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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

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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 4 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 with placebo-treated *PCSK9* GOF mutation patients (P=0.0009; primary end point). 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 of premature cardiovascular disease. Alirocumab, a PCSK9 antibody, markedly lowers LDL-C levels and seems to be well tolerated in these patients.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique Identifier: NCT01604824.

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Key Words: alirocumab ■ cardiovascular diseases ■ clinical trial ■ genetics ■ hypercholesterolemia ■ PCSK9 protein, human

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

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apolipoprotein B–mediated binding of LDL particles to hepatic LDL receptors (LDLRs) 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.

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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] 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 used a novel randomized placebo-phase study design to enable a double-blinded comparison of alirocumab with placebo (based on 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 (J.D. for observational study and P.N.H. 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 coauthors 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; the 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 sex and age (±2 years) to all available FH and FDB patients from the 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-C, we only performed statistical tests for a particular variant when ≥5 individuals were observed to carry that variant, and we compared that variant with all noncarriers of that particular variant.

Treatment Study

The treatment study was conducted at 3 sites in France and 1 in Utah. We included men and women age aged 18 to 70 years with *PCSK9* 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 of 18.0 to 40.0 kg/m^2 and no cardiovascular event, heart failure, or uncontrolled diabetes within 6 months of enrollment. Patients continued to take their prestudy lipid-lowering therapies throughout the study. Additional enrollment criteria are provided in the Data Supplement.

We used a novel double-blind, randomized, placebo-phase design instead of an open-label nonrandomized study design 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 SC) 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 with group A.

The primary end point was a comparison in percent change of measured serum LDL-C from pretreatment to 2 weeks between group A (single alirocumab dose) and group B (placebo). Secondary efficacy end points included changes in other lipids at week 2 and changes in lipid measures from baseline to each study visit. Safety assessments included a physical examination, 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 ANOVA 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 previous efficacy data and suggested that ≈6 patients per dose group in this rare patient population would provide at least 80% power to detect a treatment difference of 30% (SD, 15%) versus placebo for the primary end point at a 5% significance level. Continuous primary and secondary efficacy variables were analyzed using 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 nonparametric 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 to 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 *PCSK9* 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 with patients with the same GOF mutation alone, as previously reported for the 3 Glu32Lys double-heterozygote patients.[12]

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; and PVD, peripheral vascular disease.

*Val4Ile, Glu48Lys, Pro71Leu, Arg96Cys, Asp129Asn, and 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.

‡$P<0.01$;

§$P<0.05$;

‖$P<0.001$ compared with the all other subjects combined.

