

Genotype-Dependent Effects of Dalcetrapib on Cholesterol Efflux and Inflammation

Concordance With Clinical Outcomes

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Background—Dalcetrapib effects on cardiovascular outcomes are determined by adenylate cyclase 9 gene polymorphisms. Our aim was to determine whether these clinical end point results are also associated with changes in reverse cholesterol transport and inflammation.

Methods and Results—Participants of the dal-OUTCOMES and dal-PLAQUE-2 trials were randomly assigned to receive dalcetrapib or placebo in addition to standard care. High-sensitivity C-reactive protein was measured at baseline and at end of study in 5243 patients from dal-OUTCOMES also genotyped for the rs1967309 polymorphism in adenylate cyclase 9. Cholesterol efflux capacity of high-density lipoproteins from J774 macrophages after cAMP stimulation was determined at baseline and 12 months in 171 genotyped patients from dal-PLAQUE-2. Treatment with dalcetrapib resulted in placebo-adjusted geometric mean percent increases in high-sensitivity C-reactive protein from baseline to end of trial of 18.1% ($P=0.0009$) and 18.7% ($P=0.00001$) in participants with the GG and AG genotypes, respectively, but the change was $-1.0%$ ($P=0.89$) in those with the protective AA genotype. There was an interaction between the treatment arm and the genotype groups ($P=0.02$). Although the mean change in cholesterol efflux was similar among study arms in patients with GG genotype (mean: 7.8% and 7.4%), increases were 22.3% and 3.5% with dalcetrapib and placebo for those with AA genotype ($P=0.005$). There was a significant genetic effect for change in efflux for dalcetrapib ($P=0.02$), but not with placebo.

Conclusions—Genotype-dependent effects on C-reactive protein and cholesterol efflux are supportive of dalcetrapib benefits on atherosclerotic cardiovascular outcomes in patients with the AA genotype at polymorphism rs1967309.

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High-density lipoproteins (HDLs) have multiple beneficial and potentially cardioprotective effects, including those on reverse cholesterol transport, inflammation, and oxidative stress.¹ However, several therapies targeting HDL have yielded disappointing results in recent years.²⁻⁵ This apparent discrepancy could be potentially because of differential clinical responses to such medications based on patients' genetic profiles. We have indeed shown recently that dalcetrapib effects on cardiovascular outcomes are determined by polymorphisms in the adenylate cyclase isoform 9 (*ADCY9*)

gene.^{6,7} Dalcetrapib is an inhibitor of the cholesteryl ester transfer protein (CETP), which mediates the transfer of cholesteryl esters from HDL to apolipoprotein B-containing lipoproteins.⁸ CETP inhibitors have been shown to raise the plasma level of high-sensitivity C-reactive protein (hs-CRP), a biomarker of systemic inflammation.^{5,9,10} This small but significant increase in hs-CRP was unexpected in light of the anti-inflammatory properties of HDL particles.¹¹ Whether this

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effect is generalized or limited to a subset of patients with particular characteristics is unknown. Modulation of inflammation could in turn alter drug-induced changes in HDL functions (including cholesterol mobilization), atherosclerosis, and cardiovascular outcomes.^{12,13}

The ability of HDL to efflux cholesterol from macrophages has been shown to predict the presence of coronary artery disease and incident cardiovascular events, rather than and independently of the plasma level of HDL cholesterol.^{14,15} Demonstration of genotype-dependent effects of dalcetrapib on inflammation and cholesterol mobilization would greatly strengthen the observation of the large genetically based differences in atherosclerotic cardiovascular outcomes induced by this therapy.⁶ Accordingly, we measured hs-CRP and cholesterol efflux in the dal-OUTCOMES and dal-PLAQUE-2 pharmacogenomic studies, respectively. We also evaluated the safety and tolerability profile of dalcetrapib according to patient genotype at polymorphism rs1967309 in the *ADCY9* gene.

Methods

The dal-OUTCOMES Trial

The dal-OUTCOMES study was a phase 3 clinical trial of 15 871 participants designed to test the safety and cardiovascular efficacy of the CETP inhibitor dalcetrapib (F. Hoffman-La Roche).⁵ To be eligible, patients needed to be aged ≥ 45 years; provide written informed consent; be hospitalized with an acute coronary syndrome defined either as elevated cardiac biomarkers with symptoms of acute myocardial ischemia, new or presumed new ischemic electrocardiographic abnormalities, or loss of viable myocardium based on imaging; symptoms of acute myocardial ischemia in the absence of elevated cardiac biomarkers if accompanied by new or presumed new ECG changes and additional evidence of obstructive coronary disease; or myocardial infarction associated with percutaneous coronary intervention. Patients with serum triglycerides ≥ 400 mg/dL were excluded; other exclusion criteria have been previously described.⁵ Eligible patients who were clinically stable 4 to 12 weeks after this recent acute coronary syndrome were randomly assigned to receive dalcetrapib 600 mg daily or placebo in a 1:1 ratio, in addition to evidence-based medical care. Cardiovascular events were adjudicated by an independent clinical end point committee. Dalcetrapib's safety and tolerability were evaluated through regular patient visits and systematic query of all patients concerning any side effect, as well as review of adverse events, physical examinations, and laboratory abnormalities. As previously described, 6338 patients were recruited at 461 sites in 14 countries and provided written informed consent to participate in the pharmacogenomic study of the dal-OUTCOMES trial.⁶ DNA was extracted from whole blood. After genomic data cleanup, samples from 5749 Caucasian patients were used in the genetic analysis. Plasma was collected at baseline, 3 months of follow-up, and end of trial and frozen at -80°C . Patients included in the hs-CRP analysis needed to have baseline and at least one postbaseline hs-CRP measurements.

The dal-PLAQUE-2 Trial

The dal-PLAQUE-2 study⁶ was a phase 3b multicenter, double-blind, randomized, placebo-controlled, parallel group trial designed to assess the effect of dalcetrapib on atherosclerotic disease progression in 931 patients with evidence of coronary artery disease and carotid intima-media thickness of at least 0.65 mm in the far wall of the common carotid arteries, as assessed by ultrasonography at baseline. As previously described, participants were randomized to receive dalcetrapib 600 mg daily or matching placebo until they returned for follow-up carotid imaging at 12 months. Among the 411 participants in the dal-PLAQUE-2 trial who consented to the genetic study, 386 had serial imaging measures (194 and 192 in the dalcetrapib and placebo arms, respectively). DNA was extracted from whole blood. An additional specific consent

form was signed by 171 patients who accepted to participate in the biomarkers (cholesterol efflux) substudy. Plasma was collected at baseline and at 12 months and frozen at -80°C . The research protocols were approved by the relevant institutional review boards or ethics committees, and all participants gave written informed consent.

Genotyping

Single nucleotide polymorphism rs1967309 in the *ADCY9* gene was genotyped using the Illumina Infinium HumanOmni2.5Exome-8v1_A BeadChip in dal-OUTCOMES and a Sequenom panel for dal-PLAQUE-2 samples as previously described.⁶ The rs1967309 variant was distributed according to Hardy-Weinberg proportions ($P > 0.05$) in both populations.

