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Original Research Article

# Unusual responses to PCSK9 inhibitors in a clinical cohort utilizing a structured follow-up protocol



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# ARTICLE INFO

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#### ABSTRACT

Objective: To characterize unusual responses to PCSK9 inhibitor (PCSK9i) therapy in a real-world setting, given their extremely low prevalence in clinical trials. Methods: A retrospective study of patients seen in a structured academic PCSK9i clinic who had LDL-C measurements before and after initiation of PCSK9i (up to 12 months). Unusual response was defined as: (1) no response: no changes in LDL-C level at all time points; (2) delayed response: <30% LDL-C reduction by the third dose, but achieving this threshold at a later time; (3) reduced response: <30% LDL-C reduction at all time points; and (4) lost response:  $\geq$  30% LDL-C reduction by the third dose, but displaying < 30% reduction at a later time. Results: Of the 411 patients meeting inclusion criteria, 54 were initially classified as unusual responders. After excluding those not adherent to prescribed interventions, 31 patients (7.5%) were classified as true unusual responders. These included: 2 with no response, 12 with delayed response, 3 with reduced response, 6 with delayed or reduced response, 4 with lost response, and 4 with delayed and lost response. Response to PCSK9i therapy at all time points revealed higher on-treatment LDL-C values (94-100 vs. 47-51 mg/dL, p < 0.001) and lower degree of percent reduction in LDL-C (23.3–34% vs. 61.1–64.5%, p < 0.001) in the unusual versus usual responders. Lipoprotein (a) (Lp[a]) values were consistently higher in the unusual responders (81-92.5 vs. 28.5-52 mg/dL, p < 0.01). Fold change in post-versus pre-treatment PCSK9 plasma results was similar between the two cohorts (p >0.05), suggesting that unusual responses were not due to insufficient plasma PCSK9 blockade. Multiple logistic regression analysis identified clinical FH (OR 2.9, 95% CI 1.27-7.24) and no ezetimibe therapy (OR 0.334, 95% CI 0.150-0.728) as factors related to true unusual response. Conclusions: Unusual responses to PCSK9i in a clinical cohort are more common than reported in clinical trials. Of the suspected unusual responders, nearly half were the result of adherence issues, and thus careful medication

the suspected unusual responders, nearly half were the result of adherence issues, and thus careful medication reconciliation should be the first step in diagnosing an unusual response.

#### Introduction

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) inhibitors (PCSK9i) are powerful low-density lipoprotein-cholesterol (LDL-C) lowering agents that have revolutionized our ability to address residual cardiovascular (CV) risk [1]. The randomized controlled trials (RCT) that paved the way for Food and Drug Administration (FDA) approval in patients with established atherosclerotic cardiovascular disease (ASCVD) and/or familial hypercholesterolemia (FH) demonstrated consistent LDL-C reductions in the order of 50–60% [2–16]. A sub-analysis of the FOURIER trial showed that 90% of subjects experienced at least 50% LDL-C lowering with evolocumab [17]. Similarly, in the ODYSSEY trials, 98.9% of patients displayed at least 15% LDL-C reduction with alirocumab [18]. Thus, the overwhelming majority of subjects are expected to experience substantial and consistent LDL-C reductions (usual responders).

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Factors to consider when evaluating therapeutic response to pharmacotherapy entail timing, magnitude, and consistency of the effect. When considering the pharmacodynamic response to PCSK9i, peak LDL-C lowering manifests within 7 days after the first dose, with steady state reached after 2-3 doses [19,20]. Therefore, maximal LDL-C lowering capacity should be realized by the third dose, or one month after PCSK9i initiation, a common time point for first assessment of response to PCSK9i therapy. If nearly all subjects on PCSK9i demonstrate LDL-C reductions of 50-60%, what causes, and how does one define, a suboptimal response? The term hypo-responsiveness has previously been used to describe <15% LDL-C reduction [18] based on the general minimum LDL-C reduction required for drug approval by the FDA [21]. However, this is a historical definition based on the efficacy of older lipid-lowering therapies with modest LDL-C reducing capabilities. Based on the work by Qamar et al. [17], a more appropriate definition of hypo-responsiveness to PCSK9i therapy is achievement of <30% LDL-C reduction, an event that occurs infrequently and lies beyond two standard deviations of the mean for expected LDL-C reduction. Results from the large CV outcome trials using PCSK9i [15,16] firmly demonstrated sustainable LDL-C lowering throughout the duration of the trials. Assuming no changes to PCSK9i or background lipid-lowering therapy, loss of response therefore would be defined as transitioning from a standard response (50-60% LDL-C reduction from baseline) to hypo-responsiveness (<30% LDL-C reduction from baseline).

