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Plasma PCSK9 Levels and Clinical Outcomes in the TNT (Treating to New Targets) Trial

A Nested Case-Control Study

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Objectives The purpose of this study was to investigate whether high levels of circulating proprotein convertase subtilisin kexin type 9 (PCSK9) would increase cardiovascular risk in statin-treated patients.

Background Statins activate low-density lipoprotein (LDL) receptor gene expression, thus lowering plasma LDL levels. But statins also activate the expression of PCSK9, a secreted inhibitor of the LDL receptor, thereby limiting their beneficial effects.

Methods We have measured the plasma PCSK9 levels of 1,113 patients with stable coronary heart disease enrolled in the Treating to New Targets study, a randomized trial that compared the efficacy of high- versus low-dose atorvastatin. After a run-in period with atorvastatin 10 mg daily, patients were randomized to either continue with 10 mg or be up-titrated to 80 mg atorvastatin and followed during 5 years for major cardiovascular events (MCVEs).

Results Circulating PCSK9 levels measured at randomization were predictive of clinical outcomes in the group randomized to remain on atorvastatin 10 mg ($p = 0.039$), but not in the group that intensified atorvastatin treatment to 80 mg ($p = 0.2$). Further, PCSK9 levels measured 1 year post-randomization did not change upon increase of the statin dose.

Conclusions PCSK9 levels predict cardiovascular events in patients treated with low-dose atorvastatin. (A Study to Determine the Degree of Additional Reduction in CV Risk in Lowering LDL Below Minimum Target Levels [TNT]; NCT00327678) (J Am Coll Cardiol 2012;59:1778–84) © 2012 by the American College of Cardiology Foundation

The concentration of low-density lipoprotein cholesterol (LDL-C) is a significant predictor of atherosclerotic cardiovascular disease. This relationship is causal with overwhelming evidence that reducing LDL-C levels will also reduce cardiovascular risk. The extent of reduction in risk is a function of how much LDL-C levels are reduced, with several trials now clearly demonstrating greater benefits with more intensive LDL-C lowering (1,2). This has led to recommendations of strict LDL-C targets in high-risk patients. Given that many people cannot achieve these targets even with the highest dose of the most effective statins, there is an obvious need for additional LDL-C lowering therapies (3,4).

Statins inhibit the rate-limiting enzyme involved in cholesterol biosynthesis, thereby decreasing intracellular cholesterol levels. This activates a feedback mechanism that up-regulates LDL receptor gene expression, resulting in lower circulating LDL-C levels. However, statins also up-regulate the expression of proprotein convertase subtilisin kexin type 9 (PCSK9), a known inhibitor of the LDL

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receptor. This up-regulation of PCSK9 has the potential to limit the efficacy of statin-induced LDL-C lowering (5,6). The reason for this is that PCSK9 binds to the LDL receptor and directs it toward lysosomal degradation rather than normal recycling to the cell membrane (7,8). Inhibiting PCSK9 is thus a logical strategy for enhancing statin-induced LDL-C lowering to maximize the reduction in cardiovascular risk (9). Prospective studies are therefore needed to determine whether PCSK9 holds a predictive value for cardiovascular risk. We have conducted a case-control study nested in the TNT (Treating to New Targets) study, a randomized, controlled trial of patients with stable coronary heart disease (CHD) to examine these questions.

Methods

Study design. The current study is a case-control substudy nested in the TNT trial in which the data analysis was per intention-to-treat principle. The study protocol and outcomes of the TNT study have been published previously (10,11). In brief, patients with clinically manifest CHD commenced 8 weeks of open-label treatment with atorvastatin 10 mg daily. After this run-in period, 10,001 patients with LDL-C levels <130 mg/dl were randomized in a double-blind design to therapy with either 10 or 80 mg atorvastatin per day. Patients were followed for a median of 4.9 years. The pre-planned selection of the nested case-control study population is described in detail in a previous publication (12). Only patients from whom informed consent was obtained for measuring additional biomarkers were eligible for this substudy. We included all patients who developed major cardiovascular events (MCVEs) during follow-up (cases). MCVEs were defined as CHD death, nonfatal, non-procedure-related myocardial infarction, resuscitated cardiac arrest, and fatal or nonfatal stroke. We then randomly selected 1,1[illegible] patients who did not develop MCVE during follow-up (controls), matching with the case patients within each treatment group. We calculated that by assuming a common standard deviation of 150 ng/ml in plasma PCSK9 levels; the nested case-control substudy had 90% power to detect a difference of 26 ng/ml between the cases and the controls group.