Biomarker Measurements

All biomarker measurements were made without knowledge of genotypes. hs-CRP concentration in serum was assessed by immunonephelometry using a BNII analyzer (Dade Behring, Deerfield, IL).

In samples from 171 patients of the dal-PLAQUE-2 trial, cholesterol efflux capacity was measured in vitro with J774 macrophages (treated with cAMP) grown for 24 hours in presence of tritiated (^3H) cholesterol. After equilibration during 18 hours, cholesterol efflux was initiated by adding individual patient's serum (depleted of apolipoprotein B-containing lipoproteins with PEG6000) or control serum in triplicate wells for 4 hours. The concentration of patient's serum tested was carefully selected from dose-response curves obtained from pooled human plasma to avoid saturation of the efflux signal and to allow measuring both increases and decreases. Tritiated cholesterol counts were measured in aliquots of cell-free culture medium and J774 cell homogenates with a beta counter (Tricarb, Perkin-Elmer). Cholesterol efflux capacity was defined as the fraction of ^3H -cholesterol found in the medium relative to the total cholesterol label in each well. Each sample batch was tested in parallel with the same pool of normolipidemic serum to calculate the sample/control ratio. Control serum cholesterol efflux capacity values needed to be within the limits defined by the historical mean $\pm 2\text{SD}$.

Statistical Analysis

Baseline and safety data are reported using descriptive statistics. hs-CRP and cholesterol efflux were analyzed as changes from baseline using a general linear model. A log transformation was applied to hs-CRP because of skewed distribution. Differences between dalcetrapib and placebo groups were, therefore, described through placebo-adjusted geometric mean percent change. The geometric mean is obtained by the antilog of mean of the log-transformed data. Placebo-adjusted geometric mean percent change at the time point t was calculated as $[(\text{geometric mean for change at } t \text{ with dalcetrapib} - \text{geometric mean for change at } t \text{ with placebo}) / \text{geometric mean for change at } t \text{ with placebo}] \times 100$. The 1-degree of freedom additive genetic test was used to test for association of rs1967309 with cholesterol efflux, whereas the 2-degree of freedom genetic test was used for hs-CRP because of the nonlinear effect of rs1967309 genotypes on hs-CRP or by pairwise testing between genotypes as specified. All tests were 2-sided and conducted at the 0.05 significance level. Statistical analyses were performed using SAS statistical software version 9.4 (SAS Institute Inc, Cary, NC).

Results

Characteristics of the Study Populations

The patient flows in the studies are depicted in Figure 1. Baseline characteristics of the study participants were well balanced among study groups (Tables 1 and 2). Characteristics of participants included in the current analysis were similar to those of patients involved in the global studies (Table I in the [Data Supplement](#)). Baseline characteristics segregated by

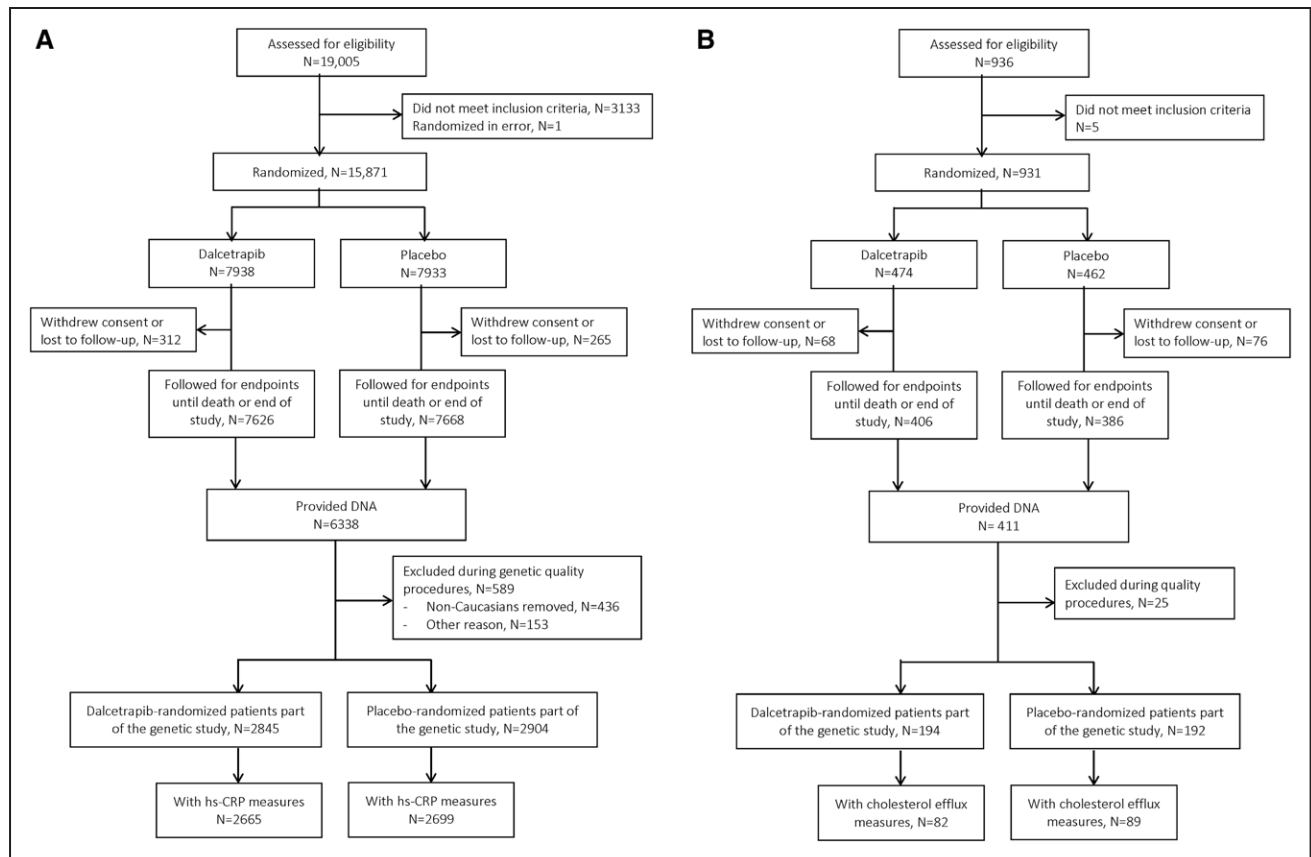


Figure 1. A, Flow diagram of dal-OUTCOMES. B, Flow diagram of dal-PLAQUE-2.

genotype group are presented in Tables II and III in the [Data Supplement](#).

