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# Artificial intelligence-enabled retinal vasculometry for prediction of circulatory mortality, myocardial infarction and stroke

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## ABSTRACT

**Aims** We examine whether inclusion of artificial intelligence (AI)-enabled retinal vasculometry (RV) improves existing risk algorithms for incident stroke, myocardial infarction (MI) and circulatory mortality.

**Methods** AI-enabled retinal vessel image analysis processed images from 88 052 UK Biobank (UKB) participants (aged 40–69 years at image capture) and 7411 European Prospective Investigation into Cancer (EPIC)-Norfolk participants (aged 48–92). Retinal arteriolar and venular width, tortuosity and area were extracted. Prediction models were developed in UKB using multivariable Cox proportional hazards regression for circulatory mortality, incident stroke and MI, and externally validated in EPIC-Norfolk. Model performance was assessed using optimism adjusted calibration, C-statistics and $R^2$ statistics. Performance of Framingham risk scores (FRS) for incident stroke and incident MI, with addition of RV to FRS, were compared with a simpler model based on RV, age, smoking status and medical history (antihypertensive/cholesterol lowering medication, diabetes, prevalent stroke/MI).

**Results** UKB prognostic models were developed on 65 144 participants (mean age 56.8; median follow-up 7.7 years) and validated in 5862 EPIC-Norfolk participants (67.6, 9.1 years, respectively). Prediction models for circulatory mortality in men and women had optimism adjusted C-statistics and $R^2$ statistics between 0.75–0.77 and 0.33–0.44, respectively. For incident stroke and MI, addition of RV to FRS did not improve model performance in either cohort. However, the simpler RV model performed equally or better than FRS.

**Conclusion** RV offers an alternative predictive biomarker to traditional risk-scores for vascular health, without the need for blood sampling or blood pressure measurement. Further work is needed to examine RV in population screening to triage individuals at high-risk.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Population screening for myocardial infarction (MI) and stroke using risk prediction tools exist but have limited uptake; risk scores for circulator mortality do not exist.

### WHAT THIS STUDY ADDS

⇒ Risk models developed in UK Biobank (validated in European Prospective Investigation into Cancer-Norfolk) using artificial intelligence (AI)-enabled retinal vasculometry (RV), age, history of cardiovascular disease, use of hypertensive medication and smoking yielded high predictive test performance for circulatory mortality.

⇒ Risk scores for MI and stroke performed similarly to established risk scores.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ AI-enabled RV extraction offers a non-invasive prognostic biomarker of vascular health that does not require blood sampling or blood pressure measurement, and potentially has greater community reach to identify individuals at medium-high risk requiring further clinical assessment.

## INTRODUCTION

Circulatory mortality, including cardiovascular disease (CVD), coronary heart disease (CHD), heart failure and stroke, is a major cause of morbidity and mortality worldwide.[1 2] A large number of risk algorithms exist to predict CVD,[3] and the addition of fixed and modifiable risk factor phenotypes have been evaluated, but have so far shown little improvement in CVD prediction.[4–6] Machine learning techniques incorporating 473 potential risk factors for the prediction CVD in the UK Biobank (UKB) cohort yielded areas-under-the-curve (AUC) from receiver operating characteristic curve of 0.774, compared with AUC of 0.724 for Framingham risk scores (FRS).[7] Other CVD risk scores, using different CVD outcome definitions, have already been evaluated in UKB including the European Systemic Coronary Risk Evaluation (SCORE),[8] QRISK3[9] and American College of Cardiology/American Heart Association[10] risk score with C-statistic values of 0.775, 0.739 and 0.736, respectively.[6]

Examination of retinal blood vessels (arterioles and venules) may offer a microvascular phenotype more indicative of the presence of early circulatory related disease processes, providing a non-invasive window on the circulatory system. Narrow retinal arterioles show a clear association with higher blood pressure (BP), hypertension and with incident CVD.[10] Arteriolar vessel width narrowing

and venular widening may be important for mortality, stroke[10] and CHD incidence,[11] but there are inconsistencies in the literature,[12 13] such as retinal vessel associations with CVD risk in women but not in men.[10 14] Other features of retinal vasculometry (RV), such as vessel tortuosity, may offer more discerning markers of vascular status but remain little studied at scale.[15 16] Unfortunately, machine learning approaches do not currently clarify which features of RV are important, although they may do in the future.

We developed a fully automated artificial intelligence(AI)-enabled system (QUantitative Analysis of Retinal vessels Topology and siZe (QUARTZ)) for examining the retinal vascular tree, which overcomes many of the difficulties of earlier approaches, allowing detailed vasculometry quantification in large population studies.[17–19] In the subset of UKB who underwent retinal imaging,[20] and in the European Prospective Investigation into Cancer (EPIC)-Norfolk[15] cohorts, we examine detailed characterisation of RV as a non-invasive maker of vascular health in relation to circulatory mortality prediction. In addition, we provide findings for FRS for stroke,[21] and myocardial infarction (MI)[22] in the same subset that underwent retinal imaging, and assess the incremental value of adding RV to FRS for incident stroke and MI.

## MATERIALS AND METHODS

*UKB* is a prospective cohort study for which baseline biomedical and physical assessments were carried out 2006–2013, in 502 682 adults aged 40–69 years recruited from 22 UK centres.[23] Ocular assessments occurred during the latter phase (2009–2013; seven centres) and included visual acuity, autorefraction, digital fundus photography with the Topcon 3D-OCT 1000 Mark 2.[20] Non-mydriatic 45° digital colour images, centred on the fovea were available for 88 052 participants.

*EPIC-Norfolk* was the UK component of the European Prospective Investigation into Cancer (EPIC) study.[24 25] Here, we focus on data from the third clinical follow-up (2004–2011)[25] on 8603 participants aged 48–92 years who underwent a biomedical and eye examination similar to that of UKB (online supplemental material for further details).[15]

### Health outcomes

The primary outcome was circulatory mortality as defined using International Classification of Diseases (ICD) (ICD-10 codes I00-I99 and ICD-9 390-459) coded death registry data from the Office for National Statistics and the Health and Social Care Information Centre (now NHS Digital) for England and Wales, and the Information Services Department for Scotland, provided information on date and cause(s) of death to 31 January 2018 for UKB and 31 March 2018 for EPIC-Norfolk. Incident MI and stroke events after retinal image capture were based on medical records linkage with hospital diagnoses of non-fatal events, supplemented with participant health and lifestyle questionnaire data from repeat surveys in UKB and EPIC-Norfolk (2012–2018). ICD-10 codes I21-I25 (or ICD-9 codes 410, 411, 412 429.79) were used for fatal and non-fatal MI; and ICD-10 codes I60, 61, 63, 64 (or ICD-9 codes 430, 431, 434, 436) for ischaemic and haemorrhagic stroke (see Algorithmically defined health outcomes at https://www.ukbiobank.ac.uk/enable-your-research/about-our-data/health-related-outcomes-data).

### AI-enabled retinal image processing

A validated, fully automated AI-enabled system (QUARTZ)[17–19] extracted thousands of measures of retinal vessel width, tortuosity and area from the whole retinal image. Supervised machine learning techniques were used within QUARTZ; with a support vector machine used to create an image quality score[17] and deep learning was used to develop an algorithm to distinguish between arterioles and venules.[18] QUARTZ measures of width (μm[26]), total vessel area (mm$^2$), tortuosity (arbitrary units)[15 27] and variance of widths along a vessel segment, were averaged for each image (weighted by the length of each vessel segment), separately for arterioles and venules. Person level averages were obtained by averaging across right and left eyes.

### Statistical analysis

Statistical analyses were carried out using STATA software (V.16, StataCorp LP). Retinal vessel widths and area showed normal distributions, tortuosity required log-transformation and within-vessel-width-variance required inverse square-root transformation to normalise distributions. Models were developed in UKB for men and women separately throughout, and externally validated in EPIC-Norfolk. We hypothesised that retinal vessel characteristics in relation to circulatory mortality might be modified by age, smoking status, presence of CVD/diabetes and use of BP lowering medications. Hence, two-way interactions between RV and age, smoking status and self-reported use of BP medication, prevalent diabetes and CVD were first examined in mutually adjusted Cox proportional hazard[28] models for circulatory mortality. Interaction terms with p values <0.2 were then included along with main effects in Cox regressions models using backward elimination (p value set to 0.1).

Bootstrapping with 100 replications was used for internal validation to adjust model performance measures for optimism, including Harrel's C-statistic for discrimination, $R^2$ statistic (representing a measure of explained variation)[29] and calibration slope (where a slope of 1.0 is ideal).[30] The original beta coefficients were adjusted for shrinkage by multiplying the beta-coefficients by the optimism-adjusted calibration slope (presented online supplemental table S3), applied to the EPIC-Norfolk cohort to estimate C-statistic, $R^2$ and calibration slopes and baseline hazard. Model performance was graphically assessed from plots of the observed probability of event at 5 years by deciles of predicted risk at 5 years in UKB and by octiles in EPIC-Norfolk.

FRS for incident fatal and non-fatal stroke use age, systolic BP, treatment of hypertension, presence of diabetes and smoking status[21] and for MI risk scores additionally include total and high-density lipoprotein (HDL) cholesterol levels,[22] with separate risk equations in men and women; there risk scores were applied to UKB and EPIC-Norfolk cohorts and were recalibrated to the baseline survival function within each cohort according to the 5-year survival rates. Following FRS criteria, participants reporting use of cholesterol lowering medications, diabetes or missing data on total or HDL cholesterol were excluded from all MI analyses.[22] FRS models were also extended to include RV. Alternative models for incident fatal and non-fatal stroke and MI using age, smoking status, medical history (self-reported history of heart attack, stroke or diabetes and use of BP lowering medications) and RV only were developed in UKB following the same approach as for circulatory mortality. A medical history of MI did not preclude inclusion in models for incident stroke events, and vice-versa.

