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The roles of apo(a) size, phenotype, and dominance pattern in PCSK9-inhibition-induced reduction in Lp(a) with alirocumab^S

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Abstract An elevated level of lipoprotein (a) [Lp(a)] is a risk factor for CVD. Alirocumab, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9, is reported to reduce Lp(a) levels. The relationship of Lp(a) reduction with apo(a) size polymorphism, phenotype, and dominance pattern and LDL cholesterol (LDL-C) reduction was evaluated in a pooled analysis of 155 hypercholesterolemic patients (75 with heterozygous familial hypercholesterolemia) from two clinical trials. Alirocumab significantly reduced total Lp(a) (pooled median: -21% , $P = 0.0001$) and allele-specific apo(a), an Lp(a) level carried by the smaller (median: -18% , $P = 0.002$) or the larger (median: -37% , $P = 0.0005$) apo(a) isoform, at week 8 versus baseline. The percent reduction in Lp(a) level with alirocumab was similar across apo(a) phenotypes (single vs. double bands) and carriers and noncarriers of a small size apo(a) (≤ 22 kringle). The percent reduction in LDL-C correlated significantly with the percent reduction in Lp(a) level ($r = 0.407$, $P < 0.0001$) and allele-specific apo(a) level associated with the smaller ($r = 0.390$, $P < 0.0001$) or larger ($r = 0.270$, $P = 0.0183$) apo(a) sizes. **In conclusion**, alirocumab-induced Lp(a) reduction was independent of apo(a) phenotypes and the presence or absence of a small size apo(a).—Enkhmaa, B., E. Anuurad, W. Zhang, K. Yue, C-S. Li, and L. Berglund. **The roles of apo(a) size, phenotype, and dominance pattern in PCSK9-inhibition-induced reduction in Lp(a) with alirocumab.** *J. Lipid Res.* 2017. 58: 2008–2016.

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An elevated level of lipoprotein (a) [Lp(a)] has been established as an independent causal risk factor for CVD (1–4). Lp(a) contains a lipid core and two different types of apolipoproteins, apoB-100 and apo(a) (5). The apo(a) consists of repeated loop structures, termed as kringles, where copy numbers differ considerably between individuals. More than 40 different sized apo(a)s have been described in humans and, in general, smaller apo(a) sizes with a smaller number of kringle repeats are associated with higher plasma Lp(a) levels (6–9). Individuals with smaller apo(a) isoforms have a 2-fold increased risk for CVD compared with those with larger isoforms (10). Multiple clinical trials have shown that inhibition of proprotein convertase subtilisin/kexin type 9 (PCSK9) with monoclonal antibodies reduces Lp(a) levels (11–16). However, whether reduction of Lp(a) levels depends on apo(a) size, a major predictor of Lp(a) level and associated atherogenicity, is unknown. As the majority of individuals that might benefit from an Lp(a)-lowering intervention are expected to be carriers of atherogenic smaller apo(a) sizes, it is important to assess the effects of PCSK9 inhibitors across the apo(a) size spectrum.

In the current study, we investigated the effects of alirocumab, a monoclonal antibody directed against PCSK9, on Lp(a) level in relation to apo(a) size polymorphism using existing datasets and cohorts of patients with either heterozygous familial hypercholesterolemia (HeFH) or primary hypercholesterolemia (HC) (11, 13). By taking apo(a) molecular properties into account, we were able to explore interactions between Lp(a) and PCSK9 inhibitors in more detail. In a previous study, we demonstrated that the allele-specific apo(a) level (ASL), taking both the genotypic and

Abbreviations: ASL, allele-specific apo(a) level; FCR, fractional catabolic rate; HC, hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; LDL-C, LDL cholesterol; Lp(a), lipoprotein (a); PCSK9, proprotein convertase subtilisin/kexin type 9; PR, production rate; % Ratio, apo(a) protein expression ratio.

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phenotypic characteristics of apo(a) into account, informed coronary artery disease risk assessment (17). Thus, Lp(a)-associated cardiovascular risk in two individuals with similar Lp(a) levels and apo(a) isoform sizes may differ depending on the relative apo(a) allele expression and/or dominance pattern. To assess these issues, we compared the effects of alirocumab on Lp(a) and ASLs in individuals with single versus double expressed apo(a) protein isoforms, as well as across a range of apo(a) isoform dominance patterns.

MATERIALS AND METHODS

Study design and subjects

Details on the study design and cohort have been described previously (11, 13). Briefly, samples from a total of 155 subjects with available specimens for the required time points were analyzed in the current study. The pooled analyses included 80 patients with primary HC (HC study) (13) and 75 patients with HeFH (HeFH study) (11) enrolled in two phase II randomized double-blind placebo-controlled studies. Of the HC patients, 25 patients received placebo with atorvastatin (80 mg) and 55 subjects received 150 mg alirocumab with 10 or 80 mg of atorvastatin every 2 weeks for 8 weeks. HeFH patients were on a stable statin dose with ~77% of patients taking a high dose (maximum dose: simvastatin, 80 mg; atorvastatin, 40 or 80 mg; rosuvastatin, 20 or 40 mg) and 72% ($n = 54$) also taking ezetimibe. Alirocumab was administered every 2 weeks at a dose of 150 mg ($n = 16$) or every 4 weeks at a dose of 150 mg ($n = 15$), 200 mg ($n = 16$), and 300 mg ($n = 14$) for 12 weeks. For the purpose of this study, we analyzed data at two time points, baseline and week 8, for both studies. The current study was approved by the Institutional Review Board at University of California Davis and all study participants provided informed consent at the time of their enrollment in the clinical trials.