PCSK9 measurements. PCSK9 concentration was measured in fasting plasma samples collected at the time of randomization (after the 8-week atorvastatin 10 mg run-in period) and again 1 year after randomization. Blood samples were collected in 10-ml lavender-top EDTA Vacutainer (BD, Franklin Lakes, New Jersey) venous blood collection tubes using standard phlebotomy practices. Immediately after collection, tubes were gently inverted 6 times, and centrifuged at 2,000 g for 10 min. Plasma samples were transferred into 8-ml freezer vials, frozen at −70°C, shipped on dry ice, and thawed for biomarker analysis (12). Lipid levels had already been measured (10). PCSK9 concentrations were measured in duplicate after a second thaw, using the CY-8079 enzyme-linked immunosorbent assay (ELISA) kit (Cyclex, Nagano, Japan), as per the manufacturer's instructions. We determined the inter- and intraplate variability to be less than 5.1% and 4.3%, respectively. To ascertain the stability of PCSK9 in frozen plasma samples, we subjected 6 randomly chosen aliquots stored at −80°C to 2 freeze-thaw cycles over 4 months. PCSK9 levels were measured and found to be within the assay intraplate variability range. PCSK9 concentration of plasma aliquots from a compound heterozygote individual lacking PCSK9 (Genetic Technology provided by Dr. Horton) was measured and found below the detection threshold.

Statistical analyses. Patient characteristics at randomization were compared between treatment groups or between outcomes using a chi-square test for categorical variables, and a Wilcoxon rank sum test for continuous variables. Similarly, characteristics of substudy patients at time of randomization were compared between those who did and those who did not experience a cardiovascular event during the study follow-up. Changes in PCSK9 levels were tested with a signed rank test, and compared between treatment groups with a Wilcoxon rank sum test. Spearman rank correlation analyses were used to assess the correlation between PCSK9 and lipids. The association between PCSK9 levels at time of randomization and the primary outcomes was assessed using a Cox proportional hazards model, using time to outcome as the dependent variable. The association between PCSK9 levels at year 1 and subsequent primary event was analyzed similarly while excluding patients with any prior event during year 1. Baseline PCSK9 levels measured at time of randomization were not included in the model. Hazard ratios (HRs) were calculated using an unadjusted model and also a multivariate model adjusted for age, sex, treatment group, smoking status (current smoker or not), hypertension, diabetes, body mass index, and LDL-C levels. To examine whether the association between PCSK9 and outcome differed between patients who did or did not intensify atorvastatin treatment, the treatment by PCSK9 interaction was examined in the same analysis models incorporating the interaction term. Analyses were also repeated within each individual treatment group. To compare the strength of association between PCSK9 and MCVE relative to other biomarkers, including lipids and lipoprotein cholesterol, the van der Waerden normal scores based on ranks were calculated for each of the biomarker by taking the inverse cumulative normal (PROBIT) function of $r_i/(n + 1)$. These scores were then used in the Cox proportional hazards model to assess the effect of biomarker concentration on clinical outcome.

Abbreviations and acronyms

CHD = coronary heart disease
HDL-C = high-density lipoprotein cholesterol
HR = hazard ratio
IQR = interquartile range
[illegible]-C = [illegible]ensity lipoprotein [illegible]esterol
MCVE = major [illegible]iovascular event(s)
PC[illegible] = [illegible]rotein convertase subtilisin kexin type 9

In the Cox proportional hazards analyses, PCSK9 level was not log-transformed, as the transformation did not improve the normality (the Shapiro-Wilk statistics worsened slightly from 0.94 to 0.84). In a Supremum test for proportional hazards assumption, the proportional hazards assumption was not violated. We considered a p value <0.05 to indicate statistical significance.

Results

Patient population and characteristics. Characteristics of patients in the present study were similar to those in the entire TNT population (12). Of the 496 patients who experienced an MCVE, 110 (22.8%) died due to a CHD event, 288 (58.1%) had a nonfatal myocardial infarction, 142 (28.6%) had a stroke, and 23 (4.6%) were resuscitated after cardiac arrest. This distribution mirrors that of the 982 patients who suffered an event in the entire TNT population, in which 23% died due to a CHD event, 56% had nonfatal myocardial infarction, 28% had stroke, and 5% were resuscitated after cardiac arrest. Of the 496 MCVEs that occurred during follow-up, 107 (22%) occurred in the first year. The characteristics of patients by treatment groups and by subgroups who experienced an MCVE and those who did not are shown in Table 1. Baseline characteristics were similar in the group randomized to rem 10 mg atorvastatin and in the group randomized to re atorvastatin 80 mg. Patients who had an MCVE during trial were on average older and more likely e hype tension and/or diabetes at baseline co ed n thos who did not experience an event. At omiz n levels of LDL-C and triglycerides were ign ig d levels of high-density lipopro choles (HDL-C) were significantly lower in c an in contr Changes in plasma lipids over the 8-week run-in period were similar in cases and controls, with LDL-C levels reduced by 38% and 40%, respectively.