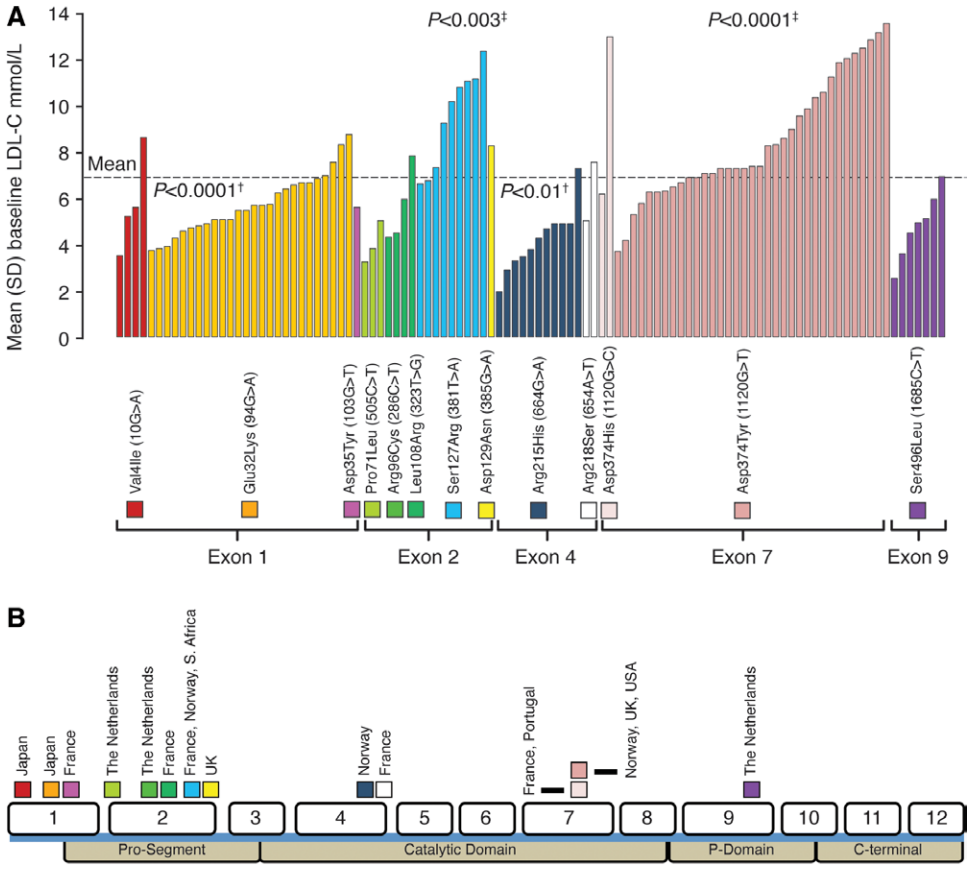

Figure 1. Distribution of untreated low-density lipoprotein cholesterol (LDL-C) for patients with familial GOF mutations in *PCSK9* without *LDLR* mutations (**A**) and position of the mutations and the 12 exons of the *PCSK9* gene relative to the protein domains (**B**). †*P* value indicates reduction 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. 1.81 mmol/L=70 mg/dL; 2.59 mmol/L=100 mg/dL.

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 with the entire *PCSK9* GOF mutation population: Asp374Tyr and Ser127Arg carriers had severe dyslipidemia, whereas 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; 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-C 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 with control *PCSK9* GOF mutation patients treated with placebo for 2 weeks (*P*=0.0009; primary end point). Changes in LDL-C levels in response to alirocumab were similar but temporally delayed by 2 weeks in group B compared with group A because of 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 of <70 mg/dL (Table 4). Reductions of LDL-C in the 2 groups were temporally related to reductions of free PCSK9 (Figure 2B). In a pooled analysis of 8-week lipid changes apolipoprotein B, triglycerides, very low-density lipoprotein cholesterol, and Lp(a) were significantly reduced (Table 4). In an exploratory analysis, we

Table 2. Comparison of Untreated Lipid Profiles (Mean±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, y	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; and LDLR, low-density lipoprotein receptor.

*$P<0.001$;

†$P<0.01$;

‡$P<0.05$ when compared with *PCSK9* GOF mutation carriers. The 11 patients who were double heterozygotes for mutations in *PCSK9* and *LDLR* were excluded from the analysis.

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 seemed to correlate with kinetics of free PCSK9 reduction (Figure 2D).

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, y	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
Previous history of diabetes mellitus	0	3
Previous 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; and GOF, gain of function.

Safety

No patient discontinued early from the study for any reason. The most common treatment-emergent adverse events were infections and included nonserious 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 ≥1 fasting blood glucose levels >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 myocardial infarction was not found, and a follow-up stress test did not reveal cardiac ischemia.

Discussion

PCSK9 GOF 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 with 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 4 different *PCSK9* GOF mutations achieved a marked additional reduction in LDL-C (≤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. Although GOF mutations were found throughout the *PCSK9* coding sequence, our study confirms that carriers of

