Association of ambient air pollution with age-related macular degeneration and retinal thickness in UK Biobank

Chua, S. Y. L., Warwick, A., Peto, T., Balaskas, K., Moore, A. T., Reisman, C. A., Desai, P., Lotery, A. J., Dhillon, B., Khaw, P. T., Owen, C. G., Khawaja, A. P., Foster, P. J., & Patel, P. J. (2021). Association of ambient air pollution with age-related macular degeneration and retinal thickness in UK Biobank. *British Journal of Ophthalmology*. Advance online publication. https://doi.org/10.1136/bjophthalmol-2020-316218

Published in:
British Journal of Ophthalmology

Document Version:
Peer reviewed version

Association of ambient air pollution with age-related macular degeneration and retinal thickness in UK Biobank

Sharon YL Chua PhD (1,2); Alasdair Warwick (3); Tunde Peto (4);Konstantinos Balaskas (1,5) Anthony T Moore FRCOphth (2,6); Charles Reisman (7); Parul Desai PhD FRCOphth (8); Andrew J Lotery (9); Baljean Dhillon FRCS(Ed) FRCOphth(10,11); Peng T Khaw PhD FMedSci (1,2); Christopher Owens (12); Anthony P Khawaja (1,2,8); Paul J Foster* PhD FRCS(Ed) (1,2,8); Praveen J Patel* MD FRCOphth (1,8); on behalf of The UK Biobank Eye and Vision Consortium

Author affiliations:

1. NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust & UCL Institute of Ophthalmology, London, UK
2. UCL Institute of Ophthalmology, London, UK
3. UCL Institute of Cardiovascular Science, UK
4. School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, Belfast, UK.
5. School of Biological Sciences, University of Manchester, Manchester, UK
6. Ophthalmology Department, University of California, San Francisco, USA
7. Topcon Healthcare Solutions Research & Development, Oakland, New Jersey, USA
8. Moorfields Eye Hospital, London, UK
9. Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, UK
10. Centre for Clinical Brain Sciences, School of Clinical Sciences, University of Edinburgh, Edinburgh, UK
11. NHS Lothian Princess Alexandra Eye Pavilion, Edinburgh, UK
12. Population Health Research Institute, St. George's, University of London, London, UK

* Joint senior authors taking joint credit and responsibility

Correspondence to: Paul Foster

Address: UCL Institute of Ophthalmology, 11-43 Bath Street, London, EC1V 9EL

Email: p.foster@ucl.ac.uk

Telephone: 07971 663189

Word count: 3,779

SYNOPSIS

Age-related macular degeneration (AMD) is the leading cause of vison loss among the elderly in high income countries. Increased exposure to air pollution may be associated with AMD and differences in retinal layer thickness.

ABSTRACT

Aim: To examine the associations of air pollution with both self-reported age related macular degeneration (AMD), and in vivo measures of retinal sub-layer thicknesses.

Methods: We included 115,954 UK Biobank participants aged 40 to 69 years old in this cross-sectional study. Ambient air pollution measures included particulate matter, nitrogen dioxide (NO_2) and nitrogen oxides (NO_x). Participants with self-reported ocular conditions, high refractive error (< -6 or > +6 diopters) and poor spectral-domain optical coherence tomography (SD-OCT) image were excluded. Self-reported AMD was used to identify overt disease. Spectral-domain optical coherence tomography (SD-OCT) imaging derived photoreceptor sub-layer thickness and retinal pigment epithelium (RPE) layer thickness were used as structural biomarkers of AMD for 52,602 participants. We examined the associations of ambient air pollution with self-reported AMD and both photoreceptor sub-layers and retinal pigment epithelium (RPE) layer thicknesses.

Results: After adjusting for covariates, people who were exposed to higher fine ambient particulate matter with an aerodynamic diameter <2.5μm ($PM_{2.5}$) (per interquartile range [IQR] increase) had higher odds of self-reported AMD (OR= 1.08, p=0.036), thinner photoreceptor synaptic region (β= -0.16μm, $p=2.0X10^{-5}$), thicker photoreceptor inner segment layer (β= 0.04μm, p=0.001) and thinner RPE (β= -0.13μm, p=0.002). Higher levels of $PM_{2.5}$ absorbance and nitrogen dioxide (NO_2) were associated with thicker photoreceptor inner and outer segment layers, and a thinner RPE layer. Higher levels of PM_{10} (PM with an aerodynamic diameter <10μm) was associated with thicker photoreceptor outer segment and thinner RPE, while higher exposure to NO_x was associated with thinner photoreceptor synaptic region.

Conclusion: Greater exposure to $PM_{2.5}$ was associated with self-reported AMD, while $PM_{2.5}$, $PM_{2.5}$ absorbance, PM_{10}, NO_2 and NO_x were all associated with differences in retinal layer thickness.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in adults 50 years and above in high income countries.[1] Dry AMD is characterized by progressive dysfunction of the retinal pigment epithelium (RPE), photoreceptor loss and retinal degeneration.[2] By 2020, the global projected number of people with AMD is approximately 200 million, increasing to nearly 300 million by 2040.[3] Well-known risk factors include older age, smoking and genetic factors.[1] A constellation of adverse factors (both risk genotypes, smoking and body mass index [BMI] ≥25) together increases the risk 19-fold.[4] As smoking tobacco is a risk factor, it is plausible that ambient air pollution may also be a modifiable risk factor.

Air pollution is one of the world's most important environmental health risks. It is associated with increased mortality and morbidity.[5] Exposure to air pollution is associated with pulmonary and cardiovascular disease[6] and eye diseases including glaucoma[7] and AMD.[8] The mechanisms of air-pollution-induced health effects may likely involve oxidative stress and inflammation.[9] The retina is one of the highest oxygen-consuming tissues in the human body and resides in an environment that is primed for the generation of reactive oxygen species (ROS) and resultant oxidative damage.[10] Oxidative damage increases with age, resulting in retinal dysfunction and cell loss. Rapid, non-invasive optical coherence tomography (OCT) imaging of the retina is now commonly used by community opticians and hospital eye clinics and to assess retinal structural changes associated with AMD, and to guide its management.[11]

If air pollution has an adverse effect on AMD risk, this may offer a new range of interventions for controlling this important condition. We examined data from UK Biobank, a large community-based cohort study. The aim of our study was to evaluate the relationship between ambient air pollution, AMD status and OCT imaging derived structural features of the disease: photoreceptor sub-layer and RPE layer thickness.

