Hyperglycaemia-associated Caspase-3 predicts diabetes and coronary artery disease events

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Abstract

Background—Apoptosis is central in both diabetes and atherosclerosis, linked to pancreatic beta cell death and plaque progression. Circulating Caspase-3 has also been associated with diabetes and coronary calcium score. Here, we explored if soluble Caspase-3 (sCaspase-3) is associated with cardio-metabolic risk factors and predicts incidence of diabetes and coronary artery disease (CAD).

Methods—Clinical data and plasma from 4637 individuals from the Malmö Diet and Cancer cohort were studied. Plasma sCaspase-3 was measured by a Proximity Extension Assay. National registers were used to identify diabetes and CAD events during follow-up. Type 2 diabetes risk variants and expression quantitative trait loci (eQTL) for sCaspase-3 were retrieved from the DIAGRAM consortium and the Genotype-Tissue Expression project.

Results—HbA1c was the factor with the strongest association with sCaspase-3 ($r = 0.18$, $P = 1.3 \times 10^{-36}$). During follow-up 666 individuals developed diabetes and 648 individuals suffered from CAD. Increasing sCaspase-3 was associated with a higher risk of developing diabetes

(hazard ratio (HR) 1.18 per 1unit; $P = 7 \times 10^{-5}$) and CAD (HR 1.2 per 1 unit, $P = 1 \times 10^{-4}$) during follow-up. A genetic variant rs60780116, located upstream of CASP3, showed strong association with type 2 diabetes (OR 1.06, 95%CI 1.04–1.07, $P = 8.4 \times 10^{-11}$). An eQTL was identified between this variant and gene expression of CASP3, where the allele positively correlated with type 2 diabetes was associated with increased CASP3 expression in blood.

Conclusions—The present study provides evidence for plasma sCaspase-3 as a marker of cardio-metabolic risk factors and as a predictor of future diabetes and CAD in a cohort without cardiovascular disease or diabetes at baseline.

Keywords

apoptosis; atherosclerosis; biomarker; diabetes

Introduction

Apoptosis is a central process in both diabetes and atherosclerosis. The activation of apoptosis can occur through the extrinsic death receptor pathway or the intrinsic mitochondrial pathway leading to the activation of effector caspases, including Caspase-3. Soluble Caspase-3 (sCaspase-3) is detectable in the circulating blood, most likely released from cells exposed to pro-apoptotic stimuli or from cells undergoing apoptosis [1–3].

Studies on human pancreatic tissue have revealed that apoptotic beta cells are more common in patients with type 2 diabetes (T2D) and that Caspase-3 activation might be induced by metabolic stress which could be an important pathophysiological mechanism in T2D [4–7]. In line with this, plasma levels of Caspase-3 were shown, in the Dallas Heart study, to be increased in patients with diabetes, but if Caspase-3 could predict future diabetes remains unexplored [8].

The Dallas Heart study also showed that plasma Caspase-3 was associated with coronary calcium score, suggesting that it could be used as a stage marker of coronary atherosclerotic disease [8]. As apoptosis is increased in atherosclerotic plaques from patients with T2D and apoptosis may contribute to both plaque growth and ruptures, sCaspase-3 might also be a potential marker of atherosclerotic complications [9–13]. Recent studies from the population-based Malmö Diet and Cancer cohort reported non-significant relationships between sCaspase-3 and incidence of coronary events [14] and ischaemic stroke [15]. However, non-acute coronary interventions were not included. Since sCaspase-3 has been linked to diabetes and atherosclerosis, the latter being the underlying reason for most non-acute coronary interventions, we examined the relationship between sCaspase-3 and incidence of diabetes and coronary artery disease events (CAD), which also includes non-acute coronary interventions, over an extended 27-year follow-up period.

Methods

Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to Anders Dahlin at Lund University.

Description of the study cohort

The Malmö Diet and Cancer Study Cardiovascular Cohort (MDCS-CC) was used and is based on 6103 randomly selected samples from the Malmö Diet and Cancer Study (collected between 1991 and 1994), as described previously [16–18].

All participants provided written informed consent. The study was approved by the Regional Ethical Review Board in Lund, Sweden (LU51/90), and was carried out in accordance with the Helsinki Declaration.

The plasma levels of sCaspase-3 were assessed in 4637 of the 6103 patients. All patients without assessed sCaspase-3 were excluded prior to analysis.

To explore whether sCaspase-3 predicted incidence of diabetes, all patients with a previous diagnosis of diabetes or fasting whole blood glucose 6.1 mmol/L (corresponding to plasma glucose 7.0 mmol/L) were excluded ($n = 384$) [19]. To explore whether sCaspase-3 predicted incidence of CAD, all patients with prevalent CAD at baseline were excluded ($n = 94$).

Information regarding clinical risk factors

Clinical information regarding medications (lipid lowering, antihypertensive), diabetes, (defined as fasting whole blood glucose 6.1 mmol/L or previous diabetes diagnosis), current smoking and blood pressure (defined as blood pressure >159/94 mmHg or treatment for high blood pressure) was collected using self-administered questionnaires at inclusion. Waist circumference, weight, standing height, body mass index (BMI) and blood pressure were measured as previously described [20]. Fasting levels of cholesterol (total cholesterol, high-density lipoprotein (HDL), triglycerides, glucose and HbA1c) were measured in blood samples according to the standard procedures at the Department of Clinical Chemistry, Skåne University Hospital, Malmö. Friedewald's formula was used to calculate low-density lipoprotein (LDL) levels. Insulin was measured together with fasting glucose to assess Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) as previously described [20–22]. HbA_{1c} (percentage) was determined by using ion-exchange chromatography, and C-reactive protein (CRP; milligrams per litre) was analysed with a Tina-quant CRP latex assay (Roche Diagnostics, Basel, Switzerland) as previously described [20].