Sensitivity analyses restricted model development and validation to white ethnicity. Using EPIC-Norfolk, external validation was extended to a broader spectrum of incident cerebrovascular disease (ICD-10 I60-69; ICD-9 430-438) and incident ischaemic

**Table 1** Clinical characteristics at baseline eye assessment in UK Biobank (2009–2013) and from the third health check phase for EPIC-Norfolk (2004–2011)

| | Mean (SD) or (%) | |
|---|---|---|
| **Baseline characteristic** | UK Biobank N=**66 326** | EPIC N=5955 |
| Median duration of follow-up (years) | 7.7 | 9.1 |
| Age (years) | 56.8 (8.2) | 67.6 (7.6) |
| Female (%) | 55.0% | 57.1% |
| Ethnicity (%) | | |
| White | 92.0% | 99.5% |
| Black | 2.5% | 0.1% |
| Asian | 2.5% | 0.0% |
| Other | 2.5% | 0.2% |
| Unknown/did not answer | 0.6% | 0.0% |
| Smoking (%) | | |
| Never smoker | 56.7% | 49.7% |
| Occasionally | 2.6% | N/A |
| Ex-smoker | 34.0% | 44.2% |
| Current smoker | 6.1% | 4.5% |
| Prefer not to say/missing | 0.5% | 1.6% |
| BMI (kg/m$^2$) | 27.2 (4.7) | 26.8 (4.3) |
| Systolic BP (mm Hg) | 136.8 (18.3) | 135.7 (16.6) |
| Diastolic BP (mm Hg) | 81.5 (10.0) | 78.5 (9.3) |
| Total cholesterol (mmol/L) | 5.7 (1.1) | 5.4 (1.1) |
| LDL cholesterol (mmol/L) | 3.5 (0.9) | 3.2 (1.0) |
| HDL cholesterol (mmol/L) | 1.5 (0.4) | 1.5 (0.4) |
| Triglycerides (mmol/L) | 1.7 (1.0) | 1.7 (0.9) |
| | UK Biobank | EPIC |
| Image quality* | 0.9 (0.1) | 0.9 (0.1) |
| Arteriolar width (µm) | 86.9 (7.9) | 75.1 (6.4) |
| Venular width (µm) | 103.1 (13.0) | 91.4 (10.8) |
| Arteriolar tortuosity† | 4.4 (1.6) | 4.3 (1.6) |
| Venular tortuosity† | 3.1 (1.4) | 3.2 (1.3) |
| Arteriolar vessel area (mm$^2$) | 1.8 (0.8) | 2.0 (0.7) |
| Venular vessel area (mm$^2$) | 2.5 (0.9) | 2.6 (0.7) |
| Arteriolar segment SD$^{-1}$ (µm$^{-1}$) | 0.08 (0.03) | 0.13 (0.03) |
| Venular segment SD$^{-1}$ (µm$^{-1}$) | 0.10 (0.03) | 0.14 (0.04) |
| Use of medications | | |
| Blood pressure lowering | 19.6% | 35.1% |
| Cholesterol lowering | 17.9% | 22.3% |
| Prefer not to report/missing | 1.2% | N/A |
| Self-reported history | | |
| Heart attack (%) | 1.9% | 3.1% |
| Stroke (%) | 1.4% | 2.0% |
| Prefer not to report/missing | 1.2% | N/A |
| Diabetes (%) | 4.9% | 4.0% |

Values are mean (SD) or (%).
Missing data were BMI n=287, blood pressure n=207 in UK Biobank only. Framingham risk score-based models for incident MI that used lipids missing data were as follows after excluding those with prevalent events (MI or diabetes) or using lipid lowering therapy, n= 6805 for total cholesterol or HDL cholesterol for UK Biobank. Other missing variables for UK Biobank were LDL cholesterol n=4628; triglycerides n=4576; and for EPIC-Norfolk—total cholesterol n=428, LDL cholesterol n=510, HDL cholesterol n=427, triglycerides n=428)
**Image quality score generated by QUARTZ, values range from 0.6 to 1.0, higher values indicate higher image quality.*
*†Geometric mean exponentiated SD of the log-transformed values; the 95% range for the geometric mean is from (geometric mean÷GSD$^2$) to (geometric mean×GSD$^2$).*
BMI, body mass index; BP, blood pressure; EPIC, European Prospective Investigation into Cancer; HDL, high-density lipoprotein; LDL, low-density lipoprotein; QUARTZ, QUantitative Analysis of Retinal vessels Topology and siZe.

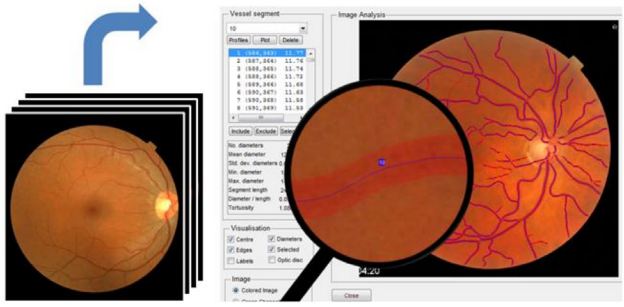
heart disease (ICD-10 I20-I25; ICD-9 410-414). We followed Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis guidelines for reporting of model development and validation.[31]

## RESULTS

Table 1 shows for UKB mean age at baseline was 56.8 years with median duration of follow-up 7.7 years after retinal image capture (maximum 8.2 years), and for EPIC-Norfolk, mean age was older (67.6 years) and median follow-up 9.1 years (maximum 12.4 years). Figure 1 is a visual representation of retinal image analysis using the QUARTZ software. Online supplemental figure S1 shows the number of UKB and EPIC-Norfolk participants and events available for circulatory mortality, incident stroke and incident MI analyses.

### Circulatory mortality

64 144 UKB participants with 327 circulatory deaths and 5862 EPIC-Norfolk participants with 201 circulatory deaths were included. In men, arteriolar and venular width, tortuosity and



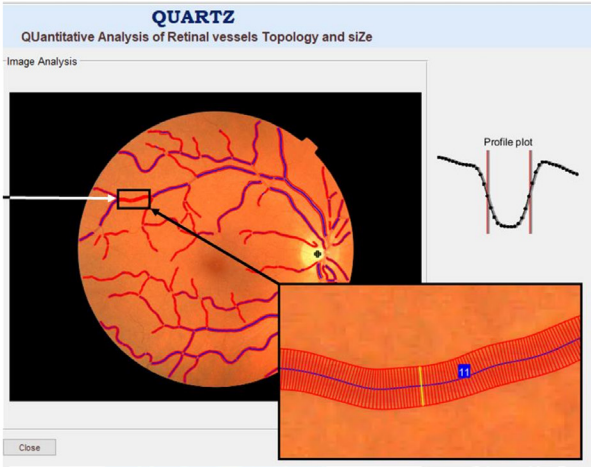




**Figure 1** Fully automated retinal image processing of the vascular tree using artificial intelligence-enabled QUARTZ software.

width-variance were identified as statistically significant predictors of circulatory mortality. In women, arteriolar and venular area and width, venular tortuosity and venular width-variation contributed to risk prediction. RV effects on circulatory mortality were modified by smoking status, BP medications and history of MI. In men and women, optimism adjusted C-statistics (0.75–0.77) and $R^2$ (0.33–0.44) statistics in UKB and EPIC-Norfolk, were reasonably high (table 2, online supplemental table S1 for full model diagnostics; online supplemental tables S2 and S3 for regression coefficients). In UKB men, predicted risks were closely aligned with observed risks. A similar picture emerged for EPIC-Norfolk men cohort, with about double the risk of circulatory mortality, half the numbers of events and being about a decade older at retinal image capture (figure 2). UKB women showed a wide separation of risk groups and close alignment of predicted and observed risks even, at low risks (<0.5%). Calibration plots for EPIC-Norfolk women were less clear due to the lower number of events available, hence 95% CI around predictions were wider (figure 2).

### Incident stroke

63 839 UKB participants with 446 incident strokes and 5708 EPIC-Norfolk participants with 211 incident stroke events after retinal image capture were included (online supplemental figure S1). In UK-Biobank, FRS C-statistic was 0.74 in men and 0.74 in women (table 2) with lower values in EPIC-Norfolk; approximately one-third of the variation in stroke-risk incidence was explained by $R^2$ (less so in EPIC-Norfolk men). Observed risks were more aligned with predicted risks in men than in women (online supplemental figure S2). Addition of RV to FRS did not improve model performance statistics overall (online supplemental table S4, figure S2).

Models based on age, smoking status, medical history and RV showed similar performance to FRS with C-statistic of 0.73 in men and 0.75 in women and marginally improved $R^2$ values in UKB (table 2; full model diagnostics online supplemental table S4). As for FRS, performance metrics were lower in EPIC-Norfolk. Multivariable models (online supplemental tables S2 and S3) showed venular and arteriolar tortuosity and width were predictors of stroke in men and women and additionally venular/arteriolar area in women with some modification by smoking status, BP medications and history of MI. Calibration plots showed risk predictions closer to the 45° line particularly at lower levels of predicted risk in women (online supplemental figure S2).

### Incident MI

45 734 UKB participants with 393 incident MI and 4062 in EPIC-Norfolk with 265 incident MI after retinal image capture were included (online supplemental figure S1). In UKB, FRS C-statistics were 0.71 in men and 0.76 in women with approximately one-quarter (24%) of the variation in MI risk explained by FRS in men and 35% in women (table 2). In EPIC-Norfolk, with approximately 5× the risk of MI, performance statistics were lower. Calibration plots for FRS showed better alignment of observed and predicted risks in men compared with women (online supplemental figure S3). Addition of RV to FRS did not improve model performance overall (online supplemental table S5, figure S3). Compared with FRS alone a simpler model based on age, smoking status, medical history and RV performed marginally less well in men and women in both cohorts (online supplemental tables S2 and S5, figure S3). Multivariable models for MI using RV (online supplemental tables S2 and S3) showed arteriolar and venular width, venular width variability and arteriolar area were predictors in men, whereas for women venular tortuosity, venular/arteriolar area and venular width variability were predictors. RV effects were modified by smoking status.

### Cases in top quintile of risk scores

For circulatory mortality models based on age, smoking status medical history and RV captured between 52% and 65% of cases of circulatory mortality in the top quintile of the risk score distribution (table 3). For incident stroke, RV based models compared with FRS captured about 5% more cases in UKB men and 8% more cases in UKB women and 3% more EPIC-Norfolk men in the top quintile of risk scores (table 3) but 1.8% fewer EPIC-Norfolk women. However, for MI, FRS captured more cases of MI in the top quintile of risk. Considering stroke and MI scores combined, the simpler RV models captured more cases in the top quintile than FRS for UKB men and women, and similar proportions in EPIC-Norfolk men and women.

### Sensitivity analyses

Restricting model development and validation to those of white ethnicity did not materially alter model performance for any of the models presented. FRS and all RV models for stroke showed systematically improved external validation for outcomes based on inclusion of all incident cerebrovascular disease in EPIC-Norfolk (online supplemental table S4) far right-hand column and (online supplemental figure S4), especially in women. In contrast, for *all* incident ischaemic heart disease in

**Table 2** Optimism adjusted model performance (95% CIs) for prediction of circulatory mortality, incident stroke and myocardial infarction models developed in UK Biobank cohort (2009–2018) with external validation in EPIC-Norfolk cohort (2004–2018)