METHODS

Study population

UK Biobank (UKBB) is a very large community-based cohort of 502,656 UK residents registered with the National Health Service (NHS) and aged 40–69 years at enrolment. Baseline examinations were carried out between 2006-2010 at 22 study assessment centres. The North West Multi-centre Research Ethics Committee approved the study in accordance with the principles of the Declaration of Helsinki. The overall study protocol (http://www.ukbiobank.ac.uk/resources/) and protocols for individual tests (http://biobank.ctsu.ox.ac.uk/crystal/docs.cgi) are available online. Participants answered a wide-ranging touch-screen questionnaire covering demographic, socioeconomic, lifestyle, systemic and ocular diseases information. Definition of hypertension was based on self-reported. Physical measures included height and weight. Body mass index (BMI) was defined as weight divided by height squared.

Ocular assessment

Ocular assessment was introduced as an enhancement in 2009 for six assessment centers which are spread across the UK.[12] Habitual visual acuity (VA) was measured

using a logarithm of the minimum angle of resolution (LogMAR) chart (Precision Vision, LaSalle, Illinois, USA) on a computer screen under standard illumination.[12,13] Refractive error was measured using an autorefractor (Tomey RC 5000, Nagoya, Japan).[14] High resolution OCT imaging was performed using the Topcon 3D OCT 1000 Mk2 (Topcon Inc, Oakland, NJ, USA) in a dark room, without pupillary dilation using the 3D macular volume scan (scan settings: 512 horizontal A scans per B scan; 128 B scans in a 6 x 6 mm raster pattern). The Topcon Advanced Boundary Segmentation (TABS) Algorithm (Version 1.6.1.1) [15] was used to detect retinal layer boundaries and measure the thickness of the RPE[16] and photoreceptor sub-layers. (**Supplementary Figure 1**). The TABS segmentation algorithm has been validated previously showing a high degree of precision and reproducibility compared to manual segmentation methods.[15] Strict quality control was implemented to exclude images of poor quality as described in detail previously.[17] OCT scans with image quality score (signal strength) < 45 were excluded. Several segmentation indicators were calculated to identify poor scan quality or segmentation failures. Participants with the poorest 20% of images for each of these indicators were also excluded. These indicators included an inner limiting membrane (ILM) indicator, a validity count, and motion indicators. The ILM indicator was a measure of the minimum localized edge strength around the ILM boundary across the entire scan. It is useful for identifying blinks, scans that contain regions of severe signal fading, and segmentation errors. The validity count indicator is used to identify scans with a significant degree of clipping in the OCT scan's z-axis dimension. The motion indicators use both the nerve fibre layer and the full retinal thicknesses, from which Pearson correlations and absolute differences between the thickness data from each set of consecutive B-scans are calculated. The lowest correlation and the highest absolute difference in a scan serve as the resulting

indicator scores and identify blinks, eye motion artifacts, and segmentation failures. The image quality score and the aforementioned indicators usually are highly correlated.[18]

Definition of AMD status

Definition of AMD status was based on self-reported data. AMD status was determined as those who selected “macular degeneration” from a predefined list of eye disorders to the question “Has a doctor ever told you that you have any of the following problems with your eyes?” We also carried out a validation of self-reported AMD status by carrying out masked grading of the retinal OCT and fundus images for features of AMD based on the Beckman AMD classification on a random subset of age-matched participants.[19]

Estimates of air Pollution

The air pollution estimates were provided by the Small Area Health Statistics Unit (http://www.sahsu.org/) as part of the BioSHaRE-EU Environmental Determinants of Health Project (http://www.bioshare.eu/), and were linked centrally to the assessment data by UK Biobank analysts (http://biobank.ctsu.ox.ac.uk/crystal/docs/EnviroExposEst.pdf). Detailed estimates of air pollution parameters have been published.[20] The annual average concentration of $PM_{2.5}$ (aerodynamic diameter of less than 2.5μm), PM_{coarse} (aerodynamic diameter between 2.5 and 10μm, PM_{10} (aerodynamic diameter of less than 10μm), $PM_{2.5}$ absorbance (a measurement of the blackness of $PM_{2.5}$ filter – a proxy for elemental or black carbon), nitrogen dioxide (NO_2) and nitrogen oxides (NO_x) were calculated centrally by the UK Biobank using a land use regression model developed by the

European Study of Cohorts for Air Pollution Effects (ESCAPE) project (http://www.escapeproject.eu/).[21] By using the predictor variables obtained from the Geographic Information System such as traffic, land use, and topography, the land use regression models calculate the spatial variation of annual average air pollution concentration at participants' residential addresses given at baseline visit. NO_2 annual concentration data were available for four years (2005, 2006, 2007 and 2010), while PM_{10} data was available for 2007 and 2010. We averaged the values to obtain the mean estimate. All other particulate matter and nitrogen pollutants had the exposure data for a single year (2010).

Inclusion and exclusion criteria

A uniform set of exclusion criteria was applied in analysis of AMD status, photoreceptor layer and RPE thickness (**Figure 1**). We excluded data from: (1) participants who withdrew consent; or (2) had self-reported diabetes-related eye disease, eye injury resulting in vision loss or other serious eye conditions; high refractive error (< -6 diopters [D] or > +6D) or (3) participants who had poor OCT image scans using TABS software.[16,22] These participants were excluded because of the well-recognized impact these factors have on retinal layer thickness.[23]

Statistical analysis

The present analysis was based on cross-sectional data collected at one point in time. For this analysis, if both eyes of a patient were eligible for inclusion in the analysis, one eye was randomly selected using STATA software (version 13, StataCorp LP, College Station, TX, USA). We examined the baseline characteristics of participants

included for each specific outcome (self-reported AMD and retinal layers). Descriptive statistics for continuous variables are presented as mean (standard deviation [SD]), whereas categorical variables are presented as number (percentage). We examined the associations of each air pollutant (independent variables) with self-reported AMD (dependent variable) using logistic multivariable regression models, adjusted for age, sex, race, Townsend deprivation index, BMI, smoking status, and refractive error. The associations of air pollutants with photoreceptor sub-layers and RPE thicknesses (dependent variables) were adjusted for the same variables, using linear multivariable regression models. The effect estimates represent the change in self-reported AMD and retinal layers variables per interquartile range (IQR) increment in air pollution. Statistical significance was set at $p < 0.05$ for the outcomes self-reported AMD and RPE thickness. When photoreceptor sub-layer thickness was analyzed as an outcome, statistical significance was set at $p<0.002$ after Bonferroni correction as we examined six different types of air pollutants with four distinct photoreceptor related layers. In sensitivity analysis, we examined the associations of air pollutants with visually significant self-reported AMD. Visually significant self-reported AMD was defined as self-reported AMD participants with VA worse than LogMAR 0.3 (equivalent to Snellen 20/40), while non-visually significant self-reported AMD was defined as those with VA of LogMAR 0.3 or better.

