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Retinal Vascular Caliber, Iris Color, and Age-Related Macular Degeneration in the Irish Nun Eye Study

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1 **Retinal vascular caliber, iris color and age-related macular degeneration in the Irish**
2 **Nun Eye Study**

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11

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16

17 **ABSTRACT (250 words)**

18 **PURPOSE.** To evaluate the relationship between retinal vascular caliber (RVC), iris color
19 and age-related macular degeneration (AMD) in elderly Irish nuns.

20 **METHODS.** Data from 1233 participants in the cross-sectional observational Irish Nun Eye
21 Study were assessed from digital photographs with a standardized protocol using computer-
22 assisted software. Macular images were graded according to the modified Wisconsin age-

23 related maculopathy grading system. Regression models were used to assess associations,
24 adjusting for age, mean arterial blood pressure, body mass index, refraction and fellow RVC.

25 **RESULTS.** In total, 1122 (91%) participants had gradable retinal images of sufficient quality
26 for vessel assessment (mean age: 76.3 years [range: 56-100 years]). In an unadjusted
27 analysis, we found some support for a previous finding that individuals with blue iris color
28 had narrower retinal venules compared to those with brown iris color ($P<0.05$) but this was
29 no longer significant after adjustment. AMD status was categorized as no AMD, any AMD
30 and late AMD only. Individuals with any AMD (early or late AMD) had significantly narrower
31 arterioles and venules compared to those with no AMD in an unadjusted analysis but this
32 was no longer significant after adjustment. A non-significant reduced risk of any AMD or
33 late AMD only was observed in association with brown compared to blue iris color, in both
34 unadjusted and adjusted analyses.

35 **CONCLUSIONS.** RVC was not significantly associated with iris color or early/late AMD after
36 adjustment for confounders. A lower but non-significant AMD risk was observed in those
37 with brown compared to blue iris color.

38

39 **Introduction**

40 In recent years, studies have shown that non-invasive measurement of retinal blood vessels
41 may offer insights into certain systemic diseases^{1–8}, highlighting the eye as a unique window
42 to view microvascular health elsewhere within the body. This opportunity may afford
43 clinicians and other health professionals mechanistic insight into diseases with a
44 microvascular component⁵.

45 Previous studies have examined ethnic and racial variation in retinal microvascular
46 characteristics, identifying an association between retinal vessel caliber (RVC) and ethnicity,
47 principally on the basis of a darker iris colour^{9–12}. The reason for the observed racial and
48 ethnic differences in RVC is uncertain but several underlying factors have been proposed
49 including genetic variation, anthropometric and ocular biometrics, varying susceptibility to
50 vascular risk factors, or perhaps measurement error as a result of reduced vessel contrast
51 against a more pigmented epithelium¹⁰. Furthermore, it has also been reported that
52 individuals with a lighter iris color were more likely to have a higher prevalence and a greater
53 likelihood of progression to late AMD than those with a darker iris color¹³.

54 Age-related macular degeneration (AMD; MIM# 603075) is the most common form of visual
55 impairment among older people of European descent¹⁴, accounting for more than half of all
56 new cases of registered blindness¹⁵. Several epidemiological studies have suggested
57 common mechanistic processes and risk factors shared between AMD and cardiovascular
58 disease (CVD)^{16–18} and some have examined the relationship between RVC and AMD,
59 although the findings reported have been inconsistent^{19–25}. It has been suggested that
60 changes in RVC may be a risk factor for AMD, and that factors involved in the pathogenesis
61 of AMD such as inflammation may influence RVC at an earlier stage before AMD
62 manifestations are observed clinically. As such, changes in RVC may potentially provide

63 some prognostic indication for better risk stratification in individuals with an increased risk
64 of developing AMD later in life.

65 As such, we sought to evaluate the relationship between RVC, iris color and AMD status
66 using cross-sectional data from the Irish Nun Eye Study (INES) which included 1233 white
67 participants aged 56-100 years.