Plasma sCaspase-3 levels

The Proseek® Multiplex Oncology I 96×96 reagent kit (proximity extension assay technology) was used to measure plasma levels of sCaspase-3 as previously described [15]. Plasma sCaspase-3 is expressed in arbitrary units, and the measuring range was 61 to 62500 pg/mL for Caspase-3. The coefficient of variation was 16% for sCaspase-3.

Genome-wide association study (GWAS) and genetic regulation of Caspase-3

The GWAS summary statistics for T2D were retrieved from the DIAGRAM GWAS meta-analysis ($N = 898,130$), and results on expression quantitative trait loci (eQTL) in human whole blood were retrieved from the Genotype–Tissue Expression (GTEx) Project ($N = 670$) [23,24]. Genetic variants in cis-regulatory elements of Caspase-3 were restricted to a

maximum of 250 kb from the transcription start site of Caspase-3 and with a minor allele frequency greater than 0.01.

Follow-up

The Swedish Hospital Discharge Register, the hospital-based outpatient care, the National Cause-of-Death Register and the Swedish Coronary Angiography and Angioplasty Register were used to identify CAD during the follow-up period to 31 December 2018. CAD included non-fatal myocardial infarction ($n = 212$), fatal myocardial infarction ($n = 94$), death by ischaemic heart disease ($n = 44$), coronary artery bypass graft surgery ($n = 92$) and percutaneous coronary intervention ($n = 206$) based on the International Classification of Diseases 9th and 10th revisions (ICD-9 and ICD-10) as previously described [22].

To identify diabetes events, both local and national registers were used, including the Swedish National Diabetes Register, the Regional Diabetes 2000 Register of the Scania Region, the Malmö HbA_{1c} Register, the Swedish Inpatient Register, the Swedish Outpatient Register and the nationwide Swedish drug prescription register as previously described [20,25]. Diabetes events were classified as two occasions of fasting plasma glucose concentration 7.0 mmol/L in the Swedish National Diabetes Register and the Diabetes 2000 Register. In the Malmö HbA_{1c} Register, a new diabetes event was identified by two HbA_{1c} measurements 42 mmol/mol (6.0%). Finally, new-onset diabetes was identified using the Swedish Inpatient and Outpatient Registers and the nationwide prescription register by prescriptions of insulin or other antidiabetic medication (Anatomical Therapeutic Chemical Classification System Code A10).

Statistical analysis

A Mann–Whitney U-test was used for two group comparisons and Spearman's correlation coefficient for correlations. A multiple linear regression model was used to identify which factors were associated with sCaspase-3 levels. To achieve that, we compared the change in R2 in a leave-one-out (LOO) analysis for each variable in the model. Since total cholesterol, HDL, LDL and triglycerides were highly correlated, we only considered LDL to evaluate importance of age, sex, smoking, BMI, HOMA-IR and HbA1c and hypertension by comparing models with and without each factor. In the same way, the importance of LDL, HDL, TC and TG was analysed by adding each variable in the model with age, sex, smoking, BMI, HOMA-IR, HbA1c and hypertension.

We employed Cox proportional hazards regression to explore the associations between baseline sCaspase-3, in tertiles or per 1-unit increment, and incidence of diabetes and incidence of CAD, respectively. The Kaplan–Meier curves were used to illustrate the relation between tertiles of baseline sCaspase-3 and incidence of both diseases. Hazard ratios (HRs) with 95% confidence interval were calculated in 3 separate models: 1) unadjusted; 2) adjusted for age and sex; and 3) adjusted for common risk factors for type 2 diabetes (smoking, age, sex, hypertension, body mass index and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)) and cardiovascular risk factors according to Framingham (smoking, diabetes, age, sex, total cholesterol, high-density lipoproteins, low-density lipoproteins, diabetes and hypertension) and body mass index for incident

diabetes and incident CAD, respectively. A sensitivity analysis was performed to examine whether an association between sCaspase-3 and incidence CAD was mediated by diabetes, including all covariates adjusted for in the Cox regression analysis in predicting CAD (age, sex, hypertension, LDL, HDL, TG, total cholesterol and smoking).

$P < 0.05$ was considered significant. SPSS Statistics 22 (Chicago, IL, USA) was used for the statistical analyses.

Results

Plasma sCaspase-3 is associated with cardiometabolic factors

First, associations between baseline sCaspase-3 and clinical characteristics were assessed in all patients with measured plasma sCaspase-3 ($n = 4637$). A study cohort flow chart is presented in Fig. 1. Baseline plasma sCaspase-3 was significantly higher in active smokers (10.8 (IQR 10.1–11.4) au/mL vs 10.7 (IQR 10.0–11.3) au/mL, $P = 0.002$), male participants (10.8 (IQR 10.1–11.4) au/mL vs 10.7 (IQR 10.0–11.3) au/mL, $P = 0.00008$) and individuals with hypertension (10.8 (IQR 10.1–11.4) au/mL vs 10.7 (IQR 10.0– 11.3) au/mL, $P = 0.0003$). sCaspase-3 correlated with HbA1c ($r = 0.18$, $P = 1.3 \times 10^{-36}$), fasting glucose ($r = 0.12$, $P = 7.8 \times 10^{-16}$), total cholesterol ($r = 0.05$, $P = 0.001$), triglycerides ($r = 0.1$, $P = 6.8 \times 10^{-12}$), HDL ($r = –0.07$, $P = 1\ 9\ 10^{-5}$), LDL ($r = 0.05$, $P = 0.001$), age ($r = 0.04$, $P = 0.04$), body mass index (BMI; $r = 0.06$, $P = 1\ 9 \times 10^{-4}$) and C-reactive protein (CRP; $r = 0.08$, $P = 4.9 \times 10^{-7}$). A multiple linear regression model including the risk factors adapted from the Framingham risk score (HbA1c, smoking, age, sex, hypertension, total cholesterol, triglycerides and high-density lipoproteins), BMI and HOMA-IR was used toexamine which factor was most important in the association with sCaspase-3. HbA1c was identified as the factor with the greatest influence on sCaspase-3 levels ($R^2 = 0.028$; Fig. 2a).

