ATP-binding cassette G1 membrane transporter-mediated cholesterol efflux capacity influences coronary atherosclerosis and cardiovascular risk in Rheumatoid Arthritis

Running title: Associations of ABCG1-CEC with atherosclerosis and cardiovascular risk in RA

ATP-Binding Cassette G1 membrane transporter-mediated Cholesterol Efflux Capacity Influences Coronary Atherosclerosis and Cardiovascular Risk in Rheumatoid Arthritis

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ABSTRACT

Objectives. Cholesterol efflux capacity (CEC) measures the ability of high-density lipoprotein (HDL) to remove cholesterol from macrophages and reduce the lipid content of atherosclerotic plaques. CEC inversely associated with cardiovascular risk beyond HDL-cholesterol levels. CEC through the ATP-binding-cassette G1 (ABCG1) membrane transporter is impaired in rheumatoid arthritis (RA). We evaluated associations of ABCG1-CEC with coronary atherosclerosis, plaque progression and cardiovascular risk in RA.

Methods. Coronary atherosclerosis (noncalcified, partially, fully-calcified, low-attenuation plaque) was assessed with computed tomography angiography in 140 patients and reevaluated in 99 after 6.9±0.3 years. Cardiovascular events including acute coronary syndromes, stroke, cardiovascular death, claudication, revascularization and hospitalized heart failure were recorded. ABCG1-CEC was measured in Chinese hamster ovary cells as percentage of effluxed over total intracellular cholesterol.

Results. ABCG1-CEC inversely associated with extensive atherosclerosis (≥5 plaques) (adjusted odds ratio 0.50 [95% CI 0.28-0.88]), numbers of partially-calcified (rate ratio [RR] 0.71 [0.53-0.94]) and low-attenuation plaques (RR 0.63 [0.43-0.91] per standard deviation increment). Higher ABCG1-CEC predicted fewer new partially-calcified plaques in patients with lower baseline and time-averaged CRP and fewer new noncalcified and calcified plaques in those receiving higher mean prednisone dose. ABCG1-CEC inversely associated with events in patients with but not without noncalcified plaques, with <median but not higher CRP and in prednisone users but not nonusers (p-for-interaction=0.021, 0.033 and 0.008 respectively).

Conclusion. ABCG1-CEC inversely associated with plaque burden and vulnerability, and plaque progression conditionally on cumulative inflammation and corticosteroid dose. ABCG1-CEC inversely associated with events specifically in patients with noncalcified plaques, lower inflammation and in prednisone users.

Keywords: Rheumatoid arthritis, coronary atherosclerosis, cardiovascular events, cholesterol efflux capacity, ABCG1.

Observational studies described an inverse association between high-density lipoprotein cholesterol (HDL-C) levels and cardiovascular events (1). Yet, genetic mutations and variants affecting HDL-C levels had no impact on cardiovascular risk (2) and HDL-C raising medications failed to lower it (3,4). These observations suggested that perhaps HDL function, rather than levels, may preferentially underlie its cardioprotective benefits (5). HDL removes cholesterol from foam cells in atherosclerotic lesions—a property known as cholesterol efflux capacity (CEC)—and may prevent formation and progression of atherosclerosis (5). In recent meta-analyses, CEC inversely related with cardiovascular risk independently of HDL-C levels in general patients (6). Additionally, CEC negatively associated with atherosclerotic lesion size and was a better predictor of plaque severity than HDL-C levels (7). Furthermore, CEC inversely related with lipid-rich plaque burden and macrophage density in atherosclerotic lesions (8).

