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1 Genetic and dietary factors influencing the progression of nuclear cataract

2 Ekaterina Yonova-Doing, MSc¹, Zoe A. Forkin, BSc^{1,3}, Pirro G. Hysi, MD, PhD¹, Katie M.
3 Williams, MPhil, FRCOphth², Tim D. Spector, MD, PhD¹, Clare E Gilbert, FRCOphth, MD⁴,
4 Christopher J. Hammond, MD, FRCOphth^{1,2}

5
6 1. Department of Twin Research and Genetic Epidemiology, Kings College London

7 2. Department of Ophthalmology, Kings College London

8 3. University of Warwick Medical School

9 4. London School of Hygiene and Tropical Medicine

10 Corresponding author: Christopher J. Hammond, MD, FRCOphth, Departments of
11 Ophthalmology & Twin Research, King's College London, 3rd Floor, Block D, South Wing, St.
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21 Address for reprints: Christopher J. Hammond, MD, FRCOphth, Departments of Ophthalmology
22 & Twin Research, King's College London, 3rd Floor, Block D, South Wing, St. Thomas'
23 Hospital, Westminster Bridge Rd., London SE1 7EH, UK.

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33 Abstract

34 Purpose: To determine the heritability of nuclear cataract progression and to explore
35 prospectively the effect of dietary micronutrients on the progression of nuclear cataract.

36 Study design: Prospective cohort study

37 Participants: Cross-sectional nuclear cataract and dietary measurements were available for 2054
38 white female twins from the TwinsUK cohort. Follow-up cataract measurements were available
39 for 324 of the twins (151 monozygotic and 173 dizygotic twins).

40 Methods: Nuclear cataract was measured using a quantitative measure of nuclear density
41 obtained from digital Scheimpflug images. Dietary data was available from EPIC food frequency
42 questionnaires. Heritability modelling was carried out using maximum likelihood structural
43 equation twin modelling. Association between nuclear cataract change and micronutrients was
44 investigated using linear and multinomial regression analysis. The mean interval between
45 baseline and follow-up examination was 9.4 years.

46 Main outcome measures: nuclear cataract progression

47 Results: The best fitting model estimated that the heritability of nuclear cataract progression was
48 35% (95% CI: 13%-54%); individual environmental factors explaining the remaining 65% (95%
49 CI 46-87%) of variance. Dietary vitamin C was protective against both nuclear cataract at
50 baseline and nuclear cataract progression ($\beta=-0.0002$, $p=0.01$ and $\beta=-0.001$, $p=0.03$
51 respectively), while manganese and intake of micronutrient supplements were protective against
52 nuclear cataract at baseline only ($\beta=-0.009$, $p=0.03$ and $\beta=-0.03$, $p=0.01$ respectively).

53 Conclusions: Genetic factors explained 35% of the variation in progression of nuclear cataract
54 over a 10 year period. Environmental factors accounted for the remaining variance, and in
55 particular dietary vitamin C protected against cataract progression assessed almost 10 years after
56 baseline.

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73 Age-related cataract is the leading causes of blindness in the world, affecting about 20 million
74 people, particularly in Sub-Saharan Africa¹. Its prevalence increases from 2.9% in the 43-54 age
75 group to 40% in the over 75 years old group². As the world's population ages, cataract will
76 remain a serious healthcare and socioeconomic burden, both in terms of healthcare provision,
77 and blindness in less developed countries.

78 Nuclear cataract is the most common form of age-related cataract². Apart from age, other factors
79 associated with nuclear cataract are smoking, oxidative stress and dietary antioxidant intake³⁻⁵.
80 However, studies of the effect of dietary vitamin C intake⁶⁻¹¹, serum vitamin C levels^{6, 9, 11-13} or
81 vitamin C supplementation^{6, 10, 14} on nuclear cataract formation have given often conflicting
82 results. Case-control studies^{7, 11, 12, 14} and some cohort studies^{6, 9, 10} have found protective effects.
83 Other prospective cohort studies have either found no effect overall^{8, 13, 15} or protective effects
84 only in subgroups^{8, 15}. Similarly to vitamin C, dietary^{6, 16} and supplemental^{14, 17} vitamin E intake
85 as well as vitamin E blood levels^{6, 13} have been shown to be inversely related with nuclear
86 cataract. Randomised clinical trials of vitamins C and E supplementation alone or in combination
87 with other vitamins failed to find an effect^{18, 19}. Vitamin A has been associated with reduced risk
88 of nuclear cataract^{9, 20, 21}, as have been lutein and zeaxanthin²²⁻²⁴. The studies exploring dietary
89 nutrients and cataract progression have similar findings to those looking at prevalent cataract,
90 with cohort studies finding a protective effect^{16, 25}. However, supplement trials have largely
91 failed to find an effect while supplement trials have failing to find an effect^{18, 26, 27}.

92 As opposed to vitamins and micronutrients²⁸, the role of minerals in cataract formation in general
93 and in nuclear cataract in particular is poorly studied.

94 Together with epidemiological factors, genetic factors also play role in cataract formation. We
95 have previously reported that genetic factors explain 48% of cross-sectional variance in age-
96 related nuclear cataract²⁹. In a recent genome-wide meta-analysis, variants in two genes, *CRYAA*
97 and *KCNABI*, were found to be associated with nuclear cataract in Asian populations³⁰ but no
98 findings are available for populations of European origin. In comparison to epidemiological
99 factors, little is known about genetic susceptibility factors in age-related cataract.

