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Papotti, Bianca
Ormseth, Sarah
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Publication Date

2023-12-01

DOI

10.1016/j.jtauto.2023.100206

Peer reviewed

Journal of Translational Autoimmunity 7 (2023) 100206

Contents lists available at ScienceDirect

Journal of Translational Autoimmunity

journal homepage: www.sciencedirect.com/journal/journal-of-translational-autoimmunity

Statins influence the relationship between ATP-binding cassette A1 membrane transporter-mediated cholesterol efflux capacity and coronary atherosclerosis in rheumatoid arthritis

George A. Karpouzas [a,*], Bianca Papotti [b], Sarah R. Ormseth [a], Marcella Palumbo [b], Elizabeth Hernandez [a], Maria Pia Adorni [c], Francesca Zimetti [b], Matthew J. Budoff [d], Nicoletta Ronda [b]

[a] Division of Rheumatology, Harbor-UCLA Medical Center and the Lundquist Institute for Biomedical Innovation, Torrance, CA, USA
[b] Department of Food and Drug, University of Parma, Parma, Italy
[c] Department of Medicine and Surgery, University of Parma, Parma, Italy
[d] Division of Cardiology, Harbor-UCLA Medical Center and the Lundquist Institute for Biomedical Innovation, Torrance, CA, USA

ARTICLE INFO

Handling Editor: Y Renaudineau

Keywords:
Rheumatoid arthritis
Coronary atherosclerosis
Cardiovascular events
Cholesterol efflux capacity
ABCA1
Statins

ABSTRACT

Objectives: Cholesterol efflux capacity (CEC) is the main antiatherogenic function of high-density lipoprotein (HDL). ATP-binding-cassette A1 (ABCA1) membrane transporter initiates cholesterol export from arterial macrophages to pre-β HDL particles fostering their maturation; in turn, those accept cholesterol through ABCG1-mediated export. Impaired pre-β HDL maturation may disrupt the collaborative function of the two transporters and adversely affect atherosclerosis. Statins exert atheroprotective functions systemically and locally on plaque. We here evaluated associations between ABCA1-CEC, coronary atherosclerosis and cardiovascular risk and the influence of statins on those relationships in rheumatoid arthritis (RA).

Methods: Evaluation with computed tomography angiography was undertaken in 140 patients and repeated in 99 after 6.9 ± 0.3 years. Events comprising cardiovascular death, acute coronary syndromes, stroke, claudication, revascularization and heart failure were recorded. ABCA1-CEC and ABCG1-CEC were evaluated in J774A.1 macrophages and Chinese hamster ovary (CHO) cells respectively and expressed as percentage of effluxed over total intracellular cholesterol. Covariates in all cardiovascular event risk and plaque outcome models included atherosclerotic cardiovascular disease (ASCVD) risk score and high-density lipoprotein cholesterol.

Results: ABCA1-CEC negatively correlated with ABCG1-CEC ($r = -0.167$, $p = 0.049$). ABCA1-CEC associated with cardiovascular risk (adjusted hazard ratio 2.05 [95%CI 1.20–3.48] per standard deviation [SD] increment). There was an interaction of ABCA1-CEC with time-varying statin use ($p = 0.038$) such that current statin use inversely associated with risk only in patients with ABCA1-CEC below the upper tertile. ABCA1-CEC had no main effect on plaque or plaque progression; instead, ABCA1-CEC (per SD) associated with fewer baseline total plaques (adjusted rate ratio [aRR] 0.81, [95%CI 0.65–1.00]), noncalcified plaques (aRR 0.78 [95%CI 0.61–0.98]), and vulnerable low-attenuation plaques (aRR 0.41 [95%CI 0.23–0.74]) in statin users, and more low-attenuation plaques (aRR 1.91 [95%CI 1.18–3.08]) in nonusers (p-for-interaction = 0.018, 0.011, 0.025 and < 0.001 respectively). Moreover, ABCA1-CEC (per SD) associated with greater partially/fully-calcified plaque progression (adjusted odds ratio 3.07 [95%CI 1.20–7.86]) only in patients not exposed to statins during follow-up (p-for-interaction = 0.009).

Conclusion: In patients with RA, higher ABCA1-CEC may reflect a proatherogenic state, associated with enhanced cardiovascular risk. Statin use may unmask the protective impact of ABCA1-mediated cholesterol efflux on plaque formation, progression and cardiovascular risk.

* Corresponding author. 1124 West Carson Street, Building E4-R17, Torrance, CA, 90502, USA.
E-mail address: gkarpouzas@lundquist.org (G.A. Karpouzas).

https://doi.org/10.1016/j.jtauto.2023.100206
Received 3 May 2023; Received in revised form 23 June 2023; Accepted 4 July 2023
Available online 7 July 2023

1. Introduction

The cholesterol content of arterial wall macrophages is the end result of cholesterol loading via native or modified low-density lipoprotein (LDL) and its exit from the cells—the first step in the process known as reverse cholesterol transport [1]. Cholesterol efflux capacity (CEC) measures the ability of high-density lipoprotein (HDL) to remove excess cholesterol from macrophages and protect against atherosclerosis [1]. Transporter proteins on the cell surface actively export cholesterol to various acceptor HDL particles according to their composition and maturation [1]. CEC negatively associated with atherosclerotic plaque size and severity, lipid burden and macrophage density [2,3], as well as cardiovascular risk and mortality independently of HDL-C levels in general patients [4]. Although recent meta-analyses were inconclusive on whether CEC was altered compared to controls or influenced by disease activity [5,6], they did show it improved with therapy and inversely associated with carotid plaque presence in RA [7].

The ATP-binding-cassette membrane transporter A1 (ABCA1) initiates the process by exporting cholesterol to circulating apo-A1 or lipid-poor discoidal pre-β HDL particles; lecithin cholesterol acyl transferase (LCAT) in serum promotes their maturation to spherical HDL, which in turn receive more cholesterol through the ABCG1 membrane transporter. Yet, the relative contributions and associations of these individual CEC pathways with cardiovascular risk and coronary atherosclerosis are largely unknown [8]. Our group reported that ABCG1-CEC in particular was lower in RA, negatively associated with disease activity [9], and improved with therapy [5,10]. Moreover, ABCG1-CEC inversely associated with coronary plaque burden and vulnerability and attenuated atherosclerosis progression conditionally on cumulative inflammation and corticosteroid dose [11]. Lastly ABCG1-CEC was inversely linked to cardiovascular risk specifically in patients with lipid-rich noncalcified plaques, lower inflammation and in prednisone users [11].

