from an older population-based cohort. The Copenhagen City Eye Study[13] reported the 14-year incidence of early and late AMD as 31.5% and 14.8%, respectively, considerably higher than our incidence estimates observed in the BMES. The older age range (60–80 years) of the Copenhagen study sample and the low 14-year follow-up rate of 38%[13] could explain the relatively high incidence found in this study. In the BDES, the 15-year early AMD incidence was 14.3%, whereas the late AMD incidence was 3.1%,[11] comparable to our 15-year incidence estimates in the BMES after age standardization to the BDES population.

We found that the overall 15-year incidence of both early and late AMD was substantially higher compared with the 10-year incident rates in the BMES cohort. However, the 15-year incidence of early AMD was relatively lower among participants aged 80 years or older, dissimilar to previous findings of 10-year early AMD incidence in the same cohort.[10] The lower incidence in this

oldest old age group may be due to high mortality and the low number of subjects who were at risk of early AMD at this age.

The AREDS simplified severity scale was previously validated using the 10-year incidence of late AMD data from the BMES.[10] We found that the probabilities of developing late AMD by baseline AREDS scale Steps 0 and 1 over 15 years were twice as high as the 10-year late AMD incidence from the same steps, whereas the probabilities of developing late AMD by baseline AREDS scale Steps 2, 3, or 4 (when both eyes had large soft drusen or retinal pigmentary changes) over 15 years were somewhat similar to the 10-year late AMD incident rates.[10] This observation could suggest that the time needed for progression from severe early AMD to late AMD likely may be within 10 years or less, whereas the duration of 15 years is more applicable to those with less severe stages of early AMD at baseline.

Second-eye incidence of late AMD has been reported in a number of clinic-based studies.[23–26] In the BMES, we found a substantially higher incidence of early or late AMD in the second eye of persons with early or late AMD in the first eye at baseline. This is comparable to previous observations in the BDES, which reported a 39% second-eye incidence of late AMD in those with unilateral late AMD at baseline.[11]

We previously demonstrated that the incidence of specific early AMD lesions and late AMD was greater in women compared with men.[9,10] Although we found a similar pattern in this report, the association between female sex and 15-year incident AMD was only significant for early but not for late AMD. We also found that current smoking at baseline was significantly associated with incident late but not incident early AMD over 15 years. This is in keeping with previous findings from the BMES and other studies of white populations, including the Rotterdam study.[12,27–29] Weekly fish consumption of at least 1 or more servings was associated with a reduced risk of late but not early AMD over the 15-year follow-up period. Similar findings were reported in a meta-analysis of pooled data from 9 studies, in that fish intake at least twice a week was associated with reduced risks of early and late AMD.[30] Consistent with genetic knowledge of AMD, 2 risk alleles of *CFH* and 1 or 2 risk alleles of *ARMS2* were significantly associated with increased risk of early and late AMD over the longer term, with a greater risk magnitude for late AMD.[31]

Increasing severity of baseline early AMD lesions, including larger drusen size ($\geq$125 μm), drusen location closer to the fovea, larger area involved by drusen, and the presence of retinal pigment epithelial abnormalities, has been well recognized to predict the incidence of late AMD.[6,7,10,11] We also previously reported a high risk of developing GA from eyes with indistinct soft and reticular drusen, central location, and larger area involved by drusen over 15 years.[32] These associations were generally consistent across the 10- and 15-year AMD incidence, although with different risk magnitude: Magnitude of the risk of late AMD associated with baseline early AMD lesion characteristics was higher for the 10-year than for the 15-year incidence, particularly so for central location of soft drusen. This observation may suggest that early AMD characteristics are indicative of a more severe stage likely leading to late AMD within 10 years rather than 15 years.

Study Limitations

We were able to follow approximately 75% of survivors of this older cohort at the 5- and 10-year visits. However, follow-up rate reduced to only 56% by the time the 15-year examinations were performed. This could have introduced selection bias due to selective follow-up and survival. Persons who died were older and more likely to have chronic systemic conditions and disabilities. Participants who were lost to follow-up were likely to have been younger (mean age, 61 vs. 64 years) and more likely to smoke (23% vs. 13%) at baseline. It is thus likely that our estimates of the 15-year incidence of AMD could have been underestimated, although the "true incidence" would not be more useful than the current estimate, because only those who survived demand eye health and aged care services.

Strengths of this study include the relatively long-term follow-up of a population-based cohort, the use of retinal photographs to document macular conditions, and a validated AMD grading system to assess the size and location of AMD lesions. A major limitation of our study is the substantial number of participants lost to follow-up at the 15-year visit, as mentioned previously. A further limitation includes the lack of high-resolution imaging (e.g., spectral-domain optical coherence tomography), unavailable at the time of the BMES examinations, which might have increased the ability to detect some AMD lesions, such as early-stage GA.

In summary, we documented the 15-year incidence of early and late AMD in an older Australian cohort, particularly among persons with no lesions or only early-stage lesions at baseline. Our incidence rates for early and late AMD were comparable to those reported in the BDES population over the same follow-up period. Current smoking at baseline was a stronger risk factor for 15-year incidence of late AMD than early AMD.

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **AREDS** = Age-Related Eye Diseases Study; ***ARMS2*** = *age-related maculopathy susceptibility 2*; **BDES** = Beaver Dam Eye Study; **BMES** = Blue Mountains Eye Study; ***CFH*** = *complement factor H*; **CI** = confidence interval; **GA** = geographic atrophy; **OR** = odds ratio; **SNP** = single nucleotide polymorphism.

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Incidence and Progression of Geographic Atrophy

Observations from a Population-based Cohort

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Purpose: To examine early age-related macular degeneration (AMD) lesion characteristics and risk factors associated with the long-term development and progression of geographic atrophy (GA).

Design: Population-based cohort.

Participants: Of 3654 participants aged ≥49 years in the Blue Mountains Eye Study, 75.8%, 76.7%, and 56.1% of survivors attended the 5-, 10-, and 15-year follow-up examinations, respectively.

Methods: Retinal photographs were taken at each visit. Incident GA was confirmed using a side-by-side grading method. Computer planimetry was used to measure the area involved by GA. Fast and slow/normal progression rates were defined as GA area enlargement by ≥2 and <2 mm^2/year, respectively. Incident GA was estimated using the Kaplan–Meier product-limit method. Early AMD lesion characteristics were assessed for association with GA incidence using eye-specific data and generalized estimating equation models adjusting for age, current smoking, and presence of risk alleles of the complement factor H (*CFH*) or age-related maculopathy susceptibility 2 (*ARMS2*) genes, genotyped or imputed using genome-wide scan data.

Main Outcome Measures: Incidence and progression of GA.

Results: By excluding 41 subjects with GA at baseline, of 2503 participants at risk of GA, incident pure GA (without coexisting neovascular AMD lesions) was confirmed in 57 participants, with a 15-year incidence of 3.6%. Baseline early AMD lesion characteristics associated with GA incidence included drusen type (soft indistinct: odds ratio [OR], 59.0; 95% confidence interval [CI], 20.4–171.0; reticular drusen: OR, 13.9; 95% CI, 4.0–47.6); drusen location within a 500-μm radius of the fovea (OR, 15.1; 95% CI, 7.4–30.8); drusen area greater than 375 μm in diameter (OR, 10.1; 95% CI, 4.0–25.6); presence of retinal pigment epithelial depigmentation (OR, 9.0; 95% CI, 4.1–19.8); or hyperpigmentation (OR, 12.0; 95% CI, 6.1–23.5), referenced to subjects with no or hard drusen only. Fast progression was more frequent among current smokers at baseline, subjects with the *CFH* or *ARMS2* risk genotypes, and pseudophakic eyes.

Conclusions: Early AMD lesion characteristics (type, location, area involved) were strongly associated with higher long-term risk of developing GA independent of age, smoking, and AMD genetic susceptibility from the *CFH* or *ARMS2* genes. Known AMD risk factors also were more frequently present among quickly progressing GA cases.

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Geographic atrophy (GA) is 1 of 2 types of age-related macular degeneration (AMD) and is characterized by a sharply defined area of retinal pigment epithelium (RPE) degeneration in which choroidal blood vessels are visible.[1] It accounts for approximately 35% to 40% of late-stage AMD cases.[2] There is currently no effective treatment for GA, and once the foveal center is involved, affected patients are deprived of central vision and may develop legal blindness.[3–5]

Although the short-term (e.g., 2–5 years) incidence of GA is available from population-based studies,[6–9] there are limited data available on long-term incidence (over 10 to 15 years). Two large population-based studies with long-term follow-up, the Beaver Dam Eye Study (BDES) and the Blue Mountains Eye Study (BMES), reported that the 10-year incidence of pure GA was 0.8%[10] and 1.7%,[11] respectively. The 15-year incidence of pure GA in the BDES was 1.3%.[12] In both studies, the presence of large drusen and retinal pigmentary abnormalities was found to be associated with increased long-term incidence of late AMD, including GA and neovascular AMD.[10,11,13,14] However, population-based data for the associations of early AMD lesions with long-term incidence of pure GA are limited.

The natural history and progression of GA have been assessed in both the BDES and BMES populations[4,15] and clinic-based cohort studies.[16,17] Larger atrophic areas at baseline were found to be associated with fast progression of GA lesions,[3,16] and the shape of the atrophic area, termed "GA configuration" (classic, multifocal, and merged), also appears to have different progression rates.[4] In a previous BMES report, 5-year progression of GA occurred in 43%

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of eyes with GA at baseline, where GA progression was defined as an increase in the atrophic area by ≥2 subfields of the Wisconsin grading grid or by extension of atrophy into the foveal center without quantitative measures.[15]

This report examines the 15-year incidence of pure GA and associations of early AMD lesion characteristics with GA incidence in the BMES cohort. We also assess the 5-year GA progression rate, indicated by quantitative measures of the size of atrophic areas and GA involvement with respect to the fovea, and explore factors associated with fast versus slow progression of prevalent and incident cases of pure GA.

Methods

Study Population

The BMES is a population-based cohort study of vision and eye disease in persons aged 49 years or older residing in the Blue Mountains region west of Sydney, Australia.[18,19] The baseline study recruited and examined 3654 participants (82.4% of those eligible) between 1992 and 1994 (BMES I). Of these, 2334 participants (75.8% of survivors) attended the 5-year follow-up examinations (1997−1999; BMES II). The 10-year (2002−2004; BMES III) and 15-year (2007−2009; BMES IV) follow-up examinations were attended by 1952 participants (76.7% of survivors) and 1149 participants (56.1% of survivors), respectively. All examinations were approved by the Western Sydney Area Health Service and University of Sydney Human Research Ethics Committees and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

At each visit, a comprehensive questionnaire was administered and eye examinations were performed, as previously described.[18] This included 30° stereoscopic retinal fundus photographs of the macula and other retinal fields of both eyes using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany) at the BMES I, II, and III examinations and 40° degree digital photographs with a Canon CF-60 DSi (Canon Inc., Tokyo, Japan) at the BMES IV examination. Visual acuity was assessed following the Early Treatment Diabetic Retinopathy Study protocol.[19] Blood samples were collected at the BMES II examination. DNA extraction and genotyping were performed in more than 80% of the BMES participants.

Photographic Grading

The details of photographic grading have been reported elsewhere[18] and closely followed the Wisconsin Age-Related Maculopathy Grading System (WARMGS) protocol.[1] For this report, side-by-side grading of retinal photographs of each subject taken at the BMES I, II, III, and IV was performed to confirm incident pure GA cases. Incident pure GA was defined as the first appearance of GA in either eye of subjects who had no sign of GA at the previous visit and no neovascular AMD (including pigment epithelial or sensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars) at all previous and current examinations. All incident late AMD cases including incident GA cases also were confirmed by BMES, BDES, and Rotterdam Eye Study principal investigators.

Circles of defined diameters (63, 125, 250, 350, and 644 μm and 0.5 disc area, and 1 disc area) were used to estimate the size of drusen, RPE depigmentation and hyperpigmentation, and the areas involved by each lesion.[1] The locations of lesions were defined as within 500 μm, between 500 and 1500 μm, and between 1500 and 3000 μm radius of the foveal center, as defined by the WARMGS grid.[1] The presence and absence of drusen and pigmentary abnormalities were graded first, followed by detailed grading of size, location, and area of lesions. Early AMD was defined as the presence of large (>125-μm diameter), indistinct soft or reticular drusen or both large distinct soft drusen and retinal pigmentary abnormalities within the macular area.

Computer Planimetry

Geographic atrophy progression was assessed in both prevalent (from baseline examination, BMES I) and incident pure GA cases (detected at follow-up visits, BMES II or III) that had retinal photographs available at subsequent visits. Progression was measured as increasing size of the atrophic areas over 5 years from one BMES examination to the next and converted to progression rate in millimeters squared per year. The retinal photographs from the BMES I, II, and III, on 35-mm film, were scanned using a CanoScan 5600F scanner (Canon Inc.) at 2400 dots per inch. The digital format of the WARMGS grid was enlarged according to the resolution of the scanned photographs using Adobe Photoshop CS4 (Adobe Systems Inc., San Jose, CA) so that the measurements could be performed digitally. The resolution of the grid was amended according to the resolution of the BMES IV digital photographs when used for images taken at the BMES IV. A random sample of retinal photographs without pathology from each BMES examination was used to obtain the scaling factor (microns per pixel) using Photoshop. This was calculated by dividing 4500 μm, taken as the constant distance between the center of the optic disc to the center of the fovea, by the same distance measured in pixels to obtain the number of microns per pixel. This scale factor was then set into ImageJ software (Rasband WS. ImageJ software [1997−2012]. Bethesda, MD: US National Institutes of Health; available at: http://imagej.nih.gov/ij/; accessed August 10, 2011) to allow measurements to be read in microns. Tracings were made along the margin of GA and area measurements were obtained using the "region of interest" manager.

Definition of Geographic Atrophy Progression

The average progression of GA was calculated using data from the BMES, BDES,[4] and Age-Related Eye Disease Study (AREDS)[20] and was found to be an area between 1 and 2 mm^2 per year. We defined this as the normal progression rate. If the GA progression rate was greater than 2 mm^2/year, this was defined as fast progression. If the GA progression rate was less than 1 mm^2/year, this was defined as slow progression. Figure 1 shows examples of cases with different progression rates.

The configuration of GA has been used and described by previous studies.[4,21] The "classic" configuration is defined as a single round atrophic area, the "multifocal" configuration is defined as 2 or more areas of atrophy within the macula region, and the "merged" configuration is defined as 2 or more initially separate areas (multifocal) that subsequently amalgamated into a large irregular atrophic area. We added a "mixed" configuration in our report to include eyes with 2 or more configuration types described earlier.

Geographic atrophy progression was assessed using 2 indicators: enlargement of the atrophic area and progression to involve the fovea associated with worsened best-corrected visual acuity (BCVA) in eyes that had not yet developed GA at the fovea when it was first detected.

Genotyping

The complement factor H (*CFH*) single nucleotide polymorphism (SNP) rs1061170 and the age-related maculopathy susceptibility 2 (*ARMS2*) SNP rs10490924 were genotyped in 1874 and 593

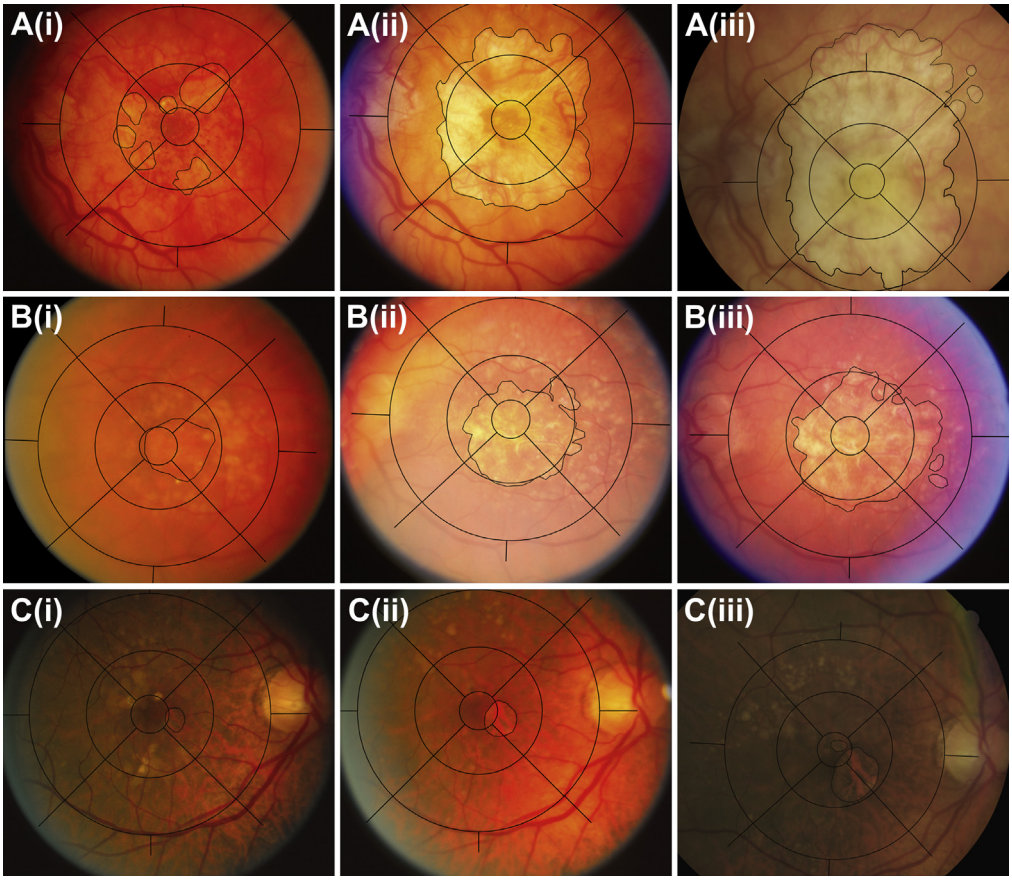

Figure 1. Examples of the fast **(A)**, normal **(B)**, and slow **(C)** progression of geographic atrophy (GA) by enlargement of atrophic areas. Tracings around the GA perimeter show the increase in area from the first detection of GA (i) to 5 years (ii) and 10 years after the first detection of GA (iii).

participants, respectively, using TaqMan assays (Applied Biosystems, Foster City, CA)[22] and restriction fragment length polymorphism analysis.[23] In the remaining subjects, imputed SNPs of these 2 genes were obtained from BMES genome-wide association scan data with imputation. For the latter, genotyping was performed using the Illumina Human 670-Quad custom array (version 1), and stringent quality control was performed using PLINK (PLINK v1.07. Available at: http://pngu.mgh.harvard.edu/purcell/plink/. Accessed June 7, 2012).[24] Imputation from the 1000 Genomes was performed using IMPUTE 2.0.[25] The imputation r^2 was 0.968 for *CFH*-rs1061170 and 0.996 for *ARMS2*-rs10490924. In our study sample, 1501 and 509 participants had both typed and imputed rs1061170 and rs10490924, respectively, and the concordance rates between typed and imputed SNPs were 99.6% for rs1061170 and 99.2% for rs10490924. Of 2503 participants used in our analyses, 151 had rs1061170 imputed and 1287 had rs10490924 imputed.