100 Factors that lead to development of a phenotype may be different from factors underlying
101 change, such as progression of lens opacity. We therefore set out to establish the relative
102 importance of genes on progression of nuclear cataract using a classical twin model with a highly
103 quantitative measure of nuclear cataract. We also examined how intake of micronutrients and
104 supplements associated with nuclear cataract at baseline affects nuclear cataract progression over
105 a decade.

106 Methods

107 Subjects

108 Nuclear cataract data at baseline were available for 2515 white female twins (mean age of 62.3,
109 range 50.1-83.1) from the TwinsUK cohort, 2054 of whom had also completed a food frequency
110 questionnaire (FFQ) around the time of their eye-examination (median=2 years). The 461 twins
111 with cataract data but without FFQ data were 2.5 years younger on average and were less
112 affected by cataract. Cataract progression data was collected in 324 twins (151 monozygotic
113 (MZ) twins and 173 dizygotic (DZ) twins with a mean age at follow-up of 69.8±5.4 years (range:

114 58.3-83.6 years) as part of the Healthy Ageing in Twins (HATS) study between 2006 and 2010³¹.
115 Individuals included in the follow up were all part of our original cataract heritability study of
116 1012 twin participants assessed in 1998 and 1999²⁹. The mean time between baseline and second
117 visits was 9.4 years (range: 7-12 years). The smaller number of individuals with follow up data is
118 mainly due to the fact that the HATS study (where the follow up data was collected) was not
119 designed specifically as a cataract follow-up study, and had different selection criteria:
120 participants were over 40 years of age and had to have previously attended clinical phenotyping
121 irrespective of whether they had an eye examination or not (N=4610). The TwinsUK study
122 started in 1992, but eye measures were only performed on subjects over 50 years of age in 1998-
123 1999, and subsequently from 2006. That meant that individuals (age \geq 50) who attended the
124 HATS visit who did not have eye examinations in 1989-1999 had their baseline cataract
125 assessment during HATS (2006-2011, N=1523). Reasons for only having longitudinal data for
126 324 of the original 1012 twins included: deceased (N=52), withdrawn participation from the
127 TwinsUK registry (N=169), non-contactable (N=30), refused further phenotyping (N=82);
128 cataract surgery (N=11), refusal of dilating drops or unavailability of ophthalmic testing at
129 HATS visit (344).”

130 Both the baseline study and HATS study received local research ethics approval and were
131 conducted according to the tenets of the Declaration of Helsinki. All the participants gave written
132 informed consent.

133 Phenotyping

134 Nuclear cataract scores

135 Digital black and white lens photographs were taken using a Scheimpflug camera (Case 2000,
136 Marcher Enterprises Ltd, Worcester, UK) and same camera was used at both baseline and
137 follow-up. Nuclear cataract was measured quantitatively by calculating the pixel density in the
138 centre of the lens nucleus, also known as the central nuclear dip score (NDS)²⁹. This score
139 measures the amount of white scatter (opalescence) and more opacification results in higher
140 pixel density. As NDS uses black-and-white images, it does not assess the brunescence of the
141 lens. Nuclear cataract progression was measured as the difference in measurements between the
142 visits: $\Delta\text{NDS} = \text{NDS at follow-up} - \text{NDS at baseline}$. Both NDS and ΔNDS were not normally
143 distributed and were therefore transformed using natural logarithm prior to the analysis.

144 Nutrient intake

145 Intake of micronutrients (vitamins and minerals) and supplements intake was estimated using the
146 self-administered EPIC FFQ taken at the baseline visit. This questionnaire explored the average
147 frequency of intake of 131 foods and supplements over 1 year period^{32, 33}. Nutrient intake was
148 calculated using an established nutrient database and the dietary variables were adjusted for
149 calorie intake, yielding an energy-adjusted mg/ug of each nutrient per person per day^{32, 34, 35}. We
150 considered the following micronutrients in the analysis: sodium, potassium, calcium,
151 magnesium, phosphorus, iron, copper, zinc, chloride, manganese, iodine, retinol, carotene,
152 vitamin D, vitamin E, thiamine, riboflavin, niacin, tryptophan, vitamin B6, vitamin B12, folate,
153 pantothenate, biotin and vitamin C.

154 Data on supplement intake were available for 33 different supplements. However, the percentage
155 of individuals taking any single supplement was 10% or less. Supplements were, therefore,
156 grouped as follows: *any supplements*, *micronutrient supplements* (vitamins and mineral in any

157 combination), *micronutrient supplements excluding multivitamins* (eg. vitamin C only, vitamin D
158 only, iron only, ACD complex), *minerals only* (eg. iron only, calcium only), and *other*
159 *supplements* (eg. Aloe Vera, Echinacea, Ginkgo, omega-3). Each supplement group was coded
160 as binary variable, with yes indicating that they took one or more of the supplements in a specific
161 group.

162 Statistical Analysis

163 Modelling of Heritability

164 Heritability analyses were performed on 310 twins (155 pairs: 72MZ and 83DZ) as data were
165 missing on 14 co-twins. Zygosity was determined by a standardised questionnaire and confirmed
166 using genome-wide single nucleotide polymorphism genotyping data or DNA short tandem
167 repeat fingerprinting.

168 Twin studies are able to estimate the heritability of a trait (the amount of variance explained by
169 genetic factors) using maximum likelihood structural equation modelling. The variance of the
170 trait and the covariance within twin pairs are used to estimate additive genetic effects (A),
171 shared/family environmental effects (C), and individual environmental effects (E). We
172 implemented the modeling in the OpenMx package (<http://openmx.psyc.virginia.edu>). The
173 goodness of fit of the full ACE model and sub-models were compared with the observed data
174 and the best fitting model was selected.