CEC is facilitated by several membrane transporter proteins on macrophages that export free cholesterol to various acceptor HDL particles according to their size, protein and lipid composition (5). CEC through the aqueous diffusion and the scavenger receptor type B class 1 (SR-B1) pathways depend on free cholesterol gradient between cells and mature HDL particles (5). The ATP-binding cassette A1 (ABCA1) membrane transporter actively and unidirectionally exports free cholesterol exclusively to lipid-free or lipid-poor apo-A1, discoidal HDL particles (5). The ATP-binding cassette G1 (ABCG1) membrane transporter on the other hand actively promotes efflux predominantly to mature, spherical HDL particles (5). Therefore, comprehensive assessment of HDL-CEC across these specific pathways individually may provide relevant details about

HDL quality, maturation and function. ABCG1-CEC in particular, is specifically coupled with the elimination of 7-ketocholesterol (9), which induces apoptosis/necrosis in endothelial cells and macrophages (10) and is the most abundant oxysterol in oxidized LDL and in human atherosclerotic plaques (11). Cell cholesterol efflux through the ABC transporters is also associated with lower plaque inflammation (10,12). On the other hand, systemic inflammation induces HDL dysfunction (13). ABCG1-CEC was shown to be lower in RA, inversely related with disease activity (14), and improving with treatment (15).

It is currently unknown whether CEC associates with cardiovascular event risk independently of HDL-C levels or with coronary atherosclerosis in RA. It is also unclear whether CEC at large- much less individual CEC pathways- themselves or their impact on such outcomes are influenced by disease activity, systemic inflammation or RA-specific therapies (16,17). In recent reports, corticosteroids fostered cholesterol accumulation in macrophages *in vitro* (18) and independently promoted coronary atherosclerosis progression in RA (19). In the present study we explored the link between ABCG1-CEC and coronary atherosclerosis burden, its progression and long-term incident cardiovascular risk in RA. We additionally explored whether inflammation and corticosteroid exposure influenced the relationship between ABCG1-CEC and atherosclerosis progression or cardiovascular risk.

MATERIALS AND METHODS

Patient recruitment

One hundred forty patients enrolled in the *PRO*spec*T*ive *E*valuation of Latent *C*oronary A*T*herosclerosis in *R*heumatoid *A*rthritis [PROTECT RA] cohort (20) and serum

available for CEC evaluations were included. The original cohort encompassed 150 patients receiving care in a single center, who underwent atherosclerosis evaluation with coronary computed tomography angiography (CCTA) between March 2010 and March 2011. Patients were prospectively followed for cardiovascular events and 99 of them underwent coronary atherosclerosis reevaluation 6.9±0.3 years later. Participants were between 18 and 75 years old, fulfilled 2010 classification criteria for RA and had no history of cardiovascular disease such as angina, myocardial infarction, stroke, transient ischemic attack, claudication, revascularization, or heart failure. Patients with concurrent autoimmune syndromes (other than Sjogren's), active or chronic infections, malignancy within 5 years, glomerular filtration rate <60 mL/min, body weight exceeding 147.7 kg, or allergy to iodine were excluded. The study was approved by the local Institutional Review Board and participants signed informed consent in accordance with the Declaration of Helsinki.

Coronary Computed Tomography Angiography

Baseline atherosclerosis assessments were carried out in a 64-multidetector row scanner between March 2010 and March 2011. Follow-up scans were carried out in a 256-multidetector row scanner between March 2017 and March 2018. Image acquisition and processing protocols (21) as well as scoring reproducibility (22) have been previously described. Coronary atherosclerosis was evaluated on contrast-enhanced scans according to a standardized 17-segment American Heart Association model (22). Both sets of images were analyzed at the same time and in random order by a single, experienced interpreter, blinded to patients' clinical information (MJB) (21). For longitudinal comparisons, coronary segments in baseline and follow-up scans were

coaligned using fixed anatomic landmarks as fiducial points. Segment involvement score reported the total number of segments with plaque in a single patient (0-17). Presence of ≥5 segments with plaque per patient constituted extensive atherosclerotic disease and associated with greater cardiovascular risk (23,24). Plaque composition was reported as noncalcified, partially and fully calcified as previously described (25). Plaques were also evaluated for low-attenuation areas (≤30 Hounsfield units, low-attenuation plaques) which correspond to necrotic lipid cores and considered high-risk features for plaque rupture (26).

