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Characterization of Autosomal Dominant Hypercholesterolemia Caused by *PCSK9* Gain of Function Mutations and its Specific Treatment with Alirocumab, a PCSK9 Monoclonal Antibody

Running title: *Hopkins et al.; PCSK9 Gain of Function Mutations and Treatment*

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Abstract:

Background - Patients with *PCSK9* gene gain of function (GOF) mutations have a rare form of autosomal dominant hypercholesterolemia. However, data examining their clinical characteristics and geographic distribution are lacking. Furthermore, no randomized treatment study in this population has been reported.

Methods and Results - We compiled clinical characteristics of *PCSK9* GOF mutation carriers in a multinational retrospective, cross-sectional, observational study. We then performed a randomized placebo-phase, double-blind study of alirocumab 150 mg administered subcutaneously every 2 weeks to 13 patients representing four different *PCSK9* GOF mutations with low-density lipoprotein cholesterol (LDL-C) >70 mg/dL on their current lipid-lowering therapies at baseline. Observational study: Among 164 patients, 16 different *PCSK9* GOF mutations distributed throughout the gene were associated with varying severity of untreated LDL-C levels. Coronary artery disease was common (33%; average age of onset 49.4 years) and untreated LDL-C concentrations were higher compared with matched carriers of mutations in the *LDLR* (n=2126) or apolipoprotein B (n=470) genes. Intervention study: In *PCSK9* GOF mutation patients randomly assigned to receive alirocumab, mean percent reduction in LDL-C at 2 weeks was 62.5% ($P<0.0001$) from baseline, 53.7% compared to placebo-treated *PCSK9* GOF mutation patients ($P=0.0009$; primary endpoint). After all subjects received 8 weeks of alirocumab treatment, LDL-C was reduced by 73% from baseline ($P<0.0001$).

Conclusions - *PCSK9* GOF mutation carriers have elevated LDL-C levels and are at high risk for premature cardiovascular disease. Alirocumab, a PCSK9 antibody, markedly lowers LDL-C levels and appears to be well tolerated in these patients.

Clinical Trial Registration - www.clinicaltrials.gov; Unique Identifier: NCT01604824

Key words: hypercapnia; hypercholesterolemia; cardiovascular disease; genetics; PCSK9, clinical trial, alirocumab

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Introduction

Autosomal dominant hypercholesterolemia (ADH), which features high levels of low density lipoprotein cholesterol (LDL-C), is a common monogenic disorder (estimated prevalence 1 in 250–500) that substantially contributes to the worldwide burden of premature cardiovascular disease (CVD).[1,2] Plasma levels of LDL-C are regulated primarily by apolipoprotein B-mediated binding of LDL particles to hepatic LDL receptors (LDLR) followed by cellular internalization and metabolism. Patients with genetic defects in this pathway have high levels of LDL-C and early-onset CVD, as evident in patients with *LDLR* (OMIM #606945) or *APOB* mutations (OMIM #107730) causing familial hypercholesterolemia (FH) and familial defective apolipoprotein B (FDB), respectively.

DNA recombinant mapping in families in France and Utah in which ADH did not cosegregate with markers for *LDLR* or *APOB* identified 1p34 as the responsible locus.[3,4] Shortly thereafter, several gain of function (GOF) mutations in the *PCSK9* gene (OMIM #607786) were identified as a third cause of ADH: Ser127Arg and Phe216Leu in 3 French families,[5] and Asp374Tyr in the Utah family[6] and later in Norwegian and English families.[7,8] Additional *PCSK9* GOF mutations were later identified in several small studies from various geographical locations.[9–12]

Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates serum LDL catabolism by binding and targeting LDLR to lysosomal degradation.[13–17] Thus, increased PCSK9 function leads to reduced hepatic LDLR levels and concomitant high plasma LDL-C levels[13] and vice versa.[18] In several patient populations who cannot achieve target LDL-C levels with currently available lipid-lowering therapies, blockade of PCSK9 with alirocumab, or other human PCSK9 monoclonal antibodies, has demonstrated significant LDL-C reductions.[19–23]

Despite growing awareness that PCSK9 mutations may cause ADH, no global study has been performed that examines and compares the clinical characteristics of the rare patients with different *PCSK9* GOF mutations to each other or to patients with FH and FDB. We report a worldwide comparative compilation of patients known to have varying *PCSK9* GOF mutations so as to describe their physical and laboratory manifestations, prevalence of CVD, and lipid response to therapy. We also report results from the first randomized intervention trial in *PCSK9* GOF mutation patients treated with alirocumab for which we employed a novel randomized placebo-phase study design to enable a double-blinded comparison of alirocumab with placebo (based upon differential onset of effect between study arms) and the opportunity for all subjects to receive active study medication, and contribute to the analysis of safety and efficacy.[24]

Methods

Study Designs

The studies were designed by Regeneron Pharmaceuticals Inc. in collaboration with one of the authors (JD for observational study, PNH for treatment study). The study protocols were approved by the investigational review board at each study center and all subjects in the treatment study provided written informed consent. Data were collected at the study sites by several of the co-authors and were analyzed by representatives of Regeneron Pharmaceuticals Inc.

Comparative Observational Study

We conducted a retrospective global comparative compilation study in which individuals known to have *PCSK9* GOF mutations were categorized so as to associate mutations with lipid profiles, comorbidity, and response to therapy. All of these patients had also been previously characterized for functional mutations in *LDLR,* and *APOB* exons 26 and 29. Data were collected

by supplying the collaborators with a uniform data collection sheet that included untreated and on-treatment lipid profiles; lipid-lowering therapy at the time of treated lipid profiles; presence of xanthoma, xanthelasma, and arcus lipoides corneae; and occurrence and age of onset of CVD.

We compared lipid profiles and other clinical characteristics of patients with *PCSK9* GOF mutations to patients with FH and FDB. For this comparison we selected molecularly proven carriers of pathological *LDLR* or *APOB* mutations from the Dutch Familial Hypercholesterolemia Registry who had untreated lipid levels available.[25,26] Each patient with a *PCSK9* GOF mutation was matched by gender and age (± 2 years) to all available FH and FDB patients Dutch Familial Hypercholesterolemia Registry. This approach yielded a cohort with an average of 3 FDB and 16 FH patients for each *PCSK9* carrier. *LDLR* mutations were characterized as 'defective' (missense, small in-frame indel, synonymous with added splice site) or 'deficient' (large or frame-shifting indel, nonsense, splice site, promoter variant). In comparisons of the effect of different *PCSK9* GOF mutations on LDL cholesterol, we only performed statistical tests for a particular variant when five or more individuals were observed to carry that variant, and we compared that variant to all non-carriers of that particular variant.