C-Reactive Protein

Geometric mean hs-CRP concentrations at baseline were 1.7, 1.6, and 1.6 mg/L for patients with the GG, AG, and AA genotypes, respectively, in dal-OUTCOMES (n=5364). Treatment with dalcetrapib resulted in placebo-adjusted geometric mean percent increases in hs-CRP from baseline to end of trial of 18.1% ($P=0.0009$) and 18.7% ($P=0.00001$) in participants with the GG and AG genotypes, respectively, but the change was -1.0% ($P=0.89$) in those with the AA genotype (Table 3). This decrease in placebo-adjusted geometric mean percent change in hs-CRP with dalcetrapib in patients with the AA genotype was significantly different from the increase observed in those with the AG genotype ($P=0.03$). There was also a significant statistical interaction between the treatment arm and the genotype groups on change in hs-CRP (log scale; $P=0.02$; interaction term in the linear model).

At 36 months, placebo-adjusted geometric mean percent changes with dalcetrapib were 17.7% ($P=0.02$), 20.4% ($P=0.002$), and -9.7% ($P=0.34$) for the GG, AG, and AA genotypes, respectively (Figure 2). Similar directional results were observed at 3 and 24 months, as detailed in Table 3 and Figure 2.

Cholesterol Efflux Capacity of Serum HDL

Although the mean change in cholesterol efflux from baseline to 12 months was similar between study arms in

dal-PLAQUE-2 patients with the GG genotype ($7.8\pm 18.0\%$ and $7.4\pm 10.8\%$; $P=0.93$), increases were $22.3\pm 22.3\%$ and $3.5\pm 12.3\%$ with dalcetrapib and placebo for those with the AA genotype ($P=0.005$; Table 4). Increases in efflux were $7.8\pm 18.0\%$, $12.9\pm 16.9\%$, and $22.3\pm 22.3\%$ with dalcetrapib in participants with the GG, AG, and AA genotypes, respectively; when testing for an additive genetic effect of the A allele at rs1967309 on change in cholesterol efflux within study arms, we saw a significant association with increased efflux in the dalcetrapib arm ($P=0.02$; Figure 3), but not in the placebo group.

Safety Profile

Dalcetrapib was well tolerated irrespective of genotype (Table 5). Gastrointestinal side effects were reported in 26.4%, 25.1%, and 23.3% of participants with the GG, AG, and AA genotypes in the placebo group and in 30.2%, 30.9%, and 30.5% of participants in the dalcetrapib group, respectively. Diarrhea occurred in 6.1%, 4.8%, and 5.0% of patients in the placebo group with the GG, AG, and AA genotypes, respectively, and in 7.3%, 8.4%, and 9.7% of participants in the dalcetrapib group. The rates of treatment discontinuation because of diarrhea were 1.0%, 1.6%, and 2.3% in patients with the 3 genotypes in the dalcetrapib group. Hypertension was reported as an adverse event in 9.1% and 10.0% of patients with the GG genotype in the placebo and dalcetrapib groups, 8.4% and 9.2% with the AG genotype in the placebo and dalcetrapib groups, and 10.3% and 9.5% of participants with the AA genotype for

Table 1. Demographics of the dal-OUTCOMES Population

Variable	Dalcetrapib (n=2665)	Placebo (n=2699)
Age, y	60.5 (±9.0)	60.5 (±9.0)
Female sex, n (%)	554 (20.8)	530 (19.6)
Race, n (%)		
White	2661 (99.9)	2694 (99.8)
Region of enrollment, n (%)		
North America	1287 (48.3)	1320 (48.9)
Eastern Europe	664 (24.9)	678 (25.1)
Western Europe	714 (26.8)	701 (26.0)
rs1967309 MAF, %	41.5	40.9
Cardiovascular risk factors, n (%)		
Hypercholesterolemia	2069 (77.6)	2111 (78.1)
Hypertension	1799 (67.5)	1844 (68.3)
Diabetes mellitus	578 (21.7)	580 (21.5)
Current smoker	519 (19.5)	549 (20.3)
CVD history, n (%)		
Coronary heart disease	720 (27.0)	723 (26.8)
Angina pectoris	434 (16.3)	447 (16.6)
PCI	414 (15.5)	428 (15.9)
Previous MI	430 (16.1)	417 (15.5)
CHF class I	355 (13.3)	344 (12.8)
CHF class II	75 (2.9)	80 (3.1)
CABG	162 (6.1)	185 (6.9)
Stroke	73 (2.7)	71 (2.6)
PAD	233 (8.7)	201 (7.5)
Index diagnosis, n (%)		
NSTEMI	1148 (43.1)	1162 (43.1)
STEMI	1187 (44.5)	1188 (44.0)
Hospitalization for ACS	249 (9.3)	272 (10.1)
Procedural MI	81 (3.0)	76 (2.8)
Medication, n (%)		
Aspirin	2576 (96.7)	2638 (97.7)
P2Y12 inhibitor	2390 (89.7)	2423 (89.8)
Atorvastatin	1189 (44.6)	1247 (46.2)
Other statin	1406 (52.8)	1385 (51.3)
ACE inhibitor	1751 (65.7)	1760 (65.2)
ARB	347 (13.0)	321 (11.9)
Beta-blocker	2385 (89.5)	2453 (90.9)
eGFR <60 mL/min/1.73 m ² , n (%)	259 (9.7)	286 (10.6)
hs-CRP, mg/L, median (IQR)	1.5 (0.7, 3.7)	1.5 (0.8, 3.5)
Lipids, lipoproteins, and apolipoproteins, mg/dL		
Total cholesterol	146.4 (±32.0)	145.4 (±33.1)
Triglycerides	133.1 (±73.3)	133.1 (±78.4)

(Continued)

Table 1. Continued

Variable	Dalcetrapib (n=2665)	Placebo (n=2699)
HDL cholesterol	43.3 (±11.7)	43.0 (±11.7)
LDL cholesterol	76.6 (±25.5)	76.1 (±25.6)
Apo B/Apo A1	0.6 (±0.2)	0.6 (±0.3)

Plus-minus values are means±SD. ACE, angiotensin-converting enzyme; ACS, acute coronary syndrome; Apo B/Apo A1, apolipoprotein B/apolipoprotein A1; ARB, angiotensin receptor blockers; CABG, coronary artery bypass graft surgery; CHD, coronary heart disease; CHF, congestive heart failure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; MAF, minor allele frequency; MI, myocardial infarction; NSTEMI, non-ST-segment-elevation MI; PAD, peripheral arterial disease; PCI, percutaneous coronary intervention; P2Y12 inhibitors, antiplatelet agents blocking ADP-induced aggregation; and STEMI, ST-segment-elevation MI.

placebo and dalcetrapib groups, respectively. Systolic blood pressure was increased by 0.0 and 0.7 mmHg after 3 months of treatment with placebo and dalcetrapib, respectively, in patients with the GG genotype, 1.2 and 1.1 mmHg for those with the AG genotype, and 1.0 and 0.9 mmHg with the AA genotype, without a genetic effect ($P>0.05$; Table 6).

