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### Authors

Ceponiene, Indre  
Li, Dong  
El Khoudary, Samar R  
et al.

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## Association of Coronary Calcium, Carotid Wall Thickness, and Carotid Plaque Progression with Low Density Lipoprotein and High Density Lipoprotein Particle Concentration Measured by Ion Mobility (From Multiethnic Study of Atherosclerosis [MESA])

Indre Ceponiene, MD<sup>a,b</sup>, Dong Li, MD<sup>a,c</sup>, Samar R. El Khoudary, MD<sup>d</sup>, Rine Nakanishi, MD<sup>a</sup>, James H. Stein, MD<sup>e</sup>, Nathan D. Wong, PhD<sup>f</sup>, Negin Nezarat, MD<sup>a</sup>, Mitsuru Kanisawa, MD<sup>a</sup>, Sina Rahmani, MD<sup>a</sup>, Kazuhiro Osawa, MD<sup>a</sup>, Matthew Tattersall, MS<sup>e</sup>, Matthew J. Budoff, MD<sup>a</sup>

<sup>a</sup>The Lundquist Institute at Harbor UCLA Medical Center, 1124 W. Carson Street, Torrance, CA 90502, USA

<sup>b</sup>Lithuanian University of Health Sciences, Medical Academy, Department of Cardiology, 2 Eiveniu Street, Kaunas, Lithuania

<sup>c</sup>Emory University School of Medicine, Division of Hospital Medicine, 100 Woodruff Circle Atlanta, GA 30322 USA

<sup>d</sup>University of Pittsburgh, Graduate School of Public Health, Department of Epidemiology, 130 De Soto Street, Pittsburgh, PA 15261, USA

<sup>e</sup>University of Wisconsin School of Medicine and Public Health, Department of Medicine, 750 Highland Ave, Madison, WI 53726, USA

<sup>f</sup>University of California, Irvine, Division of Cardiology, 333 City Blvd W, Orange, CA 92868, USA

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**Corresponding author and requests for reprints:** Matthew J. Budoff, MD, Los Angeles Biomedical Research Institute at Harbor-UCLA, 1124 W. Carson Street, Torrance, California, USA, Tel: +1-310 2224107, Fax: +1-3107829652, mbudoff@lundquist.org.

Author statement

**Indre Ceponiene:** Conceptualization, Methodology, Writing – Original draft presentation, Visualization, **Dong Li:** Formal analysis, Writing – Review & Editing, **Samar R. El Khoudary:** Supervision, Writing – Review & Editing, **Rine Nakanishi:** Supervision, Writing – Review & Editing, **James H. Stein:** Supervision, Writing – Review & Editing, **Nathan D. Wong:** Writing Review & Editing, **Negin Nezarat:** Writing – Review & Editing, **Mitsuru Kanisawa:** Writing Review & Editing, **Sina Rahmani:** Writing – Review & Editing, **Kazuhiro Osawa:** Writing – Review & Editing, **Matthew Tattersall:** Writing – Review & Editing, **Matthew J. Budoff:** Conceptualization, Methodology, Validation, Investigation, Resources, Supervision, Writing – Review & Editing, Project Administration.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Abstract

Current risk stratification strategies do not fully explain cardiovascular disease (CVD) risk. We aimed to evaluate the association of low-density lipoprotein (LDL-P) and high-density lipoprotein (HDL-P) particles with progression of coronary artery calcium and carotid wall injury. All participants in the Multi-Ethnic Study Atherosclerosis (MESA) with LDL-P and HDL-P measured by ion mobility, coronary artery calcium score (CAC), carotid intima-media thickness (IMT) and carotid plaque data available at Exam 1 and 5 were included in the study. CAC progression was annualized and treated as a categorical or continuous variable. Carotid IMT and plaque progression were treated as continuous variables. Fully adjusted regression models included established CVD risk factors, as well as traditional lipids. Mean ( $\pm$ SD) follow-up duration was  $9.6\pm 0.6$  years. All LDL-P subclasses as well as large HDL-P at baseline were positively and significantly associated with annualized CAC progression, however, after adjustment for established risk factors and traditional lipids, only the association with medium and very small LDL-P remained significant ( $\beta -0.02$ ,  $p=0.019$  and  $\beta 0.01$ ,  $p=0.003$ , per 1 nmol/l increase, respectively). Carotid plaque score progression was positively associated with small and very small LDL-P ( $p<0.01$  for all) and non-HDL-P ( $p=0.013$ ). Only the association with very small LDL-P remained significant in a fully adjusted model ( $p=0.035$ ). Mean IMT progression was not associated with any of the lipid particles. In conclusion, in the MESA cohort, LDL-P measured by ion mobility was significantly associated with CAC progression as well as carotid plaque progression beyond the effect of traditional lipids.