ABCA1-mediated cholesterol efflux on pre-β HDL is the pivotal and most time-consuming step in reverse cholesterol transport; pre-β HDL concentration correlates with ABCA1-CEC [12]. Loss-of-function mutation in ABCA1 protein in humans associated with marked reduction in serum HDL and premature coronary artery disease [13]. ABCA1 heterozygotes experience three times greater incidence of coronary artery disease, especially those with decreased CEC [14]. In contrast, overexpression of human ABCA1 in mice fed a high-fat diet increased HDL-C and suppressed atherosclerosis by promoting ABCA1-CEC [15]. Nevertheless, the relationships between ABCA1-CEC, coronary atherosclerosis and cardiovascular risk in RA are unknown.

Statins decrease cardiovascular risk [16,17] and coronary atherosclerosis progression in both general [18] and RA patients [19] by lowering LDL-C, decreasing inflammation and LDL oxidation and increasing levels of HDL-C [16,20,21]. Yet, conflicting data regarding the effect of statins on HDL-CEC have been reported [22]. The impact of statin therapy on ABCA1-CEC itself or more importantly its relationship with coronary atherosclerosis in RA is unknown. Hence, in the present study we explored associations between ABCA1-CEC, coronary atherosclerosis, and long-term cardiovascular risk in RA and characterized the influence of statins on those relationships.

2. Material and methods

2.1. Patient recruitment

We evaluated 140 patients originally enrolled in the *PRO*spec*T*ive *E*valuation of Latent *C*oronary A*T*herosclerosis in *R*heumatoid *A*rthritis (PROTECT RA) cohort [23] with available serum samples for CEC measurements. The entire cohort included 150 patients from a single center, undergoing coronary atherosclerosis evaluation with computed tomography angiography (CCTA) between March 2010 and March 2011. Participants were subsequently followed for incident cardiovascular events and 99 were re-evaluated for coronary atherosclerosis progression 6.9 ± 0.3 years later. Upon enrollment, subjects were between 18 and 75 years old, satisfied 2010 classification criteria for RA and reported no prior diagnosis of cardiovascular disease including angina, myocardial infarction, transient ischemic attack, stroke, claudication, revascularization, or heart failure. Patients with coexisting inflammatory rheumatic diseases (except for Sjogren's), infections, malignancy within 5 years, body weight exceeding 147.7 kg, glomerular filtration rate <60 mL/min, or iodine allergy were excluded. The local Institutional Review Board approved the study and all participants signed informed consent in compliance with the Declaration of Helsinki.

2.2. Coronary computed tomography angiography

Baseline coronary anatomy evaluations were completed in a 64-multidetector row scanner between March 2010 and March 2011. Serial evaluations occurred in a 256-multidetector row scanner between March 2017 and March 2018. Protocols for image acquisition and processing and scoring reproducibility have been previously illustrated [23]. Plaque presence and morphology was assessed on contrast-enhanced scans using a standardized 17-segment American Heart Association model [24]. Baseline and serial images were evaluated at the same sitting and in random order by an experienced interpreter, blinded to patients' clinical data (MJB). Coronary segment coalignment using fixed anatomic landmarks as fiducial points allowed for longitudinal plaque comparisons. Segment involvement score described the number of segments harboring plaque in an individual patient (0-17). Each segment was scored for stenosis severity on a scale from 0 to 4 as previously described [23]. Segment stenosis score represented the cumulative stenotic severity of all evaluable segment per patient (0-68). Presence of >4 segments with plaque per patient was considered extensive disease and lesions rendering greater than 50% luminal obstruction were considered obstructive. The composition of atherosclerotic lesions was described as noncalcified, partially and fully calcified as previously outlined [23]. Plaques were further assessed for areas of low-attenuation (≤ 30 Hounsfield units, low-attenuation plaques) which represent necrotic lipid cores and regarded as high-risk features for rupture [25]. The presence of extensive or obstructive disease, noncalcified and low attenuation plaque have all been linked to greater cardiovascular risk.

2.3. Laboratory evaluations

Metabolic panel, complete blood counts, c-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) evaluations were completed at the time of each CCTA examination and every clinic visit. Fasting lipid assessments were similarly completed during baseline and follow-up scans and according to EULAR recommendations throughout the follow-up [26]. Additional serum was collected and biobanked as previously described [23].

Cholesterol concentration of all lipoprotein classes [HDL, Lp(a), LDL, IDL and VLDL] and their respective subclasses (HDL_2, HDL_3, LDL_1-LDL_4, $VLDL_1$-$VLDL_3$) were directly measured with single vertical spin density gradient ultracentrifugation (VAP-II, Atherotec Birmingham, AL, USA) [27]. Measurements of serum levels of ApoA1 and ApoB100 apoproteins were included in the VAP test and expressed as mg/dl.

2.3.1. Serum cholesterol loading capacity

Serum cholesterol loading capacity (CLC) as a measure of overall serum lipoproteins loading cells with cholesterol, was evaluated as intracellular cholesterol content in human THP-1 monocyte-derived macrophages using a fluorimetric assay [28]. THP-1 cells were cultured with 100 ng/mL PMA for 72 h allowing differentiation into macrophages and then incubated with 5% v/v lipoprotein-depleted serum (LPDS; Sigma-Aldrich, Darmstadt, Germany) for 24 h. Next, patient sera

were added to the culture at 10% dilution in cell culture medium for 24 h. Cells were then washed and lysed, cholesterol and protein content was measured in cell lysates with the Amplex Red® Cholesterol Assay and BCA assay, respectively (both from ThermoFisher Scientific, MA, USA). CLC values were expressed as micrograms of cholesterol per milligram of proteins.