| Model | UK Biobank men | UK Biobank women | EPIC-Norfolk men | EPIC-Norfolk women |
|---|---|---|---|---|
| | Circulatory mortality (number of events/sample size) | | | |
| **Age, smoking, medical history+RV** | (227/29 257) | (100/35 887) | (114/2516) | (87/3346) |
| Calibration slope | 0.913 (0.800 to 1.026) | 0.857 (0.732 to 0.982) | 1.084 (0.888 to 1.279) | 0.872 (0.674 to 1.070) |
| C-statistic | 0.749 (0.720 to 0.779) | 0.763 (0.717 to 0.810) | 0.774 (0.732 to 0.815) | 0.748 (0.692 to 0.805) |
| $R^2$ | 0.369 (0.310 to 0.427) | 0.443 (0.369 to 0.518) | 0.392 (0.302 to 0.482) | 0.333 (0.228 to 0.438) |
| | Stroke (number of events/sample size) | | | |
| **FRS for stroke** | (245/28 573) | (201/35 266) | (98/2432) | (113/3276) |
| Calibration slope | 0.908 (0.769 to 1.047) | 0.919 (0.764 to 1.074) | 0.819 (0.552 to 1.087) | 0.943 (0.734 to 1.152) |
| C-statistic | 0.736 (0.706 to 0.766) | 0.736 (0.702 to 0.770) | 0.682 (0.629 to 0.735) | 0.732 (0.682 to 0.781) |
| $R^2$ | 0.295 (0.233 to 0.358) | 0.310 (0.240 to 0.379) | 0.199 (0.098 to 0.300) | 0.309 (0.215 to 0.402) |
| **Age, smoking, medical history+RV** | | | | |
| Calibration slope | 0.896 (0.767 to 1.025) | 0.860 (0.729 to 0.991) | 0.808 (0.571 to 1.045) | 0.780 (0.603 to 0.958) |
| C-statistic | 0.729 (0.699 to 0.759) | 0.753 (0.721 to 0.784) | 0.691 (0.637 to 0.746) | 0.714 (0.660 to 0.768) |
| $R^2$ | 0.315 (0.256 to 0.375) | 0.352 (0.289 to 0.416) | 0.213 (0.113 to 0.314) | 0.274 (0.179 to 0.369) |
| | Myocardial infarction (number of events/sample size) | | | |
| **FRS for confirmed MI** | (275/19 150) | (118/26 584) | (166/1622) | (99/2440) |
| Calibration slope | 1.216 (0.994 to 1.439) | 1.036 (0.813 to 1.260) | 1.567 (1.210 to 1.924) | 0.834 (0.583 to 1.085) |
| C-statistic | 0.706 (0.678 to 0.734) | 0.758 (0.718 to 0.798) | 0.689 (0.650 to 0.728) | 0.688 (0.640 to 0.737) |
| $R^2$ | 0.235 (0.175 to 0.295) | 0.345 (0.256 to 0.433) | 0.233 (0.153 to 0.312) | 0.208 (0.109 to 0.308) |
| **Age, smoking, medical history+RV** | | | | |
| Calibration slope | 0.836 (0.673 to 0.999) | 0.803 (0.590 to 1.016) | 0.905 (0.655 to 1.156) | 0.786 (0.517 to 1.054) |
| C-statistic | 0.675 (0.647 to 0.703) | 0.709 (0.669 to 0.749) | 0.641 (0.598 to 0.683) | 0.650 (0.593 to 0.707) |
| $R^2$ | 0.178 (0.118 to 0.238) | 0.226 (0.136 to 0.316) | 0.150 (0.077 to 0.224) | 0.162 (0.067 to 0.256) |

Framingham risk scores (FRS) for incident stroke and myocardial infarction are also presented.
Estimates for calibration slope, C-statistic and $R^2$ values are given with bootstrapped 95% CI in parenthesis.
FRS, Framingham risk score; RV, retinal vasculometry.

EPIC-Norfolk, performance of FRS and RV models remained remarkably unchanged in men but marginally improved in women (online supplemental table S5 far right-hand column and online supplemental figure S5).

## DISCUSSION

This study compares risk predictions using AI-enabled RV with established CVD risk-algorithms. To the best of our knowledge it represents the largest population-based study of RV. Importantly, external validation of the prediction models was carried out in a separate large cohort, which is uncommon in this field. Our automated AI-enabled system extracts the retinal vascular tree over the entire retinal image (figure 1), distinguishes between arterioles and venules and provides measures of tortuosity, width-variance and area, in addition to vessel width. Risk models showed that all RV components contributed to risk prediction. Adding RV to FRS resulted in marginal changes in the prediction of stroke or MI. However, a simpler non-invasive risk score based on age, sex, smoking status, medical history and RV yielded comparable performance to FRS, without the need for blood sampling or BP measurement. Prediction of circulatory mortality using age, sex, smoking status, medical history and RV has not been reported previously, and yielded the highest model performance in terms of C-statistics $R^2$ statistics and agreement between observed and predicted risks, even at lower levels of risk, in both the internal and external validation cohorts.

### Comparisons with other studies

Prospective associations have been largely based on retinal vessel width with mortality,[12] incident stroke[12] and with CHD (in women, not men),[10 32] from restricted measurement areas of the retina.[10 33] Measurements are often not automated, requiring operator involvement, which limits application to large populations. In agreement with others, our models show that both arteriolar and venular vasculometry contribute to risk prediction,[10 34] and this aligns with our previous work.[15 27 35] Seidelmann *et al* reported that narrower central retinal artery and wider central retinal vein equivalent dimensions offered significant additional information to equations for incident atherosclerotic CVD risk,[10] especially in women, but C-statistics were modest (between 0.55 and 0.57) compared with the much higher levels in the current study (ie, between 0.70 and 0.77). Our RV models generally performed better in women and may indicate that microvascular dysfunction contributes more to CHD pathogenesis in women than in men, as they have smaller coronary arteries exhibiting more diffuse 'non-obstructive' atherosclerosis,[36] with a larger burden of coronary microvascular disease,[37] leading to higher morbidity and mortality.[38] A recent study using the UKB data source in fewer participants (54 813 vs 65 144 in this study), showed that retinal vessel density and fractal dimensions (extracted from the entire image after deep learning vessel segmentation without distinction between arterioles and venules) were associated with other health outcomes, including overall mortality, hypertension and congestive heart failure, but did not report on risk prediction performance.[39] Moreover, there was no consistent evidence of associations with incident circulatory disease, and cerebrovascular disease and associations with incident MI were null.[39] Another study in a sub-set of UKB participants (n=5663) with both retinal and cardiovascular MRI used deep learning/AI approaches to estimate structural cardiac

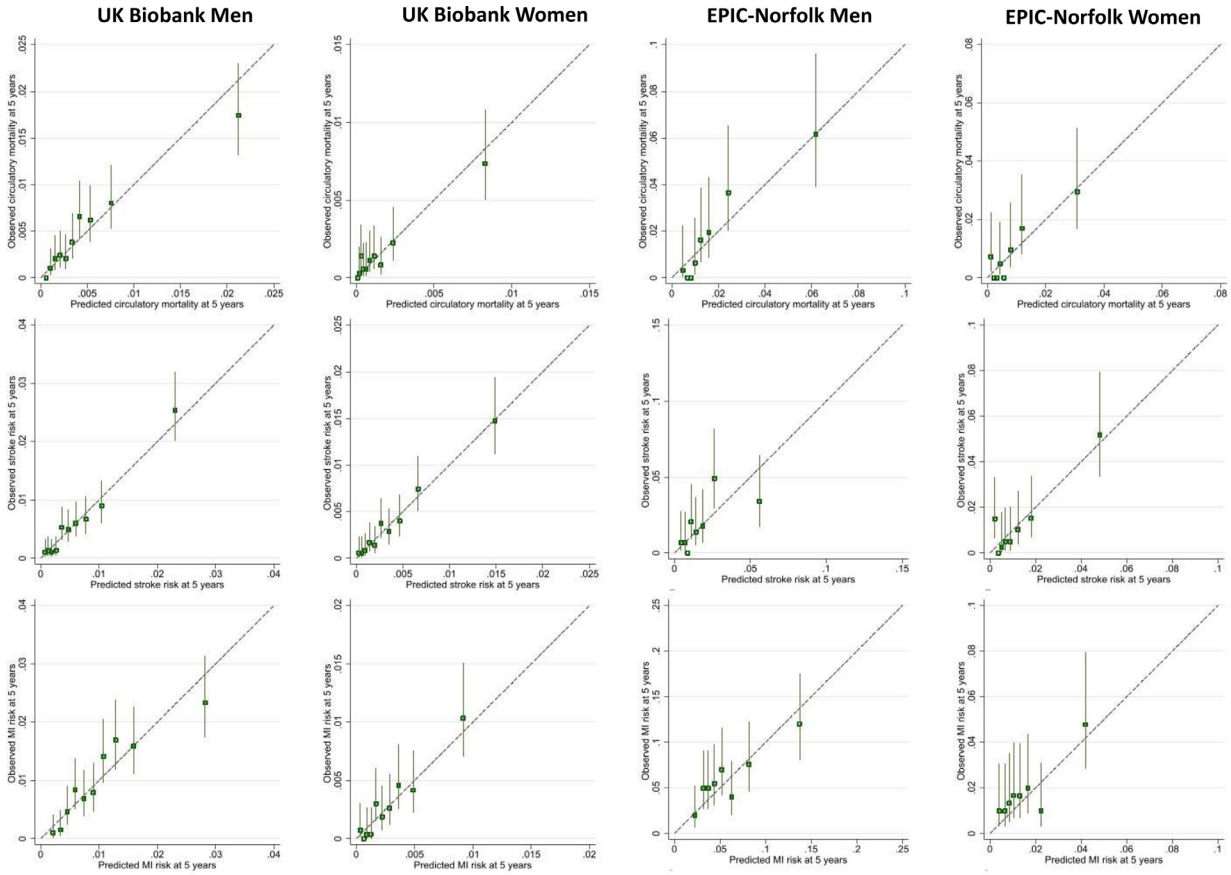



**Figure 2** Observed risk of outcome at 5 years by deciles of predicted risk in Biobank and eights of predicted risk in EPIC-Norfolk. Predicted risk based on model using age, smoking, medical history and retinal vasculomatry . EPIC, European Prospective Investigation into Cancer. Vertical lines around symbols are the 95% confidence intervals. Dotted lines represent perfect calibration. The scale of the vertical and horizontal axes represent the probability e.g., 0.1 equates to a 10% risk of event by 5 years.

indices as intermediaries for predicting MI.[40] However, given their approach, specific retinal features of importance remain unclear.

European SCORE CVD risk score,[8] QRISK3 risk score[41] and the American College of Cardiology/American Heart Association CVD risk score algorithms have already been evaluated in UKB. The published C-statistics for these three risk scores were 0.77, 0.74 and 0.74, respectively, with 95% CI that overlap with values for the simpler RV model presented in this study. However, the novel C-statistics for circulatory mortality reported in this study are higher. Our approach of focussing on the retinal microvasculature as a key prognostic marker of incident cardiovascular outcomes and circulatory mortality is supported by saliency maps presented in a study using end-to-end AI of retinal images to estimate the extent of coronary artery calcium scores in cross-sectional associations,[42] with C-statistics for incident CVD

**Table 3** Percentage of circulatory mortality, incident stroke and incident MI events (after retinal image capture) in top quintile of risk score distributions for UK Biobank and EPIC-Norfolk

| Model | UK Biobank Men | | UK Biobank Women | | EPIC-Norfolk Men | | EPIC-Norfolk Women | |
|---|---|---|---|---|---|---|---|---|
| | **Number, % of all circulatory mortality in top quintile of circulatory mortality risk score distribution** | | | | | | | |
| Age, smoking, medical history+RV | 126 | 55.5% | 65 | 65.0% | 63 | 55.3% | 45 | 51.7% |
| | **Number, % of all incident stroke in top quintile of stroke risk score distribution** | | | | | | | |
| FRS stroke | 115 | 46.9% | 100 | 49.8% | 37 | 37.8% | 55 | 48.7% |
| Age, smoking, medical history+RV | 133 | 54.3% | 114 | 56.7% | 40 | 40.8% | 53 | 46.9% |
| | **Number, % of all incident MI in top quintile of MI risk score distribution** | | | | | | | |
| FRS confirmed MI | 116 | 42.2% | 59 | 50.0% | 68 | 41.0% | 37 | 37.4% |
| Age, smoking, medical history+RV | 109 | 39.6% | 58 | 49.2% | 65 | 39.2% | 33 | 33.3% |
| | **Number, % of all incident stroke or MI in top quintile of stroke or MI risk score distribution** | | | | | | | |
| FRS confirmed MI or FRS stroke | 259 | 49.8% | 181 | 56.7% | 120 | 45.5% | 108 | 50.9% |
| Age, smoking, medical history+RV | 264 | 50.8% | 190 | 59.6% | 119 | 45.5% | 104 | 49.9% |

FRS, Framingham risk scores; MI, myocardial infarction; RV, retinal vasculometry.

varying between 0.68 and 0.76. Our model using RV together with easily attainable data including age, smoking status, sex and a brief medical history, is simple, non-invasive and exhibits performance that is comparable, or even better than, current risk algorithms, including end-to-end AI approaches.