When referring to those with an "unusual" PCSK9i response, data from prior studies [17,18] have shown this to be a rare event in the RCT setting. However, these studies solely focused on the degree of LDL-C reduction and did not identify types of unusual response or delve into, and suggest, possible biologic causes. The objective of this study was to characterize the full spectrum of unusual responses to PCSK9i therapy, to determine its prevalence in a real world setting, and to identify potential biologic causes that explain this rare outcome and should enhance our understanding of cholesterol homeostasis.

#### Methods

This was a retrospective study of patients receiving medical care at the Center for Preventive Cardiology of Oregon Health & Science University (OHSU) between July 2015 and February 2020. One of the patients received care at the Joslin Diabetes Center. This study was conducted under the approval of our Institutional Review Board (IRB #00018643). To be included in the study, patients had to have LDL-C measurements before and at least once after initiation of PCSK9i, and all provided signed informed consent to participate even though their treatment strategy was designed and delivered according to standards of care. Patients seen in our PCSK9i clinic undergo a structured protocol that consists of clinic visits prior to PCSK9i initiation and every 6 months while on therapy, with plasma samples (lipid panel, lipoprotein (a) [Lp(a)], and PCSK9 level) obtained at baseline and post-PCSK9i initiation, within 5 days after an injection, at 1, 6, and 12 months [22,23].

A usual response was classified as a sustained  $\geq$  30% LDL-C reduction after the first time point of treatment. Of the usual responders, the term 'suboptimal response' was used to define those with less than the expected 50-60% reduction. Unusual responses were classified as: (1) no response - no apparent reduction in LDL-C at all time points; (2) delayed response – failing to achieve  $\geq$  30% LDL-C reduction by the third dose (one month post-PCSK9i initiation), but achieving this threshold at a later time; (3) reduced response - <30% LDL-C reduction at all time points; and (4) lost response – achieving >30% LDL-C reduction by the third dose (one month post-PCSK9i initiation), but displaying <30% reduction at a later time (Fig. 1). A patient could be counted for more than one unusual response type. Patients were excluded from the true unusual responder cohort if there was evidence of non-adherence to lifestyle habits, background lipid-lowering therapy, or PCSK9i therapy (including appropriateness of injection technique). Adherence to lifestyle habits and background therapeutics was assessed in all patients by direct interview and by review of the medial record and pharmacy fill records. Adherence to PCSK9i and appropriateness of injection technique was assessed in all patients by direct interview and monitored by review of pharmacy fill records and by analyzing changes in plasma PCSK9 levels, a method we previously published [24]. Briefly, if plasma PCSK9 levels rise by less than two-fold the antibody has not distributed appropriately in the plasma compartment (perhaps due to non-adherence, ineffective injection technique, dermatologic or lymphatic issues, etc). Non-adherence included: significant worsening of lifestyle habits while on PCSK9i; discontinuing background lipid-lowering therapy when PCSK9i was initiated; discontinuing PCSK9i at any time during the observation period; and/or following a non-standard dosing regimen (e.g., alirocumab 75/150 mg or evolocumab 140 mg given every 4 weeks).

Statistical analysis for comparisons between true unusual responders and usual responders was done by chi-squared or unpaired Student's ttest run with a two-tail distribution when comparing one variable at a time. Lp(a) values were additionally tested by Mann-Whitney non parametric test. A multiple logistic regression analysis with stepwise selection was performed using the Hosmer-Lemeshow goodness of fit test to test the null. We screened for the variables with stronger contribution ( $p \leq$ 0.15) within the cohort characteristics, demographics (age, sex), past medical history (ASCVD, FH, hypertension, diabetes, obesity, and tobacco use), baseline laboratory values (LDL-C, Lp(a), and PCSK9) and baseline lipid lowering therapy (statins and ezetimibe), each category independently. The variables selected through the first-pass filter (age, FH, LDL-C and ezetimibe use) were reevaluated under a second screen (p



Fig. 1. Response types to PCSK9i therapy

(A) Usual LDL-C reduction to PCSK9i therapy. Black line = usual response (n = 357). (B) Unusual LDL-C reduction to PCSK9i therapy. Blue line = no response (n = 2); Green line = delayed response (n = 12); Red line = reduce response (n = 3); Yellow line = lost response (n = 4). Not depicted, 6 patients had either delayed or reduced response and 4 had delayed and lost response. Shaded area = expected LDL-C reduction. Dashed line = threshold for hypo-responsiveness.

 $\leq$  0.08). All statistical analyses were ran with Prism 8.3 (GraphPad Software, LLC). Differences with p-values <0.05 were considered statistically significant.