Treatment Study

The treatment study was conducted at 3 sites in France and one in Utah. We included men and women age 18–70 with *PSCK9* GOF mutations verified by DNA sequencing and serum LDL-C levels ≥70 mg/dL at screening on a stable lipid-lowering regimen, and considered not at goal by the investigator. Subjects had body mass index 18.0–40.0 kg/m^2 and no cardiovascular event, heart failure, or uncontrolled diabetes within 6 months of enrolment. Patients continued to take their pre-study lipid-lowering therapies throughout the study. Additional enrolment criteria are provided in the Data Supplement.

We utilized a novel double-blind, randomized, placebo-phase design instead of an open-label non-randomized study design in order to enable a double-blinded comparison of alirocumab with placebo (Figure I in the Data Supplement). This study design also provided on-drug treatment data for all subjects in this small group of unique patients.[24] All participants received a single-blind dose of placebo at week 2. After subsequent randomization, group A received alirocumab (150 mg subcutaneously) at weeks 0, 2, 4, 6, and 10 and placebo at weeks 8, 12, and 14; group B received alirocumab at weeks 2, 4, 6, 8, and 12 and placebo at weeks 0, 10, and 14 (Figure I in the Data Supplement). Follow-up visits were conducted at weeks 16, 18, 20, and 22. Accordingly, the number of alirocumab doses was equal in the 2 groups but the dosing schedule for group B was shifted by 2 weeks compared to group A.

The primary endpoint was a comparison in percent change of measured serum LDL-C from pre-treatment to 2 weeks between group A (single alirocumab dose) and group B (placebo). Secondary efficacy endpoints included changes in other lipids at week 2 and changes in lipid measures from baseline to each study visit. Safety assessments included a physical exam, the evaluation of vital signs, electrocardiography, and blood tests. Further details and the schedule of assessments are provided in the Data Supplement.

Statistical Analysis

Comparative Observational Study

For the comparative observational study, we used analysis of variance to assess differences in mean lipoprotein levels between each of the individual *PCSK9* mutations and all other *PCSK9* GOF mutations combined. This methodology was also used to compare lipoprotein levels in all patients with *PCSK9* GOF mutations (without LDLR mutations) combined and patients with FH and FDB. To determine the effect of medication, a paired t-test was performed on lipoprotein

levels before and after treatment.

Treatment Study

Power analysis for the treatment study were based on prior efficacy data and suggested that approximately 6 patients per dose group in this rare patient population would provide at least 80% power to detect a treatment difference of 30% (standard deviation [SD]15%) versus placebo for the primary endpoint at a 5% significance level. Continuous primary and secondary efficacy variables were analyzed using an analysis of covariance (ANCOVA) model with treatment arm as the fixed effect and using the relevant baseline value as a covariate. The rank-based ANCOVA was used for triglycerides and lipoprotein (a) [Lp(a)]. The results for the remaining lipid parameters were also confirmed using a non-parametric method (Kruskal-Wallis). There were no missing data points.

Results

Comparative Observational Study

During 2012, 200 lipid specialty centers around the world were contacted and 164 patients (83 men and 81 women, aged <1–79 years) heterozygous with previously identified *PCSK9* GOF mutations were compiled from 12 centers in 8 countries (Table 1). The patients carried 16 different missense mutations, 6 of which were previously undescribed (Table 1). Individual *PCSK9* GOF mutations generally had restricted geographic distributions and were found in a small number of pedigrees (Figure II in the Data Supplement). Examples include 22 patients with Arg215His found only in 2 pedigrees in Norway, and 12 patients with Val4Ile and 30 patients with Glu32Lys found only in Japan. Obligate carrier founders in the Utah pedigree were migrants from the United Kingdom. For pooled *PSCK9* GOF mutation patients, mean untreated total and LDL-C were 359 and 272 mg/dL, respectively. Eleven patients were double

heterozygotes for mutations in *PCSK9* and *LDLR*; these patients tended to have higher untreated lipids compared to patients with the same GOF mutation alone, as previously reported for the three Glu32Lys double heterozygote patients.[12]

GOF mutations were found in all structural protein domains and 5 of 9 coding exons (Figure 1), and were associated with varying degrees of lipid abnormalities (Table 1). Untreated lipid levels associated with each mutation were compared to the entire *PCSK9* GOF mutation population: Asp374Tyr and Ser127Arg carriers had severe dyslipidemia while Glu32Lys, Arg215His, and Ser465Leu carriers were comparatively mild, although substantial variation was present in patients carrying the same mutation (Figure 1).

The physical stigmata of elevated cholesterol were frequent (Table 1), with prevalence similar to previous reports for FH and FDB (Table I in the Data Supplement). Also similar to FH and FDB,[3,27] 44% of patients had a history of CVD. Coronary artery disease was the most prevalent manifestation (33%) with an average age of onset of 49.4±13.8 years (Table 1 and Table I in the Data Supplement).

In a comparison with FH and FDB patients drawn from the Dutch Hypercholesterolemia Registry, *PCSK9* GOF mutation patients had the highest, and FDB patients the lowest, mean untreated LDL-C levels (Table 2). Among patients with FH, those with deficient mutations had higher untreated LDL-C levels than those with defective mutations (Table 2). Although lipid-lowering therapy (primarily statins, Figure III in the Data Supplement) improved lipid profiles, a substantial proportion failed to achieve guideline LDL cholesterol levels (Figure III in the Data Supplement).

Treatment Study

Six Asp374Tyr mutation carriers were enrolled in Utah, and 4 Ser127Arg, 2 Leu108Arg, and 1

Arg218Ser carriers in France (Figure IV in the Data Supplement). Baseline characteristics of the subjects in groups A and B were mostly similar (Table 3) although some differences are apparent.

