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Association of apolipoproteins C-I and C-II truncations with coronary heart disease and progression of coronary artery calcium: Multi-Ethnic Study of Atherosclerosis

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Abstract

Background and aims: Higher truncated-to-native proteoform ratios of apolipoproteins (apo) C-I (C-I'/C-I) and C-II (C-II'/C-II) are associated with less atherogenic lipid profiles. We examined prospective relationships of C-I'/C-II and C-II'/C-II with coronary heart disease (CHD) and coronary artery calcium (CAC).

Methods: ApoC-I and apoC-II proteoforms were measured by mass spectrometry immunoassay in 5790 MESA baseline plasma samples. CHD events (myocardial infarction, resuscitated cardiac

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arrest, fatal CHD, n = 434) were evaluated for up to 17 years. CAC was measured 1–4 times over 10 years for incident CAC (if baseline CAC = 0), and changes (follow-up adjusted for baseline) in CAC score and density (if baseline CAC>0).

Results: C-II'/C-II was inversely associated with CHD (n = 434 events) after adjusting for non-lipid cardiovascular risk factors (Hazard ratio: 0.89 [95% CI: 0.81–0.98] per SD), however, the association was attenuated after further adjustment for HDL levels (0.93 [0.83–1.03]). There was no association between C-I'/C-I and CHD (0.98 [0.88–1.08]). C-II'/C-II was positively associated with changes in CAC score (3.4% [95%CI: 0.6, 6.3]) and density (6.3% [0.3, 4.2]), while C-I'/C-I was inversely associated with incident CAC (Risk ratio: 0.89 [95% CI: 0.81, 0.98]) in fully adjusted models that included plasma lipids. Total apoC-I and apoC-II concentrations were not associated with CHD, incident CAC or change in CAC score.

Conclusions: Increased apoC-II truncation was associated with reduced CHD, possibly explained by differences in lipid metabolism. Increased apoC-I and apoC-II truncations were also associated with less CAC progression and/or development of denser coronary plaques.

1. Introduction

Increased risk of atherosclerosis is linked to alterations in plasma lipid levels, including increased plasma triglycerides, and higher LDL and lower HDL cholesterol levels [1]. The changes in lipid levels are in turn related to perturbations in multiple apolipoproteins (apo) that influence lipoprotein metabolism and vascular biology [2]. Among those, apoC-I and apoC-II directly regulate triglyceride clearance and HDL metabolism [3–7]. In preclinical studies, higher total apoC-I and apoC-II were associated with the development of atherosclerosis and vulnerable atherosclerotic plaque [8–11]. In humans, several cross-sectional studies described positive associations between apoC-I content on triglyceride-rich lipoproteins and carotid atherosclerosis [12–14].

Both apoC-I and C-II appear in the circulation predominantly as full-length native proteins along with minor truncated proteoforms [15,16]. In our recent analysis from the Multi-Ethnic Study of Atherosclerosis (MESA), we found a strong relationship of apoC-I and apoC-II proteoform composition with plasma lipids that were independent of total apoC-I and apoC-II concentrations. Greater amounts of truncated apoC-I (C-I′) and apoC-II (C-II') were associated with lower triglycerides levels and higher HDL levels while higher total apoC-I and apoC-II concentrations were associated with higher triglycerides levels [17].

To test whether distribution of apoC-I and apoC-II proteoforms among individuals may have implications for vascular disease, we examined the association of proteoform composition with CHD risk. We also explored whether apoC-I and apoC-II proteoforms are associated with progression of subclinical coronary atherosclerosis measured by coronary artery calcium (CAC). CAC score, reflecting both volume and density of calcified plaque, has been closely associated with atherosclerotic burden and future CHD events [18–20]. Increases

in CAC scores over time are directly related to future clinical CHD [21]. However, higher levels of plaque density -possibly indicating more mature and stable plaques- have been linked with reduced cardiovascular risk, especially in those with lower plaque volume [22,23]. Thus, analyses of calcific atherosclerosis must consider effects both on plaque volume and plaque density.

2. Patients and methods

2.1. Study population

Data used in this study were obtained from the Multi-Ethnic Study of Atherosclerosis (MESA) (https://www.mesa-nhlbi.org) in accordance with their published data access policies, including an approved written proposal. The MESA is a multicenter longitudinal study examining factors associated with subclinical CVD and the progression from subclinical to clinical CVD [24]. In total, MESA enrolled 6814 participants, all free of CVD at recruitment. Institutional review boards at each MESA study site approved the study protocol and informed consent was obtained from all study participants. The present study included 5790 participants with available plasma samples from Exam 1 (characteristics in Supplement Table 1).

2.2. Clinical and demographic characteristics

Information about participant demographics, medical history, and medication usage was obtained by standardized questionnaires. Resting blood pressure was measured three times, with the average of the last two measurements recorded in the database. Blood samples were obtained after a 12-h fast. Diabetes was defined as fasting glucose >6.99 mmol/l or use of hypoglycemic medications. Blood samples were obtained after a 12-h fast. Blood biomarkers were measured at the MESA central laboratory at the University of Minnesota.

