Application of a deep-learning marker for morbidity and mortality prediction derived from retinal photographs: a cohort development and validation study

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Summary

Background Biological ageing markers are useful to risk stratify morbidity and mortality more precisely than chronological age. In this study, we aimed to develop a novel deep-learning-based biological ageing marker (referred to as RetiPhenoAge hereafter) using retinal images and PhenoAge, a composite biomarker of phenotypic age.

Methods We used retinal photographs from the UK Biobank dataset to train a deep-learning algorithm to predict the composite score of PhenoAge. We used a deep convolutional neural network architecture with multiple layers to develop our deep-learning-based biological ageing marker, as RetiPhenoAge, with the aim of identifying patterns and features in the retina associated with variations of blood biomarkers related to renal, immune, liver functions, inflammation, and energy metabolism, and chronological age. We determined the performance of this biological ageing marker for the prediction of morbidity (cardiovascular disease and cancer events) and mortality (all-cause, cardiovascular disease, and cancer) in three independent cohorts (UK Biobank, the Singapore Epidemiology of Eye Diseases [SEED], and the Age-Related Eye Disease Study [AREDS] from the USA). We also compared the performance of RetiPhenoAge with two other known ageing biomarkers (hand grip strength and adjusted leukocyte telomere length) and one lifestyle factor (physical activity) for risk stratification of mortality and morbidity. We explored the underlying biology of RetiPhenoAge by assessing its associations with different systemic characteristics (eg, diabetes or hypertension) and blood metabolite levels. We also did a genome-wide association study to identify genetic variants associated with RetiPhenoAge, followed by expression quantitative trait loci mapping, a gene-based analysis, and a gene-set analysis. Cox proportional hazards models were used to estimate the hazard ratios (HRs) and corresponding 95% CIs for the associations between RetiPhenoAge and the different morbidity and mortality outcomes.

Findings Retinal photographs for 34 061 UK Biobank participants were used to train the model, and data for 9429 participants from the SEED cohort and for 3986 participants from the AREDS cohort were included in the study. RetiPhenoAge was associated with all-cause mortality (HR 1·92 [95% CI 1·42–2·61]), cardiovascular disease mortality (1·97 [1·02–3·82]), cancer mortality (2·07 [1·29–3·33]), and cardiovascular disease events (1·70 [1·17–2·47]), independent of PhenoAge and other possible confounders. Similar findings were found in the two independent cohorts (HR 1·67 [1·21–2·31] for cardiovascular disease mortality in SEED and 2·07 [1·10–3·92] in AREDS). RetiPhenoAge had stronger associations with mortality and morbidity than did hand grip strength, telomere length, and physical activity. We identified two genetic variants that were significantly associated with RetiPhenoAge (single nucleotide polymorphisms rs3791224 and rs8001273), and were linked to expression quantitative trait locis in various tissues, including the heart, kidneys, and the brain.

Interpretation Our new deep-learning-derived biological ageing marker is a robust predictor of mortality and morbidity outcomes and could be used as a novel non-invasive method to measure ageing.

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Introduction

Globally, the number of people aged 80 years or older is projected to reach 425 million by 2050,[1] which is likely to result in an increased prevalence of several diseases, including cardiovascular and other chronic diseases.[2–4] Identifying robust ageing biomarkers for disease risk stratification could allow early implementation of health interventions to reduce the burden of these diseases. In this context, the concept of biological age is useful for examining differences in ageing rates among individuals and to identify the physiological changes associated with the ageing process.

Different measurements have been used to estimate biological ageing, including clinical and serum-based

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Research in context

Evidence before this study

Biological ageing markers are useful to risk stratify morbidity and mortality more precisely than chronological age. We searched PubMed and Google Scholar from database inception to Sept 30, 2023, for research articles written in English using the search terms: "biological age" and "risk stratification" and "mortality" or "morbidity". Different measurements have been used to estimate biological ageing such as serum-based biomarkers. However, data collection methods are usually relatively invasive and samples require laboratory processing, thus limiting their clinical application.

Added value of this study

We developed and validated a novel marker of biological ageing, RetiPhenoAge, which was developed and trained on retinal photographs using a deep learning algorithm with composite clinical phenotypic information. We showed that RetiPhenoAge is strongly associated with several mortality and morbidity outcomes, including cardiovascular disease and renal outcomes, independent of confounders. We found similar findings in independent cohorts from Singapore and the USA. We also showed that RetiPhenoAge had stronger associations with mortality and morbidity than hand grip strength, telomere length, and physical activity. Compared with most previous biological ageing markers, this novel marker is non-invasive and does not require any laboratory processing.

Implications of all the available evidence

RetiPhenoAge is a useful marker that could be used for additional screening opportunities with the purpose of identifying people with physiological alterations that could lead to increased risk of mortality and morbidity. Providing early recommendations in the context of ageing populations could have major public health benefits.

biomarkers.[5–8] PhenoAge is an example of a biological ageing marker that combines different measures including albumin, glucose, C-reactive protein, and chronological age.[9] PhenoAge has been shown to predict differences in the risk of all-cause mortality, cause-specific mortality, physical functioning, and cognitive performance measures among individuals with the same chronological age. This biological ageing marker can thus be used to approximate individual differences in biological ageing. However, PhenoAge and other similar markers are based on the measurement of blood parameters, which is relatively invasive and requires laboratory processing, thus limiting their clinical application.

See Online for appendix

The retina of the eye is amenable to non-invasive imaging and rapid assessment with digital photography and provides direct, non-invasive visualisation of the neural tissue and microvasculature. For example, changes in retinal blood vasculature might reflect a range of subclinical pathophysiological responses to hyperglycaemia and inflammation,[10] and thus these changes are associated with increased risk of chronic and age-related diseases.[11,12] The geometrical complexity of the retinal vasculature and the vessel calibres are strongly affected by age.[13] The retina also provides information about neuronal structures, and changes in nerve fibre layers are associated with other ageing disorders including Alzheimer's disease.[14]

We hypothesised that ageing-associated features (ie, neural tissue and microvasculature) visualised in the retina could facilitate the development of a composite non-invasive biological ageing marker. We therefore developed a retinal photograph-based deep-learning algorithm to estimate biological ageing based on PhenoAge measures. First, we aimed to determine whether the performance of this new biological ageing marker (referred to as RetiPhenoAge hereafter) can robustly stratify risks for mortality (all-cause, cardiovascular disease, and cancer) and disease events (cardiovascular disease and cancer) independent of chronological age, in three independent cohorts across three continents. Second, we aimed to compare the performance of this marker with the performance of other known ageing biomarkers, such as hand grip strength and telomere length. Third, we explored the underlying biology of RetiPhenoAge by assessing its associations with different systemic characteristics, blood metabolite levels, and genetic variants.