68

69 **Methods**

70 **Study Population**

71 The INES was a cross-sectional observational study of eye health in white Irish nuns
72 selected from convents across Ireland, with recruitment between 2007 and 2009. It was
73 designed specifically to examine the prevalence of AMD in a population with a restricted
74 lifestyle and also to examine the relationship between light exposure and AMD.

75 **Study characteristics**

76 Contact was made with 152 convents, of which 126 (82.9%) responded and agreed to
77 participate. One thousand, five hundred nuns in these 126 convents were invited to take
78 part in the study and 1233 (82.2%) agreed. Those who did not participate tended to be ill
79 or unavailable on the day of examination. The inclusion criteria mandated participants to be
80 of Irish descent, aged over 55 years and have lived in a convent for at least 25 years. There
81 were no specific exclusion criteria. In order to maximise recruitment and minimise disruption
82 to participant routines, all examinations were carried out within the community. The study
83 was approved by the Institutional Review Board and the Office for Research Ethics
84 Committee Northern Ireland and adhered to the tenets of the Declaration of Helsinki.
85 Informed written consent was obtained from all participants prior to participation.

86 **Demographic Data**

87 Demographic data were obtained from interviews by a trained field worker using a structured
88 questionnaire. This study was specific to one ethnicity as only white Irish nuns were
89 included.

90 **Anthropometric and Blood Pressure Measurements**

91 Blood pressure was measured in a seated position with an oscillometric blood pressure
92 aneroid sphygmomanometer (Speider and Keller) after the questionnaires had been
93 completed. Mean arterial blood pressure (MABP) was calculated as one third of the systolic
94 (SBP) plus two thirds of the diastolic blood pressure (DBP). Height, weight, and waist
95 circumference were measured and body mass index (BMI) calculated as weight (in
96 kilograms) divided by height (in meters) squared.

97 **Ocular Examination and Retinal Photography.**

98 Each individual underwent a comprehensive ophthalmic examination. Medical and
99 ophthalmic questionnaires covered areas such as medical and ocular history and relevant
100 risk factors for AMD. Iris color was determined from the undilated pupil of each eye by a
101 single examiner (EM) and categorized by comparison with four standard photographs prior
102 to pupil dilation as blue, brown, hazel or green. Refractive error was recorded either from a
103 recent prescription or from the participant's glasses. Where glasses were not available,
104 corrected visual acuity was achieved by pinhole correction: refraction was not carried out.
105 Each individual had anterior segment photography, photography of the skin around the eyes
106 and forehead and retinal findings were recorded by stereoscopic retinal imaging using the
107 NIDEX AFC 210 digital camera. Fields 1 and 2 were captured following dilation of the pupils
108 with 1% tropicamide.

109 **AMD Characterization**

110 All images were independently graded by the Network of Ophthalmic Reading Centres UK
111 (NetwORC UK) in Belfast. Anonymized images were submitted to NetwORC UK and trained
112 graders followed a standardized procedure to identify the characteristics of early and late
113 AMD using the definitions of the Wisconsin Age Related Maculopathy Grading System²⁶.
114 The definitions for AMD were based on the International Classification for Age-related
115 Macular Degeneration²⁷. The presence of features within a 6000µm circle centered on the
116 fovea was recorded. Drusen were classified according to size, characteristics of
117 homogeneity of surface features and outline. Pigmentary changes were classified into two
118 categories; hyperpigmentation and hypopigmentation. These features were used to assign
119 each eye to a severity grade as follows: No AMD: no features of AMD or the presence of
120 soft distinct drusen ($>63\mu\text{m}$ and $\leq 125\mu\text{m}$) or pigmentary abnormalities only; early AMD: soft,
121 indistinct ($\geq 125\mu\text{m}$) or reticular drusen only or soft distinct drusen with pigmentary
122 abnormalities; late AMD: either geographic atrophy; (well demarcated area of retinal pigment
123 atrophy with visible choroidal vessels) or neovascular AMD (presence of any of the
124 following): serous or hemorrhagic retinal or retinal pigment epithelial detachment, subretinal
125 neovascular membrane, or periretinal fibrous scar.