2.3. CHD follow-up

The primary outcome for this study was "hard" coronary heart disease (CHD), which included definite myocardial infarction, resuscitated cardiac arrest and fatal CHD. The present analysis includes events reported through end of 2017, i.e., for up to 17 years of follow-up. Details on surveillance of cardiovascular events have been previously reported [25]. Additional details on MESA follow-up methods and event adjudication are available at http://www.mesa-nhlbi.org.

2.4. CAC measurement

All participants had a CT scan of the chest for CAC during Exam 1 (2000–2002). Approximately half of the participants were then randomly selected to undergo a CT scan during Exam 2 (2002–2004), and the other half during Exam 3 (2004–2006). Exam 4 scans (2005–2007) were preferentially taken in participants without Exam 3 scans while Exam 5 (2010–2012) scans were preferentially performed in participants with scans from Exam 3 and/or Exam 4 [20]. CAC was measured by electron-beam (3 sites), or multi-detector (3 sites) CT [25]. The methodology for acquisition and interpretation of the scans has been published [26]. Briefly, calcified lesions of at least 4 adjacent voxels above a threshold of 130 Hounsfield units (HU) were identified; below this, scans were assigned a zero

value. Each discrete plaque area was then multiplied according to the Agatston's method [27], depending on the highest voxel density value anywhere in the plaque, i.e., 130–199 HU by 1, 200–299 HU by 2, 300–399 by 3, and 400 HU by 4. The CAC (Agatston) score was calculated as the sum of the within-plane scores across all calcified lesions. As noted previously [22], CAC density data was back-calculated from CAC scores using the appropriate slice thickness and plaque volume information and reflect the average CAC density for all CT slices from that participant. Scans obtained subsequent to coronary revascularization procedures that were performed after Exam 1 were excluded from this analysis.

2.5. Measurement of apoC-I and apoC-II proteoforms and total plasma concentrations

ApoC-I and apoC-II proteoform composition was measured in Exam 1 plasma samples from 5790 participants by mass-spectrometry immunoassay (MSIA) as described previously [17]. Relative abundance of apoC-I and apoC-II proteoforms was obtained by division of their peak area by the sum of peak areas of both truncated and native proteoform. Samples were run in batches of 96; each batch contained 90 analytical samples and 6 quality control samples (two different pooled plasma samples each aliquoted in triplicate). Mean intra-assay coefficients of variation were 4.6% and 1.5% for truncated and native apoC-I, and 7.3% and 0.5% for truncated and native apoC-II, respectively. Mean between-assay coefficients of variation were 5.5% and 1.8% for truncated and native apoC-I, and 9.1% and 0.6% for truncated and native apoC-II, respectively.

Total apoC-I and apoC-II concentrations were determined by sandwich ELISAs in a subset of 3851 participants who had paired Exam 1 and Exam 5 plasma samples as part of an ancillary study on cognitive function (characteristics in Supplement Table 1). Replicates with a coefficient of variation >15% were repeated. Mean intra-assay coefficients of variation were 6% for apoC-I, and 4% for apoC-II. Mean between-assay coefficients of variation were 7% for both apoC-I and apoC-II.

Both MSIA and ELISA used identical apoC-I and apoC-II detection antibodies (Academy Biomedical Co, Houston, TX) and are described in detail in the Supplemental Methods.

2.6. Statistical analyses

Statistical analyses were conducted using SAS v9.4 (SAS Institute, Cary, NC). The data are presented as mean ± standard deviation for normally distributed continuous variables, median (interquartile range) for non-normally distributed continuous variables, and numbers (percentages) for categorical variables. ApoC-I and apoC-II proteoform composition was expressed as truncated-to-native proteoform ratios (C-I'/C-I and C-II'/C-II, respectively). All apoC-I and apoC-II measures were natural log transformed to approximate normal distribution and scaled to a mean equal to zero and an SD equal to one to allow direct comparison of effects.

For MESA, and the current analysis, Exam 1 data was considered baseline. Baseline characteristics between the groups who did and did not develop CHD were compared using Student's t-test for continuous variables and by χ^2 or Fisher's tests for categorical variables.

The associations between baseline apoC-I and apoC-II continuous measures and incident CHD were tested by Cox proportional hazard regression models. Proportional hazard assumptions were assessed by inspecting Kaplan-Meier curves (above *vs.* below the median) and formally tested by cumulative sums of Martingale residuals with *p*-values of Kolmogorov-type supremum test. Proportional hazard models were adjusted for age, sex, and race/ethnicity (Model 1), and then adjusted for baseline smoking status, BMI, diabetes, hypertension medications, systolic blood pressure, eGFR and use of statins (Model 2), and then for baseline plasma triglycerides, and non-HDL and HDL cholesterol (Model 3). Associations that were attenuated in Model 3 (after additional adjustment for multiple lipid measures) were additionally tested with separate inclusion of triglycerides, and HDL and non-HDL cholesterol in the model to ascertain which of these lipids accounted for this attenuation. In additional analyses, we explored the interactions of apoCs measures with age (by median), sex, race/ethnicity, and prevalent nonzero CAC. In the subset with available total apoC-I and apoC-II concentrations, we also tested the relationship of CHD with apoC-I and apoC-II proteoform concentrations combined in the same model for each apolipoprotein. To account for potential selection bias of requiring availability of a matching Exam 5 sample, all analyses assessing total apoC-I and apoC-II measures were weighed by inverse probability of attrition by Exam 5 calculated from the propensity scores derived from multivariate logistic regression models that included study site, age, race, sex, urine albumin-creatinine ratio, tobacco smoking status, presence of diabetes, primary language, education level and income category. Missing covariate values were imputed by multiple imputation using a chained equation approach with a set of 20 plausible substitutes consistent with the observed values [28]. Balance of propensity scores in "exposed" *versus* "unexposed" participants was inspected using histograms of propensity scores stratified by quartiles of baseline apoC-I and apoC-II concentrations (Supplement Fig. 1A and B).