sCaspase-3 predicts future diabetes

Next, to investigate whether sCaspase-3 levels predicted diabetes incidence, all patients with prevalent diabetes and/or fasting blood glucose 6.1mmol/L were removed ($n = 384$; flow chart in Fig. 1). Among the remaining 4253 patients, 666 patients developed diabetes during the follow-up (median 25.1 (IQR 17.5–26.1 years)). Baseline levels of sCaspase-3 were significantly higher among individuals who developed diabetes during the follow-up (10.8 (IQR 10.2–11.5) vs 10.7 (IQR 10.0–11.3) au/mL, $P = 0.003$). Next, to assess whether plasma sCaspase-3 predicted incidence of diabetes, plasma levels of sCaspase-3 were divided into tertiles. The risk of future diabetes increased with higher levels of plasma Caspase-3 (Kaplan–Meier curves in Fig. 2b; log-rank test, $P = 0.001$; Fig. 2b). Significant differences in clinical characteristics are presented in Table 1.

The calculated hazard ratio (HR) per 1-unit increase of sCaspase-3 was 1.16 (95% CI 1.07–1.26, $P = 0.0003$) according to a univariate Cox regression model. sCaspase-3 also remained a significant predictor of future diabetes upon adjusting for age and sex (HR 1.15, CI 1.06–1.25, $P = 0.0005$) and when adjusting for factors associated with diabetes: sex, age, hypertension, body mass index (BMI), HOMA-IR and smoking (HR 1.12, CI 1.03–1.21, $P = 0.006$; Table 2).

A variant rs60780116 is associated with type 2 diabetes (T2D) and gene expression of CASP3

Next, we examined whether any variants in the genomic region of CASP3 (maximum 250 kb to the transcription start site) were associated with T2D using the latest GWAS [24]. We found that the T allele of rs60780116 (allele frequency 0.83) showed genome-wide significant association with T2D (beta = 0.056, SE = 0.009, $P = 8.4 \times 10^{-11}$; Fig. 3a). Furthermore, gene expression of *CASP3* in whole blood was associated with rs60780116 and thus was identified as an eQTL (from the GTEx project). As shown in Fig. 3b, Tallele of rs60780116 was associated with increased gene expression of CASP3 (the normalized effect size = 0.11, $P = 7.2 \times 10^{-6}$).

Baseline sCaspase-3 levels are increased in individuals who suffered from CAD during follow-up and predict future CAD

To explore whether sCaspase-3 could also predict future CAD, all patients with prevalent CAD at baseline were excluded ($n = 94$, study cohort flow chart presented in Fig. 1). A total of 4543 patients were then included for the follow-up analysis. During the follow-up (median 25.2 (IQR 18.7–26.2 years)), 648 patients suffered from CAD events and baseline plasma sCaspase-3 levels were observed to be significantly higher among individuals who developed CAD (10.8 (IQR 10.1–11.4) au/mL vs (10.7 (IQR 10.0–11.3) au/mL, $P = 0.002$). To explore whether sCaspase-3 could predict future CAD, the cohort was divided into tertiles based on sCaspase-3 levels. The Kaplan–Meier curves revealed that the risk of suffering from CAD increased with higher levels of sCaspase-3 (log-rank test, $P = 0.002$; Fig. 4a). Significant differences in clinical characteristics comparing individuals who suffered from a CAD event or not are presented in Table 3.

Using a univariate Cox regression model, the calculated hazard ratio (HR) for suffering from a CAD event per 1-unit increase of sCaspase-3 was 1.17 (95% CI 1.08–1.27, $P = 0.0001$; Table 2). sCaspase-3 remained a significant predictor of future CAD upon adjustment for age and sex (HR 1.13, 95% CI 1.04–1.22, $P = 0.004$; Table 2) and when adjusting for risk factors according to the Framingham risk score (sex, age, hypertension, LDL, HDL, total cholesterol, triglycerides, smoking and diabetes) and BMI (HR 1.09, 95% CI 1.003–1.18, $P = 0.04$; Table 4).

Finally, we performed a sensitivity analysis and confirmed that the associations remained significant when excluding patients with prevalent (at baseline) and incident (during follow-up) diabetes before the CAD events (HR 1.12, 95% CI 1.03–1.23, $P = 0.01$) when adjusting for cardiovascular risk factors (age, sex, LDL, HDL, TG, total cholesterol, smoking, BMI, hypertension and HOMA-IR).

Discussion

Circulating Caspase-3 has previously been shown, in the Dallas Heart Study, to be increased in plasma from patients with diabetes and to correlate with coronary calcium [8]. Furthermore, reduction of beta-cell mass mediated by Caspase-3-dependent apoptosis has been suggested as a potential pathophysiological mechanism in T2D [5–7]. Based on these

previous findings, we aimed to explore potential associations between sCaspase-3 levels and cardiometabolic risk factors and incidence of diabetes and CAD.