Results

Of the 133,964 participants who completed ocular assessment, 24 participants withdrew their consent. Of the 133,940, we excluded 13,329 participants according to the exclusion criteria (**Figure 1**), leaving data on 120,611 participants. There were

complete data (age, sex, race, Townsend deprivation index, BMI, smoking status, refractive error, self-reported AMD and air pollution measures) for 115,954 participants. Of the 115,954, there was complete OCT imaging data on retinal layers for 68,088 participants. We excluded 15,486 participants according to the exclusion criteria for OCT. Hence, 52,062 participants were included in the analysis for examining RPE and photoreceptor layer thickness. This large number of exclusions for retinal layers was because of a later start for OCT imaging in UK Biobank, meaning a smaller number of people were scanned.

The characteristics of participants with data on self-reported AMD and a sub-group with data on retinal layer are shown in **Table 1**. Both groups had similar sociodemographic and clinical characteristics. Compared to participants with self-reported AMD, those without self-reported AMD were more likely non-white (9.1% vs 7.0%; p=0.01), younger (56.8 years vs 61.6 years), more likely male (46.0% vs 40.9%), more likely to come from a more deprived area (less negative Townsend deprivation index) (-1.1 vs -1.4) and more likely to be smokers (9.7% vs 7.6%) (all p<0.001) (**Supplementary Table 1**). The distribution of ambient air pollution exposure of participants with data on self-reported AMD and a sub-group with retinal layer data are shown in **Supplementary Table 2**. The mean [SD] of the various retinal layers are as follows: total length of photoreceptor (142.1µm [8.2µm]), photoreceptor synaptic region (80.4µm [6.6µm]), photoreceptor inner segment (23.8µm [2.0µm]), photoreceptor outer segment (37.9µm [4.3µm]) and RPE (25.6µm [7.2µm]). Of the 115,954 participants, 1,286 (1.1%) were diagnosed with AMD. Masked grading of OCT and retinal fundus images from 119 participants (60 with self-reported AMD and 59 without self-reported AMD) showed that 75% of those with self-reported AMD had

OCT features of AMD while only 12% of those without self-reported AMD had OCT features of AMD.

Participants exposed to higher levels of $PM_{2.5}$ concentration were 8% more likely to have self-reported AMD (OR 1.08, 95% CI 1.01 to 1.16; p=0.036, per IQR increase) (**Table 2**). Following Bonferroni correction, higher levels of $PM_{2.5}$ and NO_x were associated with thinner photoreceptor synaptic region (**Table 3**). In contrast, per IQR increase in $PM_{2.5}$, $PM_{2.5}$ absorbance and NO_2 were associated with a thicker photoreceptor inner segment layer. Exposure to higher levels of $PM_{2.5}$ absorbance, PM_{10} and NO_2 were associated with a thicker photoreceptor outer segment layer (**Table 3**). Higher concentration of $PM_{2.5}$, $PM_{2.5}$ absorbance, PM_{10} and NO_2 were associated with a thinner RPE layer (**Table 4**). In addition, we examined the association of smoking status with self-reported AMD. Among participants with self-reported AMD, 510/1,286 (39.7%) and 101/1,286 (7.9%) were previous and current smokers, respectively. After adjusting for age, sex, race, Townsend deprivation index, BMI, SER and $PM_{2.5}$, compared to never smoking, previous and current smokers were not associated with self-reported AMD (p>0.05). We have additionally adjusted for hypertension in the multivariable models in view of its relationship with AMD[24] and air pollution.[25] The associations of air pollutants with self-reported AMD, photoreceptor sub-layers and RPE thickness did not differ after additional adjustment for hypertension. Sensitivity analysis showed that participants with higher exposure to $PM_{2.5}$ was marginally associated with visually significant self-reported AMD (n=167) (OR 1.18, 95% CI 0.98 to 1.41; p=0.08, per IQR increase) compared to participants with either no self-reported AMD or those with non-visually significant self-reported AMD, although it was not statistically significant. None of the other air pollutants were

statistically significant with visually significant self-reported AMD. In the sensitivity analysis, we have also additionally adjusted for smoking pack years and there was a borderline significant association between $PM_{2.5}$ and self-reported AMD (OR 1.07, 95% CI 0.99 to 1.16; p=0.07, per IQR increase).

Discussion

In this large study of UK Biobank participants, we have identified novel associations between ambient outdoor air pollutant levels at participants' residential addresses with self-reported AMD, and also with retinal structure (including thickness of photoreceptor and RPE layers on OCT imaging).

Our results showed that greater ambient $PM_{2.5}$ exposure was associated with increased odds of AMD and corresponding retinal thicknesses (specifically photoreceptor sub-layer and RPE). No such significant associations were observed for PM_{coarse}. This may be explained by differences in the sites of deposition in the respiratory tract and the sources and chemical composition for these different-sized PM.[26] PM_{coarse} are primarily produced from mechanical grinding, windblown dust, and agricultural activities, and mainly deposit in the upper and larger airways. In contrast, $PM_{2.5}$ particles are mainly from combustion process and are able to reach the smaller airways and alveoli and are transmitted to the blood,[27] causing a cascade of physiological events associated with morbidity and mortality.[5,28] The deeper penetration of $PM_{2.5}$ may account for the stronger associations of $PM_{2.5}$ with self-reported AMD and structural biomarkers observed in our study.