## Results

Of the 411 patients who met our inclusion criteria, 357 patients (86.9%) were classified as usual responders, and 54 (13.1%) as unusual responders (Table 1). Of the unusual responders, 23 did not adhere to therapy -2 with non-adherence to lifestyle habits, 14 with non-adherence to background lipid-lowering therapy, and 9 with non-adherence to PCSK9i. Some patients had more than one cause of non-

# Table 1

Baseline characteristics.

Variable	Usual	All Unusual	True Unusual
	Responders	Responders	Responders <sup>a</sup>
N	257	54	21
Age (mean $\pm$ SD)	537 638 + 99	$565 \pm 135$	57 5 $\pm$ 12 8 (NS)
Age (mean $\pm 3D$ ) Male cex N (%)	172(49.5)	$30.3 \pm 13.3$	$37.3 \pm 12.0$ (NS)
ACCUD N $(04)$	200 (01)	20 (37)	12(30.7)(NS)
ASCVD, N (%)	269 (61)	42 (77.0) 27 (69 E)	24(77.4)(NS)
CVD, N (%)	200 (72.6)	37(00.3)	20(04.3)(NS)
CVD, N (%)	24(0.7)	4(7.4)	3(9.7)(NS)
PAD, N (%)	51(17.1)	12 (22.2)	9(29)(N3)
N(%)	54 (15.1)	10 (18.5)	8 (25.8) (193)
ASCVD risk factors, N	N (%)		
Hyperlipidemia	357 (100)	54 (100)	31 (100)
FH	175 (49)	33 (61.1)	22 (71) (p < 0.05)
HoFH	8 (2.2)	1 (1.9)	0 (0) ( <i>NS</i> )
HeFH	167 (46.8)	32 (59.3)	22 (71) (p < 0.05)
Hypertension	215 (60.2)	30 (55.6)	17 (54.8) (NS)
Diabetes	58 (16.2)	9 (16.7)	3 (9.7) (NS)
Obesity	137 (38.4)	24 (44.4)	14 (45.2) (NS)
Current tobacco	12 (3.4)	4 (7.4)	1 (3.2) ( <i>NS</i> )
Family history ASCVD	283 (79.3)	47 (87)	27 (87.1) ( <i>NS</i> )
Lipid parameters at h	aseline (median [IOF	81)	
LDL-C <sup>b</sup>	132 (102–170)	121.5	133 (104.5–191.5)
	(,	(91 5-169 8)	(NS)
Lp(a) <sup>b</sup>	30 (11–97)	80 (23-127)	73 5 (27 5–118 3)
2p(d)	00(11 )/)	00 (10 12/)	(NS)
PCSK9 <sup>c</sup>	361.1	408	358 5 (253 5-460)
1 0010	(284 5-500 8)	(267 3-523 3)	(NS)
Baseline linid-loweri	of the range N (%)	(10,10 01010)	(10)
Statins	165 (46 2)	26 (48 1)	9 (29) (NS)
High-intensity	109 (30 5)	15 (27.8)	8 (25 8) (NS)
Moderate-	33 (9 2)	5 (9 3)	0(20.0)(NS)
intensity	33 (3.2)	0 (5.0)	0 (0) (10)
Low-intensity	23 (6 4)	6 (11 1)	1(32)(NS)
Ezetimibe	219 (61.3)	29 (53 7)	13(41.9) (n <
Eletimbe	219 (01.5)	29 (00.7)	0.05)
BAS	33 (9.2)	3 (5.6)	0.00)
Niacin	24 (6 7)	4 (7 4)	2(65)(NS)
Fibrates	19 (5 3)	5 (9 3)	4(12.9)(NS)
Supplements	71 (19 9)	12 (22 2)	6 (19 4) (NS)
None	71 (19.9)	14 (25.9)	11 (35.5) (n < 1)
	, - (1), )	1, (20.7)	0.05)

ASCVD, atherosclerotic cardiovascular disease; BAS, bile acid sequestrants; CAD, coronary artery disease; CVD, cardiovascular disease; FH, familial hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); N, number; NS, not significant; PAD, peripheral artery disease; PCSK9, protein convertase subtilisin/kexin type 9; SD, standard deviation.

P values show significance of differences between true unusual responders and usual responders.

<sup>a</sup> Without adherence complications: regression of lifestyle interventions, discontinuing background lipid-lowering therapy, discontinuing PCSK9i, and/or suboptimal dose of PCSK9i.

adherence. Lack of adherence to PCSK9i therapy was confirmed by patient report in 2 cases, and by inappropriate change (<2-fold rise [1]) in PCSK9 levels in 7 cases. The remaining 31 patients (7.5% of the entire cohort) were classified as true unusual responders, without apparent cause justifying the poor response. Hereafter, the designation of unusual responders refers to this cohort of 31 patients, which included: 2 with no response, 12 with delayed response, 3 with reduced response, 6 with delayed or reduced response, 4 with lost response, and 4 with delayed and lost response.

