University of Dundee

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Mordi, Ify R.; Trucco, Emanuele; Syed, Mohammad Ghouse; MacGillivray, Tom; Nar, Aditya; Huang, Yu

Prediction of Major Adverse Cardiovascular Events from Retinal, Clinical and Genomic Data in Individuals with Type 2 Diabetes: A Population Cohort Study

Ify R Mordi MD[1], Emanuele Trucco PhD[2], Mohammad Ghouse Syed MTech[2], Tom MacGillivray PhD [3], Adi Nar MSc[4], Yu Huang PhD[4], Gittu George MSc[4], Stephen Hogg PhD[2], Radha Venkateshan PhD[5], Vijayaraghavan Prathiba MBBS[5], Ranjit Mohan Anjana MD[5], Viswanathan Mohan PhD[5], Colin NA Palmer PhD[4], Ewan R Pearson PhD[4], Chim C Lang MD[1], Alex SF Doney PhD[4] and the INSPIRED Investigators

1. Division of Molecular and Clinical Medicine, School of Medicine, University of Dundee, Dundee, United Kingdom

2. VAMPIRE project, Computing, School of Science and Engineering, University of Dundee, Dundee

3. VAMPIRE project, Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, United Kingdom

4. Division of Population Health and Genomics, School of Medicine, University of Dundee, Dundee, United Kingdom

5. Madras Diabetes Research Foundation, Chennai, India.

Short Title: Retinal and Genomic Prediction of MACE

Corresponding Authors: Ify Mordi and Alex Doney, School of Medicine, University of Dundee, Dundee, United Kingdom, DD1 9SY

Email: i.mordi@dundee.ac.uk; a.doney@dundee.ac.uk

Telephone: +44 1382 383106 (IM), +44 1382 388006 (AD)

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ABSTRACT

Objectives

Improved identification of individuals with type 2 diabetes at high cardiovascular risk could help in selection of newer cardiovascular risk-reducing therapies. The aim of this study was to determine whether retinal vascular parameters, derived from retinal screening photographs, alone and in combination with a genome-wide polygenic risk score for coronary heart disease (CHD PRS) would have independent prognostic value over traditional CV risk assessment in patients without prior cardiovascular disease.

Research Design and Methods

Patients in the GoDARTS study were linked to retinal photographs, prescriptions, and outcomes. Retinal photographs were analysed using VAMPIRE software, a semi-automated AI platform, to compute arterial and venous fractal dimension, tortuosity and diameter. CHD PRS was derived from previously published data. Multivariable Cox regression was used to evaluate the association between retinal vascular parameters and major adverse cardiovascular events (MACE) at 10 years compared to the pooled cohort equations (PCE) risk score.

Results

5,152 individuals were included. 1,017 individuals suffered a MACE. Reduced arterial fractal dimension and diameter and increased venous tortuosity each independently predicted MACE. A risk score combining these parameters significantly predicted MACE after adjustment for age, sex, PCE and the CHD PRS (HR 1.11 per SD increase; 95% CI 1.04-1.18, p=0.002) with similar accuracy to PCE (AUC 0.663 vs. 0.658, p=0.33). A model incorporating retinal parameters and PRS improved MACE prediction compared to PCE (AUC 0.686 vs. 0.658, p<0.001).

Conclusions

Retinal parameters alone and in combination with genome-wide CHD PRS have independent and incremental prognostic value compared to traditional CV risk assessment in type 2 diabetes.

Patients with type 2 diabetes have a disproportionately higher risk of atherosclerotic cardiovascular disease (ASCVD) morbidity and mortality compared with individuals without type 2 diabetes.[1, 2] Most of this excess risk has been attributed to the higher prevalence of conventional CV risk factors in patients with type 2 diabetes. Currently, risk estimates using scores, such as the American College of Cardiology/American Heart Association atherosclerotic CVD Pooled Cohort Equation (PCE) risk score, are recommended in clinical guidelines to identify at-risk patients for primary prevention therapy.[3, 4] While these risk scores are useful in identifying high-risk patients, they can overestimate CVD risk across the population and have poor calibration in those with type 2 diabetes.[5] It is also worth noting that the relationship between the presence of traditional CVD risk factors and atherosclerosis development is not necessarily direct and CVD events can occur despite effective traditional CVD risk management. Thus, substantial improvement in identifying individuals remaining at high CVD risk in type 2 diabetes is urgently needed, particularly in light of novel therapies such as sodium-glucose co-transporter 2 inhibitors and glucagon-like peptide-1 receptor agonists which reduce CVD risk.[6, 7]

Recent studies have shown that assessment of genomic risk of coronary heart disease (CHD) using genome-wide polygenic risk scores (PRS) can be integrated into traditional clinical risk prediction models.[8] These CHD-PRS have shown equivalent predictive accuracy to clinical risk scores, though they have not specifically been examined in the higher-risk type 2 diabetes population.[9, 10]

In the United Kingdom patients with type 2 diabetes undergo regular diabetes retinal screening (DRS) to manage risk of diabetic retinopathy. There is increasing interest in the potential use of DRS screening photographs as a source of screening for more global risk of diabetes complications beyond diabetic retinopathy including CV disease. Such a non-invasive direct assessment of global vascular risk would greatly enhance the efficiency of DRS and might provide incremental value beyond traditional CV risk markers. Previous work has demonstrated that the presence of retinopathy is associated with adverse CV events in individuals with and without type 2 diabetes.[11-14] Studies have also reported the association of specific retinal vascular morphometric parameters such as retinal vessel diameter[15, 16], tortuosity[17] and

fractal dimension (a measure of branching complexity)[18] with both CVD risk factors and CVD events. Recently, automated deep-learning approaches have been used to predict the presence of CVD risk factors on the basis of a retinal photograph alone[19] further indicating the retina can provide relevant information on CVD risk. Promisingly, this approach was also able to predict CV outcomes with a similar accuracy as traditional risk factors.