246 NO_2 is a product of combustion, primarily from traffic- and industrial sources, and one
247 of the most notable ambient air pollutants associated with health effects.[29,30] Similarly,
248 NO_x is produced from the reaction of nitrogen and oxygen gases in the air during
249 combustion.[31] NO_x contributes to the formation of fine particles and ground level
250 ozone. $PM_{2.5}$ absorbance, a measurement of the blackness of PM2.5 filter – a proxy
251 for elemental or black carbon, is also an indicator of combustion particles. Since the
252 major source of NO_2, NO_x and $PM_{2.5}$ absorbance is from combustion particles, it may
253 explain the similar associations observed between these air pollutants with the retinal
254 structures. A recent longitudinal population-based study using data from the Taiwan
255 National Health Insurance Program between years 2000-2010 included 39,819 AMD-
256 free participants, with 1442 participants developing AMD during the 11-year follow up.
257 AMD status was defined via International Classification of Diseases, Ninth Revision,
258 Clinical Modification (ICD-9-CM). Compared to participants in the lowest exposure
259 quartile, those in the highest quartile of NO_2 and carbon monoxide (CO) had increased
260 risk of self-reported AMD (NO_2: HR=1.91, 95% CI 1.64-2.23, p<0.001 and CO:
261 HR=1.84, 95% CI 1.50-2.15, p<0.001, respectively).[8] The difference in findings
262 between ours and the Taiwanese study may be related to the study population,
263 definition and proportion of AMD cases, type and method of estimating the exposure
264 of air pollutants and type of covariates adjusted in the multivariable models. Compared
265 to our study, the Taiwan study included slightly older participants (mean= 62 years vs
266 56 years), had a slightly higher proportion of AMD (3.6% vs 1.1%) and estimated a
267 smaller number of air pollutants (two air pollutants including NO_2 and CO vs six air
268 pollutants). In addition, the participant's living area was defined based on the treatment
269 venue for acute upper respiratory tract infection in the Taiwan study. The effect of

pollution on retinal structure associated with AMD were not examined in the Taiwan study.

Ambient air pollution could plausibly be associated with AMD through oxidative stress or inflammation. Oxidative damage induces many adverse biological effects including lipid, protein, deoxyribonucleic acid (DNA) oxidation, initiation of proinflammatory processes,[28] and RPE apoptosis.[32] Atrophic or “dry” AMD, also known as geographic atrophy is by degeneration of RPE cells, followed by loss of photoreceptor cells and choriocapillaris.[33] Since the RPE is involved in the turnover of photoreceptor outer segments, RPE dysfunction may lead to thickening of photoreceptor outer segments.

Our results showed that $PM_{2.5}$ and NO_x were associated with a thinner photoreceptor synaptic region. This is in agreement with a reduction in the number of photoreceptor synaptic terminals overlying drusen in AMD.[34] In contrast, $PM_{2.5}$, $PM_{2.5}$ absorbance and NO_2 were associated with thicker photoreceptor inner segment, while $PM_{2.5}$ absorbance, NO_2 and PM_{10} were associated with thicker photoreceptor outer segment. As mitochondria are prominent in photoreceptor inner segments, oxidative stress may induce mitochondrial swelling,[35] leading to a slight thickening in the photoreceptor inner segment. Abnormalities in the photoreceptor inner and outer segments have also been reported in retinal toxicity associated with hydroxychloroquine.[36] Our study did not show an association between air pollution and average total photoreceptor layer thickness, which may be explained by thinning of the synaptic region cancelling out the thickening of the inner/outer segments. In a study by Schuman *et al.*, although the authors reported decreased photoreceptor thickness over drusen, there was a lack of

widespread photoreceptor loss.[37] Hence, it is possible that there was focal loss of the photoreceptor thickness in our study but an overall loss of photoreceptor layer was not observed.

Cigarette smoking may also contribute to particulate matter air pollution.[38] Because of the previously recorded, very strong link between AMD and smoking,[39] and the plausible link between smoking and particulate air pollution, we examined the association between smoking status of participants with self-reported AMD and did not observe a significant association. This suggests that the relationship between $PM_{2.5}$ and self-reported AMD is not mediated by cigarette smoke. The prevalence of late AMD standardized to the UK population aged 50 years or more and 65 years or more was 2.4% and 4.8%, respectively. Prevalence of geographic atrophy was 1.3% and 2.5% for the respective age groups.[40] The European Eye Epidemiology (E3) Consortium performed a meta-analysis and showed that overall prevalence was 13.2% for early AMD and 3.0% for late AMD for people aged 70 years or older.[41] Compared to the E3 Consortium, participants in UK Biobank are slightly younger and include a healthier population than the rest of UK population.[42] The self-reported AMD cases in our study may represent AMD in the early stages. We compared the visual acuity between participants with and without self-reported AMD. Among those with self-reported AMD, there was a higher proportion of participants with visual impairment (VA worse than LogMAR 0.3) compared to those without visual impairment (1.8% vs 1.0%; $p<0.001$). The proportion of self-reported AMD (1.1%) in our study may have been underestimated and it is likely that the risk estimates may have been underestimated.

In addition to the increased risk of AMD associated with higher exposure to air pollution in the Taiwanese study, other studies in the UK Biobank[43] and China[7] have reported increased odds of glaucoma with higher exposure to $PM_{2.5}$. In the UK Biobank study of 111,370 participants, greater exposure to $PM_{2.5}$ was associated with both self-reported glaucoma and retinal structures associated with the disease.[43] Wang *et al*. reported that higher average levels of $PM_{2.5}$ was associated with higher burden of glaucoma disability, using national level data.[7] The New England-based Normative Aging Study showed an association between black carbon exposure with IOP that was greater in individuals with a high oxidative stress allelic score.[44] Taken together, our results support published findings of increased risk of eye diseases or association with retinal structures in participants with higher exposure to ambient air pollution. As certain groups of individuals including people with diabetes mellitus[45] or hypertension[24] may have increased risk of AMD, it will be useful to explore if these groups of individuals are at greater risk of eye disease when exposed to air pollution in future analysis.

Strength of this study include its large sample size and the highly accurate and reproducible measurements of the OCT retinal thickness. Limitations of the study include the UK Biobank is a volunteer cohort, and participants are likely healthier than the general population. Outdoor air pollution was estimated using the participants' home address and do not explain all variation in indoor concentrations. As most individuals spend a large amount of time indoors, individual exposure to all forms of air pollution may differ from that indicated by the ambient outdoor figures. This is most

likely to be non-differential between cases and controls and will therefore skew the associations towards the null. Another limitation of this analysis was the use of self-report as the sole determinant of AMD status rather than incorporating a qualitative analysis of the colour fundus photographs and SD-OCT imaging, though we did carry out masked grading of retinal imaging in a proportion of participants. This may result in non-differential misclassification bias and most likely bias the estimates towards the null. Although we applied strict automated quality control criteria including a manual check of SD-OCT scans with high and low outlying layer thickness,[17] it was not practical to manually check all OCT scans for segmentation accuracy. Selection bias may exist: out of the 115,954 participants with data on self-reported AMD, 52,602 participants had measurements on outer retinal layers. However, the baseline characteristics (Table 1) across the two AMD-associated outcome groups appear to be similar. The cross-sectional design of our study limits the ability to determine the causality between ambient air pollution and AMD-associated outcomes. Further research is needed to probe the relationship between prior air pollution exposure and risk of incident disease.