The unusual responders cohort had a mean age of 57.5 years, 38.7% were male, 77.4% had a history of ASCVD, and 71% had heterozygous FH (HeFH) (Table 1). Background lipid-lowering therapy consisted of 29% on statin therapy (majority high-intensity), 41.9% on ezetimibe, and 35.5% not on any lipid-lowering therapies. Baseline laboratory values included a median (interquartile range [IQR]) LDL-C of 133 mg/dL (104–191 mg/dL), Lp(a) 73 mg/dL (27–118 mg/dL), and plasma PCSK9 level of 358 ng/dL (253–460 ng/dL). Compared to the usual responders, the unusual responder cohort had higher prevalence of HeFH (p < 0.05). Background lipid-lowering therapy was also different, with unusual responders displaying less use of ezetimibe (p < 0.05), and being more likely to have no baseline lipid-lowering therapy at all due to medication intolerances (p < 0.05).

Among the FH cohort, genetic testing was performed in 31 of the 175 usual responders and 10 of the 22 unusual responders (Table 2). Seventeen of the 31 tested in the usual response group (54.8%) had likely causative mutations. Four of the 10 tested in the unusual response group (40%) had likely causative mutations.

Median on-treatment LDL-C values (IQR) in the unusual response cohort at 1, 6, and 12 months were 100 mg/dL (80–161 mg/dL), 94 mg/dL (68–167 mg/dL), and 96 mg/dL (71–128 mg/dL), respectively (Table 3). Corresponding median percent changes from baseline (IQR) were -23.3% (-14.2%, -26.9%), -33.7% (-25.4%, -49.5%), and -34% (-27.6%, -40.9%), respectively. Response to PCSK9i therapy at all time points revealed higher on-treatment LDL-C values (median ranges: 94–100 mg/dL versus 47–51 mg/dL, p < 0.001) and Lp(a) values (median ranges: 81–92 mg/dL versus 28–52 mg/dL, p < 0.01) in the unusual versus usual responders, respectively. Median baseline and fold change in pre-versus post-treatment plasma PCSK9 levels were similar between the two cohorts, though the unusual responders displayed regression in the magnitude of fold-increase at 6 and 12 months compared to usual responders.

Of the 12 patients with delayed response, 10 (83.3%) displayed suboptimal maximal LDL-C lowering (range: 31–46%) when they eventually exceeded the hypo-responsive threshold of <30%. Seven of the unusual response patients transitioned from one monoclonal antibody to the other, and only one (14.3%) exhibited a substantial change in maximal LDL-C response – from alirocumab 150 mg every 2 weeks with maximal LDL-C reduction of 27% to evolocumab 140 mg every 2 weeks with maximal LDL-C reduction of 42%.

A multiple logistic regression analysis identified presence of clinical FH (OR 2.9, 95% CI 1.27–7.24, p = 0.0152) and ezetimibe therapy (OR 0.334, 95% CI 0.150–0.728, p = 0.0061) as the only factors related to true unusual response through the stepwise protocol. However, the combination of these two variables failed to predict response to PCSK9i.

# Discussion

Our study is the first attempt at characterizing the frequency of inappropriate and unusual responses to PCSK9i therapy in clinical practice. First, we defined the parameters for unusual response classification, establishing a framework for future investigations. Second, we report that PCSK9i in clinical practice displays a significantly higher rate of unusual responses compared to clinical trial data (up to 13.1% vs. <2%, respectively). Third, nearly half (42.6%) of the unusual responses were due to non-adherence with prescribed therapeutic interventions (lifestyle habits, background lipid-lowering therapy, and/or PCSK9i).

<sup>&</sup>lt;sup>b</sup> mg/dL.

<sup>&</sup>lt;sup>c</sup> ng/dL.

### Table 2

Genotypi	c characteriz	ation of FI	I subjects	in the	structured	cohort.
----------	---------------	-------------	------------	--------	------------	---------

True Unusual

133 (104.5–191.5)

73.5 (27.5-118.3)

358.5 (253.5-460)

Responders<sup>a</sup>

31

(NS)

(NS)

(NS)

All Unusual

Responders

(91.5-169.8)

80 (23-127)

408 (267.3-523.3)

1215

# Table 3

Response to PCSK9 inhibitor therapy.