## Keywords

CAC progression; carotid atherosclerosis; lipoprotein particles; ion mobility

## Introduction

Cardiovascular disease (CVD) risk prediction paradigms, based on scoring systems that combine information on traditional risk factors, do not fully explain CVD risk. There is considerable interest in the use of advanced lipid testing to identify individuals who are at elevated CVD risk and who could be targeted for preventive measures<sup>1,2</sup>. Most studies have reported determination of lipoprotein subfractions without a physical separation of lipoproteins by interpreting the nuclear resonance (NMR) signal of terminal methyl groups of triglycerides and cholesterol esters. The exquisite correlation between NMR measured low density lipoprotein particles (LDL-P) and apoB suggests that apoB measurements were used as part of the calibration algorithm raising doubt whether LDL-P by NMR provided information beyond apoB measurements. In this analysis, we measured lipoprotein particles using ion mobility – a method that separates lipoproteins by size based on the movement of charged particles in a gas-phase under the influence of an electric field<sup>3</sup>. We aimed to evaluate the performance of ion mobility derived lipoprotein particle measures in determining the association of low-density lipoprotein (LDL-P) and high-density lipoprotein particles (HDL-P) with progression of coronary and carotid atherosclerosis in the MESA cohort.

## Methods

The design and objectives of the Multi-Ethnic Study of Atherosclerosis (MESA), sponsored by the National Heart, Lung, and Blood Institute, have been described elsewhere<sup>4</sup>. Briefly, MESA study is a prospective cohort study of a multiethnic population-based sample of 6814 men and women aged 45 to 84 years who were free of known CVD at baseline, recruited from 6 U.S. sites. All participants gave informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Protocols were approved by the institutional review boards of the field and reading centers.

This paper used 3 outcome variables: (1) coronary artery calcium (CAC) progression, defined as yearly absolute change in CAC between Exam 1 and 5; (2) change in mean carotid intima and media thickness (cIMT), defined as yearly absolute change in mean cIMT between Exam 1 and 5; (3) progression of carotid plaque (increase in carotid plaque score) between Exam 1 and 5. The increase in carotid plaque score was not annualized due to ordinal rather than continuous nature of the variable. Mean cIMT was calculated as the mean of the mean left and right far wall distal common carotid artery wall thicknesses.

This analysis was restricted to subjects who had baseline and follow-up measures of any of the three outcomes. Cases were treated as missing if values at baseline or follow-up were missing. Progression was calculated as follow-up minus baseline and divided by follow-up time. Subjects who underwent a coronary revascularization procedure before follow-up were excluded from the analysis of CAC progression.

Carotid artery ultrasound performed and scored at the University of Wisconsin Imaging Research Program. At baseline and follow-up, B-mode ultrasound was used to image the near and far walls of the right and left distal common carotid artery (CCA), carotid bulb, and proximal internal carotid (ICA) using a Logiq 700 ultrasound system (13 MHz transducer; General Electric Medical Systems, Wauwatosa, WI). The carotid bifurcations and internal carotid arteries were interrogated thoroughly at 9 MHz from both longitudinal and transverse approaches to identify the thickest regions. Mean and maximal IMT of the far wall of distal CCA (distal 1 cm, proximal to the carotid bifurcation point, where the distal CCA diameter remains uniform) and the proximal 1 cm of the proximal internal carotid were measured in triplicate using a semiautomated border detection program (Syngo Arterial Health package; Siemens Medical Solutions, Malvern, PA) blinded to subject demographic and medical information<sup>5</sup>.

Carotid plaque presence was defined as a focal abnormal wall thickness (IMT >1.5 mm) or a focal thickening of >50% of the surrounding IMT. A total carotid plaque score (range, 0–12) was calculated to describe carotid plaque burden. Of the 12 segments analyzed for each participant, 1 point per plaque was allocated for the near and far walls of each segment (CCA, bulb, and ICA) of each carotid artery that was interrogated. The excellent reproducibility of the University of Wisconsin Ultrasound Reading Center's carotid ultrasound measurements using MESA images has been previously described in detail<sup>5</sup>.

Methods for computed tomography (CT) scanning and interpretation have been reported previously<sup>6, 7</sup>. CAC was assessed at all six MESA sites at baseline by using either a cardiac-

gated electron-beam CT scanner (Chicago, Los Angeles, and New York Field Centers) or a multidetector CT system (Baltimore, Forsyth County, and St. Paul Field Centers). CAC was determined by the Agatston score with excellent reproducibility<sup>7</sup>.

Traditional lipoproteins (LDL, HDL, triglycerides) were measured as previously described<sup>3,8</sup>. Ion mobility lipoprotein particles were measured at Quest Diagnostics Nichols Institute (San Juan Capistrano, CA)<sup>9</sup>. Briefly, following isolation by dextran sulfate precipitation, the lipoproteins were fractionated and quantitated in a single scan using gas-phase electrophoresis. The analysis provided large (I, IIa, range 22.0–23.33 nm), medium (IIb, 21.41–22.0 nm), small (IIIa, 20.82–21.41 nm) and very small (IIIb, IVa to c, 18.0–20.82 nm) LDL particles, large (10.5–14.5 nm) and small HDL-P (7.65–10.5 nm), large (25.0–29.6 nm) and small (23.33–25.0 nm) intermediate density lipoprotein particles (IDL-P), and large (42.4–52.0 nm), medium (33.5–42.4), and small (29.6–33.5) very low density lipoprotein particles (VLDL-P)<sup>9</sup>

Change in CAC score between baseline and follow-up was analyzed as a continuous or categorical variable (0, 1-99, 100-199, 200-300, >300 Agatston units). Approximately half of the subjects had a CAC score and carotid plaque score of 0 at baseline, hence due to the highly skewed nature of the data, CAC score was log-transformed and carotid plaque were analyzed as  $\ln(\text{carotid plaque score} + 1)$  and as transformed and untransformed score ranging from 0 to 12 to allow for more-direct comparison. Annualized CAC change was calculated as  $(\ln \text{CAC} + 1 \text{ at follow-up} - \ln \text{CAC} + 1 \text{ at baseline}) / \text{years of follow up}$ . Yearly change in carotid IMT was analyzed as a continuous variable and estimated as  $\text{IMT at follow-up} - \text{IMT at baseline} / \text{years of follow up}$ . In cases of CAC, cIMT, carotid plaque regression, the values were treated as continuous variables in the analyses.