Methods

Study design

Full details of the study design are available in the appendix (pp 1–8). Briefly, we first trained a deep-learning algorithm to predict the composite score of PhenoAge, based on retinal photographs taken without pupil dilation, using data from the UK Biobank.[15] All UK Biobank participants with gradable retinal photographs without missing data on mortality, morbidity, or any covariate used in the analyses were included. The composite score of PhenoAge was built with nine blood biomarkers plus chronological age.[9] We chose PhenoAge as the ground truth for our deep-learning model because PhenoAge is well-validated in several populations, including Australia,[16] the UK,[17] and the USA,[18,19] and the ten variables required for the model equation are available in UK Biobank data, making the calculation feasible. We used a visual geometry group, a deep convolutional neural network architecture with multiple layers that is widely used for image recognition,[20] to develop our deep-learning-based ageing marker, referred to as RetiPhenoAge hereafter. We aimed to capture patterns and features in the retina associated with variations of blood biomarkers related to renal, immune, liver functions, inflammation, and energy metabolism, and chronological age using retinal photographs. Retinal photographs, morbidity and mortality data, and covariate data were also obtained from two independent cohorts: the Singapore Epidemiology of Eye

Diseases (SEED) study[21] (Singapore) and the Age-Related Eye Disease Study (AREDS; USA)[22] for the comparison of associations of RetiPhenoAge with mortality and morbidity. All SEED and AREDS participants with gradable retinal photographs without missing data on mortality, morbidity, or any covariate used in the analyses were included.

Informed, written consent was obtained from all the participants who participated in the SEED study, and ethical approval for SEED was obtained from the Institutional Review Board of SingHealth. Written informed consent and ethical approval were obtained from all the participants of the UK Biobank study and AREDS.

Procedures

Retinal photographs and blood samples were collected from UK Biobank participants at the baseline visit between 2009 and 2010. Morbidity and mortality outcomes were obtained from National Health Service registries up to March 18, 2020. Mortality (all-cause, cardiovascular disease, and cancer-related) and disease events (cardiovascular disease and cancer) were defined by ICD-10 codes (appendix p 2). For morbidity analyses, participants who reported having cancer or cardiovascular disease at baseline were excluded (appendix pp 2–3) to allow the calculation of the disease incidences.

All outcomes from the SEED[21] cohort were available up to Dec 31, 2019, from the National Registry of Disease office with mortality and disease events (including end-stage renal diseases [ESRD]) defined by ICD-10 codes. Outcomes from the AREDS[21] cohort were available from May 26, 1992, to Oct 20, 2005, with mortality and disease events defined by ICD-10 codes (appendix pp 3–4). In both SEED and AREDS, for morbidity analyses, participants who reported having cancer, cardiovascular diseases, or ESRD at baseline were excluded.

Furthermore, we compared the performance of RetiPhenoAge and two well-known biomarkers (hand grip strength [measured by hand grip dynamometer] and leukocyte telomere length [adjusted for the influence of technical parameters[23]]) and one lifestyle factor (physical activity [defined as minutes per week participants spent walking, and participating in moderate or vigorous activity] measured using International Physical Activity Questionnaire-Short Form[24]) for risk stratification of mortality and morbidity for the UK Biobank data (appendix p 4). Then, we explored the underlying biology of RetiPhenoAge by assessing its associations with different systemic characteristics such as diabetes and hypertension, and blood metabolite levels. We also compared the performance of RetiPhenoAge in risk-stratifying mortality and morbidity with the performance of a previous version of the biomarker, RetiAge (appendix pp 4–5).[25] We also did a genome-wide association study (GWAS), an expression quantitative trait loci mapping, a gene-based analysis, and a gene-set analysis to identify genetic variants associated with RetiPhenoAge and explore possible biological mechanisms underlying RetiPhenoAge (appendix p 5).

Statistical analysis

We assessed the correlation between RetiPhenoAge (predicted) and PhenoAge (observed) using Pearson's correlation coefficients. We used Kaplan–Meier methods to evaluate time to death and time to disease events across the quartiles of RetiPhenoAge (quartiles were built based on RetiPhenoAge to create four groups of equal number of participants based on RetiPhenoAge score). Cox proportional hazards models were used to estimate the hazard ratios (HRs) and corresponding 95% CIs for the associations between RetiPhenoAge and the different outcomes. To assess the ability of RetiPhenoAge for the risk stratification of mortality and morbidity, four models were used: model 1, no adjustment; model 2, adjusted for PhenoAge; model 3, adjusted for chronological age and sex; and model 4, adjusted for PhenoAge, chronological age, sex, BMI, systolic blood pressure, HDL cholesterol, LDL cholesterol, hypertension, diabetes, smoking, and ethnicity. In these models, RetiPhenoAge scores were grouped into quartiles from lowest to highest (quartile 1, RetiPhenoAge score 0·000–0·064; quartile 2, 0·064–0·334; quartile 3, 0·334–0·659; quartile 4, 0·659–0·997). We also considered RetiPhenoAge as a continuous variable in a sensitivity analysis. We tested if the proportional hazards assumption was met by visual inspection and by testing the Schoenfeld's residuals. We did other analyses to (1) compare the performance of RetiphenoAge with other biomarkers, (2) assess the associations between RetiPhenoAge and systemic characteristics and blood metabolite levels, and (3) to compare the performance of RetiphenoAge with our previously developed ageing marker RetiAge; full statistical methods are in the appendix (pp 5–6). p values of less than 0·05 were considered to indicate a statistically significant difference. The deep-learning model was coded and run on Python (version 3.8.18) and all statistical analyses were conducted using R (version 4.3.1).