Definition of Baseline Variables Associated with Geographic Atrophy

Smoking history was obtained from an interviewer-administered questionnaire. If participants answered "no" to smoking regularly, they were classified as nonsmokers. If participants answered "yes" and had given up smoking ≥1 year before the baseline examination, they were classified as past smokers. Current smokers were defined as participants who currently smoked or stopped smoking <1 year before baseline examinations. Regular fish consumption was assessed from a food frequency questionnaire completed by participants and defined as 1 or more servings per week, compared with infrequent consumption (<1 serving per week).

Statistical Methods

Analyses were performed using SAS version 9.1 (SAS Inc., Cary, NC). Fifteen-year person-specific GA incidence was assessed using Kaplan–Meier product limit survival estimates, including overall and incident rates by the AREDS simplified severity scale (5 steps)[26] according to baseline AMD levels. Estimates were then recalculated after adjusting for the competing risk of death. The traditional Kaplan–Meier procedure assumes that participants censored during the study, because of death or loss to follow-up, remain at similar risk of developing GA as those who completed the study (does not differentiate risk of GA between those who died and those who survived). A competing risk approach takes into account the probability of survival of participants between the 2 time points and the associated reduced risk of developing GA among those who died; because they died before developing GA, they are no longer at risk.

Associations between 15-year incidence of GA and common AMD risk factors (age, current smoking status, sex, presence of risk alleles of *CFH*-rs1061170 or *ARMS2*-rs10490924, and regular fish consumption) were assessed using age-adjusted and multivariable-adjusted discrete logistic regression models, and expressed as odds ratios (ORs). *CFH*-rs1061170 and *ARMS2*-rs10490924 were assessed categorically, in a general

model, using homozygous wild genotype (no risk alleles) as the referent and heterozygous (1 risk allele) and homozygous risk genotypes (2 risk alleles) as separate categories. These SNPs also were assessed in an additive model where genotype was treated as a continuous variable. Eye-specific data were used to assess the association between early AMD lesion characteristics (type, area, and location of early lesions) and the 15-year incidence of GA. To handle the correlation between the 2 eyes of the same individual and multiple measures of the same eyes (observations from the same individual are in a cluster), the GENMOD procedure in SAS was used to perform generalized estimation equation modeling with an exchangeable working correlation matrix, using subject identification as a cluster indicator (The GENMOD Procedure. SAS/STAT 9.1 User's Guide. Cary, NC: SAS Inc.). Because of the small number of cases available for assessment of GA progression, only descriptive data are provided.

Results

Of 3654 baseline participants, 2572 had been followed-up at least once since the baseline examination. Of these, 68 participants had late AMD at baseline or developed neovascular AMD by first follow-up after baseline (and were thus not at risk of developing GA), and 1 participant had ungradable photographs at all visits. This left 2503 participants included in the assessment of GA incidence. Table 1 presents baseline characteristics of the BMES participants with incident GA and without any late AMD. Subjects with incident GA were significantly older and had significantly worse BCVA in both their better and worse eyes compared with those without late AMD. Participants with incident GA also were more likely to have 2 risk alleles of the *CFH* gene compared with those without any late AMD. The frequency of 1 or 2 *ARMS2* gene risk alleles was slightly higher in subjects with incident GA, but the differences were not statistically significant (Table 1).

Table 1. Comparison of Baseline Characteristics between Participants with and without Incident Geographic Atrophy, Examined up to 15 years in the Blue Mountains Eye Study Cohort

Characteristic	Participants*		*P Value*†
	No Late AMD (n=2446)	*Incident GA (n=57)*	
Mean age, yrs (SD)	63.9 (8.5)	72.3 (6.7)	<0.0001
Mean BCVA, no. of letters read (SD)			
Better eye	56.2 (4.8)	52.8 (5.5)	<0.0001
Worse eye	51.5 (10.9)	46.5 (10.6)	0.0006
Sex (male)	42.6	36.8	0.4
Smoking			
Current	12.9	19.6	0.1
Past	35.7	30.4	0.4
Fish consumption (≥1 serving/wk)	60.4	43.5	0.02
CFH-rs1061170			
TT	40.3	24.0	0.0
CT	46.5	46.0	0.9
CC	13.3	30.0	0.0007
ARMS2-rs10490924			
GG	62.5	54.0	0.2
GT	33.6	40.0	0.3
TT	4.0	6.0	0.5

AMD = age-related macular degeneration; *ARMS2* = age-related maculopathy susceptibility gene 2 (T risk allele); BCVA = best-corrected visual acuity; *CFH* = complement factor H (C risk allele); GA = geographic atrophy; SD = standard deviation.
*Data shown as percentages unless otherwise indicated.
†Mantel–Haenszel chi-square.

Incidence of Pure Geographic Atrophy

Incident pure GA was identified in 57 participants (82 eyes) of 2503 subjects at risk, with an overall 15-year incidence of 3.6% (95% confidence interval [CI], 2.7–4.7). After accounting for the competing risk of death, the 15-year incidence of GA decreased to 2.2% (95% CI, 1.6–2.8). After age standardization to the Australian census 2011 population aged ≥50 years, the estimated 15-year incidence became 1.8% (95% CI, 1.2–2.4). Bilateral involvement occurred in 27 of the 57 participants (47.4%). Of the participants with bilateral involvement, 21 had bilateral involvement at the same follow-up visit, 4 had bilateral involvement at the subsequent follow-up visit, and 2 who had unilateral GA at baseline developed incident GA in the fellow eye during the follow-up period.

Table 2 presents the incidence of pure GA at the 5-, 10-, and 15-year follow-up examinations by different stages of AMD at baseline according to the AREDS 5-step severity scale.[26] Age was significantly associated with advancing severity level (*P* for trend <0.0001). At each of the follow-up visits, incidence of pure GA increased with increasing baseline AREDS severity step from 0 to 4, except for cases with AREDS scale step 3 (Table 2).

Age-related macular degeneration risk factors assessed for association with the 15-year incidence of GA are shown in Table 3. Increasing age was significantly associated with the development of GA within 15 years ($P < 0.0001$). In an age-adjusted model, current smoking (P=0.0001) and homozygous risk genotypes of *CFH*-rs1061170 (P=0.002) and *ARMS2*-rs10490924 (P=0.04) were associated with an increased risk of incident GA. There was no significant sex difference found in the long-term risk of GA. Regular fish consumption had a significant protective effect against the development of GA (P=0.02). In a model simultaneously adjusting for the above-mentioned risk factors, the significant associations of age, current smoking, and genetic risks from the *CFH* and *ARMS2* genes with GA incidence remained, and the association of weekly fish consumption with incident GA became nonsignificant (Table 3).

Table 4 demonstrates the relationship between early AMD lesion characteristics and 15-year incidence of GA using eye-specific data. Drusen characteristics that were strongly associated with increased risk of developing GA over the 15-year period included soft indistinct (OR, 28.58) and reticular drusen (OR, 14.39), drusen within a 500-μm radius of the foveal center (OR, 9.97), and a collective drusen area >375 μm in diameter (OR, 7.62–33.36). The baseline presence of RPE depigmentation (OR, 6.99) and hyperpigmentation (OR, 11.27) was significantly associated with a higher risk of developing pure GA over the same period, as was location of pigmentary abnormalities within a 1500-μm radius of the foveal center. These associations remained significant after adjusting for age, sex, smoking, regular fish consumption, and presence of the *CFH* and *ARMS2* risk alleles (Table 4).

Progression of Pure Geographic Atrophy

To investigate the progression of pure GA, both prevalent (n=7) and incident GA cases (n=12) with gradable follow-up retinal photographs were included. There were 41 baseline participants with GA, but only 7 were pure GA cases (10 eyes) and had follow-up retinal photographs available for this analysis. There were

Table 2. Incidence of Pure Geographic Atrophy at the 5-, 10-, and 15-Year Follow-up Visits of the Blue Mountains Eye Study Population by the Age-Related Eye Disease Study (AREDS) 5-Step Severity Scale[26]

AREDS Category at Baseline	No. of Participants (n=2503)	Mean Age, Yrs ± SD	Mean BCVA in Worse Eye, No. of Letters Read ± SD	Incidence GA (%)		
				5 Yrs	10 Yrs	15 Yrs
Step 0*	1873	62.6±7.9	53.4±6.8	0.0	0.2	1.0
Step 1†	207	67.4±8.4	50.0±10.8	0.5	4.7	9.6
Step 2‡	72	71.3±9.0	50.6±7.4	6.9	23.9	38.4
Step 3§	36	71.1±8.5	49.5±7.8	8.3	8.3	19.8
Step 4‖	13	70.5±7.0	48.1±9.7	30.8	60.4	100.0

BCVA = best-corrected visual acuity; GA = geographic atrophy; SD = standard deviation.
*No retinal pigment changes with none or small hard drusen in 1 or both eyes or intermediate (but not large) drusen in 1 eye only.
†Pigment changes in 1 eye with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/no pigment changes in either eye but intermediate drusen in both eyes or large drusen in 1 eye.
‡Pigment changes in both eyes with small hard drusen in 1 or both eyes or intermediate drusen in 1 eye only/pigment changes in 1 eye with intermediate drusen in both eyes or large drusen in 1 eye/no pigment changes in either eye but large drusen in both eyes.
§Pigment changes in both eyes with intermediate drusen in both eyes or large drusen in 1 eye/pigment changes in 1 eye with large drusen in both eyes.
‖Pigment changes in both eyes with large drusen in both eyes.

16 participants (23 eyes) with incident GA and follow-up retinal photographs, and 4 persons (5 eyes) were excluded because of subsequent development of neovascular AMD, leaving a total of 19 participants (28 eyes) who met the criteria for analysis of progression rate.

The average size of the GA lesions at first observation among eyes with baseline pure GA and incident pure GA was 5.0 (standard deviation [SD], 7.0) and 4.6 mm^2 (SD, 4.5 mm^2), respectively. Five years after the first detection of GA, the average size of baseline pure GA lesions had increased to 13.0 mm^2 (SD, 8.8 mm^2) and incident GA lesions had increased to 15.9 mm^2 (SD, 8.1 mm^2). The average GA progression rate for baseline and incident eyes combined (n=28) was 1.95 mm^2/year. Table 5 presents the 3 indicators of GA progression (enlargement of atrophic area, progression of atrophy into the fovea, and worsening in BCVA). Of participants with baseline GA, none survived to the 15-year follow-up. These participants were significantly older than participants without GA at baseline (mean age, 82.6 vs. 65.9 years, respectively). The multifocal configuration of GA had the fastest progression rate from the first detection to the next examination 5 years later, whereas the slowest progression rate was observed in eyes presenting with the classic configuration. Central GA (involving the fovea) was present in 57% (n=16) of 28 eyes with GA at the first detection. Of the remainder, 92% of 12 eyes with GA had progression into the fovea within the next 5 years. The mean BCVA deteriorated by an average 18 letters over the same period.

Table 3. Common Age-Related Macular Degeneration Risk Factors Associated with 15-Year Incidence of Geographic Atrophy

Risk Factor	15-Year Incidence of GA			
	Age-Adjusted (Where Appropriate)		Multivariate Adjusted*	
	OR	95% CI	OR	95% CI
Age, per year	1.18	1.14–1.22	1.22	1.16–1.27
Sex (male)	0.84	0.49–1.47	0.77	0.39–1.49
Smoking (current)	4.11	2.01–8.38	4.20	1.76–10.02
CFH-rs1061170†	1.92	1.28–2.88	1.99	1.26–3.15
TT	1.00		—	—
CT	1.66	0.82–3.38	—	—
CC	3.65	1.66–8.03	—	—
ARMS2-rs10490924†	1.66	1.02–2.70	2.38	1.41–4.02
GG	1.00		—	—
GT	1.54	0.85–2.79	—	—
TT	3.32	0.96–11.53	—	—
Fish consumption (≥1 servings/wk)	0.48	0.27–0.88	0.54	0.28–1.03

ARMS2 = age-related maculopathy susceptibility gene 2 (T risk allele); *CFH* = complement factor H (C risk allele); CI = confidence interval; GA = geographic atrophy; OR = odds ratio.
*Multivariate model adjusted for age, sex, smoking, *CFH*, *ARMS2*, and regular fish consumption.
†Computed using additive model.

Table 6 presents the frequencies of risk factors in fast or slow/normal progression groups. Fast, slow, and normal progression were observed in 10, 7, and 2 participants, respectively, according to the worse eye if the cases were bilateral (n=2). A larger proportion of participants with fast progressing GA were current smokers (30% vs. 0% in the slow/normal progression group), had 1 or both eyes pseudophakic before or at the first detection of GA in the same eye (30% vs. 11%), and had the homozygous risk genotype of the *CFH* (50% vs. 22%) or the *ARMS2* (10% vs. 0%) alleles compared with subjects who had slow/normal progress. Participants who regularly consumed fish were more frequently represented in the slow/normal progress groups compared with the fast progress group (57% vs. 38%).

Discussion

In this older Australian cohort, we found an overall 3.6% incidence of pure GA over 15 years. Common AMD risk factors, including increasing age, history of smoking, genetic risk from the *CFH* and *ARMS2* alleles, and regular fish consumption, were associated with long-term incidence of pure GA. In addition, early AMD lesion characteristics strongly predicted risk of GA independent of the known risk factors discussed earlier. We also found that fast progression of GA was more likely to occur in affected individuals who were current smokers or possessed at least 1 risk allele of the *CFH* gene or both risk alleles of the *ARMS2* gene, and in pseudophakic eyes, whereas slow progression of GA was

Table 4. Relationship between Baseline Drusen and Retinal Pigmentary Abnormalities and the 15-Year Incidence of Geographic Atrophy, Analyzed by Eye

Early AMD Characteristics	No. Who Developed GA	No. at Risk	15-Year Incidence of GA			
			Age-Adjusted		*Multivariate Adjusted**	
			OR	*95% CI*	*OR*	*95% CI*
Drusen type						
None or hard drusen only	27	4094	1.00	—	1.00	—
Intermediate	24	488	3.92	2.36–6.52	5.30	2.67–10.49
Soft distinct	2	100	1.61	0.41–6.26	3.02	0.70–13.08
Soft indistinct	24	100	28.58	12.66–64.53	59.02	20.38–170.95
Reticular	15	44	14.39	5.56–37.22	13.85	4.03–47.64
Drusen location						
None or ≥3000 μm from foveal center	27	4094	1.00	—	1.00	—
1500–3000 μm from foveal center	1	42	1.93	0.38–9.92	3.40	0.49–23.73
500–1500 μm from foveal center	4	188	1.83	0.61–5.50	3.65	1.21–10.99
Within a 500-μm radius of foveal center	48	451	9.97	5.88–16.90	15.08	7.38–30.82
Drusen area						
None or <375 μm in diameter	39	4535	1.00	—	1.00	—
≥375 to <0.5 disc area	16	156	7.62	3.76–15.4	10.11	4.00–25.57
≥0.5 disc area	25	87	33.36	13.65–81.56	61.12	18.37–203.34
RPE depigmentation						
Present vs. absent	20	144	6.99	3.87–12.32	9.02	4.10–19.80
Location						
None or ≥1500 μm from foveal center	62	4881	1.00	—	1.00	—
500–1500 μm from foveal center	8	72	5.24	2.31–11.90	8.04	2.84–22.80
Within a 500-μm radius of foveal center	12	67	10.35	4.74–22.67	11.20	3.61–34.75
Area						
None to <375 μm in diameter	70	4932	1.00	—	1.00	—
≥375 μm to <2 disc areas[†]	12	88	8.40	4.04–17.46	5.62	2.07–15.28
Hyperpigmentation						
Present vs. absent	41	317	11.27	6.70–18.96	12.02	6.14–23.53
Location						
None or ≥1500 μm from foveal center	41	4723	1.00	—	1.00	—
500–1500 μm from foveal center	16	98	13.17	6.33–27.38	14.91	6.47–34.37
Within a 500-μm radius of foveal center	25	146	16.37	8.55–31.33	14.97	5.95–37.63
Area						
None or <64 μm in diameter	45	4727	1.00	—	1.00	—
≥64 μm in diameter	37	240	12.21	7.08–21.05	11.18	5.65–22.10

AMD = age-related macular degeneration; CI = confidence interval; GA = geographic atrophy; OR = odds ratio; RPE = retinal pigment epithelium.
*Multivariate model adjusted for age, sex, smoking, regular fish consumption, and the *CFH* and *ARMS2* risk alleles.
[†]There were no cases ≥2 disc areas.

more likely to occur in those who consumed 1 or more servings of fish weekly.

The BMES and BDES[12] are the only 2 studies to have reported GA incidence over a 15-year follow-up period in population-based older samples. Both study protocols and methods used, including examination procedures and retinal photographic grading of AMD signs, were similar, whereas the BDES population had a slightly younger age limit (≥43 years) than the BMES population (≥49 years). The BMES found an incidence of 3.6% that was more than double the 1.3% rate observed in the BDES[12] over the 15-year period. The 10-year cumulative incidence of GA was also double in the BMES (1.7%)[11] compared with the BDES (0.8%).[10] After accounting for the competing risk of death and age standardization of the BMES population to the general Australian population, the 15-year incidence of pure GA was 1.8%; however, the 95% CI of 1.2 to 2.4 is consistent with the finding of a 1.3% 15-year incidence reported from the BDES. Apart from the different age ranges of the 2 study samples, there are likely different environmental exposures (smoking, sunlight exposure) that could explain the difference in GA incidence between the 2 studies. The association between smoking and incidence of late AMD was somewhat dissimilar in the BMES compared with the BDES, as previously reported.[27–29] There was a higher frequency of baseline current smokers in the BDES (men 21%, women 18%) compared with the BMES (men 15%, women 11%). However, current smokers had a 4-fold greater risk of incident late AMD compared with nonsmokers in the BMES,[28] whereas no similar association was found between past or current smoking and incident late AMD (or GA) in the BDES.[29,30]

A Danish study of 946 subjects aged ≥60 years, which used a similar grading system to detect AMD from color fundus photographs, reported a 14-year incidence of GA of 4.9%,[31] substantially higher than the 15-year incidence found in the BDES and BMES. The difference could be due to an older baseline age (≥60 years) of the Danish study sample compared with the baseline ages of those in the

Table 5. Progression in Atrophic Area, Foveal Involvement, and Best-Corrected Visual Acuity Over 5 and 10 Years in Eyes with Pure Geographic Atrophy

	GA Progression					
	First Observation to 5 Yrs		Between 5 and 10 Yrs		First Observation to 10 Yrs	
Characteristics of Progression	n	Mean mm² (SD)	n	Mean mm² (SD)	n	Mean mm² (SD)
Change in mean GA area						
Prevalent (baseline) GA eyes (n=10)	9	7.7 (5.2)	3	4.5 (2.9)	4	7.5 (4.4)
Incident GA eyes (n=18)	18	11.2 (6.0)	3	8.6 (6.3)	3	20.3 (16.2)
All GA eyes (n=28)	27	10.1 (5.9)	6	6.6 (4.9)	7	13.0 (12.0)
Change in mean GA area (by GA configuration)*						
Classic	7	5.5 (5.3)	4	4.7 (4.6)	5	9.3 (10.3)
Merged	7	10.3 (6.0)	0	—	0	—
Multifocal	6	13.8 (6.2)	3	13.7 (1.0)	3	31.1 (1.9)
Mixed	7	11.2 (3.8)	1	—	1	—
	n	%	n	%	n	%
Change in foveal involvement*	11	92	0	0	2	67
	n	Difference in the mean no. of letters read correctly (SD)	n	Difference in the mean no. of letters read correctly (SD)	n	Difference in the mean no. of letters read correctly (SD)
Change in BCVA (later visit minus previous visit)*	27	−18.6 (17.9)	8	1.25 (6.6)	8	−9.5 (21.6)

BCVA = best-corrected visual acuity; GA = geographic atrophy; SD = standard deviation.
For foveal involvement, n = number of eyes with GA that progressed and number of eyes with GA that were at risk of progression into the fovea (eyes that had already developed GA in the fovea were not included).
*Prevalent and incident GA eyes were combined.