Online supplemental tables S2 and S3 present the regression-coefficients for the RV models for circulatory mortality, incident stroke and incident MI. Beta-coefficients with p values ≤0.1 (as defined by our backward stepwise elimination for model development) are retained in the risk prediction equation. Regression coefficients with p>0.1 were therefore not included in the model. It is usual to present both main effects and interaction effects in the same model even if the main effect is not statistically significant. However, in risk prediction only coefficients that contribute to risk discrimination are retained and coefficients that are not formally statistically significant, as defined a priori, will not add to discrimination, and are therefore not included in the final risk equation. This may at first seem counterintuitive, but it is evident that certain RV features are important in risk prediction because they are related to (or potentially affected by) other factors such as smoking, presence of CVD and BP lowering medications, which is biologically plausible and supported by other evidence.[12 14 15 43–48]

## Strengths and limitations

Model development in UKB provided a large sample size and number of prospective events. QUARTZ successfully processed a high percentage (77%) of retinal images captured by non-experts providing 'vasculomic' indices of vascular health. External validation in an older higher risk cohort (EPIC-Norfolk) replicated the findings, and models were also robust to inclusion of a wider spectrum of cerebrovascular and ischaemic heart disease events.

UKB and EPIC-Norfolk are 'healthy' cohorts with relatively low event rates compared with other geographically similar middle-aged cohorts.[49] Prevalence of current smoking was very low in UKB (6%) and limited the ability to examine interactions with RV. Although we did not find limiting the analysis to those of white ethnicity materially altered the results, the proportion of non-white participants in UKB is low. RV may relate to microvascular endothelial function elsewhere in the body and may underpin the causal pathways behind prognostic models, which may differ with ethnicity. Confirmation of model performance in other cohorts with higher CVD rates and in different (especially non-white) ethnic groups would be informative.

## Implications and conclusions

Retinal imaging is established within clinic and hospital eye care and in optometric practices in the US and UK. AI-enabled vasculometry risk prediction is fully automated, low cost, non-invasive and has the potential for reaching a higher proportion of the population in the community because of 'high street' availability and because blood sampling or sphygmomanometry are not needed. RV is a microvascular marker, hence offers better prediction for circulatory mortality and stroke compared with MI which is more macrovascular, except perhaps in women. In the general population it could be used as a non-contact form of systemic vascular health check, to triage those at medium-high risk of circulatory mortality for further clinical risk assessment and appropriate intervention. In 2017–2018 in the UK, 41% of 40–74 years old attended their primary care NHS Health Check, which includes QRISK based screening for CVD.[50] With a trend towards lower attendance in more recent years (ie, from 2012 onwards), and socioeconomic inequalities in attendance (where younger ages, males, those more deprived and certain ethnic groups were less likely to attend),[50] this 'high street' RV approach could directly feed into primary medical services and help achieve greater screening coverage (under the assumption that this age group are likely to attend optometric practice for visual correction, especially with the onset of presbyopia). In addition, this would offer a novel approach to identify those at high risk of circulatory mortality, which are not currently screened for. While a high percentage of retinal images in this study captured by non-expert personnel were of sufficient quality to be used for RV quantification (~80%), we would expect this to be improved with fundus imaging carried out by healthcare practitioners, such as those working in optometric practice. However, moving forward experimental evidence would be needed to formally assess the effectiveness on CVD prevention before advocating implementation. Despite this, having a further low cost, accessible, non-invasive screening test in the community to encourage clinical risk assessment uptake in the community (in addition to current screening approaches), is highly likely to help prolong disease-free status in an ever-ageing population with increasing comorbidities, and assist with minimising healthcare costs associated with lifelong vascular diseases.

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**Collaborators** UK Biobank Eye and Vision Consortium.

**Contributors** ARR, CGO, PW, DS, SB, PJF designed the present study and raised funding. ARR, CGO, RW, SB, RL, SH, K-TK, PJF collected data for the study and undertook data management. ARR, RW, SB analysed the data. ARR and CGO are responsible for data integrity and are guarantors.

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**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and the UK Biobank study was approved by the North West Multi-Centre Research Ethics Committee (11/NW/03820). The EPIC-Norfolk study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk and Waveney NHS Research Governance Committee (2005EC07L). Participants gave informed consent to participate in the study before taking part.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data may be obtained from a third party and are not publicly available. The data supporting the results reported here are available through the UK Biobank https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access.

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# Supplementary Materials

**Artificial intelligence enabled retinal vasculometry for prediction of circulatory mortality, myocardial infarction and stroke**

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## UK Biobank biomedical examination

Baseline assessments were carried out 2006-2010, in 22 UK recruitment centres, in 502,682 adults aged 40-69 years.[1] Study participants had a detailed examination (including anthropometry, blood pressure, urine and venous blood sampling) and self-completed questionnaire about health (including information on pre-existing CVD, self-reported heart attack, stroke, angina, type 2 diabetes, and other medical conditions), and lifestyle (with a particular focus on dietary habits and smoking status) as well as medication usage (including lipid lowering, antihypertensives and insulin). Weight and height, were measured in participants after removal of heavy clothing and without shoes. Weight was measured using digital scales (Tanita BC-418MA, Tanita UK Ltd, Middlesex, UK) and height with a stadiometer (Seca 202, Seca, Birmingham, UK). Seated blood pressure was measured twice 1 minute apart using an automated blood pressure monitor (Omron HEM-7015IT, Omron Electronics Ltd, Milton Keynes, UK); the mean of both measures was used. A non-fasting venous blood sample was collected; details of the analytic measures have been published previously.[2] Blood samples were processed and analysed by a single laboratory between 2014-2017, and included serum total cholesterol, and HDL-cholesterol;[3] LDL-cholesterol was calculated using the Fredrickson–Friedewald equation,[4] except in 10,884 patients where triglycerides were >400 mg/dL (2.2%) where a direct measure was used.[3]

UK Biobank eye examination occurred at baseline in a subset of participants[5] from December 2009 to July 2010 towards the latter end of recruitment in 6 UK Biobank centres. Participants attended for repeat assessment 1 to 5 years after recruitment and ocular assessments in this latter phase (August 2012-June 2013) were largely from individuals that had not undergone an ocular assessment on entry into UK Biobank. Both phases included visual acuity, autorefraction, intraocular pressure and corneal biomechanics.[5] Digital fundus photography and spectral domain OCT images were taken using the Topcon 3D-OCT 1000 Mark 2. Non-mydriatic 45° digital colour images, centred on the fovea were captured from 68,550 participants in the first phase and 19,502 from the second phase. Overlap with baseline ocular assessment was minimal.

## EPIC-Norfolk biomedical examination at 3rd Health Check

Between 2004 and 2011 8,623 participants took part in the third health check. Weight and height, were measured with participants in light clothing without shoes. Weight was measured to the nearest 0.1 kg using regularly calibrated digital scales (Tanita TBF-300, Tanita UK Ltd, Middlesex, UK) and height to the last complete 0.1 cm using a stadiometer (Chasmors, UK). Seated blood pressure was measured twice using an automated blood pressure monitor (Accutorr PlusTM, Datascope Patient Monitoring, Huntington,

UK); the mean of both measures was used. A non-fasting venous blood sample was collected; details of the analytic measures have been published previously.[6] Serum total cholesterol and HDL-cholesterol were measured using an auto-analyser (RA 1000 Technicon, Bayer Diagnostics, Basingstoke, UK); LDL-cholesterol was calculated using the Fredrickson–Friedewald equation.[4]

EPIC- Norfolk eye examination. Ophthalmic tests included measurement of vision, visual acuity (LogMAR acuity), and closed field auto-refraction (Humphrey model 500, Humphrey Instruments, San Leandro, California, USA), which was used to estimate axial length. Macular centred 45° digital fundus photographs were taken using a TRC-NW6S non-mydriatic retinal camera and IMAGEnet Telemedicine System (Topcon Corporation, Tokyo, Japan) with a 10 megapixel Nikon D80 camera (Nikon Corporation, Tokyo, Japan) without pharmacological dilation of the pupil.

## Health outcomes

The primary outcome was circulatory mortality as defined using International Classification of Diseases (ICD-10 codes I00-I99 and ICD9 390-459) coded death registry data from the Office for National Statistics and the Health and Social Care Information Centre (now NHS Digital) for England and Wales, and the Information Services Department for Scotland, provided information on date and cause(s) of death to 31st January 2018 for UK Biobank and 31st March 2018 for EPIC-Norfolk. Incident MI and stroke events after retinal image capture were based on medical records linkage with hospital diagnoses of non-fatal events, supplemented with participant health and lifestyle questionnaire data from repeat surveys in UK Biobank and EPIC-Norfolk (2012-2018). ICD-10 codes I21-I25 (or ICD-9 codes 410, 411,412 429.79) were used for fatal and non-fatal MI; and ICD-10 codes I60,61,63,64 (or ICD-9 codes 430, 431,434,436) for ischaemic and haemorrhagic stroke.

## Statistical Analysis

### Development of circulatory mortality models in UK Biobank

Statistical analyses were carried out using STATA software (version 16, StataCorp LP, College Station, TX). Retinal vessel widths and area showed normal distributions, tortuosity required log-transformation and within-vessel-width-variance required inverse square-root transformation to normalize distributions. Throughout models were developed in UK Biobank for men and women separately, and externally validated in EPIC-Norfolk. We hypothesized that retinal vessel characteristics in relation to disease incidence, might be modified by age, smoking status, presence of CVD/diabetes and use of BP lowering

medications. Hence, two-way interactions between retinal vasculometry and age, smoking status and self-reported use of blood pressure medication, prevalent diabetes and CVD were first examined in mutually adjusted Cox proportional hazard[7] models for circulatory mortality. Interaction terms with p values <0.2 were then included along with main effects in Cox regressions models using backward elimination (p value set to 0.1).

Bootstrapping with 100 replications was used for internal validation to adjust model performance measures for optimism, including Harrel's C-statistic for discrimination, $R^2$ statistic (representing a measure of explained variation) and calibration slope (where a slope of 1.0 is ideal).[8] The model from the bootstrapped sample was applied to the bootstrapped sample to estimate *apparent performance* and to the original dataset to test *model performance*. Optimism was estimated within each bootstrapped sample as the difference in performance parameters (C-statistic, $R^2$ and calibration slope) between *model performance vs apparent performance*. The overall (average) optimism across all bootstrapped samples was determined to adjust measures of model performance (C-statistic, $R^2$ and calibration slope).

### External validation of circulatory mortality models in EPIC-Norfolk cohort

The original beta coefficients from the prognostic models were adjusted for shrinkage to allow for over-fitting using the calibration slopes adjusted for optimism from the bootstrapped sampling. The adjusted linear predictor was then applied to the EPIC-Norfolk cohort and C-statistic, $R^2$ and calibration slope estimated. Calibration plots of the observed vs expected event probability by octiles of predicted risk of an event were calibrated to the average 5-year baseline survival in the EPIC-Norfolk cohort.