126 **Retinal vessel caliber assessment**

127 Retinal arteriolar and venular calibers were measured using Interactive Vessel ANalysis
128 software (IVAN; University of Wisconsin, Madison, WI) according to a standardized protocol
129 for all retinal vessels located between a half and one disc diameter distance from the optic
130 disc margin in the digitized image. The revised Knudston-Hubbard formula²⁸, was used to
131 summarize these measurements as CRAE (Central Retinal Arteriolar Equivalent) and CRVE
132 (Central Retinal Venular Equivalent), which represents the average caliber of the arterioles
133 and venules in each examined eye. A single trained grader (AMG), blinded to the
134 participants' characteristics, conducted all retinal measurements. Reproducibility of retinal

135 vascular measurements was high with intragrader reliability assessed in 200 randomly
136 selected retinal photographs and an intraclass correlation coefficient (95% confidence
137 interval) calculated as 0.975 (0.967 – 0.981) for CRAE and 0.993 (0.990 – 0.994) for CRVE,
138 respectively. A high correlation between the right and left eyes in retinal vascular
139 measurements has been reported elsewhere²⁸. Data from the right eye was used and when
140 unavailable, was replaced by the left eye.

141 **Statistical Analysis**

142 All statistical analyses were performed using IBM SPSS Statistics version 21 (IBM Corp.,
143 Armonk, NY). Quantitative retinal vascular caliber was assessed as a continuous variable.
144 One-way analysis of variance and multiple linear regression analyses were used to compare
145 the mean CRAE and CRVE by iris color in both unadjusted (Model 1) and adjusted analyses
146 (Models 2, 3 and 4). The minimally adjusted model (Model 2) included covariates for
147 refraction, age, BMI, and MABP. The model was not adjusted for gender as all participants
148 were female. The fully adjusted model (Model 4) also included the covariates from the
149 minimally adjusted model in addition to diabetes mellitus status, hypertension, ever smoker,
150 ischemic heart disease (IHD), cerebrovascular accident (CVA), alcohol consumption
151 (yes/no) and the fellow vessel (venule or arteriole) caliber (i.e. CRAE as a covariate in the
152 analysis of CRVE and vice versa) as suggested previously²⁹. The same approach was used
153 to compare CRAE and CRVE according to AMD status with adjustment for potential
154 confounders in a minimally and fully adjusted model as described for iris color. Logistic
155 regression was used to assess the significance of iris color as a predictor of AMD status.
156 Separate analyses of any AMD (early and late AMD) versus no AMD and of late AMD only
157 versus no AMD were performed each with adjustment for confounders. A P value < 0.05
158 was regarded as statistically significant.

159

160 **Results**

161 In total, gradable retinal images of sufficient quality for vessel assessment were available in
162 1122 (91%) of the 1233 participants. Images were not available for 111 participants, in part
163 as a consequence of difficulties with image acquisition due to postural complications with
164 the elderly participant, poor pupillary dilation, the presence of an artificial eye or an out of
165 focus image. Participants with missing retinal vessel caliber measurements (n=111) were
166 significantly older and more likely to have moderate to severe cataract than those with retinal
167 images captured ($P<0.001$; 84.8 years vs 76.3 years; Table 1); 11 had no retinal images
168 captured and 60 had macula-centered images only, which were not amenable to
169 measurement using the IVAN software. The mean age of the 1122 participants included
170 was 76.3 years (range: 56-100 years).