BDES or BMES. Participants aged <60 years at baseline comprised 32.5% of the BMES population compared with none in the same age group in the Danish Study, which could have accounted for the differences in the incidence rates between the 2 studies. We do not have directly comparable data from the Danish study, so direct age standardization between the 2 study samples is not possible. Because of relatively high proportions of study participants who had died or were lost to follow-up (37.9%) in the Danish study[31] and in the 15-year follow-up examinations of our study cohort, survival bias also could have affected the incidence estimates of the 2 studies.

Previous studies have documented that eyes with early AMD lesions had a substantially higher risk of progression to late AMD, including GA and neovascular AMD.[13,15,32] Therefore, the strong association between early AMD lesion characteristics and risk of developing GA found in our study is expected.

Table 6. Proportion of Participants Presenting with the Selected Risk Factors by Fast and Slow Progression of Pure Geographic Atrophy

	Participants with GA Progression (%)		
Risk Characteristics	Fast (≥2 mm²/yr) (n=10)	Slow (<1 mm²/yr) (n=7)	Slow/Normal (<2 mm²/yr) (n=9)
Mean baseline age (yrs)	71.9	72.4	75.2
Sex (male)	30.0	28.6	33.3
Smoking			
Past	40.0	28.6	33.3
Current	30.0	0.0	0.0
Fish consumption (≥1 serving/wk)	37.5	50.0	57.1
Pseudophakic*	30.0	14.3	11.1
CFH-rs1061170			
TT	10.0	71.4	55.6
CT	40.0	14.3	22.2
CC	50.0	14.3	22.2
ARMS2-rs10490924			
GG	50.0	57.1	55.6
GT	40.0	42.9	44.4
TT	10.0	0.0	0.0

ARMS2 = age-related maculopathy susceptibility gene 2; *CFH* = complement factor H; GA = geographic atrophy.
*Pseudophakia detected before or at the same examination as GA detected.

We found that average progression of GA, measured as enlargement in GA area, was 1.95 mm^2/year, which is similar to the average progression found in other population-based studies (1.2−1.8 mm^2/year)[4,20] but lower than the progression rate found in a clinic-based sample (2.6 mm^2/year).[16] In defining the progression rate in terms of enlargement in area, we have assumed a steady, similar enlargement rate in GA area across all stages of GA progression. Whether such an assumption holds is unclear, as indicated by Klein et al.[4] We also found a faster progression rate among cases with multifocal compared with classic GA configuration, consistent with the BDES finding in pure GA cases.[4] However, multifocal GA configuration usually indicates a more advanced stage of GA, and therefore the different progression rates observed may be due to differing stages of GA rather than the morphologic differences of GA configurations per se. Nevertheless, these findings were based on a small number of GA cases and should be interpreted with caution.

Older age and history of smoking have been consistently identified as risk factors for late AMD in population-based studies.[9,33−35] The association between smoking and 15-year incident GA documented in this study is in keeping with previous observations.[27,28] Past smokers in the BMES were shown to have a 3-fold higher risk of incident GA after adjusting for age, sex, and other factors as previously reported.[28] We found that similar risk factors associated with a high risk of developing GA also were associated with fast progression of GA, including genetic influence from the *CFH* and *ARMS2* genes, current smoking, and less frequent consumption of fish. The higher proportion of participants who consumed fish at least weekly in the slow/normal progression group compared with the fast progression group is consistent with evidence suggesting a beneficial effect of omega 3 fatty acids on the development and progression to either form of late AMD.[36−38] The higher proportion of pseudophakic participants in the fast progression group than in the slow/normal progression group lends support to the hypothesis that sunlight exposure also could be a risk factor for GA.[39,40]

Study Strengths and Limitations

Strengths of our study include its long-term follow-up of an older predominantly Caucasian cohort that is comparable to the BDES cohort. However, the low follow-up rate at the 15-year examinations may have led to an over- or underestimation of GA incidence. Our findings from all GA cases, including both clinical and subclinical cases, without apparent visual symptoms reflect the natural history of the condition. We were able to assess GA progression and risk characteristics associated with the progression by comparing fast with slow/normal progression groups. However, we cannot exclude the possibility that GA progresses differently at different stages of the disease and that fast progress cases may occur in a more advanced stage. Limitations of this study include the lack of optical coherence tomography measures because of timing (early 1990s, when optical coherence tomography was not available). We also could not statistically validate the association between certain risk factors and progression of GA lesions because of the small number of cases available to analyze progression. Concordance studies have shown differences in progression between bilateral GA cases and unilateral GA cases with early AMD in the fellow eye[41]; however, we were unable to assess this because of the small number of GA cases in these subgroups.

In conclusion, we report the 15-year incidence of GA to be 3.6% (2.2% after adjustment for competing risk of death) in this older Australian cohort and confirm strong associations between baseline early AMD lesion characteristics and higher risk of developing GA over the long term, independent of age, smoking, and genetic susceptibility from the 2 major AMD genes (*CFH* and *ARMS2*). Similar AMD risk factors were also found to be associated with fast progression of GA over 5 to 10 years.

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Incidence and Progression of Reticular Drusen in Age-related Macular Degeneration

Findings from an Older Australian Cohort

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Purpose: To assess the 15-year incidence and progression of reticular drusen and associations of this lesion with age-related macular degeneration (AMD) risk factors.

Design: Population-based cohort.

Participants: Blue Mountains Eye Study participants (n = 3654) 49 years of age and older attended baseline examinations; of these, 75.8%, 76.7%, and 56.1% of survivors attended 5-year, 10-year, and 15-year follow-up examinations, respectively.

Methods: Color retinal photographs were obtained and comprehensive questionnaires were administered at each visit, and DNA samples were genotyped. Fundus autofluorescence images were not available. Reticular drusen identified from photographs were confirmed with side-by-side grading using the Wisconsin AMD grading protocol. Incidence was assessed using Kaplan-Meier product limit survival methods, controlling for competing risk of death. Associations between smoking, fish consumption, serum lipids, systemic and dietary factors, the *CFH* single nucleotide polymorphism (SNP) rs1061170 and *ARMS2* SNP rs10490924, and the 15-year incidence of reticular drusen were analyzed in discrete logistic regression models. Generalized estimating equation models were used to analyze eye-specific relationships between these risk factors and 5-year progression from reticular drusen to late AMD.

Main Outcome Measures: Incidence and progression of reticular drusen.

Results: The 15-year cumulative incidence of reticular drusen was 4.0% (n = 95). Increasing age (per decade increase; odds ratio [OR], 3.4; 95% confidence interval [CI], 2.6–4.4), female sex (OR, 2.0; 95% CI, 1.3–3.2), and presence of risk alleles of *CFH-rs1061170* (OR, 1.8; 95% CI, 1.3–2.4) or *ARMS2-rs10490924* (OR, 3.0; 95% CI, 2.1–4.4) were associated with higher reticular drusen incidence. Current smoking at baseline predicted higher reticular drusen incidence (OR 2.1, 95% CI 1.0–4.5) after adjusting for age, sex, *CFH-rs1061170* and *ARMS2-rs10490924* polymorphisms. Of 118 eyes with reticular drusen, 40 (33.9%) developed late AMD over 5 years. A higher proportion of eyes with reticular drusen located outside versus within the macular area progressed to late AMD (50.0% vs. 37.8%). Dietary lutein–zeaxanthin intake was associated with decreased likelihood of progression from reticular drusen to late AMD (adjusted OR, 0.5; 95% CI, 0.3–1.0).

Conclusions: Known AMD risk factors were associated with greater long-term risk of reticular drusen. Neither total area nor central location of reticular drusen predicted 5-year progression to late AMD. Increased consumption of lutein–zeaxanthin predicted a lower risk of progression. *Ophthalmology 2014;121:917-925 © 2014 by the American Academy of Ophthalmology.*

Described as soft, confluent drusen forming ill-defined networks of interlacing ribbons,[1] reticular drusen have been relatively underresearched, and their incidence, prognosis, and risk factors have not been well characterized. Reticular drusen are also termed *reticular pseudodrusen* and *subretinal drusenoid deposits*, as a result of observations using different imaging methods.[2–4] For the purpose of this report, we retain the term *reticular drusen* designated by Klein et al[1] for reticular drusen lesions detected using only color fundus photographic grading.

In most previous studies, reticular drusen were grouped together with large drusen into the category of indistinct soft drusen, with only a few studies reporting the prevalence and incidence of reticular drusen as a separate lesion. The Beaver Dam Eye Study (BDES) reported a reticular drusen prevalence of 0.7% and an overall 15-year incidence of 3.0% in a population 43 to 86 years of age at the baseline examination.[5] We previously reported a 2.0% 5-year incidence of reticular drusen in the Blue Mountains Eye Study (BMES) population.[6] In a report of BMES 10-year age-related macular degeneration (AMD) incidence, we included both reticular drusen and indistinct soft drusen in the incidence of 11.7%.[7]

Findings from clinic-based samples that used multimode imaging methods have shown strong associations between reticular drusen and late AMD.[2,8–10] Some studies report

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that reticular drusen were detected commonly coexisting with geographic atrophy (GA),[3,11] whereas others suggest that reticular drusen commonly accompanied neovascular AMD.[9] Lack of longitudinal data in these studies, however, precludes important prognostic information that ophthalmologists need in providing advice to patients found to have signs of reticular drusen, for example, about the timeframe of progression from reticular drusen to late AMD.

In this report, we aimed to assess the prevalence and 15-year incidence and progression of reticular drusen as a standalone lesion in a well-characterized older Australian population sample. We also aimed to assess associations of common AMD risk factors and other early AMD lesion characteristics with the incidence of reticular drusen, as well as its progression to late AMD, over a 15-year period.

Methods

Population

Details of the BMES have been reported previously.[12,13] Briefly, 3654 permanent residents (82.4% of those eligible) living in 2 postcodes of the Blue Mountains region, west of Sydney, Australia, participated in the study from 1992 through 1994 (baseline examination [BMES I]). Of these, 2334 participants (75.8% of survivors) were examined from 1997 through 1999 (BMES II); 1952 participants (76.7% of survivors) were re-examined from 2002 through 2004 (BMES III); and 1149 participants (56.1% of survivors) were re-examined from 2007 through 2009 (BMES IV). All examinations were approved by the Western Sydney Area Health Service and University of Sydney Human Research Ethics Committees and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Procedures

A comprehensive questionnaire was administered and an eye examination was conducted at each visit. In BMES I, II, and III, 300 stereoscopic retinal fundus photographs were obtained using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochon, Germany). In BMES IV, 400 digital photographs were obtained using a Canon CF-60 DSi with DS Mark III body (Canon, Tokyo, Japan). Retinal photographs centered on the optic disc (Diabetic Retinopathy Study standard field 1), macula (Diabetic Retinopathy Study standard field 2), upper and lower arcades, and fields nasal to the optic disc and temporal to the macula were obtained from both eyes of each participant.

Systolic and diastolic blood pressure measurements were recorded from the first and fifth Korotkoff sounds using a mercury sphygmomanometer after participants were seated for at least 5 minutes.[14] Diet was assessed from a self-administered food frequency questionnaire completed by participants at each examination, which included questions on dietary supplement use.[15] The Australian tables of food composition[16,17] and the United States Department of Agriculture carotenoid food composition database[18] were used to calculate or estimate, respectively, the intake of nutrients, including lutein and zeaxanthin, in micrograms.

Fasting blood samples were collected from 3222 participants at BMES I to assess white cell count (WCC) and cholesterol levels, as previously described.[19–21] Briefly, WCC ($\times 10^9$/l) was determined using Coulter Counter methods (Beckman Coulter, Inc, Fullerton, CA)[19] or an Advir 120 autoanalyzer (Bayer, Leverkusen, Germany),[22] and total cholesterol, high-density lipoprotein (HDL), and triglyceride concentrations (mmol/l) were measured on a Reflotron reflectance photometric analyser (Roche Diagnostics, Mannheim, Germany).[20] Blood samples collected from 2272 participants during the BMES II and III examinations additionally were used for genotyping.

Photographic Grading

Retinal photographic grading was conducted by 2 senior graders using the Wisconsin Age-Related Maculopathy Grading System (WARMGS) protocol, as described previously.[12] The presence and location of reticular drusen within or outside the WARMGS grid was recorded. The WARMGS grid consists of 3 concentric circles with a radius of 500, 1000, and 3000 μm, termed the central, inner, and outer subfields of the grid, respectively. The inner and outer circles are subdivided by diagonal lines that demarcate the superior, inferior, nasal, and temporal quadrants. The WARMGS grid is superimposed over the macular and centered on the fovea prior to grading (Fig 1A). Reticular drusen was defined as confluent drusen forming an interlacing ribbon-like network, with individual lesions usually more than 125 μm in diameter (Fig 1).[1] One of the authors (N.J.) regraded cases with confirmed or questionable reticular drusen in a side-by-side manner using all available retinal photographs from baseline and follow-up visits. Red-free images were used, if available, to distinguish between reticular and indistinct soft drusen. Location of reticular drusen was recorded as presence in each subfield of the WARMGS grid. Reticular drusen area was measured within a 500-, 1000-, and 3000-μm radius of the foveal center. Difficult cases, where the presence of reticular drusen was questionable or where discrepancies existed between the original and subsequent side-by-side grading, were adjudicated and verified by a senior researcher with grading experience (J.J.W.) and a retinal specialist (P.M.).

Late AMD was defined as GA involving the fovea or the presence of any sign of neovascular AMD (pigment epithelial or neurosensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars), as described in the International Age-Related Maculopathy Classification.[23] Early AMD was defined as the presence of either large (>125-μm diameter) indistinct soft or reticular drusen or of distinct soft drusen with retinal pigmentary abnormalities (hyperpigmentation and retinal pigment epithelial depigmentation) within the macula, in the absence of any late AMD. Early AMD lesion type, area, and location were defined using the WARMGS protocol.[1] Briefly, individual lesion size and the collective area of drusen and pigmentary abnormalities were estimated using circles of defined diameters (63, 125, 250, 350, and 644 μm, 0.5 disc area [DA] and 1 DA). Location of lesions was defined as within a 500-μm radius, between a 500- and 1500-μm radius, and between a 1500- and 3000-μm radius of the foveal center in the WARMGS grid.[1]

Definition of Reticular Drusen Incidence and Progression

Incident reticular drusen was defined as the first occurrence of reticular drusen at the 5-, 10-, or 15-year follow-up in eyes without reticular drusen or any late AMD at previous visits. For cases where reticular drusen and late AMD both were present for the first time in a follow-up visit, we assumed that reticular drusen developed first, before the occurrence of late AMD during the 5-year interval between the 2 visits.

Progression from reticular drusen to late AMD was defined as the development of any late AMD (GA or neovascular AMD) that followed the development of reticular drusen. Progression was assessed for baseline and incident reticular drusen cases where

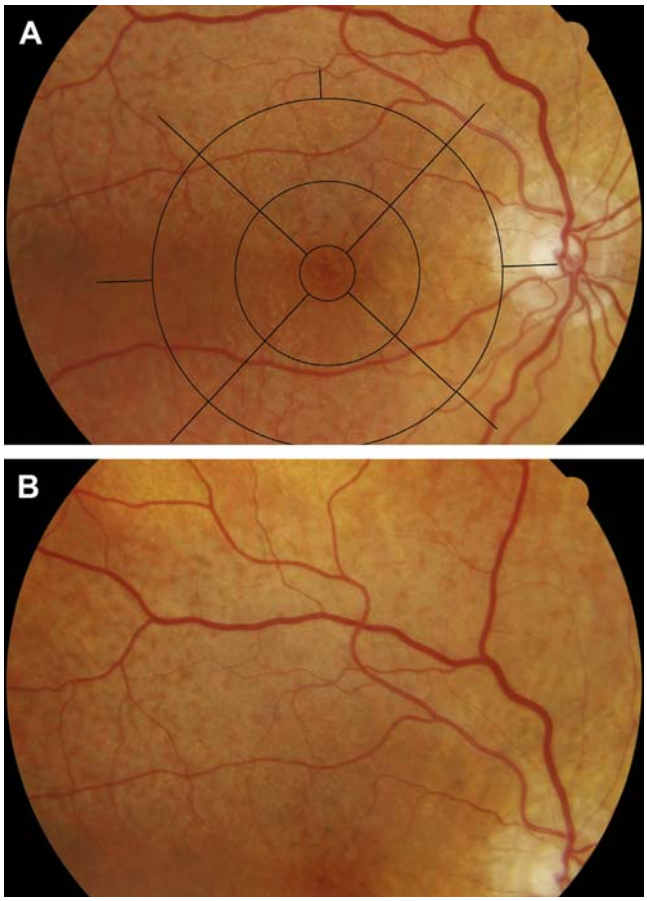

Figure 1. **A**, Example of reticular drusen (distinguished as soft confluent drusen that form broad ribbon-like networks) in the upper and lower arcades of the macula with the Wisconsin Age-Related Maculopathy Grading System grid centered on the fovea. **B**, Reticular drusen visible in the upper arcade of the same eye in (**A**).

reticular drusen progression to late-stage AMD could be assessed for at least 5 years among cases with 5 or more years of follow-up. Location of reticular drusen was defined by subfields of the WARMGS grid, and the area of reticular drusen defined as small if less than 2 DA and large if 2 DA or more, within the confines of the WARMGS grid.[1]

Genotyping

The *complement factor H* (*CFH*) single nucleotide polymorphism (SNP) *rs1061170* was genotyped in 1874 participants using TaqMan assays (Applied Biosystems, Foster City, CA).[24] The *age-related maculopathy susceptibility gene 2* (*ARMS2*) SNP *rs10490924* was genotyped in 593 participants using restriction fragment length polymorphism analysis.[22] In the remaining participants, imputed SNPs of the 2 genes were obtained from BMES genome-wide association scan data. Genotyping of the entire BMES sample was performed using an Illumina Human 670-Quad custom array (version 1) (Illumina Inc., San Diego, CA) and stringent quality control testing was performed using PLINK (Purcell S. PLINK version 1.07; available at: http://pngu.mgh.harvard.edu/purcell/plink/; accessed July 12, 2012). Imputation then was performed from 1000 genomes using IMPUTE version 2.0 (Department of Statistics, University of Oxford, Oxford, UK).[25] The imputation r^2 was 0.968 for *CFH* SNP rs1061170 and 0.996 for *ARMS2* SNP *rs10490924*. In our study sample, 1501 and 509 participants had both typed and imputed rs1061170 and rs10490924, respectively, and the concordance rates between typed and imputed SNPs were 99.6% for rs1061170 and 99.2% for rs10490924.