Usual Responders	True Unusual Responders <sup>a</sup>	Variable	Usual Responders	All I Rest
31	10			
17 (54 8%)	4 (40%)	IN Lipid paramete	30/ are at baceline (median	54 [[0]])
17 (34.8%)	+ (+070)	LDL-C <sup>b</sup>	132 (102–170)	121 (91.
		Lp(a) <sup>b</sup>	30 (11–97)	80 (
13	3	PCSK9 <sup>c</sup>	361.1	408
4	1		(284.5–500.8)	
		Lipid paramete	ers post 3rd dose (at 1 i	month)
		LDL-C <sup>D</sup>	50 (31–74)	95 (
c.7981 > A	c.798T > A (p.Asp266Glu),	a. 1		
(p.Asp266Glu),	heterozygous	% change	-61.1 (-49,	-24
heterozygous		from	-72.4)	-37
c.223T > A	c.156/G.A (p.Val523Met,	baseline		
(p.Cys75Ser),	chr19.GRCH37:g.11224419G > A),	Lp(a)	28.5 (7-88.3)	66 (
heterozygous	heterozygous	% change	-16 (-0.3, -31.1)	-6.
deletion (exons 11–12),	c.1964del (p.Phe655Serfs*10),	from		
heterozygous	heterozygous	baseline	05045	
c.10901.C		PCSK9	3584.5	363
(p.Cys364Arg),		6 1 1 1	(2712-4613.9)	(23)
neterozygous		rold change	9.9 (7.6, 12.4)	9.5
p.D90E (also known as $a 270T > A$ )		from		
C.2/UT > A,		Jasenne Lipid paramata	ma at 6 months (modio	n [IOD
$a_{121C} > A (n Tro 44^{a})$			47 (20, 60, 2)	
heterozygous		LDL-C	47 (30-09.3)	09(
Two mutations: LDLR		% change	-64 5 (-50 8	-35
A391T LDLR M652T		from	-74.6)	-51
heterozygous		baseline	, 110)	01
$c.682G > T (p.Glu228^{a}).$		Lp(a) <sup>b</sup>	52 (9-95)	80 (
heterozygous		- <b>F</b> (-)		
c.259T > G		% change	-21.1 (-10,	-12
(p.Trp87Gly),		from	-33.2)	
heterozygous		baseline		
$c.501C > A (p.Cys167^{a}),$		PCSK9 <sup>c</sup>	3713.6	349
heterozygous			(2759.1-4519.4)	(23)
c.858C > A		fold increase	10.3 (7.7, 13.1)	8.3
(p.Ser286Arg),		from		
heterozygous		baseline		
		Lipid paramete	ers at 12 months (media	an [IQI
c.10580G > A	10580G > A (p.Arg3527Gln),	LDL-C <sup>b</sup>	51 (35–68)	87 (
(p.Arg3527Gln),	heterozygous			
heterozygous (two		% change	-62 (-51.4,	-35
		c	<b>TO 1</b>	40
patients)		from	-72.1)	-49
	13 17 (54.8%) 13 17 (54.8%) 13 4 c.798T > A (p.Asp266Glu), heterozygous c.223T > A (p.Cys75Ser), heterozygous deletion (exons 11–12), heterozygous c.1090T.C (p.Cys364Arg), heterozygous p.D90E (also known as c.270T > A), heterozygous c.131G > A (p.Trp44°), heterozygous c.131G > A (p.Trp44°), heterozygous c.391T; LDLR M652T, heterozygous c.259T > G (p.Trp87Gly), heterozygous c.501C > A (p.Cys167°), heterozygous c.858C > A (p.Ser286Arg), heterozygous c.858C > A (p.Ser286Arg), heterozygous c.10580G > A (p.Arg3527Gln), heterozygous	Usual RespondersTrue Unusual Responders311017 (54.8%)4 (40%)13341c.798T > Ac.798T > A (p.Asp266Glu), heterozygousheterozygousc.223T > Ac.223T > Ac.1567G.A (p.Val523Met, (p.Cys75Ser), heterozygouschr19.GRCH37:g.11224419G > A), heterozygousdeletion (exons 11-12), heterozygousc.1964del (p.Phe655Serfs*10), heterozygousc.1090T.C (p.Cys364Arg), heterozygousc.131G > A (p.Trp44*), heterozygousc.331T > A (p.Gu1228*), heterozygousc.682G > T (p.Glu228*), heterozygousc.501C > A (p.Cys167*), heterozygousc.501C > A (p.Cys167*), heterozygousc.10580G > A (p.Ser286Arg), heterozygousc.10580G > A (p.Arg3527Gln), heterozygous	Usual RespondersTrue Unusual RespondersVariable3110N17 (54.8%)4 (40%)Lipid parameter LDL-Cb133PCSK9 <sup>C</sup> 41Lipid parameter LDL-Cb(p.Asp266Glu), (p.Asp26GGlu), (p.Asp26GGlu), heterozygous% change fromc.798T > Ac.798T > A (p.Asp266Glu), (p.Asp26GGlu), heterozygous% change fromheterozygousc.1567G.A (p.Val523Met, heterozygousbaseline (p.Cys75ser), heterozygousheterozygousc.1567G.A (p.Val523Met, heterozygousbaseline (p.Cys75ser), (hr19.GRCH37:g.11224419G > A), heterozygousheterozygousheterozygous% change from heterozygousc.1090T.C (p.Cys364Arg), heterozygousFold change from baselineheterozygousfold change from heterozygousc.131G > A (p.Trp44 <sup>a</sup> ), heterozygousLDL-C <sup>b</sup> Two mutations: LDLR A391T; LDL RM652T, heterozygous% change from heseline c.682G > T (p.Glu228 <sup>a</sup> ), heterozygousc.682G > T (p.Glu228 <sup>a</sup> ), heterozygousfold increase from haselinec.585C > A (p.Arg3527Gln), heterozygousfold increase from haselinec.10580G > A (p.Arg3527Gln), heterozygous10580G > A (p.Arg3527Gln), heterozygous	Usual Responders     True Unusual Responders     Variable     Usual Responders       31     10     N     357       17 (54.8%)     4 (40%)     Lipid parameters at baseline (median LDLC <sup>6</sup> 132 (102-170)       13     3     PCSK9 <sup>C</sup> 361.1       4     1     (284.5-500.8)     Lipid parameters post 3rd dose (at 1 metrozygous cost 1-19.GRCH373; 11224419G > A), heterozygous     % change     -61.1 (-49, from -72.4)       c.232T > A     c.1567G.A (p.Val523Met, (p.Cys75Ser), chr19.GRCH373; 11224419G > A), heterozygous     % change     -16 (-0.3, -31.1)       heterozygous     neterozygous     1.96464 (p.Phe655Serfs*10), heterozygous     baseline       c.1090T.C     (p.Cys75Ser), chr19.GRCH373; 11224419G > A), heterozygous     fod change     -16 (-0.3, -31.1)       p.D90E (also known as c.270T > A), heterozygous     1.96464 (p.Phe65Serfs*10), heterozygous     baseline       c.270T > A), heterozygous     Grow -74.6     baseline     1.00.LC <sup>6</sup> 47 (30-69.3)       heterozygous     from -74.6     baseline     1.01.LC <sup>6</sup> 47 (30-69.3)       c.289T > G     (p.Arg3527Gin), heterozygous     % change     -21.1 (-10, from -33.2)     baseline <tr< td=""></tr<>