To investigate whether the performance of RetiPhenoAge for predicting mortality and morbidity was affected by age-related and non-age-related diseases, we did several sensitivity analyses. In the SEED cohort, the performance of RetiPhenoAge was estimated in individuals with and in individuals without cataract (defined using the Wisconsin grading system[26]), age-related macular degeneration (defined using a simplified Beckman grading system[27]), and high myopia (defined as a spherical equivalent of ≤0·50 dioptres). In the UK Biobank cohort, the performance of RetiPhenoAge was estimated according to high myopia status (defined as a spherical equivalent of ≤0·50 dioptres). To investigate the ability of RetiPhenoAge to predict the risks of ocular age-related diseases, in the SEED cohort, we estimated the association of RetiPhenoAge with two outcomes: age-related cataract (defined using the Wisconsin grading system) and visual impairment (using the WHO

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definition:[28] visual acuity worse than 6/12 [20/40] in the better eye with the best possible correction).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Retinal photographs for 34 061 UK Biobank participants were included in the testing dataset (mean age 56·9 years [SD 8·3]; 18 322 [53·8%] females and 15 739 [46·2%] males; table 1). During the 10-year follow-up period, 1310 (3·8%) of the 34 061 participants died from all causes, 356 (1·0%) died from cardiovascular disease, and 545 (1·6%) died from cancer. Among the participants free of cardiovascular disease (n=30 024) or cancer events (n=30 277) before the beginning of the follow-up, 736 (2·5%) had developed cardiovascular disease and 5190 (17·1%) had cancer events during the follow-up. The correlation between RetiPhenoAge (predicted) and PhenoAge (observed) was strong with a Pearson's correlation coefficient of 0·71 (mean absolute error 5·52 [95% CI 5·49–5·56]; root mean squared error 7·28 [7·20–7·35]; appendix p 25).

We included data for 9429 participants from the SEED cohort. The mean age of participants was 58·6 years (SD 10·3) and 4753 (50·4%) of 9429 participants were female and 4676 (49·6%) were male (appendix p 9). During follow-up, 1778 (18·9%) of 9429 participants died from any cause and 574 (6·1%) died from cardiovascular disease related causes. Among the participants free of disease events at the beginning of the follow-up, 467 (5·1%) of 9184 participants had a stroke, 714 (8·1%) of 8794 had a myocardial infarction, 834 (9·2%) of 9077 participants had cancer, and 159 (1·7%) of 9405 participants had (ESRD; data were not available for AREDS participants).

We included data for 3950 participants from the AREDS cohort (34 individuals died before the beginning of the follow-up and were thus excluded from 3984 individuals initially selected). The mean age of participants was 69·4 years (SD 5·1) and 2174 (55·0%) were female and 1776 (45·0%) were male (appendix p 9). During follow-up, 235 (5·9%) participants died from any cause and 97 (2·5%) of 3950 participants died from cardiovascular disease related causes. Among the participants free of disease events at the beginning of the follow-up (cardiovascular disease or cancer), 282 (8·0%) of 3541 participants had a cardiovascular disease event, and 346 (10·6%) of 3250 patients had cancer.

In the unadjusted model (model 1), the magnitudes of effect for all outcomes were high. For example, the risk of cardiovascular disease mortality was 15 times higher in RetiPhenoAge quartile 4 than RetiPhenoAge quartile 1 (HR 15·4 [95% CI 9·1–26·0]; table 2, figure 1). The magnitudes of effect decreased after adjustment for PhenoAge (model 2; eg, for cardiovascular disease mortality the HR for quartile 4 was 4·27 [2·47–7·36] compared with quartile 1); however, all the HRs corresponding to RetiPhenoAge quartile 4 remained significant, with the exception of those for cancer events. In the fully adjusted model (model 4; adjusted for PhenoAge, chronological age, sex, BMI, systolic blood pressure, HDL cholesterol, LDL cholesterol, hypertension, diabetes, smoking, and ethnicity), all the HRs corresponding to RetiPhenoAge quartile 4 were significant, with the exception of those for cancer events. The highest magnitude of effect for the fully adjusted model (model 4) was for cancer mortality (HR 2·07 [95% CI 1·29–3·33]). The results when considering RetiPhenoAge as a continuous variable were similar (appendix p 10). The sensitivity analysis by high myopia status showed that the magnitudes of effect were similar in individuals with high myopia and the whole set of UK Biobank participants with an albeit non-significant effect for cardiovascular events in individuals with high myopia, indicating that myopia did not affect the performance of RetiphenoAge (appendix p 11). Finally, to assess the proportional hazard assumption, we plotted the estimates of the time-dependent coefficients of the survival models, adjusted for age and sex, for the five outcomes, and tested the Schoenfeld's residuals (appendix p 26). All

	UK BioBank population (n=34 061)
Age, years	56·9 (8·3)
Sex	
Female	18 322 (53·8%)
Male	15 739 (46·2%)
Albumin, g/L	45·7 (2·6)
Creatinine, µmol/L	73·2 (17·5)
Glucose, mmol/L	5·1 (1·0)
C reactive protein, mg/L	2·4 (4·0)
Lymphocytes, %	29·3% (7·6)
Mean corpuscular volume, fL	91·9 (4·5)
Red blood cell distribution width, %	13·5% (0·9)
Alkaline phosphatase, U/L	83·3 (24·9)
White blood cell count, × 10^9 cells per L	6·977 (2·0)
PhenoAGE	50·973 (10·0)
RetiPhenoAge	50·436 (6·7)
RetiAGE	0·339 (0·3)
Mortality outcome (n=34 061)	
Follow-up, years	9·9 (9·8–10·1)
All-cause mortality	1310 (3·8%)
Cardiovascular disease mortality	356 (1·0%)
Cancer mortality	545 (1·6%)
Cancer event outcome (n=30 277)*	
Follow-up, years	9·9 (9·7–10·0)
Cancer events	5190 (17·1%)
Cardiovascular disease event outcome (n=30 024)*	
Follow-up, years	9·8 (9·7–10·0)
Cardiovascular disease events	736 (2·5%)

Data are mean (SD), n (%), or median (IQR). *Individuals who had an event before the beginning of follow-up were excluded from the analysis.