Other Risk Factors

Smoking status was ascertained from an interviewer-administered questionnaire and participants were classified as nonsmokers if they answered "no" to smoking regularly. If participants had quit smoking more than 1 year before the examination, they were classed as past smokers. Current smokers were defined as participants who currently smoked or had stopped smoking less than 1 year before the examination. Regular fish consumption was defined as consuming 1 or more serving of fish per week. Alcohol consumption (including beer, wine, or spirits) was categorized as none, more than 0 to 2 or fewer standard drinks, and more than 2 standard drinks per day. These categories were formulated based on Australian National Health and Medical Research Council recommendations of up to 2 standard drinks per day.[26]

Statistical Analyses

SAS software version 9.1 (SAS Inc., Cary, NC) was used. The 15-year incidence of reticular drusen was estimated using the Kaplan-Meier product limit survival method controlling for competing risk of death. Age was treated categorically (49–54, 55–64, 65–74, and 75 or more years), and the 15-year incidence of reticular drusen was considered by sex within each age-group. Age- and sex-adjusted discrete logistic regression models were used to assess associations between well-known AMD risk factors (age, sex, smoking, presence of risk alleles for *CFH* SNP rs1061170 and *ARMS2* SNP rs10490924, blood pressure, WCC, total cholesterol, HDL, triglycerides, fish consumption, alcohol consumption, vitamin supplementation, and lutein–zeaxanthin dietary intake) and the 15-year development of reticular drusen using person-specific data. If risk factors were associated significantly with the 15-year incidence of reticular drusen in age- and sex-adjusted models, they were adjusted for further in a multivariate-adjusted model and were expressed as odds ratios (ORs). The 2 SNPs (rs1061170 in *CFH* and rs10490924 in *ARMS2*) were assessed additively, where genotype (none, 1, or 2 risk alleles) was treated as a continuous variable.

Generalized estimating equation models, using the GENMOD procedure in SAS,[27] were used to analyze eye-specific data for associations between other early AMD lesion characteristics and the 15-year incidence of reticular drusen. This method also was used to assess the relationship between the common AMD risk factors and 5-year progression of reticular drusen to late AMD using eye-specific data. Each risk factor was defined using information collected at the time when reticular drusen was detected. Estimates are expressed as age- and sex-adjusted or multivariate-adjusted OR (age, sex, smoking, rs1061170 in *CFH* and rs10490924 in *ARMS2* polymorphisms) and 95% confidence intervals (CIs).

Results

Prevalence and Incidence of Reticular Drusen

Reticular drusen were present in 1.95% of the baseline BMES population sample. Of the 65 participants with reticular drusen, in 38 (58.5%), this sign was present in both eyes (bilateral). The copresence of reticular drusen with late AMD occurred in 7 of the total 103 eyes (6.8%) with prevalent reticular drusen.

Incident reticular drusen were identified in 95 (152 eyes) of 2738 participants at risk, with an overall 15-year incidence of 4.0% (95% CI, 3.2–4.8) after controlling for the competing risk of death. Of the 95 cases, 57 (60.0%) were bilateral. Baseline characteristics of

Table 1. Comparison of Baseline Characteristics in Participants with and without Incident Reticular Drusen

Characteristic	Participants (%)		P Value
	No Reticular Drusen (n = 2135)	*Incident Reticular Drusen (n = 95)*	
Mean age (SD), yrs	63.4 (8.2)	69.5 (6.4)	<0.0001
Female sex	56.6	73.7	0.001
Smoking status			
Nonsmoker	51.8	57.5	0.6*
Former smoker	35.3	30.9	
Current smoker	13.0	11.7	
CFH: rs1061170			
TT	40.3	22.0	0.0008*
CT	46.2	53.7	
CC	13.5	24.4	
ARMS2: rs10490924			
GG	62.9	37.0	<0.0001*
GT	33.2	51.9	
TT	3.8	11.1	
Mean blood pressure (SD), mmHg			
Systolic	144.6 (20.4)	147.7 (20.4)	0.1
Diastolic	83.5 (9.9)	83.2 (8.4)	0.7
Mean white cell count (SD), $\times 10^9$cells/l	6.4 (1.7)	6.5 (1.5)	0.3
Mean total cholesterol (SD), mmol/l	6.0 (1.0)	6.2 (1.1)	0.3
Mean high-density lipoprotein (SD), mmol/l	1.4 (0.4)	1.5 (0.4)	0.01
Mean triglycerides (SD), mmol/l	1.8 (1.1)	1.6 (0.8)	0.02
Fish consumption (≥1 servings/wk)	60.1	52.0	0.2
Daily alcohol consumption			
None	20.0	18.2	0.6*
>0 to ≤2 standard drinks	59.7	64.9	
>2 standard drinks	20.2	16.9	
Any current vitamin supplement intake	39.6	40.3	0.9
Mean dietary lutein and zeaxanthin intake (SD), μg	829.0 (480.2)	907.5 (691.4)	0.3

ARMS2 = *age-related maculopathy susceptibility gene 2* (T risk allele); *CFH* = *complement factor H* (C risk allele); SD = standard deviation.
*Unadjusted tests for heterogeneity used to calculate *P* values.

participants with and without incident reticular drusen are presented in Table 1. Participants who demonstrated reticular drusen were more likely to be women, be older at baseline, have at least 1 risk allele of rs1061170 in *CFH* or rs10490924 in *ARMS2*, and have higher mean HDL and lower mean triglyceride levels than participants who did not demonstrate reticular drusen.

The 15-year cumulative incidence of reticular drusen by age and sex is presented in Table 2. It was associated significantly with increasing age ($P < 0.0001$ for trend) in both men and women. After adjusting for age, the 15-year cumulative incidence of reticular drusen was twice as likely in women than in men (5.6% [95% CI, 5.59–5.61] vs 2.2% [95% CI, 2.19–2.21]; Table 2).

In an age- and sex-adjusted model (Table 3), each additional risk allele of rs1061170 in *CHF* and rs10490924 in *ARMS2* also was associated with a greater 15-year risk of incident reticular drusen ($P = 0.0004$ and $P < 0.0001$, respectively). Increasing WCC was associated positively with reticular drusen incidence, but this association was marginally nonsignificant ($P = 0.07$). Current smoking at baseline was not associated with the 15-year development of reticular drusen in the age- and sex-adjusted model, but became so after adjusting for age, sex, and presence of the rs1061170 in *CFH* and rs10490924 in *ARMS2* polymorphisms. In the multivariate-adjusted model, increasing age, female sex, and presence of risk alleles for rs1061170 in *CFH* and rs10490924 in *ARMS2* remained significantly associated with an increased risk of incident reticular drusen, in addition to current smoking status (Table 3).

The association between early AMD lesion characteristics and the 15-year incidence of reticular drusen is shown in Table 4. After adjusting for age and sex, the presence of indistinct soft drusen (excluding reticular drusen; OR, 4.3), location of any soft drusen within a 500-μm radius of the foveola (OR, 2.6), and a collective area of any soft drusen of 375 μm or more in diameter (ORs, 3.1–6.3) were predictive of the development of reticular drusen. After further adjusting for smoking and the rs1061170 in *CFH* and rs10490924 in *ARMS2* risk alleles, only the location of any soft drusen at close proximity to the fovea (OR, 2.2) and an area of soft drusen of 375 μm or more but less than 0.5 DA in diameter (OR, 3.2) were associated significantly with a greater risk of reticular drusen (Table 4).

Progression of Reticular Drusen

Of the 65 participants with prevalent reticular drusen, 37 had retinal photographs available from at least 1 follow-up visit. Of these, 18 participants (48.6%) progressed to late AMD within 15 years in at least 1 eye. Five-year progression from reticular drusen to late AMD occurred in 14 of the 37 participants (37.8%; 18 of 59 eyes [30.5%]).

Of the 95 participants (152 eyes) with incident reticular drusen detected at either of the 5-, 10-, or 15-year follow-up visits, only 40 participants (59 eyes) had follow-up information. Overall, 18 of 40 participants (26 of 59 eyes) progressed to late AMD within 5 to 10 years. Of the 26 eyes with reticular drusen that progressed, 22 eyes progressed to late AMD in 5 years. This included eyes in which both reticular drusen and late AMD were detected at the same visit and in which reticular drusen was assumed to have developed before the late AMD.

Table 2. Fifteen-Year Cumulative Incidence of Reticular Drusen by Age and Sex

	15-Year Incidence of Reticular Drusen					
	Women		*Men*		*Both*	
Age (yrs)	*No. of Cases/No. at Risk*	*% (95% Confidence Interval)*	*No. of Cases/No. at Risk*	*% (95% Confidence Interval)*	*No. of Cases/No. at Risk*	*% (95% Confidence Interval)*
49–54	0/205	0	1/162	0.8 (−0.8 to 2.4)	1/367	0.4 (−0.4 to 1.2)
55–64	13/519	3.1 (1.4–4.8)	4/420	1.1 (0.0–2.2)	17/939	2.2 (1.2–3.2)
65–74	42/530	10.3 (7.4–13.2)	12/414	3.2 (1.4–5.0)	54/944	7.0 (5.2–8.8)
75+	15/259	6.2 (3.3–9.1)	8/229	3.5 (1.2–5.8)	23/488	4.9 (3.0–6.8)
P trend	<0.0001		<0.0001		<0.0001	
Total	70/1513	5.6 (4.3–6.9)*	25/1225	2.2 (1.3–3.1)*	95/2738	4.0 (3.2–4.8)

*Age-adjusted difference between sexes, $P = 0.0032$.

Of a total 118 eyes with either prevalent or incident reticular drusen that were available for assessment of progression, 33.9% (40 eyes: 18 with prevalent and 22 with incident reticular drusen) progressed to late AMD over 5 years. Of these, 23 (57.5%) progressed to GA, 12 (30.0%) progressed to neovascular AMD, and 5 (12.5%) demonstrated both GA and neovascular AMD in the same eye. By comparison, of 722 eyes with other early AMD lesions (excluding reticular drusen) that were available for assessment of progression, 62 eyes (7.9%) progressed to late AMD over 5 years, including 27 (43.6% of the 62 eyes) in which GA developed, 30 (48.4%) in which neovascular AMD developed, and 5 (8.1%) in which both late AMD lesions developed. Further, of 4176 eyes with no prior early AMD lesions, 6 (0.1%) progressed to late AMD over 5 years, in all of which neovascular AMD developed.

A higher proportion of eyes with reticular drusen outside the WARGMS grid progressed to late AMD compared with eyes with reticular drusen in the central, inner, or outer circles (50.0% vs. 23.5%–37.8%). Of eyes with reticular drusen present within the grid, there was no difference in the proportion of eyes that progressed to late AMD between eyes with small (28.6%) or large (37.9%) areas of reticular drusen.

No significant associations were found between the 5-year progression from reticular drusen to late AMD and most known AMD risk factors assessed (age, female sex, smoking status, genetic risk from rs1061170 in *CFH* and rs10490924 in *ARMS2* polymorphisms, blood pressure, WCC, total cholesterol, HDL, triglycerides, fish consumption, alcohol consumption, and vitamin supplementation; data not shown). However, a significantly lower

Table 3. Associations between Well-Known Age-Related Macular Degeneration Risk Factors and the 15-Year Incidence of Reticular Drusen

	15-Year Incidence of Reticular Drusen			
	Age- and Sex-Adjusted (Where Appropriate)		*Multivariate Adjusted**	
Risk Factor	*Odds Ratio*	*95% Confidence Interval*	*Odds Ratio*	*95% Confidence Interval*
Age per 10 yrs	3.4	2.6–4.4	4.3	3.1–5.9
Female sex	2.0	1.3–3.2	2.2	1.3–3.8
Smoking status				
Former smoker	1.0	0.6–1.7	1.3	0.7–2.1
Current smoker	1.7	0.9–3.4	2.1	1.0–4.5
CFH allele rs1061170†	1.8	1.3–2.4	1.8	1.3–2.6
ARMS2 allele rs10490924†	3.0	2.1–4.4	3.2	2.2–4.6
Blood pressure per 10 mmHg				
Systolic	1.0	0.9–1.1	—	—
Diastolic	1.0	0.8–1.3	—	—
White cell count‡	1.2	1.0–1.5	—	—
Total cholesterol‡	1.0	0.8–1.3	—	—
High-density lipoprotein‡	1.2	0.9–1.4	—	—
Triglycerides‡	0.8	0.6–1.1	—	—
Fish consumption (≥1 servings/wk)	0.8	0.5–1.2	—	—
Daily alcohol consumption				
None	0.7	0.4–1.2	—	—
>0 to ≤2 standard drinks (referent)	1.0			
>2 standard drinks	1.0	0.5–1.9	—	—
Vitamin supplementation (current baseline intake)	1.0	0.6–1.5	—	—
Lutein-zeaxanthin intake‡	1.1	0.9–1.4	—	—

ARMS2 = age-related maculopathy susceptibility gene 2; *CFH = complement factor H*.
*Multivariate odds ratios adjusted for age, sex, past and current smoking status, the *CFH* and *ARMS2* risk alleles as additive variables.
†Analyzed using additive models; estimates represent risk associated with each additional single nucleotide polymorphism of the minor variants.
‡Per standard deviation increase in each risk factor.

Table 4. Relationship between Baseline Early Age-Related Macular Degeneration Lesion Characteristics and the 15-Year Incidence of Reticular Drusen, Analyzed by Eye

Early Age-Related Macular Degeneration Characteristics	No. of Eyes That Progressed/ No. of Eyes at Risk of Progression to Reticular Drusen	15-Year Incidence of Reticular Drusen			
		Age and Sex Adjusted		*Multivariate Adjusted**	
		Odds Ratio	*95% Confidence Interval*	*Odds Ratio*	*95% Confidence Interval*
Drusen type					
None or hard distinct	99/3823	1.0		1.0	
Intermediate	21/439	1.1	0.7–1.8	1.1	0.6–1.8
Distinct soft	9/94	2.0	0.9–4.3	1.8	0.7–4.4
Indistinct soft	12/73	4.3	1.7–10.8	2.1	0.6–7.7
Drusen location					
None or ≥500-μm radius of foveal center	101/4034	1.0		1.0	
<500-μm radius of foveal center	40/385	2.6	1.7–4.1	2.2	1.3–3.7
Drusen area					
None or <375 μm in diameter	111/4238	1.0		1.0	
≥375 μm to <0.5 disc area in diameter	21/143	3.1	1.8–5.4	3.2	1.7–5.8
≥0.5 disc area in diameter	8/40	6.3	2.2–18.1	2.3	0.4–12.2
RPE depigmentation					
Absent	141/4402	1.0		1.0	
Present	9/131	1.3	0.6–2.7	1.5	0.6–3.4
Location					
None or ≥1500-μm radius of foveal center	141/4405	1.0		1.0	
≥500- to <1500-μm radius of foveal center	4/67	1.4	0.6–3.1	1.8	0.7–4.5
<500-μm radius of foveal center	5/61	1.3	0.4–4.5	1.2	0.3–5.6
Area					
None or <375 μm in diameter	146/4451	1.0		1.0	
≥375 μm in diameter	4/82	1.2	0.4–3.7	1.3	0.4–4.7
Hyperpigmentation					
Absent	135/4260	1.0		1.0	
Present	15/274	1.2	0.6–2.2	1.2	0.6–2.7
Location					
None or ≥1500-μm radius of foveal center	135/4278	1.0		1.0	
≥500- to <1500-μm radius of foveal center	4/86	1.1	0.5–2.5	1.4	0.6–3.3
<500-μm radius of foveal center	10/124	1.4	0.5–3.7	1.3	0.4–4.4
Area					
None or <64 μm in diameter	137/4280	1.0		1.0	
≥64 μm in diameter	12/208	1.1	0.5–2.3	1.1	0.5–2.6

RPE = retinal pigment epithelium.
*Multivariate odds ratios adjusted for age, sex, current smoking, and the *CFH* and *ARMS2* risk alleles.

risk of progression to late AMD was evident in the eyes of subjects with increased intake of dietary lutein–zeaxanthin (OR, 0.5; 95% CI, 0.3–1.0; $P = 0.046$) after adjusting for age, sex, current smoking, and presence of the rs1061170 in *CFH* and rs10490924 *ARMS2* polymorphisms.

Discussion

We found an overall 15-year incidence of reticular drusen of 4.0% in this older Australian cohort, with more than 50% of incident cases developing bilaterally. The incidence of reticular drusen rose with increasing age and was significantly higher in women than in men. Current smoking and presence of the rs1061170 in *CFH* and rs10490924 in *ARMS2* risk alleles also were associated independently with higher 15-year incidence of reticular drusen. A substantially higher proportion (34%) of eyes with reticular drusen (with or without early AMD lesions) compared with eyes without reticular drusen but with other early AMD lesions (8%) progressed to late-stage AMD within 5 years. Increased dietary lutein–zeaxanthin intake was associated with a lower risk of progression from reticular drusen to late AMD over 5 years.

The BDES and the BMES are the only 2 longitudinal population-based studies to have reported 15-year incidence of reticular drusen in older cohorts to date. Although the prevalence of reticular drusen in the BMES (1.95%) was more than double the prevalence reported in the BDES (0.7%), the 15-year incidence of reticular drusen in the BMES, after accounting for competing risk of death (4.0%), was similar to the incidence in the BDES (3.0%), given that the BMES population was slightly older than the BDES population at baseline (43–86 years for the BDES and 49–97 years for the BMES). In addition to age, differences in environmental exposures (smoking, diet, sunlight exposure) could also account for the differences in prevalence and incidence of reticular drusen between these 2 studies.

We confirmed that older age and female sex are associated with a greater risk of reticular drusen, consistent with the BDES[5] and previous cross-sectional studies that

reported a female preponderance for reticular drusen.[3,8] Smoking was associated independently with incident reticular drusen, as it was with late AMD in the BMES.[28] The risk of incident reticular drusen for current smokers was 2.1-fold the risk for nonsmokers in our cohort, which was similar to that found in the BDES (current smokers had 1.9 times the risk of reticular drusen compared with noncurrent smokers),[5] although no similar association was found between smoking and incident late AMD in the BDES.[29–31]

The BDES previously reported a higher prevalence of reticular drusen in those with than without the rs1061170 in *CFH* polymorphism[5]; however, the increased risk of reticular drusen associated with the presence of either or both the rs1061170 in *CFH* and rs10490924 in *ARMS2* minor alleles, found in our study, has not been demonstrated previously.[5] An inflammatory basis has been hypothesized for the development of reticular drusen[8,32]; however, we did not observe a significant relationship between WCC levels and 15-year incident reticular drusen.