### Framingham Risk Scores for stroke and MI in UK Biobank and EPIC-Norfolk cohorts

Framingham risk scores (FRS) for incident fatal and non-fatal stroke[9] and MI[10] were applied to UK Biobank and EPIC-Norfolk cohorts and recalibrated to baseline survival function within each cohort. Following FRS criteria, participants reporting use of cholesterol lowering medications, diabetes or missing data on total or HDL cholesterol were excluded from all MI analyses.[10] Those reporting a history of heart attack or stroke or those with a date of event stroke or MI prior to retinal image capture were excluded from the corresponding prognostic modelling for that outcome. FRS models were also extended to include retinal vasculometry. Model development and validation followed a similar approach as described for circulatory mortality.

### Retinal vasculometry models for stroke and MI in UK Biobank and EPIC-Norfolk cohort

Alternative models for incident fatal and non-fatal stroke and MI using age, smoking status, medical history (self-reported history of heart attack, stroke or diabetes and use of blood pressure lowering medications) and retinal vasculometry only were developed in UK Biobank following the same approach as for circulatory mortality. A medical history of MI did not preclude inclusion in models for incident stroke events and vice-versa. Participants reporting diabetes or use of blood pressure lowering medications were included in stroke analyses. Participants with missing data on smoking status or self-report on medications for lowering blood pressure or lipids, or those that preferred not to report a history of heart attack or stroke were excluded from all FRS analyses (UK Biobank n= 1182 (1.8%); EPIC-Norfolk n=93 (1.6%)).

Prognostic models using retinal vasculometry included up to 26 candidate predictors in men and up to 28 in women, in the stepwise procedure based on inclusion of main effects and interactions with retinal vasculometry with $p<0.2$. A maximum of 16 predictors were identified by the stepwise procedure with $p<0.1$ in any single model. Retinal vasculometry measures excluded by the stepwise procedure were re-inserted back into the model to check whether they became statistically significant. Fractional polynomial models were used to examine presence of non-linear associations but none were identified.

### Sensitivity analyses

Sensitivity analyses restricted the entire model development and validation to the white ethnic group to check for systematic differences in model performance. With the EPIC-Norfolk cohort having a relatively smaller number of incident events, we assessed the external validation of models to a broader spectrum of incident cerebrovascular disease (ICD10 I60-69; ICD 9 430-438) and incident ischaemic heart disease (ICD10 I20-I25; ICD9 410-414).

## Sample size considerations

Prediction models considered the following variables: retinal vessel width, tortuosity, area, width variance [arteriolar and venular], age, sex, smoking status [current, former and never], blood pressure, serum lipids [total and HDL cholesterol] Framingham risk scores, history of diabetes / stroke / heart attack, use of blood pressure lowering medications plus significant two-way interactions with retinal vasculometry (described above). This yielded between 26 to 28 candidate predictor parameters for consideration in the stepwise regression procedure. With 65,000 UK Biobank participants, 327 circulatory deaths, 446 incident strokes and 393 incident MI events provided sufficient sample size to ensure model shrinkage factor (to allow for over-fitting) was in the region of 0.9 and that absolute differences in model's apparent vs an adjusted $R^2$ (hypothesized to be ~0.2), was approximately 0.1.[11] UK Biobank provided an unprecedented sample size in

terms of retinal imaging on a population based sample. It encompassed a wide range of patient characteristics for model development and it has been shown that risk factor associations in the UK Biobank seem to be generalisable.[12]

## Ethics, governance and consent

The UK Biobank and EPIC-Norfolk studies were carried out following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The UK Biobank study was approved by the North West Multi-Centre Research Ethics Committee (11/NW/03820). All participants gave written, informed consent.

The EPIC-Norfolk study was approved by the Norfolk Local Research Ethics Committee (05/Q0101/191) and East Norfolk and Waveney NHS Research Governance Committee (2005EC07L). All participants gave written, informed consent.

The data reported in this article are available via application to the UK Biobank to other researchers for purposes of reproducing the results or replicating the procedure.

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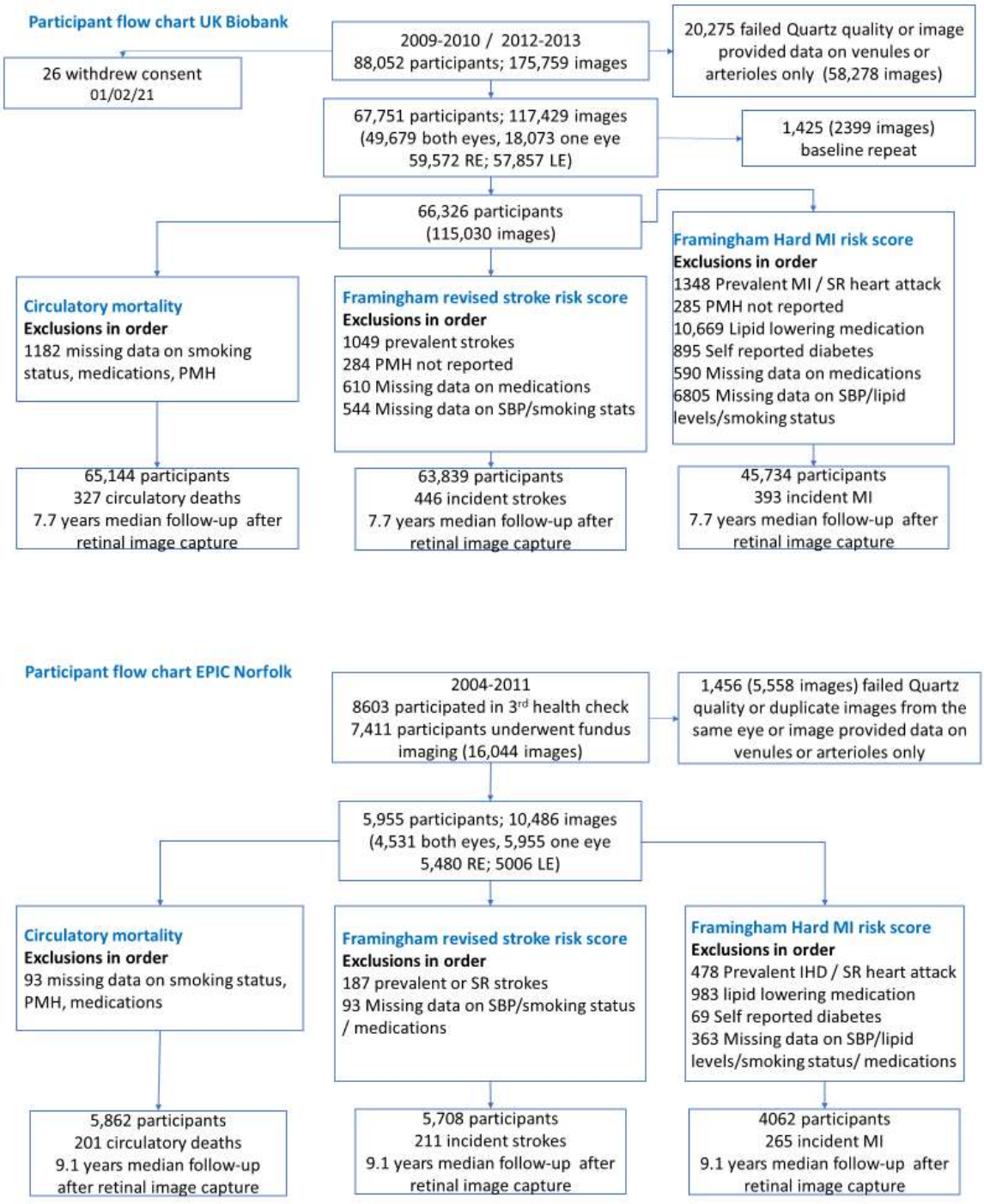
# Supplemental Figures



Rudnicka AR, *et al. Br J Ophthalmol* 2022;0:1–8. doi: 10.1136/bjo-2022-321842

## Figure S1 Participant flow chart in UK Biobank and EPIC cohorts




SR= self-reported; PMH previous medical history; SBP systolic blood pressure

Figure S2 Observed risk of incident stroke at 5 years by deciles of predicted risk in UK Biobank and octiles of predicted risk in EPIC-Norfolk

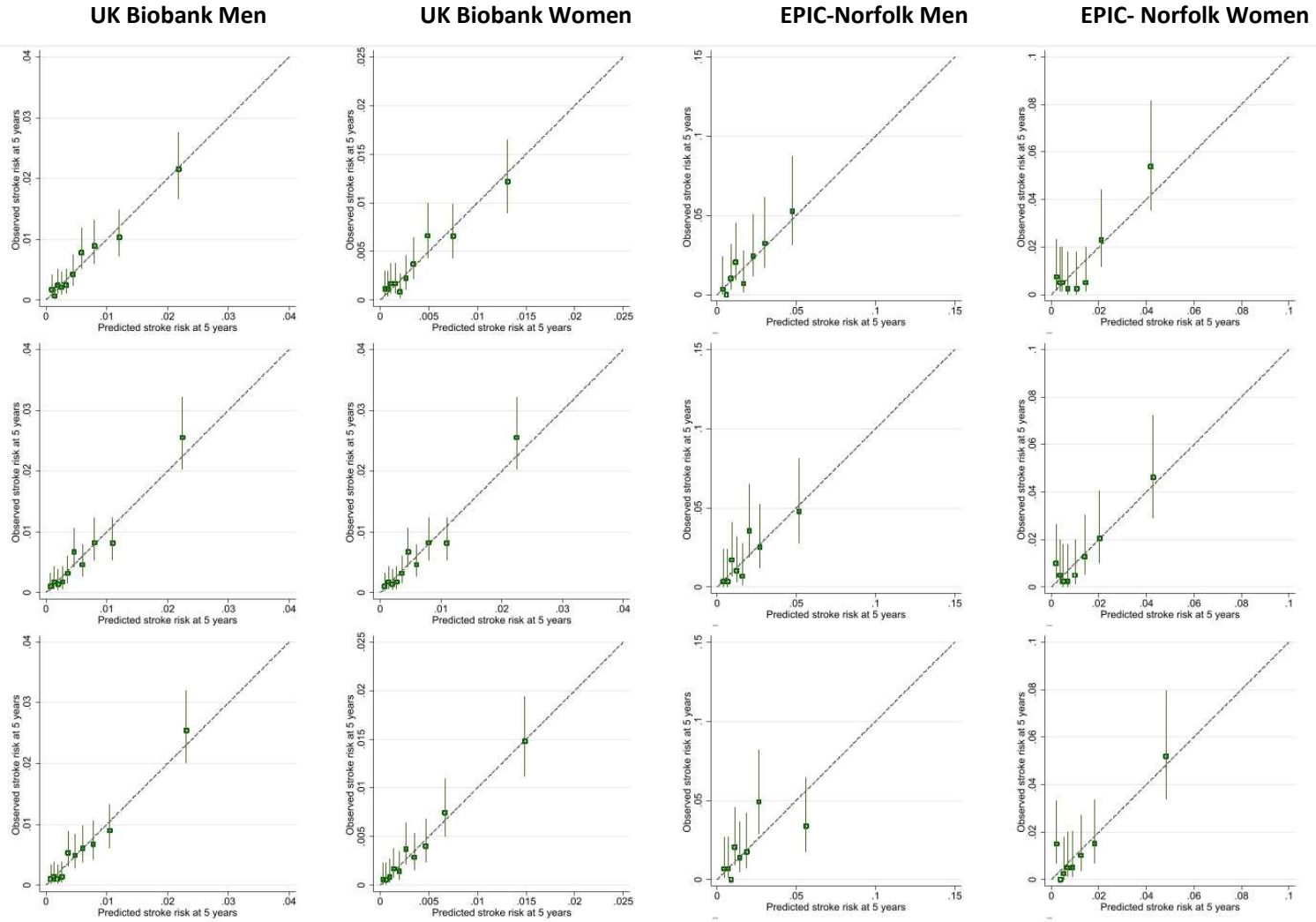



***Figure S2 footnote:***

*Top row: revised Framingham stroke risk score (after recalibration for baseline survival within each cohort)*