Because a large proportion of participants had CAC values of zero (about half), the association between apoC measures and follow-up CAC progression was tested in two parts depending on baseline CAC, as previously suggested in MESA [29]. In those with zero CAC, the association between apoC-I and apoC-II measures and incident CAC was tested by log-binomial regression models with robust standard errors. In those with nonzero baseline CAC, the changes in continuous CAC score and CAC density, modeled as their follow-up values adjusted for baseline value, were analyzed by mixed linear regression for repeated measures. CAC density was additionally adjusted for CAC volume [22]. CAC scores were log-2 transformed to approximate normal distribution. All regression models were initially adjusted for age, sex, race/ethnicity, and follow-up time (Model 1), then adjusted for baseline body-mass index (BMI), diabetes, use of antihypertensive medications, systolic blood pressure, tobacco smoking and use of statins (Model 2), and additionally adjusted for baseline plasma triglycerides, and HDL and non-HDL cholesterol levels (Model 3). Covariates in Model 2 were chosen based on their known prior associations with CAC and retained (those noted above) if they remained associated with CAC progression. Covariates subsequently excluded were eGFR, physical activity level, and sedentary lifestyle estimates. All models for CAC outcomes were weighted by inverse probability scores for study attrition derived for each follow-up visit from multivariate logistic regression models as described above. Histograms of propensity scores for each follow-up visit by quartiles of

baseline C-I'/C-I and C-II/C-II were plotted to confirm the balance of probability weights between exposed and unexposed individuals (Supplement Fig. 1C–J). Beta-estimates for CAC score and CAC density were also present as percent difference to ease interpretation of results. To account for a recently observed interaction of CAC density with CAC volume in predicting CHD risk in MESA [23], the association between apoC-I and apoC-II measures and changes in CAC score and CAC density was stratified by baseline CAC volume of 130 mm^3.

3. Results

3.1. Baseline characteristics of study participants

Measurement of apoC-I and apoC-II proteoforms via MSIA was completed for 5790 MESA participants, including 5766 participants with available data for CHD assessment (and 3847 with total apoC-I and apoC-II concentrations) (Fig. 1). The baseline characteristics of all participants with baseline apoC-I and apoC-II proteoform measures, and of the subgroup with baseline total apoC-I and apoC-II concentration measurements, are shown in Supplement Table 1. The average age was lower and some of the cardiovascular risk factors were more favorable in the subset with total apoC-I and apoC-II concentrations.

3.2. Association of apoC-I and apoC-II proteoforms with CHD risk

A total of 434 CHD events occurred over a median time to an event of 9 years, including 217 events in those with measurements of total apoCs (median time to an event of 12 years). Those who developed CHD were on average older, more likely men, and had higher blood pressure and use of antihypertensive medications, higher fasting glucose levels and prevalence of diabetes, higher use of statins, higher triglycerides and lower HDL cholesterol levels, worse kidney function and greater prevalence of nonzero CAC (Table 1).

Those with higher C-II'/C-II (above the median) had reduced time to a CHD event (Fig. 2A, right panel). This inverse association between C-II'/C-I and CHD persisted after adjustment for clinical and demographic covariates (Fig. 2B, Hazard ratio: 0.89 [95% CI: 0.81–0.98] per 1 SD, Model 2), but was attenuated after adjustment for baseline plasma lipids (0.91 [0.81–1.02], Model 3). After examining each lipid measure separately, the association of C-II'/C-II with CHD was not independent of baseline HDL cholesterol (0.93 [0.83–1.03]). In subgroup analyses, the results did not appear to differ (*p*-values for interaction >0.05) by sex, age, or prevalent CAC, however, the inverse association between C-II'/C-II was present in White, Black and Hispanic persons, but not in Chinese Americans (*p* for interaction 0.02) (Fig. 2C).

No associations were noted between the C-I'/C-I ratio or total apoC-I and apoC-II concentrations and CHD events (Fig. 2B). As there was a violation of the proportional hazard assumption over 17 years for the CI'/CI (Fig. 2A, left panel), the association between C-I'/C-I and CHD was also reassessed for the initial 10 years of follow-up. Again, the 10-year CHD risk was not related to baseline C-I'/C-I (Hazard ratio: 1.07 [95% CI: 0.94–1.21], Model 1, n = 247 events).