Laboratory evaluations

Complete blood counts, comprehensive metabolic panel, erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP) were completed on the day of CCTA assessments and on every subsequent clinic visit. Fasting lipid evaluations were carried out on the days of both scans and according to EULAR recommendations for cardiovascular risk assessment during the follow-up period (26). Additional serum was collected on the day of CCTA for biomarker studies and aliquots were frozen to -80^{0}C until assayed in batches as previously described (27).

Serum cholesterol efflux capacity

ABCG1-CEC was measured in Chinese hamster ovary cells either untransfected or transfected with the ABCG1 gene as formerly reported (28). Briefly, after labeling with 1,2-^{3}H-cholesterol for 24 hours, cells were incubated in medium with 0.2% bovine serum albumin for 24 hours, and subsequently treated with 1% (volume/volume) whole serum for 4 hours. Serum CEC was expressed as the percentage of radioactivity released in the supernatant over the total intracellular radioactivity. The ABCG1-specific

CEC contribution was calculated as the difference in CEC between ABCG1-transfected and untransfected cells. To correct for interassay variability, a pool of human normolipidemic sera was tested in each assay, and the CEC from these was used to normalize the patient sample values across different experiments (29). The normalized CEC of a second pool of normolipidemic sera concurrently tested in each assay provided an index of intra-assay variability.

Covariates and outcomes

All participants had a 10-year atherosclerotic cardiovascular disease (ASCVD) risk score computed at baseline using the American Heart Association pooled cohort equation calculator (30). Disease activity based on a standard 28-joint examination for tenderness and swelling and C-reactive protein (DAS28-CRP) was calculated at all clinic visits (every three to four months) throughout the follow-up duration. Medications including conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs), biologic DMARDs (bDMARDs), prednisone, and statins were recorded and reconciled against pharmacy prescriptions on each visit.

A prespecified composite cardiovascular endpoint including cardiovascular death, acute coronary syndrome, stroke, transient ischemic attack, peripheral arterial disease, coronary or peripheral revascularization and heart failure was the clinical outcome of interest. All events were adjudicated by the respective specialists, who were blinded to CCTA results, after review of electronic medical records and based on standard definitions (31). Only first cardiovascular events were analyzed.

Coronary atherosclerosis outcomes of interest included numbers of segments with any, noncalcified, partially, fully calcified, low-attenuation plaque, presence of extensive disease (≥5 plaques) at baseline and new plaque formation at follow-up.

Statistical analysis

Categorical variables were presented as frequencies with percentages and continuous variables as means with standard deviations (SD). The effect of ABCG1-CEC on presence of extensive disease at baseline was evaluated with robust logistic regression. Robust negative binomial regression was used for count outcomes (numbers of any, noncalcified, partially-calcified, fully-calcified, and low-attenuation plaques) both at baseline and for plaque change at follow-up. The influence of baseline CRP, time-varying CRP, baseline prednisone use and time-weighted average daily prednisone dose during follow-up on the relationship between ABCG1-CEC and plaque progression was evaluated by adding the respective moderators and their products with ABCG1-CEC as interaction terms to the corresponding models. Multivariable baseline plaque outcome models adjusted for ASCVD risk score and statin use. Multivariable plaque progression outcome models adjusted for ASCVD risk score, number of the respective plaque types at baseline, as well as covariates significant in the corresponding multivariable models. The impact of ABCG1-CEC on cardiovascular event risk was evaluated in a Cox regression model adjusting for ASCVD risk score and plaque burden. The influence of moderators such as presence of noncalcified plaque at baseline, high (≥median) versus lower baseline CRP and prednisone exposure on the relationship between ABCG1-CEC and cardiovascular risk was examined in separate cox regression models including the respective moderators and their corresponding

interaction terms with ABCG1-CEC. SPSS version 27 and Stata version 15 were used. P values <0.05 were considered significant.