*Middle row: prediction model based on revised Framingham stroke risk score plus retinal vasculometry*

*Bottom row: prediction model based on retinal vasculometry, age, smoking and medical history*

*Vertical lines around symbols are 95% confidence intervals. Dotted line represents perfect calibration.*

*Incident stroke codes: ICD10: I60,I61,I63,I64, ICD9: 430,431,434,436*

*The scale of the vertical and horizontal axes is a probability e.g., 0.1 equates to a 10% risk of event by 5 years.*



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Figure S3 Observed risk of confirmed MI at 5 years by deciles of predicted risk in UK Biobank and octiles of predicted risk in EPIC-Norfolk

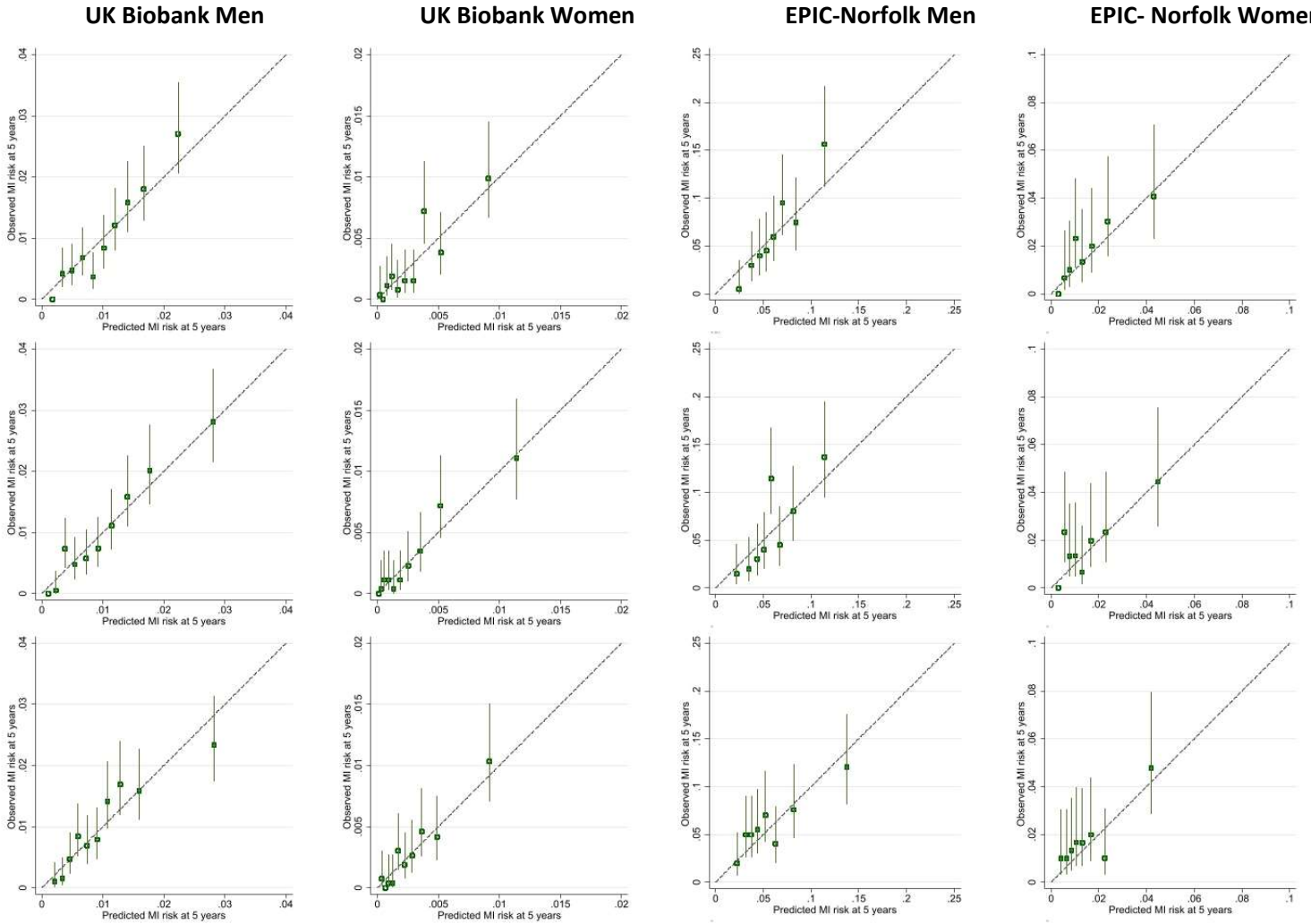


***Figure S3 footnote**:*

*Top row: Framingham risk score for confirmed MI (after recalibration for baseline survival within each cohort)*

*Middle row: prediction model based on Framingham risk score for confirmed MI plus retinal vasculometry*

*Bottom row: prediction model based on retinal vasculometry, age, smoking and medical history*

*Vertical lines around symbols are 95% confidence intervals. Dotted line represents perfect calibration.*

*Incident MI codes: ICD10: I21-I25, ICD9: 410,411,412,429.79*

*The scale of the vertical and horizontal axes is a probability e.g., 0.1 equates to a 10% risk of event by 5 years.*

Figure S4 Observed risk of incident cerebrovascular disease at 5 years by eighths of predicted risk in EPIC-Norfolk cohort

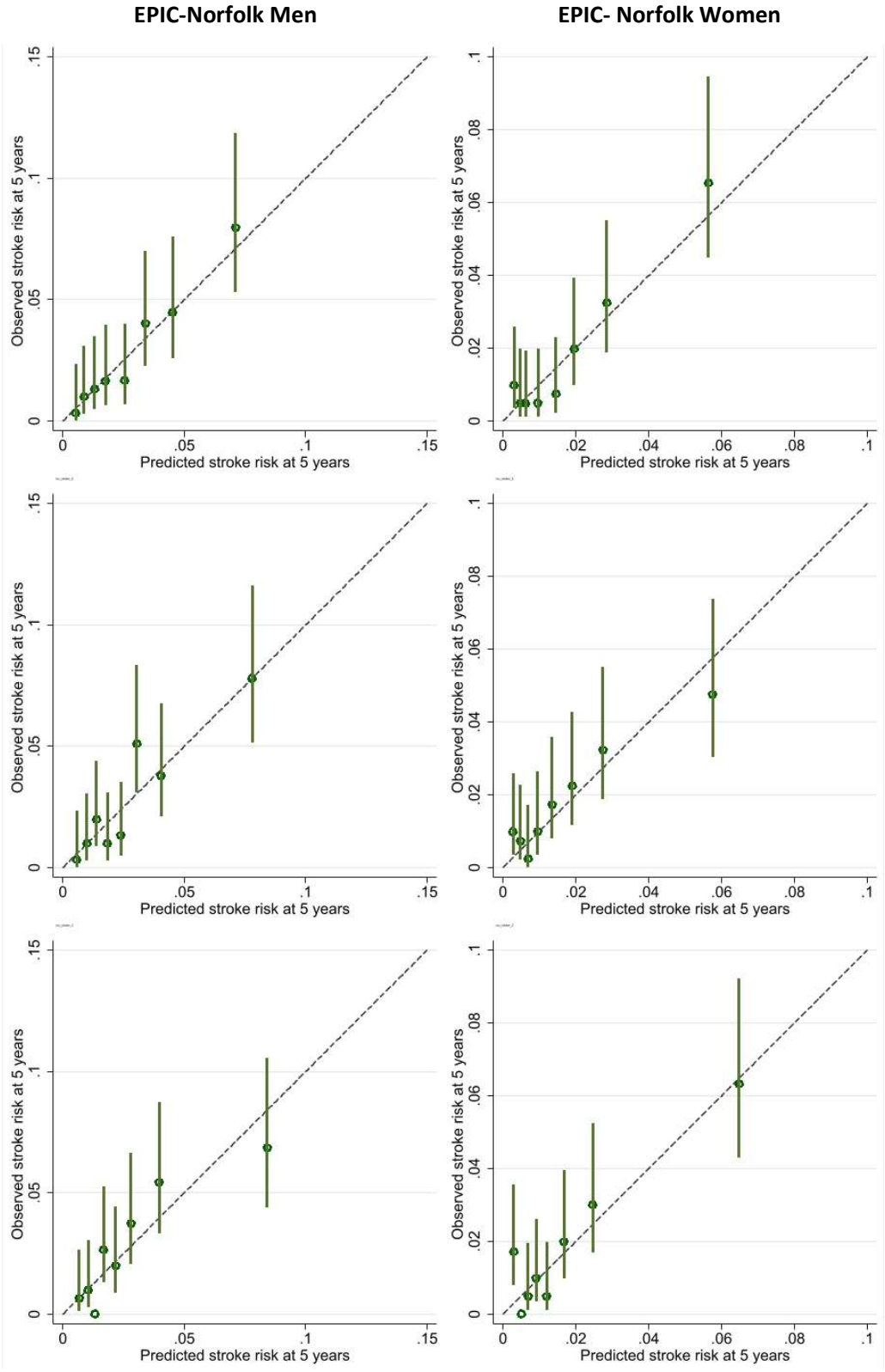

***Figure S4 footnote**:*

*Incident cerebrovascular disease ICD9 430-438; ICD10 I60-I69*

*Top row: revised Framingham stroke risk score (after recalibration for baseline survival in EPIC-Norfolk)*

*Middle row: prediction model based on revised Framingham stroke risk score plus retinal vasculometry*

*Bottom row: prediction model based on retinal vasculometry, age, smoking and medical history*

*Vertical lines around symbols are 95% confidence intervals. Dotted line represents perfect calibration.*



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Figure S5 Observed risk of ischaemic heart disease at 5 years by eighths of predicted risk for in EPIC-Norfolk cohort