Measurement of Lp(a) and LDL cholesterol levels

Plasma Lp(a) levels were measured by rate immunonephelometry, as described (13, 18). LDL cholesterol (LDL-C) levels were calculated by the use of the Friedewald formula (19). In additional analyses, LDL-C levels were corrected for Lp(a) contribution and the level of Lp(a) mass (in milligrams per deciliter) multiplied by 0.3 subtracted from LDL-C values (20–23).

Determination of apo(a) isoform sizes, apo(a) dominance patterns, and ASLs

The apo(a) isoform sizes were analyzed by SDS-agarose gel electrophoresis of plasma samples, followed by immunoblotting (24). For the HeFH study, due to the unavailability of previously unthawed samples at week 8, we used plasma samples from baseline and week 6. ASLs were determined based on computerized scanning of apo(a) protein bands, as previously described (17, 25, 26). Briefly, in subjects with double expressed apo(a) isoforms, protein dominance was determined by optical analysis of the apo(a) protein bands validated by computerized scanning. Each subject was classified as: *a*) larger band dominating; *b*) smaller band dominating; or *c*) neither band dominating (i.e., codominating). As in previous studies, an apo(a) protein band was defined as dominating if it carried $\geq 70\%$ of the total level (17, 25, 27). Two apo(a) proteins were defined as codominating if each band carried $\geq 40\%$, but $\leq 60\%$, of the total Lp(a).

Assessment of the presence of preferential changes in apo(a) expression and ASLs in subjects with double apo(a) isoforms

In order to assess whether changes in total Lp(a) levels were preferentially associated with changes in apo(a) expression levels of a defined apo(a) isoform (larger, smaller, or both), we calculated the apo(a) protein expression ratio (% Ratio) within a given allele pair as [% Ratio = expression level (%) of the larger apo(a) isoform/expression level (%) of the smaller apo(a) isoform] and compared these ratios at baseline versus week 8. In order to robustly detect a difference and to minimize misclassification, we defined an expression change as greater than $\pm 20\%$ in % Ratio. Based on the extent of change in % Ratio from baseline to week 8, we classified changes in apo(a) expression into three patterns: *a*) equally changed or no change group (% Ratio change within $\pm 20\%$); *b*) larger preferentially changed (change in % Ratio greater than -20%); and *c*) smaller preferentially changed (change in % Ratio greater than $+20\%$).

Statistics

All analyses were performed with SAS version 9.4 (SAS Institute Inc., Cary, NC). The two-sided Wilcoxon rank-sum test was used to test two group medians. The Kruskal-Wallis test was used to test three group medians. The Spearman's rank correlation coefficient was used to assess the correlation between two numerical variables, and Fisher's *r*-to-*z* transformation was used to test the difference between two independent correlation coefficients. Fisher's exact test was used to test the association between categorical variables. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline demographic, metabolic, and Lp(a)/apo(a)-related characteristics

Baseline clinical characteristics for all subjects and separately for the HC and HeFH study are shown in supplemental Table S1. A total of 116 patients (75%) received alirocumab and 39 patients (25%) received placebo. There were no significant differences in the distributions of age, gender, ethnicity/race, and lipid levels between the two arms. Analyses for each study across treatment arms revealed similar findings (supplemental Table S1).

In all subjects, the baseline median Lp(a) level did not differ significantly between the placebo (20 mg/dl) and treatment (32 mg/dl) arms (Table 1). The prevalence of a high Lp(a) level (>30 mg/dl) was 41% and 52% in the placebo and treatment arms, respectively. The apo(a) isoform sizes were detected in all subjects, except for one hypercholesterolemic male subject, and the majority of subjects in both placebo (64%) and treatment (67%) groups had double apo(a) protein bands. The median sizes for the larger and smaller apo(a) were similar between the two groups. In all subjects, baseline Lp(a) levels were significantly and inversely correlated with both smaller ($r = -0.553$, $P < 0.0001$) and larger ($r = -0.195$, $P < 0.05$) apo(a) sizes. These associations remained significant at week 8 ($P < 0.05$). The prevalence of small size apo(a) (≤ 22 kringsles) was 26% and 35% in the placebo and treatment arms, respectively. The majority of subjects with double apo(a) protein bands ($n = 103$) had smaller-dominating (i.e., greater expression intensity for the smaller vs. larger allele) (40% and 42%,