Table 1: **Characteristics of the UK Biobank study population**

	N (N cases) n/N	Model 1			Model 2			Model 3			Model 4		
		HR (95% CI)	p value	p_{trend}	HR (95% CI)	p value	p_{trend}	HR (95% CI)	p value	p_{trend}	HR (95% CI)	p value	p_{trend}
All-cause mortality				<0·0001			<0·0001			<0·0001			<0·0001
Q1	88/8834	1 (ref)			1 (ref)			1 (ref)			1 (ref)		
Q2	221/8629	2·67 (2·08–3·42)	<0·0001		1·65 (1·29–2·12)	<0·0001		1·44 (1·1–1·89)	0·0077		1·38 (1·05–1·81)	0·0216	
Q3	373/8488	4·67 (3·7–5·89)	<0·0001		2·07 (1·62–2·63)	<0·0001		1·72 (1·29–2·29)	0·0002		1·53 (1·14–2·03)	0·0042	
Q4	628/8110	8·51 (6·81–10·64)	<0·0001		2·9 (2·28–3·68)	<0·0001		2·4 (1·77–3·24)	<0·0001		1·92 (1·42–2·61)	<0·0001	
Cardiovascular disease mortality				<0·0001			<0·0001			<0·0001			0·0248
Q1	15/8834	1 (ref)			1 (ref)			1 (ref)			1·0 [Reference]		
Q2	53/8629	3·77 (2·12–6·69)	<0·0001		2·15 (1·21–3·83)	0·0089		1·7 (0·92–3·14)	0·0890		1·52 (0·82–2·8)	0·1812	
Q3	96/8488	7·09 (4·11–12·21)	<0·0001		2·73 (1·57–4·74)	0·0004		1·94 (1·03–3·66)	0·0409		1·51 (0·8–2·86)	0·2034	
Q4	192/8110	15·38 (9·09–26·01)	<0·0001		4·27 (2·47–7·36)	<0·0001		2·9 (1·5–5·6)	0·0015		1·97 (1·02–3·82)	0·0440	
Cancer mortality				<0·0001			<0·0001			0·0001			0·0093
Q1	35/8834	1 (ref)			1 (ref)			1 (ref)			1 (ref)		
Q2	102/8629	3·0 (2·04–4·4)	<0·0001		1·89 (1·28–2·78)	0·0013		1·71 (1·13–2·6)	0·0115		1·66 (1·09–2·52)	0·0176	
Q3	159/8488	4·77 (3·31–6·88)	<0·0001		2·17 (1·49–3·18)	<0·0001		1·93 (1·24–3·02)	0·0037		1·73 (1·11–2·72)	0·0160	
Q4	249/8110	7·91 (5·56–11·27)	<0·0001		2·78 (1·9–4·06)	<0·0001		2·52 (1·58–4·04)	0·0001		2·07 (1·29–3·33)	0·0027	
Cardiovascular disease events													
Q1	69/8351	1 (ref)		<0·0001	1 (ref)		<0·0001	1 (ref)		0·0002	1 (ref)		0·0099
Q2	159/7800	2·57 (1·94–3·41)	<0·0001		1·72 (1·29–2·29)	0·0002		1·55 (1·13–2·12)	0·0062		1·42 (1·04–1·95)	0·0288	
Q3	200/7296	3·53 (2·69–4·64)	<0·0001		1·79 (1·34–2·39)	<0·0001		1·52 (1·07–2·15)	0·0189		1·32 (0·93–1·87)	0·1217	
Q4	308/6577	6·26 (4·82–8·13)	<0·0001		2·57 (1·92–3·44)	<0·0001		2·08 (1·43–3·01)	0·0001		1·70 (1·17–2·47)	0·0053	
Cancer events				<0·0001			<0·0001			0·0614			0·5888
Q1	917/8140	1 (ref)			1 (ref)			1 (ref)			1 (ref)		
Q2	1189/7713	1·4 (1·29–1·53)	<0·0001		1·07 (0·97–1·17)	0·1598		0·99 (0·89–1·09)	0·8110		0·98 (0·89–1·08)	0·6675	
Q3	1477/7417	1·86 (1·72–2·02)	<0·0001		1·18 (1·07–1·3)	0·0008		1·06 (0·95–1·19)	0·3120		1·02 (0·91–1·15)	0·6927	
Q4	1607/7007	2·2 (2·03–2·39)	<0·0001		1·21 (1·1–1·35)	0·0002		1·09 (0·96–1·24)	0·1870		1·02 (0·89–1·15)	0·8156	

Model 1, unadjusted; model 2, adjusted for PhenoAge (coded as continuous); model 3, adjusted for chronological age and sex; model 4, adjusted for PhenoAge (coded as continuous), chronological age, sex, BMI, systolic blood pressure, HDL cholesterol, LDL cholesterol, hypertension, diabetes, smoking status, ethnicity. RetiPhenoAge quartiles: Q1, RetiPhenoAge score 0·000–0·064; Q2, 0·064–0·334; Q3, 0·334–0·659; Q4, 0·659–0·997. HR=hazard ratio. Q=quartile.

Table 2: **Risk of mortality and morbidity associated with RetiPhenoAge by quartile in the UK Biobank study**

Schoenfeld's residuals were visually independent of time and were non-significant, indicating that the assumption was met for all outcomes.

To localise the retinal features contributing to RetiPhenoAge, we generated saliency maps (appendix p 27), which indicated that RetiPhenoAge commonly focuses on the macula, optic disc, and retinal vessels.