Here, we showed that circulating levels of sCaspase-3 are associated with cardiometabolic risk factors and predict incidence of both diabetes and CAD in a population cohort without known cardiovascular disease nor diabetes.

sCaspase-3 predicts incidence of diabetes

Caspase-3 is a well-described effector caspase known to regulate cell apoptosis, both through the intrinsic and the extrinsic apoptosis pathways [26]. Beta-cell apoptosis has been shown to occur more frequently in pancreatic islets from patients with T2D compared with controls [4]. The increased apoptosis of beta cells may be due to glucotoxic or ER stress-induced mitochondrial dysregulation and downstream activation of Caspase-3 [5]. Previous studies have also suggested that Caspase-3 could regulate T2D onset and that decreasing beta-cell mass due to apoptosis could be an important pathological mechanism in T2D [5–7]. These previous studies have suggested that Caspase-3 activation could play a role also in the early stages of T2D development. On the contrary, circulating sCaspase-3 has remained largely unexplored and the present study is the first to describe that sCaspase-3 predicts incidence of diabetes. Importantly, sCaspase-3 levels remained a significant predictor of diabetes after adjusting for common risk factors linked to T2D development. We also identified higher baseline levels of sCaspase-3 in plasma from patients who developed T2D compared with patients who did not. Even though the predictive role of sCaspase-3 has not been explored previously, plasma levels of sCaspase-3 were shown, in the Dallas Heart Study, to be increased in plasma from patients with diabetes [8].

Furthermore, we found that a genetic variant rs60780116 was associated with T2D. The T allele of rs60780116 was associated with increased risk of T2D. The T allele of rs60780116 was also associated with increased gene expression of Caspase-3 in blood. Taken together, these findings support a potential role of sCaspase-3 as a marker to identify individuals who will develop T2D and imply that Caspase-3 may also play a potential role in the development of T2D. However, due to lack of gene and protein expression data on the same individuals, the present study cannot determine the effect of the genetic variant on protein expression levels, and the specific role of Caspase-3 in T2D development needs to be further studied.

sCaspase-3 is associated with cardiometabolic factors and especially HbA1c

Baseline levels of sCaspase-3 were associated with several cardiometabolic risk factors including hypertension, smoking, male sex, triglycerides, CRP, total cholesterol and HbA1c in the present study. Yet, most of the associations were weak, and according to the regression model, HbA1c was identified as the factor with the strongest association with sCaspase-3 levels. This finding is of certain interest as hyperglycaemia is a condition that has been shown to induce apoptosis in vascular endothelial cells [27,28] This may imply that patients with higher glucose levels may have a higher risk of suffering from endothelial cell apoptosis, leading to a dysfunctional endothelium, which in turn is an important factor for atherosclerotic plaque development [29]. Beside endothelial cells, other cell types

including circulating leucocytes could also be potential sources of circulating sCaspase-3. sCaspase-3 has previously been shown to be released from peripheral blood mononuclear cells upon activation of the extrinsic apoptosis pathway [14]. In further support for apoptosis as biological processes associated with circulating Caspase-3 levels, the same study by Xue *et al.* showed that plasma levels of soluble Fas-associated protein with death domain (FADD) strongly correlated with plasma levels of sCaspase-3. However, determining the major cellular sources of sCaspase-3 is beyond the scope of this study.

sCaspase-3 predicts atherosclerotic coronary artery disease events

Cell apoptosis is a common feature of human atherosclerosis, suggested to contribute to plaque growth in the later stage of the disease [10–12]. Apoptotic cells are also present at the sites of plaque ruptures, the most common cause of coronary events [13]. Therefore, markers of cell apoptosis have gained interest as candidate markers of the atherosclerotic disease and events.

Caspase-3 is an executioner enzyme that regulates cell apoptosis. Circulating Caspase-3 may be released from either apoptotic cells or live cells exposed to cell stress stimuli, potentially as a response to avoid cell apoptosis [1–3,14].

In the Dallas Heart Study, Matulevicius *et al* showed that high levels of sCaspase-3 were associated with a higher coronary calcium score, potentially reflecting late-stage atherosclerosis [8]. Therefore, sCaspase-3 levels could potentially be associated with coronary atherosclerotic disease rather than the presence of vulnerable plaques causing acute complications. A previous study of sCaspase-3 in the MDC-CC cohort did not find any significant relationship between Caspase-3 and incidence of acute coronary events [14]. However, that study had a shorter follow-up period and did not include non-acute coronary interventions, and the absence of a significant difference could be related to lower statistical power and a different end-point definition compared with the present study. The present study showed that sCaspase-3 levels predict future CAD (including acute coronary events and non-acute coronary interventions) in a cohort without known cardiovascular disease. sCaspase-3 remained a significant predictor of future CAD after adjusting for major cardiovascular risk factors according to the Framingham risk score, BMI and diabetes. The association between sCaspase-3 and CAD could be due to the pre-CAD diabetes. However, sCaspase-3 was associated with CAD both through diabetes and independently of diabetes according to our sensitivity analysis. This suggests that sCaspase-3 is a marker of coronary atherosclerotic events in general and may not be specific to the acute cardiovascular events.

Limitations of the study

A limitation of the present study is that the PEA methodology cannot differentiate between active and inactive sCaspase-3 or between free proteins or proteins bound to extracellular vesicles. Moreover, we cannot separate different forms of diabetes, yet the vast majority of cases included will be T2D.

Conclusions

In conclusion, the present study provides evidence for plasma sCaspase-3 as a marker-associated cardiometabolic risk factor, which predicts both diabetes and CAD among patients without previous cardiovascular events.