Lipid and Lipoprotein Response

In *PCSK9* GOF mutation patients randomly assigned to receive alirocumab, mean percent reduction in LDL-C at 2 weeks was 62.5% ($P<0.0001$) from baseline and 53.7% compared to control *PCSK9* GOF mutation patients treated with placebo for 2 weeks ($P=0.0009$; primary endpoint). Changes in LDL-C levels in response to alirocumab were similar but temporally delayed by 2 weeks in group B compared to group A due to the placebo-phase study design (Figure 2A). After 8 weeks of alirocumab treatment, mean percent change in LDL-C was 73.3% ($P<0.0001$) and 12 of 13 subjects achieved an LDL-C level <70 mg/dL (Table 4). Reductions of LDL-C in the 2 groups were temporally related to reductions of free PCSK9 (Figure 2B). Pooled analysis of 8-week lipid changes in apolipoprotein B, triglycerides, very low-density lipoprotein (VLDL) cholesterol, and Lp(a) were significantly reduced (Table 4). In an exploratory analysis, we examined changes in levels of LDL-C and free PCSK9 from baseline as a function of *PCSK9* GOF genotype. Alirocumab treatment resulted in marked reductions in LDL-C levels from baseline in all patients with all *PCSK9* genotypes (Figure 2C). Potential differences in the rate of LDL-C reduction between the genotypes appeared to correlate with kinetics of free PCSK9 reduction (Figure 2D).

Safety

No patient discontinued early from the study for any reason. The most common treatment-emergent adverse events were infections and included non-serious upper and lower respiratory tract infections and gastroenteritis (Table II in the Data Supplement). No patient experienced an

elevation of hepatic enzymes or creatinine kinase 3-fold above the upper limit of normal; no trends were observed in hepatic enzymes, creatinine kinase, or fasting blood glucose over the course of the study. Five patients experienced one or more fasting blood glucose levels above 126 mg/dL during the course of the trial. All of these patients had a history of abnormal fasting blood glucose or an elevated level at screening. One subject experienced a serious adverse event of chest pain. Evidence for a myocardial infarction was not found and a follow-up stress test did not reveal cardiac ischemia.

Discussion

Gain of function *PCSK9* mutations are a third, rare cause of ADH, but knowledge of the clinical attributes of mutation carriers and their response to therapy have heretofore been limited. In an observational study we characterized the *PCSK9* GOF mutation phenotype. Compared to FH or FDB, these patients had similarly frequent physical stigmata and premature CVD, but higher LDL-C levels. Although we report evidence that these patients respond to available lipid-lowering treatments, most did not attain optimal lipid profiles on their current regimen of statins plus other lipid-lowering therapies, thus establishing the need for additional therapies. We then demonstrated in a clinical intervention trial that patients with four different *PCSK9* GOF mutations achieved a marked additional reduction in LDL-C (up to 73%) after the addition of alirocumab to their current regimen, and nearly all attained the goal of 70 mg/dL. The results of this small, randomized, placebo-phase trial suggest that PCSK9 antibodies may become a specific and effective treatment for *PCSK9* GOF mutation patients.

Our observational study demonstrated that *PCSK9* GOF variants had mostly restricted geographical distributions, were found in a limited number of pedigrees, and exhibited significant phenotypic variability in associated disease severity. While GOF mutations were

found throughout the *PCSK9* coding sequence, our study confirms that carriers of either Asp374Tyr or Ser127Arg mutations had significantly higher untreated LDL-C levels than the other *PCSK9* GOF mutation carriers. This result is not unexpected given that these 2 mutations were among the first to be described, and extends the results from a smaller study suggesting that the Asp374Tyr variant may be associated with a severe form of ADH.[7] However, because most mutations were reported in a limited number of pedigrees, in our comparison of the different variants we are unable to define the portion of the phenotype contributed by background genetics. The geographic isolation of GOF variants suggests they are likely due to private mutations in different populations, and is consistent with a relatively recent origin of many or all of them. As cascade screening[28] was used to enrich for the presence of *PCSK9* mutations, we are unable to obtain a true prevalence of GOF mutations in the general population. However, extensive efforts were undertaken to define the genetic architecture of ADH in Holland and Japan but no overlap in the variants was found, supporting the geographical isolation of these mutations.

Despite variability in disease severity of individual mutations, pooled analyses revealed significantly greater LDL-C levels in *PCSK9* GOF mutation patients compared to patients with FH or FDB. FH patients are found worldwide, and *LDLR* variants causing FH are distributed throughout the gene (over 1700 reported) with greater disease severity associated with individual mutations. In contrast, FDB is also found worldwide, though a single *APOB* variant (Arg3527Gln), found primarily in northern Europeans, is responsible for the vast majority of FDB cases (>95%). For our comparison, we matched the *PCSK9* GOF mutation patients with FH and FDB patients from the Dutch Familial Hypercholesterolemia Registry, the largest such resource in the world. It is possible that these patients have more or less severe disease than

patients from other parts of the world due to genetics or shared environment, and additional comparisons to other large collections of patients will be of interest. However, because this registry includes a large number of patients identified by cascade screening, it may better reflect the phenotype of patients with FH and FDB in a population-based sample than many other registries that consist mostly of index patients and their first-degree relatives. Although relative severity of these patients bear future investigation, it is clear that the severity of the *PCSK9* GOF phenotype warrants maximizing lipid-lowering therapies in these patients.

In our intervention study, alirocumab administration significantly reduced LDL-C levels in all patients enrolled, and this was temporally correlated with free PCSK9 reductions (Figure 2B and 2D). The magnitude of LDL-C reduction was similar to that observed in previous studies of PCSK9 monoclonal antibodies administered to different patient populations. By utilizing a randomized placebo-phase design,[24] each patient contributed to the safety and efficacy data while still enabling comparison of alirocumab administration to placebo. During the 2-week placebo-controlled portion of the trial, alirocumab administration also significantly reduced apolipoprotein B and triglycerides. While some difference in the baseline LDL-C levels and other characteristics was present between groups A and B (not unexpected given the small size and international design of the study), a post-hoc pooled analysis of all subjects after 8 weeks of alirocumab treatment revealed statistically significant reductions of LDL-C, apolipoprotein B, triglycerides, Lp(a), and VLDL cholesterol levels. We conclude that inhibition of PCSK9 in patients with *PCSK9* GOF mutations greatly reduces LDL-C levels. While all mutation carriers responded to treatment, our results suggest that the rate of reduction in LDL-C may differ in patients carrying different GOF mutations, and this may correlate with the rate of free PCSK9 reduction after alirocumab administration, providing interesting future avenues of research into

the biochemical mechanisms of PCSK9.