In analyses of apoC-II proteoform *concentrations*, CHD risk was inversely associated with truncated apoC-II in Models 1 and 2, and positively associated with native apoC-II in Models 1 and 2. CHD risk was inversely associated with truncated apoC-I and positively associated with native apoC-I concentration in Model 1 only (Supplement Figure 2).

3.3. Association of apoC-I and apoC-II proteoforms with CAC outcomes

A total of 5063 participants had at least one follow-up CAC measurement with a median follow-up time of 8.4 years. In those with CAC score of zero at baseline (n = 2580), incident CAC (n = 902 events) was inversely associated with C-I'/C-I in Model 1 (Risk ratio 0.87 [95% CI: 0.80, 0.95] per 1 SD) and Model 3 (0.91 [0.82, 1.00]) (Table 2). In those with non-zero CAC at baseline, change in CAC score was inversely associated with C-I'/C-I in Model 1 only (−2.7% [−5.2, −0.2]) and positively associated with C-II'/C-II in all models (3.4% [0.6, 6.3] in Model 3) (percent changes calculated from the β-estimates for $\log_2$ CAC scores as indicated in Table 2). Change in CAC *density* was positively associated with C-I'/C-I in Model 1 only (a difference of 8.4% [2.1, 14.7] on the density scale from 1 to 4) and with C-II'/C-II in all models (6.3% [0.3, 12.6] in Model 3) (Table 2). The positive associations between C-II'/C-II and changes in CAC score and CAC *density* were present in those with CAC volume 130 mm^3 (n = 1449; 10.7% [4.1, 17.9] and 11.1% [3.6, 18.6], respectively, Model 3), but not in those with CAC volume >130 mm^3.

Total apoC-I was not associated with incident CAC or change in CAC score; however, it was positively associated with change in CAC *density* in all models (6.3% [0.3, 12.6], Model 3) (Table 2). Total apoC-II was positively associated with change in CAC *density* in Model 3 only (8.4% [1.5, 15]) (Table 2).

4. Discussion

In the present study, apoC-I and apoC-II truncations showed prospective relationships with CHD risk and several measures of coronary calcific atherosclerosis (Fig. 3). Increased apoC-II truncation was associated with reduced CHD risk; however, the association was attenuated after adjustment for plasma HDL cholesterol. It was also associated with greater increases in CAC score, but also in CAC density in those with prevalent CAC. While higher apoC-I truncation was not associated with development of CHD, it was associated with reduced risk of incident CAC. Importantly, total concentrations of apoC-I and apoC-II showed no consistent association with CHD risk or changes in CAC measures.

Understanding how these truncations may differ from native proteins in their effects on cardiovascular risk is limited in part by a paucity of relevant clinical data on apoC-I and apoC-II. *In vitro*, total apoC-I and apoC-II were shown to induce proinflammatory and proapoptotic responses in vascular smooth muscle cells and macrophages [8, 9]. In murine models of atherosclerosis, overexpression of apoC-I facilitated development of atherosclerosis and atherosclerotic plaque inflammation [10,11]. In humans, higher total apoC-I concentrations on VLDL particles were present in those with greater carotid intima-media thickness [12–14]. The lack of association between total apoC-I and apoC-II and future coronary events or changes in CAC score upon adjustment for cardiovascular risk factors in the current study suggests the few preclinical studies may have been misleading.

The cross-sectional studies in humans may have reflected, and been confounded by, high-risk phenotypes already present in those with advanced atherosclerosis.

In contrast, ratios of truncated-to-native proteoforms for both apolipoproteins showed relationships with CHD risk and/or different measures of CAC progression. Higher C-II'/C-II was associated with lower CHD risk in Models 1 and 2. Weakening of this association in Model 3, particularly after adjusting for HDL cholesterol levels, suggests that HDL metabolism may be a partial link between increased apoC-II truncation and cardiovascular risk. The latter notion is further supported by our recent report of strong cross-sectional and longitudinal inverse associations between C-II'/C-II and plasma HDL cholesterol levels in this same cohort [17]. On the other hand, higher C-II'/C-II was also associated with greater increases in CAC score in those with prevalent CAC at baseline. These seemingly contradictory findings, i.e., an increase in CAC score in tandem with reduction in cardiovascular risk, are similar to those observed with statins therapy [30]. Importantly, as with statin therapy [31], higher C-II'/C-II was also associated with increases in CAC density. This pattern of plaque change is consistent with generation of a more diffusely calcified and less vulnerable plaque [32]. Previous analyses in MESA demonstrated that increases in plaque density were associated with reduced cardiovascular risk [22]. Recent analysis in MESA showed that the inverse association between CAC density and CHD risk was present only in participants with low plaque volume only [23]. In agreement with these findings, the positive association between C-II'/C-II and changes in CAC density in the present study was confined to those with low CAC volume. Importantly, the positive association between C-II'/C-II and changes in CAC score was also limited to those with low baseline plaque volume, further supporting the density component of CAC score as primary driver of this relationship.