Plasma PCSK9 levels at randomization. PCSK9 levels at randomization had a slight right-skewed distribution and ranged between 1 and 1538 ng/ml (Fig. 1). Median PCSK9 levels at randomization were similar (p = 0.89) for the group randomized to remain on atorvastatin 10 mg (309 ng/ml, interquartile range [IQR]: 228 g/ml) and the group randomized to receive 80 g of ato tatin daily (309 ng/ml, IQR: 228 to 397 ml). Nor e median PCSK9 levels at randomiz on si cantly d rent between cases (311 ng/m QR: 236 40 g/ml) and controls (308 ng/ml, I 226 394 ng/ p = 0.42). In the group randomize ain on atorvastatin 10 mg throughout the , PC evels sured at the time of randomizatio ere higher nts who subsequently suffered a uring follo up (318 ng/ml, IQR: 240 to 425 ng/ml in t ses vs. 305 ng/ml, IQR: 221 to 384 ng/ e controls; 0.026) (Table 1). Similarly, in the g p randomized to the 10-mg dose, those who experi- ed a majo CHD event during follow-up had higher n an PCSK evels at randomization (322 ng/ml, IQR: 24 439 ml) than controls (305 ng/ml, IQR: 221 to 384 ng/ , p = 0.025). In contrast to the group randomized ain on 10 mg atorvastatin, plasma PCSK9 levels at and nization did not differ between the case and controls in the group that intensified atorvastatin treatment to 80 mg (300 ng/ml, IQR: 233 to 369 ng/ml vs. 313 ng/ml, IQR: 227 to 410 ng/ml; p = 0.22).

PCSK9 levels after 1-year treatment. PCSK9 levels distribution after 1 year of treatment was similar to that observed at time of randomization and did not differ

Table 1 Patient Char istics at Tim Randomization*

	Atorvastatin 10 mg				Atorvastatin 80 mg				
		ith MCVE	Without MCVE	p Value†	All	With MCVE	Without MCVE	p Value†	p Value‡
n	49	262	587		764	234	530		
Age, yrs		62 ± 8	60 ± 9	0.0009	62 ± 8	63 ± 8	61 ± 8	0.0027	0.15
Male	700 (	213 (81)	487 (83)	0.56	639 (84)	199 (85)	440 (83)	0.49	0.53
Curr oker	121 (14)	49 (18)	72 (12)	0.046	110 (14)	44 (19)	66 (12)	0.054	0.98
Hyperte	0 (55)	172 (66)	298 (51)	0.0001	425 (56)	144 (62)	281 (53)	0.029	0.91
Diabetes me	136 (16)	64 (24)	72 (12)	0.0001	122 (16)	50 (21)	72 (14)	0.0068	0.98
Total cholesterol, l	174 ± 24	176 ± 25	173 ± 24	0.080	174 ± 23	176 ± 24	174 ± 23	0.23	0.89
Delta TC, mg/ml		−71 ± 22	−73 ± 24	0.147		−71 ± 22	−71 ± 23	0.60	
LDL cholesterol, mg/dl	97 ± 18	100 ± 18	96 ± 18	0.0062	97 ± 17	100 ± 16	96 ± 17	0.0056	0.89
Delta LDL-C, mg/ml		−63 ± 21	−66 ± 21	0.89		−63 ± 21	−65 ± 20	0.28	
HDL-C, mg/dl	46 ± 10	44 ± 9	47 ± 10	0.0002	47 ± 11	45 ± 11	48 ± 11	0.0004	0.32
Delta HDL-C, mg/ml		−1 ± 4	−1 ± 5	0.36		−0.5 ± 5	−1 ± 6	0.10	
Triglycerides, mg/dl	136 (102–186)	143 (107–197)	133 (100–181)	0.024	133 (99–181)	135 (98–188)	132 (99–179)	0.46	0.14
Delta TG, mg/ml		−47 ± 61	−46 ± 61	0.19		−46 ± 52	−44 ± 59	0.62	
PCSK9, ng/ml	309 (228–396)	318 (240–425)	305 (221–384)	0.026	309 (228–397)	300 (233–369)	313 (227–410)	0.22	0.89

Values are mean ± SD, n (%), or median (interquartile range). Deltas indicate changes in plasma lipids after the 8-week run-in period (i.e., before randomization). *At the time of randomization, all participants had been on 10 mg atorvastatin for at least 8 weeks (run-in period). †p value for patients who experienced an event versus those who did not in both treatment groups. ‡p value for patients randomized to remain on 10 mg atorvastatin versus patients up-titrated to 80 mg atorvastatin.