2.3.2. Serum cholesterol efflux capacity

Specific CEC pathways were assessed using previously published cell models [28]. ABCA1-CEC was evaluated in J774 macrophages cultured with or without c-AMP. Cells were first labeled with 1,2–^{3}H(N)-cholesterol for 24 h and then incubated in medium with 0.2% bovine serum albumin for an additional 18 h. Apo-B depleted serum 2% (vol/vol) was added next for 4 h. CEC was expressed as the percentage of radioactivity present in the supernatant over the total intracellular radioactivity. ABCA1- CEC was the difference in serum-induced cholesterol efflux between cells cultured in the presence or absence of c-AMP. A pool of human normolipidemic sera and 10 μg/mL of human isolated apo-AI (Merk Life Science, Darmstadt, Germany) were also tested in each assay to control for inter-assay variability and therefore to normalize sample values across various experiments. The normalized CEC from human normolipidemic sera and human apoA-I also provided an index of intra-assay variability (calculated as <10%).

For ABCG1-CEC, Chinese hamster ovary cells both untransfected and transfected with the ABCG1 gene were used. Cells were labeled with 1,2–^{3}H(N)-cholesterol for 24 h and then incubated in medium with 0.2% bovine serum albumin for 24 h. Then, 1% (vol/vol) apo-B depleted serum was added for 6 h. CEC was expressed as the percentage of radioactivity released in the supernatant over the total intracellular radioactivity. ABCG1-CEC was the difference in serum-induced cholesterol efflux between ABCG1-transfected and untransfected cells. In order to adjust for inter-assay variability, a pool of human normolipidemic sera and 12.5 μg/mL of human isolated HDL (Merk Life Science, Milano, Italy) were tested in each assay, and the CEC obtained was used to normalize sample values across different experiments. The normalized CEC of a separate pool of normolipidemic sera concurrently tested in each assay provided an index of intra-assay variability. Intra-assay coefficient of variation was <10%.

2.4. Covariates and outcomes

A 10-year atherosclerotic cardiovascular disease (ASCVD) risk score based on the American Heart Association pooled cohort equation was estimated for all patients at baseline [29]. Anthropometric measures including height, weight and waist circumference were collected. Disease activity was calculated based on a 28-joint clinical examination for tenderness, swelling and C-reactive protein (DAS28-CRP) at all clinic visits (every three to four months) during follow-up. Conventional synthetic disease modifying anti-rheumatic drug (csDMARD), biologic DMARD (bDMARD), prednisone and statin use and dosing was captured on every visit and cross-referenced against pharmacy records. Statin doses throughout follow-up were converted to atorvastatin equivalent average daily dose based on standard conversion tables [30].

The clinical outcome of interest was a prespecified composite endpoint including cardiovascular death, acute coronary syndrome, stroke, transient ischemic attack, peripheral arterial disease, revascularization, and heart failure. Incident events were adjudicated upon electronic medical record review by specialists blinded to CCTA results, and according to standard definitions [31].

Baseline coronary atherosclerosis outcomes were segment involvement score, segment stenosis score, numbers of segments with non-calcified, partially/fully calcified, and low-attenuation plaque per-patient. Plaque progression was an increase in number of total, non-calcified and partially/fully calcified plaques at follow-up compared to baseline.

2.5. Statistical analysis

Categorical variables were reported as frequencies with percentages and continuous variables as means with standard deviations (SD). Non-normally distributed variables were natural log transformed. The influence of ABCA1-CEC on cardiovascular event risk was evaluated in a Cox regression model adjusting for ASCVD risk score and baseline extensive or obstructive coronary plaque presence. The potential moderating role of baseline statin therapy on the relationship between ABCA1-CEC and cardiovascular risk was examined in a separate Cox regression model additionally including statin use and its interaction term with ABCA1-CEC.

We evaluated the effect of the interaction of ABCA1-CEC with time-varying statin use on cardiovascular risk with marginal structural models using inverse probability of treatment and censoring weighting to increase comparability between statin exposure groups as previously described [19]. Data were discretized into monthly intervals and time-varying covariates not measured in a given interval were imputed using last observation carried forward. Pooled logistic regression models computed inverse probability of treatment and censoring weights as a function of baseline covariates (including ASCVD score, segment involvement score, CRP and bDMARD use) and time-varying CRP. Stabilized weights were calculated by multiplying the treatment and censoring weights. These weights were used in a weighted pooled logistic regression model with cardiovascular risk as the outcome and ABCA1-CEC, time-varying statin use, ABCA1 x statin use interaction term, ASCVD score, time-varying bDMARD use, and the cubic spline for months since baseline as predictors. Robust standard errors accounted for clustering within patients. If the P-for-interaction was <0.05, marginal structural models evaluated the effect of time-varying statin use on cardiovascular risk in patients with ABCA1-CEC≥5.7% (upper tertile) and patients with ABCA1-CEC levels below the upper tertile.

The effect of ABCA1-CEC on baseline plaque and plaque progression outcomes was evaluated with robust negative binomial regression and robust binary logistic regression, respectively. The influence of baseline statin use, statin exposure during follow-up, and time-weighted average atorvastatin equivalent dose on the relationship between ABCA1-CEC and plaque outcomes was evaluated by adding the corresponding moderators and their products with ABCA1-CEC as interaction terms to the respective models. All models adjusted for ASCVD score, HDL-C, and covariates significant in the corresponding multivariable models. Plaque progression models additionally adjusted for number of the respective plaques at baseline and time between scans. SPSS version 27 and Stata version 15 were used. P values < 0.05 were considered significant.

3. Results

Patients were largely middle-aged women with long-standing, seropositive and erosive disease (Table 1). All were treated with an average of two concurrent csDMARDs (80% methotrexate), 86/140 (61%) additionally received bDMARDs (all TNF-α inhibitors) at baseline. Patient characteristics according to screening and follow-up CCTA status are shown in Table 1. Mean (SD) ABCA1-CEC was 5.14 (1.07)% and ABCG1-CEC was 4.71 (0.92)%. ABCA1-CEC did not correlate with ApoA1 ($r = -0.009$, $p = 0.914$), total HDL-C ($r = -0.084$, $p = 0.324$), HDL_2-C ($r = -0.133$, $p = 0.118$), or HDL_3-C ($r = -0.038$, $p = 0.658$). ABCA1-CEC positively correlated with ApoB100 ($r = 0.221$, $p = 0.009$), LDL_3-C ($r = 0.228$, $p = 0.007$) and triglycerides ($r = 0.239$, $p = 0.004$). In contrast, ABCG1-CEC inversely correlated with ApoB100 ($r = -0.319$, $p < 0.001$), LDL_3-C ($r = -0.377$, $p < 0.001$), LDL_4-C ($r = -0.232$, $p = 0.006$) and serum CLC ($r = -0.256$, $p = 0.002$), while it correlated positively with ApoA1 ($r = 0.284$, $p = 0.001$), total HDL-C ($r = 0.354$, $p < 0.001$), HDL_2-C ($r = 0.377$, $p < 0.001$) and HDL_3-C ($r = 0.310$, $p < 0.001$). Notably, ABCA1-CEC inversely correlated with ABCG1-CEC ($r = -0.167$, $p = 0.049$).