TABLE 1. Baseline Lp(a)- and apo(a)-related variables

	All		HC Study ^a		HeFH Study ^b	
	Placebo	Alirocumab	Placebo	Alirocumab ^c	Placebo	Alirocumab ^d
Plasma level	n = 39	n = 115 ^e	n = 25	n = 54 ^e	n = 14	n = 61
Lipoprotein(a) (mg/dl)	20 (2; 299; 71)	32 (1; 308; 69)	12 (2; 132; 43)	22 (1; 172; 51)	34 (2; 299; 119)	43 (2; 308; 99)
ASL, larger (mg/dl)	16 (0.7; 135; 25)	7 (0.1; 109; 17)	13 (2; 53; 22)	7 (0.7; 75; 13)	21 (0.7; 135; 26)	9 (0.1; 109; 26)
ASL, smaller ^f (mg/dl)	11 (0.3; 164; 50)	24 (0.2; 308; 63)	10 (0.3; 123; 23)	15 (0.2; 169; 51)	12 (0.8; 164; 106)	32 (0.8; 308; 69)
Apo(a) expression	n = 39	n = 116	n = 25	n = 55	n = 14	n = 61
Single isoform, n (%)	14 (36)	37 (32)	12 (48)	19 (35)	2 (14)	18 (30)
Double isoforms, n (%)	25 (64)	78 (67)	13 (52)	35 (64)	12 (86)	43 (70)
No isoform, n (%)	0 (0)	1 (1)	0 (0)	1 (2)	0 (0)	0 (0)
Apo(a) dominance	n = 25	n = 78	n = 13	n = 35	n = 12	n = 43
Co-dominating, n (%)	10 (40)	39 (50)	5 (38)	21 (60)	5 (42)	18 (42)
Larger dominating, n (%)	5 (20)	6 (8)	4 (31)	4 (11)	1 (8)	2 (5)
Smaller dominating, n (%)	10 (40)	33 (42)	4 (31)	10 (29)	6 (50)	23 (54)

Data are expressed as median (minimum; maximum; interquartile range) or number (percent).

^aEighty hypercholesterolemic patients on atorvastatin (10 or 80 mg) were evaluated.

^bSeventy-five HeFH patients on stable statin dose with (n = 54) and without ezetimibe (n = 21) were evaluated.

^cAlirocumab was administered every 2 weeks at a dose of 150 mg for 8 weeks.

^dAlirocumab was administered every 2 weeks at a dose of 150 mg (n = 16) or every 4 weeks at a dose of 150 mg (n = 15), 200 mg (n = 16), and 300 mg (n = 14) for 8 weeks.

^eOne person is missing the baseline value; the data is based on n = 115 or n = 54.

^fGiven the high proportion of Whites in the current cohort and the evidence that the vast majority of individuals are heterozygotes for apo(a) alleles, it is highly likely that the apo(a) bands on Western blots are products of the smaller rather than the larger apo(a) allele within a given individual (25, 42). For these reasons, for subjects with a single expressed apo(a) band, it is entered as smaller apo(a) band (kringles) and accounted for the size of smaller apo(a), and thus also for the ASL, smaller.

respectively) or codominating (i.e., similar expression intensity for both larger and smaller alleles) (40% and 50%, respectively) isoforms in both placebo and treatment arms. There were no significant differences in the distribution of baseline apo(a) dominance patterns and ASLs carried by the larger or smaller apo(a) sizes between the placebo and treatment groups (Table 1).

Effect of treatment on total plasma Lp(a) and ASLs

In all subjects, treatment with alirocumab resulted in a significantly greater change (-7 mg/dl vs. -1 mg/dl, respectively, $P = 0.0001$) and percent change (-20.8% vs. -2.7% , respectively, $P = 0.0001$) in Lp(a) level compared with placebo (Table 2). Similarly, reductions in ASLs associated with the larger ($P = 0.0277$) or smaller ($P = 0.0002$) apo(a) sizes were significantly greater in the treatment versus placebo group. In the treated arm, percent changes in ASLs associated with the larger and smaller apo(a) sizes were -37% and -18% , respectively. Furthermore, greater reductions in the Lp(a) level (-19.0 mg/dl vs. -3.0 mg/dl, $P < 0.0001$) and the ASL for larger (-6.8 mg/dl vs. -1.1 mg/dl, $P = 0.0326$) or smaller apo(a) sizes (-12.7 mg/dl vs. -1.8 mg/dl, $P < 0.0001$) were observed in subjects with high (>50 mg/dl) versus low (≤ 50 mg/dl) baseline Lp(a) levels, respectively. Taking advantage of the availability of week 6 samples for the HeFH study, we also examined the relationship between alirocumab-induced Lp(a) reductions at week 6 and week 8. The extent of Lp(a) reductions was similar at these two time points [median Lp(a) change: -12% vs. -13% at week 6 vs. week 8, respectively, $P > 0.05$].

Impact of the presence of single versus double apo(a) bands on change and percent change in total plasma Lp(a) levels

For all subjects within each arm, the change (in milligrams per deciliter) and percent change in Lp(a) level were comparable across single versus double apo(a) phenotypes

(data not shown). This observation was similar when analyzed separately for the two studies. For the HC patients, treatment with alirocumab resulted in a significantly greater reduction in Lp(a) levels compared with placebo, regardless of apo(a) phenotypes. For the HeFH patients, although a greater reduction was seen with alirocumab versus placebo for both apo(a) phenotypes, the difference did not reach statistical significance (data not shown).