Lipoprotein particles were analyzed as continuous variables. Univariate comparisons (ANOVA analysis) and multivariate comparisons using robust linear regression analysis were performed. Robust linear regression, which down-weights observations with large residuals, was used for the analysis of CAC, cIMT and carotid plaque.

Model 1 included baseline (Exam 1) parameters such as age (years), race/ethnicity (white, Chinese, Black, Hispanic), body-mass index, current cigarette smoking (compared to never/former), parent history of myocardial infarction, systolic blood pressure, diastolic blood pressure, diabetes status (insulin use or fasting glucose >140), fasting glucose, high sensitivity C-reactive protein, lipid-lowering medication use, and family income. Model 2 (fully adjusted model) included Model 1 plus LDL-C, HDL-C, and triglycerides.

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, North Carolina, USA). A p value of <0.05 was considered significant.

## Results

Mean follow-up was  $9.6 \pm 0.6$  years. 2,510 and 3,305 subjects were included in the analyses for cIMT and carotid plaque, and CAC, respectively (Figure 1).

Baseline characteristics of these subgroups are provided in Table 1. In addition, 715 (21.6%) subjects had zero CAC both at baseline and follow-up.

Higher concentrations of total LDL-P, very small (IIIb to IVc) to small (IIIa) LDL-P, and non-HDL-P, also lower levels of large LDL-P (I to IIa) and large HDL-P subfractions were significantly associated with greater CAC progression, defined as the increase in absolute CAC per year (Figure 2). Although medium LDL-P (IIb) was also associated with annual CAC progression, the trend was non-linear.

Annual change in CAC was significantly associated with all baseline LDL-P subfractions in unadjusted analyses (Table 2). When adjusted for major CVD risk factors (Model 1) and additionally for conventional lipids (Model 2), annual change in CAC retained significant positive associations with very small LDL-P (IIIb to IVc) and an inverse association with large and medium LDL-P, but not with IDL-P or HDL-P (Table 2).

In unadjusted or adjusted (both Model 1 and Model 2) analyses, annual change in mean cIMT was not associated with any of the lipoprotein particles (Table 3).

In unadjusted and Model 1 adjusted linear regression analyses, change in carotid plaque score was significantly associated with total LDL-P, as well as small to very small LDL-P (IIIa to IVc), but not large or medium LDL-P, or HDL-P (Table 4). In the fully adjusted model (Model 2), additionally adjusted for traditional lipoproteins, change in carotid plaque score was significantly associated only with very small LDL-P (LDL-P IIIb,  $p=0.035$ ).

## Discussion

As previously in most of the studies lipoprotein particle testing was performed with NMR, this study is one of the few that used ion mobility to determine lipoprotein particle concentration. Rather than imputing particle data based on its presumed composition, ion mobility separates and analyzes the lipoprotein particles themselves. Ion mobility measures lipoprotein concentration for the entire size spectrum of lipoprotein particles ranging from 5 nm to 53 nm at a high size resolution ( $<0.1$  nm diameter on average)<sup>3</sup>.

In this study, we observed a significant positive association of small and very small LDL-P with two measures of atherosclerosis: CAC progression and carotid plaque progression even after adjustment for traditional lipids. It is known that small LDL particles contain substantially less cholesterol than large LDL-P, such that at the same serum concentration of LDL cholesterol, individuals with predominantly small LDL have greater total concentration of LDL particles than those with predominantly large LDL, and thus may have greater CVD risk<sup>10</sup>. This may explain why LDL particles are associated with atherosclerosis and CVD outcomes independently from total LDL cholesterol that is a general measure of lipid pool. Small LDL-P may be more atherogenic due to their susceptibility to oxidation and greater affinity for proteoglycans, increasing subendothelial permeability and accumulation<sup>11</sup>. Moreover, oxidized LDL may promote an inflammatory response that could lead to plaque formation and vulnerability<sup>12</sup>.

This is in agreement with the Malmo Prevention Project study indicating that very small LDL-P measured by ion mobility were significantly associated with CVD events independently of traditional risk factors and traditional lipids<sup>13</sup>. Moreover, the simulation model was constructed to further suggest that lipid particles included into a functional risk score were associated with CVD events after adjustment for traditional risk factors<sup>14</sup>. Furthermore, a recent cross-sectional study reported an association between ion-mobility measured lipid particles with the presence of CAC in subjects with diabetes or metabolic syndrome<sup>15</sup>.