The aim of this study was to determine whether retinal vascular parameters measured from DRS photographs, alone and in combination with a genome-wide CHD-PRS would have independent and incremental prognostic value over traditional CVD risk assessment.

RESEARCH DESIGN AND METHODS

Study Cohort

Individuals with type 2 diabetes from the Genetics of Diabetes Audit and Research Tayside Scotland (GoDARTS) study were used for this study. GoDARTS has been previously described.[20] In brief, GoDARTS is a cohort study in the Tayside region of Scotland (population ~400,000) that began recruiting in 1996 and up to 2015 included 10,149 individuals with type 2 diabetes and 8,157 controls without type 2 diabetes at the time of recruitment. Data on clinical and lifestyle parameters were collected at the time of recruitment, and participants also provided consent to electronic health record linkage for past and future clinical events including laboratory tests, eye screening, hospital admissions and death. Patients also provided a sample of blood for genotyping, and genome-wide association was performed using a number of separate genotyping arrays including the Affymetrix Genome-Wide Human SNP (single nucleotide polymorphism) Array 6.0, the Illumina HumanOmniExpress, Immunochip, Metabochip or the Human Exome array. The GoDARTS study and electronic health record (EHR) linkage has been approved by the East of Scotland Research Ethics Committee. The EHR is fully anonymized and provided to researchers through robust information governance protocols administered by the Health

Informatics Centre (HIC) Safehaven, including research ethics approval for studies conducted within the Safehaven environment.

Derivation of the cohort for this study is summarised in **Supplementary Figure 1**. As per current recommendations for use of the PCE risk score we selected patients aged between 40 and 79 years old with no prior history of hospitalisation for myocardial infarction (MI) or stroke using ICD-10 codes I21-I23 and I60-I63. We used the date of the retinal photograph used for obtaining vascular parameter as the study entry date. For clinical measurements e.g. blood pressure, glycated haemoglobin (HbA1c), cholesterol where there was more than one measure prior to the date of retinal imaging the median of values within the preceding 3 years was used to provide a measurement reflective of the true value. These values were used to calculate the PCE risk score for 10-year risk of CVD events at the time of retinal imaging.[21] The genome-wide CHD-PRS was assembled for each GoDARTS participant by integrating data across the various genotyping platforms based on the genome-wide analysis and data provided by Khera *et al*.[22] We used the "score" function in plink 1.9 to generate the PRS (the file was preprocessed to include one line per scored variant). To avoid issues of variable availability of single nucleotide polymorphism (SNP) assays between genotyping arrays, giving rise to individual variability in score simply due to available SNP numbers, the CHD-PRS was *z*-transformed.

Retinal Vascular Parameters

The Scottish National DRS uses standardised protocols that are used across all participating centres. Further details are available at https://www.ndrs.scot.nhs.uk/. In the Scottish DRS the retina is photographed with a 45° view centred on the macula. The earliest available DRS digital retinal photographs were obtained for patients with type 2 diabetes in GoDARTS. These photographs had been previously reported for the presence of retinopathy by trained ophthalmologists for the purposes of clinical management at the time. No further analysis or selection was performed prior to assessment using the VAMPIRE (Vascular Assessment and Measurement Platform for Images of the Retina; version 3.1, Universities of Edinburgh and Dundee, UK) software platform. Further details on the VAMPIRE analysis

pipelines including inter-observer variability have been published previously. The right eye was prioritised for measurement of vascular parameters. Where the right eye photograph was not of sufficient quality, based on the related Scottish DRS data, the left eye photograph was selected. The measurement methodology, variability and workflow for VAMPIRE has been previously described.[23-27] While VAMPIRE measures a comprehensive range of retinal vascular parameters using a semi-automated AI approach, for this analysis we considered three of the most widely investigated retinal vascular parameters for both retinal arterioles and venules - fractal dimension of the retinal vascular pattern (FDa and FDv), tortuosity (torta and tortv), and central retinal artery and vein equivalent (CRAE and CRVE) which summarise vessel calibre.

Clinical Outcomes

The primary outcome for this study was the time-to-first incidence of a composite 3-point major adverse cardiovascular event (MACE) comprising CV death, non-fatal myocardial infarction (MI) and non-fatal stroke after the date of the retinal photograph. Patients were followed up to December 2017 for a maximum of 10 years up from the date of the analysed retinal photograph until the first qualifying event (CV death, MI or non-fatal stroke) or censored at non-CV death. Follow-up was limited to 10 years to correspond to the duration of CVD prediction from the PCE risk score. Cause and date of death was obtained from the General Register of Scotland, with any ICD-10 code from I00-I99 within the first 2 causes of death recorded as a CV death. Non-fatal MI and stroke events were determined from the Scottish Morbidity Record of hospitalisations using the same ICD codes.