Fifty two of 140 patients (37.1%) received statin therapy at baseline.

Table 1
Baseline characteristics.

	Total Sample (n = 140)		No follow-up CCTA (n = 41)		Follow-up CCTA (n = 99)	
Age (years)*	52.91	±10.52	56.36	±10.24	51.48	±10.35
Female, no. (%)	123	(87.86%)	38	(92.68%)	85	(85.86%)
RA duration (years)	10.58	±7.61	11.61	±8.76	10.15	±7.09
Age at diagnosis (years)	42.33	±11.15	44.75	±12.58	41.33	±10.41
RF positive, no. (%)	121	(86.43%)	32	(78.05%)	89	(89.90%)
ACPA positive, no. (%)	120	(85.71%)	34	(82.93%)	86	(86.87%)
Erosions, no. (%)	92	(65.71%)	29	(70.73%)	63	(63.64%)
CRP (ln) (mg/dL)	1.49	±1.15	1.51	±1.20	1.49	±1.13
DAS28-CRP	2.53	±0.99	2.77	±1.13	2.43	±0.92
Total Cholesterol (mg/dL)	169.25	±35.25	175.63	±35.92	166.61	±34.81
LDL-c (mg/dL)	95.04	±28.37	97.88	±29.48	93.87	±27.96
LDL_1-c (mg/dL)	13.35	±6.55	14.19	±6.93	13.01	±6.39
LDL_2-c (mg/dL)	22.28	±12.07	21.21	±13.34	22.71	±11.54
LDL_3-c (mg/dL)	31.32	±12.45	30.86	±12.64	31.51	±12.43
LDL_4-c (mg/dL)*	9.24	±6.48	11.22	±8.50	8.42	±5.26
HDL-c (mg/dL)	51.49	±14.08	52.88	±16.44	50.92	±13.03
HDL_2-c (mg/dL)	14.31	±6.61	15.07	±8.26	14.00	±5.82
HDL_3-c (mg/dL)	37.17	±7.93	37.78	±8.52	36.92	±7.70
Systolic BP**	128.35	±15.34	134.07	±14.40	125.98	±15.17
Diastolic BP	73.17	±8.91	74.00	±8.73	72.83	±9.00
Diabetes, no. (%)	22	(15.71%)	8	(19.51%)	14	(14.14%)
Current smoking, no. (%)	12	(8.57%)	4	(9.76%)	8	(8.08%)
Body mass index (kg/m^2)	28.95	±5.60	29.17	±5.90	28.86	±5.50
Waist circumference (inches)	36.73	±4.84	36.46	±5.11	36.84	±4.74
ASCVD risk score**	4.96	±6.83	7.47	±9.72	3.92	±4.89
Statin use, no. (%)	52	(37.14%)	13	(31.71%)	39	(39.39%)
Prednisone use, no. (%)	48	(34.29%)	17	(41.46%)	31	(31.31%)
Methotrexate use, no. (%)	112	(80.00%)	33	(80.49%)	79	(79.80%)
No. Concurrent csDMARDs	1.94	±0.80	2.05	±0.84	1.89	±0.78
bDMARD use, no. (%)	86	(61.43%)	23	(56.10%)	63	(63.64%)
Plaque presence (any), no. (%)	98	(70.00%)	30	(73.17%)	68	(68.69%)
Number of plaques total	1.96	±2.27	2.20	±2.30	1.86	±2.26
Number of noncalcified plaque	1.01	±1.16	1.10	±1.39	0.97	±1.05
Number of partially calcified plaques	0.56	±1.30	0.71	±1.42	0.51	±1.25
Number of fully calcified plaques	0.42	±1.05	0.51	±1.21	0.38	±0.98
Number of low attenuation plaques	0.29	±0.77	0.27	±0.67	0.30	±0.81
Extensive plaque (≥5), no. (%)	19	(13.57%)	8	(19.51%)	11	(11.11%)
CLC (μg/mg protein)	12.67	±2.83	12.88	±2.67	12.58	±2.90
ABCA1-CEC (%)	5.14	±1.07	5.19	±1.02	5.11	±1.10
ABCG1-CEC (%)	4.71	±0.92	4.75	±0.90	4.69	±0.93

Values are mean ± standard deviation unless otherwise indicated. RF: rheumatoid factor, ACPA: anti-citrullinated protein antibodies, CRP: C-reactive protein, DAS28-CRP: disease activity score based on 28 joint counts and CRP, LDL-c: low-density lipoprotein, HDL-c: high density lipoprotein, BP: blood pressure, ASCVD: atherosclerotic cardiovascular disease score, cs-DMARDs: conventional synthetic disease modifying anti-rheumatic drugs, bDMARD: biologic disease modifying anti-rheumatic drugs, CLC: cholesterol loading capacity, ABCA1-CEC: Cholesterol efflux capacity through the ABCA1 transporter, ABCG1-CEC: Cholesterol efflux capacity through the ABCG1 transporter.
*$p < 0.05$, **$p < 0.01$ for between-group comparisons using independent samples *t*-test.

Statin users were more commonly male, older, with longer disease duration, had higher prevalence of diabetes, hypertension and higher ASCVD scores (all $p < 0.05$, not shown). Moreover, statin users exhibited greater plaque burden and likelihood of extensive and obstructive disease (Supplementary table 1). No difference in ABCA1-CEC was seen between baseline statin users and nonusers, even after adjusting for ASCVD score and DAS28-CRP (not shown).