Our study has limitations. While we endeavored to obtain all available *PCSK9* GOF mutation carriers from a wide selection of lipid research and specialty clinics around the world, we believe that additional *PCSK9* GOF mutations will be found. Furthermore, the collection of clinical information on the *PCSK9* GOF mutation carriers was necessarily limited by the retrospective study design. While additional information regarding other CAD risk factors and course of lipid management would be desirable, such data may best be collected in the setting of prospective follow-up. While we found that carriers of either Asp374Tyr or Ser127Arg mutations had higher LDL cholesterol levels than carriers of other mutations as a whole, our analysis was constrained by available sample size, which may limit the generalizability of our findings. Finally, we note some clinical differences in the two randomized intervention groups, a result not surprising given the relatively small group of mutation carriers included in the intervention trial. While imbalance in baseline factors may have had some unexpected effect on lipid response at 2 weeks (the time for the placebo-controlled primary endpoint determination), the large and essentially universal change from baseline at 8 weeks make an important contribution to responses at 2 weeks less likely.

In conclusion, *PCSK9* GOF mutation is characterized by a high prevalence of premature CVD and higher untreated LDL-C levels than FH and FDB. Intervention in these patients with alirocumab, a monoclonal antibody against PCSK9, was well-tolerated and resulted in marked reductions in LDL-C levels, suggesting PCSK9 antibodies may become an important targeted treatment option for these patients.

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References:

1. Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J.* 2013;34:3478–3490a.

2. Sjouke B, Kusters DM, Kindt I, Besseling J, Defesche JC, Sijbrands EJ, et al. Homozygous autosomal dominant hypercholesterolaemia in the Netherlands: prevalence, genotype-phenotype relationship, and clinical outcome. *Eur Heart J.* 2014;36:560–565.

3. Varret M, Rabes JP, Saint-Jore B, Cenarro A, Marinoni JC, Civeira F, et al. A third major locus for autosomal dominant hypercholesterolemia maps to 1p34.1-p32. *Am J Hum Genet.* 1999;64:1378–1387.

4. Hunt SC, Hopkins PN, Bulka K, McDermott MT, Thorne TL, Wardell BB, et al. Genetic localization to chromosome 1p32 of the third locus for familial hypercholesterolemia in a Utah kindred. *Arterioscler Thromb Vasc Biol*. 2000;20:1089–1093.

5. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet*. 2003;34:154–156.

6. Timms KM, Wagner S, Samuels ME, Forbey K, Goldfine H, Jammulapati S, et al. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. *Hum Genet.* 2004;114:349–353.

7. Leren TP. Mutations in the PCSK9 gene in Norwegian subjects with autosomal dominant hypercholesterolemia. *Clin Genet*. 2004;65:419–422.

8. Sun XM, Eden ER, Tosi I, Neuwirth CK, Wile D, Naoumova RP, et al. Evidence for effect of mutant PCSK9 on apolipoprotein B secretion as the cause of unusually severe dominant hypercholesterolaemia. *Hum Mol Genet*. 2005;14:1161–1169.

9. Allard D, Amsellem S, Abifadel M, Trillard M, Devillers M, Luc G, et al. Novel mutations of the PCSK9 gene cause variable phenotype of autosomal dominant hypercholesterolemia. *Hum Mutat*. 2005;26:497.

10. Homer VM, Marais AD, Charlton F, Laurie AD, Hurndell N, Scott R, et al. Identification and characterization of two non-secreted PCSK9 mutants associated with familial hypercholesterolemia in cohorts from New Zealand and South Africa. *Atherosclerosis*. 2008;196:659–666.

11. Miyake Y, Kimura R, Kokubo Y, Okayama A, Tomoike H, Yamamura T, et al. Genetic variants in PCSK9 in the Japanese population: rare genetic variants in PCSK9 might collectively contribute to plasma LDL cholesterol levels in the general population. *Atherosclerosis*. 2008;196:29–36.

12. Noguchi T, Katsuda S, Kawashiri MA, Tada H, Nohara A, Inazu A, et al. The E32K variant of PCSK9 exacerbates the phenotype of familial hypercholesterolaemia by increasing PCSK9 function and concentration in the circulation. *Atherosclerosis*. 2010;210:166–172.

13. Horton JD, Cohen JC, Hobbs HH. PCSK9: a convertase that coordinates LDL catabolism. *J Lipid Res*. 2009;50 (Suppl):S172–S177.

14. Lo SP, Bottomley MJ, Calzetta A, Settembre EC, Cirillo A, Pandit S, et al. Mechanistic implications for LDL receptor degradation from the PCSK9/LDLR structure at neutral pH. *EMBO Rep*. 2011;12:1300–1305.

15. Lambert G, Sjouke B, Choque B, Kastelein JJ, Hovingh GK. The PCSK9 decade. *J Lipid Res*. 2012;53:2515–2524.

16. Wang Y, Huang Y, Hobbs HH, Cohen JC. Molecular characterization of proprotein convertase subtilisin/kexin type 9-mediated degradation of the LDLR. *J Lipid Res*. 2012;53:1932–1943.

17. Seidah NG, Awan Z, Chretien M, Mbikay M. PCSK9: a key modulator of cardiovascular health. *Circ Res.* 2014;114:1022–1036.

18. Maxwell KN, Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc Natl Acad Sci U S A*. 2004;101:7100–7105.

19. Stein EA, Mellis S, Yancopoulos GD, Stahl N, Logan D, Smith WB, et al. Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. *N Engl J Med*. 2012;366:1108–1118.

20. Stein EA, Gipe D, Bergeron J, Gaudet D, Weiss R, Dufour R, et al. Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolaemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial. *Lancet*. 2012;380:29–36.

21. Giugliano RP, Desai NR, Kohli P, Rogers WJ, Somaratne R, Huang F, et al. Efficacy, safety, and tolerability of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 in combination with a statin in patients with hypercholesterolaemia (LAPLACE-TIMI 57): a randomised, placebo-controlled, dose-ranging, phase 2 study. *Lancet*. 2012;380:2007–2017.

22. Sullivan D, Olsson AG, Scott R, Kim JB, Xue A, Gebski V, et al. Effect of a monoclonal antibody to PCSK9 on low-density lipoprotein cholesterol levels in statin-intolerant patients: the GAUSS randomized trial. *JAMA*. 2012;308:2497–2506.

23. Raal F, Scott R, Somaratne R, Bridges I, Li G, Wasserman SM, et al. Low-density lipoprotein cholesterol-lowering effects of AMG 145, a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease in patients with heterozygous familial hypercholesterolemia: the Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder (RUTHERFORD) randomized trial. *Circulation*. 2012;126:2408–2417.