175 Nutrient factor analysis

176 Comparisons of means and proportions for all variables between individuals with or without
177 follow-up data, or between MZ and DZ twins per group in terms of age, nuclear cataract scores,

178 nutrient and supplement intake were performed using two-sample two-tailed t-tests or z-tests,
179 assuming equal variance.

180 Association was assessed using linear regression analyses. Univariable linear regression was
181 firstly carried out where each factor or supplement group was individually regressed against
182 NDS at baseline. All nutrients or supplement groups showing significant univariable association
183 ($p < 0.05$) were then included in a multivariable linear regression model; independent variables
184 were identified using stepwise backwards procedure with threshold for removal set at 0.05.
185 Factors showing significant ($p < 0.05$) association in the multivariable model were tested for
186 association with progression. We used linear models to establish the relationship between NDS
187 (continuous variable) and nutrients but because NDS had to be normalised, giving a clinical
188 interpretation of the betas becomes more difficult. Therefore, in addition to the linear models we
189 calculated risk reduction by calculating relative risk ratios (RRR) using multinomial regression.
190 In this case NDS, Δ NDS and the associated nutrients were divided into tertiles and the first tertile
191 was set as reference while supplement intake per supplement group was kept binary. In all cases,
192 models were adjusted for family structure and for age, either at the first visit only (baseline
193 analysis) or for both age at baseline and Δ age=age at follow-up – age at baseline. All analyses
194 were carried using STATA10 statistical package (www.stata.com).

195 Results

196 Cross-sectional data were available for 2054 white female twins (827 MZ and 916 DZ), 324
197 (151 MZ and 173 DZ) of whom also had nuclear cataract measured at follow-up. Baseline
198 characteristics, nutrient and supplement intake are shown in Table 1 and an example of a lens
199 image is available in Figure 2. The twins with follow-up data were on average 1.1 years younger

200 at baseline (60.4 vs 61.5 years) and, given their younger age, had less cataract (mean NDS scores
201 of 55.3 and 60.4 respectively) compared to those with only cross-sectional data. In both cases
202 these differences were not statistically significant ($p>0.05$). The MZ and DZ twins with follow-
203 up data were similar in terms of age and NDS scores ($p>0.05$). The MZ and DZ twins with cross-
204 sectional data only were similar in terms of age but the MZ twins had slightly higher NDS score
205 (61.6 versus 59.3, $p=0.02$).

206 There were also no statistically significant differences between groups in terms of micronutrient
207 intake except for iron ($p=0.02$), thiamine ($p=0.04$) and biotin ($p=0.01$). The twins with follow-up
208 data had slightly lower iron and thiamine intake (mean of 12.6 mg and 1.7mg respectively) and
209 slightly higher biotin intake (mean of 49.7mg) compared individuals without follow-up data.

210 There were also no significant differences in supplement intake between the two groups
211 ($p>0.05$). There were no statistically significant differences between MZ and DZ twins in terms
212 of nutrient or supplement intake ($p>0.05$).

213 As expected, nuclear cataract scores progressed in all participants (Figure 1). The mean baseline
214 central nuclear dip score was 55 ± 11 (range: 32-99) with the score increasing by an average of
215 19.9 ± 16.9 (range 1-137) over the period of follow-up. The heritability analysis, conducted on
216 155 twin pairs (72MZ and 83DZ pairs), showed that the best fitting model was one explained by
217 additive genetic factors and unique (individual) environment, with no significant effect of
218 common environment or non-additive genetic factors. Calculations estimated the heritability to
219 be 0.35, meaning that genetic factors explained 35% (95% CI: 13-54%) of variance in
220 progression of nuclear cataract with, individual environmental factors accounting for the
221 remaining 65% (95% CI: 46-87%).

222 To test associations between micronutrient intake and cataract progression we used univariable
223 regression (Table 2) followed by stepwise regression in 2054 female twins who had baseline data
224 on nutrient intake. Seven micronutrients showed significant association ($p < 0.05$) with NDS and
225 were used in multivariable analysis: these were potassium, magnesium, manganese, phosphorus,
226 the vitamins C and E, and folate. Following stepwise multivariable regression, two factors
227 remained significantly associated with NDS at baseline: vitamin C ($\beta = -0.0002$, $SD = 6.3E-05$,
228 $p = 0.01$) and manganese ($\beta = -0.009$, $SD = 0.04$, $p = 0.03$). From these two nutrients only vitamin C
229 showed association with cataract progression ($\beta = -0.001$, $SD = 0.001$, $p = 0.03$). A sensitivity
230 analysis, excluding subjects with greatest progression (> 100 units of change), did not alter the
231 result. Comparing people in the highest and the lowest tertiles of vitamin C intake was associated
232 with 19% risk reduction at baseline (relative risk ratios (RRR) of 0.81, 95% CI: 0.68-0.96) and a
233 33% risk reduction of cataract progression (RRR of 0.66 [0.47-0.91]) (Table 3). Manganese
234 intake was associated with 20% risk reduction (RRR of 0.80, 95% CI: 0.67-0.95) at baseline
235 (Table 3).

236 Two supplement groups, *micronutrient supplements* and *minerals only*, showed significant
237 association with NDS ($p < 0.05$) (Table 2) but only *micronutrient supplements* stayed significant in
238 the multivariate model ($\beta = -0.03$, $SD = 0.01$, $p = 0.01$) and their intake led to 18% risk reduction in
239 people within the highest compared to the lowest tertile of nutrient intake (RRR=0.82, 95% CI:
240 0.57-1.20) (Table 3). We found no statistically significant association between taking
241 micronutrients in supplemental form and progression of nuclear cataract.