3.1. Association of ABCA1-CEC with cardiovascular risk

Fifteen patients suffered 18 cardiovascular events over 6.03 ± 2.42 years (incidence rate of 2.08 [1.31–3.30] events/100 patient-years, Supplementary Table S2). Patients experiencing cardiovascular events had higher ABCA1-CEC compared to those without (5.58 [95% CI 5.20–5.95]% vs. 5.09 [95% CI 4.90–5.28]%, p = 0.023). In a Cox regression model adjusting for ASCVD score and baseline extensive or obstructive plaque, higher ABCA1-CEC associated with greater cardiovascular event risk (HR 2.05 [95% CI 1.20–3.48] per 1-SD higher ABCA1-CEC, p = 0.008). Event free survival at high (+1 SD) and low (-1SD) ABCA1-CEC are shown in Fig. 1. Statin treatment at baseline did not influence this relationship (p-for-interaction = 0.705). In contrast, in marginal structural models covarying for ASCVD score and time-varying

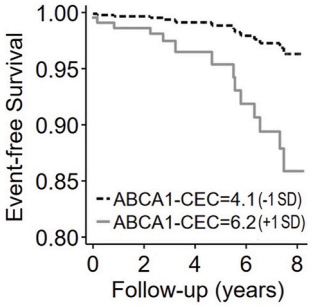

Fig. 1. Association of ABCA1-CEC with risk of incident cardiovascular events. ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 transporter; SD, standard deviation. Model adjusted for atherosclerotic cardiovascular disease (ASCVD) risk score and baseline extensive or obstructive coronary plaque presence.

bDMARD use, the interaction between ABCA1-CEC and time-varying statin use was significant (p = 0.038). Statin use associated with lower cardiovascular event risk in patients with ABCA1-CEC<5.7% (OR 0.11 [0.03–0.39], p = 0.001) but not those with ABCA1-CEC ≥ 5.7% (OR

2.51 [95%CI 0.50–12.71], p = 0.267).

3.2. Relationships between ABCA1-CEC and coronary atherosclerosis are influenced by statin use

Ninety-eight patients (70%) had coronary atherosclerosis on screening CCTA. ABCA1-CEC was not different in patients with vs. without atherosclerosis (5.10 [4.88–5.32]% vs. 5.22 [4.91–5.53]%, p = 0.53) or those with vs. without specific plaque subtypes (not shown). There was no main effect of ABCA1-CEC on baseline plaque (Fig. 2). Instead, its impact on atherosclerosis was moderated by statin use (Fig. 2). Specifically, ABCA1-CEC predicted lower segment involvement score (Fig. 3A), segment stenosis score (Fig. 3B), number of noncalcified plaques (Fig. 3C), and number of vulnerable low-attenuation plaques (Fig. 3D) in statin users, yet higher number of low-attenuation plaques in statin nonusers. Statin use did not moderate the effect of ABCA1-CEC on number of partially/fully-calcified plaques (p-for-interaction = 0.162).

Serial atherosclerosis evaluation was completed in 99 of 140 patients within 6.9 ± 0.3 years. Of the 41 remaining, four had no clinic follow-up after the screening assessment, two expired, six migrated, and 29 declined reevaluation. Although patients without serial assessments were older, had higher systolic blood pressure and cardiovascular risk, the differences in ASCVD scores were no longer significant after adjusting for age (Table 1). A total of 68 (68.7%) patients had coronary atherosclerosis at follow-up; 10 (10.1%) without plaque at baseline showed 15 new segments with plaque. The remaining 58 (58.6%) patients with baseline atherosclerosis developed 84 new plaques.

ABCA1-CEC showed no main effect on the plaque progression outcomes, and statin exposure during follow-up did not moderate the effect of ABCA1-CEC on progression of total or noncalcified plaque (Fig. 4). However, statin exposure did influence the relationship between ABCA1-CEC and partially/fully-calcified plaque progression such that higher ABCA1-CEC associated with an increased likelihood of partially/fully-calcified plaque progression only in statin unexposed patients (Figs. 4 and 5A). In a sensitivity analysis evaluating statin exposure as a continuous variable, the interaction between ABCA1-CEC and weighted daily-average atorvastatin equivalent dose predicting partially/fully-calcified plaque progression was also significant (p-for-interaction = 0.019). Specifically, ABCA1-CEC inversely associated with the probability of partially/fully-calcified plaque progression at very high (+2 SD) and high (+1 SD) but not lower levels of time-weighted average atorvastatin equivalent dose (Fig. 5B). Additionally, at higher levels (≥1 SD) of ABCA1-CEC, the probability of partially/fully-calcified plaque progression was lower at high and very high compared to low (unexposed) weighted average atorvastatin equivalent dose. In contrast, the interaction of ABCA1-CEC with weighted average atorvastatin equivalent dose was not significant in predicting noncalcified plaque progression (p-for-interaction = 0.120).