In the SEED cohort, findings were similar: all HRs corresponding to RetiPhenoAge quartile 4 in the model adjusted for chronological age and sex were significant, with the exception of those for cancer events (appendix p 12). PhenoAge could not be calculated in SEED because some variables were not available. The highest HR corresponded to ESRD (HR 4·15 [95% CI 2·20–7·85]). When fully adjusted (model 3), RetiPhenoAge quartile 4 was not significant for all outcomes, however, the risks of myocardial infarction (p_{trend}=0·040) and ESRD events (p_{trend}=0·046) were increased for each quartile increase in RetiPhenoAge. The sensitivity analysis of cataract status showed that the magnitudes of effect were decreased in individuals with cataract when compared with the whole set of SEED participants, except for cancer events (appendix pp 13–14). However, in individuals with cataract, increase in RetiPhenoAge SD was significantly associated with increased risks of cardiovascular disease mortality (p=0·010), stroke events (p=0·016), myocardial infarction (p=0·038), and ESRD (p=0·003) (appendix pp 13–14). The findings of the sensitivity analyses by age-related macular degeneration (appendix pp 15–16) and high myopia status (appendix p 17) were similar; in individuals with age-related macular degeneration, RetiPhenoAge (per SD increase) was associated with cardiovascular mortality (p=0·041), stroke events (p=0·005), myocardial infarction (p=0·053), and ESRD (p=0·002). In individuals with high myopia, RetiPhenoAge per SD increase was associated with cardiovascular mortality (p=0·007) and myocardial infarction (p=0·001; appendix pp 13–16). Furthermore, RetiPhenoAge was associated with age-related cataract and visual impairment. For each 1-SD increase in RetiPhenoAge, the sex and age-adjusted odds ratios were 1·93 (95% CI 1·80–2·07; p<0·0001) for age-related cataract and 1·27 (1·21–1·34; p<0·0001) for visual impairment (appendix p 28). In the AREDS cohort, in model 3, the risk of cardiovascular disease mortality was almost two times higher in RetiPhenoAge quartile 4 than RetiPhenoAge quartile 1 (HR 1·92 [95% CI 1·01–3·64]; appendix p 18). The risk of cardiovascular events

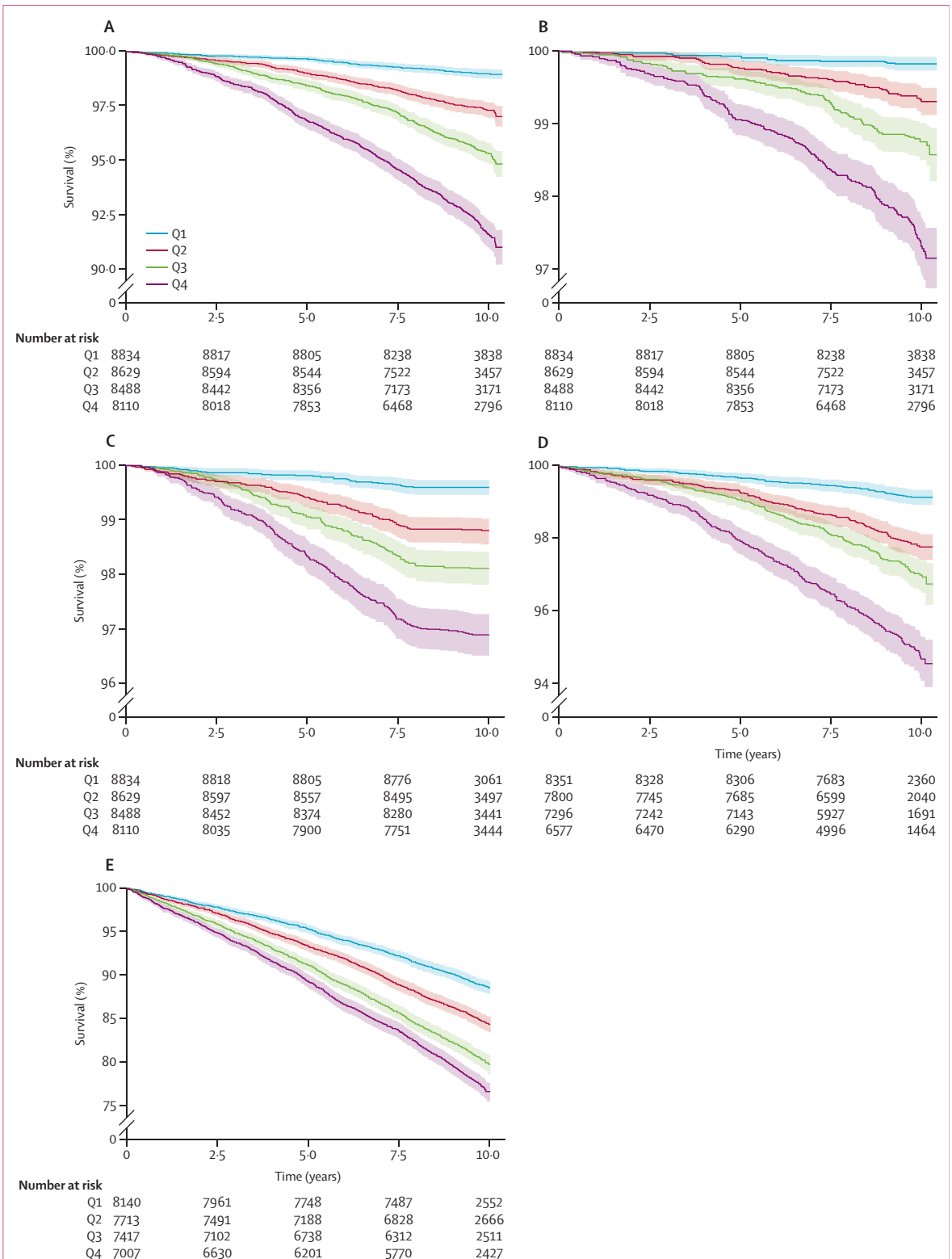

Figure 1: **Kaplan–Meier estimates of mortality and morbidity events by RetiPhenoAge quartiles in UK Biobank participants**
(A) All-cause mortality. (B) Cardiovascular disease mortality. (C) Cancer mortality. (D) Cardiovascular disease events. (E) Cancer events. Shading indicates 95% CIs. Q=quartile.