PCSK9, protein <sup>†</sup>Description of specific gene mutation was not available for all patients in whom genetic testing was performed.

<sup>a</sup> Without adherence complications: regression of lifestyle interventions, discontinuing background lipid-lowering therapy, discontinuing PCSK9i, and/or suboptimal dose.

This observation implies that a detailed clinical evaluation of medication adherence is essential when an unusual response is suspected. Fourth, the most common type of unusual response was a delayed response, meaning that the attainment of  $\geq$ 30% LDL-C lowering effect was eventually obtained, but in a longer time frame than is typical. Thus, if a hyporesponsive effect is experienced early in the course of treatment, the best strategy is to simply allow more time for PCSK9i-mediated LDL-C lowering to take effect. However, it is important to note that the majority of delayed responders still exhibited an overall suboptimal response, less than the expected 50-60% reduction. Finally, in most patients, switching the monoclonal antibody did not seem to confer additional LDL-C lowering when an unusual response was noted.

Explanations for unusual response to PCSK9i can be categorized in two broad areas: 1) impaired monoclonal antibody entry into the systemic circulation, and 2) abnormally low effect of the monoclonal antibody in the circulation. Reduced entry of a PCSK9i into the circulation may be related to any of the following: 1) poor or no adherence to PCSK9i

Lipid parameter	s post 3rd dose (at 1 n	ionth) (median [IQR])	
LDL-C <sup>b</sup>	50 (31–74)	95 (71–127)	100 (80.3–160.8) (p < 0.001)
% change	-61.1 (-49,	-24.7 (-14,	-23.3 (-14.2, -26.9)
from	-72.4)	-37.5)	(p < 0.001)
baseline			
Lp(a)	28.5 (7–88.3)	66 (32–109)	81 (15–129) ( <i>NS</i> )
% change from baseline	-16 (-0.3, -31.1)	-6.5 (2.3, -14.9)	0 (14.7, -11.1) (p < 0.01)
PCSK9 <sup>c</sup>	3584.5	3635.5	3762.4
	(2712 - 46139)	(2337 2-5165)	(2212 5-5161 3) (NS)
fold change	9.9 (7.6, 12.4)	9.5 (6.6, 13.3)	10.4 (7.9, 14.4) ( <i>NS</i> )
from baseline			
Linid narameter	s at 6 months (median	[IOR])	
LDL-C <sup>b</sup>	47 (30–69.3)	69 (52–113)	94 (68.5–167.5) (p < 0.001)
% change	-64.5 (-50.8.	-35.2 (-21.9.	-33.7(-25.4, -49.5)
from	-74.6)	-51.5)	(p < 0.001)
L p(a) <sup>b</sup>	E2 (0, 0E)	90 (00 11E)	92 = (40.9, 111.2)
Lp(a)	32 (9-93)	80 (23–113)	( <i>NS</i> )
% change	-21.1 (-10,	-12.5 (1.8, -17.8)	-3 (3.1, -12.5) (p <
from	-33.2)		0.01)
baseline			
PCSK9 <sup>c</sup>	3713.6	3493.4	1415.8
	(2759.1-4519.4)	(2321.1-4196.7)	(1126.5-2170.4) (NS)
fold increase from baseline	10.3 (7.7, 13.1)	8.3 (4.6, 12.5)	7 (3.4, 11.1) ( <i>NS</i> )
Lipid parameter	s at 12 months (media	n [IQR])	
LDL-C <sup>b</sup>	51 (35–68)	87 (60.5–110)	96 (71–128) (p < 0.001)
% change	-62 (-51 4	-35 (-21.3	-34(-276 - 409)
from	-72 1)	_49 3)	(n < 0.001)
baseline	-/2.1)	-+5.5)	(p < 0.001)
Jaseinie	42 E (7 100 0)	00 (40 104)	0.0 5 (0.0 0 1.00 5) (-
Lp(a)	43.5 (7-109.8)	83 (48–124)	< 0.05)
% change	-18.5 (-3.5,	-10 (-3.6, -16.8)	-12.5 (3.5, -17.3)
from baseline	-33.3)		(NS)
DCSVOC	3602.2	2412 5	2802
L COVA	(2027.2 4002.0)	(1227 7 4067 A)	(1704 0 2E62 0) (MC)
6-14-1	(2027.3-4903.9)	(233/./-400/.4)	(1/04.9-3003.9) (NS)
from	10.5 (7.4, 12.9)	5.8 (4.9, 11.3)	7.3 (4.5, 12.4) (NS)
baseline			

IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); N, number; NS, not significant; PCSK9, protein convertase subtilisin/kexin type 9.

P values show significance of differences between true unusual responders and usual responders.

<sup>a</sup> Without adherence complications: regression of lifestyle interventions, discontinuing background lipid-lowering therapy, discontinuing PCSK9i, and/or suboptimal dose. of PCSK9i.

<sup>b</sup> mg/dL.

<sup>c</sup> ng/dL.

therapy; 2) improper PCSK9i administration technique; 3) dermatological factors impairing systemic absorption of drug; and 4) inappropriate antibody disposition through the lymphatic system [18,24]. As seen previously [18] and confirmed in our study, the most common cause of unusual response to PCSK9i is related to discontinuation of background

lipid-lowering therapies (e.g., statins) after initiation of a PCSK9i. Our group proposed an algorithm to act as a first-line diagnostic tool to assess possible PCSK9i resistance by measuring plasma PCSK9 concentrations before and after treatment with a PCSK9i [1,24]. Measuring PCSK9 levels is a quick and easy method to check for proper adherence without measuring plasma antibody (mAb) levels, a process that requires the use of proprietary and generally unavailable idiotypic antibodies against the mAb inhibitor. Since PCSK9i binds PCSK9 molecules, our assay measures the progressively and dramatically rising plasma PCSK9 levels caused by the delayed clearance of monoclonal antibody-PCSK9 complexes, and is very useful to confirm adherence to therapy. If the patient with unusual response has at least a 2-fold increase in plasma PCSK9 level, then adherence and technique issues are not at play, and investigations into biologic reasons for resistance should commence. Using this method in conjunction with patient questioning and chart review, we were able to positively identify 31 of the 54 patients with unusual responses to PCSK9i as likely to have true biologic mechanisms at play.