Assessment of the presence of preferential changes from baseline in apo(a) expression in subjects with double apo(a) protein bands

Based on the extent of changes from baseline in % Ratio for a given allele-pair, we assessed whether apo(a) expression changes differed across apo(a) allele sizes. There was a significant difference in the distribution pattern of changes between the placebo and treatment arms ($P = 0.023$) (Table 3). While the majority of subjects experienced equal or no changes from baseline in the relative apo(a) expression within a given individual in the treatment arm (63%), a greater proportion of subjects in the treatment arm experienced preferential changes in the larger apo(a) expression compared with the placebo arm (35% vs. 8%). A limited number of subjects experienced preferential changes in the smaller apo(a) expression in both placebo (4%) and treatment (3%) arms. Analyses by each study revealed that these findings were more pronounced in the HC study (Table 3). To further analyze the alirocumab-induced distribution pattern, we focused on alirocumab-responders in the HC study. Subjects with double apo(a) isoforms experiencing any degree of change in Lp(a) level were included in these analyses. Out of 30 alirocumab-responders with double apo(a) isoforms, only two subjects experienced a preferential change in the smaller apo(a) expression, while 17 subjects experienced a preferential change in the larger apo(a) expression (Fig. 1).

TABLE 2. Change and percent change in Lp(a) and ASLs by treatment arms

	All			HC Study			HeFH Study		
	Placebo	Alirocumab	P	Placebo	Alirocumab	P	Placebo	Alirocumab	P
Lp(a) level									
Change (mg/dl)	n = 39 -1 (-25; 37; 7)	n = 115 -7 (-58; 37; 16)	0.0001	n = 25 -1 (-25; 27; 6)	n = 54 -8 (-50; 19; 18)	0.0001	n = 14 -2 (-17; 37; 10)	n = 61 -5 (-58; 37; 11)	0.086
Percent change	n = 39 -3 (-55; 100; 37)	n = 115 -21 (-79; 100; 43)	0.0001	n = 25 -3 (-50; 33; 37)	n = 54 -35 (-79; 14; 35)	<0.0001	n = 14 -3 (-56; 100; 31)	n = 61 -13 (-75; 100; 24)	0.065
ASL, larger									
Change (mg/dl)	n = 25 -0.2 (-12; 14; 4)	n = 77 -2 (-25; 28; 7)	0.0277	n = 13 -2 (-12; 8; 4)	n = 34 -3 (-25; 10; 7)	0.247	n = 12 -0.02 (-8; 14; 4)	n = 43 -1 (-25; 28; 6)	0.043
Percent change	n = 25 -2 (-76; 59; 26)	n = 77 -37 (-91; 100; 54)	0.0005	n = 13 -8 (-76; 38; 33)	n = 34 -57 (-91; 80; 55)	0.0139	n = 12 -0.7 (-61; 59; 20)	n = 43 -35 (-82; 100; 50)	0.008
ASL, smaller									
Change (mg/dl)	n = 39 0 (-15; 23; 5)	n = 114 -3 (-40; 23; 10)	0.0002	n = 25 0 (-13; 19; 4)	n = 53 -4 (-36; 9; 15)	0.0001	n = 14 -1 (-15; 23; 6)	n = 61 -3 (-40; 22; 8)	0.136
Percent change	n = 39 0 (-55; 100; 35)	n = 114 -18 (-78; 318; 36)	0.0020	n = 25 0 (-51; 34; 38)	n = 53 -30 (-78; 318; 30)	0.0011	n = 14 -3 (-55; 100; 23)	n = 61 -11 (-75; 218; 21)	0.168

Data are expressed as median (minimum; maximum; interquartile range).

Impact of the presence of a small size apo(a) (≤ 22 kringle repeats) on change and percent change in Lp(a) and LDL-C levels

Next, we investigated whether alirocumab-induced Lp(a) reduction differed between carriers and noncarriers of small size apo(a). In all patients, a greater reduction in Lp(a) level was seen in small size apo(a) carriers versus noncarriers (-8.5 vs. -3.5 mg/dl, respectively, $P = 0.013$). However, while the absolute levels differed, the corresponding percent change in Lp(a) level, although smaller in carriers versus noncarriers (-19% vs. -26%, respectively), did not differ significantly. Moreover, in all patients, regardless of the presence (or absence) of small size apo(a), alirocumab treatment resulted in a greater change and percent change in Lp(a) level compared with placebo. Similar findings were observed in the HC study. When examining the effects of the presence of small size apo(a) on LDL-C reduction, we did not find any significant difference in LDL-C reduction between carriers and noncarriers of small size apo(a).