Discussion

The *ADCY9* gene rs1967309 genotype-dependent effects of dalcetrapib on hs-CRP and cholesterol efflux from macrophages are supportive of its effects on cardiovascular outcomes and atherosclerosis. Although patients with the AA genotype

Table 2. Demographics of the dal-PLAQUE-2 Population

Variable	Dalcetrapib (n=82)	Placebo (n=89)
Age, y	61.1 (±8.1)	59.7 (±8.6)
Female sex, n (%)	29 (35.4)	17 (19.1)
Body mass index, kg/m ²	29.7 (±5.4)	30.6 (±6.4)
Race, n (%)		
White	81 (98.8)	88 (98.9)
Region of enrollment, n (%)		
North America	82 (100.0)	89 (100.0)
rs1967309 MAF, %	37.8	43.8
Cardiovascular risk factors, n (%)		
Hypertension	47 (57.3)	67 (75.3)
Current smoker	15 (18.3)	15 (16.9)
Lipids, mg/dL		
Total cholesterol	144.1 (±28.1)	144.9 (±34.0)
Triglycerides	135.4 (±76.1)	122.9 (±62.7)
HDL cholesterol	45.1 (±13.3)	46.3 (±13.0)
LDL cholesterol	71.9 (±20.9)	74.0 (±26.5)
hs-CRP, mg/L, median (IQR)	1.7 (0.6, 4.0)	1.6 (0.6, 4.2)

Plus-minus values are mean±standard deviation. HDL indicates high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; MAF, minor allele frequency; and N, number of patients.

Table 3. hs-CRP in dal-OUTCOMES

	GG		AG		AA	
	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo
n at baseline	917	933	1285	1323	463	443
Baseline, GM, mg/L (IQR)	1.72 (0.7, 4.0)	1.77 (0.8, 3.8)	1.65 (0.7, 3.5)	1.66 (0.8, 3.5)	1.71 (0.8, 3.6)	1.44 (0.6, 3.1)
3 mo, GM, mg/L (IQR)	1.77 (0.8, 3.6)	1.57 (0.7, 3.4)	1.74 (0.8, 3.7)	1.51 (0.7, 3.1)	1.69 (0.8, 3.7)	1.30 (0.6, 2.6)
GM % change at 3 mo	3.7 (n=883)	-11.9 (n=912)	6.5 (n=1240)	-8.2 (n=1290)	-3.0 (n=454)	-10.8 (n=432)
Placebo-adj GM % change (95% CI) at 3 mo	17.8 (7.2, 29.4)		15.9 (7.7, 24.9)		8.7 (-4.9, 24.2)	
P value*	0.0007		0.00009		0.22	
24 mo, GM, mg/L (IQR)	1.50 (0.7, 3.1)	1.40 (0.7, 2.6)	1.67 (0.8, 3.5)	1.32 (0.6, 2.9)	1.55 (0.7, 3.4)	1.26 (0.6, 2.2)
GM % change at 24 mo	-3.0 (n=303)	-21.3 (n=321)	5.3 (n=396)	-12.6 (n=416)	-9.3 (n=131)	-13.5 (n=134)
Placebo-adj GM % change (95% CI) at 24 mo	23.3 (3.7, 46.5)		20.5 (4.7, 38.7)		4.8 (-20.2, 37.7)	
P value*	0.02		0.009		0.73	
36 mo, GM, mg/L (IQR)	1.70 (0.8, 3.5)	1.46 (0.6, 3.1)	1.63 (0.7, 3.4)	1.45 (0.7, 3.1)	1.57 (0.8, 3.1)	1.31 (0.6, 3)
GM % change at 36 mo	-1.5 (n=402)	-16.3 (n=411)	3.8 (n=575)	-13.8 (n=614)	-9.8 (n=212)	-0.1 (n=210)
Placebo-adj GM % change (95% CI) at 36 mo	17.7 (2.2, 35.5)		20.4 (7.0, 35.4)		-9.7 (-26.6, 11.2)	
P value*	0.02		0.002		0.34	
End of trial, GM, mg/L (IQR)	1.69 (0.8, 3.6)	1.51 (0.7, 3.2)	1.72 (0.8, 3.6)	1.44 (0.7, 3.1)	1.61 (0.8, 3.4)	1.36 (0.6, 3)
GM % change at EOT	-1.5 (n=895)	-16.6 (n=916)	3.7 (n=1248)	-12.6 (n=1294)	-8.1 (n=455)	-7.1 (n=435)
Placebo-adj GM % change (95% CI) at EOT	18.1 (7.1, 30.2)		18.7 (9.9, 28.3)		-1.0 (-13.9, 13.9)	
P value*	0.0009		0.00001		0.89	
Interaction P value†	0.02					

CI indicates confidence interval; EOT, end of trial; GM, geometric mean; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; and n, number of patients. *P value for dalcetrapib versus placebo differences in log-transformed change in hs-CRP between baseline and time point. †Interaction P value for interaction term of treatment and genotype effects on log-transformed change in hs-CRP between baseline and end of trial.

benefited from a significant reduction in cardiovascular events when treated with dalcetrapib compared with placebo, heterozygous patients (AG genotype) had an intermediate response,

and those with the GG genotype were exposed to an increase in risk.⁶ This is concordant with the increase of 18% in hs-CRP and lack of improvement in cholesterol efflux capacity

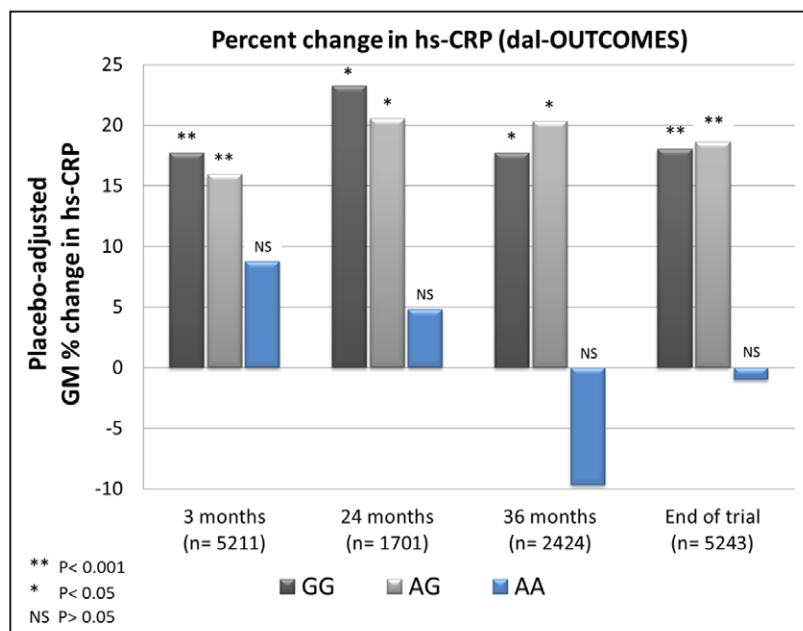


Figure 2. Placebo-adjusted geometric mean percent change from baseline in hs-CRP for the 3-month, 24-month, 36-month, and end of trial hs-CRP measures in dal-OUTCOMES for each genotype of rs1967309 in the *ADCY9* gene. P values for dalcetrapib versus placebo differences in change in log-transformed hs-CRP between baseline and time point are shown. hs-CRP indicates high-sensitivity C-reactive protein.