The HDL Atherosclerosis Treatment Study that measured lipoprotein subfractions using four methods, including NMR and ion mobility, found that small dense LDL were independently related to coronary artery stenosis progression, although the extent of these associations differed depending on the method used<sup>16</sup>. In a previous cross-sectional MESA baseline analysis (n=5538), an association of NMR-derived LDL size and small LDL-P with carotid intima-media thickness was no longer significant after accounting for lipoprotein subclasses and risk factors<sup>17</sup>. However, Otvos et al. demonstrated that in case of discordance between LDL-C and NMR measured LDL-P, LDL-P better predicted incident CVD events and cIMT in a MESA cohort, compared to LDL-C<sup>18</sup>. Moreover, in a post-hoc analysis of the JUPITER trial that investigated the association of ion mobility measured lipoprotein particles with CVD events, LDL-P and smaller subfractions of LDL-P and VLDL-P, but not baseline LDL-C, were related to CVD events<sup>9</sup>.

In our analysis, large, but not small, HDL-P were significantly negatively associated with CAC progression, but this association became insignificant after adjustment for standard lipid measures. In the Malmö Prevention Project Study, high levels of HDL-C, but not HDL-P subfractions, were associated with incident CVD<sup>13</sup>. The varying association of HDL particles with progression of subclinical atherosclerosis may be in part explained by the heterogeneity of the HDL particles, genetic factors, and also impacted by cholesterol efflux capacity that was not measured in this study<sup>19</sup>.

A possible limitation for this study was that ion mobility was performed on samples that were previously frozen and stored for a prolonged period of time. The impact of storage and freezing on lipoprotein analysis is not known. However, lipoprotein profiles from MESA stored samples have been shown to be consistent with those obtained from fresh frozen specimens per Quest Diagnostics laboratory. Moreover, multiple comparisons were performed increasing the probability of a Type I error.

In agreement with previously reported data from outcome studies<sup>9</sup>, this analysis demonstrated that lipoprotein particles measured with ion mobility were associated with atherosclerosis progression in the coronary and carotid vessels independently from conventional methods. The importance of this finding in overall and residual cardiovascular risk prediction should be further evaluated in prospective studies using ion mobility testing.

In conclusion, LDL particles measured with ion mobility were independently associated with progression of atherosclerosis in a MESA cohort. A significant positive association of small and very small LDL-P with progression of CAC and carotid plaque score was

observed. Large HDL particles were significantly inversely associated with CAC and carotid plaque progression; however, this association was attenuated after adjustment for traditional lipids.