Of other early AMD lesions and early AMD lesion characteristics, we found that soft drusen location close to the fovea and an intermediate area of soft drusen was associated with 15-year incident reticular drusen. This finding is inconsistent with the BDES finding that presence of more severe drusen type, but not drusen location or area, predicted 15-year development of reticular drusen.[5]

Approximately 1 in 3 eyes with reticular drusen progressed to late AMD in 5 years in our study, compared with more than 50% of eyes that progressed to late AMD in a cohort of the Nutritional AMD Treatment 2 study.[33] The Nutritional AMD Treatment 2 study, however, assessed participants who had unilateral neovascular AMD already present, which may explain the higher frequency of eyes that progressed from reticular drusen to late AMD.[7] We found that progression from reticular drusen to pure GA was slightly more frequent than the progression to neovascular AMD, a finding that seems to conflict with other reports, although the previous findings were based on associations between reticular drusen and late AMD detected at the same visit.[2,9,11]

In the Geographic Atrophy Progression study population,[34] the average growth rate of reticular drusen area was found to be 4.4 mm^2/year over 18 months, although this observation was obtained from 16 eyes only. We did not conduct quantitative measures of areas of reticular drusen because most reticular drusen were located outside the WARMGS grid, and therefore the photographic fields obtained may not include all of the reticular drusen in the eye.

We found that the proportion of eyes with reticular drusen that progressed to late AMD in 5 years was 4-fold the corresponding proportion of eyes without reticular drusen but with other early AMD lesions. This finding is consistent with clinicians' experience as well as observations from other studies. Klein et al[5] demonstrated that eyes with reticular drusen conferred a 6-fold risk of late AMD compared with eyes with indistinct soft drusen but no reticular drusen. We also observed that reticular drusen progressed to retinal pigment epithelial depigmentation followed by GA. Fading of reticular drusen as neovascular AMD developed also has been documented in previous studies.[33,34]

Although we observed a decreased risk of 5-year progression from reticular drusen to late AMD associated with increased dietary intake of lutein–zeaxanthin, this observation was based on a relatively small sample of eyes of participants who had dietary intake information available. Nevertheless, our observation is in keeping with findings from the Age-Related Eye Disease Study that showed a protective effect of dietary lutein–zeaxanthin intake on the development of late AMD[35] and a similar protective effect for treatment with lutein–zeaxanthin supplements in participants with low dietary intake of lutein–zeaxanthin (Age-Related Eye Disease Study 2).[36]

Strengths of this study include long-term follow-up of participants in an older Australian cohort and side-by-side grading of retinal photographs obtained over the follow-up period of the same participants. The low follow-up rate in the 15-year visits, however, could have led to an overestimation or underestimation of reticular drusen incidence. Limitations include that only color retinal fundus photographs were used to detect reticular drusen, resulting in substantial underestimation of reticular drusen prevalence and incidence, compared with multimodal imaging methods (fundus autofluorescence, infrared reflectance, blue light photography, and spectral-domain optical coherence tomography), as used in other studies.[3,11,37,38] The distinction between indistinct soft drusen and reticular drusen can be differentiated well with monochromatic blue light as well as red-free images, and therefore misclassification of drusen is unlikely to have occurred in our study.

In summary, the 15-year incidence of reticular drusen was 4.0% in this older Australian cohort and was associated with well-known AMD risk factors. Reticular drusen, however, were documented only from standard color photography, which could have underestimated the prevalence and incidence substantially compared with that found using multimodal imaging methods. The proportion of eyes that progressed from reticular drusen to late AMD over 5 years was 4-fold that in eyes without reticular drusen, suggesting that this sign portends a higher risk. High dietary intake of lutein–zeaxanthin was associated with a lower risk of progression from reticular drusen to late AMD over 5 years, although confirmation of this finding in future studies will be important.

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Original Investigation

Incidence, Progression, and Associated Risk Factors of Medium Drusen in Age-Related Macular Degeneration
Findings From the 15-Year Follow-up of an Australian Cohort

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IMPORTANCE The natural course and prognosis of medium drusen and risk factors associated with the incidence and progression of this lesion type in age-related macular degeneration (AMD) are not well understood.

OBJECTIVE To assess the 15-year incidence and progression of medium drusen and associated risk factors.

DESIGN, SETTING, AND PARTICIPANTS Population-based cohort in the Blue Mountains region, west of Sydney, Australia. Included in the study were 3654 participants 49 years or older who attended baseline examinations of the Blue Mountains Eye Study (1992-1994), and 75.8%, 76.7%, and 56.1% of survivors who attended the 5-year, 10-year, and 15-year follow-up examinations, respectively.

MAIN OUTCOMES AND MEASURES Color retinal fundus photographs were obtained at each examination. The incidence and progression of medium drusen (maximum diameter, 63 to <125 μm) were assessed using Kaplan-Meier product-limit survival methods, controlling for competing risk of death. Factors associated with a 15-year incidence of medium drusen were assessed using discrete logistic regression models after adjusting for age, sex, smoking status, serum lipid levels, systemic and dietary factors, and *CFH* rs1061170 and *ARMS2* rs10490924 polymorphisms. Associations between lesion characteristics and the progression to late AMD were assessed using generalized estimating equation models and eye-specific data.

RESULTS Among 1317 participants at risk, the 15-year cumulative incidence of medium drusen was 13.9% (n = 281). Increasing age (per decade older) (odds ratio [OR], 1.4; 95% CI, 1.2-1.8) and the presence of at least 3 risk alleles of the *CFH* rs1061170 or *ARMS2* rs10490924 genes (OR, 2.1; 95% CI, 1.1-4.1) were associated with a higher incidence. There was no association between past smoking (OR, 0.8; 95% CI, 0.6-1.1) or current smoking (OR, 0.6; 95% CI, 0.4-1.1) and the development of medium drusen. The progression rate to late AMD in eyes with both medium drusen and retinal pigmentary abnormalities was 4-fold higher than that in eyes with medium drusen alone. Larger total area and central location of medium drusen were associated with a greater likelihood of the progression to worse stages of AMD.

CONCLUSIONS AND RELEVANCE Older age and the presence of *CFH* and *ARMS2* risk alleles are 2 main risk factors associated with the development of medium drusen. The copresence of medium drusen plus retinal pigment epithelium abnormalities signals a greater risk of the progression to late AMD than the presence of medium drusen alone.

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Early age-related macular degeneration (AMD) is characterized by the presence of drusen and retinal pigmentary abnormalities.[1,2] Drusen vary in size (diameter range, ≤63 to ≥250 µm) and type (hard, soft, distinct, and indistinct). Pigmentary abnormalities include clusters of pigment granules within the sensory retina (increased pigmentation) and sharply demarcated areas of retinal pigment epithelium (RPE) depigmentation.

The international classification and grading system for AMD categorizes medium drusen as intermediate soft drusen, defined as drusen with a maximum diameter of 63 to less than 125 µm, larger than the maximum diameter of hard drusen (<63 µm) but smaller than the minimum diameter of large soft drusen (≥ 125 µm).[1] A similar definition of this drusen type was used by the Age-Related Eye Disease Study[2] and clinical classification system,[3] categorized as medium drusen. Furthermore, the Wisconsin Age-Related Maculopathy Grading System[4] defines medium drusen by the maximum diameter, although the categorization of medium drusen is not used. In this study, we describe this type of drusen as medium drusen.

Despite recent interest in medium drusen and their inclusion in AMD incidence studies,[5,6] knowledge of the associated risk factors and the progression of medium drusen is limited. Medium drusen have been underrepresented in studies[3,7-9] compared with large drusen, soft drusen, and pigmentary lesions. In this study, we aimed to assess the 15-year incidence and progression of medium drusen in an older Australian cohort, as well as associations between common AMD risk factors and the development and progression of medium drusen.

Methods

Population

The Blue Mountains Eye Study (BMES) is a population-based cohort study of vision and eye disease in Australians 49 years or older residing in the Blue Mountains region, west of Sydney. The survey methods and the BMES baseline population have been previously described.[10,11] Briefly, 3654 residents (82.4% of those eligible) participated in baseline examinations from 1992 to 1994 (BMES I). Of these, 2334 (75.8% of survivors) were reexamined from 1997 to 1999 (BMES II), 1952 (76.7% of survivors) were reexamined from 2002 to 2004 (BMES III), and 1149 (56.1% of survivors) were reexamined from 2007 to 2009 (BMES IV). Baseline and all follow-up examinations were approved by human research ethics committees of the Western Sydney Area Health Service and the University of Sydney and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants at each visit.

Procedures

At each visit, a comprehensive interview with questionnaires on demographic and lifestyle factors and medical histories was conducted. A validated Food Frequency Questionnaire was self-administered by participants before each visit. Comprehensive eye examinations were performed as described previously.[10] Briefly, 30° stereoscopic retinal photographs of the macula and other retinal fields of both eyes were obtained using a fundus camera (FF3; Carl Zeiss) at the BMES I, II, and III examinations, and 40° photographs were obtained using a digital camera (CF-60 DSi with Ds Mark III body; Canon) at the BMES IV examination.

Photographic Grading

Retinal photographic grading of both eyes of each participant was conducted by 2 senior graders using a modified Wisconsin Age-Related Maculopathy Grading System protocol.[10] The Wisconsin Age-Related Maculopathy Grading System grid consists of 3 concentric circles that subdivide the macula from the foveal center, with radii of 500, 1500, and 3000 µm that demarcate the central, inner, and outer subfields of the macula, respectively. The grid was superimposed on the macula during the grading. The grading procedures and intergrader and intragrader agreements have been previously described,[10] with quadratic weighted κ values ranging from 0.64 to 0.93 and 0.54 to 0.94, respectively. Adjudication was provided by a senior retinal specialist (P.M.) if needed.

Late AMD was defined per an international age-related maculopathy classification[1] as the presence of neovascular AMD (indicated by RPE or neurosensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis or old atrophic disciform scars, or photocoagulation scars) or the presence of geographic atrophy. Early AMD was defined as the presence of large (diameter, ≥125 µm) indistinct soft drusen or reticular drusen or the presence of large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation and depigmentation of RPE cells) within the macula, in the absence of any late AMD lesions. The maximum diameters of individual drusen and collective macular areas involved by drusen and pigmentary abnormalities within the eye were estimated as specified in the Wisconsin Age-Related Maculopathy Grading System[4] using circles with diameters of 63, 125, 250, 350, and 644 µm, 0.5 disc area, and 1 disc area.

Definition of Medium Drusen Incidence and Progression

The incidence of medium drusen was defined as its presence at the 5-year, 10-year, or 15-year follow-up visit in persons who had no drusen or hard drusen only in any eye at baseline visits. The progression of medium drusen was defined as a progression to worse AMD lesions, including large soft drusen, retinal pigmentary abnormalities, or late AMD at follow-up visits, in eyes with medium drusen as the most severe lesion at baseline visits.

Genotyping

Genotyping data were available in 2761 participants of the BMES cross-section II examinations, which included the original cohort and BMES extension survey (1999-2000) samples. A custom array (Human 670-Quad, version 1; Illumina Inc) was used, with stringent quality control testing using a whole-genome association analysis tool set (PLINK, version 1.07; http://pngu.mgh.harvard.edu/purcell/plink/). After quality control checking, 2534 participants with genome-wide association study data

Table 1. Fifteen-Year Incidence of Medium Drusen by Age and Sex[a]

	Women		Men		Both	
Age, y	No. of Cases/No. at Risk	% (95% CI)	No. of Cases/No. at Risk	% (95% CI)	No. of Cases/No. at Risk	% (95% CI)
15-Year Kaplan-Meier Incidence of Medium Drusen						
49-54	32/145	28.5 (20.8-38.3)	17/110	19.9 (12.7-30.3)	49/255	24.7 (19.1-31.6)
55-64	68/319	28.9 (23.3-35.4)	49/257	27.0 (20.8-34.6)	117/576	28.1 (23.8-32.9)
65-74	59/219	41.1 (31.3-52.7)	43/167	43.9 (32.8-57.0)	102/386	42.7 (35.1-51.3)
≥75	8/51	15.7 (8.2-28.9)	5/49	15.8 (5.5-40.5)	13/100	16.2 (8.6-29.4)
Total	**167/734**	32.2 (28.0-36.9)	**114/583**	29.0 (24.5-34.3)	**281/1317**	30.8 (27.7-34.3)
15-Year Cumulative Incidence of Medium Drusen After Controlling for Competing Risk of Death						
49-54	13/145	24.1 (17.0-31.2)	8/110	16.8 (9.7-23.9)	21/255	20.9 (15.8-25.9)
55-64	37/319	22.2 (17.6-26.7)	48/257	17.6 (13.3-21.9)	85/576	20.0 (16.9-23.2)
65-74	63/219	17.8 (13.9-21.8)	79/167	13.0 (9.7-16.4)	142/386	15.5 (13.0-18.1)
≥75	40/51	2.5 (0.8-4.1)	39/49	1.8 (0.3-3.3)	79/100	2.2 (1.0-3.3)
Total	**153**/734	15.8 (13.7-17.9)	**174/583**	11.8 (9.9-13.7)	**327/1317**	13.9 (12.5-15.3)

[a] The definition of medium drusen herein excludes larger soft drusen, retinal pigmentary abnormalities, and late age-related macular degeneration.

were imputed with a genetic variation catalog (1000 Genomes, version 1; http://www.1000genomes.org/) using a software program (IMPUTE, version 2.0; https://mathgen.stats.ox.ac.uk/impute/impute_v2.html).[12] The imputation r^2 coefficients were 0.968 for *CFH* (OMIM 134370) rs1061170 and 0.996 for *ARMS2* (OMIM 611313) rs10490924.

The *CFH* single-nucleotide polymorphism rs1061170 was also genotyped in 1928 participants using an assay (TaqMan; Applied Biosystems),[13] and the *ARMS2* single-nucleotide polymorphism rs10490924 was genotyped in 638 participants using restriction fragment length polymorphism analysis.[14] Imputed single-nucleotide polymorphisms of these 2 genes were used for the remaining participants. Of the 1544 and 547 participants who had both typed and imputed rs1061170 and rs10490924, respectively, the concordance rates between typed and imputed single-nucleotide polymorphisms were 99.6% for rs1061170 and 99.2% for rs10490924.

Other Risk Factors

Participants were classified as nonsmokers if they answered no to the question of whether they smoke regularly. Past smoking was recorded if participants had smoked regularly but quit smoking at least 1 year before the examination. Current smoking was recorded if participants were current smokers or had stopped smoking less than 1 year before the examination. Alcohol consumption (including beer, wine, or spirits) was categorized as none, 1 to 2, or more than 2 standard drinks per day. These categories were based on Australian National Health and Medical Research Council[15] recommendations of no more than 2 standard drinks per day.

Systolic and diastolic blood pressure measurements were recorded from the first and fifth Korotkoff sounds using a mercury sphygmomanometer after participants had been seated for at least 5 minutes.[16] Dietary consumption and supplement use were extracted from the self-administered Food Frequency Questionnaire.[17] The Australian tables of food composition[18,19] and the US Department of Agriculture carotenoid food composition database[20] were used to estimate the intake of nutrients, including lutein and zeaxanthin intake in micrograms. Regular fish consumption was defined as consuming at least 1 serving of fish per week.

Fasting blood samples were collected from 3222 baseline participants to assess white blood cell count and cholesterol levels as previously described.[21-23] Briefly, white blood cell count was determined using cell counting methods (Coulter Counter; Beckman Coulter, Inc).[21] Total cholesterol, high-density lipoprotein cholesterol, and triglycerides concentrations were measured on a reflectance photometric analyzer (Reflotron; Roche Diagnostics).[22]

Statistical Analysis

A software package (SAS, version 9.3; SAS Institute Inc) was used for statistical analyses. The 15-year incidence of medium drusen was estimated using Kaplan-Meier product-limit survival estimates and competing risk analyses that control for competing risk of death among persons at risk of medium drusen, after excluding participants with any worse stage of early or late AMD lesions at baseline. Associations between common AMD risk factors (age, sex, smoking status, blood pressure, white blood cell count, fish consumption, alcohol consumption, antioxidant and zinc supplementation intake, *CFH* rs1061170 and *ARMS2* rs10490924 risk alleles, and total cholesterol, high-density lipoprotein cholesterol, and triglycerides concentrations) and the 15-year incidence of medium drusen were assessed using age- and sex-adjusted discrete logistic regression models. If these risk factors reached $P \leq .09$ in the age- and sex-adjusted regression models, they were included in the multivariable-adjusted logistic regression model. The final multivariable-adjusted logistic regression model included age, sex, past and current smoking, zinc supplementation, and the combined *CFH* rs1061170 and *ARMS2* rs10490924 risk alleles as covariables. The combined *CFH* rs1061170 and *ARMS2* rs10490924 risk alleles were categorized as none, 1, 2, or 3 or more.

Frequencies of the progression from medium drusen alone, as well as from medium drusen plus RPE abnormalities, to worsening stages of AMD were reported. Generalized estimating equation models using the GENMOD procedure

Table 2. Comparison of Baseline Characteristics of Participants With vs Without Incident Medium Drusen

Baseline Characteristic	Without Incident Medium Drusen	With Incident Medium Drusen	*P* Value
Age, mean (SD), y	62.1 (8.1)	62.3 (7.5)	.65
Female sex, %	54.7	59.4	.16
Smoking status, %			<.001[a]
Never smoker	47.8	61.8	
Past smoker	37.3	28.7	
Current smoker	14.8	9.5	
CFH rs1061170, %			.40[a]
TT	42.3	38.2	
CT	47.0	49.0	
CC	10.6	12.9	
ARMS2 rs10490924, %			.09[a]
GG	65.6	57.9	
GT	30.4	37.8	
TT	4.0	4.3	
CFH and *ARMS2* combined risk, %			.15[a]
No risk alleles	28.7	21.3	
1 Risk allele	40.6	44.0	
2 Risk alleles	25.5	27.6	
≥3 Risk alleles	5.2	7.1	
Blood pressure, mean (SD), mm Hg			
Systolic	143.4 (19.9)	144.6 (21.1)	.39
Diastolic	83.2 (10.0)	83.8 (9.1)	.38
White blood cell count, mean (SD), /μL	6371.8 (1760.9)	6175.4 (1409.6)	.06
Total cholesterol concentration, mean (SD), mg/dL	232.4 (40.7)	233.9 (42.2)	.60
High-density lipoprotein cholesterol concentration, mean (SD), mg/dL	55.3 (17.4)	56.4 (14.7)	.29
Triglycerides concentration, mean (SD), mg/dL	155.3 (90.8)	147.7 (97.9)	.23
Fish consumption of ≥1 serving per wk, %	60.3	60.2	.98
Alcohol consumption, standard drinks per d, %			.65[a]
None	29.6	31.6	
1-2	58.6	55.5	
>2	11.8	12.9	
Any antioxidant supplementation, %	36.1	35.2	.79
Any zinc supplementation, %	16.9	11.6	.04
Dietary lutein and zeaxanthin intake, mean (SD), μg	0.8 (0.5)	0.8 (0.4)	.55

SI conversion factors: To convert white blood cell count to $\times 10^9$/L, multiply by 0.001; to convert total and high-density lipoprotein cholesterol concentrations to millimoles per liter, multiply by 0.0259; to convert triglycerides level to millimoles per liter, multiply by 0.0113.

[a] Unadjusted tests for heterogeneity were used to calculate these *P* values.

(SAS, version 9.3; SAS Institute Inc)[24] were applied to eye-specific data to assess associations between medium drusen area and location characteristics and the progression to early or late AMD. Association estimates are presented as age- and sex-adjusted or multivariable-adjusted odds ratios (ORs) and 95% CIs.