171

Characteristic	All participants	Gradable (n=1122)	Ungradable (n=111)	P
Mean Age (SD, years)	77.1 (8.4)	76.3 (8.1)	84.8 (7.4)	<0.001
Mean BMI (SD, kg/m ²)	24.5 (5.1)	24.6 (5.1)	23.8 (5.0)	0.09
Mean MABP (SD, mmHg)	92.5 (10.6)	92.4 (10.4)	93.2 (12.3)	0.47
IHD n(%)	137 (11%)	120 (11%)	17 (15%)	0.20
CVA n(%)	40 (3%)	36 (3%)	4 (3%)	0.79
Ever Smoked n(%)	57 (5%)	49 (4%)	8 (7%)	0.22
Diabetes n(%)	37 (3%)	34 (3%)	3 (3%)	1.00
Hypertension n(%)	504 (41%)	454 (40%)	50 (43%)	0.59
Chronic Kidney disease	705 (60%)	623 (59%)	82 (74%)	0.004
Alcohol consumption	81 (7.9%)	77 (8.2%)	4 (3.6%)	0.20
1-7 measures/week	78 (7.6%)	74 (7.9%)	4 (3.6%)	
>7 measures/week	3 (0.3%)	3 (0.3%)	0 (0%)	
Osteoporosis	402 (33%)	365 (33%)	37 (31%)	0.78
*Statins	482 (39%)	448 (40%)	34 (28%)	0.01
*Asprin	420 (34%)	369 (33%)	51 (42%)	0.03
*Diuretics	281 (23%)	240 (21%)	41 (34%)	0.001
*Beta Blockers	215 (17%)	192 (17%)	23 (19%)	0.57
*Calcium channel blockers	169 (14%)	142 (13%)	27 (22%)	0.003
*Ace inhibitors	145 (12%)	134 (12%)	11 (9%)	0.37
*Corticosteroids	66 (5%)	62 (5%)	4 (3%)	0.31
*NSAIDs	62 (5%)	53 (5%)	9 (7%)	0.18

SD: standard deviation; BMI: body mass index; MABP: mean arterial blood pressure (one third of the systolic blood pressure plus two thirds of the diastolic blood pressure); IHD: ischemic heart disease; CVA: cerebrovascular accident; NSAIDs: non-steroidal anti-inflammatory drugs; *Medications with a frequency >5%.

Iris color and retinal vessel caliber

Iris color was characterized as blue (59.0%), brown (20.2%), hazel (14.4%) and green (6.5%). CRAE and CRVE were normally distributed, with means and standard deviations (SD) of 120.4 (12.6) μm and 169.0 (18.3) μm respectively. Table 2 shows the diameters (μm) for retinal vessel caliber categorised by iris color. Initial one-way analysis of variance showed no significant differences in mean CRAE ($P=0.34$) and CRVE ($P=0.15$) between the iris color groups.

Table 2. Summary retinal vessel caliber measurements: CRAE and CRVE by iris color.

	Color	N	Mean (μm)	SD
Central Retinal Arteriolar Caliber (CRAE)	Blue	662	120.0	12.5
	Brown	227	121.6	12.7
	Hazel	161	119.6	12.8
	Green	72	121.1	12.4
Central Retinal Venular Caliber (CRVE)	Blue	662	168.0	18.3
	Brown	227	170.8	18.1
	Hazel	161	169.3	18.2
	Green	72	171.3	19.1

185 SD: Standard deviation

186 Although an unadjusted analysis, suggested that individuals with brown iris color had
187 significantly broader retinal venules ($P=0.05$) compared to those with blue iris color (Table
188 3), this finding was not corrected for multiple comparisons. Iris color was no longer
189 significantly associated with vascular caliber following adjustment for refraction, age, BMI,
190 and MABP (Model 2), and additional covariates diabetes mellitus, hypertension, ever
191 smoker, IHD, CVA, alcohol consumption (yes/no) and fellow vessel (Models 3 and 4),. All
192 comparisons for arterioles were non-significant.