242 Discussion

243 This study has found that progression of nuclear cataract over a ten year period in a group of UK

244 female twins is influenced by genetic factors which explain 35% of variance. The heritability
245 estimate of cataract progression is lower than our previous cross-sectional estimates of
246 susceptibility to development of nuclear cataract in this cohort²⁹ and it is also lower than the
247 heritability estimated in the 324 individuals estimated from the nuclear score measurement at
248 follow-up (61%, 95%CI: 45%-72%). This is consistent with previous studies showing
249 heritability is generally lower when examining change, compared to cross-sectional studies³⁶⁻³⁸.
250 In addition to early developmental differences and the body's response to environmental factors
251 in adulthood, environmentally driven processes or accumulated 'errors' (such as somatic gene
252 mutation and epigenetic remodeling) might play a greater role in determining change during
253 ageing than genetic factors³⁸.

254 This study has also identified vitamin C as a micronutrient affecting nuclear cataract progression.
255 We also replicate the previously found association between cross-sectional cataract and vitamin
256 C intake. Vitamin C intake has long been studied in relation to age-related cataract as it is the L-
257 enantiomer of ascorbate. Ascorbate is present in significant concentration in the aqueous humour
258 that bathes the lens and may reduce oxidation products in the lens, thus reducing oxidative
259 stress^{39, 40}. However the conclusions of the many studies into its effects on cataract development
260 are inconsistent and often conflicting⁶⁻¹⁵. Many of these studies have been in relatively well-
261 nourished populations, and are cross-sectional, though cross-sectional studies in India where
262 overall antioxidant levels may be lower have found an inverse relationship between vitamin C
263 and cataract^{9, 20}. Our results are similar to the CAREDS study that showed vitamin C intake,
264 assessed with food frequency questionnaire 10 years prior to cataract assessment, to be protective
265 of nuclear cataract prevalence¹⁵. The Blue Mountains Eye Study also found that vitamin C
266 intake, both dietary and supplements together, resulted in a lower nuclear cataract incidence over

267 10 years¹⁰. This study is the first, to our knowledge, to show that dietary vitamin C intake
268 protects against progression of nuclear lens opacity.

269 We also found dietary manganese to be protective against cross-sectional nuclear cataract
270 independently of vitamin C. We cannot exclude that this association was a type I error, given we
271 did not find an association between dietary manganese and nuclear cataract progression and the
272 lack of dose-response (Table 3), although factors associated with incidence and progression do
273 not always overlap. Manganese is an important antioxidant present in the human lens⁴¹⁻⁴³, and
274 its concentration has been reported to be lower in cataractous lenses in comparison with normal
275 lenses^{43, 44}. This study was not designed to elucidate the cause-effect relationship underlying the
276 associations we found and we, therefore, cannot distinguish whether manganese depletion is a
277 cause or effect of cataractogenesis. Further studies are needed to answer this question. We also
278 detected an association between supplemental intake of micronutrients and cross-sectional
279 nuclear cataract but not between supplemental nutrients and cataract progression. These results
280 are similar to those reported in the Blue Mountain Eye Study⁴⁵. As only 10% or fewer
281 participants in our study took any single supplement, we had to group supplements together and,
282 therefore, we could not draw conclusions on the effect on any single supplement or of
283 components of supplements (eg. supplemental vitamin C).

284 We used a highly quantitative measure of cataract from digital images (NDS), which essentially
285 measures the nuclear opalescence (or “white scatter”) of the lens. The measure was also highly
286 reproducible: the intraclass correlation coefficient for the worse eye, in 30 subjects from our
287 original study²⁹ who came for repeat measurements, was 0.93. The fact that every subject
288 measured showed progression suggests that NDS is sensitive to change. Many epidemiological
289 studies have used the Lens Opacity Classification System (LOCS) grading scale, comparing

290 phenotype to standardised photographs of 6 stages of lens opacification, which includes both
291 nuclear opalescence and nuclear colour or brunescence⁴⁶. LOCS III was developed to increase
292 steps between scores to allow greater sensitivity to change, accepting a lower inter-grader
293 reproducibility. Longitudinal studies using the LOCS III scale show relatively little change: in a
294 Longitudinal Study of Cataract Group only 24% of participants had an increase in nuclear
295 opacities over an average of 4.6 years²⁵. Although our central NDS is not the same measure, it is
296 highly correlated with average nuclear opalescence graded digitally or at the slit lamp²⁹. Digital
297 image-derived nuclear dip scores using pixel density counts may be better suited for measuring
298 progression, and allowed our study the power to detect associations with a relatively small
299 sample size.

300 A potential limitation is that our cohort is based on twin volunteers rather than a population
301 study, but they are unselected and from across the UK and unlikely to significantly differ from
302 the UK general population⁴⁷. Twin studies use the “Equal Environment Assumption”, that the
303 degree of shared family environment is the same for both monozygotic and dizygotic twin pairs.
304 This is generally found to be true, though there are few studies of elderly subjects which explore
305 this assumption. In addition, the TwinsUK cohort is predominantly a female cohort and we could
306 not assess any gender differences in risk factors. The findings of this study can only be
307 generalizable to Caucasian women of similar age as it reflects cataract progression in a group of
308 white British women between, on average, the ages of 60 and 70, and so may not reflect other
309 population groups or age ranges. In this article, we aimed to explore the effect on nuclear
310 cataract formation of all micronutrients, however we had no data on carotenoid (lutein and
311 zeaxanthin) intake. We also lacked power to explore the effects of smoking on cataract
312 progression as 85% of participants have never smoked.