Statistical Analysis

Continuous variables are reported as mean ± standard deviation or median and interquartile range as appropriate and categorical variables as number and percentage. Retinal vascular parameters were standardised by z-transformation to facilitate comparisons. The correlations between continuous clinical variables, the PCE 10-year CV risk score and the CHD PRS and retinal vascular parameters were

assessed using Pearson's correlation coefficient; t-tests were performed to assess differences between categorical clinical variables and retinal parameters.

Associations between the PCE risk score, CHD PRS and retinal vascular parameters and time-to-first-MACE were evaluated using Cox proportional hazards regression with adjustment for age and sex. Hazard ratios for retinal vascular parameters and the CHD PRS are reported per standard deviation (SD) increase in z-transformed value. We created a combined retinal risk score based on the beta coefficients from the adjusted Cox regression model of retinal vascular parameters that were significantly associated with MACE. The independent association of the retinal risk score with MACE was assessed using Kaplan-Meier analysis and multivariable Cox regression with adjustment for age, sex, duration of diabetes, glycated haemoglobin, PCE risk score and the CHD PRS. The incremental predictive value of the retinal risk score and the CHD PRS in addition to PCE risk score was assessed using receiver-operator characteristic (ROC) curves, with the area under the curve (AUC) compared. Additionally, we calculated the continuous net reclassification index (NRI) and integrated discrimination index (IDI). All tests were two-sided and a p value <0.05 was considered significant. All analyses were performed using R version 3.5.1.

RESULTS

Baseline Characteristics

In total 5,152 individuals with type 2 diabetes without a prior MACE were included in the primary analysis **(Supplementary Figure S1)**. Baseline cohort characteristics are summarised in **Table 1**. The selected cohort was similar to the overall type 2 diabetes population in GoDARTS **(Supplementary Table S1)**. On the date of the photograph used for measurement and entry into the study the mean age of the cohort was 65.2 ± 9.3 years and 43.9% of the cohort was female. The median duration since diagnosis of diabetes was 6.7 years, and mean glycated haemoglobin was 59.1 ± 12.7 mmol/l (7.6 ± 1.2%). 1,130 individuals (21.9%) had any level of diabetic retinopathy at the time of retinal screening, while only 11

individuals (1.9%) had proliferative retinopathy. Median total cholesterol was 4.4 mmol/l. As expected, the population was at a relatively high CV risk, with a median PCE 10-year risk of ASCVD estimated at 29%. The majority of the population (74%) were on statin therapy at the time of retinal imaging.

Association of Individual Retinal Vascular Morphological Parameters with Clinical Variables

Correlations between retinal vascular parameters and continuous clinical and genomic risk were weak, with all *r* values between -0.1 and 0.1 **(Supplementary Figure S2)**. Each individual retinal parameter was only weakly correlated with PCE 10-year ASCVD risk (r values between -0.07 and 0.05) and the CHD PRS (r values between -0.03 and 0.05).

Association between Individual Retinal Vascular Parameters and Cardiovascular Outcomes

At 10 years (median follow-up 9.8 years), 1,017 individuals had a MACE occurrence (19.7% of the whole cohort), including 794 CV deaths (15.4%), 274 non-fatal MI (5.3%) and 151 non-fatal stroke (2.9%).

After adjustment for age and sex, increased arterial fractal dimension (FDa), decreased venular tortuosity (tortv) and increased CRAE were all significantly associated with MACE incidence **(Table 2)**. After additional adjustment for PCE risk score and the CHD PRS these variables remained independently associated with MACE with little change in the HR estimates (FDa HR 0.93 per SD increase; 95% CI 0.86-1.00, p=0.040; tortv HR 1.08; 95% CI 1.01-1.15, p=0.019; CRAE HR 0.90; 95% CI 0.83-0.98, p=0.015).

Combined Retinal Score and Association with Clinical Risk Factors and Outcomes

Using the beta coefficients from the Cox model adjusted for age, sex, PCE risk score and CHD PRS (shown in Table 2) for the association of FDa (β=-0.08), tortv (β=0.07) and CRAE (β=-0.10) with MACE we constructed an overall retinal risk score as follows:

$$Retinal\ risk\ score = (-0.08*FDa) + (0.07*tortv) + (-0.10*CRAE)$$

Males had higher retinal risk scores than females (1.99 vs. 1.79, p<0.001). There was no difference in the retinal risk score between smokers and non-smokers (1.90 vs. 1.91 respectively, p=0.89). Similar to individual retinal vascular parameters, correlations between the retinal risk score and continuous clinical risk factors were weak **(Supplementary Figure S2)** with the greatest correlation being with systolic blood pressure (r=0.084), followed by duration of diabetes (r=0.074) and age (r=0.062). While the retinal risk score was weakly correlated with the PCE risk score (r=0.094) there was no correlation with CHD PRS (r=-0.021).

After adjustment for age, sex, glycated haemoglobin, diabetes duration, PCE risk score and the CHD PRS the retinal risk score was significantly associated with incidence of MACE (HR 1.11; 95% CI 1.04-1.18, p=0.002) **(Table 3)**. Patients in the highest tertile of retinal risk score had significantly increased likelihood of MACE incidence than those in the lowest tertile (HR 1.32; 95% CI 1.13-1.55, p<0.001) **(Supplementary Figure S3)**. There was a significant interaction between the retinal risk score and age, with the retinal risk score being more strongly associated with outcome in younger patients (median age 66.3 years – <66.3 years old HR 1.23; 95% CI 1.09-1.38, p<0.001; ≥66.3 years HR 1.05; 95% CI 0.97-1.14, p=0.20; interaction p value 0.012). The retinal risk score was more strongly associated with MACE in individuals at the lowest genetic or clinical risk, however the interaction between the retinal risk score and the PRS or PCE scores did not reach statistical significance (p=0.14 and p=0.09 respectively) **(Supplementary Table S2)**.