24. Feldman B, Wang E, Willan A, Szalai JP. The randomized placebo-phase design for clinical trials. *J Clin Epidemiol.* 2001;54:550–557.

25. Fouchier SW, Kastelein JJ, Defesche JC. Update of the molecular basis of familial hypercholesterolemia in The Netherlands. *Hum Mutat*. 2005;26:550–556.

26. Umans-Eckenhausen MA, Defesche JC, Sijbrands EJ, Scheerder RL, Kastelein JJ. Review of first 5 years of screening for familial hypercholesterolaemia in the Netherlands. *Lancet*. 2001;357:165–168.

27. Haddad L, Day IN, Hunt S, Williams RR, Humphries SE, Hopkins PN. Evidence for a third genetic locus causing familial hypercholesterolemia. A non-LDLR, non-APOB kindred. *J Lipid Res*. 1999;40:1113–1122.

28. Leren TP. Cascade genetic screening for familial hypercholesterolemia. *Clin Genet.* 2004;66:483–487.

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Table 1: Summary of Clinical Data of Patients with a Familial GOF Mutation in *PCSK9*

Mutation				Total Cholesterol (mg/dL)	LDL-C (mg/dL)	Number Affected/Number Assessed				
Protein (DNA)	**Exon**	**N**	**Countries (n)**	**Mean±SD (n)**	**Mean±SD (n)**	**CAD**	**Stroke**	**PVD**	**Arcus**	**Xanthoma**
Val4Ile (10G>A)†	1	12	Japan	365.0±103.2 (12)	274.9±99.8 (10)	4/12	0/12	1/12	3/12	10/12
With *LDLR* mutation		8	Japan	386.7±108.3 (8)	309.4±100.5 (6)	4/8	0/8	1/8	2/8	7/8
No *LDLR* mutation		4	Japan	313.6±84.7 (4)	222.7±82.4 (4)	0/4	0/4	0/4	1/4	3/4
Glu32Lys (94G>A)	1	30	Japan	329.1±87.4** (29)	242.1±89.7** (28)	7/30	4/30	0/30	8/29	13/29
With *LDLR* mutation		3	Japan	580.1±69.6 (2)	495.0±119.9 (2)	1/3	0	0/3	2/3	3/3
No *LDLR* mutation		27	Japan	308.2±52.2 (27)	221.2±51.4 (26)	6/27	4/27	0/27	6/26	10/26
Asp35Tyr (103G>A)	1	1	France	300.5 (1)	218.1 (1)	0/1	0/1	0/1	0/1	0/1
Glu48Lys (142G>A)†,‡	1	1	Netherlands	232.8 (1)	164.3 (1)	-/0	-/0	-/0	-/0	-/0
Pro71Leu (212C>T)†	2	6	Netherlands	227.4±33.3 (3)	156.2±35.6 (3)	1/6	2/6	0/6	-/0	-/0
Arg96Cys (286C>T)†	2	3	Netherlands	271.5±46.0 (3)	191.4±34.4 (3)	2/3	0/3	0/3	0/2	0/2
Leu108Arg (323T>G)	2	1	France	365.4 (1)	303.6 (1)	1/1	0/1	0/1	0/1	0/1
Ser127Arg (381T>A)	2	17	France (12) Norway (3) South Africa (2)	419.2±80.4* (16)	368.5±82.4** (9)	2/15	0/14	0/12	4/15	8/15
Asp129Asn (385G>A)†	2	1	United Kingdom	399.1 (1)	321.3 (1)	0/1	0/1	0/1	0/1	0/1
Arg215His (664G>A)	4	22	Norway	287.3±102.9* (20)	163.6±53.8** (11)	4/4	-/0	-/0	-/0	-/0
Phe216Leu (646T>C)‡	4	1	France	263.0 (1)	169.0 (1)	0/1	0/1	0/1	0/1	0/1
Arg218Ser (654A>T)	4	2	France	340.3±87.4 (2)	244.0±68.8 (2)	0/1	0/1	0/1	1/1	1/1
Asp374His (1120G>C)	7	4	France (1) Portugal (3)	408.7±123.4 (4)	372.0±185.2 (2)	3/4	0/4	0/4	1/4	1/4
Asp374Tyr (1120G>T)	7	44	Norway (11) United Kingdom (13) United States (20)	419.6±105.2*** (42)	329.1±102.5*** (35)	13/39	0/10	1/10	3/22	14/22
Ser465Leu (1685C>T)†	9	10	Netherlands	269.9±58.8* (7)	186.8±56.8* (7)	4/10	0/10	0/10	-/0	-/0
Arg496Trp (1777C>T)	9	9	Netherlands	300.5±48.3 (3)	337.6±184.5 (3)	0/9	0/4	0/4	-/0	-/0
All mutations		164	All countries	358.9±107.9 (144)	272.2±109.8 (116)	41/126 (33%)	6/98 (6.1%)	2/96 (2%)	20/89 (22)	47/89 (53%)

CAD indicates coronary artery disease; LDL-C, low-density lipoprotein cholesterol; PVD, peripheral vascular disease; and SD, standard deviation.
**P*<0.05; ***P*<0.01; ****P*<0.001 compared the all other subjects combined.
†Val4Ile, Glu48Lys, Pro71Leu, Arg96Cys, Asp129Asn, Ser465Leu mutations were previously unreported.
‡LDL-C levels for 2 mutations (Glu48Lys and Phe216Leu) are provided on lipid-lowering therapy because either the patient's medication history was unknown or the only data available were on medication. Cholesterol levels refer to untreated values. To convert values for cholesterol to mmol/L, multiply by 0.02586.