193 **AMD status and retinal vessel caliber**

194 AMD status was categorized as no AMD ($n=975$), early AMD ($n=99$) and late AMD ($n=27$).
195 The summary statistics for those with any AMD and those without AMD are displayed in
196 Table 4. In an unadjusted analysis, individuals with any AMD had significantly narrower
197 CRAE ($P<0.05$) and CRVE ($P=0.03$) compared to those without AMD. Following adjustment
198 for refraction, age, BMI, MABP (minimally adjusted, Model 2), and also for additional
199 covariates (Models 3 and 4), AMD status was no longer significantly associated with vessel
200 caliber (Table 5).

201 **Table 3.** Difference in mean retinal vessel caliber (μm) for each iris color compared to blue (reference group) in unadjusted (Model 1),
 202 minimally adjusted (Model 2) and fully adjusted (Models 3 and 4) analyses.

	Model 1 (95% CI)	P	Model 2 (95% CI)	P	Model 3 (95% CI)	P	Model 4 (95% CI)	P
CRAE								
Brown	1.6 (-0.4, 3.5)	0.11	1.2 (-0.6, 3.0)	0.19	0.8 (-1.2, 2.8)	0.46	-0.1 (-1.6, 1.5)	0.92
Hazel	-0.4 (-2.6, 1.8)	0.72	0.2 (-1.9, 2.3)	0.84	-0.9 (-3.2, 1.4)	0.44	-1.0 (-2.8, 0.7)	0.25
Green	1.1 (-2.0, 4.1)	0.49	-0.3 (-3.2, 2.6)	0.83	-1.1 (-4.2, 2.1)	0.50	-1.4 (-3.8, 1.0)	0.26
CRVE								
Brown	2.8 (0.1, 5.6)	0.05	2.3 (-0.3, 4.9)	0.09	2.0 (-1.0, 4.9)	0.19	1.3 (-1.0, 3.5)	0.28
Hazel	1.3 (-1.8, 4.5)	0.41	1.8 (-1.2, 4.8)	0.25	0.3 (-3.0, 3.7)	0.85	1.2 (-1.4, 3.8)	0.39
Green	3.3 (-1.1, 7.8)	0.14	1.4 (-2.8, 5.6)	0.53	0.8 (-3.8, 5.3)	0.75	1.7 (-1.8, 5.3)	0.34

203 Model 1 – unadjusted

204 Model 2 – adjusted for refraction, age, BMI, and MABP

205 Model 3 – adjusted for refraction, age, BMI, MABP, diabetes mellitus, hypertension, ever smoker, IHD, CVA, alcohol consumption (yes/no)

206 Model 4 – adjusted for refraction, age, BMI, MABP, diabetes mellitus, hypertension, ever smoker, IHD, CVA, alcohol consumption (yes/no)

207 and fellow vessel caliber

208 CRAE: Central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; BMI: body mass index; MABP: mean arterial blood
 209 pressure; IHD: ischemic heart disease; CVA: cerebrovascular accident; 95% CI: 95% confidence interval.

210

211 **Table 4.** Summary statistics of participants included for retinal vessel assessment by AMD
 212 status.

Characteristic	No AMD (n= 976) mean (SD)	Any AMD (n= 126) mean (SD)	P
Mean Age (SD, years)	75.6 (8.0)	81.3 (6.7)	<0.001
Mean BMI(SD, kg/m ²)	24.7 (5.1)	24.5 (4.8)	0.70
Mean MABP (SD, mmHg)	92.1 (10.3)	94.5 (10.9)	0.02
IHD n(%)	99 (10%)	14 (11%)	0.74
CVA n(%)	28 (3%)	7 (6%)	0.11
Ever Smoked n(%)	42 (4%)	7 (6%)	0.53
Diabetes n(%)	28 (3%)	6 (5%)	0.27
Hypertension n(%)	390 (40%)	57 (45%)	0.26

213 Any AMD is composed of early and late AMD. SD: standard deviation; BMI: body mass
 214 index; MABP: mean arterial blood pressure; IHD: ischemic heart disease; CVA:
 215 cerebrovascular accident.