Results

Prevalence of Medium Drusen

Of 3654 baseline participants, we excluded persons with late AMD (n = 75), early AMD (n = 185), or large soft distinct drusen (n = 113), and we included 3281 for the assessment of medium drusen. The status of medium drusen could be clearly defined in 2959 participants, among whom 534 (18.0%) had medium drusen, including 445 (83.3%) with medium drusen alone and 89 (16.7%) with medium drusen plus RPE abnormalities. Medium drusen was bilateral in 16.6% (74 of 445).

Incidence of Medium Drusen

Among 1317 persons without any AMD lesions at baseline who had been followed up, the 5-year, 10-year, and 15-year cumulative incidences of medium drusen were 10.1% (95% CI, 8.6%-11.9%), 17.7% (95% CI, 15.6%-20.1%), and 30.8% (95% CI, 27.7%-34.3%), respectively. After controlling for competing risk of death, the 5-year, 10-year, and 15-year cumulative incidences of medium drusen were 5.7% (95% CI, 4.8%-6.6%), 9.2% (95% CI, 9.1%-9.3%), and 13.9% (95% CI, 12.5%-15.3%). The 15-year incidences of medium drusen by age and sex are listed in **Table 1**. The incidence rates across all age groups were comparable, except for those 75 years or older. The incidence of medium drusen was slightly lower in men compared with women.

Table 3. Associations Between Known Age-Related Macular Degeneration Risk Factors and the 15-Year Incidence of Medium Drusen

Risk Factor	15-Year Incidence of Medium Drusen, Odds Ratio (95% CI)	
	Age and Sex Adjusted, Where Appropriate	Multivariable Adjusted[a]
Age, per decade older, y	1.4 (1.2-1.7)	1.4 (1.2-1.8)
Male sex	0.8 (0.6-1.0)	0.9 (0.6-1.2)
Smoking status		
Never smoker	1 [Reference]	1 [Reference]
Past smoker	0.7 (0.5-0.9)	0.8 (0.6-1.1)
Current smoker	0.6 (0.4-1.0)	0.6 (0.4-1.1)
CFH and *ARMS2* combined risk[b]		
No risk alleles	1 [Reference]	1 [Reference]
1 Risk allele	1.4 (1.0-2.0)	1.6 (1.1-2.4)
2 Risk alleles	1.4 (0.9-2.1)	1.5 (0.9-2.2)
≥3 Risk alleles	2.1 (1.1-3.9)	2.1 (1.1-4.1)
Zinc supplementation	0.7 (0.5-1.1)	0.7 (0.4-1.0)

[a] Adjusted for age, sex, past and current smoking, any zinc supplementation, and the combined *CFH* and *ARMS2* risk alleles.

[b] Single-nucleotide polymorphisms *CFH* rs1061170 and *ARMS2* rs10490924.

Baseline characteristics of participants with and without incident medium drusen are listed in **Table 2**. There were no significant differences in the mean age or the frequency of female sex between participants with and without incident medium drusen. However, participants with incident medium drusen were marginally more likely to have at least 1 risk allele of *ARMS2* rs10490924 and have a lower mean white blood cell count and were less likely to be past or current smokers or to take zinc supplementation.

Table 3 lists associations between known AMD risk factors and the incidence of medium drusen. Each decade increase in age was significantly associated with a 15-year incidence of medium drusen after adjusting for sex. The presence of at least 3 risk alleles of *CFH* rs1061170 and *ARMS2* rs10490924 combined was significantly associated with an increased risk of developing medium drusen (OR, 2.1; 95% CI, 1.1-3.9) after adjusting for age and sex. These associations remained similar after additional adjustment for past and current smoking and for zinc supplementation. The 15-year incidence of medium drusen was inversely associated with a higher intake of zinc; however, this association was marginally nonsignificant in both age- and sex-adjusted and multivariable-adjusted models. Risk factors, including smoking status, blood pressure, white blood cell count, serum lipid levels, fish and alcohol consumption, antioxidant supplementation, and lutein and zeaxanthin intake, were not significantly associated with the 15-year incidence of medium drusen in the age- and sex-adjusted models.

Progression of Medium Drusen

The frequency of the progression to large soft drusen in eyes with medium drusen alone was similar to that in eyes with medium drusen plus RPE abnormalities (41.8% and 50.0%, respectively; $P = .20$). However, the progression to late-stage AMD or late AMD lesions (geographic atrophy or neovascular AMD) was 4-fold higher in eyes with medium drusen plus RPE abnormalities compared with eyes with medium drusen alone (23.0% vs 5.0%). An example of medium drusen progression is shown in the **Figure**.

Table 4 summarizes the 15-year progression of medium drusen to early and late AMD in relation to total area and location of medium drusen. Eyes with medium drusen plus RPE abnormalities were excluded. After adjusting for age and sex, a large total area (≥375 µm) compared with a small total area (<375 µm) of medium drusen was significantly associated with high risk of developing early AMD (OR, 2.9; 95% CI, 1.5-5.4) and any (early or late) AMD (OR, 3.0; 95% CI, 1.6-5.5). However, it was not significantly associated with risk of late AMD (OR, 2.3; 95% CI, 0.8-6.8). After further adjusting for smoking status, fish consumption, and the presence of the *CFH* and *ARMS2* risk alleles, these associations remained. Similarly, the association between a central location of medium drusen and the development of early AMD (OR, 2.6; 95% CI, 1.4-4.8) and any AMD (OR, 2.4; 95% CI, 1.3-4.5) was significant after adjusting for these covariables.

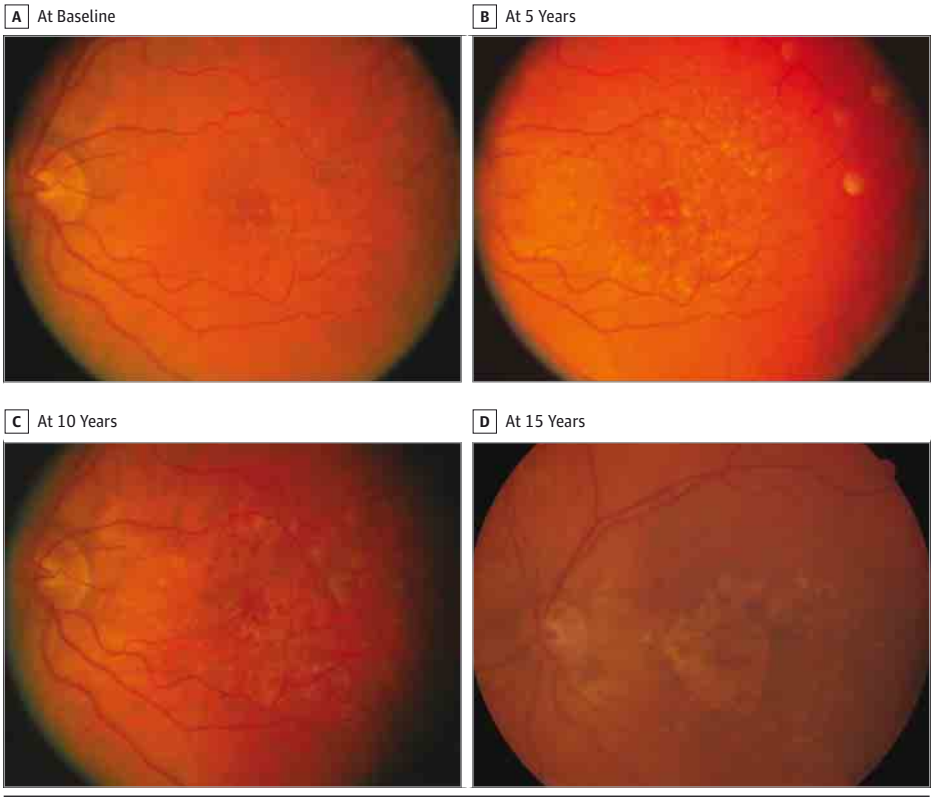
Discussion

We found that 13.9% of at-risk persons 49 years or older developed medium drusen over 15 years. The incidence of medium drusen did not appear to differ across the 3 age groups from 49 to 74 years, but it was slightly higher in women compared with men. The lower incidence of medium drusen observed in participants 75 years or older at baseline is likely because most persons in this age group had died or passed this early stage of AMD, and few participants in this age group were considered at risk of developing medium drusen.

A per decade increase in age and the presence of at least 3 risk alleles of *CFH* rs1061170 and *ARMS2* rs10490924 combined were independently associated with an increased risk of developing medium drusen. No other known AMD risk factors were found to be associated with the incidence.

Few population-based reports on the incidence of medium drusen are available in the literature. The 5-year, 10-year, and 15-year incidence rates of medium drusen in the BMES were 5.7%, 9.2%, and 13.9%, respectively. In the Reykjavik Eye Study,[25] a population-based study of residents in Iceland, the 5-year incidence of medium drusen increased with older age, ranging from 5.0% in individuals 50 to 59 years old to 22.7% in individuals 70 to 79 years old. In comparison, the overall 5-year incidence of medium drusen was 10.1% in the BMES (using the Kaplan-Meier method), or 5.7% after accounting for competing risk of death. The Copenhagen City Eye Study[26] reported a 27.4% 14-year incidence of medium drusen among persons 60 to 80 years old at baseline. The Beaver Dam Eye Study[27,28] found 14.0% and 23.9%, respectively, 10-year and 15-year cumulative incidences of medium drusen after adjusting for competing risk of death, higher than the 9.2% and 13.9% incidences at 10 years and 15 years, respectively, in our cohort study. Sources of disparity between our findings and the results of the Reykjavik Eye Study[25] and Copenhagen City Eye Study[26] likely include variations in defining medium drusen

Figure. An Example of the Progression of Medium Drusen



A, Left eye with medium drusen plus retinal pigment epithelium abnormalities at baseline. B, Large soft drusen at 5 years. C, More apparent retinal pigmentary abnormality at 10 years. D, Geographic atrophy at 15 years.

Table 4. Fifteen-Year Progression of Medium Drusen to Worse Age-Related Macular Degeneration (AMD) Stages by Medium Drusen Area and Location at Baseline[a]

	15-Year Progression to Worse AMD Stage								
	Early AMD			Any Late AMD			Any Early or Late AMD		
		aOR (95% CI)			aOR (95% CI)			aOR (95% CI)	
Medium Drusen Characteristic	No. of Cases/ No. at Risk	Age and Sex Adjusted	Multivariable Adjusted	No. of Cases/ No. at Risk	Age and Sex Adjusted	Multivariable Adjusted	No. of Cases/ No. at Risk	Age and Sex Adjusted	Multivariable Adjusted
Area									
Diameter <375 μm	73/308	1.0	1.0	13/327	1.0	1.0	76/311	1.0	1.0
Diameter ≥375 μm	41/68	2.9 (1.5-5.4)	3.2 (1.4-7.2)	9/71	2.3 (0.8-6.8)	1.6 (0.4-6.6)	44/71	3.0 (1.6-5.5)	3.3 (1.5-7.4)
P value	<.001	NA	NA	.03	NA	NA	<.001	NA	NA
Location									
Radius of the foveal center ≥500 μm	30/150	1.0	1.0	5/160	1.0	1.0	32/152	1.0	1.0
Radius of the foveal center <500 μm	82/222	2.6 (1.5-4.3)	2.6 (1.4-4.8)	17/235	3.1 (0.8-11.4)	3.5 (0.6-20.4)	86/226	2.5 (1.5-4.2)	2.4 (1.3-4.5)
P value	<.001	NA	NA	.06	NA	NA	<.001	NA	NA

Abbreviations: aOR, adjusted odds ratio; NA, not applicable.

[a] As defined in the Blue Mountains Eye Study, early AMD includes large indistinct soft or reticular drusen or large distinct soft drusen with retinal pigmentary abnormalities. Odds ratios are adjusted for age, sex, past and current smoking, fish consumption, and increasing numbers of *CFH* and *ARMS2* gene risk alleles (0, 1, or 2). *P* values are for differences in the number of cases between small vs large areas or between farther vs closer locations of intermediate drusen by AMD stage.

and different methods used to calculate the incidence estimates (eg, the competing risk approach was not used in the Reykjavik Eye Study or the Copenhagen City Eye Study). While the Beaver Dam Eye Study had a lower age limit (43 years at baseline) for their study sample, variations in other environmental exposures, as well as grading variations in defining medium drusen, could also explain the difference in incidence between our study and the BDES.[27,28]

The age-related increase in medium drusen incidence before adjusting for competing risk of death found herein is consistent with other AMD stages and lesions.[7,29,30] Past or current smoking was not associated herein with the 15-year

incidence of medium drusen as in previous BMES observations.[31,32] Although current smoking was previously strongly associated with the prevalence and incidence of late AMD, the association between smoking and early AMD was much weaker than that between smoking and late AMD.[31,32] Smoking may likely be a promoter, having a greater role in the progression from early to late AMD than in the initiation of early AMD.

We demonstrated that risk of the progression of medium drusen to late AMD was substantially higher for medium drusen plus RPE abnormalities. This parallels previous findings of faster progression from early to late AMD in eyes with large drusen plus RPE abnormalities compared with eyes with large drusen alone.[27,30,33] This observation supports the use of severity scales that incorporate multiple lesion types to better classify risk of the progression to late AMD.[2,3,34]

We also found that eyes with medium drusen located closer to the fovea or eyes with large total macular areas involved by medium drusen were more likely to progress to early AMD. The nonsignificant association of these characteristics with the progression to late AMD was likely due to the few incident late AMD cases in this cohort. These findings are consistent with the Beaver Dam Eye Study[28] in that the 15-year incidences of both early and late AMD were higher in eyes with a large total area of medium drusen at baseline compared with a small total area of medium drusen at baseline.

The strengths of this study include its long-term follow-up of an older Australian cohort, the use of retinal photographs, and the availability of a validated AMD grading system to assess the size and location of AMD lesions. Its limitations include the low follow-up rate at the 15-year examination, which could have led to an overestimation or underestimation of the incidence. Because only color fundus photographs rather than high-resolution imaging (eg, spectral-domain optical coherence tomography) were available during baseline and follow-up examinations, this may have led to an underestimation of the prevalence and incidence. However, we used only color photographs among the entire BMES cohort to ensure that the comparisons were valid. Although the medium drusen category has been included in retinal photographic grading since the 5-year follow-up examinations, side-by-side grading of baseline and follow-up retinal images provided precise classification of this drusen type.

Conclusions

In summary, the 15-year cumulative incidence of medium drusen was 13.9% in this at-risk older Australian cohort. The proportion of eyes that progressed to late AMD was significantly higher in eyes with medium drusen plus RPE abnormalities compared with eyes with medium drusen alone. Larger total area and central location of medium drusen were associated with a greater likelihood of the progression to worse stages of AMD. These findings are informative for the monitoring and management of patients at risk of early and late AMD.

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Ethnic Variation in Early Age-Related Macular Degeneration Lesions Between White Australians and Singaporean Asians

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Purpose. We compared early age-related macular degeneration (AMD) lesion characteristics between white Australians and Singaporean Asians.

Methods. Participants of the Blue Mountains Eye Study (BMES; whites, $n = 3508$) and the Singapore Epidemiology of Eye Disease Study (SEED; Malay, $n = 3280$, Indian, $n = 3400$, and Chinese, $n = 3353$) underwent examinations, including retinal photography. The AMD lesions were assessed following the Wisconsin AMD grading protocol by the same photographic grader. Prevalence and characteristics of early AMD lesions were compared between the BMES and the SEED. The associations between ethnicity and early AMD lesion types were analyzed using logistic regression models adjusting for age, sex, smoking status, lipids, and genetic polymorphisms associated with AMD.

Results. After age-standardization to the BMES population, the prevalence of distinct soft drusen was significantly higher in Singaporeans compared to Australians (23.9%, 95% confidence interval [CI] 22.9–25.0 vs. 6.2%, 95% CI 5.3–7.0), with an adjusted odds ratio (OR) of 4.6 (95% CI 3.4–6.0). In contrast, the prevalence of indistinct soft or reticular drusen was significantly lower in Singaporeans compared to Australians (6.5%, 95% CI 5.9–7.1 vs. 8.3%, 95% CI 7.4–9.3, with nonsignificant adjusted OR of 1.2, 95% CI 0.8–1.7). Soft drusen of any type were present frequently at the inner and outer macula (within a zone $\geq$500 to $<$3000 μm radius from the foveal center) among Singaporeans, while among Australians soft drusen were present more frequently at the central macula ($<$500 μm radius).

Conclusions. Singaporean Asians had a milder spectrum of early AMD lesions and lesion characteristics (predominantly distinct soft drusen and noncentral location) compared to white Australians.

Keywords: age-related macular degeneration, AMD, Asian, Australian, early AMD, late AMD, drusen, pigment

Differences in the prevalence of early and late signs of age-related macular degeneration (AMD) and specific AMD lesions, between whites and blacks residing in the United States long have been observed and documented; these findings have been suggested to reflect underlying ethnic predisposition to AMD.[1–3] For example, larger drusen and retinal pigmentary abnormalities have been reported to be present more frequently, and the advanced forms of AMD more prevalent, in whites compared to blacks.[1,3]

Emerging data on AMD in Asians now are available.[4,5] Although it was suggested that AMD was not common in Asians,[4] the pooled prevalence of early (6.8%) and late AMD (0.56%) in four Asian populations in a meta-analysis was comparable to the prevalence of early (8.8%) and late AMD (0.59%) in white populations,[6] challenging this previous assumption that AMD is less frequent in Asians than in whites.[7] However, these previous studies comparing differences in prevalence of AMD between white and Asian populations have focused only on the overall prevalence of any, early, or late AMD. The prevalence of individual early AMD lesions in particular, may exhibit ethnic variability. Similar to white populations, the type and frequency of different early AMD lesions in Asians may indicate a certain risk of progression to late AMD, for example, eyes with indistinct soft drusen having a higher risk of developing late AMD compared to those with distinct soft drusen, in mainly Caucasian populations.[8,9] Few studies have assessed the differences in specific AMD lesion characteristics and distributions between different ethnic

groups.[10,11] Such information may yield further insights into the early pathogenesis and presentation of AMD in diverse ethnic groups.

In this report, we aimed to compare directly the frequencies of different types of early AMD lesions, bilateral involvement, and lesion location between population-based samples of white Australians and Asians living in Singapore.

Methods

Study Populations

The Blue Mountains Eye Study (BMES). The BMES is a longitudinal population-based study of vision and eye disease in a predominantly white population aged 49 years and older residing in the Blue Mountains region, west of Sydney Australia. Details of the study methods and procedures have been described previously.[12,13] Briefly, of 4433 eligible persons identified in a door-to-door census of the study area, 3654 (82.4%) persons participated in the baseline study conducted from 1992 to 1994 (BMES I). Of these, 2334 participants (75.8% of survivors) were examined 5 years later, from 1997 to 1999 (BMES II-a). An additional 1378 eligible permanent residents were identified following a second door-to-door census in 1999. This included residents who had moved into the study area or had reached 49 years of age between BMES I and II. Of these newly eligible persons, 1174 participated in the study (85.2%, BMES II-b). Prevalence of AMD was derived from BMES II survey sample with a total number of 3508 participants from BMES II-a and b.