Table 4. Cholesterol Efflux in dal-PLAQUE-2

	GG		AG		AA	
	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo
N	30	30	42	40	10	19
Baseline, mean (SD)	0.54 (0.11)	0.51 (0.11)	0.55 (0.11)	0.53 (0.11)	0.53 (0.11)	0.53 (0.14)
12 mo, mean (SD)	0.58 (0.11)	0.54 (0.12)	0.61 (0.12)	0.54 (0.12)	0.64 (0.12)	0.54 (0.13)
% change (SD) at 12 mo	7.8 (18.0)	7.4 (10.8)	12.9 (16.9)	1.7 (13.6)	22.3 (22.3)	3.5 (12.3)
<i>P</i> value*	0.93		0.001		0.005	

n indicates number of patients; and SD, standard deviation.

**P* value for dalcetrapib versus placebo differences in percent change in cholesterol efflux between baseline and 12 mo.

of HDL with dalcetrapib when compared with placebo in patients with the GG genotype.

We have shown that dalcetrapib reduced clinical cardiovascular events by 39% compared with placebo and induced carotid atherosclerosis regression in patients with the AA genotype at polymorphism rs1967309.⁶ This contrasts with the failure of torcetrapib and evacetrapib, as well as that of dalcetrapib in GG patients.^{4,6,16} Interestingly, CETP inhibitors have been shown to raise the level of hs-CRP.^{5,9,10} This is surprising given the effects of CETP inhibition on HDL and the known anti-inflammatory properties of these particles.¹¹ Whether this apparently proinflammatory response to CETP inhibitors contributed to their disappointing results was not clear before our study. The fact that only patients with the AA genotype at polymorphism rs1967309 both did not increase their CRP level and benefited from improved cardiovascular outcomes when treated with dalcetrapib suggests that differential effects of CETP inhibitors on the inflammatory status likely contribute to their impact on clinical events. Promotion of inflammation may perhaps counterbalance the benefits on clinical outcomes of the increased cellular cholesterol efflux induced by CETP inhibitors.¹⁷⁻¹⁹

Inflammation is also known to negatively impact HDL functions. The cholesterol efflux assay used in our study measures the effect of the patient's serum (and particularly the HDL particles) on efflux from standard J774 macrophages. The genotype-dependent effects on cholesterol efflux observed, therefore, mean that dalcetrapib affected HDL function according to the A/G alleles at rs1967309. Increased level of serum amyloid A in HDL particles impairs their cholesterol efflux capacity.¹² Furthermore, patients treated for an extracardiac inflammatory disease like rheumatoid arthritis and who experience reductions in hs-CRP also benefit from a concomitant improvement in cholesterol efflux capacity.²⁰ C-reactive protein has been shown to inhibit efflux of cholesterol from macrophages,²¹ whereas the expression of ATP-binding cassette transporters ABCA1 and ABCG1 has also been shown to be decreased by chronic inflammation.²² We have shown in this study clear genotype-dependent effects of dalcetrapib on the inflammatory marker hs-CRP, with increases in patients with the GG and AG genotypes but not in those with the AA genotype at rs1967309. In addition, the placebo-adjusted geometric mean percent change in the concentration of hs-CRP appeared to decrease gradually over time during the follow-up in patients with the AA genotype randomly assigned to receive dalcetrapib. Thus, dalcetrapib resulted in the lack of increase in hs-CRP in patients with

the AA genotype, unlike those with the GG and AG genotypes at rs1967309. Of note, the hs-CRP level at baseline was lower in patients of the placebo group with the AA genotype.

Systolic blood pressure varied slightly (<1 mmHg) between study groups, with a small but not statistically significant increase that appeared to be more consistent at 1 and 3 months in patients with the GG genotype at polymorphism rs1967309 treated with dalcetrapib compared with placebo (Table 6). Hypertension was reported as an adverse event more frequently in the dalcetrapib group than with placebo both in patients with the GG and AG genotypes, but this was reversed (less hypertension-adverse event with dalcetrapib than placebo) in those with the AA genotype. Diarrhea occurred in 5% to 6% of patients in the placebo group, and in 7.3%, 8.4%, and 9.7% of participants with the GG, AG, and AA genotypes, respectively, in the dalcetrapib group. This apparent genotype-dependent increase could be related to the threefold increase in cholesterol efflux, and, perhaps, associated increased lipid excretion, in dalcetrapib-treated patients with the AA genotype (versus the GG genotype). Nevertheless, diarrhea led to treatment discontinuation in 1.0%, 1.6%, and 2.3% of patients with the 3 genotypes in the dalcetrapib group.

ADCY9 is an isoform of the membrane-bound enzyme adenylate cyclase responsible for the formation of cAMP

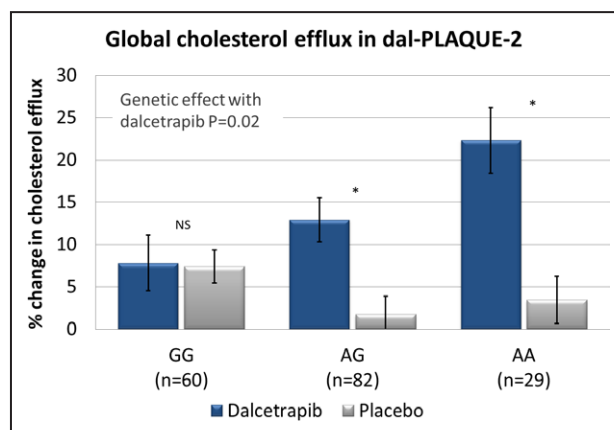


Figure 3. Global cholesterol efflux percent change from baseline to 12 months by treatment arm and for each genotype of rs1967309 in the *ADCY9* gene in the dal-PLAQUE-2 population. Showing mean percent change \pm standard error. * indicates *P* values <0.05 for dalcetrapib versus placebo differences in percent change in cholesterol efflux between baseline and 12 months. NS indicates not significant.

