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RESEARCH LETTER
Lipids & Atherosclerosis

Serum modified high-density lipoprotein and risk of atherosclerotic cardiovascular disease in a Japanese community-based nested case–control study

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High-density lipoprotein cholesterol (HDL-C) is inversely associated with the risk of coronary heart disease (CHD). However, persons with abnormally high HDL-C levels reportedly have a paradoxically higher risk of developing atherosclerotic cardiovascular disease (ASCVD) or related mortality.[1,2] HDL that undergoes oxidation and glycation loses its anti-atherosclerotic function, and its excess increases the risk of developing atherosclerosis.[3] Modified HDL is suspected of contributing to the progression of atherosclerosis by binding to the lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1).[4] A novel method for measuring various types of modified HDL that bind to LOX-1, designated as LOX-1 ligand containing apoAI (LAA), was developed.[4] The present study aimed to investigate the effect of LAA as a marker of dysfunctional HDL on the incidence of ASCVD in a large-scale Japanese community-based cohort study.

The Tsuruoka Metabolomics Cohort Study is a prospective study involving 11 002 dwellers aged 35–74 years in Tsuruoka City, Yamagata Prefecture, Japan. The baseline survey was conducted from April 2012 until March 2015. We conducted a nested case–control study, including 52 new ASCVD cases developed from the baseline survey to 31 December 2017. The Medical Ethics of the Keio University School of Medicine, Tokyo, Japan, approved the study (approval no. 20110264), and all participants provided written informed consent. To determine the incidence of ASCVD events, i.e. CHD and atherothrombotic cerebral infarction, the medical records of participants, suspected to have ASCVD were reviewed, and the final diagnoses of the first incidence were made by a panel of experienced physicians including at least two more of cardiologists and neurologists based on patient's symptoms, electrocardiogram, coronary angiography, computed tomography, and magnetic resonance imaging. We also performed a search of public death certificates to identify fatal ASCVD-related events. For each ASCVD case, three controls without a history of cardiovascular diseases were randomly selected from the participants by matching them with the baseline year, sex, age (within three years), and health check-up facility.

Of all participants in Tsuruoka Metabolomics Cohort Study, a total of 208 participants (52 cases and 156 controls) were selected in the present nested case–control study. The baseline serum samples of participants had been stored at −80°C until the analysis was conducted in Hokenkagaku West Co. Ltd., Kyoto, Japan. According to the previously described method, LAA was measured using a LOX-1 binding-based enzyme-linked immunosorbent assay with recombinant LOX-1 and anti-apoAI antibodies.[4] The intra-assay and inter-assay coefficients of variance were 6.9% ($n = 16$) and 12.7% ($n = 13$), respectively. Conditional logistic regression models were used to calculate the odds ratio (OR) and 95% confidence interval (CI) of the incidence of ASCVD according to quartiles and 1-standard deviation (SD) increment of log-transformed LAA levels adjusted for body mass index, hypertension, diabetes, anti-hypercholesterolaemia medication, LDL-C, HDL-C, chronic kidney disease, current smoking, current drinking, and modified LDL, designated as LOX-1 ligand containing

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Table 1 Risk characteristics among cases and control participants

	Cases	Controls	*P* for mean difference
No.	52	156	
Age, years, mean ± SD	64.6 ± 6.9	64.6 ± 6.8	
Men, %	71.2	71.2	
Body mass index, kg/m^2, mean ± SD	23.9 ± 3.0	23.7 ± 3.1	0.61
Hypertension, %	73.1	54.5	0.018
Anti-hypertensive mediation, %	48.1	38.5	0.22
Diabetes, %	30.8	9.6	<0.001
Medication for diabetes, %	23.1	5.1	<0.001
Anti-hypercholesterolaemia mediation, %	30.8	18.6	0.065
LDL cholesterol, mg/dL, mean ± SD	123 ± 32	116 ± 33	0.23
HDL cholesterol, mg/dL, mean ± SD	58 ± 15	65 ± 18	0.010
Chronic kidney disease, %	21.2	17.3	0.54
Current smoking, %	21.2	20.5	0.92
Current drinking, %	46.2	60.9	0.063
LAB[a], ng cs/mL (geometric min.–max.)	700–3500	500–3200	0.22

Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, and/or use of anti-hypertensive medication. Diabetes was defined as fasting glucose ≥126 mg/dL, glycated haemoglobin ≥6.5%, and/or medication use for diabetes. Chronic kidney disease was defined as positive for proteinuria and/or an eGFR of <60 mL/min per 1.73 m^2.
[a]LAB, lectin-like oxidized low-density lipoprotein receptor-1 ligand containing apoB.