A model combining age, sex and the retinal risk score had similar predictive performance to the PCE risk score (AUC 0.663 vs. 0.658 respectively) **(Figure 1 and Supplementary Table S3)**. There was a small improvement in NRI (0.080, 95% CI 0.010-0.150, p=0.024), though there was no improvement in the IDI. A model including age, sex, retinal risk score and the CHD PRS performed significantly better than the PCE risk score (AUC 0.686 vs. 0.658 respectively, p<0.001; IDI 0.019, 95% CI 0.013-0.025; NRI 0.240, 95% CI 0.147-0.285, both p<0.001). The addition of the PCE score to a model with age, sex, retinal risk

score and the CHD PRS did provide modest improvement (AUC 0.690 vs. 0.686, p=0.033; IDI 0.004, 95% CI 0.002-0.006, p<0.001; NRI 0.07, 95% CI 0.00-0.14, p=0.05).

CONCLUSIONS

We have identified several key findings in this analysis of patients with type 2 diabetes without a prior history of myocardial infarction or stroke. First, we have shown that a simple retinal risk score based on these retinal parameters is independently associated with MACE and has similar performance to an established clinical risk score for prediction of 10-year MACE incidence. Second, we found that the combination of the retinal risk score plus a CHD polygenic risk score had incremental prognostic value for prediction of MACE over the PCE risk score alone. These findings raise the possibility that clinical CV risk prediction could potentially be achieved using routinely obtained retinal photographs, obviating the need for logistically more complex, costly and inconvenient clinic attendances for blood sampling and blood pressure assessments and clinical history taking etc. Such an approach may have particular benefits in remote rural communities where access to healthcare is limited as retinal photography can be relatively easily acquired, using mobile phone technology and portable cameras.[28] Genome-wide data also provides improved prediction, only needs to be obtained once, is increasingly cheap and convenient to obtain and also can be obtained remotely by relatively simple procedures. Use of routinely obtained retinal photographs may be a particularly efficient method of determining CV risk in patients with type 2 diabetes.

Previous studies have shown that some retinal vascular parameters are associated with increased CV risk, although many of these studies are limited by their cross-sectional study design. Most have reported only associations of retinal vascular diameter (CRAE and CRVE) with CV risk factors. A recent cross-sectional study of over 50,000 individuals from UK Biobank found an association between narrower retinal arterioles and higher systolic blood pressure and arterial stiffness.[29] This study also reported opposing results for retinal venous diameter, which replicated results from previous studies.[30, 31]

Consistent with other studies, we found that narrower retinal arterioles are associated with worse outcome.[15, 16, 32, 33]

Fewer studies have evaluated the association of fractal dimension with CV risk. Importantly very few studies have considered the arterial and venous fractal dimensions separately, with most combining the two for an overall assessment. We found that arterial but not venous fractal dimension was independently associated with MACE. Our finding that increased arterial fractal dimension was associated with reduced MACE incidence is supported by other studies showing that lower overall fractal dimension was associated with older age and higher mean arterial blood pressure.[34-36] Liew et al. evaluated 3303 individuals and reported a U-shaped association between overall fractal dimension and CV outcomes, with those with fractal dimension in the lowest and highest quartiles having the highest risk of CHD mortality.[18] This has not been replicated elsewhere; we found that arterial and venous fractal dimension had opposing associations with MACE, which may explain their findings. A small cross-sectional study of 55 individuals did document opposing associations between retinal arterial and venous fractal dimension and cerebrovascular magnetic resonance imaging findings.[37]

A key novel aspect of our study is the fact that we have simultaneously considered multiple retinal parameters, and this allowed us to combine the independent features into a simple retinal risk score, and compared this with the PCE risk score. While we only found very weak correlations between the retinal risk score and conventional clinical risk factors the retinal risk score was independently associated with MACE even after adjustment for clinical and genetic risk. This may indicate that the information provided by the retina is, to an extent, independent of and additional to conventional clinical and genomic risk factors, and may indicate endogenous phenotypic susceptibility to lifestyle and genetic background. Our finding that the retinal risk score was performed at least as well as the PCE score further underscores the potential for routinely obtained retinal photographs to provide an assessment of global CV health, providing added value to diabetes retinal screening programmes. The ability to directly image the

vasculature via the retina is particularly attractive, and assessment of the retina appears to provide novel information related to CV risk.

A further unique aspect to our study is the incorporation of a large genome-wide polygenic risk score for CHD. These PRS are becoming increasingly available (https://www.pgscatalog.org), and recent studies have tested their ability to provide incremental risk prediction in addition to traditional CV risk markers. Inouye et al showed that a large CHD PRS was able to predict adverse outcome, and had a higher C-statistic than any individual risk factors.[8] As in our study, a CHD PRS has also been shown to have incremental value over the PCE risk score[10]. Taking this approach one step further, we have shown that the combination of retinal and polygenic risk scores (in addition to age, sex, HbA1c and duration of diabetes) performs significantly better for prediction of MACE than the PCE risk score.