In our previous analysis in this cohort, higher C-I'/C-I was associated with a less atherogenic lipid profile, including lower triglycerides and higher HDL cholesterol [17]. In the present study, however, the inverse relationship between C-I'/C-I and incident CAC persisted after adjustment for plasma lipids. It is plausible that these traditional lipid measures do not fully reflect the complexity of apoC-I proteoforms action on proatherogenic lipoproteins. Besides activation of lipoprotein lipase (LPL), apoC-I inhibits hepatic lipase and cholesteryl ester transferase protein (CETP), and activates lecithin-cholesterol acyl transferase (LCAT) [33–35]. These pathways may contribute to atherosclerosis via remodeling of HDL and LDL particles in the absence of apparent changes in their overall cholesterol content [36,37]. Nonetheless, the inverse association between C-I'/C-I and incident CAC was not corroborated by a reduction in clinical coronary events. Consistent with this, in those with prevalent CAC at baseline, i.e., those with greater CHD risk [21], C-I'/C-I was not associated with changes in CAC measures upon adjustment for clinical covariates. Thus, our data suggest that apoC-I truncation may be more important in the initial development of atherosclerosis but less prominent in progression of existing atherosclerotic plaque and development of cardiovascular events.

A major strength of the study is the unique ability to concurrently assess the relationships of apoC-I and apoC-II total concentrations and relative proteoform composition measures with repeated CT scans of coronary arteries over 10 years of follow-up and with clinical

cardiovascular events for up to 17 years. This approach allowed us to demonstrate their distinct associations with changes in CHD risk and coronary atherosclerosis after adjustment for relevant covariates. The ability to calculate plasma concentrations of individual proteoforms helped to demonstrate more favorable associations of truncated proteoforms (versus native) with both clinical and subclinical coronary atherosclerosis.

A major limitation was the observational nature of the study, which limits conclusions regarding underlying causality. The prerequisite of having a paired follow-up sample available approximately 10-years after baseline for the subset with total apoC-I and apoC-II concentrations measurement might have favored selection of relatively healthier sub-cohort with less advanced atherosclerosis. Nevertheless, even in this "healthier" subset, the patterns of the associations of individual apoC proteoforms concentrations with CHD risk and CAC measures was very similar to that shown in the complete cohort. As pointed out previously [22], the use of an arbitrary 4-point scale curtailed CAC densities greater than 400 Hounsfield units. On the other hand, the presence of less dense plaques might have been underestimated by recording only the maximum density for each scan per study protocol. These potential shortcomings might have increased the variation and therefore weakened the relationships between apoC-I and apoC-II measures and CAC density. Although the findings are relatively consistent and biologically plausible, we acknowledge a chance of false positive results, i.e., type I error, from use of multiple exposure variables. Thus, confirmation in another cohort may be needed to draw more definite conclusions.

The current results suggest that posttranslational truncations of apoC-I and apoC-II provide additional information beyond that indicated by total apoC-I and apoC-II concentrations in predicting coronary events and calcified coronary atherosclerotic progression. Of particular importance, increased apoC-II truncation, possibly through its favorable effect on HDL cholesterol levels, was associated with reduced CHD risk and development of more dense coronary plaque. Greater understanding of the factors regulating apoC-I and apoC-II truncations and mechanisms underlying their associations with vascular disease may facilitate development of new therapeutic strategies for prevention and treatment of atherosclerosis and reducing cardiovascular risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Abbreviations

apoC-I	apolipoprotein C-I
apoC-I′	truncated apoC-I
C-I'/C-I	truncated-to-native apoC-I ratio
apoC-II	apolipoprotein C-II
apoC-II'	truncated apoC-II
C-II'/C-II	truncated-to-native apoC-II ratio
CAC	coronary artery calcium
CHD	coronary heart disease
HR	hazard ratio
MESA	Multi-Ethnic Study of Atherosclerosis
MSIA	mass spectrometry immunoassay