Change and the percent change in Lp(a) and ASLs across LDL-C tertiles at week 8

To test whether the LDL-C level after alirocumab treatment was associated with changes in Lp(a) and ASLs, we analyzed data across tertiles of LDL-C at week 8. In all alirocumab-treated patients, distributions of change ($P = 0.0007$) and percent change ($P = 0.0001$) in Lp(a) levels differed significantly across LDL-C tertiles (Fig. 2). Thus, in alirocumab-treated patients, both the change (-8.0 mg/dl vs. -2.0 mg/dl, respectively, $P = 0.0033$) and percent change (-36% vs. -6%, respectively, $P = 0.0001$) in Lp(a) levels were significantly greater in the lowest versus the highest tertile of LDL-C. Similarly, in the alirocumab-treated arm of the pooled set, distributions of change ($P = 0.0006$) and percent change ($P = 0.0003$) for ASLs carried by the smaller apo(a) sizes differed significantly across LDL-C tertiles. Furthermore, a greater change (-4.8 mg/dl vs. -1.0 mg/dl, respectively, $P = 0.0021$) and percent change (-27.9% vs. -3.3%, respectively, $P = 0.0005$) were seen for the ASL carried by the smaller apo(a) sizes in the lowest versus the highest tertile of LDL-C. Similar findings were observed for change in ASLs carried by the larger apo(a) sizes (-3.0 mg/dl vs. -0.7 mg/dl, respectively, $P = 0.0488$).

Correlations between changes in LDL-C, Lp(a), and ASLs

To assess the relationship between reductions in LDL-C and Lp(a) or ASLs in more detail, we estimated Spearman's rank order correlation coefficients. The relationship was also examined after taking the contribution of Lp(a) to LDL-C concentration into account. In the treatment group, both change and percent change in LDL-C were significantly and positively correlated with change and percent change in Lp(a) and ASLs, respectively. The strongest significant correlation was observed for the association between percent reductions in LDL-C and Lp(a) levels ($r = 0.407$; $P < 0.0001$). Further, percent changes in corrected LDL-C levels were significantly and positively associated with percent changes in Lp(a) ($r = 0.308$; $P = 0.0009$) or

TABLE 3. Changes in expression levels of the larger versus smaller apo(a) isoforms in subjects with double protein bands after 8 weeks of treatment

Change in apo(a) Expression	All			HC Study			HeFH Study		
	Placebo (n = 25)	Alirocumab (n = 78)	<i>P</i>	Placebo (n = 13)	Alirocumab (n = 35)	<i>P</i>	Placebo (n = 12)	Alirocumab (n = 43)	<i>P</i>
Equally changed or no change, n (%)	22 (88)	49 (63)	0.023	10 (77)	15 (43)	0.055	12 (100%)	34 (79)	0.181
Larger preferentially changed, n (%)	2 (8)	27 (35)		2 (15)	18 (51)		0 (0%)	9 (21)	
Smaller preferentially changed, n (%)	1 (4)	2 (3)		1 (8)	2 (6)		0 (0%)	0 (0)	

The % ratio for a given allele-pair was calculated as described in the Materials and Methods and was compared between baseline and week 8. In the HeFH study, apo(a) isoforms were determined using plasma samples at week 6. *P* value indicates differences in the distribution pattern of changes across the three apo(a) expression groups between the placebo and treatment arms.

ASLs for smaller apo(a) sizes ($r = 0.318$; $P = 0.0006$). In the HC study, percent change in LDL-C level was significantly correlated with percent change in ASL carried by the larger allele ($r = 0.376$; $P = 0.031$). In this study, change in corrected LDL-C level was significantly and positively correlated with the change in Lp(a) and ASLs for the smaller apo(a) sizes. In the HeFH study, both change and percent change in LDL-C level were significantly correlated with change and percent change in Lp(a) and ASLs carried by the smaller allele. In this study, both change and percent change in Lp(a) or ASLs for smaller apo(a) sizes were significantly and positively correlated with change and percent change, respectively, in the corrected LDL-C levels. Alirocumab-induced percent reductions in LDL-C levels were significantly correlated with LDL-C levels at week 8 in both the HC ($r = 0.952$, $P < 0.0001$) and HeFH ($r = 0.876$, $P < 0.0001$) studies.

Characteristics of patients experiencing no changes in Lp(a) levels

Participants with identical Lp(a) values at baseline and week 8 were considered to be experiencing no changes in Lp(a) levels. Thus, these patients had “0” for both change and percent change entries in the dataset. A total of 20 patients, nine in the HC study and 11 in the HeFH study,