Our study may have useful clinical implications. While we do not suggest that retinal and polygenic risk score could completely replace clinical risk factor measurements, their use could have additional clinical value in identifying higher-risk patients who could benefit from cardio-protective therapies such as SGLT2 inhibitors and GLP-1 receptor agonists. Recent work has demonstrated the use of a CHD PRS in the ODYSSEY-OUTCOMES trial independent of LDL-cholesterol, and those with higher PRS derived greater benefit from alirocumab.[38] With low-cost genome-wide genotyping becoming increasingly available, and routine type 2 diabetes retinal screening conducted in many countries, it is likely that these could be readily incorporated into routine clinical practice.

Our study does have some limitations. Despite our large sample size and longitudinal data incorporating genetics, it remains an observational study with its inherent limitations. Our cohort only includes individuals with type 2 diabetes and so cannot necessarily be extrapolated to individuals without type 2 diabetes. It would be interesting in future work to apply this approach individuals without diabetes. Similarly, our cohort is predominantly Caucasian, so the influence of the CHD PRS may be different in other ethnicities. We only used three retinal parameters – it is possible that other retinal parameters may also provide independent prognostic value. The retinal risk score was created and validated within one

cohort, and thus lacks external validation. Finally, we used specific software to analyse retinal images, and parameter values may not be the same as those obtained from other retinal image analysis.[26] Further work needs to be undertaken to standardise and refine analytical pathways to allow adoption into clinical practice.[26, 39]

In individuals with type 2 diabetes with no prior history of MACE, a simple retinal risk score obtained from routine retinal photographs using a semi-automated AI approach to artery and vein classification was able to predict incident MACE at 10 years with similar performance to the pooled cohort equations ASCVD risk score, particularly in younger individuals. The combination of retinal and genomic risk scores had independent and incremental prognostic value over the clinical risk score. Incorporation of these measures into routine clinical practice might help identify individuals at high CV risk over and above traditional clinical risk factors who might benefit from intensified CV-protective therapy, and may even represent a feasible alternative to traditional clinical risk assessment.

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Conflicts of Interest: None

A list of abbreviations is provided as Supplementary Table S4.

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FIGURE LEGEND

1. Area Under the ROC Curve Analysis.

   Comparison of PCE, retinal and genetic risk models for prediction of MACE at 10 years showing

   the area under the curve and 95% confidence intervals. p values all vs. PCE risk score alone.

Table 1. Baseline Characteristics

	Total number of patients = 5,152
Age (years)	65.2 ± 9.3
Female number (percent)	2,263 (43.9)
Diabetes duration (years), median (IQR)	6.7 (3.8-10.6)
Smoking history number (percent)	2602 (50.5)
Glycated haemoglobin (mmol/mol)	59.1 ± 12.7
Glycated haemoglobin (%)	7.6 ± 1.2
Systolic blood pressure (mmHg)	139 ± 11
Diastolic blood pressure (mmHg)	77 ± 8
Body mass index (kg/m^2)	32 ± 6
Total cholesterol (mmol/l)	4.4 ± 0.9
HDL cholesterol (mmol/l)	1.3 ± 0.3
PCE 10-year ASCVD risk (%), median (IQR)	29 (16-42)
Oral Anti-hyperglycaemic therapy only	1935 (37.6)
Insulin use	1408 (27.3)
Aspirin use	2,243 (43.5)
Statin use	3,817 (74.1)
Any retinopathy	1,229 (23.9)

Continuous variables reported as mean ± standard deviation unless otherwise stated.

IQR – interquartile range; PCE – pooled cohort equations; ASCVD – atherosclerotic cardiovascular disease

Table 2. Association of Individual Retinal Vascular Parameters with Incidence of Major Adverse Cardiovascular Events at 10 years.

	Hazard Ratio Adjusted for Age and Sex (95% CI)	p value	Hazard Ratio Adjusted for Age, Sex, PCE and CHD PRS (95% CI)	Beta (se)	p value
Fractal Dimension (arterial)	0.92 (0.85-0.99)	**0.020**	0.93 (0.86-1.00)	-0.077 (0.038)	**0.040**
Fractal Dimension (venous)	1.01 (0.94-1.09)	0.77	1.02 (0.94-1.10)	0.016 (0.039)	0.69
Tortuosity (arterial)	0.99 (0.93-1.05)	0.64	0.97 (0.91-1.04)	-0.030 (0.033)	0.37
Tortuosity (venous)	1.08 (1.01-1.15)	**0.022**	1.08 (1.01-1.15)	0.077 (0.033)	**0.019**
Central Retinal Artery Equivalent	0.88 (0.81-0.96)	**0.003**	0.90 (0.83-0.98)	-0.104 (0.043)	**0.015**
Central Retinal Venous Equivalent	1.06 (0.98-1.14)	0.16	1.05 (0.97-1.14)	0.047 (0.040)	0.25

All hazard ratios per standard deviation increase. CHD – coronary heart disease; CI – confidence interval; PCE – pooled cohort equations risk score; PRS – polygenic risk score, se – standard error

Table 3. Association of Retinal, Clinical and Polygenic Risk Scores with Incidence of Major Adverse Cardiovascular Events at 10 years.