Acknowledgements

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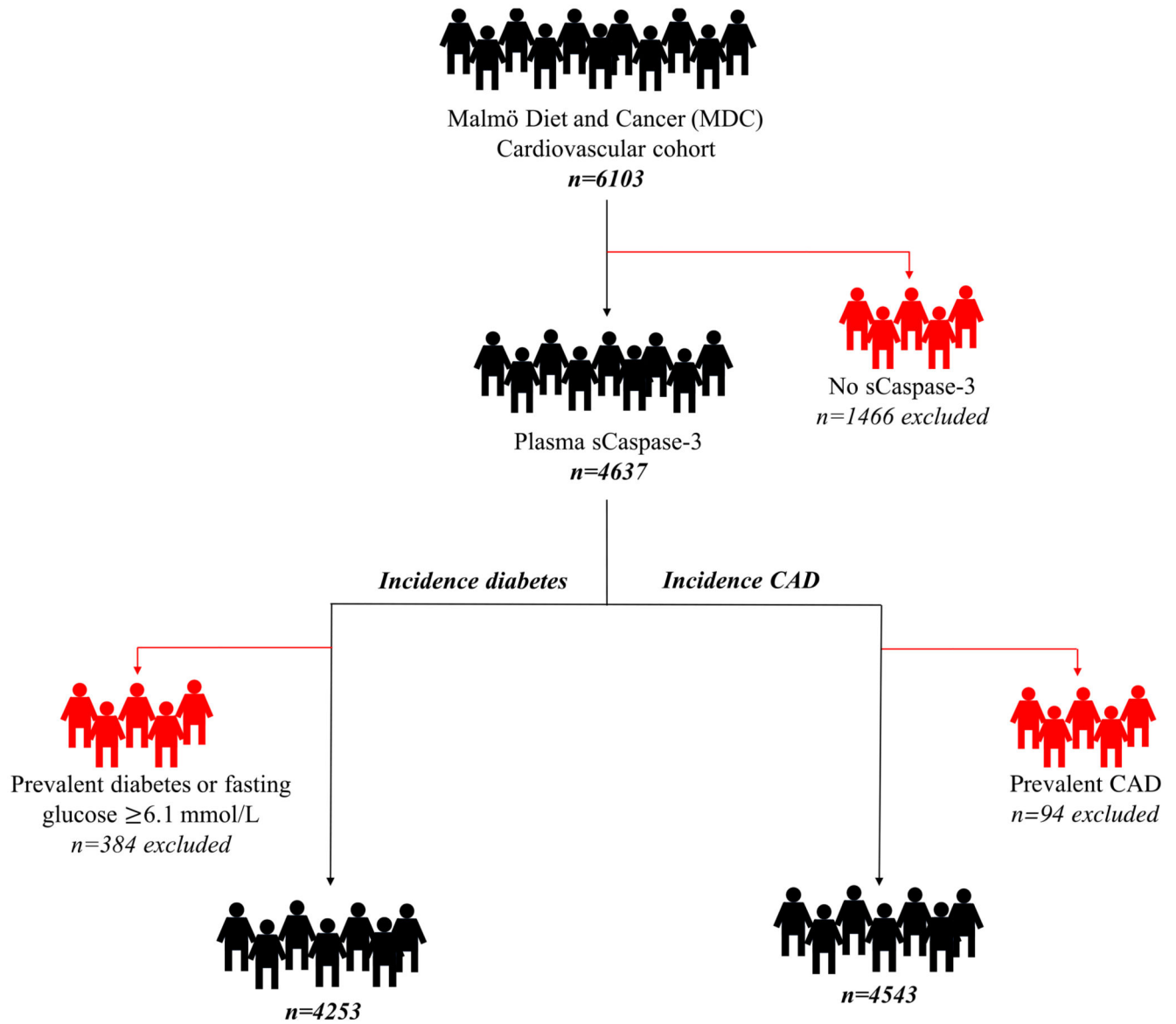
Fig. 1. Flow chart describing the study design and exclusion criteria.
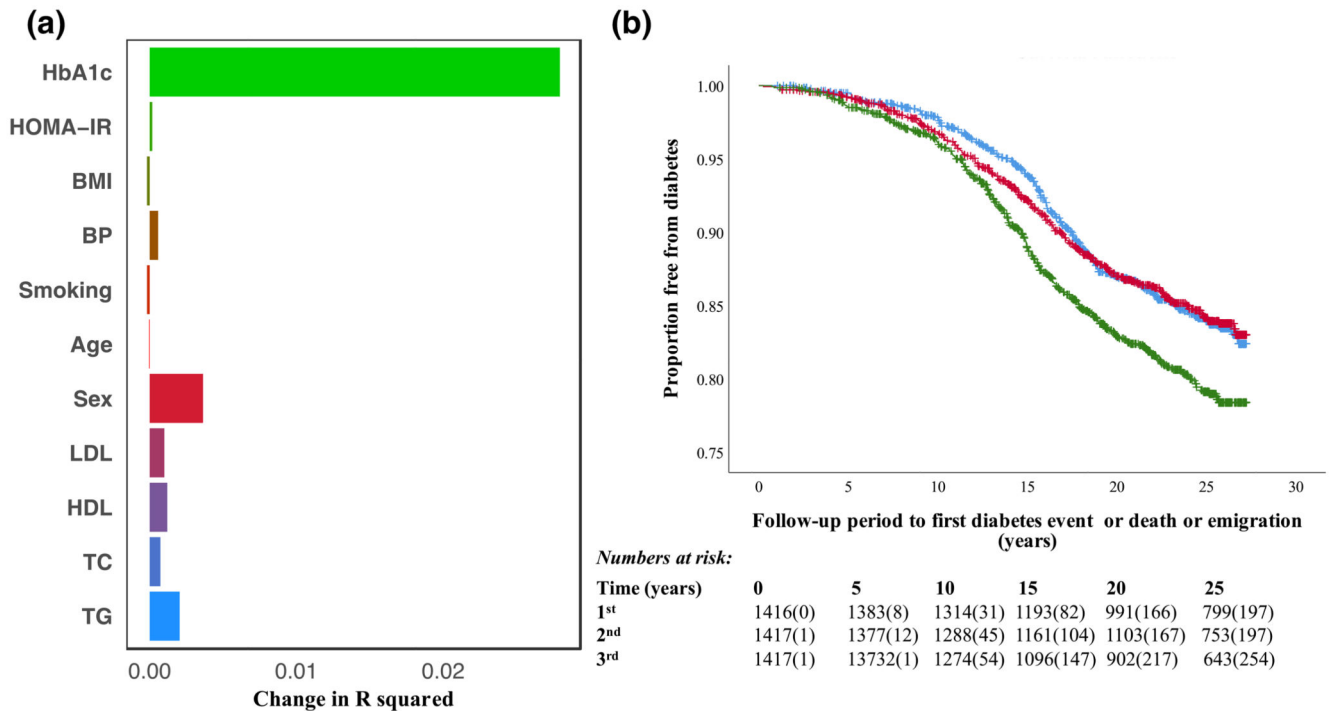
(a)