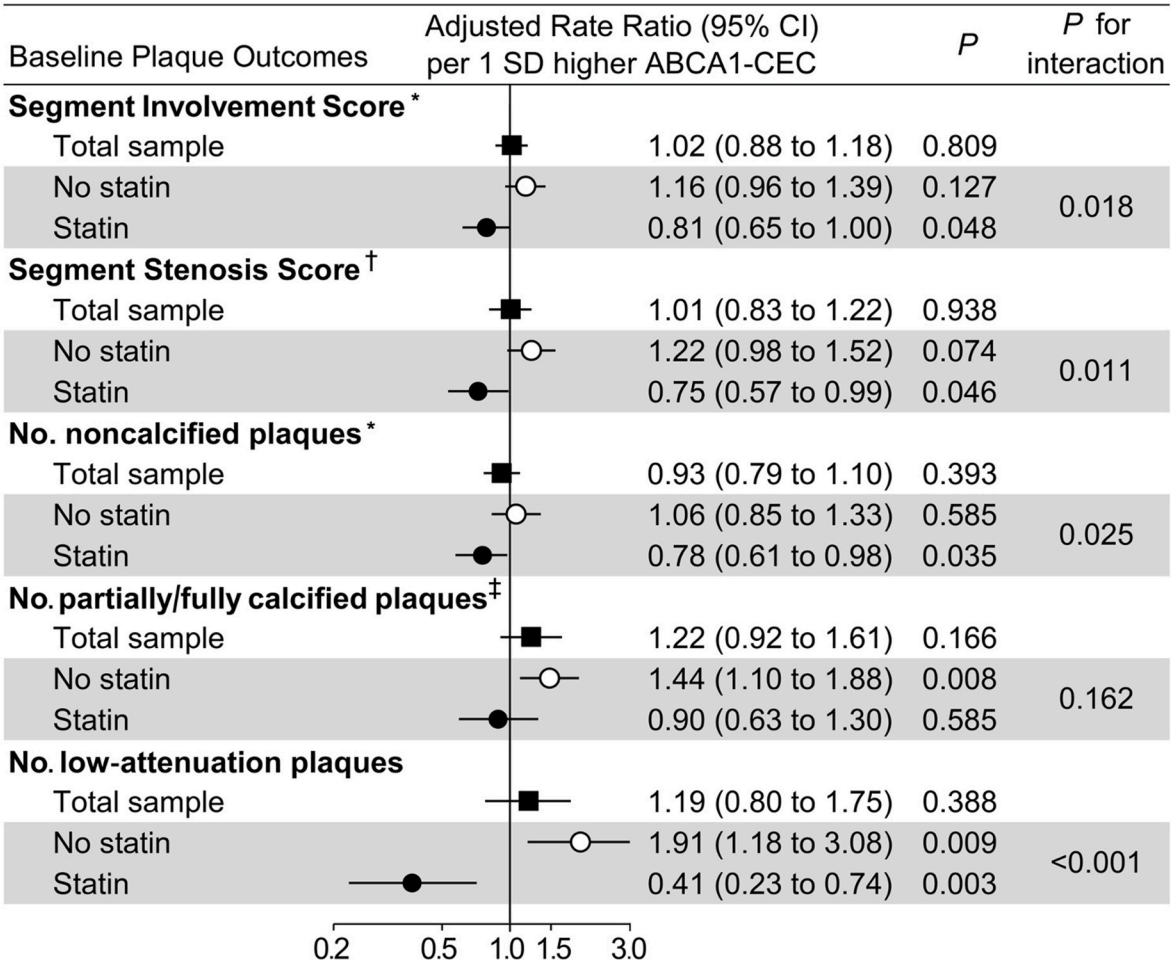

Fig. 2. Associations of ABCA1-CEC with baseline atherosclerosis outcomes for the total sample and stratified by statin use. ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 transporter; 95% CI, 95% confidence interval; SD, standard deviation. Rate ratios derived from negative binomial regression models indicate the percent change in the outcome associated with one standard deviation unit increase in ABCA1-CEC. All models controlled for atherosclerotic cardiovascular disease (ASCVD) risk score and high-density lipoprotein cholesterol. *Additionally adjusted for waist circumference. †Additionally adjusted for waist circumference and age at diagnosis. ‡Additionally adjusted for age at diagnosis.

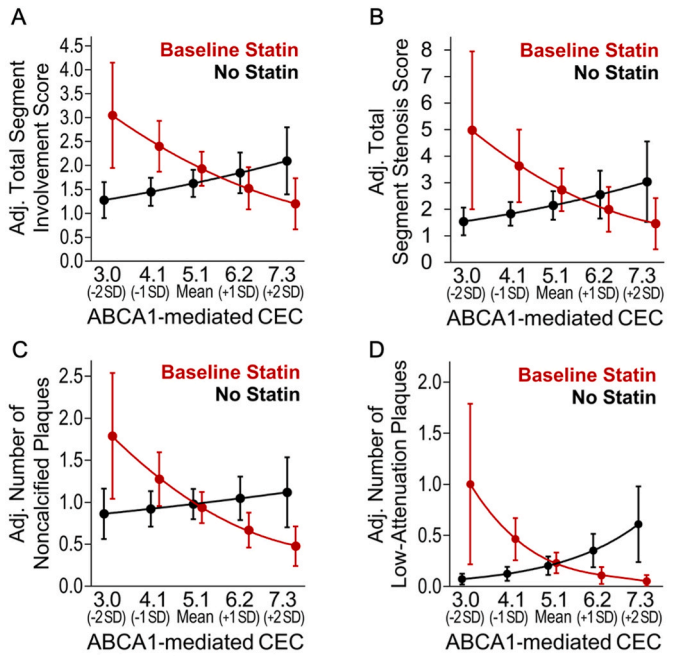

Fig. 3. Baseline coronary plaque outcomes across levels of ABCA1-CEC stratified by statin use. (A) Total number of plaques (B) Cumulative stenotic plaque severity (C) Number of noncalcified plaques (D) Number of low-attenuation plaques. ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 transporter; SD, standard deviation; Adj., adjusted. Estimates derived from negative binomial regression models adjusted for the same covariates as corresponding models in Fig. 2.

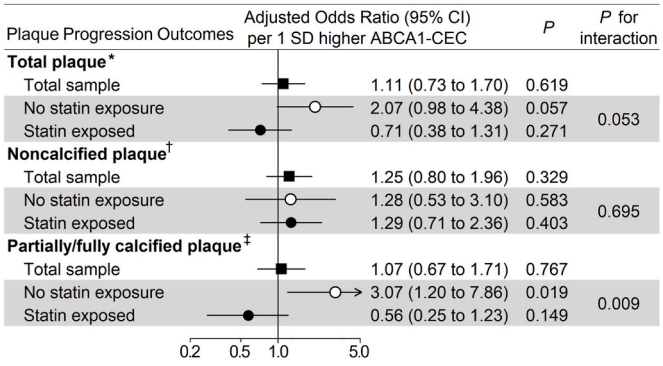

Fig. 4. Associations of ABCA1-CEC with coronary atherosclerosis progression for the total sample and stratified by statin exposure during follow-up. ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 transporter; 95% CI, 95% confidence interval; SD, standard deviation. Odds ratios derived from binary logistic regression models adjusted for atherosclerotic cardiovascular disease (ASCVD) risk score, high-density lipoprotein cholesterol, baseline plaque burden, and time between scans. *Additionally adjusted for time-averaged C-reactive protein. †Additionally adjusted for time-weighted average prednisone dose. ‡Additionally adjusted for time-averaged C-reactive protein, waist circumference, and age at diagnosis.