RESULTS

Participants were mostly middle-aged women with established, seropositive and erosive disease (Table 1). All were treated with a mean of two concurrent csDMARDs (80% methotrexate) and 86/140 (61%) additionally received bDMARDs (all TNF-α inhibitors) upon enrollment. Mean ASCVD score was low.

ABCG1-CEC associates with baseline coronary atherosclerosis presence and burden

Coronary atherosclerosis was present in 90 (70%) and extensive disease was seen in 19 (13.6%) patients at baseline. Mean (standard deviation [SD]) ABCG1 was 4.71 (0.92)%. ABCG1 inversely associated with likelihood of extensive atherosclerosis (≥5 plaques) at baseline (adjusted odds ratio 0.50 [95% CI 0.28-0.88] per 1SD increase in ABCG1). Likewise, each SD increase in ABCG1-CEC associated with 29% fewer partially-calcified (rate ratio [RR] 0.71 [95% CI 0.53-0.94]) and 37% fewer low-attenuation plaques (RR 0.63 [95% CI 0.43-0.91]) independently of ASCVD score and statin use (Figure 1).

Associations of ABCG1-CEC with coronary atherosclerosis progression

Ninety nine of 140 patients with baseline scans had repeat atherosclerosis evaluation within 6.9±0.3 years. Of the remaining 41, four had no follow-up after baseline evaluation, two expired, six migrated, and 29 declined reassessment. Although patients with no follow up atherosclerosis assessment had higher average age, systolic blood

pressure and ASCVD risk, differences in ASCVD scores were no longer significant after adjusting for age (Table S1). A total of 68 (68.7%) patients had coronary atherosclerosis at follow-up and 10 (10.1%) showed new segments with plaque compared to baseline. ABCG1-CEC showed no main association with plaque progression in adjusted models (results not shown). However, ABCG1-CEC influenced the effect of baseline CRP on plaque progression in models adjusting for ASCVD score, baseline plaque numbers, HDL-C, duration of statin exposure, time-averaged CRP and age at RA diagnosis (p-for-interaction=0.001). Specifically, increasing ABCG1-CEC associated with fewer new plaques— particularly fewer new partially-calcified plaques— when baseline CRP was low (-1 standard deviation [SD]) but not when CRP was high (+1SD, Figure 2A). Additionally, RA patients with low baseline CRP formed significantly fewer partially calcified plaques compared to those with high CRP at high (above mean) but not low (below mean) ABCG1-CEC. Likewise, ABCG1-CEC influenced the relationship between time-averaged CRP and partially-calcified plaque progression in models adjusting for ASCVD score, baseline plaque numbers, duration of statin exposure and time-weighted average daily prednisone dose (p-for-interaction=0.021). Specifically, higher ABCG1-CEC predicted fewer new partially-calcified plaques in patients with low (-1SD) but not high (+1SD) time-averaged CRP (Figure 2B).

Moreover, higher ABCG1-CEC predicted fewer new noncalcified and calcified plaques exclusively in patients receiving the highest time-weighted mean daily prednisone dose (+2SD) —but not lower doses— during follow-up in models adjusting for ASCVD score, baseline plaque numbers, HDL-C, statin exposure duration and time-averaged CRP (p-for-interaction= 0.008 and 0.002 respectively, Figure 2C,D). Likewise,

prednisone users on the highest time-weighted mean daily dose stratum formed a significantly higher number of new noncalcified and calcified plaques compared to nonusers when ABCG1-CEC was low (<mean) but not when it was high (≥mean).