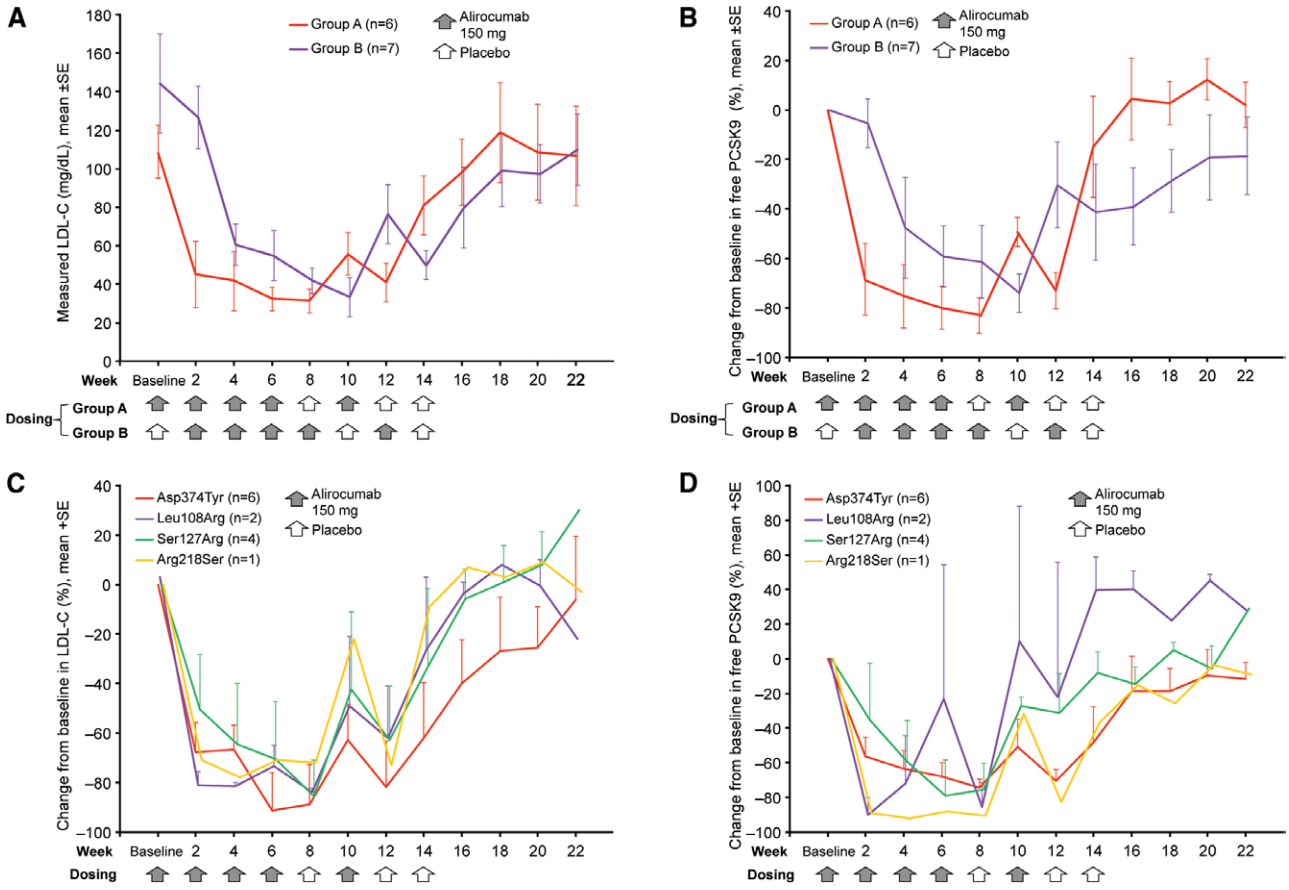

Figure 2. Change in low-density lipoprotein cholesterol (LDL-C) and free PCSK9 for patients with familial gain of function mutation in *PCSK9* in the randomized alirocumab study. (**A**) Mean (±SE) LDL-C values and (**B**) mean (±SE) percent change from baseline in free plasma PCSK9 are shown by study group together with an indication of the dosing schedules. Mean (+SE) percent change from baseline in LDL-C (**C**) and free plasma PCSK9 (**D**) are shown by *PCSK9* GOF mutation. **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.