The Singapore Epidemiology of Eye Disease (SEED) Study. The SEED studies include three population-based studies, the Singapore Malay Eye Study (SiMES), the Singapore Indian Eye Study (SINDI), and the Singapore Chinese Eye Study (SCES), which are cross-sectional studies of the Malay, Indian, and Chinese populations aged 40+ years residing in southwestern Singapore. Detailed study methods for the SiMES, SINDI, and SCES have been reported previously.[14,15] Briefly, age-stratified random sampling was used to select 5600 Malay, 6350 Indian, and 6752 Chinese names from the Ministry of Home Affairs. From the number of eligible persons, a total of 3280 Malays[16] participated in the SiMES, 3400 Indians[17] participated in the SINDI, and 3353 Chinese[18] participated in the SCES from 2004 to 2006, 2007 to 2009, and 2009 to 2011, respectively.

Examinations of the BMES were approved by the Western Sydney Area Health Service and University of Sydney, and adhered to the tenets of the Declaration of Helsinki. The SEED (SiMES, SINDI, and SCES) study protocols were approved by the SingHealth Institutional Review Board, and all examinations were conducted at the Singapore Eye Research Institute in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before examinations of each study.

Examination Procedures

Each participant in the BMES and the SEED study cohort underwent a comprehensive ocular examination and completed a detailed largely identical interviewer-administered questionnaire pertaining to their socio-demographic and lifestyle factors, and medical history. The SEED cohort questionnaires and examination protocols were adapted from the BMES questionnaire.

In the BMES, 30° stereoscopic retinal fundus photographs of both eyes of each participant were taken using a Zeiss FF3 fundus camera (Carl Zeiss Meditec, Oberkochon, Germany) and processed on color film (Kodachrome; Eastman Kodak, Rochester, NY, USA). Similarly, in the SEED cohort, 45° digital retinal fundus photographs of the macular and optic disc were taken with a Canon CR-DGi with a 10D SLR digital camera backing (Canon Tokyo, Japan) after dilation.

Blood samples were collected from 3222 BMES and 9670 SEED participants at examination. Total cholesterol and high density lipoprotein (HDL) concentrations (mmol/L) were measured on a Reflotron reflectance photometric analyzer (Roche Diagnostics, Manheim, Germany) in the BMES, or were obtained from the biochemistry tests conducted at the Singapore National University Hospital Reference Laboratory. Blood samples collected also were used for genotyping.

AMD Grading and Definitions

Retinal photographs taken from participants of the BMES and SEED studies were graded by a single senior grader (MM) using a masked manner at the Centre for Vision Research, University of Sydney, Australia, and closely followed the Wisconsin Age-Related Maculopathy Grading System (WARMGS) protocol.[19] Adjudication of early AMD lesions was provided by a senior researcher (JJW). All late AMD cases were confirmed by a retinal specialist (PM). Briefly, the presence and location of AMD lesions were graded using the WARMGS grid containing three concentric circles corresponding to a distance of 500, 1000, and 3000 μm radius from the foveal center, superimposed over the macula. Lesion area was graded using the WARMGS standard circles with defined diameters of 63, 125, 250, 375, and 660 μm, and 0.5 and 1 disc area (DA).

Late AMD was defined as the presence of any sign of neovascular AMD (pigment epithelial or neurosensory subretinal detachment, retinal or subretinal hemorrhage, subretinal fibrosis, or old atrophic disciform scars, or photocoagulation scars) as described in the International Age-Related Maculopathy classification[20] or geographic atrophy (GA). Early AMD lesions were defined by the same classification system. Distinct soft drusen were distinguished as discrete whitish-yellow nodules >125 μm in diameter with uniform density and sharp edges. Indistinct soft drusen were described as >125 μm in diameter with decreasing density from center outwards to the periphery and fuzzy edges. Confluent indistinct soft drusen with the appearance of broad interlacing ribbons were distinguished as reticular drusen. The retinal pigment epithelial (RPE) depigmentation was defined as faded, but sharply demarcated areas of the RPE without visible choroidal vessels underneath. Granules of gray or black pigment within the retina were distinguished as hyperpigmentation.[19] In this report, early AMD was defined as the presence of either large (>125 μm diameter) indistinct soft or reticular drusen, or distinct soft drusen with retinal pigmentary abnormalities (RPE depigmentation and hyperpigmentation). In the following analyses we have included reticular drusen under the broader category of indistinct soft drusen.

Macular areas involved by early AMD lesions were categorized as small, intermediate, and large areas for each lesion type; and location of early AMD lesions categorized as within the central macula, inner macula, outer macula, or outside the macula area, as shown in Table 1.

Bilateral involvement of early or late AMD was defined among persons with early or late AMD, respectively. Participants with early AMD in one eye and late AMD in the fellow eye were considered bilateral for any AMD, but unilateral for early or late AMD. If maculopathy data were missing in one eye, the study subject was excluded from analysis of bilateral involvement.

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Table 1. Definitions of the Area and Location of the Early AMD Lesions Assessed

Area and Location	Definitions
Drusen area	
Small	None or <375 μm in diameter
Intermediate	≥375 μm to <0.5 disc area in diameter
Large	≥0.5 disc area in diameter
RPE depigmentation area	
Small	None or <375 μm in diameter
Intermediate	≥375 μm to <2 disc area in diameter
Large	≥2 disc area in diameter
Hyperpigmentation area	
Small	None or <64 μm in diameter
Intermediate	≥64 to <660 μm in diameter
Large	≥660 μm in diameter
Location (all lesions)	
Central macula	<500 μm radius from the foveal center
Inner macula zone	≥500 to <1500 μm radius from the foveal center
Outer macula zone	≥1500 to <3000 μm radius from the foveal center
Outside macula	≥3000 μm radius from the foveal center

Genotyping

In the BMES, genotyping of the *complement factor H* (*CFH*) single nucleotide polymorphism (SNP) *rs1061170* and the *age-related maculopathy susceptibility gene 2* (*ARMS2*) SNP *rs10490924* was performed using TaqMan assays (Applied Biosystems, Foster City, CA, USA) and restriction fragment length polymorphism analysis, respectively. Both SNPs also were imputed using the BMES genome wide association scan data. Genome wide genotyping was first performed using an Illumina Human 670-Quad custom array version 1; Illumina, Inc., San Diego, CA, USA and stringent quality control applied. Imputation was then performed using the 1000 Genomes penal and IMPUTE 2.0 (Department of Statistics, University of Oxford, Oxford, UK). In the SEED, genotyping was performed using the Illumina Human 610-Quad array (Illumina, Inc.). Similar quality control procedures were applied in the SEED as in the BMES before analysis. Imputation then was performed using the 1000 Genomes penal and IMPUTE 2.0 (Department of Statistics, University of Oxford).

Definitions of Other Variables

Smoking status was obtained from the interviewer-administered questionnaire in the BMES and the SEED cohort. Participants categorized as nonsmokers were those who answered "no" to smoking regularly. If participants answered "yes," but had stopped smoking ≥1 year before the examination they were categorized as past smokers. Participants who currently smoked or had stopped smoking <1 year before the examination were categorized as current smokers. In the BMES, hypertension was considered present if participants were taking antihypertensive medication at the time of examination, or blood pressure was ≥140 mm Hg, or diastolic blood pressure was ≥90 mm Hg at examination. In the SEED, hypertension was considered present if systolic blood pressure was ≥140 mm Hg, diastolic blood pressure was ≥90 mm Hg, or with previous physician diagnosis reported by the participant. Body mass index (BMI) was calculated from weight and height measurements taken at examination.

Statistical Analyses

The program SAS (version 9.3; SAS Institute, Inc., Cary, NC, USA) was used for all analyses. We included participants aged 50 years or older only, with 3508 from the BMES, 2453 from the SiMES, 2427 from the SINDI, and 2633 from the SCES. The worse eye prevalence of late and early AMD in the BMES were compared to worse eye prevalence estimates of the SiMES, SINDI, and SCES, respectively, after direct age-standardization to the BMES population. Comparisons also were performed within subgroups stratified by smoking status after age-standardization to the BMES population. Differences in the frequencies of bilateral involvement of early AMD lesions between the ethnic groups were assessed using Fisher's exact test. Logistic regression models adjusted for age, sex, smoking, lipids, hypertension, BMI, and the *CFH* and *ARMS2* polymorphisms were used to estimate association magnitudes between each Asian ethnicity, and the presence of distinct soft drusen and indistinct soft drusen, with reference to the BMES.

Early AMD lesion characteristics, including the areas involved by the lesions and location of the lesions within the central, inner, and outer zones were compared between the BMES and the Singaporean Asian samples combined. Data of both eyes and generalized estimating equation (GEE) models were used in these analyses.

Results

Table 2 shows the characteristics of participants without AMD and with early AMD in the BMES compared to the SiMES, SINDI, SCES, or these Asian samples combined. Among participants with and without early AMD, the mean age and frequency of women were lower in the three Asian populations combined compared to the BMES ($P < 0.0001$ for age and sex). There was a lower frequency of past smokers and higher frequency of current smokers in the combined Singaporean Asian sample compared to the BMES (both $P < 0.0001$). The mean ages of participants with early AMD were higher than participants without any AMD across all the study samples (Table 2). The frequency of participants with 1 or 2 risk alleles of *CFH* was significantly lower in the SiMES, SINDI, and SCES compared to the BMES, whereas there was a higher frequency of 2 risk alleles for *ARMS2* across the Singaporean Asian samples compared to the BMES sample (all $P < 0.0001$). Mean cholesterol, high density lipoprotein, and BMI were significantly lower in the Asian samples compared to the BMES in both subgroups with and without early AMD lesions (Table 2).

The crude prevalence of AMD in each study population is presented in Table 3 and age-standardized prevalence is presented in Table 4. After direct age-standardization, late AMD prevalence was nonsignificantly lower in the combined Asian sample compared to the BMES, whereas the prevalence of early AMD was significantly higher in the combined Asian samples compared to the BMES.

The prevalence of distinct soft drusen was substantially higher in each of the Asian samples or in the combined Asian samples, compared to the BMES. In contrast, the prevalence of indistinct soft drusen was significantly lower in SiMES and SCES, or in the combined Asian samples, compared to the BMES sample. The prevalence of indistinct soft drusen in SINDI was similar to the BMES, after age-standardization to the BMES sample (Table 4). There was no difference in the prevalence of retinal pigmentary abnormalities in the combined Asian samples compared to the BMES sample.

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Table 2. Characteristics in Participants Without AMD and With Early AMD in the BMES Compared to Participants of the SiMES, SINDI, SCES, and the Three Asian Samples Combined

Characteristic	BMES, n = 2867	SiMES, n = 1746	P Value*	SINDI, n = 1806	P Value*	SCES, n = 2046	P Value*	Combined Asian, n = 5598	P Value†
	% Participants With No AMD								
Mean age, y (SD)	65.1 (8.7)	61.2 (8.1)	<0.0001	60.0 (7.8)	<0.0001	60.9 (8.2)	<0.0001	60.7 (8.0)	<0.0001
Sex, female	56.5	51.7	0.002	48.3	<0.0001	52.2	0.003	50.8	<0.0001
Smoking status									
Nonsmoker	49.2	62.3	<0.0001‡	73.1	<0.0001‡	74.8	<0.0001‡	70.4	<0.0001‡
Past smoker	40.1	18.5		12.6		12.9		14.5	
Current smoker	10.8	19.2		14.3		12.4		15.1	
Hypertension, present	75.5	75.3	0.9	62.7	<0.0001	61.9	<0.0001	66.3	<0.0001
CFH, rs1061170-BMES/ rs1080155-SiMES, SINDI, SCES									
No risk alleles	38.4	83.0	<0.0001‡	54.0	<0.0001‡	93.7	<0.0001‡	75.7	<0.0001‡
1 risk allele	47.3	15.8		37.9		6.2		21.0	
2 risk alleles	14.4	1.2		8.1		0.1		3.4	
ARMS2, rs10490924-BMES/ rs3750847-SiMES, SINDI, SCES									
No risk alleles	62.6	37.8	<0.0001‡	40.8	<0.0001‡	31.5	<0.0001‡	37.1	<0.0001‡
1 risk allele	32.7	47.4		47.7		49.3		48.0	
2 risk alleles	4.8	14.8		11.5		19.2		14.9	
Mean total cholesterol, mmol/L (SD)	5.8 (1.1)	5.7 (1.2)	<0.0001	5.2 (1.1)	<0.0001	5.5 (1.1)	<0.0001	5.5 (1.1)	<0.0001
Mean HDL, mmol/L (SD)	1.5 (0.4)	1.4 (0.3)	<0.0001	1.1 (0.3)	<0.0001	1.3 (0.4)	<0.0001	1.3 (0.4)	<0.0001
Mean BMI, kg/m^2 (SD)	27.8 (4.9)	26.4 (4.9)	<0.0001	26.3 (4.7)	<0.0001	23.7 (3.7)	<0.0001	25.4 (4.6)	<0.0001
	% Participants With Early AMD								
	n = 284	n = 147		n = 166		n = 219		n = 532	
Mean age, years (SD)	75.1 (8.4)	67.6 (7.8)	<0.0001	67.0 (8.6)	<0.0001	66.9 (8.1)	<0.0001	67.1 (8.2)	<0.0001
Sex, female	62.7	39.5	<0.0001	44.0	0.0001	39.3	<0.0001	40.8	<0.0001
Smoking status									
Nonsmoker	53.0	57.9	0.01‡	68.7	0.0002‡	69.9	<0.0001‡	66.2	<0.0001‡
Past smoker	38.8	26.9		19.9		18.7		21.3	
Current smoker	8.2	15.2		11.5		11.4		12.5	
Hypertension, present	82.0	85.7	0.3	78.9	0.4	78.1	0.3	80.5	0.6
CFH, rs1061170-BMES/ rs1080155-SiMES, SINDI, SCES									
No risk alleles	26.6	80.5	<0.0001‡	52.0	<0.0001‡	90.3	<0.0001‡	74.6	<0.0001‡
1 risk allele	50.0	19.5		35.0		9.0		20.8	
2 risk alleles	23.4	0.0		13.0		0.8		4.6	
ARMS2, rs10490924-BMES/ rs3750847-SiMES, SINDI, SCES									
No risk alleles	48.8	32.7	<0.0001‡	43.9	<0.0001‡	20.2	<0.0001‡	31.9	<0.0001‡
1 risk allele	47.3	41.6		37.4		54.5		44.9	
2 risk alleles	4.0	25.7		18.7		25.4		23.2	
Mean total cholesterol, mmol/L (SD)	5.9 (1.0)	5.6 (1.2)	0.01	4.8 (1.1)	<0.0001	5.3 (1.1)	<0.0001	5.2 (1.2)	<0.0001
Mean HDL, mmol/L (SD)	1.5 (0.4)	1.4 (0.4)	<0.0001	1.1 (0.3)	<0.0001	1.3 (0.4)	<0.0001	1.3 (0.4)	<0.0001
Mean BMI, kg/m^2 (SD)	27.0 (4.5)	26.1 (5.5)	0.09	26.2 (4.8)	0.1	23.2 (3.7)	<0.0001	24.9 (4.8)	<0.0001

* *P* value for comparison between participants of the BMES and SiMES or SINDI or SCES.
† *P* value for comparison between participants of the BMES and participants of the SiMES, SINDI, and SCES combined.
‡ Unadjusted tests for heterogeneity used to calculate *P* values.

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TABLE 3. Comparison of the Crude Prevalence of AMD in the BMES to the SiMES, SINDI, and SCES Samples, and the three Asian Samples Combined

Lesion	BMES	SiMES		SINDI		SCES		Combined Asian	
	Prevalence % (No. Affected/ Total No.)	Prevalence % (No. Affected/ Total No.)	P Value*	Prevalence % (No. Affected/ Total No.)	P Value*	Prevalence % (No. Affected/ Total No.)	P Value*	Prevalence % (No. Affected/ Total No.)	P Value*
Any late AMD	1.76 (60/3410)	0.97 (20/2060)	0.02	0.54 (13/2427)	<0.0001	0.95 (25/2633)	0.008	0.81 (58/7120)	<0.0001
Pure GA	0.67 (23/3410)	0.39 (8/2060)	0.2	0.00 (0/2427)	<0.0001	0.19 (5/2633)	0.006	0.18 (13/7120)	<0.0001
Neovascular AMD	1.09 (37/3409)	0.58 (12/2059)	0.06	0.54 (13/2427)	0.02	0.76 (20/2633)	0.2	0.63 (45/7119)	0.01
Any early AMD	9.01 (284/3152)	7.76 (147/1894)	0.1	8.42 (166/1972)	0.5	9.67 (219/2265)	0.4	8.68 (532/6132)	0.6
Any large drusen†	13.06 (412/3154)	20.62 (398/1930)	<0.0001	21.11 (422/1999)	<0.0001	26.83 (620/2311)	<0.0001	23.07 (1440/6241)	<0.0001
Soft distinct drusen	6.00 (194/3235)	17.02 (326/1915)	<0.0001	17.54 (348/1984)	<0.0001	24.12 (556/2305)	<0.0001	19.82 (1230/6205)	<0.0001
Soft indistinct or reticular drusen	7.65 (241/3149)	4.35 (82/1886)	<0.0001	4.33 (85/1964)	<0.0001	3.63 (82/2258)	<0.0001	4.08 (249/6109)	<0.0001
Any pigment abnormality	15.08 (487/3229)	15.65 (323/2064)	0.6	14.98 (319/2129)	0.9	16.04 (385/2400)	0.3	15.57 (1027/6594)	0.5
RPE depigmentation	9.34 (301/3221)	11.49 (236/2054)	0.01	7.91 (169/2136)	0.07	7.71 (185/2398)	0.03	8.95 (590/6589)	0.5
Hyperpigmentation	14.33 (463/3230)	11.25 (231/2053)	0.001	12.71 (270/2124)	0.09	14.11 (338/2395)	0.8	12.76 (839/6573)	0.03

* *P* value for crude prevalence comparison between participants of the BMES and the SiMES, SINDI, SCES, or Combined Asian sample.
† Includes soft distinct, soft indistinct, and reticular drusen.

Compared to the BMES, the combined Singaporean Asian sample had a lower frequency of bilateral late AMD (17.24% vs. 58.33%, $P < 0.0001$) and bilateral early AMD (30.31% vs. 48.13%, $P < 0.0001$). Bilateral retinal pigment abnormalities also were less frequent in the SiMES, SINDI, and SCES compared to the BMES (all $P < 0.0001$). However, there was no difference in the frequencies of bilateral reticular drusen or other soft drusen between the Asian and BMES samples (all $P > 0.05$, data not shown).

After stratifying by smoking status, the age-standardized prevalence of early AMD was not significantly different between the combined Singaporean Asian and BMES sample in each category of nonsmokers and current smokers. However, there was a significantly higher prevalence of early AMD in past smokers in the combined Asian sample compared to the BMES (Table 5). Distinct soft drusen prevalence remained significantly higher in all three Asian ethnicities compared to the BMES, regardless of smoking status, whereas indistinct soft drusen prevalence remained significantly lower in the combined Asian samples compared to the BMES in the nonsmoking subgroup. In the two subgroups of past smokers and current smokers, the differences in the prevalence of indistinct soft drusen between the combined Asian samples and the BMES were nonsignificant (Table 5).