313 Those participants with follow-up data collected were seen as part of the HATS study which was
314 not designed as a cataract follow up study. This meant that the number of subjects fell to 324
315 individuals, thus reducing the amount of data we could analyse and our power. The individuals
316 who were lost to follow up in HATS were in general of lower socioeconomic status, had higher
317 self-rated health status and were less health aware³¹. Any introduced bias would have probably
318 resulted in loss of power as this group of individuals are more likely to have less healthy diets and
319 more cataract. For this reason we decided to test the association with progression only for
320 nutrients which were associated with NDS at baseline. Those with follow-up data were on
321 average 1.8 years younger than the original cohort, but they were in general not significantly
322 different in other respects or in nutrient or supplement intake, hopefully reducing potential
323 selection bias in the progression data. As in any observational study, ours is potentially
324 susceptible to residual confounding, missing data or misspecification of variables.

325 In summary, this study has shown that progression of nuclear cataract over a 10 year period is
326 influenced by genetic factors with a heritability of 35%. Dietary vitamin C and manganese, both
327 factors related to oxidative stress, appear to influence cross-sectional nuclear cataract and
328 vitamin C intake also significantly influences nuclear cataract progression.

329

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344 Figures

345 Figure 1: Consort diagram of the study

346 Legend: This figure shows the number of individuals that participated in the different parts of the
347 study and reasons for none-participation at follow-up

348 Figure 2: Black and white Scheimpflug lens images

349 Legend: This figures shows Scheimpflug lens images of a healthy lens (left) and a lens with
350 nuclear cataract (right). The centre of the lens (lens nucleus) on the right is much whiter than the
351 one on the left.

352 Figure 3: Progression of nuclear cataract between the two visit dates

353 Legend: This figure is graphical representation of the progression of nuclear cataract (deltaNDS)
354 between the two visits. NDS – nuclear dip score, $\text{deltaNDS} = \text{NDS at follow-up} - \text{NDS at}$
355 baseline. The y-axes show frequency of deltaNDS per bins with width of 6.25 points.

356 Tables

357 Table 1: Baseline sample characteristics and nutrient intakes in individuals with or without
358 follow-up data

359 Legend: This table shows the baseline characteristics for the participants as well as the baseline
360 intake of micronutrients (mean \pm standard deviation) and supplements per supplement group (%
361 of users). The supplement groups studied are as follows: any supplement, micronutrient
362 supplements (vitamins and mineral in any combination), micronutrient supplements excluding
363 multivitamins (eg. vitamin C only, vitamin D only, iron only, ACD complex), minerals only (eg.
364 iron only, calcium only), and other supplements (eg. Aloe Vera, Echinacea, Ginkgo, omega-3).
365 The * denotes statistically significant difference ($p < 0.05$) between subjects with and without and
366 without follow-up.

367 Table 2: Results from univariable regression models of nuclear cataract scores and nutrient
368 intake of micronutrients and supplement groups

369 Legend: This table shows the results of the univariable linear regression analysis between
370 nuclear cataract (natural logarithm transformed nuclear dip score) and energy adjusted
371 micronutrient intakes and between nuclear cataract and supplement intake per supplement group.
372 \$ denotes that in the case of supplement groups, supplement intake was coded binary (presence
373 vs absence of intake of at least one of the components in the group). All analyses were adjusted
374 for age and family structure. * denote statistically significant associations at $p < 0.05$

375 Table 3: Results of multinomial regression analysis for factors associated with
376 cross-sectional nuclear cataract and with nuclear cataract progression

377 Legend: This table shows the results from the multinomial regression analysis for factors

378 associated with cross-sectional (vitamin C and manganese) and progression (vitamin C). The
379 relative risk ratio (RRR) with its 95% confidence intervals (95%CI) for each tertile of nuclear
380 dip score (NDS) or progression (Δ NDS) is reported. The minimum and maximum NDS score per
381 tertile are also reported.

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Table 1: Baseline sample characteristics and nutrient intakes in individuals with or without follow-up data