	Unadjusted Hazard Ratio (95% CI)	p value	Adjusted Hazard Ratio (95% CI)	p value
Retinal Risk Score (per SD increase)	1.21 (1.13-1.28)	**<0.001**	1.11 (1.04-1.18)	**0.002**
PCE (per 5% increase)	2.01 (1.86-2.17)	**<0.001**	1.40 (1.20-1.63)	**<0.001**
CHD PRS (per SD increase)	1.60 (1.44-1.79)	**<0.001**	1.68 (1.49-1.90)	**<0.001**

Combined retinal risk score = (-0.08*FDa) + (0.07*tortv) + (-0.10*CRAE).

Multivariable model included: age, sex, glycated haemoglobin, duration of diabetes, retinal risk score PCE and CHD PRS. All hazard ratios per standard deviation increase.

CHD – coronary heart disease; CI – confidence interval; PCE – pooled cohort equations risk score; PRS – polygenic risk score

Figure 1.
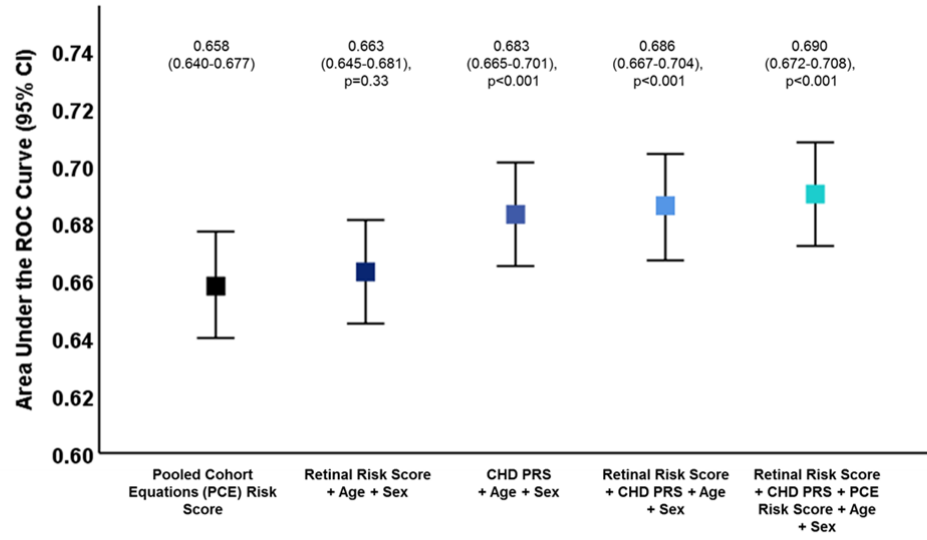
Supplementary Table S1. Comparison of Baseline Characteristics between the Selected Individuals for this Study and the Full Type 2 Diabetes Population in GoDARTS.

	Current Study (n=5,152)	GoDARTS Type 2 Diabetes Cases (n=8,698)
Age (years)	65	67
Male (%)	56.1	56.3
Body Mass Index (kg/m2)	32	31
Smoking history (%)	51	63
Systolic blood pressure (mmHg)	139	141
Diastolic blood pressure (mmHg)	77	77
Glycated Haemoglobin (mmol/mol)	59.6	60.7
Glycated Haemoglobin (%)	7.6	7.7
Total Cholesterol (mmol/l)	4.4	4.3
HDL-Cholesterol (mmol/l)	1.3	1.3

GoDARTS Type 2 diabetes baseline data taken from Hebert et al., 2018 (reference 20 in the manuscript)

Supplementary Table S2. Association between Retinal Risk Score and Major Adverse Cardiovascular

Events Across Genetic and Clinical Risk Tertiles

	Adjusted Hazard Ratio (95% CI)	p value	Interaction p value
Polygenic Risk Score Tertile			0.13
1	1.17 (1.03-1.32)	**0.014**	
2	1.08 (0.97-1.22)	0.17	
3	1.08 (0.97-1.19)	0.15	
Pooled Cohort Equations Risk Score Tertile			0.09
1	1.17 (1.01-1.35)	**0.031**	
2	1.18 (1.06-1.31)	**0.002**	
3	1.03 (0.93-1.14)	0.62	

Multivariable model included: age, sex, glycated haemoglobin, duration of diabetes, retinal risk score

PCE and CHD PRS. All hazard ratios per standard deviation increase.

Supplementary Table S3. Area Under the Curve, Integrated Discrimination Improvement and Continuous Net Reclassification for Retinal Risk

Score and CHD PRS versus the Pooled Cohort Equations ASCVD Risk Score for Prediction of Major Adverse Cardiovascular Events at 10 Years.

	Model 1	Model 2		Model 3		Model 4		Model 5	
	PCE only (baseline)	Retinal Risk Score + Age + Sex	p value	CHD PRS + Age + Sex	p value	CHD PRS + Retinal Risk Score + Age + Sex	p value	CHD PRS + Retinal Risk Score + ASCVD Risk Score + Age + Sex	p value
AUC	0.658	0.663	0.33	0.683	<0.001	0.686	<0.001	0.690	<0.001
	(0.640-0.677)	(0.645-0.681)		(0.665-0.701)		(0.667-0.704)		(0.672-0.708)	
IDI (vs. PCE only)	-	0.001	0.79	0.018	<0.001	0.019	<0.001	0.023	<0.001
		(-0.003-0.004)		(0.012-0.023)		(0.013-0.025)		(0.018-0.028)	
Continuous NRI (vs. PCE only)	-	0.080	0.024	0.216	<0.001	0.240	<0.001	0.319	<0.001
		(0.010-0.150)		(0.147-0.285)		(0.171-0.309)		(0.251- 0.388)	

AUC – Area Under the Curve; PCE – Pooled Cohort Equations Atherosclerotic Cardiovascular Disease Risk Score; PRS – Coronary Heart

Disease Genetic Risk Score; IDI – Integrated Discrimination Improvement; NRI – Net Reclassification Index.