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Table 2: Comparison of untreated lipid profiles (means±SD) of heterozygous patients with familial GOF mutation in *PCSK9*, FDB and defective and deficient *LDLR* mutations in FH

			FH by *LDLR* Mutation Class		
	***PCSK9* GOF Mutation (n)**	**FDB (n=470)**	**All FH (n=2126)**	**Defective LDLR (n=1398)**	**Deficient LDLR (n=728)**
Age (years)	36.7±18.6 (135)	32.1±16.9	28.1±16.5	29.2±16.4	26.1±16.5
Total cholesterol (mg/dL)	351.9±104.4 (134)	254.8±50.7***	290.0±82.8***	277.3±74.2***	314.8±92.8
LDL-C (mg/dL)	266.8±108.3 (108)	184.8±43.3***	219.6±76.6***	206.5±67.3***	245.2±86.2
HDL-C (mg/dL)	54.2±27.1 (108)	48.7±16.2	46.4±14.3**	46.8±15.1**	45.2±13.1***
Triglycerides (mg/dL)	150.6±115.1 (108)	111.6±65.5**	121.3±76.2	122.2±77.1*	120.5±75.3

To convert cholesterol to mmol/L, multiply by 0.02586. To convert triglycerides to mmol/L, multiply by 0.01129.
HDL-C indicates high-density lipoprotein cholesterol; FDB, familial defective apolipoprotein B; FH, familial hypercholesterolemia; GOF, gain-of-function; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; and SD, standard deviation.
*P<0.05; **P<0.01; ***P<0.001 when compared to *PCSK9* GOF mutation carriers. The 11 patients who were double heterozygotes for mutations in *PCSK9* and *LDLR* were excluded from the analysis.

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Table 3: Baseline Characteristics of Patients with Familial GOF Mutation in *PCSK9* in the Randomized Alirocumab 150 mg Study

Characteristic	Group A (n=6)	Group B (n=7)
Age (years)	42.3±14.7	46.6±13.3
Race (n)		
White	5	6
Indian Ocean Islander	1	1
Sex (n)		
Male	2	2
Female	4	5
BMI (kg/m^2)	28.5±6.6	30.4±6.7
Glucose (mg/dL)	97.7±10.8	107.6±17.8
Hemoglobin A1c (%)	5.45±0.40	6.14±0.55
Prior history of diabetes mellitus	0	3
Prior history of glucose intolerance	0	1
PCSK9 GOF mutation (n)		
Asp374Tyr	3	3
Ser127Arg	1	3
Leu108Arg	1	1
Arg218Ser	1	0
Lipid-lowering therapy (n)		
Statin	6	7
Ezetimibe	3	3
Niacin	3	2
Fibrate	0	1
Bile acid sequestrant	0	1
History of cardiovascular disease (n)	1	4

Continuous variables are shown as mean ±SD.
BMI indicates body mass index; GOF, gain-of-function; and SD, standard deviation

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Table 4: Lipid Parameters in Patients in the Randomized Study at Baseline, at Week 2, and After 8 weeks of Alirocumab 150 mg Treatment There were 6 Participants Randomized to Group A and 7 Randomized to Group B for 13 Total Combined Participants

	Baseline		Study Week 2			8 Weeks of Alirocumab Treatment
Lipid parameter	**Group A (n=6)**	**Group B (n=7)**	**Group A**	**Group B**	***P*-value**	**Combined (*P*-value)**
LDL-C (measured, mg/dL)	108.8±33.8	144.3±68.4	45.2±42.0	126.3±43.2		32.3±21.4
% change from baseline			-62.5±8.2	-8.8±7.6		-73.3±16.1 (<0.0001)
% difference group A vs B*			-53.7±11.5		0.0009	
HDL-C (mg/dL)	57.2±19.4	50.4±14.7	58.7±22.6	47.0±15.1		55.8±18.0
% change from baseline			1.0±4.6	-6.1±4.3		7.9±13.7 (0.0603)
% difference group A vs B			7.2±6.4		0.2864	
Triglycerides (mg/dL, median [IQR])	84.5 (61.0:112.0)	144.0(66.0:170.0)	55.0 (41.0:76.0)	167.0 (72.0:199.0)		64.0 (42:86)
% change from baseline			-27.9 (-33.3:-6.1)	12.9 (-27.2:29.7)		-37.8 (-46:-27) (0.0002)
% difference group A vs B			-40.8		0.0461	
VLDL-C (measured, mg/dL)	22.8±18.8	28.9±15.0	19.2±20.7	29.3±12.9		14.4±8.1
% change from baseline			-23.8±10.1	6.7±9.3		-39.5±17.5 (<0.0001)
% difference group A vs B			-30.5±13.9		0.0526	
Apo B-100 (mg/dL)	89.2±27.3	101.0±15.8	42.8±43.4	99.4±16.5		32.4±15.3
% change from baseline			-55.3±8.7	-3.8±8.0		-65.0±16.6 (<0.0001)
% difference group A vs B			-49.6±12.1		0.0021	
Lp(a) (mg/dL, median [IQR])	56.6 (34.4:69.1)	19.4 (10.0:56.1)	37.2 (16.6:77.4)	13.0 (9.0:57.6)		11.9 (4:51)
% change from baseline			-21.0	0.0 (-10.0:3.0)		-43.3 (-65:4) (0.0020)
% difference group A vs B			-21.0		0.1317	

For week 2 results, baseline refers to values obtain just prior to the first dosing of alirocumab (group A) or placebo (group B) and week 2 is the patients' nominal week 2 visit. For week 8 results, the t2wo groups are combined and baseline refers to blood drawn just prior to the first dosing of alirocumab; for group A, the baseline remains as before, but for group B, a 2-week shift is adjusted so the nominal week 2 value becomes baseline and the nominal week 10 value becomes the week 8 value.
To convert cholesterol to mmol/L, multiply by 0.02586. To convert triglycerides to mmol/L, multiply by 0.01129. To convert Lp(a) to μmol/L, multiply by 0.0357.
Apo indicates apolipoprotein; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); SD, standard deviation; SE, standard error; and VLDL-C, very low-density lipoprotein cholesterol.
*Primary endpoint. All lipid values are shown as mg/dL. Mean ±SD are given for lipid parameters at baseline, 2 weeks, and 8 weeks as well as percent changes from baseline. Least-square means ±SE are given for differences between group A and B at 2 weeks. Significance of 8-week changes from baseline were tested with 2-sided paired t-tests. Median % change from baseline and IQR (Q1:Q3) are shown for triglycerides and Lp(a).

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Figure Legends:

Figure 1: Distribution of untreated LDL-C for patients with familial gain-of-function mutations in *PCSK9* without *LDLR* mutations (**A**), and position of the mutations and the 12 exons of the *PCSK9* gene relative to the protein domains (**B**)

†*P*-value indicates reduction for mutation versus overall mean.

‡*P*-value indicates increase for mutation versus overall mean. Dotted line represents mean LDL-C level of all *PCSK9* mutation carriers from whom untreated LDL-C levels were available.