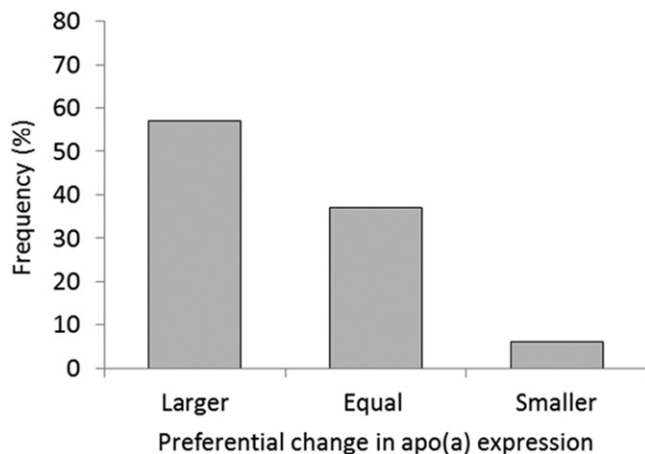


Fig. 1. Frequency of preferential changes in apo(a) expression in alirocumab-responders in the HC study. Based on the extent of change in % Ratio from baseline to week 8, changes in apo(a) expression were classified into three patterns: a) equally changed or no change group (% Ratio change is within $\pm 20\%$); b) larger preferentially changed (change in % Ratio is greater than -20%); and c) smaller preferentially changed (change in % Ratio is greater than $+20\%$). See the Materials and Methods for more information.

experienced no changes in Lp(a) levels in response to alirocumab treatment. Overall, these patients had a low baseline Lp(a) level, with only three HeFH patients having a level greater than 30 mg/dl. The majority of patients had double apo(a) protein isoforms (60% and 90% in the HC and HeFH group, respectively) with a codominating pattern. Alirocumab-induced median change (-63 mg/dl vs. -7 mg/dl) and percent change (-50% vs. -21%) in LDL-C levels were higher than those in the pooled analysis (Table 2). After excluding subjects experiencing no changes in Lp(a) levels with alirocumab, percent changes in LDL-C levels were significantly and positively correlated with percent changes in Lp(a) levels in both the HC ($r = 0.333$, $P = 0.0273$) and HeFH ($r = 0.345$, $P = 0.0142$) studies.

DISCUSSION

This is the first study assessing the role of apo(a) size, allele expression, and isoform dominance pattern during Lp(a) reduction with the PCSK9 inhibitor, alirocumab. While our approach to pool data from two studies among patients with primary HC or HeFH enabled greater statistical power, separate analyses by each parent study provided an opportunity for assessment of the effects within each condition. The major novel findings of the current study are: 1) significant reductions were seen in ASLs carried by both larger and smaller apo(a) sizes; 2) no significant differences in Lp(a) percent reductions were observed between subjects with single versus double apo(a) bands or between carriers and noncarriers of small size apo(a) (≤ 22 krings); 3) a greater Lp(a) reduction in patients with lower LDL-C levels during alirocumab treatment; and 4) significant positive associations between reductions in LDL-C and Lp(a) or ASLs.

In a pooled analysis of 10 clinical trials with bi-weekly administration of evolocumab at a dose of 140 mg, the PCSK9 inhibitor-mediated median percent reduction in Lp(a) levels at week 12 was 24.7% (16). Similarly, a recent pooled analysis of alirocumab efficacy and safety data from eight phase III trials in hypercholesterolemic patients reported 21.7–28% mean reductions in Lp(a) levels with bi-weekly administration of alirocumab [at a dose of 75/150 mg (dose increased to 150 mg at week 12 based on week 8 LDL-C level) and 150 mg] at week 12 (28). The corresponding reductions reached 25–29.1% at week 24. The alirocumab-induced median percent reduction in Lp(a) level in our combined cohort was 21% at week 8. In the