4. Discussion

This is the first study comprehensively evaluating the relationships between ABCA1-CEC, coronary atherosclerosis and cardiovascular risk and the influence of statin therapy on those relationships in patients with RA. Under physiologic conditions ABCA1 and ABCG1 transporters work coordinately in performing cholesterol efflux and HDL maturation [1]. Increasing ABCA1-mediated cell cholesterol efflux, through LCAT activity, would therefore associate with mature HDL generation and

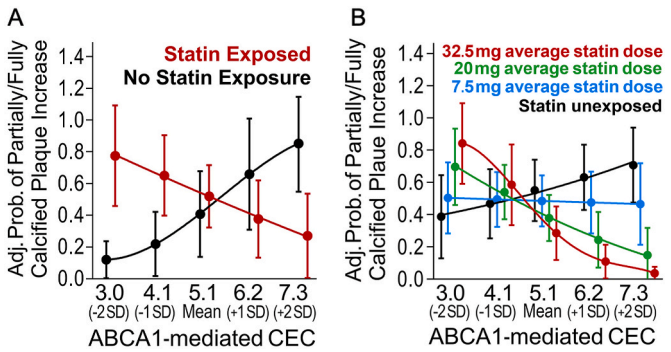

Fig. 5. Partially/fully-calcified plaque progression across levels of ABCA1-CEC (A) In patients unexposed and exposed to statins during follow-up and (B) At very high (+2 SD), high (+1 SD), mean, and low (unexposed) average daily atorvastatin equivalent dose during follow-up. ABCA1-CEC, cholesterol efflux capacity through the ATP-binding cassette A1 transporter; SD, standard deviation; Adj., adjusted; Prob., probability. Estimates derived from binary logistic regression models adjusted for atherosclerotic cardiovascular disease (ASCVD) risk score, high-density lipoprotein cholesterol, time-averaged C-reactive protein, waist circumference, age at diagnosis, baseline plaque burden, and time between scans.

greater ABCG1-CEC, enhanced cholesteryl ester transfer to ApoB100 particles—leading to larger LDL particle size and cholesterol concentration—their increased uptake through hepatic LDL-receptor, and therefore lower serum ApoB100 and LDL particle number [32]. Accordingly, we showed that ABCG1-CEC inversely related with ApoB100 levels and cholesterol concentration on smaller LDL_3 and LDL_4 particles. Consistent with the notion that ABCA1 exports cholesterol exclusively to pre-β HDL rather than mature HDL particles we found no correlation between ABCA1-CEC and HDL_2-C, HDL_3-C or total HDL-C.

More importantly, we observed an inverse correlation between ABCA1-CEC and ABCG1-CEC, consistent with a block in pre-β HDL maturation, ultimately disrupting the physiologic collaborative function of ABCA1 and ABCG1 transporters in cholesterol efflux [33]. This might be explained by decreased levels or function of LCAT, an enzyme that esterifies free cholesterol exported to pre-β HDL through ABCA1 [34]. Indeed, LCAT deficiency associated with high levels of pre-β HDL and ABCA1-CEC and low levels of HDL_3 and ABCG1-CEC [34]. Decreased LCAT levels and function have been reported in RA patients, particularly during high disease activity [35] and were shown to improve with treatment [36]. Cholesterol exported to pre-β HDL through ABCA1 can therefore not be esterified, shuttled and retained into the hydrophobic core of the particle; consequently, it may offload to apoB particles—especially smaller, more promptly oxidizable LDL particles—and then reload onto macrophages through scavenger receptors [37]. Moreover, decreased esterification would associate with lower cholesteryl ester transfer from HDL to apoB100 lipoproteins in exchange for triglycerides and therefore with decreased LDL-receptor-mediated cholesterol uptake by the liver, higher apoB100 levels and higher number of smaller LDL particles [32].

Accordingly, we found that ABCA1-CEC positively correlated with ApoB100 levels as well as cholesterol concentration on smaller and more readily oxidizable LDL_3, LDL_4 particles and triglycerides. Furthermore, in double seropositive RA patients, oxidized LDL promotes cholesterol loading on macrophages independently of inflammation [38] fostering foam cell formation and atherosclerosis [39]. Importantly, LDL oxidation has been associated with impaired CEC [40]. In fact, cholesterol loaded on macrophages through oxidized LDL is sequestered in lysosomes and effectively unavailable for efflux [40]. Correspondingly we observed that cholesterol loading capacity (CLC) inversely associated with ABCG1-CEC. Taken together, these observations indicate that in the context of a block in maturation of pre-β HDL, a rise in ABCA1-CEC may actually reflect a proatherogenic rather than atheroprotective state,

characterized by reduced formation of mature, highly efficient ABCG1-interacting HDL [11] and increase in proatherogenic, cell cholesterol loading lipoproteins. Indeed, LCAT deficient carriers showed greater increase in carotid artery wall thickness and 32% greater atherosclerotic plaque burden compared with family controls [41].

Consistent with the notion that higher ABCA1-CEC—in the context of a block in maturation of pre-β HDL—reflects a proatherogenic state, we observed that higher ABCA1-CEC associated with increased long-term cardiovascular risk independently of ASCVD score and coronary atherosclerosis burden. Additionally, patients suffering cardiovascular events had higher ABCA1-CEC compared to those without. To our knowledge, this is the first report of a direct association between ABCA1-mediated CEC specifically and cardiovascular events in any clinical setting. Our observation is in agreement with prior accounts that increasing pre-β HDL associated with presence and severity of coronary artery disease [42,43] and history of myocardial infarction [44]. Indeed, patients with prior myocardial infarction and revascularization had 87% greater pre-β HDL particle concentration and 30% higher ABCA1-CEC compared to matched controls [45]. Our findings describing the individual contribution of a defined CEC pathway and HDL population, help clarify the complex relationship between HDL-CEC and cardiovascular risk—that has been confounded by differences in cell culture systems, membrane cholesterol transporters, cholesterol tracers, and acceptors used.