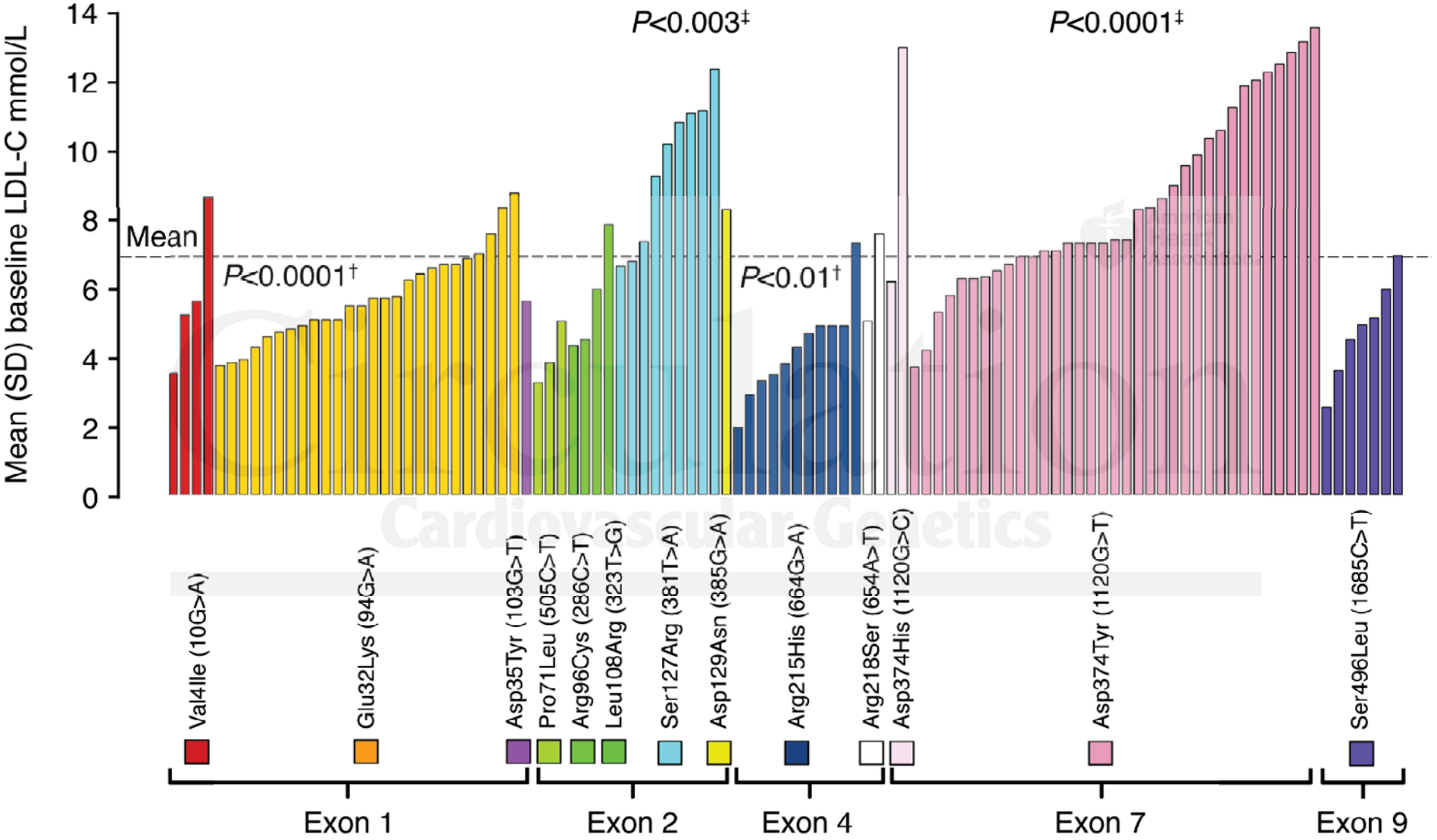
1.1 mmol/L = 70 mg/dL; 2.59 mmol/L = 100 mg/dL

LDL-C indicates low-density lipoprotein cholesterol; SD, standard deviation.

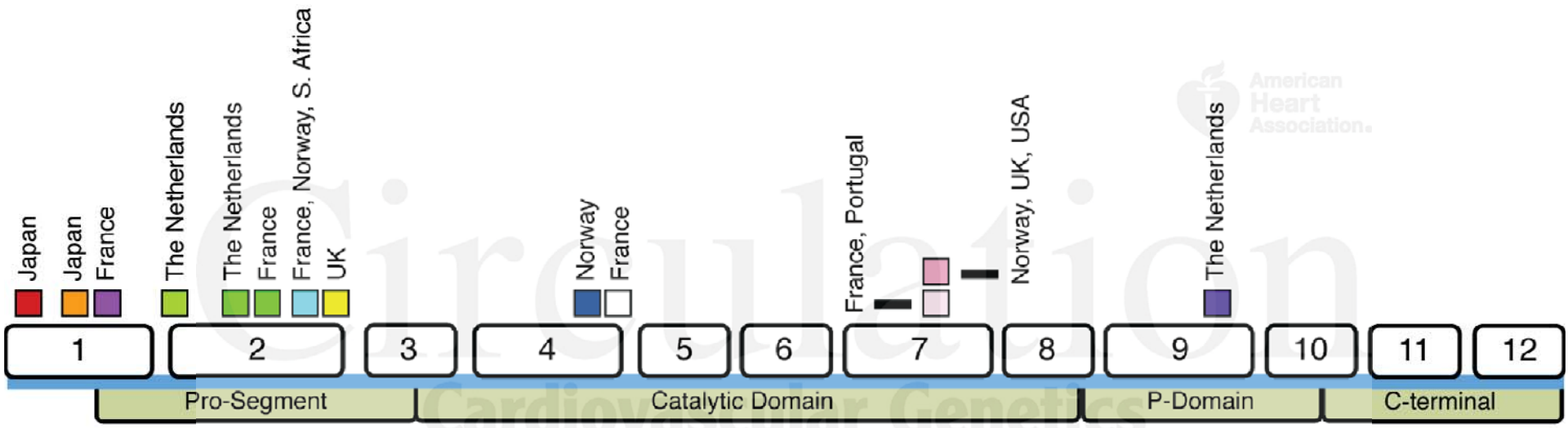
Figure 2: Change in LDL-C and free PCSK9 for patients with familial GOF mutation in *PCSK9* in the randomized alirocumab study. (**A**) Mean (±SE) LDL-C values and (**B**) mean (±SE) percent change from baseline in free plasma PCSK9 are shown by study group together with an indication of the dosing schedules. (**C**) Mean (+SE) percent change from baseline in LDL-C and (**D**) free plasma PCSK9 are shown by *PCSK9* gain of function mutation. In panels (C) and (D), results from groups A and B were combined by shifting group A visits forward 2 weeks, thereby aligning the dosing schedule in the 2 groups.