Table 6 presents a comparison of the areas and location of early AMD lesions in right eyes, between the BMES and the combined Singaporean Asian samples, shown with age-adjusted *P* values. Larger areas of soft drusen were more frequent in the SiMES, SINDI, and SCES than in the BMES (age-adjusted $P < 0.0001$). The frequencies of larger areas involved by RPE depigmentation were similar in Australian and Singaporean Asian samples, while large areas involved by hyperpigmentation were less frequent in the Asian compared to the BMES sample (6.7% vs. 9.6%, age-adjusted $P = 0.01$). Singaporean Asians were more likely to have eyes with drusen located only at the inner and outer macula zones, but significantly less likely to have eyes with drusen located at the central macula compared to the BMES sample (age-adjusted $P < 0.0001$, see Fig.). Noncentral location for hyperpigmentation was more likely in the Singaporean Asians than in the BMES (age-adjusted $P < 0.0001$).

The associations between ethnicity and soft drusen types were assessed further after adjusting for age, sex, smoking status, total cholesterol, HDL, hypertension, BMI, and the *CHF* SNPs *rs1061170* and *rs1080155*, and *ARMS2* SNPs *rs10490924* and *rs3750847* (Table 7). With reference to Australians, there was a significantly higher likelihood of having distinct soft drusen in Malays, Indians, or Chinese (ORs 4.0, 4.6, and 7.0, respectively). By comparison, there was a lower likelihood of having indistinct soft or reticular drusen among Malays, Indians, and Chinese compared to the Australian population (OR's 1.1, 1.1, and 1.3, respectively), though these associations were not significant (Table 7).

DISCUSSION

In this study, we found a significantly lower prevalence of distinct soft drusen in Australians compared to Asians (Malays, Indians, and Chinese). In contrast, we found a higher prevalence of indistinct soft drusen in Australians compared to Malays and Chinese, while Indians had a similar prevalence of indistinct soft drusen to Australians. The similarity between Indians and whites could be explained by their relatively close genetic make-up, compared to less similarity in the genome between whites and other Asian ethnicities.

Similar to the previous report by Kawasaki et al.[6] of a meta-analysis of findings from nine Asian population-based samples

Table 4. Prevalence of AMD lesions in the SiMES, SINDI, SCES, and Combined Asian Eye Study Samples Age-Standardized to the BMES

Lesion	Age-Standardized Prevalence % (95% CI)				
	BMES	SiMES	SINDI	SCES	Combined Asian
Any late AMD	1.76 (1.32–2.20)	1.09 (0.64–1.54)	0.76 (0.42–1.11)	1.52 (1.05–1.99)	1.3 (1.04–1.56)
Pure GA	0.68 (0.40–0.95)	0.43 (0.15–0.72)	0.00 (0.00–0.00)	0.28 (0.08–0.48)	0.26 (0.15–0.38)
Neovascular AMD	1.09 (0.74–1.43)	0.66 (0.31–1.01)	0.76 (0.42–1.11)	1.24 (0.82–1.67)	1.03 (0.80–1.27)
Any early AMD	9.72 (8.69–10.76)	10.98 (9.57–12.38)	13.53 (12.02–15.04)	13.01 (11.63–14.40)	12.51 (11.69–13.34)
Any large drusen*	13.88 (12.68–15.09)	26.72 (24.75–28.69)	28.35 (26.37–30.33)	32.36 (30.45–34.26)	28.84 (27.72–29.96)
Soft distinct drusen	6.15 (5.32–6.98)	22.73 (20.85–24.60)	21.75 (19.94–23.57)	28.76 (26.91–30.60)	23.93 (22.87–24.99)
Soft indistinct or reticular drusen	8.32 (7.35–9.28)	4.92 (3.94–5.90)	8.36 (7.14–9.59)	5.20 (4.28–6.11)	6.52 (5.91–7.14)
Any pigment abnormality	15.52 (14.27–16.77)	15.76 (14.19–17.34)	18.02 (16.39–19.65)	18.50 (16.95–20.06)	17.62 (16.70–18.54)
RPE depigmentation	9.63 (8.61–10.65)	10.35 (9.03–11.66)	9.07 (7.85–10.28)	7.65 (6.59–8.72)	8.97 (8.28–9.66)
Hyperpigmentation	14.78 (13.56–16.01)	12.03 (10.62–13.44)	15.64 (14.10–17.19)	16.74 (15.24–18.23)	15.06 (14.20–15.93)

* Includes soft distinct, soft indistinct, and reticular drusen.

aged 40 to 79 years, we found a 1.76% prevalence of late AMD in the BMES population to be comparable to the 1.30% prevalence in the combined Asian samples of Malays, Indians, and Chinese, all aged 50 years or older. The age-standardized prevalence of early AMD was slightly lower in Australians and Malays compared to that of Indians or Chinese (9.72% and 10.98% vs.13.53% and 13.01%, respectively).

The early AMD prevalence of 10.98% and 13.53% found in our study contrasts with previously published prevalence of 3.5% and 4.5% for the same Singaporean population of Malays[21] and Indians,[22] respectively. These differences are likely due to the age of the populations under investigation: ≥50 years in our study versus 40 to 80 years in the previous study, and age-standardization to the BMES in our study versus age-standardization to the Singapore population at the 2000 Singapore census.[21,22] Our finding also contrasts with another study of a different multiethnic cohort of Singaporean Malays, Indians, and Chinese aged 40+ years that reported similar prevalence of any AMD among the three ethnic groups (5.7%–7.7%),[11] which was comparable to white populations around the world.

Though the overall prevalence of early AMD was found to be similar between Australians and Singaporean Asians in our study, the frequency of various lesions forming early AMD were different. Malays and Chinese predominantly presented with a milder spectrum of lesions, such as distinct soft drusen and a lower frequency of advanced lesions, including indistinct soft drusen, compared to Australians. Of a few studies that have specifically investigated the prevalence of the individual lesions of late and early AMD, our observation is consistent with previous findings from the Multi-Ethnic Study of Atherosclerosis (MESA) that found higher prevalence of

Table 5. Comparison of the Age-Standardized Prevalence of Early AMD by Smoking Status in the BMES to the SiMES, SINDI, SCES, and the Three Asian Samples Combined

AMD Lesion	Age-Standardized Prevalence (95% CI)				
	BMES	SiMES	SINDI	SCES	Combined Asian
Nonsmoker					
Any early AMD	10.41 (8.88–11.94)	10.49 (8.74–12.25)	13.41 (11.64–15.17)	12.77 (11.18–14.37)	12.20 (11.22–13.18)
Any large drusen	14.72 (12.94–16.49)*	25.03 (22.57–27.48)*	28.42 (26.10–30.74)*	32.48 (30.26–34.70)*	28.86 (27.51–30.20)*
Soft distinct drusen	6.71 (5.48–7.95)*	19.64 (17.37–21.90)*	22.54 (20.39–24.70)*	29.03 (26.87–31.18)*	24.18 (22.90–25.45)*
Soft indistinct or reticular drusen	8.66 (7.25–10.07)*	6.40 (4.99–7.80)	7.69 (6.31–9.07)	4.98 (3.94–6.02)*	6.15 (5.43–6.87)*
Any pigment abnormality	15.74 (13.94–17.54)	13.49 (11.62–15.37)	18.96 (17.02–20.91)	16.51 (14.79–18.24)	16.31 (15.25–17.38)
RPE depigmentation	9.33 (7.89–10.77)*	7.87 (6.39–9.35)	8.38 (7.00–9.75)	5.94 (4.84–7.04)*	7.27 (6.52–8.02)
Hyperpigmentation	14.74 (12.99–16.50)*	10.77 (9.07–12.47)*	16.47 (14.63–18.32)	15.36 (13.68–17.04)	14.27 (13.26–15.28)
Past Smoker					
Any early AMD	9.44 (7.81–11.07)*	13.46 (9.94–16.98)	15.53 (11.14–19.93)*	14.57 (10.61–18.54)	14.30 (12.04–16.55)*
Any large drusen	13.77 (11.85–15.68)*	26.60 (22.09–31.11)*	28.42 (26.10–30.74)*	33.12 (27.94–38.30)*	29.92 (27.01–32.83)*
Soft distinct drusen	6.27 (4.94–7.61)*	21.29 (17.09–25.50)*	22.54 (20.39–24.70)*	29.78 (24.74–34.82)*	24.93 (22.17–27.69)*
Soft indistinct or reticular drusen	8.10 (6.58–9.62)	7.73 (4.97–10.50)	9.27 (5.74–12.80)	5.07 (2.61–7.54)	7.07 (5.42–8.73)
Any pigment abnormality	14.44 (12.50–16.37)*	22.07 (18.00–26.13)*	20.50 (15.76–25.23)	22.58 (18.04–27.11)*	21.74 (19.19–24.29)*
RPE depigmentation	9.15 (7.56–10.74)*	17.14 (13.43–20.85)*	12.80 (8.89–16.72)	12.29 (8.74–15.84)	14.26 (12.10–16.42)*
Hyperpigmentation	14.12 (12.20–16.03)	16.30 (12.66–19.94)	17.86 (13.37–22.36)	19.27 (14.99–23.55)	17.63 (15.27–19.99)
Current Smoker					
Any early AMD	7.66 (4.78–10.55)	7.85 (5.06–10.64)	7.86 (4.69–11.04)	10.66 (7.03–14.29)	8.82 (6.98–10.66)
Any large drusen	10.68 (7.34–14.03)*	22.77 (18.46–27.08)*	20.85 (16.08–25.61)*	30.67 (25.32–36.03)*	24.82 (22.04–27.60)*
Soft distinct drusen	3.60 (1.60–5.60)*	17.11 (13.23–20.98)*	17.44 (12.98–21.90)*	27.03 (21.86–32.21)*	20.32 (17.72–22.91)*
Soft indistinct or reticular drusen	7.37 (4.54–10.20)	5.63 (3.23–8.02)	4.24 (1.87–6.61)	5.24 (2.61–7.87)	5.21 (3.77–6.66)
Any pigment abnormality	18.99 (14.78–23.31)	19.45 (15.48–23.42)	16.37 (12.12–20.62)	21.74 (17.03–26.46)	19.42 (16.92–21.91)
RPE depigmentation	12.51 (8.96–16.07)	15.98 (12.30–19.65)	10.58 (7.06–14.10)	13.17 (9.30–17.03)	13.44 (11.29–15.59)
Hyperpigmentation	17.81 (13.70–21.92)	13.13 (9.73–16.53)	12.92 (9.07–16.77)	17.81 (13.41–22.20)	14.72 (12.48–16.96)

* Indicates significant difference between the BMES and SiMES, SINDI, SCES, or the Combined Asian samples.

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Table 6. Area and Location of Drusen and Pigmentary Abnormalities in the BMES Compared to the Combined Asian Samples (SiMES, SINDI, and SCES Combined)

Early AMD Lesion Characteristics	% Eyes			
	BMES	Combined Asian Samples*	Age-Adjusted P Value†	Odds Ratio (95% CI)‡
AREA				
Drusen				
None or <375 μm in diameter	92.4	91.8		1.0
≥375 μm in diameter	7.7	8.2	<0.0001	1.8 (1.6–2.1)
RPE Depigmentation				
None or <375 μm in diameter	96.3	96.7		1.0
≥375 μm in diameter	3.7	3.3	0.9	1.0 (0.9–1.2)
Hyperpigmentation				
None or <64 μm in diameter	90.5	93.3		1.0
≥64 μm in diameter	9.6	6.7	0.01	0.9 (0.8–1.0)
Location				
Drusen				
Central macula	87.5	59.3		1.0
Inner and outer macula zone	12.5	40.7	<0.0001	5.8 (2.7–12.3)
RPE depigmentation				
Central macula	58.4	62.4		1.0
Inner and outer macula zone	41.6	37.6	0.7	0.9 (0.5–1.5)
Hyperpigmentation				
Central macula	80.8	63.6		1.0
Inner and outer macula zone	19.2	36.4	0.03	2.0 (1.1–4.0)

Location definitions: central macula, <500 μm radius from foveal center; inner macula zone, ≥500 to <1500 μm radius from the foveal center; outer macula zone, ≥1500 to <3000 μm radius from the foveal center.

* SiMES, SINDI, and SCES samples combined.

† Unadjusted tests for heterogeneity used to calculate *P* values.

‡ ORs estimated with BMES as reference group, using generalized estimating equation models and both eyes from each participant.

distinct soft drusen among Chinese compared to black, white, or Hispanic participants.[2]

The BMES consists of participants with predominantly European ancestry and has found comparable early and late AMD prevalence and incidence to other population-based epidemiological studies, including the Beaver Dam Eye Study and the Rotterdam Eye study.[12,23–26] Singaporeans consist of many different Asian ethnicities; however, the three ethnic groups captured in the SEED study were the three predominant ethnic groups of Asians.[14,15] Similar estimates of early and late AMD prevalence were found between Singaporean Indians and Indians living in India.[22]

The SNPs at the CFH and ARMS2 loci are significant risk factors for early AMD, though they present a weaker risk for early than for late AMD, as shown in a recent genome-wide association study (GWAS) meta-analysis.[27] The differences in early AMD lesion prevalence between Australians and Asians could be explained partly by genetic differences.[27–31]

The bilateral involvement of early, late, and any AMD were shown to be comparable between the Malay and Australian populations in a previous report.[10] In the current analysis, we found that the frequency of bilateral late and early AMD was significantly lower in Malays, Indians, and Chinese compared to Australians. The higher frequencies of bilateral involvement of late and early AMD in Australians compared to Singaporean Asians in our study could have been partly due to differences in age ranges of the samples, as age-standardization was not performed in the comparison of bilateral involvement, due to small numbers. The lower frequency of bilateral early AMD in Asians also may be explained by the lower prevalence of the *CFH Y402H* polymorphism in Asian populations,[32,33] which has been found to be associated with bilateral early AMD involvement.[34,35]

In addition to increasing age, smoking is an established risk factor for AMD in many white populations.[36–38] Similar associations between smoking and an increased AMD risk also have been documented in Asians.[39–41] Among past and current smokers, we found no difference in the age-standardized prevalence of indistinct soft and reticular drusen between Australians and Singaporean Asians. This could have been due to reduced numbers of subjects in these smoking subgroups.

A higher risk of developing late AMD has been associated with a more central location of drusen and more advanced

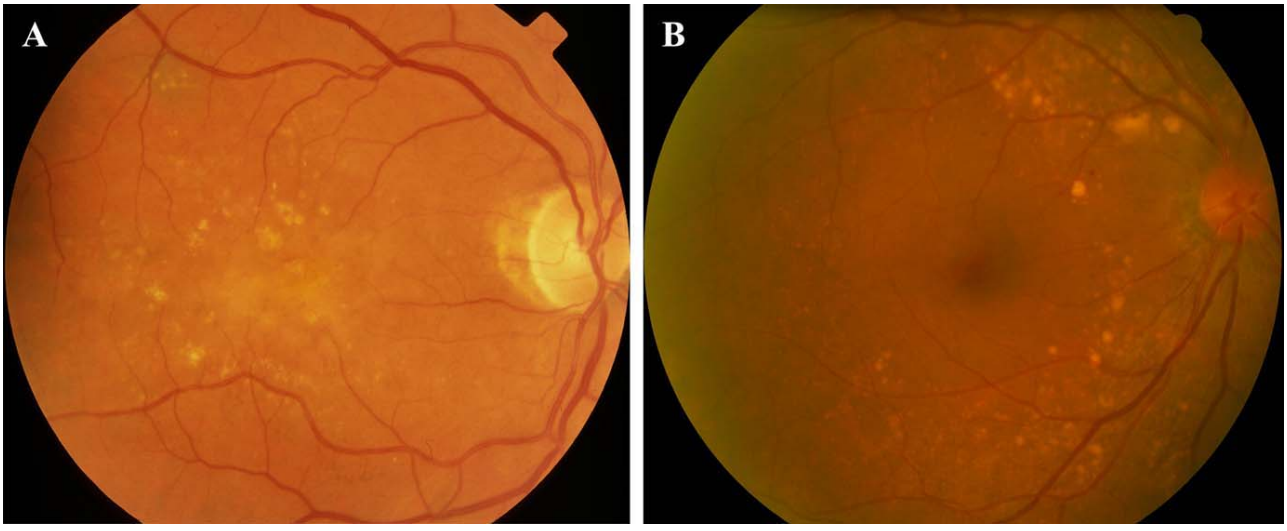

Figure. Examples of the different distribution of early AMD lesions in white Australians and Singaporean Asians. Central location of drusen in an Australian participant (**A**), compared to the more peripheral location of drusen in an Asian participant (**B**).

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Table 7. The Association Between Asian Ethnicity (SiMES, SINDI, SCES, and Combined Asian sample) and the Prevalence of Soft Drusen, With Reference to Whites (the BMES Population), Shown as ORs With 95% CIs

	Soft Drusen Type	
	Distinct Soft	Indistinct Soft and Reticular
Ethnic Group	Multivariable Adjusted OR* (95% CI)	Multivariable Adjusted OR* (95% CI)
BMES (reference group)	1.0	1.0
SiMES	4.0 (3.0, 5.4)†	1.1 (0.7, 1.7)
SINDI	4.6 (3.4, 6.3)†	1.1 (0.7, 1.8)
SCES	7.0 (5.1, 9.6)†	1.3 (0.7, 2.2)
Combined Asian sample	4.6 (3.4, 6.0)†	1.2 (0.8, 1.7)

* Adjusted for age, sex, smoking, cholesterol, high density lipoprotein, hypertension, BMI, and the *CFH* and *ARMS2* risk alleles as categorical variables.

† Indicates significant differences.

stages of early AMD lesions.[9,42] Our findings of a predominantly distinct soft drusen pattern among early AMD lesions, and relatively high prevalence of drusen and retinal pigmentary changes located away from the foveal center in Singaporean Asians compared to white Australians, reinforce the impression that Asians present a milder spectrum of early AMD lesions than whites, despite the overall lack of a substantive difference in the prevalence of early and late AMD between the Singaporean Asians and white Australians.

The strengths of this study included the large sample size of each ethnic group, and the direct comparison of AMD lesion and lesion characteristics among the studies. All studies used the same standardized grading protocol to determine AMD lesions and lesion characteristics, with AMD grading performed by the same grader (MM), adjudicated by the same senior researcher (JJW) and ophthalmologist (CY), with all late AMD cases confirmed by the same retinal specialist (PM). Limitations of the study included the small sample size in some groups when bilaterality and area and location of early AMD lesions were assessed, where it was not possible to obtain an age-standardized frequency. There is a difference in examination time between the BMES II study and the three Asian studies conducted in succession of each other, and this may have influenced the comparison between the studies if there was a temporal change in the prevalence of AMD.[10] This does not seem likely given the relatively narrow time interval. The difference in types of fundus photographs taken between the BMES and the three Asian studies (35-mm color film versus color digital images, 30° vs. 45° photographs, and stereo versus nonstereo, respectively), however, could have had subtle effects on the quantitative measurements of lesion area and location, but is unlikely to have affected the assessment of the prevalence of different AMD lesions.

In conclusion, we found that overall, Asians (from the three major Asian ethnic groups, Malays, Indians, and Chinese) living in Singapore, had a predominantly milder spectrum of early AMD lesions compared to whites living in Australia, after age standardization. Further studies of environmental and genetic risk factors, and their associations with early AMD lesions and lesion characteristics may elucidate insights into the similarities and dissimilarities in mechanisms that lead to AMD occurring in either Asians or whites.

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