ABCG1-CEC link to cardiovascular events

Fifteen patients experienced 18 cardiovascular events over 6.03±2.42 years of follow-up (incidence rate of 2.08 [1.31-3.30] events/100 patient-years, Supplementary Table S2). ABCG1-CEC did not directly associate with cardiovascular risk after adjusting for ASCVD score and number of plaques at baseline (HR 0.80 [95% CI 0.47-1.39]). However, each SD higher ABCG1-CEC associated with a 53% risk reduction in patients with low (<median) versus high (>median) baseline CRP (p-for-interaction=0.033, Figures 3 and 4A,B); a 48% lower risk in patients with versus without noncalcified plaque at baseline (p-for-interaction=0.021, Figures 3 and 4C,D); and with 50% lower event risk in patients with any versus no prednisone exposure during follow-up (p-for-interaction=0.008, Figures 3 and 4E,F).

DISCUSSION

This is the first study that formally addresses the relationships of ABCG1-CEC with coronary atherosclerosis, its progression and long-term cardiovascular risk in patients with RA. Several novel observations inform the potential clinical utility of ABCG1-CEC in future algorithms aiming to optimize cardiovascular risk stratification in this disease. First, we showed that ABCG1-CEC inversely associated with presence of extensive atherosclerotic disease and numbers of both partially-calcified and low-attenuation (lipid-rich) plaques at baseline, all of which were linked to greater

cardiovascular risk in both general and RA patients (24,32–34). Consistent with our findings, HDL cholesterol uptake inversely related with lipid-rich plaque burden, density of macrophage infiltration within plaques (8) and fibrous cap thickness in general patients (35). Likewise, one study in RA reported an inverse association between CEC at large and carotid plaque presence (36) and another, in psoriasis, showed an inverse association with noncalcified coronary plaque burden (37). This inverse association between ABCG1-CEC and high-risk plaques may be attributed to the concurrent outflow of 7-ketocholesterol and related oxysterols from plaque macrophages, along with free cholesterol to HDL, exclusively facilitated by ABCG1 transporter; 7-ketocholesterol can induce apoptosis/necrosis in endothelial cells and macrophages (9–11) and is the most prevalent oxysterol in human plaques. Alternatively, it may be explained by anti-inflammatory effects directly on lesional macrophages as a result of the cholesterol efflux process itself; including the suppression of inflammatory signals downstream of TLR2,3 and 4, modulating NFkB and type I interferon responses (38) and NLRP3 inflammasomes (12,39).

Secondly, ABCG1-CEC influenced the impact of both baseline and cumulative inflammation on atherosclerosis progression, independently of HDL-C levels; higher ABCG1-CEC associated with fewer new—particularly partially-calcified plaques—when either baseline or time-averaged CRP were low and independently of each other. In contrast, when baseline or time-averaged CRP were high, increasing ABCG1-CEC showed no association with plaque progression. Notably ~~that~~ there was no quantitative difference in ABCG1-CEC between high and low inflammation groups explaining this observation (not shown). One possible explanation may derive from the fact that CRP

itself inhibits directly the expression of ABCG1 in macrophages (40); thus, reduced ABCG1 on macrophages *in vivo* ~~in the context of high CRP — and at any given level of HDL functionality —~~may impair the disposal of cholesterol and particularly 7-ketocholesterol and related oxysterols from vascular cells, even in presence of HDL with preserved capacity to accept cholesterol. Our finding may therefore not only prove useful for the individual RA patient risk assessment, but also for the development of strategies to reduce the direct proinflammatory and proatherogenic activity of CRP, currently under investigation (42). Additionally, we recently reported that when inflammation is high in RA, higher oxidized-LDL (oxLDL) significantly enhances cholesterol loading onto macrophages both directly and indirectly—especially in double seropositive patients—leading to higher plaque burden (27,43). Indeed, in the present study, ABCG1-CEC inversely associated with serum cholesterol loading capacity (data not shown). Considering that cholesterol loaded on oxLDL is trapped in lysosomes and poorly available for efflux (44), when inflammation is high, even a good HDL function measured as ABCG1-CEC may not efficiently remove cholesterol or compensate for the proatherogenic state induced by increased cholesterol loading on macrophages; as a result, plaque progression may continue unabated. In contrast, when inflammation is controlled, oxLDL-mediated cholesterol loading declines and antiatherogenic HDL particles may efficiently perform cholesterol efflux leading to decreased plaque progression (27).