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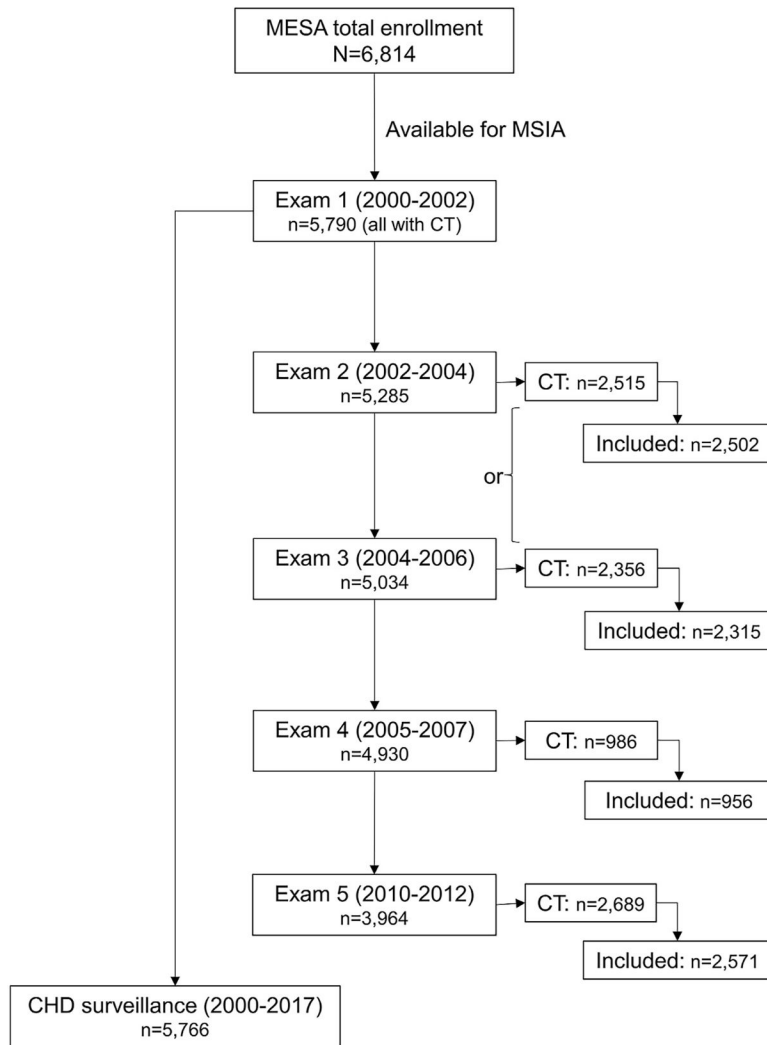
Fig. 1.
Study flow.

All enrolled participants had a CT scan performed for CAC during Exam 1 (2000–2002), with about half of these participants randomly selected for CT scan during Exam 2 (2002–2004), and the other half during Exam 3 (2004–2006). Exam 4 scans (2005–2007) were preferentially taken in participants without Exam 3 scans while Exam 5 (2010–2012) scans were preferentially performed in participants with scans from Exam 3 and/or Exam 4. CT scans with valid data and performed prior to coronary revascularization procedures were included in the present analyses.

(A)

Years	0	2	4	6	8	10	12	14	16
At risk	5,766	5,560	5,318	5,044	4,742	4,432	4,133	3,702	2,024

(B)

C-I'/C-I (n=434 events)
- Model 1 — 0.95 [0.86-1.05]
- Model 2 — 0.99 [0.89-1.09]
- Model 3 — 0.98 [0.88-1.09]

C-II'/C-II (n=434 events)
- Model 1 — 0.88 [0.80-0.97]
- Model 2 — 0.89 [0.81-0.98]
- Model 3 — 0.91 [0.81-1.02]

Total apoC-I (n=217 events)
- Model 1 — 1.00 [0.90-1.13]
- Model 2 — 0.99 [0.88-1.11]
- Model 3 — 1.02 [0.90-1.17]

Total apoC-II (n=217 events)
- Model 1 — 1.11 [1.00-1.24]
- Model 2 — 1.04 [0.93-1.16]
- Model 3 — 1.03 [0.89-1.20]

Hazard ratios (95% CI) per 1 SD

(C)

Age (median, *p-interaction=0.97*)
- ≤ 63 years (n=128 events)
- > 63 years (n=306 events)