(b)

Fig. 2.

(a) Change in R2 when the factor is added to the multiple linear regression. Since lipids are highly correlated with each other, we only consider LDL to evaluate importance of HbA1c, BMI, HOMA-IR, BP, smoking, age and sex by comparing models with and without each factor. Importance of LDL, HDL, TC and TG is measured by comparing the models with it and without (HbA1c, BMI, HOMA-IR, BP, smoking, age and sex). The adjusted R^2 from the multiple linear regression is used. Factor with an increase in R^2 means it contributes to explaining variance in sCaspase-3 compared with all other factors in the model. BP: blood pressure; TC: total cholesterol; HDL: high-density lipoprotein; TG: triglyceride; LDL: low-density lipoprotein, BMI: body mass index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. $n = 4229$, all subjects with any missing values were excluded from the analysis. (b) Kaplan–Meier survival curve per tertile of soluble Caspase-3 (sCaspase-3) visualizing the higher risk of diabetes with increasing plasma levels of sCaspase-3. T1, blue; T2, red; and T3, green. Log-rank test, $P = 0.002$. Numbers denote numbers of patients at risk per tertile and number of events in parentheses.
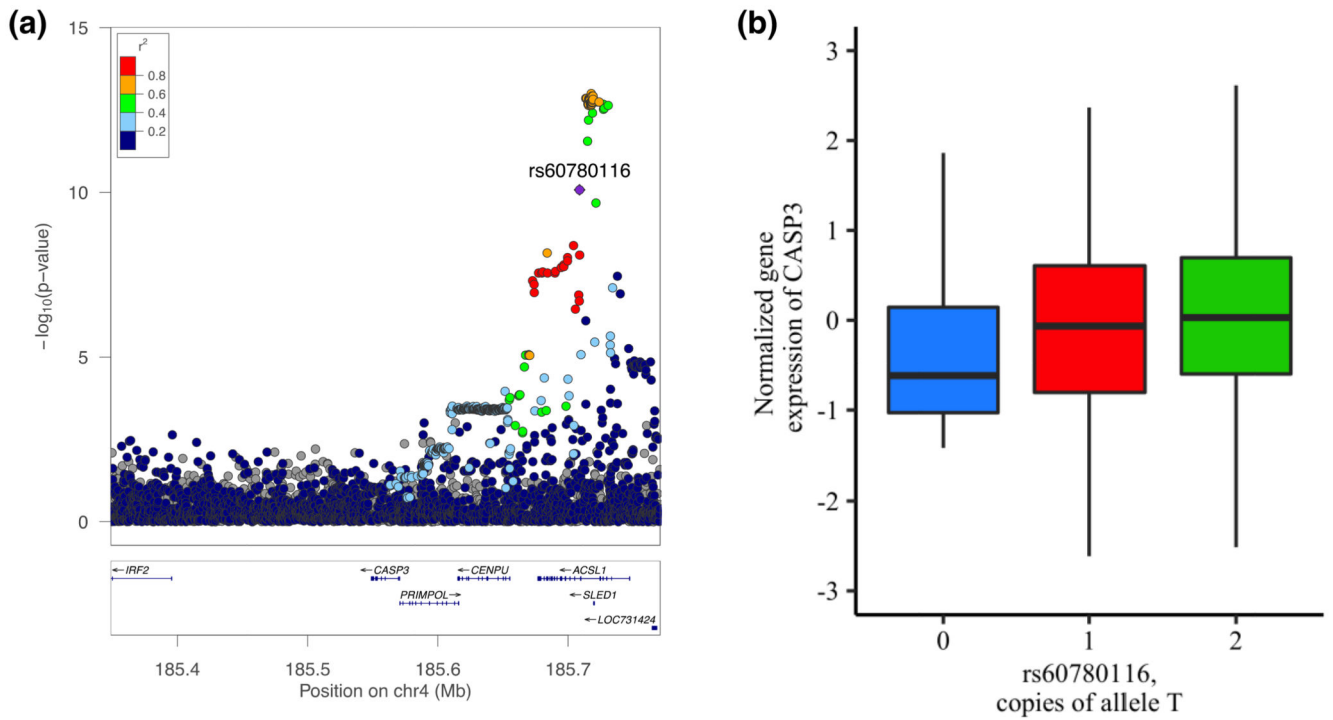
Fig. 3.
(a) Regional plot of the T2D susceptibility loci around CASP3. rs60780116 corresponds to chromosome 4 and position 185708807 (Homo sapiens (human) genome assembly GRCh37). (b) Schematic showing the eQTL SNP genotype versus eQTL gene expression of CASP3 ($n = 670$). Allele T of rs60780116 was associated with increased gene expression of CASP3, the normalized effect size = 0.11, $P = 7.2 \times 10^{-6}$.
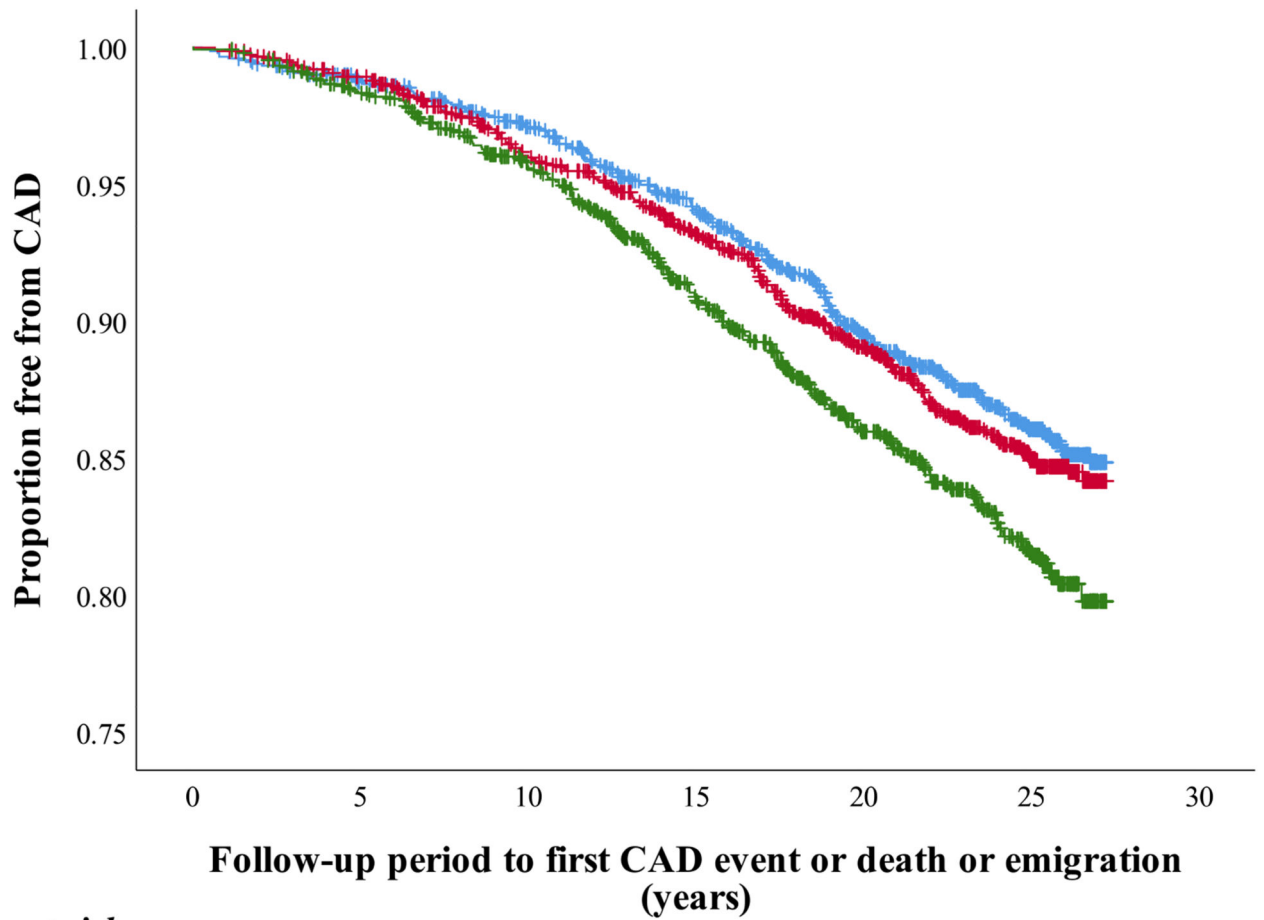
Fig. 4.