216 **AMD status and iris color**

217 Although a decrease in risk for any AMD was observed in association with brown compared
 218 to blue iris color, this was not significant in both an unadjusted analysis (OR = 0.73; C.I.:
 219 0.44-1.20; P=0.22) and also in an analysis adjusted for age, BMI, MABP and refraction (OR
 220 = 0.74; C.I.: 0.44-1.24; P=0.25). Similarly, a decrease in risk for late AMD only was observed
 221 in association with brown compared to blue iris color but again this was not significant in
 222 both an unadjusted analysis (OR = 0.35; C.I.: 0.20-1.77; P=0.35) and an analysis adjusted
 223 for age, BMI, MABP and refraction (OR = 0.61; C.I.: 0.20-1.86; P=0.39).

224

225 **Table 5.** Unadjusted (Model 1), minimally adjusted (Model 2) and fully adjusted analysis (Model 3 and 4) of retinal vessel caliber by AMD
226 status (any AMD versus no AMD).

	Model 1 (95% CI)	P	Model 2 (95% CI)	P	Model 3 (95% CI)	P	Model 4 (95% CI)	P
CRAE	-3.8 (-6.8, -1.5)	0.001	-1.3 (-3.6, 1.0)	0.27	-2.0 (-4.5, 0.6)	0.13	-1.9 (-3.9, 0.1)	0.06
CRVE	-3.7 (-7.0, -0.3)	0.03	0.1 (-3.3, 3.4)	0.98	-0.3 (-3.9, 3.4)	0.88	1.5 (-1.4, 4.4)	0.30

227 Model 1 – unadjusted
228 Model 2 – adjusted for refraction, age, BMI, MABP and iris color
229 Model 3 – adjusted for refraction, age, BMI, MABP, iris color, diabetes mellitus, hypertension, ever smoker, IHD, CVA and alcohol
230 consumption (yes/no)
231 Model 4 - adjusted for refraction, age, BMI, MABP, iris color, diabetes mellitus, hypertension, ever smoker, IHD, CVA, alcohol consumption
232 (yes/no) and fellow vessel caliber
233 CRAE: Central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; IHD: ischemic heart disease; CVA: cerebrovascular
234 accident; 95% CI: 95% confidence intervals.

235 Discussion

236 In this study, we did not find a significant association between retinal vessel caliber and iris
237 color in our study population of white Irish nuns. The Sydney Childhood Eye Study¹⁰ (SCES)
238 reported that both CRAE and CRVE were significantly wider in children of East Asian
239 ethnicity compared with white children and that white children with darker iris colour had
240 both wider CRAE and CRVE. Rochtchina and colleagues hypothesized that the ethnic
241 variability observed in association with retinal vessel caliber, may have contributed to
242 measurement error as a consequence of contrast sensitivity associated with the software's
243 ability to delineate the vessel edges against the background retinal pigment epithelium and
244 its associated level of pigmentation (iris color as a proxy for skin pigmentation). If true, this
245 could result in an over estimation of vessel caliber as the software finds it more difficult to
246 delineate the true blood vessel edges.

247 The association between RVC and ethnicity is well established with the Multi-ethnic Study
248 of Atherosclerosis¹² (MESA) concluding that blacks and Hispanics had wider CRAE and
249 CRVE than whites and Chinese. Similarly, the Singapore Childhood Study of Risk Factors
250 for Myopia¹¹ (SCORM) demonstrated that CRAE and CRVE were both significantly narrower
251 in Chinese children compared to Malay and Indian children. The findings from SCORM
252 suggested the underlying reasons for the variations between ethnic and racial groups
253 observed were unclear but perhaps reflected differences and varying susceptibility to
254 vascular risk factors such as blood pressure, anthropometric and ocular measures and/or
255 genetics. More recently, the Multi-ethnic study of Healthy Asians⁹, reported that Indians had
256 the widest CRAE and CRVE measurements, followed by the Malay and then the Chinese.