In this large study of an older middle-aged UK population, higher $PM_{2.5}$ exposure was associated with a higher risk of self-reported AMD, while all pollutants except PM_{coarse} were associated with changes in retinal structure (in either photoreceptor sublayer and/or RPE layer thickness). Overall, our findings suggest that ambient air pollution, especially fine PM or those of combustion-related particles, may affect AMD risk. It is possible that the structural features observed may be unrelated to AMD, but associated with pollution induced retinal toxicity. However, the direction of the relationships between air pollution and both AMD and associated retinal layer

thicknesses indicate higher exposure to air pollution may make the cells more vulnerable and increase the risk of AMD. Our findings add to the growing evidence of the damaging effects of ambient air pollution, even in the setting of relative low exposure of ambient air pollution. As UK Biobank is a very large prospective cohort, we anticipate being able to explore the effect of particulate matter on future risk of AMD. Further studies examining both outdoor and indoor ambient air pollution estimates on AMD and outer retinal structures may help to substantiate our findings and understand the implications for retinal disease associated with ageing. If our findings are replicated, this would support the view that air pollution is an important modifiable risk factor for AMD.

Table 1. Demographic, systemic and ocular characteristics of participants with availability of data on self-reported AMD and retinal layers.

	Participants with data on self-reported AMD (N=115,954)	Participants with data on retinal layers (N=52,602)
Sociodemographic factors		
Age	56.8 (8.0)	56.4 (8.1)
Sex		
Men	53,218 (46%)	24,753 (47%)
Women	62,736 (54%)	27,849 (53%)
Race		
White	105,465 (91%)	48,475 (92%)
Non-white	10,489 (9%)	4,127 (8%)
Townsend deprivation index	-1.1 (3.0)	-1.2 (2.9)
Clinical factors		
Body mass index (kg/m^2)	27.3 (4.5)	27.2 (4.4)
Smoking status		
Never	64,554 (56%)	29,238 (56%)
Previous	40,224 (35%)	18,421 (35%)
Current	11,176 (10%)	4,943 (9%)
Spherical equivalent (diopters)	-0.1 (2.1)	0.0 (2.0)

Numbers are mean (SD) or no. (%), unless otherwise stated.
AMD= Age-related macular degeneration, $PM_{2.5}$= Particular matter (aerodynamic diameter of less than 2.5µm), $PM_{2.5}$ absorbance= Particulate matter (a measurement of the blackness of $PM_{2.5}$ filter – a proxy for elemental or black carbon), PM_{coarse} = Particulate matter (aerodynamic diameter between 2.5 and 10µm, PM_{10}= Particulate matter (aerodynamic diameter of less than 10µm), NO2= Nitrogen dioxide, NO_x= Nitrogen oxide

377

Table 2: Association of ambient air pollution with self-reported age-relation macular degeneration (AMD)

	Multivariate regression		
	OR	*(95% CI)*	*P*-value
Air pollution factors			
$PM_{2.5}$ (μg/m^3)	1.08	(1.01, 1.16)	**0.036**
$PM_{2.5}$ absorbance (μg/m^3)	1.00	(0.93, 1.07)	0.95
$PM_{2.5-10}$ (μg/m^3)	1.01	(0.96, 1.07)	0.58
PM_{10} (μg/m^3)	0.94	(0.86, 1.02)	0.11
NO_2 (μg/m^3)	0.99	(0.91, 1.08)	0.80
NOX (μg/m3)	1.03	(0.97, 1.09)	0.34

The odds ratio represents per IQR increase in exposure variable.

Values are adjusted for age, sex, race, Townsend deprivation index, body mass index, smoking status and spherical equivalent refraction

378

Table 3: Association of ambient air pollution with thickness of the photoreceptor sub-layers

	Multivariate regression											
	Total photoreceptor			Photoreceptor synaptic region			Photoreceptor inner segment			Photoreceptor outer segment		
	β	(95% CI)	P-value	β	(95% CI)	P-value	β	(95% CI)	P-value	β	(95% CI)	P-value
Air pollution factors												
$PM_{2.5}$ (μg/m^3)	-0.07	(-0.16, 0.02)	0.15	-0.16	(-0.23, -0.09)	**2.0 X 10^{-5}**	0.04	(0.02, 0.06)	**0.001**	0.05	(0.003, 0.10)	0.04
$PM_{2.5}$ absorbance (μg/m^3)	0.06	(-0.03, 0.14)	0.22	-0.10	(-0.17, -0.03)	0.004	0.04	(0.02, 0.06)	**2.0 X 10^{-4}**	0.12	(0.07, 0.17)	**8.7 X 10^{-7}**
PM_{coarse} (μg/m^3)	-0.04	(-0.11, 0.02)	0.18	-0.03	(-0.08, 0.02)	0.21	-0.008	(-0.02, 0.007)	0.32	-0.003	(-0.04, 0.03)	0.85
PM_{10} (μg/m^3)	0.04	(-0.06, 0.14)	0.47	-0.05	(-0.13, 0.03)	0.24	-0.002	(-0.01, 0.007)	0.63	0.09	(0.04, 0.15)	**0.001**
NO_2 (μg/m^3)	0.15	(0.04, 0.26)	0.004	-0.06	(-0.14, 0.03)	0.19	0.04	(0.02, 0.07)	**0.001**	0.17	(0.11, 0.22)	**1.1 X 10^{-8}**
NO_X (μg/m^3)	-0.02	(-0.09, 0.06)	0.63	-0.10	(-0.16, -0.04)	**0.001**	0.03	(0.008, 0.04)	0.004	0.05	(0.01, 0.09)	0.009