Table 5. Adverse and Serious Adverse Events by Treatment and by rs1967309 Genotype in dal-OUTCOMES

Characteristic	GG		AG		AA	
	Dalcetrapib n=978	Placebo n=1006	Dalcetrapib n=1379	Placebo n=1417	Dalcetrapib n=485	Placebo n=476
Any adverse event, number (%)	860 (88)	859 (85)	1182 (86)	1201 (85)	415 (86)	403 (85)
Leading to discontinuation	63 (6)	42 (4)	85 (6)	61 (4)	35 (7)	20 (4)
Adverse events, number (%)						
Gastrointestinal disorders	295 (30.2)	266 (26.4)	426 (30.9)	355 (25.1)	148 (30.5)	111 (23.3)
Chest pain	126 (12.9)	118 (11.7)	170 (12.3)	167 (11.8)	57 (11.8)	58 (12.2)
Angina pectoris	101 (10.3)	94 (9.3)	109 (7.9)	122 (8.6)	32 (6.6)	35 (7.4)
Hypertension	98 (10.0)	92 (9.1)	127 (9.2)	119 (8.4)	46 (9.5)	49 (10.3)
Nasopharyngitis	65 (6.6)	81 (8.1)	95 (6.9)	105 (7.4)	35 (7.2)	31 (6.5)
Diarrhea	71 (7.3)	61 (6.1)	116 (8.4)	68 (4.8)	47 (9.7)	24 (5.0)
Diarrhea leading to discontinuation	10 (1.0)	2 (0.2)	22 (1.6)	3 (0.2)	11 (2.3)	2 (0.4)
Dizziness	78 (8.0)	82 (8.2)	87 (6.3)	109 (7.7)	32 (6.6)	34 (7.1)
Cough	59 (6.0)	51 (5.1)	74 (5.4)	91 (6.4)	27 (5.6)	28 (5.9)
Fatigue	79 (8.1)	66 (6.6)	113 (8.2)	125 (8.8)	43 (8.9)	32 (6.7)
Dyspnea	62 (6.3)	55 (5.5)	85 (6.2)	95 (6.7)	28 (5.8)	32 (6.7)
Edema peripheral	56 (5.7)	71 (7.1)	66 (4.8)	90 (6.4)	29 (6.0)	33 (6.9)
Back pain	45 (4.6)	53 (5.3)	80 (5.8)	85 (6.0)	23 (4.7)	23 (4.8)
Noncardiac chest pain	60 (6.1)	53 (5.3)	87 (6.3)	94 (6.6)	17 (3.5)	22 (4.6)
Myalgia	47 (4.8)	56 (5.6)	78 (5.7)	77 (5.4)	25 (5.2)	24 (5.0)
Pain in extremity	52 (5.3)	50 (5.0)	64 (4.6)	55 (3.9)	25 (5.2)	25 (5.3)
Arthralgia	40 (4.1)	43 (4.3)	57 (4.1)	61 (4.3)	22 (4.5)	27 (5.7)
Bronchitis	39 (4.0)	35 (3.5)	59 (4.3)	73 (5.2)	34 (7.0)	23 (4.8)
Headache	46 (4.7)	42 (4.2)	65 (4.7)	56 (4.0)	26 (5.4)	15 (3.2)
Urinary tract infection	38 (3.9)	39 (3.9)	41 (3.0)	60 (4.2)	16 (3.3)	13 (2.7)
Influenza	37 (3.8)	52 (5.2)	44 (3.2)	47 (3.3)	17 (3.5)	13 (2.7)
Upper respiratory tract infection	44 (4.5)	46 (4.6)	57 (4.1)	54 (3.8)	22 (4.5)	21 (4.4)
Diabetes mellitus	36 (3.7)	30 (3.0)	46 (3.3)	57 (4.0)	19 (3.9)	12 (2.5)
Serious adverse events, number (%)						
Any serious adverse event	234 (24)	217 (22)	323 (23)	339 (24)	96 (20)	107 (22)
Noncardiac chest pain	19 (1.9)	29 (2.9)	38 (2.8)	38 (2.7)	6 (1.2)	7 (1.5)
Angina pectoris	12 (1.2)	15 (1.5)	24 (1.7)	22 (1.6)	6 (1.2)	6 (1.3)
Chest pain	11 (1.1)	13 (1.3)	16 (1.2)	23 (1.6)	5 (1.0)	7 (1.5)
Atrial fibrillation	13 (1.3)	14 (1.4)	15 (1.1)	11 (0.8)	5 (1.0)	8 (1.7)
Unstable angina	6 (0.6)	4 (0.4)	13 (0.9)	5 (0.4)	0	0

n indicates number of patients.

from ATP.²³ Although ADCY9 is known to be regulated by different factors, including β -adrenergic receptor activation, the underlying mechanism linking that gene with cardiovascular responses to dalcetrapib is not yet clear. Interestingly, ADCY9 gene polymorphisms have been shown to affect individual response to inhaled therapy in patients with asthma, another inflammatory disease.²⁴ The basis for the pharmacogenomic interaction between ADCY9 and dalcetrapib effects is being actively investigated. Whether the

increased cellular cholesterol efflux induced by dalcetrapib could lead to changes in cholesterol content of cellular membranes, altered adrenergic signaling, and modulated production of cAMP²⁵ and whether such changes could be affected by ADCY9 gene polymorphisms is not known. Alternative hypotheses include one centered on the effects of the signaling cascade triggered by ADCY9 activation on cholesterol efflux via ADCY9-dependent cAMP production and PKA activation.^{26,27}

Table 6. Systolic and Diastolic Blood Pressure Changes by Treatment and by rs1967309 Genotype in dal-OUTCOMES

	GG		AG		AA		Genetic Association P Value†	
	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo
Change in systolic BP at 1 mo								
n	972	993	1363	1403	481	473		
Mean (SD)	0.73 (13.00)	-0.09 (13.68)	0.45 (13.20)	0.79 (13.12)	1.27 (14.65)	0.32 (13.54)		
P value*	0.38		0.53		0.47		0.69	0.63
Change in systolic BP at 3 mo								
n	951	989	1337	1385	477	468		
Mean (SD)	0.69 (14.18)	-0.02 (13.99)	1.09 (14.89)	1.23 (14.37)	0.89 (14.71)	1.02 (14.92)		
P value*	0.24		0.86		0.56		0.76	0.21
Change in diastolic BP at 1 mo								
n	972	993	1363	1403	481	473		
Mean (SD)	0.36 (8.41)	0.10 (8.55)	0.53 (8.63)	0.64 (8.21)	0.39 (8.39)	0.55 (8.85)		
P value*	0.54		0.75		0.80		0.87	0.60
Change in diastolic BP at 3 mo								
n	951	989	1337	1385	477	468		
Mean (SD)	0.50 (8.89)	0.23 (8.62)	0.42 (9.28)	1.07 (8.88)	0.53 (9.52)	1.03 (9.11)		
P value*	0.50		0.11		0.54		0.93	0.21

BP indicates blood pressure; n, number of patients; and SD, standard deviation.

*P value from Kruskal–Wallis nonparametric test.

†P value for 1-degree of freedom additive genetic effect of A allele in linear regression model with adjustment for baseline value.

rs1967309 is located in intron 2 of the *ADCY9* gene. A meta-analysis has shown that rs1967309 is a cis-acting expression quantitative trait locus for *ADCY9* in whole blood samples,²⁸ as reported through the use of HaploReg v4.1.²⁹ *ADCY9* has been shown to mediate different functions of immune cells and to modulate inflammatory responses.^{30–32} Thus, the differential mRNA expression of *ADCY9* in white blood cells (most likely accounting for changes in whole blood) supports the genotype-dependent effects on inflammation responses observed in this study. Given the known interplay of inflammation and HDL functions, the above-described *ADCY9* expression data also support the genotype-dependent effects on cholesterol efflux. Other molecular genetic approaches also suggest that rs1967309 is causal for our cholesterol efflux and other observations (unpublished), but further work is ongoing to better understand the mechanisms involved.