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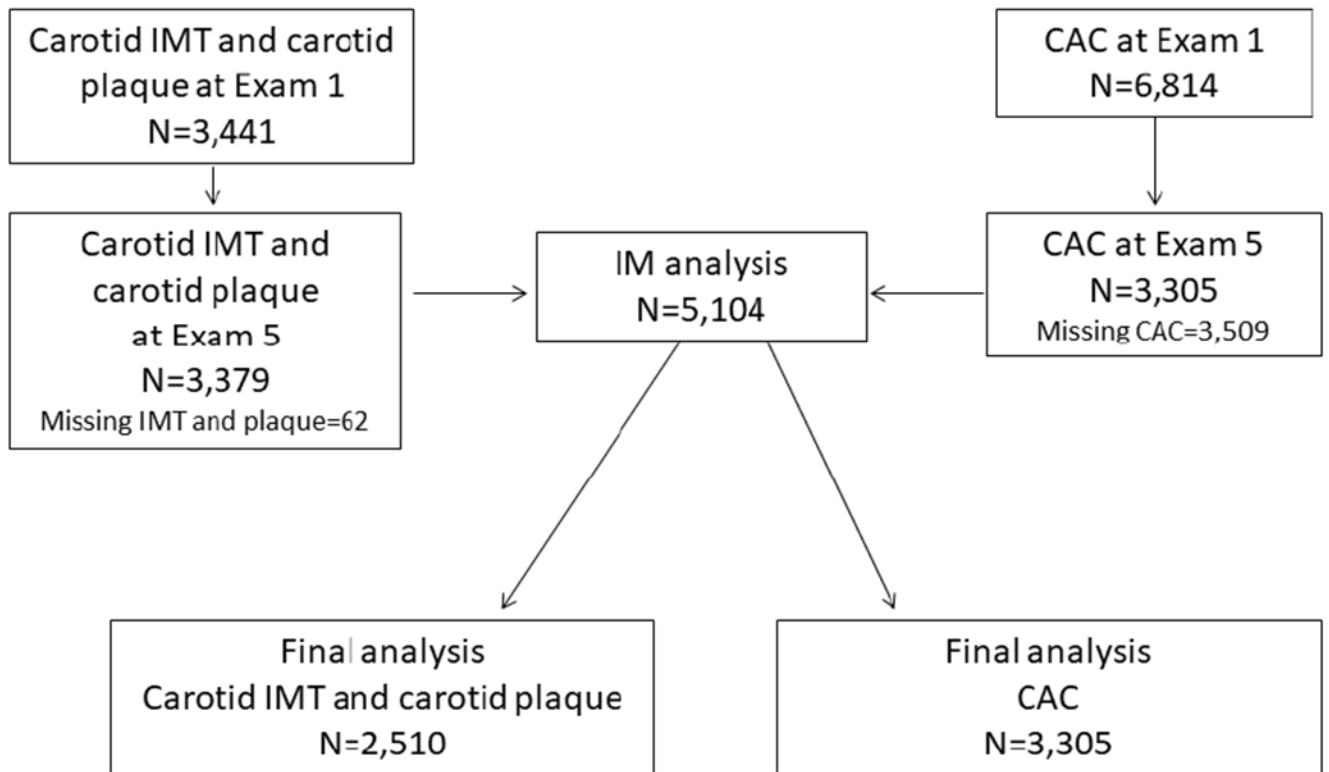
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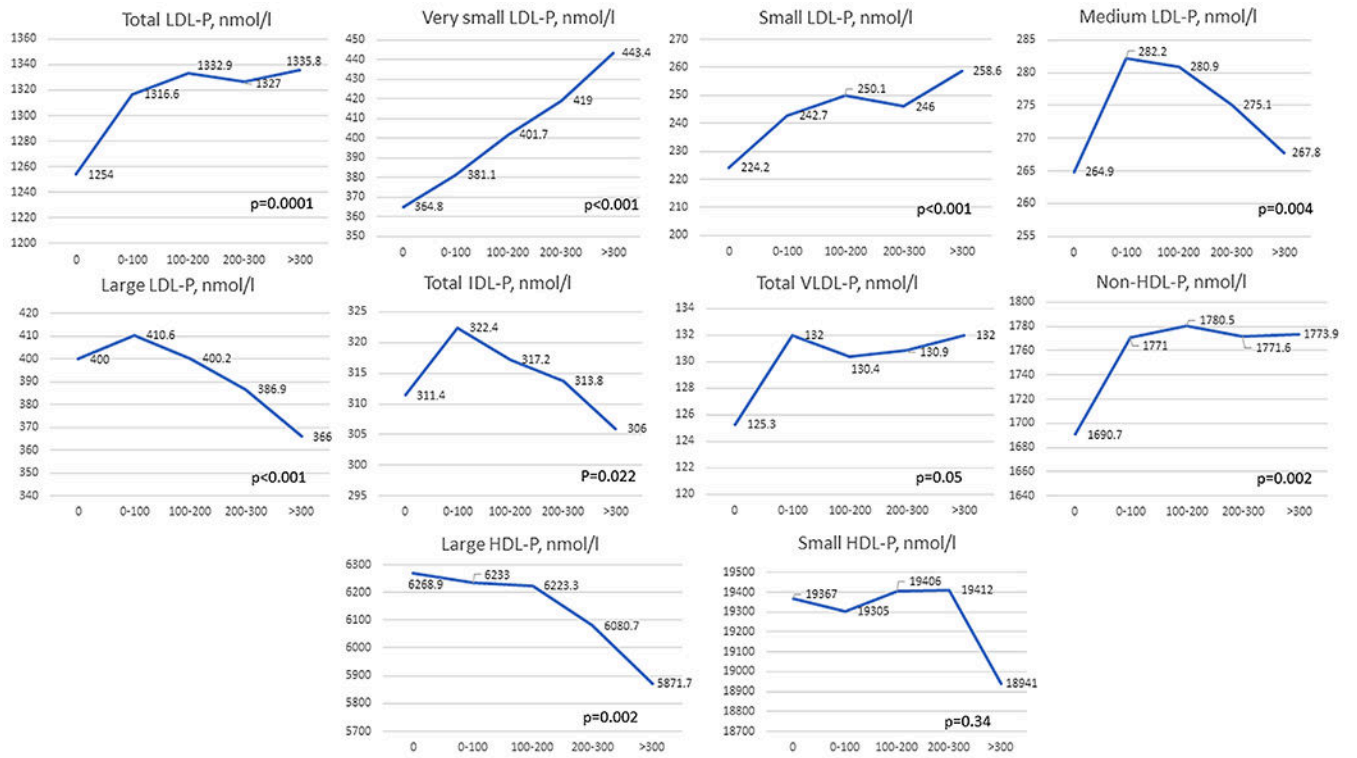
### Highlights

- Small and very small LDL-P were associated with progression of atherosclerosis.
- Advanced lipid testing may identify individuals who are at elevated CVD risk.
- Lipid ion mobility testing should be included into prospective outcome studies.

**Figure 1. Study flowchart**

Overall, 5,104 subjects with CAC (n=3,305) and carotid IMT/plaque (n=2,510) data available at Exams 1 and 5 were included in the analysis.

CAC, coronary artery calcium, IMT, intima media thickness.



**Figure 2. Association of lipoprotein subfractions with annual CAC change**

Higher concentrations of total LDL-P, very small (IIIb to IVc) to small (IIIa) LDL-P, and non-HDL-P, also lower levels of large LDL-P (I to IIa) and large HDL-P subfractions were significantly associated with greater CAC progression, defined as the increase in absolute CAC per year (Figure 2). Although medium LDL-P (IIb) was also associated with annual CAC progression, the trend was non-linear.

CAC, coronary artery calcium, LDL-P, low density lipoprotein particle, HDL-P, high density lipoprotein particle. Data presented as mean±SD.