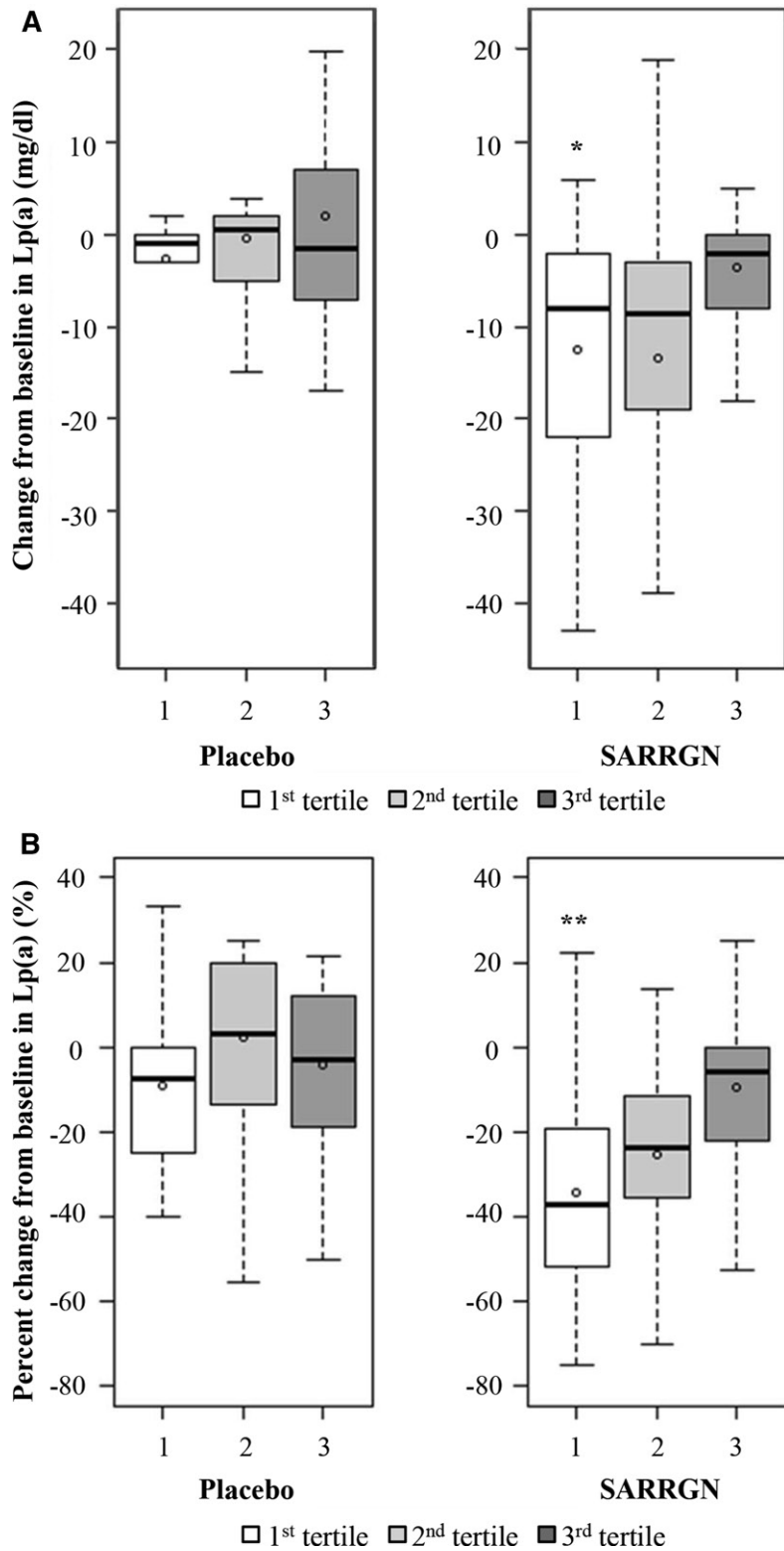


Fig. 2. Change (A) and percent change (B) in Lp(a) level by tertiles of LDL-C level at week 8 in all subjects treated with placebo or alirocumab. Both change and percent change in Lp(a) level was significantly greater in the first (the lowest) versus the third (the highest) tertile of LDL-C level at week 8 in the alirocumab (SARRGN) arm. * $P = 0.0033$ versus third tertile. ** $P = 0.0001$ versus third tertile.

alirocumab-treated arm, the median change (in milligrams per deciliter) in ASL was greater for the smaller versus the larger apo(a) isoform; however, likely due to the inverse association between apo(a) sizes and Lp(a) levels, the corresponding percent change was greater for the larger versus the smaller apo(a) isoform. Supporting this observation, in the alirocumab arm, the baseline median ASL was 3-fold higher for the smaller versus the larger apo(a) isoform.

In an earlier study, no apo(a) protein bands could be detected in 7% of whites (25). In the current study, we were able to detect apo(a) protein band(s) in each individual, except for one hypercholesterolemic subject (~0.6%) with a very low baseline Lp(a) level. The prevalence of individuals with double apo(a) isoforms was substantially higher than noted for Caucasians in other studies and approached that seen in African-Americans (25), perhaps due to a

relatively high baseline median Lp(a) level. Our findings demonstrate that alirocumab decreased Lp(a) levels irrespective of apo(a) phenotypes. Thus, the Lp(a) level was similarly reduced in subjects with single or double expressed protein isoforms. Notably, 35% of the alirocumab-treated patients with double protein isoforms experienced preferential changes in the expression level of the larger apo(a), while only 3% of patients experienced preferential changes in the expression level of the smaller apo(a).

The frequency of subjects carrying at least one small size apo(a) in whites was about 29% (17), whereas the corresponding frequency in our alirocumab-treated patients was 36%. Further, a greater change in Lp(a) level in carriers versus noncarriers of small size apo(a) was observed. This corroborates the fact that smaller apo(a) sizes usually associate with higher levels and that a larger change in level corresponded to a smaller percent change in patients with smaller apo(a) sizes due to their initially elevated levels. In addition, the distribution of apo(a) dominance pattern differed slightly from those in earlier reports (25). We found a higher frequency for codominating (~40–50% vs. 16%) and a lower frequency for larger-dominating (8–20% vs. 28%) patterns relative to those reported (25). Lp(a) nonresponders to alirocumab, in general, had low baseline Lp(a) levels.