257 Our study is limited to white Irish nuns (females only), minimizing ethnicity as a potential
258 confounder. Our findings do not indicate any significant association between RVC and iris
259 color which in part adds support to previous suggestions that variation observed in RVC may

260 be influenced by underlying ethnic differences, as opposed to iris color *per se*.
261 Nevertheless, the possibility that iris color and/or retinal pigmentation levels may influence
262 contrast sensitivity and the ability of the analysis software to delineate blood vessel edge,
263 cannot be excluded.

264 We failed to find an association with AMD following adjustment for potential confounders
265 and our findings support those from previous studies^{19–21,24,25}, but contrast those from the
266 Singapore Malay Eye Study²² and the Handan Eye Study²³. The Handan Eye Study
267 consisted of 199 individuals with early AMD and 400 age-matched controls (mean age 58.6),
268 and reported a significant association between wider retinal arteriolar caliber and early AMD
269 and soft distinct drusen. The Singapore Malay Eye Study comprised 3280 participants aged
270 between 40 and 80 years (mean age 58.7 years), and reported a wider venular caliber
271 associated with an increased prevalence of early AMD.

272 Within our study, it is important to consider the pathological pathways involved in AMD
273 etiology, such as inflammation, which may influence the retinal microvasculature, although
274 whether the retinal or choroidal circulation are more likely to influence the disease
275 processes, requires further investigation²⁵. Previous studies have implicated common
276 mechanistic processes and risk factors shared between AMD and CVD with subsequent risk
277 modification for both conditions by smoking, hypertension, inflammatory markers and
278 common genetic variants, although consistent supporting evidence from cross-sectional
279 studies has proved elusive, possibly as a consequence of potential confounding^{30–31}.
280 Studies examining the relationship between AMD and CVD risk factors have identified
281 associations between higher pulse pressure, higher systolic blood pressure and increased
282 carotid wall thickness and incident AMD, implicating a vascular re-modelling process^{32–33}.
283 Previous studies have also suggested that lighter iris color increases associated AMD risk,
284 i.e. individuals with blue iris color were more inclined to have a higher prevalence and a

285 stronger likelihood of progression to late AMD than those with a darker iris color¹³. We were
286 unable to corroborate these findings in our study.

287 The strengths of this study include the relatively large sample size and the high proportion
288 of gradable digital retinal images. Masked evaluation of RVC was performed by a single
289 trained grader. The use of a semi-automated computer based technique³⁴ to measure RVC.
290 The collection of data on potential confounders including anthropometric factors was
291 standardized and the relative uniformity of the nun's backgrounds meant fewer variations in
292 lifestyle, reducing potential confounding and provided an opportunity to examine the
293 potential complex relationship that exists between AMD and CVD risk factors, iris color and
294 the resultant effect on RVC. Importantly, our study was performed on a well-characterized
295 and aged cohort (mean age 76.3 years), free from gender or ethnicity related confounding,
296 which is particularly important for the analysis of age-related conditions such as AMD. Due
297 to the nature of their lifestyle, this novel population have lower rates of some well-recognized
298 environmental and lifestyle related risk factors, i.e. the majority were non- smokers, had
299 lower rates of alcohol consumption with reduced prevalence of cardiovascular disease and
300 diabetes, providing an opportunity to better examine lifestyle and environmental factors that
301 contribute to the etiology of complex diseases.

302 Limitations of our study include its cross-sectional design which did not let us determine
303 whether changes observed precede or are a consequence of AMD. The data available to
304 evaluate late AMD were relatively small in number, limiting the power to evaluate RVC in
305 the advanced form of this condition. Furthermore, certain data which may affect RVC
306 including intraocular pressure³⁵ was unavailable. While convent or religious orders may not
307 truly reflect the general population, they nevertheless offer an excellent opportunity to study
308 a well-characterized model of 'healthy ageing'.

309 In conclusion, our cross-sectional study of aged white Irish Nuns, has not found a significant
310 association between retinal vascular caliber and iris color, between retinal vascular caliber
311 and AMD and between iris color and AMD, following adjustment for appropriate known
312 confounders.

313

314

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