**Table 1.**

## Baseline characteristics of study participants

Variable	CAC (n=3,305)	cIMT/ Carotid plaque (n=2,510)
Age (years)	60.1±9.4	59.8±9.3
Men	1571(47.5%)	1286(47.4%)
White	1303(39.4%)	1080(39.8%)
Chinese	384(11.6%)	325(12.0%)
Black	884(26.7%)	708(26.1%)
Hispanic	734(22.2%)	600(22.1%)
Height (cm)	166.9±9.9	166.9±9.9
Weight (kg)	79.4±17.0	78.7±16.9
BMI (kg/m <sup>2</sup> )	28.4±5.3	28.2±5.1
Waist circumference (cm)	97.8±14.0	97.2±13.9
Systolic BP (mmHg)	125.8±20.3	125.0±19.9
Diastolic BP (mmHg)	72.9±10.6	72.7±10.7
Non-HDL particle number (nmol/l)	1748.4±453.6	1742.3±451.2
LDL-C (mg/dL)	117.8±30.9	117.8±30.7
HDL-C (mg/dL)	50.7±14.7	51.0±14.8
Triglycerides (mg/dl)	130.9±82.0	129.2±83.0
Fasting plasma glucose (mg/dL)	95.3±26.4	94.5±25.3
hs-CRP (mg/L)	3.5±5.2	3.4±5.0
Hypertension	1361(41.2%)	1054(38.8%)
Current cigarette smoker	392(11.9%)	327(12.1%)
Diabetes mellitus	312(9.4%)	235(8.7%)
Lipid-lowering medication	528(16.0%)	420(15.5%)
CAC=0	1011(30.6%)	1868(68.9%)
CAC (Agatston score)	287.5±595.2	273.3±587.1
Carotid plaque	2110(68.3%)	1841(67.9%)
Mean carotid plaque score	2.2±2.4	2.3±2.5
Mean cIMT (mm)	0.9±0.5	0.9±0.2

BP – blood pressure, CAC – coronary artery calcium, hs-CRP – high sensitivity C reactive protein, cIMT – carotid intima media thickness, HDL – high density lipoprotein cholesterol, LDL-P – low density lipoprotein cholesterol, HDL-P – high density lipoprotein cholesterol. Data presented as mean±SD.

**Table 2.**

Association between annual change in coronary artery calcium and lipoprotein subfractions in univariate and multivariate analyses

Lipoprotein particles	Unadjusted			Model 1			Model 2		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
LDL-P Total (nmol/l)	0.004	-0.001,0.008	0.117	0.005	0.001,0.009	<b>0.019</b>	0.001	-0.001,0.01	0.747
LDL-P I (nmol/l)	-0.033	-0.049, -0.017	<b>&lt;0.001</b>	-0.019	-0.034, -0.003	<b>0.020</b>	-0.01	-0.03,0.01	0.446
LDL-P IIa (nmol/l)	-0.047	-0.070, -0.024	<b>&lt;0.001</b>	-0.025	-0.047, -0.003	<b>0.023</b>	-0.03	-0.05, -0.01	<b>0.045</b>
LDL-P IIb (medium) (nmol/l)	-0.018	-0.034, -0.002	<b>0.028</b>	-0.006	-0.022,0.009	0.403	-0.02	-0.04, -0.00	<b>0.019</b>
LDL-P IIIa (small) (nmol/l)	0.02	0.006,0.035	<b>0.005</b>	0.019	0.006,0.033	<b>0.007</b>	0.01	-0.02,0.02	0.959
LDL-P IIIb (nmol/l)	0.059	0.037,0.082	<b>&lt;0.001</b>	0.050	0.029,0.071	<b>&lt;0.001</b>	0.03	0.01,0.06	<b>0.029</b>
LDL-P Iva (nmol/l)	0.059	0.039,0.079	<b>&lt;0.001</b>	0.050	0.030,0.067	<b>&lt;0.001</b>	0.004	0.02,0.06	<b>0.001</b>
LDL-P IVb (nmol/l)	0.08	0.047,0.113	<b>&lt;0.001</b>	0.065	0.034,0.097	<b>&lt;0.001</b>	0.05	0.01,0.09	<b>0.008</b>
LDL-P IVc (nmol/l)	0.064	0.010,0.119	<b>0.020</b>	0.054	0.002,0.105	<b>0.004</b>	0.03	-0.02,0.09	0.264
LDL-P large (I and IIa combined) (nmol/l)	-0.021	-0.030, -0.011	<b>&lt;0.001</b>	-0.011	-0.021, -0.002	<b>0.017</b>	-0.01	-0.02,0.01	0.177
LDL-P very small (IIIb to IVc combined) (nmol/l)	0.023	0.015,0.030	<b>&lt;0.001</b>	-0.011	0.011,0.026	<b>&lt;0.001</b>	0.01	0.001,0.02	<b>0.003</b>
VLDL-P total (nmol/l)	0.004	-0.029,0.036	0.834	0.021	-0.009,0.052	-0.173	-0.003	-0.07,0.01	0.204
VLDL-P large (nmol/l)	0.153	-0.027,0.333	0.096	0.206	0.037,0.375	<b>0.017</b>	-0.01	-0.34,0.14	0.419
VLDL-P medium (nmol/l)	0.029	-0.047,0.105	0.453	0.069	-0.002,0.140	<b>0.056</b>	-0.06	-0.16,0.04	0.224
VLDL-P small (nmol/l)	-0.034	-0.106,0.038	0.358	0.008	-0.059,0.076	0.806	-0.06	-0.14,0.03	0.183
IDL-P total (nmol/l)	-0.019	-0.036, -0.001	<b>0.034</b>	-0.01	-0.027,0.006	0.213	-0.02	-0.04,0.01	0.097
IDL-P large (nmol/l)	-0.006	-0.039,0.026	0.704	0.004	-0.027,0.034	0.813	-0.03	-0.07,0.01	0.091
IDL-P small (nmol/l)	-0.049	-0.079, -0.020	<b>0.002</b>	-0.033	-0.061, -0.006	<b>0.019</b>	-0.02	-0.06,0.01	0.183
Non-HDL-P (nmol/l)	0.001	-0.002,0.005	0.420	0.003	-0.000,0.006	0.089	-0.001	-0.001,0.01	0.798
HDL-P large (nmol/l)	-0.001	-0.002, -0.001	<b>0.003</b>	-0.001	-0.002,-0.001	<b>0.007</b>	0.001	-0.001,0.01	0.824
HDL-P small (nmol/l)	-0.001	-0.00,0.001	0.274	-0.001	-0.001,0.001	0.274	-0.001	-0.001,0.01	0.573