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 that 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 with patients with FH or FDB. FH patients are found worldwide, and *LDLR* variants causing FH are distributed throughout the gene (>1700 reported) with greater disease severity associated with individual mutations. In contrast, FDB is also found worldwide, although 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 because of genetics or shared environment, and additional comparisons with 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 using a randomized placebo-phase design,[24] each

Table 4. Lipid Parameters in Patients in the Randomized Study at Baseline, at Week 2, and After 8 weeks of Alirocumab 150 mg Treatment

	Baseline		Study Week 2			8 wk 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 to 112.0)	144.0 (66.0 to 170.0)	55.0 (41.0 to 76.0)	167.0 (72.0 to 199.0)		64.0 (42 to 86)
% change from baseline			−27.9 (−33.3 to 6.1)	12.9 (−27.2 to 29.7)		−37.8 (−46 to 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 to 69.1)	19.4 (10.0 to 56.1)	37.2 (16.6 to 77.4)	13.0 (9.0 to 57.6)		11.9 (4 to 51)
% change from baseline			−21.0	0.0 (−10.0 to 3.0)		−43.3 (−65 to 4) (0.0020)
% difference group A vs B			−21.0		0.1317	

There were 6 participants randomized to group A and 7 randomized to group B for 13 total combined participants.

For week-2 results, baseline refers to values obtained just before 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 2 groups are combined, and baseline refers to blood drawn just before 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. HDL-C indicates high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); and VLDL-C, very low-density lipoprotein cholesterol.

*Primary end point. All lipid values are shown as mg/dL. Mean±SD values are given for lipid parameters at baseline, 2 wk, and 8 wk, as well as percent changes from baseline. Least-square mean±SE values are given for differences between groups A and B at 2 weeks. Significance of 8-week changes from baseline was tested with 2-sided paired *t* tests. Median % change from baseline and IQR (Q1:Q3) is shown for triglycerides and Lp(a).

patient contributed to the safety and efficacy data while still enabling the 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. Although 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 very low-density lipoprotein cholesterol levels. We conclude that inhibition of PCSK9 in patients with *PCSK9* GOF mutations greatly reduces LDL-C levels. Although 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. Although we endeavored to obtain all available *PCSK9* GOF mutation carriers from a wide selection of lipid research and specialty clinics around the world, we think 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. Although additional information on other coronary artery disease risk factors and course of lipid management would be desirable, such data may best be collected in the setting of prospective follow-up. Although we found that carriers of either Asp374Tyr or Ser127Arg mutations

had higher LDL-C 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 between the 2 randomized intervention groups, a result not surprising given the relatively small number of mutation carriers included in the intervention trial. Although imbalance in baseline factors may have had some unexpected effect on lipid response at 2 weeks (the time for the placebo-controlled primary end point determination), the large and essentially universal change from baseline at 8 weeks makes an important contribution to responses at 2 weeks less likely.

In conclusion, patients with *PCSK9* GOF mutations are 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 that PCSK9 antibodies may become an important targeted treatment option for these patients.

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CLINICAL PERSPECTIVE

Gain of mutation mutations in the PCSK9 gene lead to a rare form of autosomal dominant hypercholesterolemia. However, clinical data on this condition are scarce. Our article presents the results of 2 studies. Firstly, we identified 16 different PCSK9 gain of mutation mutations from 164 patients in a multinational observational study and compared them with age- and sex-matched patients with familial hypercholesterolemia and familial defective apolipoprotein B from the Dutch Registry. The PCSK9 mutations were associated with varying degrees of low-density lipoprotein cholesterol (LDL-C) elevations, but mean untreated LDL-C levels were higher compared with those seen in patients with familial hypercholesterolemia caused by *LDLR* or *APOB* mutations. Overall, 44% of patients had cardiovascular disease, and most still presented with elevated LDL-C even after treatment with statins plus other lipid-lowering drugs. Hence, we assessed that additional treatment for these patients would be beneficial. In a second study, we subcutaneously administered the anti-PCSK9 monoclonal antibody alirocumab 150 mg 5× every 2 or every 4 weeks to patients with 4 different PCSK9 gain of mutation mutations. The randomized placebo-phase study design maximized the safety and efficacy data that could be obtained from a study with only 13 subjects. Treatment with alirocumab was well tolerated and resulted in LDL-C reductions of ≤73%. Our results suggest that anti-PCSK9 antibodies may become a targeted and effective additional treatment for these patients.