Mechanisms by which PCSK9 inhibitors reduce Lp(a) are incompletely understood. Proposed mechanisms include decreased synthesis of apoB and Lp(a), reduced availability of LDL to bind to apo(a), and enhanced Lp(a) uptake and removal by LDL-receptors or other hepatic receptors (16, 29, 30). The role of LDL-receptor-mediated uptake in Lp(a) clearance has been considered minimal (31), supported by the fact that statins have little to no effect on Lp(a) (32–36). Our findings, however, provide indirect support for recent evidence on the role of the LDL-receptor in Lp(a) clearance (16, 29). In the study by Raal et al. (16), Lp(a) cell-association was reduced by coincubation with LDL and PCSK9 and reversed by adding an antibody against PCSK9. In most cases, Lp(a) competes poorly with LDL for LDL-receptor binding and internalization. However, when LDL-receptors become more readily available due to PCSK9 inhibition, LDL-receptor-mediated Lp(a) uptake is increased, particularly in the setting of low circulating LDL-C, leading to Lp(a) reduction (29). In support of this concept, there was a larger Lp(a) percent reduction with alirocumab in the lowest versus the highest tertile of LDL-C. Findings for ASLs, particularly those associated with the smaller apo(a) sizes, were similar. Notably, nonresponders with regard to Lp(a) and with low baseline Lp(a) appeared to experience a larger LDL-C reduction. This finding may support the hypothesis that PCSK9 inhibition has a larger effect on LDL-C level in the setting of a low Lp(a) level.

A recent study using human hepatocytes and fibroblasts provides support for another mechanism by which PCSK9 inhibition reduces Lp(a) levels (37). In this study, Lp(a) cellular uptake occurred in a LDL-receptor-independent manner and neither PCSK9 nor alirocumab altered Lp(a) internalization. In contrast, the secretion of apo(a) from

human hepatocytes was increased by PCSK9, an effect that was reversed by alirocumab. It was proposed that PCSK9 does not significantly modulate Lp(a) catabolism, but rather enhances the secretion of Lp(a) from liver cells. Further, a recent kinetic study examining the individual metabolism of apo(a) and apoB-100 within plasma Lp(a) reported a significantly lower fractional catabolic rate (FCR), as well as a significantly lower production rate (PR), for Lp(a) apo(a) than for Lp(a) apoB-100 (38). Plasma residence time was, accordingly, greater than two times longer for apo(a) than for apoB-100 within Lp(a) in the fed state (38). It cannot be excluded that this possible difference in apo(a) and apoB metabolism in Lp(a) might contribute to the findings; however, additional kinetic studies are needed to establish a more explicit link and to determine any role of this differential metabolic rate in PCSK9 inhibition-induced Lp(a) reduction. More recent work provides further insights into mechanisms underlying Lp(a) and LDL-C lowering with monoclonal antibodies against PCSK9. In healthy humans, evolocumab decreased LDL-C concentration via accelerating its catabolism (39). A recent study by Reyes-Soffer et al. (40) showed that alirocumab decreased LDL-C and LDL-apoB by increasing IDL- and LDL-apoB FCRs and decreasing LDL-apoB PR. The FCR of apo(a) increased by about 25%, while the PR of apo(a) did not change. While these findings might implicate a role of the LDL-receptor in the reduction of plasma Lp(a) levels, further studies are needed to clarify how PCSK9 inhibition lowers Lp(a) levels.

There were significant correlations between alirocumab-induced reductions in LDL-C and Lp(a) levels, as well as for ASLs. After taking the contribution of Lp(a) to LDL-C concentration into account, correlations of percent reductions in corrected LDL-C with percent reductions in Lp(a) or in ASLs for smaller apo(a) sizes remained significant in the alirocumab arm. Similarly, a pooled analysis of evolocumab trials showed a significant correlation between percent reductions in Lp(a) and LDL-C (16). Further, in the latter analysis, patients with lower (≤ 40 mg/dl) LDL-C levels experienced greater Lp(a) percent reduction compared with patients with higher (> 70 mg/dl) LDL-C levels (16). HeFH patients are expected to have a smaller number of functional LDL-receptors to be affected by a PCSK9 inhibitor. In a setting where LDL-C and Lp(a) would compete for a limited number of available LDL-receptors, such as in HeFH, there is less opportunity for Lp(a) uptake, resulting in a lesser degree of reduction.

We recognize some limitations in this study. In the HeFH study, due to the unavailability of appropriate specimens from week 8, we used samples from week 6 for Western blotting; and this apo(a) phenotyping data was combined with Lp(a) data from week 8. As alirocumab-induced reductions in LDL-C level, which significantly correlated with reductions in Lp(a), reached their maximum at week 2 and were maintained consistently throughout the study period (11, 13), we did not anticipate any appreciable difference. Indeed, in additional analyses among HeFH patients, we found a similar Lp(a) reduction at week 6 versus week 8. In addition, Lp(a) levels were

measured with rate immunonephelometry, which could be affected by apo(a) sizes (41), necessitating confirmation by other studies. Furthermore, an overall smaller sample size of the cohort limited our ability to conduct analyses by atorvastatin dose, ezetimibe use, or ethnicity/race. As the current cohort consisted mainly of Caucasians, further studies are needed to explore any impact of apo(a) genetic variability on PCSK9 inhibition in other ethnicities, including African-Americans.

In conclusion, alirocumab-induced Lp(a) reduction was independent of apo(a) phenotypes, as well as the presence or absence of a small size apo(a). Reductions in ASLs were correlated with reductions in LDL-C levels. Clinical studies focused on cardiovascular outcomes with alirocumab may shed insights into the role of Lp(a) reduction in CVD prevention. **56**

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