CAC – coronary artery calcium, LDL-P – low density lipoprotein particle, VLDL-P – very low density lipoprotein particle, IDL-P – intermediate density lipoprotein particle, HDL-P – high density lipoprotein particle. CAC change shows the yearly change between the baseline coronary artery calcium and a follow-up visit approximately 10 years later. Robust linear regression, which down-weights observations with large residuals, was used due to a few cases with exceptionally large CAC values. Model 1: age (years), race/ethnicity (white, Chinese, Black, Hispanic), BMI, current cigarette smoking (compared to never/former), parent history of myocardial infarction, systolic blood pressure, diastolic blood pressure, diabetes status (insulin use or fasting glucose >140), fasting glucose, high sensitivity C-reactive protein (ln) and lipid-lowering medication use, family income. Model 2: Model 1 + LDL-C, HDL-C, triglycerides (ln)

**Table 3.**

Association between annual change in mean cIMT and ion mobility measured lipoprotein subfractions

Lipoprotein particles	Unadjusted			Model 1			Model 2		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
LDL-P total (nmol/l)	0.001	-0.001,0.003	0.232	0.001	-0.001,0.003	0.167	-0.001	-0.001,0.001	0.729
LDL-P I (nmol/l)	0.001	-0.005,0.007	0.683	0.001	-0.005,0.007	0.713	-0.001	-0.01,0.01	0.726
LDL-P IIa (nmol/l)	0.004	-0.005,0.012	0.373	0.004	-0.005,0.012	0.419	-0.001	-0.01,0.01	0.758
LDL-P IIb (medium) (nmol/l)	0.004	-0.002,0.010	0.147	0.005	-0.001,0.01	0.122	0.001	-0.01,0.01	0.981
LDL-P IIIa (small) (nmol/l)	0.003	-0.002,0.008	0.265	0.004	-0.001,0.009	0.153	-0.001	-0.01,0.01	0.963
LDL-P IIIb (nmol/l)	0.001	-0.007,0.009	0.803	0.002	-0.006,0.011	0.583	-0.001	-0.02,0.01	0.514
LDL-P IVa (nmol/l)	0.001	-0.007,0.008	0.880	0.002	-0.006,0.009	0.762	-0.001	-0.01,0.01	0.712
LDL-P IVb (nmol/l)	0.003	-0.009,0.015	0.632	0.003	-0.009,0.016	0.616	0.001	-0.01,0.02	0.669
LDL-P IVc (nmol/l)	0.001	-0.019,0.021	0.948	0.001	-0.020,0.021	0.981	-0.001	-0.01,0.01	0.785
LDL-P large (I and IIa combined) (nmol/l)	0.001	-0.002,0.005	0.530	0.001	-0.003,0.005	0.569	-0.001	-0.01,0.01	0.730
LDL-P very small (IIIb to IVc combined) (nmol/l)	0.001	-0.003,0.003	0.787	0.001	-0.002,0.004	0.661	-0.001	-0.01,0.01	0.757
VLDL-P total (nmol/l)	0.006	-0.006,0.018	0.356	0.006	-0.006,0.019	0.320	-0.001	-0.02,0.02	0.964
VLDL-P large (nmol/l)	0.029	-0.037,0.096	0.385	0.027	-0.041,0.096	0.437	0.02	-0.08,0.12	0.649
VLDL-P medium (nmol/l)	0.013	-0.015,0.041	0.379	0.013	-0.016,0.042	0.388	-0.001	-0.04,0.04	0.879
VLDL-P small (nmol/l)	0.012	-0.015,0.038	0.395	0.015	-0.013,0.042	0.287	-0.001	-0.04,0.03	0.905
IDL-P total (nmol/l)	0.001	-0.005,0.008	0.667	0.002	-0.004,0.009	0.521	-0.001	-0.01,0.01	0.511
IDL-P large (nmol/l)	0.005	-0.007,0.017	0.397	0.007	-0.005,0.019	0.273	-0.001	-0.02,0.01	0.684
IDL-P small (nmol/l)	-0.001	-0.011,0.011	0.973	0.001	-0.011,0.012	0.923	-0.001	-0.02,0.01	0.451
Non-HDL-P (nmol/l)	0.001	-0.001,0.002	0.268	0.001	-0.001,0.002	0.193	-0.001	-0.02,0.01	0.675
HDL-P large (nmol/l)	-0.001	-0.001,0.001	0.130	-0.001	-0.001,0.001	0.285	0.001	-0.001,0.01	0.911
HDL-P small (nmol/l)	-0.001	-0.001,0.001	0.797	0.001	-0.001,0.001	0.966	0.001	-0.001,0.01	0.817

IMT – intima media thickness, LDL-P – low density lipoprotein particle, VLDL-P – very low density lipoprotein particle, IDL-P – intermediate density lipoprotein particle, HDL-P – high density lipoprotein particle.