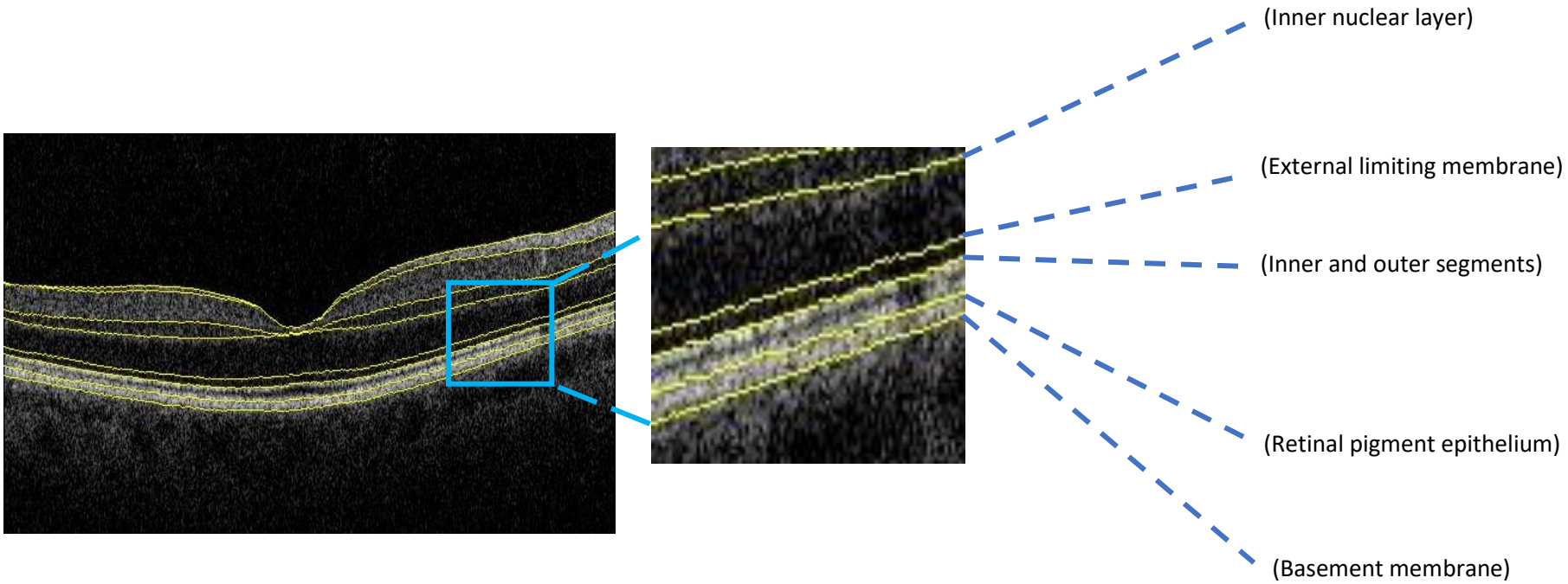
The beta coefficients represent per IQR increase in exposure variable.
Values are adjusted for age, sex, race, Townsend deprivation index, body mass index, smoking status and refractive error.
Statistical significance was set at p<0.002 after Bonferroni correction.
$PM_{2.5}$= PM<2.5μg/m^3; $PM_{2.5}$ ab= ($PM_{2.5}$ absorbance) a measurement of the blackness of $PM_{2.5}$ filter - a proxy for elemental or black carbon; PM_{coarse}= PM between 2.5 and 10μg/m^3; PM_{10}= PM <10μg/m^3; NO_2= Nitrogen dioxide; NO_x= Nitrogen oxide

Table 4: Association of ambient air pollution with thickness of the retinal pigment epithelium layer

	Multivariate regression		
	RPE		
	β	(95% CI)	P-value
Air pollution factors			
$PM_{2.5}$ (µg/m^3)	-0.13	(-0.21, -0.05)	**0.002**
$PM_{2.5}$ absorbance (µg/m^3)	-0.09	(-0.17, -0.008)	**0.03**
PM_{coarse} (µg/m^3)	-0.02	(-0.08, 0.04)	0.50
PM_{10} (µg/m^3)	-0.12	(-0.21, -0.02)	**0.01**
NO_2 (µg/m^3)	-0.12	(-0.21, -0.02)	**0.01**
NO_X (µg/m^3)	-0.05	(-0.12, 0.02)	0.17

The beta coefficients represent per IQR increase in exposure variable.
Values are adjusted for age, sex, race, Townsend deprivation index, body mass index, smoking status and refractive error.
Statistical significance was set at $p<0.05$.
RPE= Retinal pigment epithelium; $PM_{2.5}$= Particulate matter less than 2.5 µm in aerodynamic diameter; $PM_{2.5}$ ab= ($PM_{2.5}$ absorbance) a measurement of the blackness of $PM_{2.5}$ filter - a proxy for elemental or black carbon; PM_{coarse}= Particulate matter between 2.5 µm to 10 µm in aerodynamic diameter; PM_{10}= Particulate matter less than 10 µm in aerodynamic diameter; NO_2= Nitrogen dioxide; NO_x= Nitrogen oxide

380



Supplementary Figure 1. Spectral-domain optical coherence tomography images with schematic showing representative of total photoreceptor (Inner nuclear layer–Retinal pigment epithelium); photoreceptor synaptic region (Inner nuclear layer- External limiting membrane); photoreceptor inner segment (External limiting membrane-Inner and outer segments); photoreceptor outer segment (Inner and outer segments-Retinal pigment epithelium) and retinal pigment epithelium (Retinal pigment epithelium-Basement membrane).

Supplementary Table 1. Comparison of characteristics between participants with self-reported AMD and without self-reported AMD

	No self-reported AMD (N=114,668)	Self-reported AMD (N=1,286)	P-value
Sociodemographic factors			
Age	56.8 (8.1)	61.6 (5.9)	<0.001
Sex			
Men	52,692 (46.0%)	526 (40.9%)	
Women	61,976 (54.0%)	760 (59.1%)	<0.001
Race			
White	104,269 (90.9%)	1,196 (93.0%)	
Non-white	10,399 (9.1%)	90 (7.0%)	0.01
Townsend deprivation index	-1.1 (3.0)	-1.5 (2.9)	<0.001
Clinical factors			
Body mass index (kg/m^2)	27.2 (4.5)	27.4 (4.3)	0.18
Smoking status			
Never	63,879 (55.7%)	675 (52.5%)	
Previous	39,714 (34.6%)	510 (39.7%)	
Current	11,075 (9.7%)	101 (7.8%)	<0.001
Spherical equivalent (diopters)	-0.08 (2.1)	-0.03 (2.3)	0.40