Kaplan–Meier survival curve per tertile of soluble Caspase-3 (sCaspase-3) visualizing the higher risk of coronary artery disease events (CAD) with increasing plasma levels of sCaspase-3. T1, blue; T2, red; and T3, green. Log-rank test, P = 0.002. Numbers denote numbers of patients at risk per tertile and number of events in parentheses.

Table 1

Clinical characteristics of all individuals included in the analysis to investigate future diabetes and divided by incidence diabetes mellitus (Inc. DM).

	Complete n = 4253	No DM n = 3587	Inc. DM n = 666	P value
Current smokers, n (%)	923 (22)	765 (21)	158 (24)	0.17
Male sex, n (%)	1620 (38)	1335 (37)	265 (40)	0.007
Age (years), median (IQR)	57.6 (52.2–62.5)	57.8 (52.2–62.6)	56.8 (51.9–61.6)	0.01
Hypertension, n (%)	1460 (34)	1146 (32)	314 (47)	<0.001
Lipid-lowering treatment, n (%)	90 (2)	73 (2)	17 (3)	0.39
CRP (mg/L), median (IQR)	1.3 (0.6–2.6)	1.2 (0.6–2.4)	1.7 (0.8–3.5)	<0.001
HbA$_{1c}$ %, median (IQR)	4.8 (4.5–5.0)	4.7 (4.5–5.0)	4.9 (4.6–5.2)	<0.001
Fasting lipoproteins (mmol/L)				
Cholesterol, median (IQR)	6.1 (5.4–6.8)	6.1 (5.4–6.8)	6.3 (5.5–7.0)	<0.001
LDL, median (IQR)	4.1 (3.5–4.8)	4.1 (3.5–4.7)	4.3 (3.6–4.9)	<0.001
HDL, median (IQR)	1.4 (1.1–1.6)	1.4 (1.2–1.6)	1.2 (1.0–1.5)	<0.001
TG, median (IQR)	1.1 (0.9–1.5)	1.1 (0.8–1.5)	1.3 (1.0–1.8)	<0.001
Fasting glucose, mmol/L (IQR)	4.8 (4.6–5.2)	4.8 (4.5–5.1)	5.2 (4.9–5.5)	<0.001

Hypertension was defined as blood pressure >159/94 mmHg or antihypertensive treatment. CRP, C-reactive protein. HbA1c, haemoglobin A1c. LDL, low-density lipoprotein. HDL, high-density lipoprotein. TG, triglyceride.P-values describe the difference comparing individuals who did or did not develop diabetes during follow-up. Data regarding smoking were available for 4174 patients. Data regarding HbA1c data were available for 4253 patients, CRP for 4161 patients, total cholesterol for 4251 patients, TG and HDL for 4252 patients and LDL for 4247 patients.