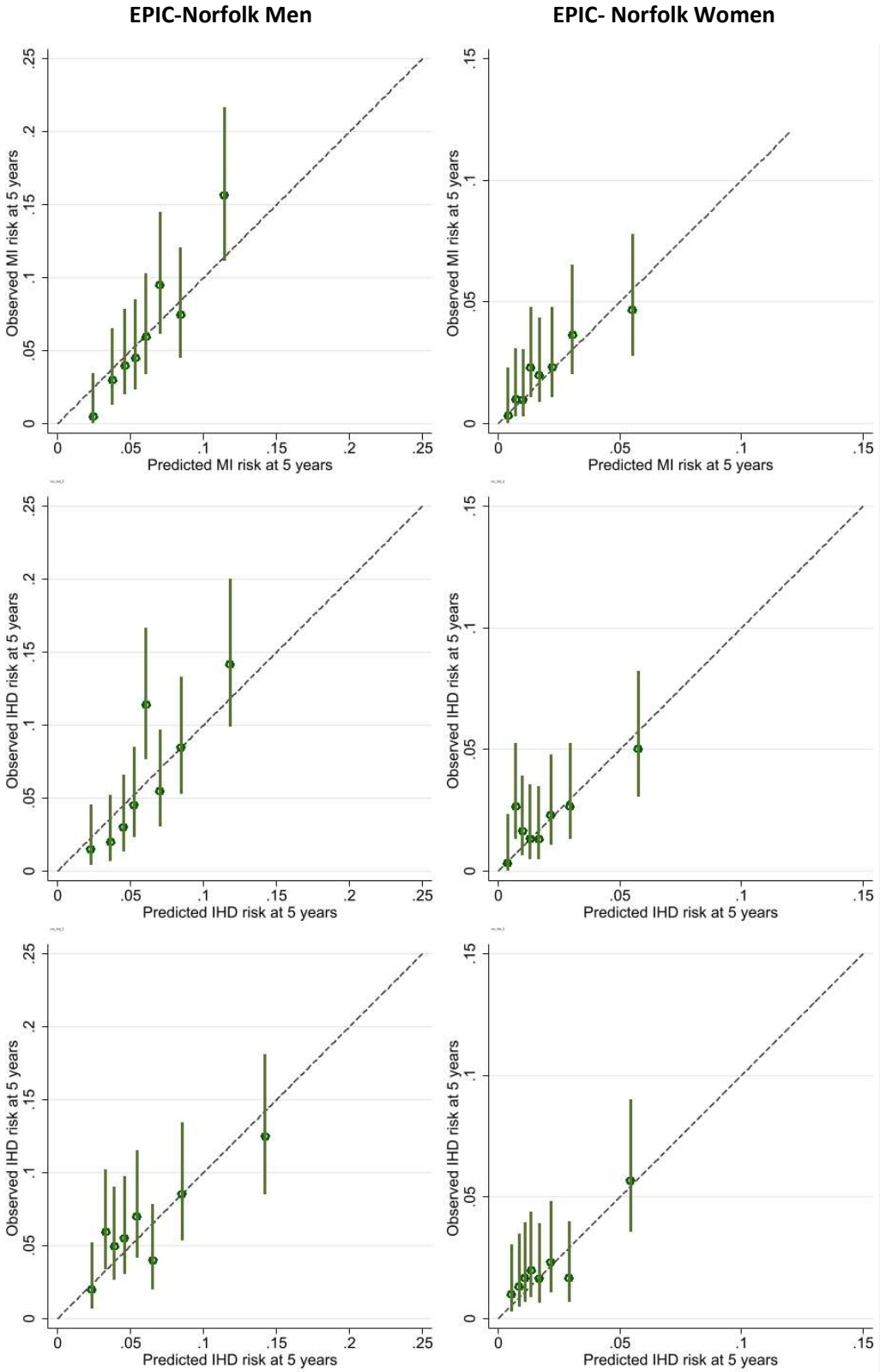

***Figure S5 footnote**:*

*Ischaemic heart disease codes ICD9 410-414; ICD10 I20-I25*

*Top row : Framingham risk score for confirmed MI (after recalibration for baseline survival in EPIC-Norfolk)*

*Middle row: prediction model based on Framingham risk for confirmed MI score plus retinal vasculometry*

*Bottom row: prediction model based on retinal vasculometry, age, smoking and medical history*

*Vertical lines around symbols are 95% confidence intervals. Dotted line represents perfect calibration.*



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# Supplementary Tables S1 to S5



Rudnicka AR, *et al. Br J Ophthalmol* 2022;0:1–8. doi: 10.1136/bjo-2022-321842

Table S1 Model diagnostics (with 95% confidence intervals) from internal validation of circulatory mortality in UK Biobank (2009-2018). External validation in EPIC- Norfolk cohort using biomedical data from the third health check (2004-2011) with circulatory mortality (ICD-10 codes I00-I99) as the health outcome (2004-2018)

| Model | Apparent performance | Test performance | Average Optimism | Optimism corrected |
|---|---|---|---|---|
| **UK Biobank Men** | | | | |
| **Age, smoking, medical history and retinal vasculometry** | | | | No. events = 114 |
| Calibration Slope | 1.000 (0.887, 1.113) | 0.913 (0.834, 0.992) | 0.087 | 0.913 (0.800, 1.026) |
| C-statistic | 0.771 (0.741, 0.800) | 0.763 (0.752, 0.773) | 0.021 | 0.749 (0.720, 0.779) |
| $R^2$ | 0.418 (0.359, 0.476) | 0.400 (0.384, 0.417) | 0.049 | 0.369 (0.310, 0.427) |
| **UK Biobank Women** | | | | |
| **Age, smoking, medical history and retinal vasculometry** | | | | No. events = 87 |
| Calibration Slope | 1.000 (0.875, 1.125) | 0.857 (0.708, 1.006) | 0.143 | 0.857 (0.732, 0.982) |
| C-statistic | 0.799 (0.753, 0.846) | 0.787 (0.766, 0.808) | 0.036 | 0.763 (0.717, 0.810) |
| $R^2$ | 0.522 (0.448, 0.597) | 0.488 (0.449, 0.526) | 0.079 | 0.443 (0.369, 0.518) |

Table S2 Final multivariable models based on retinal vasculometry, age, smoking status and medical history for circulatory mortality, incident stroke, incident myocardial infarctions (MI) in MEN. For each model the mean (standard deviation) of the linear predictor is also given

| Model | Hazard ratio (95%CI) | β coefficients |
|---|---|---|
| **Age, smoking, medical history and retinal vasculometry** | **Circulatory Mortality** | |
| Age | 1.08 (1.05, 1.10) | 0.07356 |
| Taking BP lowering Medication | 1.59 (1.18, 2.13) | 0.46127 |
| Previous MI | 3.87 (2.75, 5.45) | 1.35365 |
| Previous stroke | 2.35 (1.44, 3.84) | 0.85541 |
| Diabetes | 2.25 (1.61, 3.15) | 0.81162 |
| Current smoker | 2.33 (1.55, 3.48) | 0.84374 |
| Arteriolar InvSD | 0.93 (0.86, 1.01) | -0.07171 |
| Venular InvSD | 1.07 (1.00, 1.14) | 0.06708 |
| Age # arteriolar width | 1.00 (1.00, 1.00) | 0.00194 |
| Venular tortuosity if occasional smoker | 0.12 (0.05, 0.30) | -2.13421 |
| Venular width if non-smoker | 1.02 (1.00, 1.04) | 0.01895 |
| Arteriolar width if non-smoker | 0.96 (0.93, 0.99) | -0.04003 |
| Mean (SD) of linear predictor | 0.4066 (0.9699) | |
| | | |
| **Age, smoking, medical history + retinal vasculometry** | **Incident stroke** | |
| Age | 1.10 (1.08, 1.13) | 0.09860 |
| Current smoker | 3.10 (2.02, 4.76) | 1.13170 |
| Diabetes | 1.78 (1.26, 2.53) | 0.57847 |
| History of CVD | 2.05 (1.32, 3.18) | 0.71631 |
| Venular width | 0.99 (0.98, 1.00) | -0.01136 |
| Venular tortuosity if history of CVD | 0.40 (0.17, 0.94) | -0.90743 |
| Arteriolar tortuosity if taking BP lowering medication | 0.68 (0.46, 1.01) | -0.37987 |
| Venular width if taking BP lowering medication | 1.03 (1.01, 1.05) | 0.02592 |
| Arteriolar width if previous smoker | 0.97 (0.95, 1.00) | -0.02561 |
| Arteriolar tortuosity if occasional smoker | 0.38 (0.12, 1.18) | -0.95683 |
| Venular tortuosity width if previous smoker | 1.74 (1.02, 2.98) | 0.55405 |

| **Model** | **Hazard ratio (95%CI)** | **β coefficients** |
|---|---|---|
| Venular tortuosity if current smoker | 4.37 (1.74, 11.01) | 1.47586 |
| Mean (SD) of linear predictor | 0.2347 (0.9762) | |
| | | |
| **Age, smoking, medical history + retinal vasculometry** | **Incident MI** | |
| Age | 1.07 (1.05, 1.09) | 0.06901 |
| History of CVD | 2.39 (1.22, 4.69) | 0.87216 |
| Taking BP lowering Medication | 1.45 (1.07, 1.97) | 0.37401 |
| Current smoker | 3.19 (2.29, 4.45) | 1.16050 |
| Arteriolar width | 0.98 (0.96, 1.00) | -0.02412 |
| Age # arteriolar area | 1.02 (1.00, 1.04) | 0.02039 |
| Arteriolar width if non-smoker | 1.03 (1.00, 1.06) | 0.02702 |
| Venular width if occasional smoker | 0.92 (0.88, 0.97) | -0.07865 |
| Venular InvSD if previous smoker | 0.93 (0.87, 0.99) | -0.07380 |
| Mean (SD) of linear predictor | 0.1050 (0.7534) | |

FRS = Framingham risk score for outcomes as defined in methods

Age is in years centred to 55 years, SBP systolic blood pressure in mmHg

Arteriolar and venular widths are in microns centred to 85 microns and 100 microns respectively

Arteriolar and venular tortuosity were centred to 1.5 units.

Arteriolar and venular vessel area are in $mm^2$and centred to 1.8$mm^2$ and 2.0$mm^2$ respectively.

* InvSD is the transformed segment-width-variance values x100 (a unit increase equates to approximately 0.5 standard deviations)

# indicates interaction term between continuous variables

All regression coefficients are per unit increase in the predictors

With backward stepwise elimination for model development the p-value threshold was set to 0.1, beta-coefficients with p≤0.1 were therefore retained in the risk prediction equations. Beta-coefficients with p-values >0.1 were not included in the risk prediction equations and therefore were not included in the table.