Table 2 Univariate and matched and multivariable-adjusted odds ratios of atherosclerotic cardiovascular disease (coronary heart disease and atherothrombotic cerebral infarction) according to serum LAA levels

	Quartiles of serum total LAA,[b] ng/mL				OR per 1-SD increment of log-transformed LAA
	1 (Low)	2	3	4 (High)	
Total LAA, ng/mL					
Median	90.5	129.5	165.7	223.3	
Range	37.8–108.5	109.4–145.8	147.0–181.8	182.2–684.0	
Atherosclerotic cardiovascular disease					
No. of cases	11	8	16	17	
No. of controls	41	44	36	35	
Matched OR	1.00	0.811 (0.270–2.44)	1.88 (0.669–5.28)	2.12 (0.744–6.03)	1.64 (1.12–2.39)
Matched and multivariable-adjusted OR[a]	1.00	0.886 (0.229–3.43)	2.07 (0.597–7.16)	2.50 (0.703–8.86)	2.16 (1.28–3.64)
Coronary heart disease					
No. of cases	6	5	10	12	
No. of controls	28	28	21	22	
Matched OR	1.00	1.07 (0.248–4.65)	2.42 (0.653–9.00)	3.05 (0.805–11.6)	1.74 (1.11–2.74)
Matched and multivariable-adjusted OR[a]	1.00	1.35 (0.213–8.59)	2.98 (0.578–15.3)	4.23 (0.752–23.8)	2.43 (1.24–4.74)
Atherothrombotic cerebral infarction					
No. of cases	5	3	6	5	
No. of controls	13	16	15	13	
Matched OR	1.00	0.522 (0.094–2.88)	1.07 (0.184–6.23)	1.03 (0.177–5.99)	1.40 (0.685–2.85)
Matched and multivariable-adjusted OR[a]	1.00	0.523 (0.040–6.89)	1.59 (0.107–23.6)	2.49 (0.187–33.1)	2.63 (0.794–8.69)

[a]Multivariable adjusted for body mass index, hypertension, diabetes, anti-hypercholesterolaemia mediation, LDL cholesterol, HDL cholesterol, chronic kidney disease, current smoking, current drinking, and lectin-like oxidized LDL receptor-1 ligand containing apoB.
[b]LAA, lectin-like oxidized low-density lipoprotein receptor-1 ligand containing apoAI.

apoB.[5] All statistical analyses were performed with SAS 9.4 software (SAS Institute, Inc., Cary, NC, USA).

As for the participants' baseline characteristics in *Table 1*, the mean age was 64.6 years, and 71.2% were male. The proportion of participants with hypertension, diabetes, and use of anti-hypercholesterolaemia medication was higher in cases than in controls (73.1% vs. 54.5%, $P = 0.018$; 30.8% vs. 9.6%, $P < 0.001$; 30.8% vs. 18.6%, $P = 0.065$). The mean value of HDL-C was lower in cases than in controls (58 mg/dL vs. 65 mg/dL, $P = 0.010$). The mean follow-up period of study participants was 3.9 years. On multivariable analysis (*Table 2*), the matched OR (95% CI) of ASCVD for the highest vs. lowest quartiles of LAA was 2.50 (0.703–8.86), and that for the 1-SD increment of LAA was 2.16 (1.28–3.64). LAA was associated with the incidence of CHD or atherothrombotic cerebral infarction. The corresponding ORs (95% CIs) were 4.23 (0.752–23.8) and 2.43 (1.24–4.74) for CHD, and 2.49 (0.187–33.1) and 2.63 (0.794–8.69) for atherothrombotic cerebral infarction.

Various modified HDL types with impaired anti-atherosclerotic function lead to atherosclerosis by binding to LOX-1. Previous studies have demonstrated the effect of the specific type of modified HDL (e.g. myeloperoxidase HDL) on cardiovascular disease in haemodialysis[6] or psoriasis patients.[7] However, there has been little evidence regarding the effect of modified HDL that can bind to LOX-1 on cardiovascular events. The present study revealed that the risk of ASCVD increased with elevated LAA levels. This was in line with a previously published cross-sectional study, which positively associated LAA with coronary artery calcification, regardless of HDL-C and HDL particle concentration levels.[8] LAA reflects the total impact of modified HDL that can bind to LOX-1. This finding provided valuable evidence for the underlying mechanism of the proatherogenic effect of modified HDL.