Race/ethnicity
- White (n=163 events, *Ref*)
- Black (n=125 events, *p-interaction=0.63*)
- Hispanic (n=102 events, *p-interaction=0.35*)
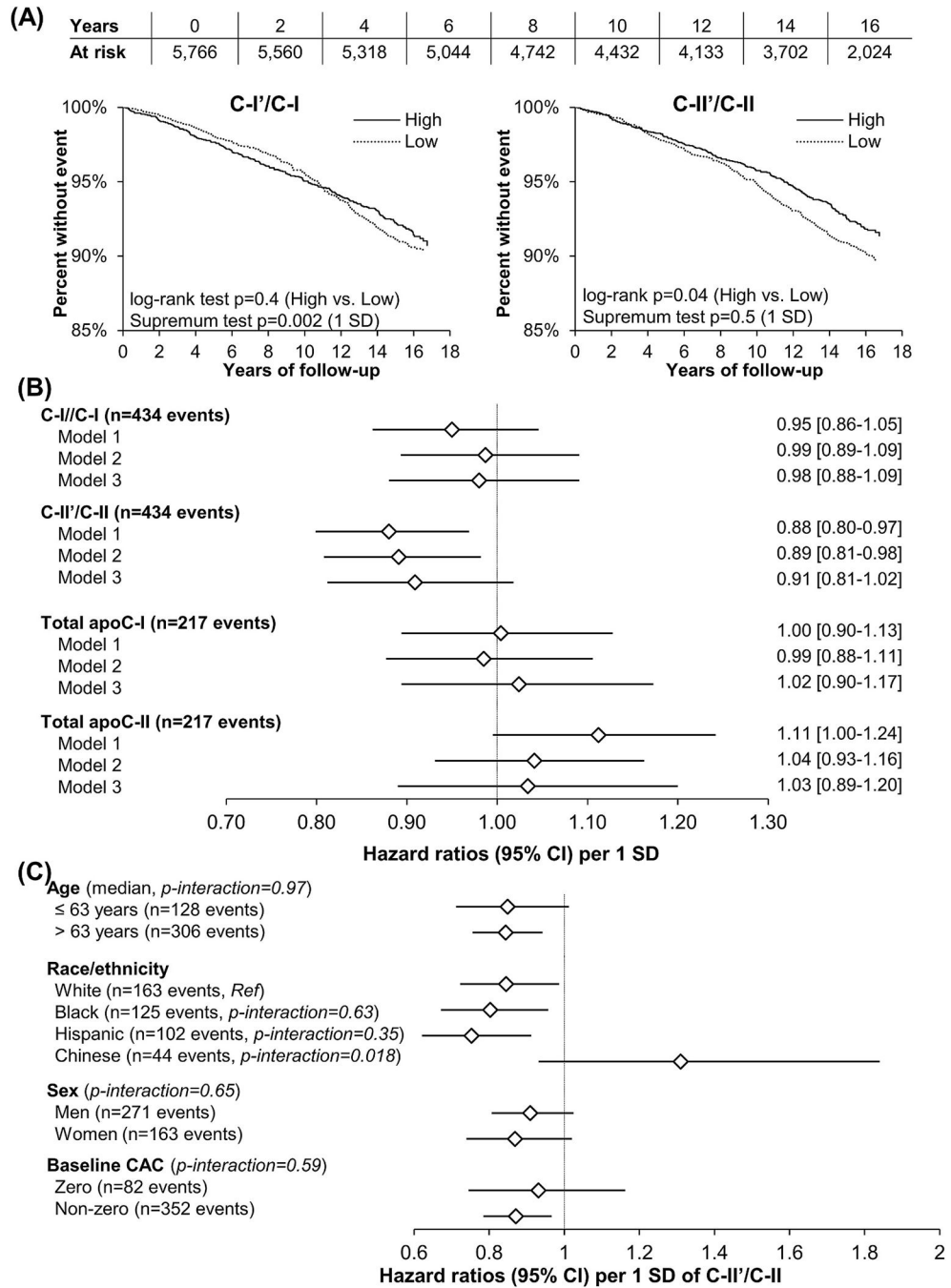- Chinese (n=44 events, *p-interaction=0.018*)

Sex (*p-interaction=0.65*)
- Men (n=271 events)
- Women (n=163 events)

Baseline CAC (*p-interaction=0.59*)
- Zero (n=82 events)
- Non-zero (n=352 events)

Hazard ratios (95% CI) per 1 SD of C-II'/C-II

Fig. 2.
Association between baseline truncated-to-native proteoform ratios and total apoC-I and apoC-II concentrations with incident coronary heart disease (CHD). (A) Kaplan-Meier curves for high and low strata (defined by median) of apoC-I and apoC-II proteoform ratios. (B) Cox proportional hazard regression models adjusted for age, sex, and race/ethnicity (Model 1), then adjusted for baseline BMI, diabetes, systolic BP, antihypertensives, tobacco smoking, eGFR and statin use (Model 2) and further adjusted for baseline triglycerides and HDL cholesterol (Model 3). (C) Relationship between C-II'/C-II and CHD risk stratified

by age, race/ethnicity, sex, and baseline CAC prevalence (unadjusted Hazard ratios). The significant supremum test indicated violation of proportional hazard assumptions.
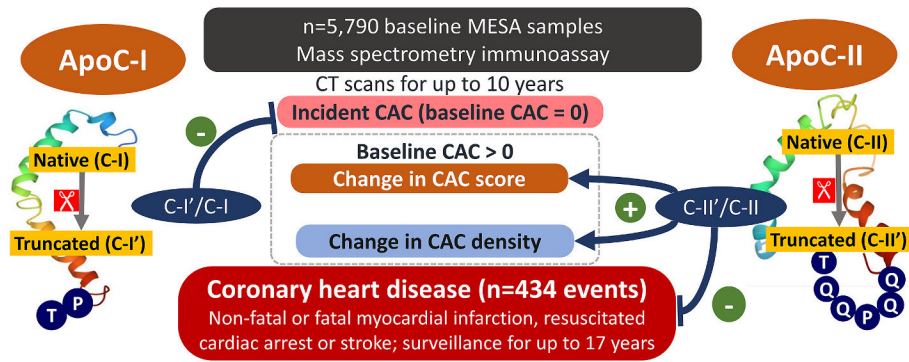
Fig. 3.
Graphical abstract.

Table 1

Baseline clinical and demographic characteristics by incident coronary heart disease (CHD).