Bold indicates p<0.05. All p values vs. Model 1.

Supplementary Table S4. List of Abbreviations.

AI – artificial intelligence

ASCVD – atherosclerotic cardiovascular disease

AUC – area under the curve

CHD – coronary heart disease

CRAE – central retinal arteriolar equivalent

CRVE – central retinal venular equivalent

CVD – cardiovascular disease

DRS – diabetes retinal screening

EHR – electronic health record

FD(a/v) – fractal dimension (arterial/venous)

GoDARTS – Genetics of Diabetes Audit and Research Tayside Study

HbA1c – glycated haemoglobin

HR – hazard ratio

ICD – International Classification of Diseases

IDI – integrated discrimination index

MACE – major adverse cardiovascular events

MI – myocardial infarction

NRI – net reclassification index

PCE – pooled cohort equations

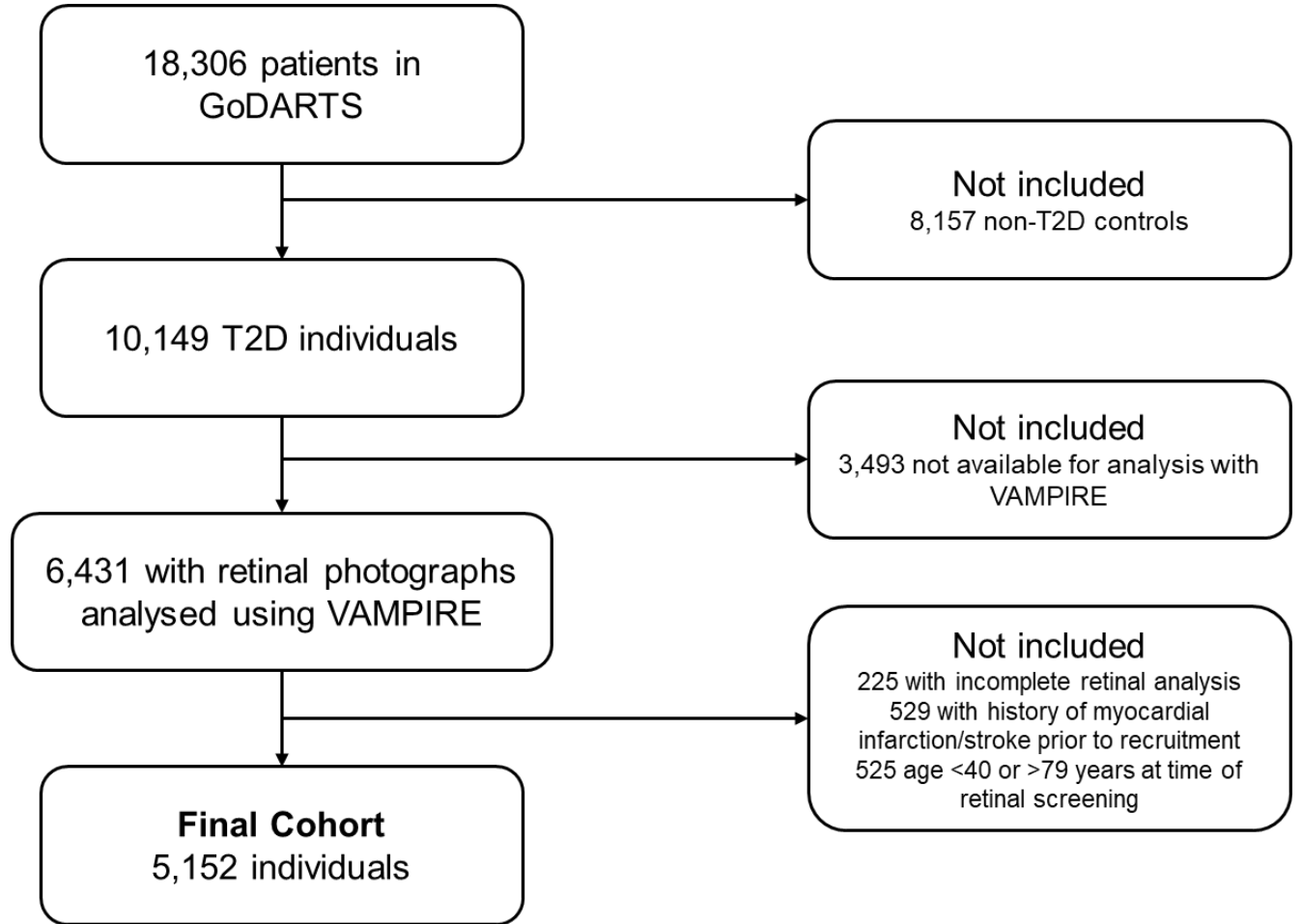PRS – polygenic risk score

ROC – receiver operator characteristic

SNP – single nucleotide polymorphism

Tort (a/v) – tortuosity (arterial/venous)