The present study had several limitations. First, the follow-up period was not long. Longer follow-up period may make more robust association between LAA and ASCVD. Second, we did not measure other lipid-related risk factors, such as lipoprotein(a) [Lp(a)], which is considered as an inherited risk factor of ASCVD.[9]

In conclusion, the present study suggested that elevated LAA affected the incidence of ASCVD events independent of HDL-C levels in Japanese community-dwellers, and LAA was a potentially useful marker of dysfunctional HDL to predict the progression of atherosclerosis. Further investigation involving a larger scale longitudinal study with a longer follow-up period is warranted.

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Data Availability

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. The data will be shared on reasonable request to the Ethics Committee for Tsuruoka Metabolomics Cohort Study via the corresponding authors.

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References

1. Hirata A, Sugiyama D, Watanabe M, Tamakoshi A, Iso H, Kotani K, Kiyama M, Yamada M, Ishikawa S, Murakami Y, Miura K, Ueshima H, Okamura T; Evidence for Cardiovascular Prevention from Observational Cohorts in Japan (EPOCH–JAPAN) Research Group. Association of extremely high levels of high-density lipoprotein cholesterol with cardiovascular mortality in a pooled analysis of 9 cohort studies including 43,407 individuals: The EPOCH-JAPAN study. *J Clin Lipidol* 2018;**12**: 674–684.
2. Zhong GC, Huang SQ, Peng Y, Wan L, Wu YQ, Hu TY, Hu JJ, Hao FB. HDL-C is associated with mortality from all causes, cardiovascular disease and cancer in a J-shaped dose-response fashion: a pooled analysis of 37 prospective cohort studies. *Eur J Prev Cardiol* 2020;**27**:1187–1203.
3. Norata GD, Pirillo A, Catapano AL. Modified HDL: biological and physiopathological consequences. *Nutr Metab Cardiovasc Dis* 2006;**16**:371–386.
4. Kakino A, Usami Y, Horiuchi S, Fujita Y, Kotani K, Chen CH, Okamura T, Sawamura T. A novel cell-free, non-fluorescent method to measure LOX-1-binding activity corresponding to the functional activity of HDL. *J Atheroscler Thromb* 2019; **26**:947–958.
5. Sato Y, Nishimichi N, Nakano A, Takikawa K, Inoue N, Matsuda H, Sawamura T. Determination of LOX-1-ligand activity in mouse plasma with a chicken monoclonal antibody for ApoB. *Atherosclerosis* 2008;**200**:303–309.
6. Honda H, Ueda M, Kojima S, Mashiba S, Michihata T, Takahashi K, Shishido K, Akizawa T. Oxidized high-density lipoprotein as a risk factor for cardiovascular events in prevalent hemodialysis patients. *Atherosclerosis* 2012;**220**:493–501.
7. Sorokin AV, Kotani K, Elnabawi YA, Dey AK, Sajja AP, Yamada S, Ueda M, Harrington CL, Baumer Y, Rodante JA, Gelfand JM, Chen MY, Joshi AA, Playford MP, Remaley AT, Mehta NN. Association between oxidation-modified lipoproteins and coronary plaque in psoriasis. *Circ Res* 2018;**123**:1244–1254.
8. Hirata A, Kakino A, Okamura T, Usami Y, Fujita Y, Kadota A, Fujiyoshi A, Hisamatsu T, Kondo K, Segawa H, Sawamura T, Miura K, Ueshima H; SESSA Research Group. The relationship between serum levels of LOX-1 ligand containing ApoAI as a novel marker of dysfunctional HDL and coronary artery calcification in middle-aged Japanese men. *Atherosclerosis* 2020;**313**:20–25.
9. Cesaro A, Schiavo A, Moscarella E, Coletta S, Conte M, Gragnano F, Fimiani F, Monda E, Caiazza M, Limongelli G, D'Erasmo L, Riccio C, Arca M, Calabrò P. Lipoprotein(a): a genetic marker for cardiovascular disease and target for emerging therapies. *J Cardiovasc Med (Hagerstown)* 2021;**22**:151–161.