Variable	No event	CHD event	p-value
n	5332	434	
Age	62 ± 10	68 ± 10	<0.0001
Women	2842 (53%)	163 (38%)	<0.0001
Race/ethnicity			0.53
White	1982 (37%)	163 (38%)	
Black	1535 (29%)	125 (29%)	
Hispanic/Latino	1157 (22%)	102 (24%)	
Chinese	658 (12%)	44 (10%)	
Tobacco use			0.22
Never	2690 (51%)	202 (47%)	
Former	1954 (37%)	177 (41%)	
Current	670 (13%)	55 (13%)	
BMI (kg/m²)	28.3 ± 5.4	28.8 ± 5.7	0.09
Antihypertensives use	1956 (37%)	227 (52%)	<0.0001
Statin use	795 (15%)	94 (22%)	0.0004
Systolic BP (mmHg)	126 ± 21	136 ± 22	<0.0001
Diastolic BP (mmHg)	72 ± 10	74 ± 11	0.0008
Diabetes	636 (12%)	102 (24%)	<0.0001
Fasting glucose (mmol/l)	5.38 ± 1.61	5.83 ± 2.33	<0.0001
Triglycerides (mmol/l)	1.47 ± 1.00	1.56 ± 0.92	0.034
Total cholesterol (mmol/l)	5.03 ± 0.93	4.95 ± 0.93	0.059
LDL-cholesterol	3.04 ± 0.82	3.01 ± 0.83	0.46
HDL-cholesterol (mmol/l)	1.33 ± 0.39	1.24 ± 0.36	<0.0001
eGFR (ml/min.1.73m²)	90 ± 21	85 ± 25	<0.0001
CAC > 0	2576 (48%)	352 (81%)	<0.0001

Data are means ± SD or n (%). Statistical differences were tested by Student's t-test for continuous variables, and Chi-square or Fisher's exact test for categories. Continuous data were natural log-transformed to approximate normal distribution.

Table 2

Relationship between total apoC-I and apoC-II concentrations and truncated-to-native proteoform ratios at baseline and coronary artery calcium (CAC) measures at follow-up.

Follow-up outcome	C-I'/C-I (1 SD)	C-II'/C-II (1 SD)	Total apoC-I (1 SD)	Total apoC-II (1 SD)
Incident CAC	**Risk Ratio [95% CI]**	**Risk Ratio [95% CI]**	**Risk Ratio [95% CI]**	**Risk Ratio [95% CI]**
n (Events/Total)	902/2838	902/2838	784/2028	784/2028
Model 1	**0.87 [0.80, 0.95]**	0.97 [0.89, 1.06]	0.96 [0.87, 1.05]	1.04 [0.95, 1.14]
Model 2	0.95 [0.87, 1.04]	1.01 [0.92, 1.10]	1.00 [0.90, 1.10]	1.01 [0.92, 1.11]
Model 3	**0.91 [0.82, 0.999]**	0.98 [0.88, 1.09]	0.94 [0.83, 1.05]	0.92 [0.82, 1.04]
$\log_2$ CAC score adj. baseline	**β-estimate [95% CI]**	**β-estimate [95% CI]**	**β-estimate [95% CI]**	**β-estimate [95% CI]**
n	2483	2483	1709	1709
Model 1	**−0.038 [−0.073, −0.003]**	**0.044 [0.008, 0.080]**	0.023 [−0.023, 0.070]	0.043 [−0.001, 0.087]
Model 2	− 0.020 [−0.055, 0.015]	**0.049 [0.013, 0.085]**	0.017 [−0.029, 0.062]	0.021 [−0.023, 0.064]
Model 3	− 0.016 [−0.053, 0.022]	**0.048 [0.008 0.088]**	− 0.007 [−0.060, 0.047]	− 0.014 [−0.072, 0.045]
CAC density adj. baseline	**β-estimate [95% CI]**	**β-estimate [95% CI]**	**β-estimate [95% CI]**	**β-estimate [95% CI]**
N	2483	2483	1709	1709
Model 1	**0.028 [0.007, 0.049]**	**0.048 [0.026, 0.070]**	**0.030 [0.005, 0.055]**	0.005 [−0.019, 0.030]
Model 2	0.009 [−0.006, 0.023]	**0.026 [0.012, 0.041]**	**0.019 [0.001, 0.036]**	0.003 [−0.014, 0.019]
Model 3	0.012 [−0.004, 0.027]	**0.023 [0.007, 0.040]**	**0.021 [0.001, 0.042]**	**0.028 [0.005, 0.050]**

Log-binomial regression with robust standard errors was used to test the association between baseline apoCs measures and incident CAC in those with baseline CAC = 0. Mixed linear regression for repeated measures with random intercept was used to test the association between baseline apoC measures and changes in CAC score and density in those with baseline CAC>0. Model 1: adjusted for age, sex, race/ethnicity, and follow-up time; Model 2: Model 1 & BMI, diabetes, systolic BP, and use of tobacco, antihypertensives and statins; Model 3: Model 2 & plasma triglycerides, and HDL and non-HDL cholesterol. CAC was also adjusted for CAC volume density and expressed on the scale from 1 to 4 density units. Differences in $\log_2$ CAC scores can be interpreted as a fold-change by 2^β. e.g., an increase of 1 SD in natural log C-II'/C-II in Model 3, β = 0.048 corresponds to $2^{0.048} = 1.034$-fold, or a 3.4% increase in CAC score. The percent changes are listed in the Results section to ease interpretation.