Table S3: Final multivariable models based on retinal vasculometry, age, smoking status and medical history for circulatory mortality, incident stroke, myocardial infarctions (MI) and in WOMEN For each model the mean (standard deviation) of the linear predictor is also given

| Model | Hazard ratio (95%CI) | β coefficients |
|---|---|---|
| | **Circulatory mortality** | |
| Age | 1.108 (1.071, 1.147) | 0.10285 |
| Taking BP lowering medication | 1.823 (1.166, 2.849) | 0.60032 |
| Diabetes | 3.754 (2.211, 6.375) | 1.32294 |
| Occasional smoker | 1.000 (0.000, 0.000) | 0.00000 |
| Current smoker | 2.755 (1.603, 4.736) | 1.01350 |
| Arteriolar area | 0.172 (0.072, 0.410) | -1.76009 |
| Venular area | 1.605 (1.092, 2.358) | 0.47298 |
| Venular InvSD | 0.676 (0.587, 0.779) | -0.39135 |
| Venular area if not taking BP lowering medication | 0.492 (0.305, 0.793) | -0.70972 |
| Arteriolar area if non-smoker | 2.638 (1.597, 4.359) | 0.97007 |
| Venular InvSD and no history of MI | 1.419 (1.222, 1.650) | 0.35028 |
| Arteriolar width and no history of stroke | 1.026 (0.999, 1.054) | 0.02603 |
| Arteriolar area and no history of stroke | 3.205 (1.354, 7.582) | 1.16461 |
| Venular width if non-smoker | 0.975 (0.954, 0.997) | -0.02489 |
| Venular tortuosity if previous-smoker | 6.168 (2.729, 13.941) | 1.81938 |
| Arteriolar width if previous-smoker | 0.950 (0.909, 0.992) | -0.05179 |
| Mean (SD) of linear predictor | 0.4356 (1.0503) | |
| | **Incident stroke** | |
| Age | 1.103 (1.077, 1.130) | 0.09808 |
| Taking BP lowering medication | 1.580 (1.141, 2.189) | 0.45746 |
| History of CVD | 2.341 (1.413, 3.879) | 0.85059 |
| Diabetes | 3.151 (2.011, 4.939) | 1.14778 |
| Venular area | 1.786 (1.111, 2.871) | 0.58011 |
| Arteriolar area | 1.707 (1.037, 2.808) | 0.53465 |
| Arteriolar tortuosity | 1.572 (1.057, 2.338) | 0.45247 |
| Venular tortuosity | 1.410 (0.903, 2.202) | 0.34357 |
| Age # arteriolar tortuosity | 0.952 (0.913, 0.993) | -0.04913 |
| Venular area if not taking BP lowering medication | 0.697 (0.481, 1.012) | -0.36031 |
| Arteriolar area if do not have diabetes | 0.453 (0.265, 0.773) | -0.79244 |

| Model | Hazard ratio (95%CI) | β coefficients |
|---|---|---|
| Venular area if do not have diabetes | 0.615 (0.365, 1.036) | -0.48565 |
| Venular tortuosity if ex-smoker | 2.764 (1.357, 5.628) | 1.01650 |
| Venular width if occasional smoker | 1.059 (1.035, 1.085) | 0.05766 |
| Mean (SD) of linear predictor | 0.0589 (1.1128) | |
| | **Incident MI** | |
| Age | 1.093 (1.063, 1.125) | 0.08936 |
| Taking BP lowering medication | 1.637 (1.045, 2.564) | 0.49259 |
| Current smoker | 3.785 (2.214, 6.468) | 1.33094 |
| Venular InvSD | 1.077 (1.009, 1.149) | 0.07400 |
| Venular tortuosity if non-smoker | 1.929 (1.007, 3.695) | 0.65682 |
| Arteriolar area if non-smoker | 0.667 (0.473, 0.940) | -0.40534 |
| Venular area if non-smoker | 0.750 (0.561, 1.004) | -0.28704 |
| Mean (SD) of linear predictor | 0.0394 (0.9406) | |

FRS = Framingham risk score for outcomes as defined in the methods

Age is in years, SBP systolic blood pressure in mmHg

Arteriolar and venular widths are in microns centred to 85 microns and 100 microns respectively.

Arteriolar and venular tortuosity were centred to 1.5 units.

Arteriolar and venular vessel area are in $mm^2$and centred to 1.8$mm^2$ and 2.0$mm^2$ respectively.

*InvSD is the transformed segment-width-variance values x100 (a unit increase equates to approximately 0.5 standard deviations)

# indicates interaction term between continuous variables

All regression coefficients are per unit increase in the predictors

With backward stepwise elimination for model development the p-value threshold was set to 0.1, beta-coefficients with p≤0.1 were therefore retained in the risk prediction equations. Beta-coefficients with p-values >0.1 were not included in the risk prediction equations and therefore were not included in the table.

Table S4 Model diagnostics (with 95% confidence intervals) for incident stroke (after retinal image capture) in UK Biobank (2009-2018) as defined in the methods. External validation in EPIC- Norfolk cohort using biomedical data from the third health check (2004-2011) with all incident cerebrovascular disease (ICD10 I60-I69) as the health outcome (2004-2018)

| Model | Apparent performance | Test performance | Average Optimism | Optimism corrected | External validation in EPIC-Norfolk |
|---|---|---|---|---|---|
| **Revised FRS stroke** | | **UK Biobank Men** | | | No. events = 176 |
| Calibration Slope | - | - | - | - | 1.019 (0.810, 1.227) |
| C-statistic | - | - | - | - | 0.711 (0.672, 0.749) |
| $R^2$ | - | - | - | - | 0.273 (0.196, 0.350) |
| **Revised FRS stroke + retinal vasculometry** | | | | | |
| Calibration Slope | 1.000 (0.869, 1.131) | 0.911 (0.790, 1.032) | 0.089 | 0.911 (0.780, 1.042) | 0.911 (0.722, 1.100) |
| C-statistic | 0.749 (0.719, 0.780) | 0.742 (0.733, 0.751) | 0.018 | 0.731 (0.701, 0.762) | 0.698 (0.658, 0.739) |
| $R^2$ | 0.359 (0.299, 0.419) | 0.342 (0.323, 0.360) | 0.042 | 0.317 (0.257, 0.377) | 0.248 (0.170, 0.325) |
| **Age, smoking, medical history + retinal vasculometry** | | | | | |
| Calibration Slope | 1.000 (0.871, 1.129) | 0.896 (0.772, 1.019) | 0.104 | 0.896 (0.767, 1.025) | 0.910 (0.736, 1.084) |
| C-statistic | 0.751 (0.721, 0.781) | 0.737 (0.727, 0.747) | 0.022 | 0.729 (0.699, 0.759) | 0.711 (0.672, 0.750) |
| $R^2$ | 0.365 (0.306, 0.425) | 0.330 (0.311, 0.348) | 0.050 | 0.315 (0.256, 0.375) | 0.262 (0.186, 0.337) |
| | | | | | |
| **Revised FRS stroke** | | **UK Biobank Women** | | | No. events = 190 |
| Calibration Slope | - | - | - | - | 1.079 (0.915, 1.242) |
| C-statistic | - | - | - | - | 0.758 (0.723, 0.794) |
| $R^2$ | - | - | - | - | 0.365 (0.296, 0.435) |
| | | | | | |
| **Revised FRS stroke + retinal vasculometry** | | | | | |

| Model | Apparent performance | Test performance | Average Optimism | Optimism corrected | External validation in EPIC-Norfolk |
|---|---|---|---|---|---|
| Calibration Slope | 1.000 (0.862, 1.138) | 0.858 (0.656, 1.061) | 0.142 | 0.858 (0.720, 0.996) | 0.923 (0.770, 1.076) |
| C-statistic | 0.771 (0.740, 0.803) | 0.762 (0.752, 0.773) | 0.021 | 0.750 (0.719, 0.782) | 0.731 (0.694, 0.768) |
| $R^2$ | 0.388 (0.323, 0.452) | 0.370 (0.351, 0.390) | 0.051 | 0.337 (0.272, 0.401) | 0.314 (0.242, 0.387) |
| **Age, smoking, medical history + retinal vasculometry** | | | | | |
| Calibration Slope | 1.000 (0.869, 1.131) | 0.860 (0.665, 1.055) | 0.140 | 0.860 (0.729, 0.991) | 0.840 (0.710, 0.971) |
| C-statistic | 0.776 (0.744, 0.807) | 0.766 (0.754, 0.778) | 0.023 | 0.753 (0.721, 0.784) | 0.734 (0.695, 0.773) |
| $R^2$ | 0.408 (0.345, 0.472) | 0.386 (0.363, 0.409) | 0.056 | 0.352 (0.289, 0.416) | 0.323 (0.251, 0.394) |

*FRS=Framingham risk score*

Table S5 Model diagnostics (with 95% confidence intervals) for incident myocardial infarction (after retinal image capture) in UK Biobank (2009-2018) as defined in the methods. External validation in EPIC- Norfolk cohort using biomedical data from the third health check (2004-2011) with all incident ischaemic heart disease (ICD10 I20-I25) as the health outcome (2004-2018)

| Model | Apparent performance | Test performance | Average Optimism | Optimism corrected | External validation in EPIC |
|---|---|---|---|---|---|
| **FRS for confirmed MI (FRS)** | | **UK Biobank Men** | | | No. events = 173 |
| Calibration Slope | - | - | - | - | 1.562 (1.212, 1.912) |
| C-statistic | - | - | - | - | 0.689 (0.651, 0.727) |
| $R^2$ | - | - | - | - | 0.231 (0.153, 0.308) |
| **FRS + retinal vasculometry** | | | | | |
| Calibration Slope | 1.000 (0.838, 1.162) | 0.887 (0.771, 1.002) | 0.113 | 0.887 (0.725, 1.049) | 1.398 (1.072, 1.723) |
| C-statistic | 0.724 (0.697, 0.751) | 0.719 (0.710, 0.728) | 0.020 | 0.704 (0.677, 0.731) | 0.683 (0.646, 0.721) |
| $R^2$ | 0.270 (0.210, 0.330) | 0.259 (0.245, 0.273) | 0.043 | 0.227 (0.167, 0.287) | 0.212 (0.136, 0.288) |
| **Age, smoking, medical history + retinal vasculometry** | | | | | |
| Calibration Slope | 1.000 (0.837, 1.163) | 0.836 (0.715, 0.957) | 0.164 | 0.836 (0.673, 0.999) | 0.910 (0.664, 1.155) |
| C-statistic | 0.704 (0.676, 0.732) | 0.689 (0.677, 0.701) | 0.029 | 0.675 (0.647, 0.703) | 0.641 (0.599, 0.683) |
| $R^2$ | 0.242 (0.182, 0.302) | 0.213 (0.189, 0.236) | 0.064 | 0.178 (0.118, 0.238) | 0.151 (0.079, 0.223) |
| | | | | | |
| **FRS for confirmed MI (FRS)** | | **UK Biobank Women** | | | No. events = 116 |
| Calibration Slope | - | - | - | - | 0.883 (0.650, 1.116) |
| C-statistic | - | - | - | - | 0.694 (0.649, 0.740) |
| $R^2$ | - | - | - | - | 0.228 (0.135, 0.320) |
| | | | | | |
| **FRS + retinal vasculometry** | | | | | |

| Model | Apparent performance | Test performance | Average Optimism | Optimism corrected | External validation in EPIC |
|---|---|---|---|---|---|
| Calibration Slope | 1.000 (0.823, 1.177) | 0.849 (0.678, 1.021) | 0.151 | 0.849 (0.672, 1.026) | 0.678 (0.467, 0.890) |
| C-statistic | 0.794 (0.756, 0.831) | 0.786 (0.769, 0.803) | 0.028 | 0.766 (0.728, 0.803) | 0.670 (0.623, 0.717) |
| $R^2$ | 0.420 (0.338, 0.501) | 0.401 (0.371, 0.430) | 0.066 | 0.354 (0.272, 0.435) | 0.167 (0.080, 0.255) |
| **Age, smoking, medical history + retinal vasculometry** | | | | | |
| Calibration Slope | 1.000 (0.787, 1.213) | 0.803 (0.635, 0.970) | 0.197 | 0.803 (0.590, 1.016) | 0.907 (0.661, 1.153) |
| C-statistic | 0.748 (0.708, 0.788) | 0.733 (0.709, 0.757) | 0.039 | 0.709 (0.669, 0.749) | 0.672 (0.620, 0.725) |
| $R^2$ | 0.315 (0.225, 0.405) | 0.292 (0.254, 0.330) | 0.089 | 0.226 (0.136, 0.316) | 0.211 (0.119, 0.303) |

*FRS=Framingham risk score*