Table 2

Hazard ratio for incident diabetes by soluble Caspase-3 (sCaspase-3)

	T1	sCaspase-3 (T1/T2/T3)	T3	*HR* per 1 unit (95% CI)	*P*
HR (95% CI) model 1	1	1.0 (0.8–1.2)	1.4 (1.1–1.6)	1.16 (1.07–1.26)	0.0003
HR (95% CI) model 2	1	1.0 (CI 0.8–1.2)	1.3 (CI 1.1–1.6)	1.15 (1.06–1.25)	0.0005
HR (95% CI) model 3	1	1.0 (CI 0.8–1.2)	1.2 (CI 1.0–1.5)	1.12 (1.03–1.21)	0.006

Model 1—unadjusted. Model 2—adjusted for sex and age. Model 3—adjusted for common risk factors for type 2 diabetes (smoking, age, sex, hypertension, body mass index and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)). HR, hazard ratio. CI, confidence interval. P-values obtained from linear regression per 1-unit increment of sCaspase-3. N for tertile =1414, N for tertile II=1418 and N for tertile III=1416 patients.

Table 3

Clinical characteristics of all individuals included in the analysis to investigate future coronary artery disease events (CAD; *n* = 4543) and divided by incidence coronary artery disease events (inc. CAD)

	Complete *n* = 4543	No CAD *n* = 3895	Inc. CAD *n* = 648	*P* value
Current smokers, *n* (%)	980 (22)	815 (21)	165 (25)	0.009
Male sex, *n* (%)	1763 (39)	1395 (36)	368 (57)	<0.001
Age (years), median (IQR)	57.7 (52.2–62.6)	57.2 (52.0–62.3)	60.2 (54.6–63.8)	<0.001
Prevalent diabetes, *n* (%)	364 (8)	272 (7)	92 (14)	<0.001
Hypertension, *n* (%)	1604 (35)	1303 (33)	301 (46)	<0.001
Lipid-lowering treatment, *n* (%)	77 (2)	57 (1)	20 (3)	0.007
CRP (mg/L), median (IQR)	1.3 (0.7–2.7)	1.3 (0.6–2.6)	1.7 (0.8–3.5)	<0.001
HbA1c %, median (IQR)	4.8 (4.5–5.1)	4.8 (4.5–5.1)	4.9 (4.6–5.2)	<0.001
Fasting lipoproteins (mmol/L)				
Cholesterol, median (IQR)	6.1 (5.4–6.8)	6.1 (5.4–6.8)	6.3 (5.6–7.0)	<0.001
LDL, median (IQR)	4.1 (3.5–4.8)	4.1 (3.5–4.7)	4.4 (3.7–5.0)	<0.001
HDL, median (IQR)	1.4 (1.1–1.6)	1.4 (1.2–1.6)	1.2 (1.0–1.5)	<0.001
TG, median (IQR)	1.1 (0.9–1.6)	1.1 (0.9–1.6)	1.3 (1.0–1.7)	<0.001
Fasting glucose, mmol/L (IQR)	4.9 (4.6–5.3)	4.9 (4.6–5.2)	5.0 (4.7–5.4)	<0.001

Hypertension was defined as blood pressure >159/94 mmHg or antihypertensive treatment. CRP, C-reactive protein. HbA1c, haemoglobin A1c. LDL, low-density lipoprotein. HDL, high-density lipoprotein. TG, triglyceride. *P* value describes the significant difference comparing individuals with or without coronary artery disease (CAD) during follow-up. The number of individual data for each variable was as follows: Smoking, *n* = 4536; CRP, *n* = 4442; HbA1c, *n* = 4543; total cholesterol, *n* = 4541, LDL, *n* = 4537, HDL, *n* = 4542; and triglycerides, *n* = 4542.

Table 4

Hazard ratio for incident coronary artery disease (CAD) by soluble Caspase-3 (sCaspase-3)

	T1	sCaspase-3 (T1/T2/T3)	T3	*HR* per 1 unit (95% CI)	*P*
HR (95% CI) model 1	1	1.0 (CI 0.9–1.3)	1.4 (1.1–1.7)	1.17 (1.08–1.27)	0.0001
HR (95% CI) model 2	1	1.0 (CI 0.8–1.2)	1.3 (CI 1.0–1.5)	1.13 (1.04–1.22)	0.004
HR (95% CI) model 3	1	1.0 (CI 0.8–1.2)	1.2 (CI 1.0–1.4)	1.09 (1.003–1.18)	0.04

Model 1—unadjusted. Model 2—adjusted for sex and age. Model 3—adjusted for cardiovascular risk factors according to Framingham (smoking, diabetes, age, sex, total cholesterol, high-density lipoproteins, low-density lipoproteins, diabetes and hypertension) and body mass index. HR, hazard ratio. CI, confidence interval. P-values obtained from linear regression per 1-unit increment of sCaspase-3. N for tertiles 1 and III = 1514 and N for tertile II= 1513 patients.