	Subject without follow-up			Subjects with follow-up		
	Total	MZ	DZ	Total	MZ	DZ
Number of individuals	1730	827	916	324	151	173
Zygoty ratio (MZ:DZ)	01:01.1	-	-	01:01.2	-	-
Age (mean \pm sd)	61.5 \pm 6.5	61.7 \pm 6.7	61.4 \pm 6.4	60.4 \pm 5.1	60.8 \pm 5.5	60.0 \pm 5.2
NDS (mean \pm sd)	60.4 \pm 17.2	61.3 \pm 17.4	59.0 \pm 14.2	55.3 \pm 11.2	55.3 \pm 11.4	55.3 \pm 11.1
Sodium (mg)	2262.8 \pm 508.7	2265.3 \pm 476.3	2258.7 \pm 535.6	2237.4 \pm 456.4	2227.7 \pm 444.4	2247.2 \pm 444.4
Potassium (mg)	4013.5 \pm 637.4	3997.0 \pm 622.4	4026.9 \pm 650.6	4033.7 \pm 580.5	4094.5 \pm 588.4	3972.5 \pm 469.4
Calcium (mg)	1117.1 \pm 284.7	1118.5 \pm 284.9	1125.1 \pm 284.6	1118.9 \pm 291.5	1138.3 \pm 295.0	1099.4 \pm 568.0
Magnesium (mg)	347.3 \pm 56.4	347.3 \pm 56.8	347.2 \pm 56.0	343.8 \pm 55.0	347.0 \pm 58.0	340.6 \pm 287.5
Phosphorus (mg)	1527.1 \pm 247.0	1527.1 \pm 234.9	1527.1 \pm 257.8	1522.0 \pm 239.3	1532.0 \pm 251.0	1512.1 \pm 227.5
Iron (mg)*	13.1 \pm 3.0	13.2 \pm 3.2	13.0 \pm 2.8	12.6 \pm 2.6	12.5 \pm 2.7	12.7 \pm 2.5
Copper (mg)	1.5 \pm 0.5	1.5 \pm 0.6	1.5 \pm 0.4	1.5 \pm 0.4	1.5 \pm 0.4	1.6 \pm 0.5
Zinc (mg)	10.2 \pm 1.7	10.2 \pm 1.8	10.1 \pm 1.7	10.2 \pm 1.7	10.2 \pm 1.8	10.1 \pm 1.6
Chloride (mg)	3629.6 \pm 792.9	3633.6 \pm 749.4	3623.0 \pm 828.6	3578.0 \pm 721.3	3566.7 \pm 690.3	3589.4 \pm 753.3
Manganese (mg)	4.2 \pm 1.2	4.1 \pm 1.1	4.2 \pm 1.2	4.2 \pm 1.1	4.3 \pm 1.1	4.2 \pm 1.1
Iodine(mg)	225.0 \pm 75.8	224.2 \pm 75.2	225.8 \pm 76.5	229.2 \pm 64.2	230.0 \pm 61.4	228.5 \pm 67.2
Retinol (ug)	579.5 \pm 817.8	569.1 \pm 570.6	554.8 \pm 496.6	611.8 \pm 472.9	588.2 \pm 422.6	635.6 \pm 519.0
Carotene (ug)	5343.4 \pm 3067.4	5503.7 \pm 3263.8	5200.4 \pm 2874.9	5305.6 \pm 3915.4	5663.8 \pm 4823.8	4945.0 \pm 2679.4
Vitamin D (ug)	2.7 \pm 1.4	2.7 \pm 1.1	2.6 \pm 1.5	2.8 \pm 1.1	3.0 \pm 1.0	2.6 \pm 1.0
Vitamin E (mg)	11.5 \pm 3.2	11.6 \pm 3.4	11.4 \pm 3.1	11.7 \pm 3.4	11.9 \pm 3.6	11.5 \pm 3.2

Thiamin (mg)*	1.8±0.4	1.8±0.4	1.8±0.4	1.7±0.3	1.7±0.3	1.7±0.3
Riboflavin (mg)	2.5±0.7	2.4±0.7	2.5±0.7	2.4±0.6	2.5±0.6	2.4±0.7
Niacin (mg)	22.0±5.7	22.2±5.1	21.8±6.2	21.3±4.5	21.3±4.6	21.2±4.4
Tryptophan (mg)	17.4±3.0	17.5±2.7	17.3±3.3	17.2±2.5	17.3±2.5	17.1±2.6
Vitamin B6 (mg)	2.6±0.6	2.6±0.6	2.5±0.5	2.5±0.5	2.5±0.5	2.5±0.5
Vitamin B12 (ug)	6.5±3.2	6.7±3.6	6.4±2.9	6.7±2.3	6.7±2.3	6.7±2.4
Folate (ug)	402.2±113.1	400.7±114.0	403.2±112.3	395.7±98.9	402.0±95.9	389.4±101.8
Pantothenate (mg)	7.4±16.0	7.5±21.3	7.2±8.6	6.8±4.2	6.5±2.1	7.1±5.6
Biotin (mg)*	48.1±10.5	47.7±10.3	48.5±10.8	49.7±10.3	50.6±10.2	48.7±10.3
Vitamin C (mg)	165.1±73.9	167.6±74.2	163.0±73.7	166.8±65.0	166.9±68.1	166.7±65.0
Any supplement (%)	55.1	54.8	55.4	55.0	54.1	55.9
Micronutrients (%)	32.57	32.4	33.2	31.7	32.8	30.8
Micronutrients excluding multivitamins (%)	23.6	24.1	23.2	21.6	24.2	19.3
Minerals only (%)	7.4	7.8	7.0	6.9	6.4	7.2
Other supplements (%)	44.9	46.2	44.4	47.1	44.2	49.5

Legend: This table shows the baseline characteristics for the participants as well as the baseline intake of micronutrients (mean ± standard deviation) and supplements per supplement group (% of users). The supplement groups studied are as follows: any supplement, micronutrient supplements (vitamins and mineral in any combination), micronutrient supplements excluding multivitamins (eg. vitamin C only, vitamin D only, iron only, ACD complex), minerals only (eg. iron only, calcium only), and other supplements (eg. Aloe Vera, Echinacea, Ginkgo, omega-3). The * denotes statistically significant difference (p<0.05) between subjects with and without and without follow-up. NDS – nuclear dip score.