AMD status was classified based on self-reporting and hospital episode statistics data (ICD10).
Numbers are mean (SD) for continuous variables and no. (%) for categorical variables.
AMD= Age-related macular degeneration

Supplementary Table 2. Distribution of $PM_{2.5}$, PM_{coarse}, PM_{10}, NO_2 and NO_X of participants with availability of data on self-reported AMD and retinal layers

	Self-reported AMD (N=115,954)		Retinal layers (N=52,602)	
	Median (IQR)	Range	Median (IQR)	Range
$PM_{2.5}$ ($\mu g/m^3$)	9.91 (1.07)	(8.17, 19.69)	9.88 (1.12)	(8.17, 19.69)
$PM_{2.5}$ absorbance ($\mu g/m^3$)	1.22 (0.33)	(0.83, 4.05)	1.22 (0.33)	(0.83, 3.71)
PM_{coarse} ($\mu g/m^3$)	6.19 (0.75)	(5.57, 12.82)	6.21 (0.77)	(5.57, 11.30)
PM_{10} ($\mu g/m^3$)	19.37 (2.67)	(13.04, 29.67)	19.33 (2.77)	(13.38, 29.30)
Nitrogen dioxide (NO_2) ($\mu g/m^3$)	31.75 (12.08)	(9.44, 102.75)	31.25 (12.63)	(9.44, 86.65)
Nitrogen oxide (NO_X) ($\mu g/m^3$)	43.66 (14.38)	(19.74, 263.96)	43.17 (14.97)	(19.74, 263.96)

AMD = Age-related macular degeneration, IQR = Interquartile range, $PM_{2.5}$= Particular matter (aerodynamic diameter of less than 2.5µm), $PM_{2.5}$ absorbance= Particulate matter (a measurement of the blackness of $PM_{2.5}$ filter – a proxy for elemental or black carbon), PM_{coarse} = Particulate matter (aerodynamic diameter between 2.5 and 10µm, PM_{10}= Particulate matter (aerodynamic diameter of less than 10µm), NO2= Nitrogen dioxide, NO_x= Nitrogen oxide

Acknowledgements & Funding

Funding:
The UK Biobank Eye and Vision Consortium is supported by grants from Moorfields Eye Charity, The NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, the Alcon Research Institute and the International Glaucoma Association (UK).
No funders had a direct role in the collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; nor in the decision to submit the manuscript for publication.

Acknowledgements
SYLC, PTK, PJF, and PJP received salary support from the NIHR BRC at Moorfields Eye Hospital.
PTK is supported in part by the Helen Hamlyn Trust. PJF received support from the Richard Desmond Charitable Trust, via Fight for Sight, London.
APK is supported by a Moorfields Eye Charity Career Development Fellowship.
The authors acknowledge a proportion of our financial support from the UK Department of Health through an award made by the National Institute for Health Research to Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology for a Biomedical Research Centre for Ophthalmology.
This research used data from the UK Biobank Resource, under data access request number 2112.

Conflict of Interest:
CR reports employment by Topcon Healthcare Solutions, Inc. outside the submitted work. PJF reports personal fees from Allergan, Carl Zeiss, Google/DeepMind and Santen, a grant from Alcon, outside the submitted work; PJP reports grants from Topcon Inc, outside the submitted work.

Ethical approval: The North West Multi-center Research Ethics Committee approved the study (reference no., 06/MRE08/65), in accordance with the tenets of the Declaration of Helsinki. Detailed information about the study is available at the UK Biobank web site (www.ukbiobank.ac.uk)

Authors' Contributions:
SYLC had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
PJF and PJP led conception and design of the study.
SYLC,PJF and PJP contributed to the data analyses, data interpretation and wrote the draft of the manuscript.
All authors reviewed the results, read and critically revised the manuscript. All authors approved the final manuscript.
The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

References

1. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *The Lancet* 2018; **392**(10153): 1147-59.
2. McLeod DS, Grebe R, Bhutto I, Merges C, Baba T, Lutty GA. Relationship between RPE and choriocapillaris in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2009; **50**(10): 4982-91.
3. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet Global health* 2014; **2**(2): e106-16.
4. Seddon JM, Francis PJ, George S, Schultz DW, Rosner B, Klein ML. Association of CFH Y402H and LOC387715 A69S with progression of age-related macular degeneration. *Jama* 2007; **297**(16): 1793-800.
5. Cohen AJ, Brauer M, Burnett R, et al. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *Lancet (London, England)* 2017; **389**(10082): 1907-18.
6. Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A. The contribution of outdoor air pollution sources to premature mortality on a global scale. *Nature* 2015; **525**(7569): 367-71.
7. Wang W, He M, Li Z, Huang W. Epidemiological variations and trends in health burden of glaucoma worldwide. *Acta ophthalmologica* 2019; **97**(3): e349-e55.
8. Chang K-H, Hsu P-Y, Lin C-J, Lin C-L, Juo S-HH, Liang C-L. Traffic-related air pollutants increase the risk for age-related macular degeneration. *Journal of Investigative Medicine* 2019: jim-2019-001007.
9. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. *J Toxicol* 2011; **2011**: 487074.
10. Jarrett SG, Boulton ME. Consequences of oxidative stress in age-related macular degeneration. *Molecular aspects of medicine* 2012; **33**(4): 399-417.
11. Puliafito CA, Hee MR, Lin CP, et al. Imaging of macular diseases with optical coherence tomography. *Ophthalmology* 1995; **102**(2): 217-29.
12. Chua SYL, Thomas D, Allen N, et al. Cohort profile: design and methods in the eye and vision consortium of UK Biobank. *BMJ open* 2019; **9**(2): e025077.
13. Cumberland PM, Rahi JS, Eye UKB, Vision C. Visual Function, Social Position, and Health and Life Chances: The UK Biobank Study. *JAMA Ophthalmol* 2016; **134**(9): 959-66.
14. Cumberland PM, Bao Y, Hysi PG, et al. Frequency and Distribution of Refractive Error in Adult Life: Methodology and Findings of the UK Biobank Study. *PLoS One* 2015; **10**(10): e0139780.
15. Yang Q, Reisman CA, Wang Z, et al. Automated layer segmentation of macular OCT images using dual-scale gradient information. *Optics express* 2010; **18**(20): 21293-307.
16. Ko F, Foster PJ, Strouthidis NG, et al. Associations with Retinal Pigment Epithelium Thickness Measures in a Large Cohort: Results from the UK Biobank. *Ophthalmology* 2017; **124**(1): 105-17.
17. Patel PJ, Foster PJ, Grossi CM, et al. Spectral-Domain Optical Coherence Tomography Imaging in 67 321 Adults: Associations with Macular Thickness in the UK Biobank Study. *Ophthalmology* 2016; **123**(4): 829-40.