VAMPIRE – Vascular Assessment and Measurement Platform for Images of the Retina
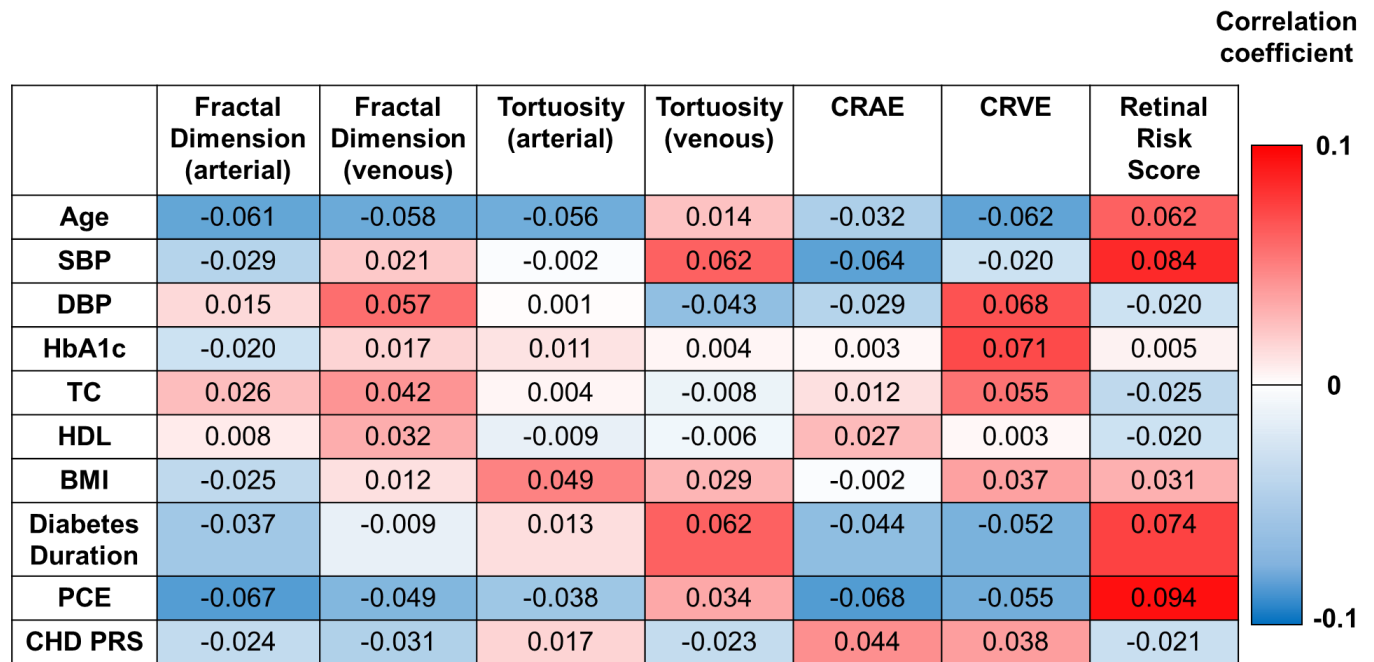
Supplementary Figure 1. Study Flowchart.

Cohort derivation.

Supplementary Figure 2. Correlations between Retinal Vascular Parameters and Continuous Clinical and Genomic Risk Factors.

Correlation matrix showing correlation coefficent (Pearson r) between Retinal Vascular Parameters and Continuous Clinical and Genomic Risk Factors.

Correlation coefficient

	Fractal Dimension (arterial)	Fractal Dimension (venous)	Tortuosity (arterial)	Tortuosity (venous)	CRAE	CRVE	Retinal Risk Score
Age	-0.061	-0.058	-0.056	0.014	-0.032	-0.062	0.062
SBP	-0.029	0.021	-0.002	0.062	-0.064	-0.020	0.084
DBP	0.015	0.057	0.001	-0.043	-0.029	0.068	-0.020
HbA1c	-0.020	0.017	0.011	0.004	0.003	0.071	0.005
TC	0.026	0.042	0.004	-0.008	0.012	0.055	-0.025
HDL	0.008	0.032	-0.009	-0.006	0.027	0.003	-0.020
BMI	-0.025	0.012	0.049	0.029	-0.002	0.037	0.031
Diabetes Duration	-0.037	-0.009	0.013	0.062	-0.044	-0.052	0.074
PCE	-0.067	-0.049	-0.038	0.034	-0.068	-0.055	0.094
CHD PRS	-0.024	-0.031	0.017	-0.023	0.044	0.038	-0.021

0.1

0

-0.1

SBP – systolic blood pressure; DBP – diastolic blood pressure; HbA1c – glycated haemoglobin; TC – total cholesterol; HDL – high-density lipoprotein cholesterol; BMI – body mass index; PCE – pooled cohort equations atherosclerotic cardiovascular risk score; CHD PRS – coronary heart disease polygenic risk score; CRAE – central retinal arterial equivalent; CRVE – central retinal venous equivalent

Supplementary Figure 3. Association between Retinal Risk Score and Major Adverse Cardiovascular Events.

Kaplan-Meier curve showing the association between tertiles of retinal risk score and time to first MACE incidence.