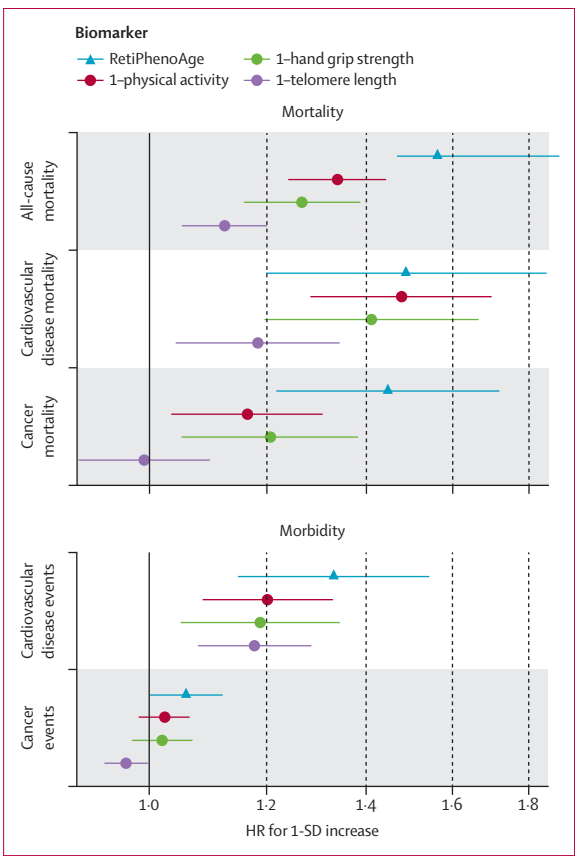

Figure 2: **Risk of mortality and morbidity associated with RetiPhenoAge, right hand grip strength, telomere length, and physical activity in UK Biobank participants**

All biomarkers were considered continuous. Their distributions were log transformed and scaled to enable comparison. HRs were considered for an increase in 1 SD in RetiPhenoAge. All models were adjusted for chronological age and sex. For right hand grip strength, telomere length, and physical activity, we inversed the distribution (1 – the actual value) to obtain the same direction of effect for that of RetiPhenoAge (increased values thus correspond to increased risk). The numbers at the right side of the plot correspond to the p values for the difference between the HRs of right-hand grip strength, telomere length, and physical activity, versus the HR of RetiPhenoAge (one-sided Z-tests). HR=hazard ratio.

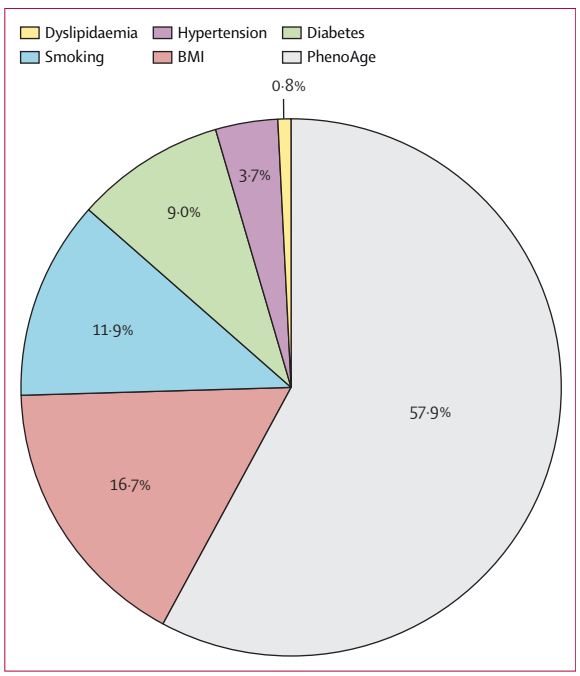

Figure 3: **Associations between RetiPhenoAge and different systemic and metabolic characteristics in UK Biobank participants**

The pie chart represents the effects estimated over the range of possible values, expressed as percentages. Effects were estimated using a linear regression model adjusted for chronological age (appendix p 19).

was increased for each quartile increase in RetiPhenoAge (p_{trend}=0·015).

RetiPhenoAge had stronger associations (absolute magnitude of effect), with all five outcomes than did physical activity, telomere length, and hand grip strength (figure 2). For all five outcomes, HRs of RetiPhenoAge were higher than HRs of hand grip strength, telomere length, and physical activity. For all-cause mortality, the HR was 1·49 (95% CI 1·34–1·67) for each 1-SD increase in RetiPhenoAge, 1·21 (1·11–1·33; p=0·013) for each 1-SD increase in hand grip strength, 1·08 (1·01–1·15; p=0·002) for each 1-SD increase in telomere length, and 1·28 (1·19–1·38; p<0·0001) each 1-SD increase in physical activity (figure 2).

Increases in RetiPhenoAge were positively associated with increased BMI and increased risk of hypertension, diabetes, and smoking (figure 3). In addition to the contribution from PhenoAge (57·9%), we found that the BMI also contributed to 16·7% of variations in RetiPhenoAge, followed by 11·9% for smoking, 9·0% for diabetes, 3·7% for hypertension, and 0·8% for dyslipidaemia (appendix p 19). RetiPhenoAge was positively associated with glucose, lactate, and glycoprotein acetyl concentrations; and negatively associated with concentrations of omega-3 fatty acid, linoleic acid, citrate, cholesterol ester in chylomicrons, and extremely large VLDL (appendix pp 20, 29). After correcting for multiple testing, only the associations with citrate, glucose, and lactate remained significant.

The GWAS performed in White individuals from UK Biobank (n=29 871) identified two genetic loci (single nucleotide polymorphisms [SNPs] rs3791224 and rs8001273) that were associated with RetiPhenoAge at the genome-wide level (appendix pp 21, 30). The summary statistics of the GWAS were not affected by population stratification, with a genome inflation indicator λ of 1·047 (intercept of linkage disequilibrium score regression 1·014). The SNP rs3791224 is located in an intron of the *SH3YL1* gene on chromosome 2 and was linked to three expression quantitative trait locis in various tissues, including the heart, kidneys, and the brain, for the genes *SH3YL1, FAM150B,* and *ACP1* (appendix pp 22–23). These three genes were also identified in gene-based analysis (appendix p 24). The SNP rs8001273 is located in an intergenic region between *LINC01072* and *GJA3* on chromosome 13 and was associated with expression quantitative trait loci of *CRYL1*, specifically in the aorta artery

(appendix pp 22–23). No pathways were identified by the gene-set analysis at the genome-wide level.