In conclusion, genotype-dependent effects on hs-CRP and cholesterol efflux are supportive of dalcetrapib benefits on atherosclerotic cardiovascular outcomes. The large-scale Dal-GenE study (ClinicalTrials.gov identifier NCT02525939) will investigate the efficacy and safety of dalcetrapib selectively in patients with the AA genotype at polymorphism rs1967309 in the *ADCY9* gene.

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CLINICAL PERSPECTIVE

The cholesteryl ester transfer protein inhibitor dalcetrapib effects on cardiovascular outcomes were determined by adenylylase cyclase 9 (*ADCY9*) gene polymorphisms in the pharmacogenomic study (n=5749 patients) of dal-OUTCOMES. In patients with genotype AA at rs1967309 in the *ADCY9* gene, there was a 39% reduction in the composite primary cardiovascular end point with dalcetrapib compared with placebo. Supportive results were obtained in the dal-PLAQUE-2 carotid imaging study. In the current study, we determined whether these clinical outcomes and imaging results are associated with concordant changes in reverse cholesterol transport and inflammation. Treatment with dalcetrapib resulted in placebo-adjusted geometric mean percent increases in high-sensitivity C-reactive protein from baseline to end of trial of 18.1% ($P=0.0009$) and 18.7% ($P=0.00001$) in participants with GG and AG genotypes, respectively, but change was -1.0% ($P=0.89$) in those with the protective AA genotype ($P=0.02$ for treatment arm–genotype interaction). Notably, cholesteryl ester transfer protein inhibitors have been shown to paradoxically increase high-sensitivity C-reactive protein when genotypes are not considered. Although the mean change in cholesterol efflux was similar among study arms in patients with GG genotype (mean: 7.8% and 7.4%), increases were 22.3% and 3.5% with dalcetrapib and placebo for those with AA genotype ($P=0.004$). There was a significant genetic effect for change in efflux for dalcetrapib ($P=0.02$), but not with placebo. Genotype-dependent effects on high-sensitivity C-reactive protein and cholesterol efflux are supportive of dalcetrapib benefits on cardiovascular outcomes in patients with the AA genotype at polymorphism rs1967309. A prospective pharmacogenomics-guided clinical trial is being conducted in these responsive patients to allow regulatory review and provide personalized therapy with dalcetrapib.

Genotype-Dependent Effects of Dalcetrapib on Cholesterol Efflux and Inflammation: Concordance With Clinical Outcomes

Jean-Claude Tardif, David Rhainds, Mathieu Brodeur, Yassamin Feroz Zada, René Fouodjio, Sylvie Provost, Marie Boulé, Sonia Alem, Jean C. Grégoire, Philippe L. L'Allier, Reda Ibrahim, Marie-Claude Guertin, Ian Mongrain, Anders G. Olsson, Gregory G. Schwartz, Eric Rhéaume and Marie-Pierre Dubé

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Comparative demographics of the dal-OUTCOMES substudy to the main study

Variable	dal-OUTCOMES substudy		dal-OUTCOMES main study	
	Placebo N=2699	Dalcetrapib N=2665	Placebo N=7933	Dalcetrapib N=7938
Age - years	60.5 ±9.0	60.5 ±9.0	60.1±9.1	60.3±9.1
Female sex - n (%)	530 (19.6)	554 (20.8)	1497 (18.9)	1573 (19.8)
Race - no (%)				
White	2694 (99.8)	2661 (99.9)	7015 (88.4)	7008 (88.2)
Region of enrollment - n (%)				
North America	1320 (48.9)	1287 (48.3)	2521 (31.8)	2522 (31.8)
Cardiovascular risk factors - n (%)				
Hypercholesterolemia	2111 (78.1)	2069 (77.6)	5753 (72.5)	5736 (72.3)
Hypertension	1844 (68.3)	1799 (67.5)	5419 (68.3)	5336 (67.2)
Diabetes	580 (21.5)	578 (21.7)	1952 (24.6)	1930 (24.3)
Current smoker	549 (20.3)	519 (19.5)	1651 (20.8)	1672 (21.1)
CVD history - n (%)				
PCI	428 (15.9)	414 (15.5)	1150 (14.5)	1159 (14.6)
Previous MI	417 (15.5)	430 (16.1)	1196 (15.1)	1276 (16.1)
CABG	185 (6.9)	162 (6.1)	462 (5.8)	432 (5.4)
Lipids and lipoproteins - mg/dl				
Triglycerides	133.1 ±78.4	133.1 ±73.3	133.0±73.6	134.2±73.6
HDL cholesterol	43.0 ±11.7	43.3 ±11.7	42.2±11.5	42.5±11.7
LDL cholesterol	76.1 ±25.6	76.6 ±25.5	75.8±25.9	76.4±26.4
hs-CRP (mg/L) - median (IQR)	1.5 (0.8, 3.5)	1.5 (0.7, 3.7)	1.5 (0.8, 3.6)	1.5 (0.7, 3.6)

Plus-minus values are means ± standard deviation. CABG: coronary artery bypass graft surgery; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; MI: myocardial infarction; PCI: percutaneous coronary intervention.

Supplemental Table 2. Demographics by rs1967309 genotype - dal-OUTCOMES

Variable	AA		AG		GG	
	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo
	N=463	N=443	N=1285	N=1323	N=917	N=933
Age - years	60.7 (±9.0)	60.8 (±8.9)	60.5 (±9.0)	60.4 (±9.2)	60.4 (±9.1)	60.4 (±8.8)
Female sex - n (%)	101 (21.8)	88 (19.9)	253 (19.7)	251 (19.0)	200 (21.8)	191 (20.5)
Race - n (%)						
White	461 (99.6)	441 (99.6)	1284 (99.9)	1321 (99.9)	916 (99.9)	932 (99.9)
Region of enrollment - n (%)						
North America	205 (44.3)	200 (45.2)	622 (48.4)	644 (48.7)	460 (50.2)	476 (51.0)
Eastern Europe	147 (31.8)	141 (31.8)	313 (24.4)	335 (25.3)	204 (22.3)	202 (21.7)
Western Europe	111 (24.0)	102 (23.0)	350 (27.2)	344 (26.0)	253 (27.6)	255 (27.3)
Cardiovascular risk factors - n (%)						
Hypercholesterolemia	364 (78.6)	341 (77.0)	990 (77.0)	1032 (78.0)	715 (78.0)	738 (79.1)
Hypertension	308 (66.5)	295 (66.6)	864 (67.2)	900 (68.0)	627 (68.4)	649 (69.6)
Diabetes	105 (22.7)	97 (21.9)	271 (21.1)	279 (21.1)	202 (22.0)	204 (21.9)
Current smoker	84 (18.1)	96 (21.7)	259 (20.2)	250 (18.9)	176 (19.2)	203 (21.8)
CVD history - n (%)						
Coronary heart disease	137 (29.6)	120 (27.1)	341 (26.5)	370 (28.0)	242 (26.4)	233 (25.0)
Angina pectoris	83 (17.9)	70 (15.8)	201 (15.6)	234 (17.7)	150 (16.4)	143 (15.3)
PCI	72 (15.6)	71 (16.0)	202 (15.7)	223 (16.9)	140 (15.3)	134 (14.4)
Previous MI	82 (17.7)	66 (14.9)	205 (16.0)	228 (17.2)	143 (15.6)	123 (13.1)
CHF class I	59 (12.7)	63 (14.2)	160 (12.5)	177 (13.4)	136 (14.8)	104 (11.2)
CHF class II	20(4.3)	16(3.6)	40(3.1)	37(2.8)	15(1.6)	27(2.9)
CABG	23 (5.0)	37 (8.3)	70 (5.5)	91 (6.9)	69 (7.5)	57 (6.1)
Stroke	13 (2.8)	9 (2.0)	35 (2.7)	33 (2.5)	25 (2.7)	29 (3.1)
PAD	51 (11.0)	41 (9.3)	98 (7.6)	96 (7.3)	84 (9.2)	64 (6.9)
Index diagnosis - n (%)						
NSTEMI	187 (40.4)	174 (39.3)	553 (43.0)	575 (43.5)	408 (44.5)	413 (44.3)
STEMI	223 (48.2)	217 (49.0)	574 (44.7)	581 (43.9)	390 (42.5)	390 (41.8)