The prevalence of HeFH was higher among the unusual responders, 71% versus 49%. In clinical trials, PCSK9i have demonstrated a magnitude of LDL-C reduction in FH subjects analogous to that seen for subjects with non-genetic hypercholesterolemia [2-14]. Among unusual responders, we found 3 mutations in LDLR and one in APOB, of which 2 LDLR mutations were not present in the group with usual responses. Table 2 shows the patient-level data for future comparison, but currently there is no clear insight into the molecular causes of poor response. Based on what we know about the central role of PCSK9 as regulator of the LDL receptor, how can a fully blocked PCSK9 not exert an effect on plasma LDL-C levels? This may be the result of: 1) mutations that cause loss of function of PCSK9; 2) anti-drug antibodies directed against PCSK9i; 3) exaggerated PCSK9 secretion in molar excess of PCSK9i; or 4) mutations in LDL receptors or its ligands apoB or apoE that render them less susceptible to PCSK9 inhibition [18,24]. Development of anti-drug antibodies (ADA) is a rare event and one that often does not translate into reduced effectiveness since the PCSK9i antibody is likely to keep its ability to bind PCSK9 even when bound to another antibody [25,26]. Nevertheless, commercially available tests to screen for the rare ADA and rarer neutralizing antibodies would be beneficial in further elucidating causes of unusual responses.

Another possible explanation for the unusual response to PCSK9i is a high concentration of Lp(a). The exact mechanism for Lp(a) clearance is not fully elucidated but is influenced by PCSK9i. It is well documented that, on average, PCSK9i reduce LDL-C and Lp(a) in a 2:1 ratio (LDL-C  $\approx$ 50–60%: Lp(a)  $\approx$ 25–30%), and often in a discordant manner (e.g., in >30% of subjects Lp(a) and LDL-C do not fall concordantly) [27,28]. Patients in our unusual response cohort trended toward a 2.5-fold higher baseline Lp(a) compared to usual responders, 73 vs. 30 mg/dL, respectively, and yet had similar baseline LDL-C, 133 vs. 132 mg/dL, respectively. Thus, the reduced LDL-C response could be accounted for by the higher proportion of reported LDL-C consisting of Lp(a) particles, which are not cleared efficiently by the LDL receptor.

Strengths of this study include the large cohort of well-characterized PCSK9i patients closely followed in a highly structured standard-of-care protocol. This entailed meticulous monitoring of medication adherence (PCSK9i and background lipid-lowering therapy), an important determinant of medication responsiveness likely less stringent in prior trials investigating PCSK9i resistance. Additionally, this study encompasses a large cohort of patients with baseline and on-treatment PCSK9 levels at regular intervals to characterize PCSK9i adherence and systemic absorption. Limitations of this study are inherent to its retrospective nature and real-world data design. As this cohort is an ongoing evaluation and routine clinical practice for our group, the entire 12 months of data was not available for all, as some patients had not been on PCSK9i for the entire duration or missed follow up visits, or had laboratory work done outside of our institution (preventing collection of plasma PCSK9 samples). For a portion of our unusual responders, this prevented the delineation between reduced response and delayed response status. Finally,

since genetic testing is not necessary for diagnosis or treatment of FH, this is performed in our practice only when the patient is eager to obtain the information, the pre-test probability is high, and we can assure cost containment. Only 21% of FH subjects in our cohort were genotyped, and although 3 unique LDLR mutations are notable in the unusual responders (Table 2) it is unclear whether these represent the cause of the poor response to PCSK9i.

# Conclusions

We have characterized unusual responses to PCSK9i and find that in a real-world population their prevalence is common, occurring at rates at least 3-fold higher than those reported in clinical trials. Of the suspected unusual responders, nearly half were patients with poor adherence to prescribed pharmacotherapy. However, the remaining subjects had true biologic reasons for the inappropriate response, and switching the monoclonal antibody did not make a difference in most patients. Additional investigation into the causes of unusual response to PCSK9i therapy may uncover novel aspects of whole body cholesterol homeostasis and new leads in the development of therapeutics.

#### Disclosures

BAW reports institutional grant from Akcea.

MDS reports advisory work for Amgen, Regeneron, Esperion; Consultant for Novartis.

SF reports advisory work for Kowa, Amgen, Novo Nordisk, Astra Zeneca and Amarin.

PBD reports institutional grants and/or advisory work for Akcea, Esperion, Regeneron, Regenzbio, and Retrophin.

JQP reports clinical trial work with Amgen, Novartis, and Akcea. The other authors have no disclosures to report.

# Authors' contributions

BAW, JRM, SF, and MDS conceived the study, BAW and JRM designed the study, performed the data collection and drafted the manuscript. CO performed the statistical analysis. All authors contributed to the writing of the manuscript and have read and approved the final version of the manuscript.

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