Model 1: age (years), race/ethnicity (white, Chinese, Black, Hispanic), BMI, current cigarette smoking (compared to never/former), parent history of myocardial infarction, systolic blood pressure, diastolic blood pressure, diabetes status (insulin use or fasting glucose >140), fasting glucose, high sensitivity C-reactive protein (ln) and lipid-lowering medication use, family income.

Model 2: Model 1 + LDL-C, HDL-C, triglycerides (ln)

**Table 4.**

Association between carotid plaque progression and ion mobility measured lipoprotein subtractions

Lipoprotein particles	Unadjusted			Model 1			Model 2		
	Coef	95% CI	P	Coef	95% CI	P	Coef	95% CI	P
LDL-P total (nmol/l)	-0.001	-0.001,0.001	<b>0.002</b>	-0.001	-0.001,0.001	<b>&lt;0.001</b>	0.001	-0.001,0.001	0.316
LDL-P I (nmol/l)	-0.001	-0.001,0.001	0.091	-0.001	-0.001,0.001	0.623	-0.001	-0.001,0.001	0.287
LDL-P IIa (nmol/l)	-0.001	-0.001,0.001	0.744	0.001	-0.001,0.001	0.503	-0.001	-0.001,0.001	0.397
LDL-P IIb (medium) (nmol/l)	0.001	-0.001,0.001	0.083	0.001	0.001,0.002	<b>0.018</b>	-0.001	-0.001,0.001	0.761
LDL-P IIIa (small) (nmol/l)	0.001	0.001,0.002	<b>&lt;0.001</b>	0.001	0.001,0.002	<b>&lt;0.001</b>	0.001	-0.001,0.001	0.057
LDL-P IIIb (nmol/l)	0.001	0.001,0.002	<b>&lt;0.001</b>	0.001	0.001,0.002	<b>&lt;0.001</b>	0.001	0.001,0.002	<b>0.035</b>
LDL-P IVa (nmol/l)	0.001	0.001,0.002	<b>0.0028</b>	0.001	0.001,0.002	<b>0.011</b>	0.001	-0.001,0.001	0.179
LDL-P IVb (nmol/l)	0.001	0.001,0.002	<b>0.0029</b>	0.001	-0.001,0.001	0.096	0.001	-0.001,0.001	0.215
LDL-P IVc (nmol/l)	0.001	-0.001,0.001	0.284	0.001	-0.001,0.001	0.330	0.001	-0.001,0.001	0.667
LDL-P large (I and IIa combined) (nmol/l)	-0.001	-0.001,0.001	0.247	-0.001	-0.001,0.001	0.995	-0.001	-0.001,0.001	0.314
LDL-P very small (IIIb to IVc combined) (nmol/l)	0.001	0.001,0.002	<b>&lt;0.001</b>	0.001	0.001,0.002	<b>&lt;0.001</b>	0.001	-0.001,0.001	0.095
VLDL-P total (nmol/l)	0.001	-0.001,0.001	0.374	0.001	-0.001,0.001	0.172	-0.001	-0.001,0.001	0.208
VLDL-P large (nmol/l)	0.001	-0.001,0.001	0.123	0.001	-0.001,0.001	0.158	-0.001	-0.001,0.001	0.574
VLDL-P medium (nmol/l)	0.001	-0.001,0.001	0.127	0.001	-0.001,0.001	0.062	-0.001	-0.001,0.001	0.486
VLDL-P small (nmol/l)	-0.001	-0.001,0.001	0.917	0.001	-0.001,0.001	0.494	-0.001	-0.001,0.001	0.067
IDL-P total (nmol/l)	-0.001	-0.001,0.001	0.798	0.001	-0.001,0.001	0.630	-0.001	-0.001,0.001	0.116
IDL-P large (nmol/l)	0.001	-0.001,0.001	0.294	0.001	-0.001,0.001	0.135	-0.001	-0.001,0.001	0.125
IDL-P small (nmol/l)	-0.001	-0.001,0.001	0.168	-0.001	-0.001,0.001	0.587	-0.001	-0.001,0.001	0.187
Non-HDL-P (nmol/l)	0.001	0.001,0.002	<b>0.013</b>	0.001	0.001,0.002	<b>0.002</b>	0.001	-0.001,0.001	0.762
HDL-P large (nmol/l)	-0.001	-0.001,0.001	0.105	-0.001	-0.001,0.001	0.06	0.001	-0.001,0.01	0.937
HDL-P small (nmol/l)	-0.001	-0.001,0.001	0.769	-0.001	-0.001,0.001	0.556	-0.001	-0.001,0.01	0.857

LDL-P – low density lipoprotein particle, VLDL-P – very low density lipoprotein particle, IDL-P – intermediate density lipoprotein particle, HDL-P – high density lipoprotein particle.

Model 1: age (years), race/ethnicity (white, Chinese, Black, Hispanic), BMI, current cigarette smoking (compared to never/former), parent history of myocardial infarction, systolic blood pressure, diastolic blood pressure, diabetes status (insulin use or fasting glucose >140), fasting glucose, high sensitivity C-reactive protein (ln if needed) and lipid-lowering medication use, family income.

Model 2: Model 1 + LDL-C, HDL-C, triglycerides (ln)