Third, the intensity of corticosteroid therapy influenced the relationship between ABCG1-CEC and coronary plaque progression, independently of inflammatory status. Higher ABCG1-CEC associated with fewer new noncalcified and calcified plaques in

patients receiving the highest mean daily prednisone dose independently of HDL-C levels and time-averaged CRP. Moreover, when ABCG1-CEC was high, no difference in new plaque formation was observed between prednisone users at the highest daily dose and nonusers; in contrast, when ABCG1-CEC was low, prednisone users formed significantly more new plaques compared to nonusers. Consistently with our findings, higher baseline ABCG1-CEC associated with greater reduction in coronary artery lipid content in general patients (45). These observations have direct clinical implications; higher prednisone dose is generally prescribed to patients with greater disease activity. Corticosteroids are proatherogenic though various mechanisms, including a negative effect on lipid levels, glycemia and blood pressure, or through direct regulation of cell functions. As our analyses were adjusted for ASCVD risk score, HDL-C levels, time-averaged CRP and statin exposure duration, our findings may specifically reflect the direct influence of prednisone on macrophage cholesterol homeostasis. Indeed, high dose steroid *in vitro* promoted cholesterol accumulation in macrophages, which associates with foam cell formation, higher atherosclerosis burden and plaque vulnerability (18,43). Interestingly, however, steroids were also shown to promote macrophage uptake of native LDL *in vitro* (18) bypassing the physiologic control of cell cholesterol influx (that normally occurs through inhibition of LDL-receptor expression) and therefore providing an additional mechanism for intracellular cholesterol accumulation. Consequently, the salient benefit of high ABCG1-CEC in prednisone treated patients, after adjusting for all other known CV risk factors, may be due to the efflux of internalized cholesterol that is readily available for membrane export, as opposed to that associated with oxLDL (44). As cumulative prednisone dose

independently predicted coronary plaque progression (19) and was linked to accelerated cardiovascular risk in RA (46), the atheroprotective effect of ABCG1-CEC particularly in this high-risk group is especially relevant.

Although ABCG1-CEC had no main effect on cardiovascular risk in adjusted analyses, higher ABCG1-CEC was linked to lower cardiovascular risk in patients with lipid-rich noncalcified plaques but not in those without. In sensitivity analyses, higher ABCG1-CEC was linked to lower incident event risk as noncalcified plaque burden increased (p-for-interaction= 0.016, not shown). Lipid-rich areas encompassing intracellular (foam cells) and extracellular cholesterol pools are characteristic of noncalcified and low-attenuation plaques and far less common in fully calcified plaques (47). CEC can therefore optimally operate in foamy macrophages in noncalcified lesions preventing atherosclerosis progression or new plaque formation. Indeed, we recently reported that cardiovascular risk reduction by bDMARDs in RA was particularly effective in patients with noncalcified and low-attenuation plaques and coincided with loss of such lipid-rich low-attenuation lesions (34). Likewise, the cardioprotective benefit of statin use in RA corresponded with noncalcified plaque resorption independently of bDMARD use (48).

Moreover, and similar to plaque progression, higher ABCG1-CEC associated with lower event risk in patients with low but not high baseline inflammation. Likewise, higher ABCG1-CEC associated with lower cardiovascular risk as CRP increased in sensitivity analyses (p-for-interaction=0.044, not shown). Lastly, the relationship between ABCG1-CEC and incident events was also influenced by glucocorticoid exposure. ABCG1-CEC inversely associated with event risk in patients receiving prednisone at any time

throughout the follow-up duration, but not in nonusers. In ancillary analyses higher ABCG1-CEC also associated with lower cardiovascular risk as time-weighted mean daily prednisone dose increased (p-for-interaction=0.009, not shown).