Statins lower LDL-C, decrease inflammation and LDL oxidation in both RA and controls [16,20,21]. The reduction of LDL and oxLDL associates both with decreased vascular cell cholesterol loading and lower fraction of intracellular cholesterol blocked in lysosomes and unavailable for efflux [40]. Therefore, statins may unmask or amplify the actual positive impact of the interaction of any given ABCA1-specific HDL with the transporter. Additionally, rosuvastatin was shown to increase ABCA1 protein levels in human carotid plaques [46] and foster ABCA1-CEC ex vivo in macrophages from mice fed a high-fat diet [47]. Similarly, simvastatin upregulated ABCA1 expression and reduced atherogenesis in apoE$^{-/-}$ mice fed a high fat diet [48]. Hence, an additional effect of statin therapy *in vivo* might be to promote actual cholesterol efflux to immature HDL by fostering ABCA1-CEC. Finally, statins also increase HDL levels and may restore its function and maturation [49] at least partially by reinstating LCAT functionality [48,50,51]. Indeed, LCAT increase promotes reverse cholesterol transport and regression of atherosclerosis [52]. Additional studies with various statins in general patients similarly reported significant improvements in CEC [53,54]. Taken together, these observations indicate that statins may unmask and bolster the positive impact of ABCA1-specific HDL efflux, as well as restore the physiologic coordinate function of ABCA1 and ABCG1 transporters in reverse cholesterol transport.

Indeed, we recently showed that ongoing statin use in RA attenuated the impact of inflammation on new coronary plaque formation and independently promoted both regression and calcification of preexistent noncalcified lesions [19]. Correspondingly, we here show that only in statin users higher ABCA1-CEC associated with fewer plaques and lower stenotic plaque severity overall, fewer noncalcified lesions with lower stenotic severity, less vulnerable low-attenuation plaques at baseline, as well as decreased likelihood of plaque progression. In contrast, in statin nonusers, higher ABCA1-CEC—as a reflection of a block in pre-β HDL maturation and proatherogenic lipoprotein increase—associated with greater stenotic plaque severity, a higher number of vulnerable, low-attenuation plaques and plaque progression. Notably, extensive or obstructive disease, presence and burden of noncalcified and low-attenuation plaques have all been associated with greater cardiovascular risk [55–57].

Although baseline statin therapy influenced the relationship of ABCA1-CEC with plaque burden and composition, it appeared to have no significant impact on its relationship with cardiovascular risk. This might be due to the contribution of other factors, such as platelet function, vasoconstriction or intraplaque hemorrhage in triggering acute events that may be more dynamic in nature, or requirement of a higher cumulative dose or longer exposure to statin treatment [58]. Accordingly, we observed that time-varying statin use throughout follow-up indeed favorably affected the relationship between ABCA1-CEC and cardiovascular event risk particularly for patients in the two lower tertiles of ABCA1-CEC but not the highest tertile. Patients exposed to statins at any time during follow-up—much like those treated with statins at baseline—have significantly higher cardiovascular risk, higher ASCVD score, obesity, greater disease duration and seropositivity for both RF and ACPA at baseline; they have lower ABCG1-CEC, numerically higher ABCA1-CEC and greater plaque prevalence and vulnerability. Likewise, throughout follow-up, they have significantly higher time-averaged systolic blood pressure, triglycerides, lower HDL-C and greater plaque and CAC progression.

Given therefore their much greater cardiovascular risk both at baseline and during follow-up, the highest ABCA1-CEC levels among them—associated with lower ABCG1-CEC—still likely reflect impaired pre-β HDL maturation; hence in those very high-risk individuals, statins may not sufficiently overcome the prevalent proatherogenic state or unmask the beneficial effect of ABCA1-CEC on cardiovascular risk. In contrast, the lower two tertiles of ABCA1-CEC may represent patients in whom statins may have been very effective and the relatively lower ABCA1-CEC may reflect restoration of pre-β HDL maturation and improved ABCG1-CEC. This is indeed consistent with our prior observation that treatment with adalimumab and methotrexate induced a transient decrease in ABCA1-CEC, likely reflecting activation of LCAT and a switch from premature pre-β to mature HDL particles and improved ABCG1-CEC [28].

Our study has certain limitations: First, the associations between ABCA1-CEC, coronary atherosclerosis and cardiovascular risk were not prespecified analyses in our original study design and therefore not powered for. Consequently our findings should be considered exploratory and validated in future adequately powered studies. Second, we did not specifically quantify LCAT levels, activity, or pre-β HDL particle concentration in our patient samples. However, the association between pre-β HDL particle concentration and ABCA1-CEC as a proxy for that, and the role of LCAT on pre-β HDL maturation are well established and reported [12,34]. Third, patients with coronary atherosclerosis at screening were prescribed statins and/or aspirin regardless of clinical indication, which may have influenced plaque progression, cardiovascular risk [19] as well as the relationship between ABCA1-CEC and those outcomes.

5. Conclusion

In the context of inflammation and impaired pre-β HDL maturation typical of RA, higher ABCA1-CEC may reflect a proatherogenic rather than atheroprotective state, associated with greater coronary atherosclerosis burden, vulnerability and enhanced cardiovascular risk. Statin use, by reducing cell cholesterol overload may unmask and promote the atheroprotective effect of ABCA1-CEC; by stimulating LCAT, it may restore pre-β HDL maturation and the coordinate antiatherogenic function of ABCA1 and ABCG1 transporters, ultimately favorably influencing the relationship of ABCA1-CEC with plaque burden, vulnerability, progression and cardiovascular risk.

Credit author statement

George Karpouzas: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. Bianca Papotti: Investigation, Writing – Review & Editing. Sarah Ormseth: Formal analysis, Data Curation, Writing – Review & Editing, Visualization. Marcella Palumbo: Investigation, Writing – Review & Editing. Elizabeth Hernandez: Investigation, Data Curation, Writing – Review & Editing. Maria Pia Adorni:

Investigation, Writing – Review & Editing. Francesca Zimetti: Investigation, Writing – Review & Editing. Matthew Budoff: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing – Review & Editing. Nicoletta Ronda: Conceptualization, Methodology Validation, Investigation, Resources, Supervision, Data Curation, Writing – Review & Editing. All authors critically revised the manuscript for important intellectual content and approved the final version to be published.

Funding

This work was supported by the American Heart Association [grant number AHA-09CRP2251004]; and Pfizer [grant numbers WI215017, 68633259].

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: George A Karpouzas reports financial support was provided by American Heart Association Inc. George A Karpouzas reports financial support was provided by Pfizer.

Data availability

Data will be made available on request.

Acknowledgments

We thank Drs. Benedict Chou, Gopika Miller and Viet Bui for assistance with clinical assessments, and Ms. Lorena Ruiz for facilitating study coordination.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2023.100206.

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