Variable	AA		AG		GG	
	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo
Hospitalization for ACS	48 (10.4)	40 (9.0)	106 (8.2)	132 (10.0)	95 (10.4)	100 (10.7)
Procedural MI	5 (1.1)	12 (2.7)	52 (4.1)	34 (2.6)	24 (2.6)	30 (3.2)
Medication - n (%)						
Aspirin	449 (97.0)	436 (98.4)	1246 (97.0)	1294 (97.9)	881 (96.1)	908 (97.3)
P2Y12 inhibitor	415 (89.6)	407 (91.9)	1162 (90.4)	1192 (90.1)	813 (88.7)	824 (88.3)
Atorvastatin	228 (49.2)	211 (47.6)	570 (44.4)	601 (45.4)	391 (42.6)	435 (46.6)
Other statin	229 (49.5)	224 (50.6)	678 (52.8)	695 (52.5)	499 (54.4)	466 (50.0)
ACE inhibitor	309 (66.7)	300 (67.7)	841 (65.5)	858 (64.9)	601 (65.5)	602 (64.5)
ARB	54 (11.7)	44 (9.9)	183 (14.2)	162 (12.2)	110 (12.0)	115 (12.3)
Beta-blocker	408 (88.1)	403 (91.0)	1161 (90.4)	1196 (90.4)	816 (89.0)	854 (91.5)
eGFR <60 mL/min/1.73m ² - n (%)	44 (9.5)	54 (12.2)	127 (9.9)	126 (9.5)	88 (9.6)	106 (11.4)
Lipids, lipoproteins and apolipoproteins - mg/dL						
Total cholesterol	145.3 (±32.6)	142.4 (±31.4)	146.6 (±32.9)	146.7 (±33.3)	146.7 (±30.3)	145.1 (±33.5)
Triglycerides	133.7 (±69.0)	126.9 (±59.9)	132.5 (±71.7)	133.0 (±79.9)	133.7 (±77.7)	136.2 (±83.7)
HDL cholesterol	42.7 (±11.5)	43.0 (±11.3)	43.2 (±11.7)	43.1 (±11.8)	43.7 (±11.9)	42.8 (±11.8)
LDL cholesterol	76.0 (±26.8)	74.1 (±24.4)	76.9 (±26.2)	77.2 (±26.0)	76.4 (±23.8)	75.4 (±25.7)
Apo B / Apo A1	0.6 (±0.2)	0.6 (±0.2)	0.6 (±0.2)	0.6 (±0.4)	0.6 (±0.2)	0.6 (±0.2)

Plus-minus values are means±SD. ACE: angiotensin converting enzyme; ACS: acute coronary syndrome; ARB: angiotensin receptor blockers; CABG: coronary artery bypass graft surgery; CHD: coronary heart disease; CHF: congestive heart failure; MI: myocardial infarction; NSTEMI: non-ST-elevation MI; PAD: peripheral arterial disease; PCI: percutaneous coronary intervention; P2Y12 inhibitors: anti-platelet agents blocking ADP-induced aggregation; STEMI: ST-elevation MI.

Supplemental Table 3. Demographics by rs1967309 genotype - dal-PLAQUE-2

Variable	AA		AG		GG	
	Dalcetrapib	Placebo	Dalcetrapib	Placebo	Dalcetrapib	Placebo
	N=10	N=19	N=42	N=40	N=30	N=30
Age - years	58.5 (±9.8)	60.9 (±7.9)	62.1 (±7.2)	59.7 (±8.8)	60.6 (±8.8)	59.0 (±8.8)
Female sex - N (%)	4 (40.0)	4 (21.1)	15 (35.7)	9 (22.5)	10 (33.3)	4 (13.3)
Body Mass Index (kg/m ²)	30.5 (±2.7)	30.9 (±7.6)	30.3 (±5.6)	31.0 (±6.8)	28.7 (±5.8)	29.9 (±4.8)
Race - N (%)						
White	9 (90.0)	18 (94.7)	42 (100.0)	40 (100.0)	30 (100.0)	30 (100.0)
Region of enrollment - N (%)						
North America	10 (100.0)	19 (100.0)	42 (100.0)	40 (100.0)	30 (100.0)	30 (100.0)
Cardiovascular risk factors - N (%)						
Hypertension	6 (60.0)	15 (79.0)	23 (54.8)	29 (72.5)	18 (60.0)	23 (76.7)
Current smoker	1 (10.0)	3 (15.8)	8 (19.1)	7 (17.5)	6 (20.0)	5 (16.7)
Lipids - mg/dl						
Total cholesterol	133.3 (±23.7)	141.9 (±24.4)	144.1 (±29.7)	150.2 (±39.3)	147.6 (±27.2)	139.7 (±31.5)
Triglycerides	150.8 (±94.6)	129.2 (±51.7)	138.0 (±80.0)	132.0 (±71.1)	126.8 (±64.8)	106.9 (±55.5)
HDL cholesterol	45.6 (±18.5)	46.2 (±13.5)	45.0 (±12.9)	45.6 (±12.5)	45.1 (±12.3)	47.1 (±13.7)
LDL cholesterol	57.6 (±10.5)	69.8 (±19.2)	71.6 (±21.0)	78.2 (±32.0)	77.1 (±21.6)	71.2 (±22.0)
hs-CRP (mg/L) - median (IQR)	2.6 (1.0, 3.2)	2.1 (0.5, 7.5)	1.7 (0.6, 5.8)	1.2 (0.5, 3.1)	1.2 (0.6, 2.8)	2.5 (1.0, 5.5)

Plus-minus values are mean±standard deviation. N: number of patients; hs-CRP: high-sensitivity C-reactive protein; IQR: interquartile range