Compared with RetiAge, the magnitudes of association for all outcomes, with the exception of cancer events, were higher for RetiPhenoAge, regardless of the adjustment strategy used (appendix p 31). For example, people in RetiPhenoAge quartile 4 (compared to quartile 1) had higher increases in all-cause mortality (HR 2·06 [95% CI 1·52–2·79]) than people in RetiAge quartile 4 (compared to quartile 1; 1·34 [1·09–1·66]), cardiovascular disease mortality (2·23 [1·15–4·33] *vs* 1·62 [1·03–2·54]), and cancer mortality (2·00 [1·36–2·95] *vs* 1·38 [1·06–1·82]), independent of chronological age, sex, BMI, systolic blood pressure, HDL cholesterol, LDL cholesterol, hypertension, diabetes, smoking, or ethnicity.

Discussion

We developed a new retinal ageing marker, RetiPhenoAge, by training a deep-learning model to predict a composite marker of biological ageing based on clinical measures (PhenoAge) using retinal photographs. First, we showed this new non-invasive biological ageing marker (RetiPhenoAge) was strongly associated with all the major ageing outcomes considered, independent of PhenoAge and other confounders. In the UK Biobank cohort, individuals in quartile 4 of RetiPhenoAge had an increased risk of all-cause mortality (HR 1·92 [95% CI 1·42–2·61]), cardiovascular disease mortality (1·97 [1·02–3·82]), cancer mortality (2·07 [1·29–3·33]), and cardiovascular disease events (1·70 [1·17–2·47]) when compared with people in RetiPhenoAge quartile 1. However, RetiPhenoAge did not perform well for cancer events (HR 1·02 [95% CI 0·89–1·15]). We found similar findings in independent cohorts from Singapore and the USA: RetiPhenoAge was associated with all-cause mortality, cardiovascular disease mortality, cardiovascular disease events, and ESRD in SEED; and with cardiovascular disease mortality and cardiovascular disease events in AREDS, independent of chronological age and sex. Second, we found that RetiPhenoAge had stronger associations with mortality and morbidity compared with hand grip strength, telomere length, and physical activity. Third, we identified BMI, smoking status, and diabetes as the most contributing factors to RetiPhenoAge variations. Fourth, RetiPhenoAge was associated with several blood glycolysis related metabolites, including citrate and lactate.

RetiPhenoAge had similar strength of associations with regard to all-cause mortality compared with other ageing measurements such as DNA methylation or accelerated ageing related to oxidative stress.[5] In our study, we further compared RetiPhenoAge with other known ageing biomarkers such as telomere length and hand grip strength. Shorter telomere length has been found to be associated with an increased overall risk of death;[7,29] and hand grip strength is highly predictive of functional limitations and disability.[8] Moreover, physical activity is strongly associated with mortality,[30] therefore we included it in the comparisons. Overall, RetiPhenoAge had stronger associations with mortality and morbidity outcomes than the other biomarkers and lifestyle factors, with 10–30% higher magnitude of effects. The largest differences were found for all-cause and cancer mortality. Furthermore, studies in ageing biomarkers have explored various image modalities, such as lens images,[31] 3D facial imaging,[32] chest x-ray,[33] and brain MRI.[34] However, the majority of these studies have relied on chronological age as the ground truth in their deep-learning models to estimate biological ageing. This approach might overlook nuanced ageing characteristics. Using PhenoAge to estimate biological ageing enabled the mitigation of this important limitation in our study. In contrast to the use of facial age, RetiPhenoAge poses fewer ethical or privacy concerns.

RetiPhenoAge, compared with other biological ageing markers such as DNA methylation or telomere length, is relatively simple and non-invasive. We also showed that RetiPhenoAge had better risk stratification abilities (for all the outcomes with the exception of cancer events) than a previous version of the ageing marker (RetiAge), which was based on estimating chronological age from retinal images. Another similar deep-learning-based retinal marker (retinal age gap), which is also based on estimating chronological age from retinal images, was found to be associated with mortality[35] and other health outcomes.[36] Based on these findings, we believe that RetiPhenoAge could be a useful marker that could be used for additional screening opportunities with the purpose of identifying people with physiological alterations that could lead to increased risk of mortality and morbidity. Such an algorithm would potentially serve as a feasible and scalable risk stratification tool in the community. Furthermore, considering the increasing availability and affordability of retinal cameras and their wide use in community screening programmes, this new algorithm could be potentially widely adopted and integrated into existing screening programmes, without the need for blood collection or time-consuming laboratory processing of samples. Providing early recommendations in the context of ageing population might have major public health benefits.

Furthermore, to investigate the underlying biology of RetiPhenoAge, we determined the associations between RetiPhenoAge and several systemic and metabolic characteristics. To do so, we regressed RetiPhenoAge with these factors while adjusting for PhenoAge (ground truth information used to build RetiPhenoAge). We determined the contributions of the systemic and metabolic characteristics to our retinal marker, after removing the effect PhenoAge. The characteristics that contributed the most were BMI, smoking, and diabetes status. Together these characteristics contributed to around 40% of the variations in RetiPhenoAge, compared with around 60% for PhenoAge. This difference reflects how RetiPhenoAge was predicted by the deep-learning algorithm with clinical biomarkers (PhenoAge) and retinal photographs. It is well established that retinal vasculature contain systemic information and that changes in this vasculature are associated with

diabetes and kidney disease.[13,37,38] This innovative method of combining anatomical (retinal) and clinical information through the deep-learning training enabled the development of a marker associated with a wide range of physiological deteriorations and thus able to discriminate for several causes of mortality and morbidity. RetiPhenoAge was associated with several blood glycolysis related metabolites. RetiPhenoAge was negatively associated with citrate and positively with glucose and lactate concentrations. The associations with glucose were expected because glucose concentrations were used to calculate PhenoAge. Regarding citrate concentrations, there is evidence that citrate can induce inflammatory cytokines before the detection of age-related disease.[39] Moreover, lactate concentrations in the brain are increased in mice during ageing.[40]