Table 2: Results from univariable regression models

	beta	standard error	p-value
	Micronutrients		
Sodium (mg)	5.41E-06	9.58E-06	0.56
Potassium (mg)*	-1.58E-05	7.54E-06	0.04
Calcium (mg)	-1.95E-05	1.52E-05	0.20
Magnesium (mg)*	-0.010	0.004	0.01
Phosphorus (mg)*	-4.01E-05	1.94E-05	0.04
Iron (mg)	-1.15E-04	0.002	0.95
Copper (mg)	0.001	0.008	0.86
Zinc (mg)	-7.76E-04	0.003	0.77
Chloride (mg)	3.79E-06	6.10E-06	0.53
Manganese (mg)*	-0.010	0.004	0.01
Iodine(mg)	-1.10E-04	6.07E-05	0.07
Retinol (ug)	2.36E-06	3.90E-06	0.55
Carotene (ug)	-1.67E-06	1.40E-06	0.23
Vitamin D (ug)	-0.004	0.003	0.22
Vitamin E (mg)*	-0.003	0.001	0.04
Thiamin (mg)	-0.013	0.013	0.30
Riboflavin (mg)	-0.011	0.006	0.08
Niacin (mg)	-1.10E-04	8.26E-04	0.89
Tryptophan (mg)	-0.001	0.001	0.27
Vitamin B6 (mg)	-0.002	0.009	0.81
Vitamin B12 (ug)	-0.001	0.001	0.50
Folate (ug)*	-9.91E-05	4.06E-05	0.02
Pantothenate (mg)	-2.81E-05	1.87E-04	0.88
Biotin (mg)	-3.01E-04	4.17E-04	0.47
Vitamin C (mg)*	-1.742E-04	6.19E-05	0.01
	Supplement groups ^s		
Any supplement	-0.015	0.009	0.12
Micronutrients*	-0.032	0.013	0.01
Micronutrients excluding multivitamins	-0.023	0.012	0.06
Minerals only*	-0.038	0.016	0.02
Any other supplement	0.005	0.014	0.72

Legend: This table shows the results of the univariable linear regression analysis between nuclear cataract (natural logarithm transformed nuclear dip score) and energy adjusted micronutrient intakes and between nuclear cataract and supplement intake per supplement

group. \$ denotes that in the case of supplement groups, supplement intake was coded binary (presence vs absence of intake of at least one of the components in the group). All analyses were adjusted for age and family structure. * denote statistically significant associations at $p < 0.05$

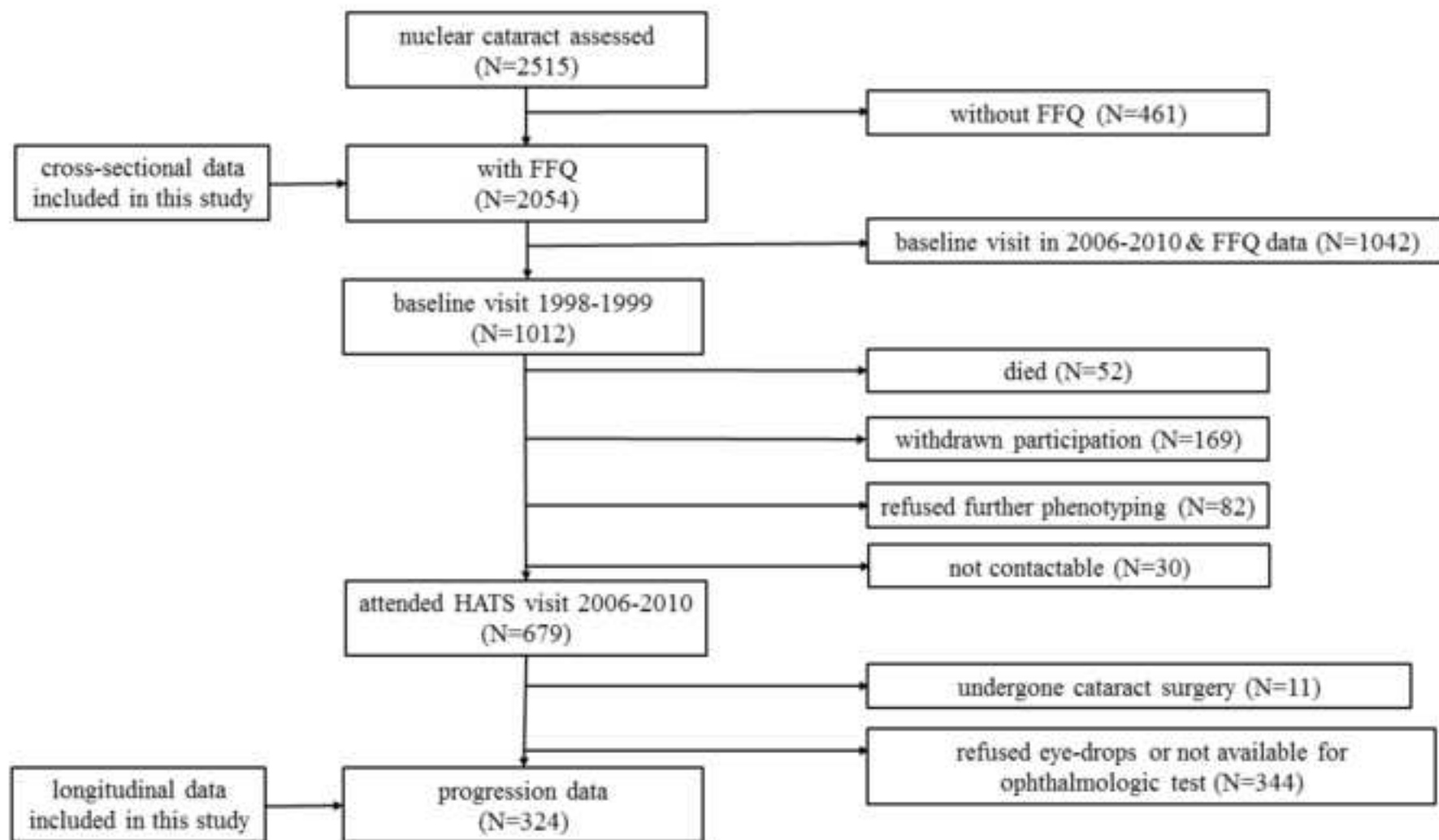
Table 3: Results of multinomial regression analysis for factors associated with cross-sectional nuclear cataract and with nuclear cataract progression