18. Khawaja AP, Chua S, Hysi PG, et al. Comparison of Associations with Different Macular Inner Retinal Thickness Parameters in a Large Cohort: The UK Biobank. *Ophthalmology* 2019.
19. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular degeneration. *Ophthalmology* 2013; **120**(4): 844-51.
20. Aung N, Sanghvi Mihir M, Zemrak F, et al. Association Between Ambient Air Pollution and Cardiac Morpho-Functional Phenotypes. *Circulation* 2018; **138**(20): 2175-86.
21. Eeftens M, Beelen R, de Hoogh K, et al. Development of Land Use Regression models for PM(2.5), PM(2.5) absorbance, PM(10) and PM(coarse) in 20 European study areas; results of the ESCAPE project. *Environmental science & technology* 2012; **46**(20): 11195-205.
22. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. *Neurology* 2016; **86**(24): 2303-9.
23. Vujosevic S, Midena E. Retinal layers changes in human preclinical and early clinical diabetic retinopathy support early retinal neuronal and Muller cells alterations. *Journal of diabetes research* 2013; **2013**: 905058.
24. Hyman L, Schachat AP, He Q, Leske MC. Hypertension, cardiovascular disease, and age-related macular degeneration. Age-Related Macular Degeneration Risk Factors Study Group. *Archives of ophthalmology (Chicago, Ill : 1960)* 2000; **118**(3): 351-8.
25. Ibald-Mulli A, Stieber J, Wichmann HE, Koenig W, Peters A. Effects of air pollution on blood pressure: a population-based approach. *Am J Public Health* 2001; **91**(4): 571-7.
26. Wilson WE, Suh HH. Fine particles and coarse particles: concentration relationships relevant to epidemiologic studies. *Journal of the Air & Waste Management Association (1995)* 1997; **47**(12): 1238-49.
27. Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K. Size-dependent proinflammatory effects of ultrafine polystyrene particles: a role for surface area and oxidative stress in the enhanced activity of ultrafines. *Toxicology and applied pharmacology* 2001; **175**(3): 191-9.
28. Brook RD, Rajagopalan S, Pope CA, 3rd, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation* 2010; **121**(21): 2331-78.
29. Gaffin JM, Hauptman M, Petty CR, et al. Nitrogen dioxide exposure in school classrooms of inner-city children with asthma. *Journal of Allergy and Clinical Immunology* 2018; **141**(6): 2249-55.e2.
30. World Health Organization. Air quality guidelines—global update 2005. http://www.who.int/phe/health_topics/outdoorair/outdoorair_aqg/en/ (accessed 20 Feburary 2019).
31. Icopal. Nitrogen Oxide (NOx) Pollution. http://www.icopal-noxite.co.uk/nox-problem/nox-pollution.aspx (accessed 15 April 2020).
32. Jiang S, Moriarty-Craige SE, Orr M, Cai J, Sternberg P, Jr, Jones DP. Oxidant-Induced Apoptosis in Human Retinal Pigment Epithelial Cells: Dependence on Extracellular Redox State. *Investigative Ophthalmology & Visual Science* 2005; **46**(3): 1054-61.
33. Sarks JP, Sarks SH, Killingsworth MC. Evolution of geographic atrophy of the retinal pigment epithelium. *Eye (Lond)* 1988; **2 (Pt 5)**: 552-77.
34. Johnson PT, Brown MN, Pulliam BC, Anderson DH, Johnson LV. Synaptic Pathology, Altered Gene Expression, and Degeneration in Photoreceptors Impacted by Drusen. *Investigative Ophthalmology & Visual Science* 2005; **46**(12): 4788-95.

35. Wilson JD, Bigelow CE, Calkins DJ, Foster TH. Light scattering from intact cells reports oxidative-stress-induced mitochondrial swelling. *Biophysical journal* 2005; **88**(4): 2929-38.
36. Rodriguez-Padilla JA, Hedges TR, 3rd, Monson B, et al. High-speed ultra-high-resolution optical coherence tomography findings in hydroxychloroquine retinopathy. *Archives of ophthalmology (Chicago, Ill : 1960)* 2007; **125**(6): 775-80.
37. Schuman SG, Koreishi AF, Farsiu S, Jung SH, Izatt JA, Toth CA. Photoreceptor layer thinning over drusen in eyes with age-related macular degeneration imaged in vivo with spectral-domain optical coherence tomography. *Ophthalmology* 2009; **116**(3): 488-96.e2.
38. Repace JL, Lowrey AH. Indoor air pollution, tobacco smoke, and public health. *Science (New York, NY)* 1980; **208**(4443): 464-72.
39. Tomany SC, Wang JJ, van Leeuwen R, et al. Risk factors for incident age-related macular degeneration: Pooled findings from 3 continents. *Ophthalmology* 2004; **111**(7): 1280-7.
40. Owen CG, Jarrar Z, Wormald R, Cook DG, Fletcher AE, Rudnicka AR. The estimated prevalence and incidence of late stage age related macular degeneration in the UK. *Br J Ophthalmol* 2012; **96**(5): 752-6.
41. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular Degeneration in Europe: The Past and the Future. *Ophthalmology* 2017; **124**(12): 1753-63.
42. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. *Am J Epidemiol* 2017; **186**(9): 1026-34.
43. Chua SYL, Khawaja AP, Morgan J, et al. The Relationship Between Ambient Atmospheric Fine Particulate Matter (PM2.5) and Glaucoma in a Large Community Cohort. *Investigative Ophthalmology & Visual Science* 2019; **60**(14): 4915-23.
44. Nwanaji-Enwerem JC, Wang W, Nwanaji-Enwerem O, et al. Association of Long-term Ambient Black Carbon Exposure and Oxidative Stress Allelic Variants With Intraocular Pressure in Older Men. *JAMA Ophthalmol* 2019; **137**(2): 129-37.
45. Choi JK, Lym YL, Moon JW, Shin HJ, Cho B. Diabetes Mellitus and Early Age-related Macular Degeneration. *Archives of Ophthalmology* 2011; **129**(2): 196-9.