We identified two genetic loci associated with RetiPhenoAge. rs8001273 was associated with the expression of *CRYL1* specifically in the aortic artery; and rs3791224 with the expression of *SH3YL1* in many tissues including heart and kidney tissues, and *ACP1* in several tissues including heart tissue. SH3YL1 protein belongs to the SH3 domain-containing family of proteins, which are involved in various cellular processes, including signal transduction, cytoskeletal organisation, and vesicular trafficking. The plasma concentration of this protein has been suggested as a novel biomarker for diabetic nephropathy in patients with type 2 diabetes.[41] Two loci located in *SH3YL1* and *ACP1* genes were associated with a retinal ageing clock.[42] The *CRYL1* gene codes for the crystallin λ 1 protein, which is mainly expressed in the lens but also in other tissues, including the aortic artery.[43] Expression of this protein in the aorta artery might be upregulated in response to pathological conditions to protect against oxidative stress and cellular damage. Furthermore, previous studies have linked *CRYL1* to Alzheimer's disease.[44] It has also been shown that levels of CRYL1 could be a novel prognostic marker of renal cell carcinoma.[45]

Strengths of this study included the utilisation of one large study (UK Biobank) for the development of our deep-learning model, and inclusion of two independent studies (SEED and AREDS) from different continents for the replication of the findings. Although smaller effect sizes were observed in SEED and AREDS, similar trends were observed, indicating the robustness of our results and the possible utility of RetiPhenoAge, regardless of ethnicity. Further studies are needed to confirm the generalisability of results to other populations. Moreover, we compared the stratification ability of our marker with several other known biomarkers. We further investigated the underlying biology by determining the associations with many systemic and metabolic characteristics, including 147 blood metabolites. We found that the performance of RetiPhenoAge in predicting mortality and morbidity in individuals with age-related macular degeneration and high myopia was similar to the performance in the whole population, suggesting that age-related and non-age-related eye diseases might not greatly alter the performance of RetiPhenoAge.

This study also has limitations. First, in the UK Biobank study, cardiovascular disease and cancer status were self-reported at baseline and thus there might be recall bias. Second, RetiPhenoAge was trained and tested using data for participants included in the UK Biobank, which could have led to overfitting issues. However, we believe this did not affect the findings since in the testing dataset we determined the associations between RetiPhenoAge and different morbidity and mortality outcomes (and not the prediction of PhenoAge). Moreover, we found similar trends in two independent datasets. Although our findings need to be further validated, this suggested that our findings might be generalisable to other populations. Third, coefficients estimated from the National Health and Nutrition Examination Survey in the USA were used to calculated PhenoAge in UK Biobank participants. Although this prevented overfitting issues, this could also have introduced a bias in our ground truth estimation. Nevertheless, PhenoAge has been validated in several populations (using the coefficients estimated in the original cohort), including Australia,[16] the UK,[17] and the USA,[17,18] demonstrating its robustness. Fourth, we found that the performance of RetiPhenoAge in predicting mortality and morbidity in individuals with cataract remained significant for most outcomes (cardiovascular disease mortality, stroke events, myocardial infarction, and ESRD), but with a smaller magnitude of effect. We acknowledge that the key determinant in obtaining clear retinal images is the transparency of the refractive media, and thus in eyes with severe cataract, the quality of retinal images would be compromised and retinal photography might fail to capture essential retinal features. Finally, the deep-learning model used to estimate RetiPhenoAge tended to overestimate the predictions for younger participants and underestimate predictions for older participants. However, older individuals are at higher risk of mortality and morbidity. If these individuals had a lower RetiphenoAge score than expected, the associations between RetiPhenoAge and the outcomes for these individuals might thus have been underestimated. Therefore, this conservative bias should not affect our conclusion. Further refinement of deep-learning models is needed to correct for this bias.

In summary, we developed and validated a novel marker of biological ageing, RetiPhenoAge, which was developed and trained on retinal photographs using deep learning algorithm with composite clinical phenotypic information. We showed this new non-invasive biological ageing marker is strongly associated with several mortality and morbidity outcomes, including cardiovascular disease and renal outcomes, independent of confounders. Our validation using two independent large cohorts validation that RetiPhenoAge is a robust predictor of mortality and morbidity outcomes, which could thus provide a novel non-invasive way to measure ageing.

Contributors

SN contributed to the conception and design of the study, the analysis of the data, the interpretation of the results, and the draft of the manuscript. THR and

C-YC contributed to the conception and design of the study, the interpretation of the results, and substantive revisions of the manuscript. HL contributed to the analysis of the data, the interpretation of the results, and substantive revisions of the manuscript. MY contributed to the acquisition of the data and substantive revisions of the manuscript. MD contributed to the analysis of the data, the interpretation of the results, and substantive revisions of the manuscript. TCQ, GL, and CCYC contributed to the analysis of the data, the interpretation of the results, and substantive revisions of the manuscript. QP, CCX, ZZ, EYC, CS, and T-YW contributed to the interpretation of the results and substantively revised the manuscript. Y-CT contributed to the conception and design of the study, the interpretation of the results, and substantively revised the manuscript. All authors have approved the submitted version. SN and MY had full access to and verified all of the data.

Declaration of interests

THR and GL own stocks in MediWhale, to whom RetiAge was licensed. ZZ holds a National Health and Medical Research Council Investigator Grant (2010072) and owns two patents for biological age prediction from ocular images (AU2023903213A0 and CN114782361A). T-YW is a consultant for Aldropika Therapeutics, Bayer, Boehringer Ingelheim, Carl Zeiss, Genentech, Iveric Bio, Novartis, Oxurion, Plano, Roche, Sanofi, and Shanghai Henlius; is an inventor, holds patents, and is a co-founder of the start-up companies EyRiS and Visre; and has interests in, and develops digital solutions for, eye diseases, including diabetic retinopathy, outside of the submitted work. C-YC holds licences and receives consultation fees from MediWhale. All other authors declare no competing interests.

Data sharing

The UK Biobank data were obtained from UK Biobank (application number 45925), and a full list of the gradable photographs and code are available online (https://github.com/medi-whale/UKBIOBANK_FUNDUS_Classifier). For the Singapore Epidemiology of Eye Disease data, since the study involves human participants, the data cannot be made freely available in the manuscript, the supplemental files, or a public repository due to ethical restrictions. Data can be requested from the Singapore Eye Research Institutional Ethics Committee for researchers who meet the criteria for access to confidential data. Interested researchers can send data access requests to seri@seri.com.sg. The Age-Related Eye Disease Study data are publicly available in the Database of Genotypes and Phenotypes and can be obtained on request from https://dbgap.ncbi.nlm.nih.gov/aa/wga.cgi?page=login.

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