		Cross-sectional results			
		vitamin C	RRR	95%CI	p-value
NDS tertiles	34.5-53.2	reference			
	53.3-54.5	0.89	0.77-1.02	0.09	
	54.6-229.2	0.81	0.68-0.96	0.01	
		manganese	RRR	95%CI	p-value
NDS tertiles	34.5-53.2	reference			
	53.3-54.5	0.76	0.66-0.87	0.001	
	54.6-229.2	0.8	0.67-0.95	0.01	
		micronutrients	RRR	95%CI	p-value
NDS tertiles	34.5-53.2	reference			
	53.3-54.5	0.82	0.60-1.12	0.82	
	54.6-229.2	0.82	0.57-1.20	0.82	
		Progression results			
		vitamin C	RRR	95%CI	p-value
Δ NDS tertiles	1.0-12.6	reference			
	12.7-19.3	0.75	0.54-1.04	0.09	
	19.4-137.1	0.66	0.47-0.91	0.01	

Legend: This table shows the results from the multinomial regression analysis for factors associated with cross-sectional (vitamin C and manganese) and progression (vitamin C). The relative risk ratio (RRR) with its 95% confidence intervals (95%CI) for each tertile of nuclear dip score (NDS) or progression (Δ NDS) is reported. The minimum and maximum NDS score per tertile are also reported.

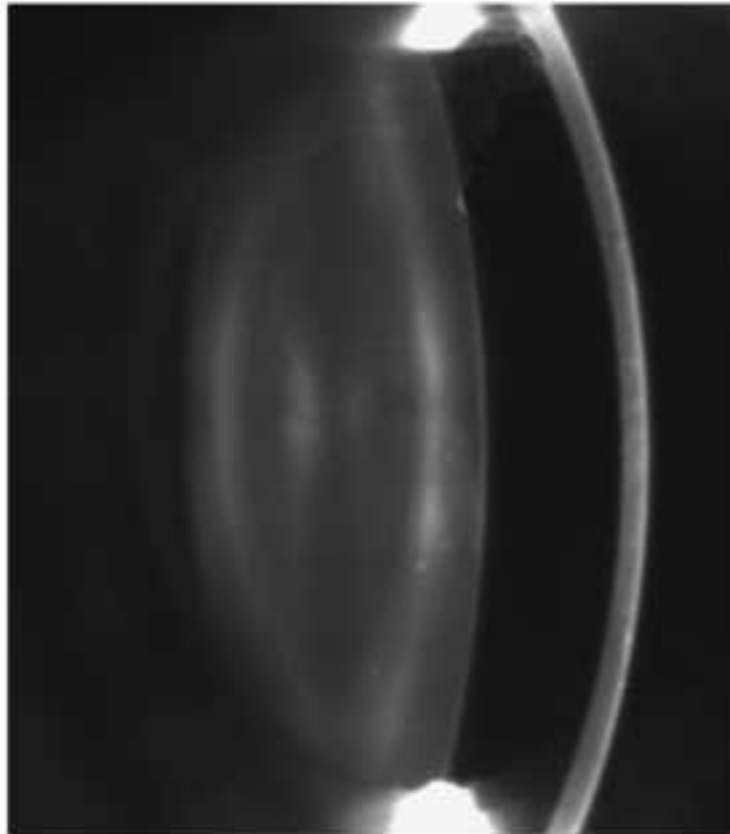
Figure

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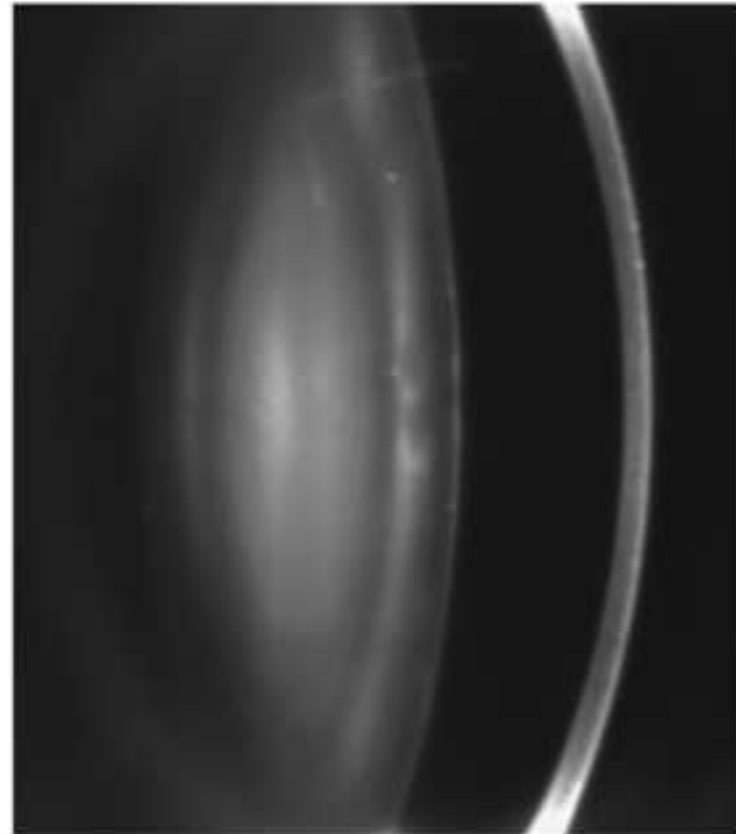


Figure

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Relatively clear nucleus



Nuclear cataract

Figure
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