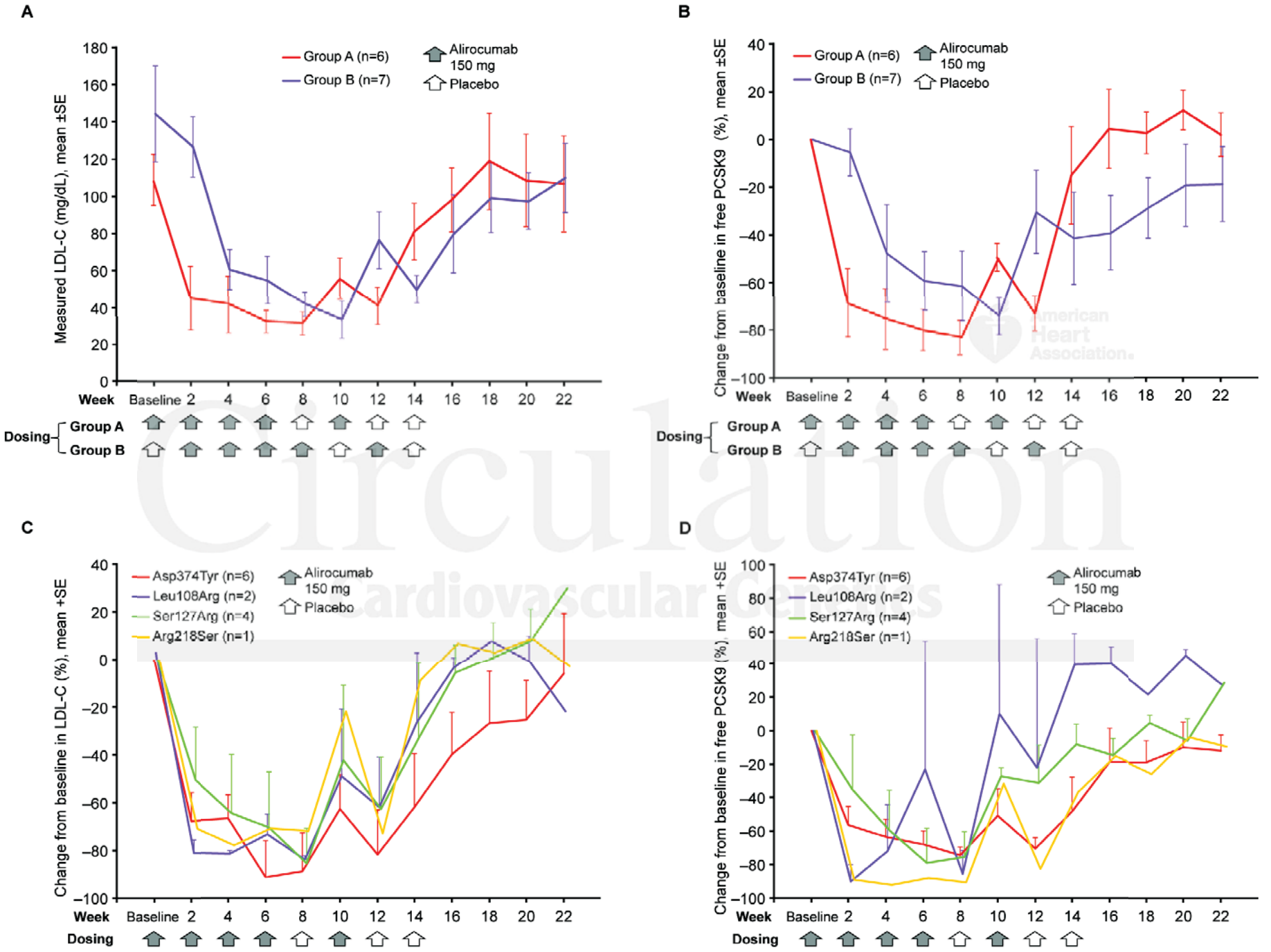
LDL-C indicates low-density lipoprotein cholesterol; and SE, standard error.

A


Mean (SD) baseline LDL-C mmol/L
14
12
10
8
6
4
2
0
Mean
P<0.0001†
P<0.003‡
P<0.01†
P<0.0001‡
Val4Ile (10G>A)
Glu32Lys (94G>A)
Asp35Tyr (103G>T)
Pro71Leu (505C>T)
Arg96Cys (286C>T)
Leu108Arg (323T>G)
Ser127Arg (381T>A)
Asp129Asn (385G>A)
Arg215His (664G>A)
Arg218Ser (654A>T)
Asp374His (1120G>C)
Asp374Tyr (1120G>T)
Ser496Leu (1685C>T)
Exon 1
Exon 2
Exon 4
Exon 7
Exon 9

B




A
Measured LDL-C (mg/dL), mean ±SE
Group A (n=6)
Group B (n=7)
Alirocumab 150 mg
Placebo
Week
Baseline 2 4 6 8 10 12 14 16 18 20 22
Dosing
Group A
Group B
B
Change from baseline in free PCSK9 (%), mean ±SE
Group A (n=6)
Group B (n=7)
Alirocumab 150 mg
Placebo
Week
Baseline 2 4 6 8 10 12 14 16 18 20 22
Dosing
Group A
Group B
C
Change from baseline in LDL-C (%), mean +SE
Asp374Tyr (n=6)
Leu108Arg (n=2)
Ser127Arg (n=4)
Arg218Ser (n=1)
Alirocumab 150 mg
Placebo
Week
Baseline 2 4 6 8 10 12 14 16 18 20 22
Dosing
D
Change from baseline in free PCSK9 (%), mean +SE
Asp374Tyr (n=6)
Leu108Arg (n=2)
Ser127Arg (n=4)
Arg218Ser (n=1)
Alirocumab 150 mg
Placebo
Week
Baseline 2 4 6 8 10 12 14 16 18 20 22
Dosing