Certain limitations of our study should be acknowledged. First, our original study design was not powered to specifically evaluate the relationships between ABCG1-CEC and coronary atherosclerosis, its progression or cardiovascular events; these findings should therefore be considered exploratory. Second, patients found to have atherosclerosis at baseline evaluation had preventive therapy with statins and/or aspirin initiated or escalated, irrespective of clinical indication; this may have influenced plaque progression and cardiovascular risk (48) as well as the relationship between ABCG1-CEC and those outcomes. Lastly, lack of standardization in methodology of HDL-CEC assessments and clarity in reporting the precise CEC pathways examined has rendered comparisons between our study and the extant literature challenging. Future standardization in reporting, streamlining of the laboratory methodology and prospective validation of individual CEC pathways in predicting atherosclerosis and clinical events in large studies may enable its uptake in clinical practice for cardiovascular risk stratification in RA.

Conclusion

ABCG1-CEC inversely associated with presence of extensive coronary atherosclerosis and burden of high-risk plaques at baseline. It associated with decreased plaque progression conditionally on baseline or cumulative inflammation and prednisone dose. ABCG1-CEC inversely associated with cardiovascular risk specifically in patients with noncalcified plaques at baseline, those with lower inflammation and in prednisone

users. Upon further validation, it may prove a useful adjunct for effective cardiovascular risk stratification and prevention strategies in the care of RA patients.

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Contributors GAK and NR conceived and designed the study. GAK, SRO, EH, and MJB collected the data. NR, BP, MP, MPA and FZ performed CEC analyses. GAK, BP, SRO, MP, EH, MPA, FZ, MJB, and NR analyzed and interpreted the data. All authors were involved in development, review and approval of the manuscript. GAK has full access to study data and is accountable for the accuracy and integrity of the work.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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FIGURE LEGENDS

Figure 1. Association of ABCG1-CEC with baseline plaque burden

ABCG1-CEC = cholesterol efflux capacity through the ATP-binding cassette G1 pathway; OR= odds ratio; RR= rate ratio; 95% CI = 95% confidence interval.

Rate ratios denote the percent change in number of segments with plaque associated with one standard deviation unit increase in ABCG1-CEC. Odds ratios or rate ratios derived from robust binary logistic regression for extensive disease and robust negative binomial regression for various plaque numbers respectively, adjusted for atherosclerotic cardiovascular disease (ASCVD) score and statin use.

Figure 2. Associations of ABCG1-CEC with coronary atherosclerosis progression. (A) ABCG1-CEC inversely associated with new partially-calcified plaque formation in patients with low but not higher baseline CRP. Multivariable model adjusted for ASCVD score, baseline plaque numbers, HDL-C, duration of statin exposure, time-averaged CRP, and age at RA diagnosis. (B) ABCG1-CEC inversely associated with new partially-calcified plaque formation in patients with low (<median) but not high time-averaged CRP. Multivariable model adjusted for ASCVD score, baseline plaque numbers, duration of statin exposure and time-weighted average daily prednisone dose. ABCG1-CEC inversely associated with new noncalcified (C) and calcified (D) plaque formation in individuals receiving the highest time-weighted mean daily prednisone dose. Multivariable models adjusted for ASCVD score, baseline plaque numbers, HDL-C, statin exposure duration and time-averaged CRP.

ABCG1-CEC = cholesterol efflux capacity through the ATP-binding cassette G1 pathway; SD = standard deviation.

Figure 3. Association of ABCG1-CEC with long-term incident cardiovascular event risk. All models adjusted for ASCVD risk score and number of coronary segments with plaque.

Figure 4. Higher ABCG1-CEC attenuates cardiovascular risk in patients with low (<median) vs. high baseline CRP **(A, B)**, those with versus without noncalcified plaque at baseline **(C, D)**, and those with any versus no prednisone exposure during follow-up **(E, F)**. All models adjusted for ASCVD risk score and number of coronary segments with plaque at baseline.