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MCVE = major cardiovascular event(s); PCSK9 = proprotein convertase subtilisin kexin type 9; TC = total cholesterol; TG = triglycerides.

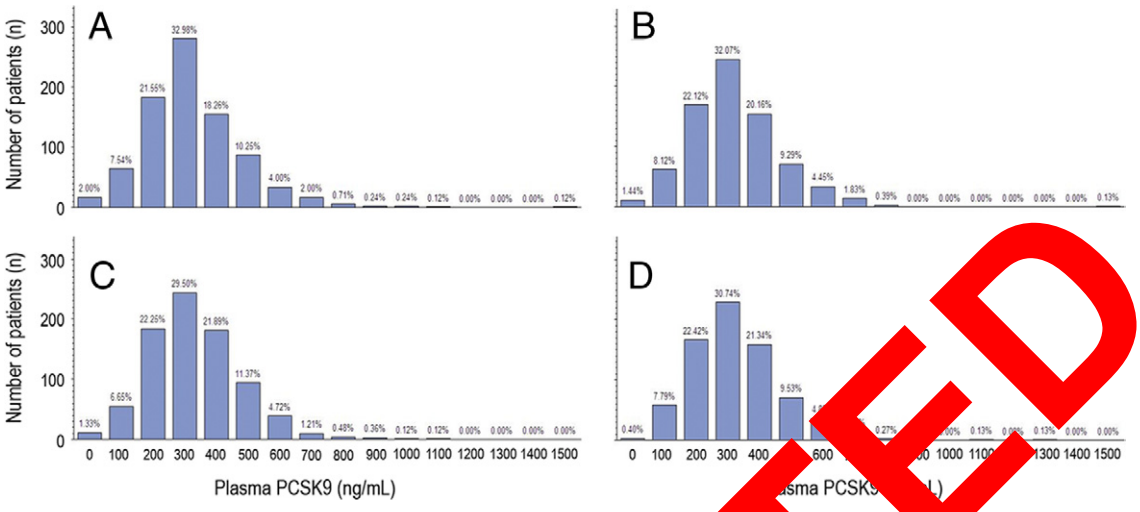

Figure 1 **Frequency Distribution of PCSK9 Plasma Levels in the TNT Trial**

Distribution of proprotein convertase subtilisin kexin type 9 (PCSK9) levels in the 10 mg ato group at randomiz **(A)**, in the 80 mg atorvastatin group at randomization **(B)**, in the 10 mg atorvastatin group after 1 year of treatment **(C)**, and in the g atorvastatin group after 1 year of treatment **(D)**.

between both treatment arms of the study (Fig. 1). Overall, PCSK9 levels 1 year after randomization did not significantly between the 10 and 80 mg atorvastatin t ment groups (318 ng/ml, IQR: 231 to 417 ng/ml vs. ng/ml, IQR: 231 to 407 ng/ml; p = 0.82). N ere the significant differences between the 2 tre ent oups i terms of change in PCSK9 between th ndom ion and 1-year post-randomization levels (t si) total group and both treatment s, there e no signif-icant correlations between PCSK9 le (at randomization and 1 year), L L-C ndomization and 1 year), HDL-C (at ran ization an ear), triglycerides (at randomization d 1 year), lipid anges between pre–run-in and omiz n, and lipid changes between randomization and post-ra omization. PCSK9 levels did no sig ntly tween sexes. There were also no gnifi t corre s between PCSK9 levels at rand zation d age or body mass index (not shown).

Re nship etween PCSK9 plasma levels measured at time omization or after 1 year of treatment and s of major cardiovascular or CHD events. Table 2 lo the relationship between PCSK9 levels at randomization and risk of MCVE or CHD events. Plasma PCSK9 levels measured at randomization were not associated with the risk of MCVE or CHD events (HR per 100 ng/ml increase: 1.03 [95% CI: 0.97 to 1.09], p = 0.39; HR per 100 ng/ml increase: 1.05 [95% CI: 0.98 to 1.12], p = 0.18, respectively). The treatment by PCSK9 interaction on MCVE was statistically significant (p = 0.03), however, the treatment by PCSK9 interaction on CHD was not statistically significant (p = 0.11). Separate analyses were performed for each treatment group. In the group randomized to remain on 10 mg atorvastatin, PCSK9 levels measured at randomization correlated significantly with both MCVE and major CHD events (HR per 100 ng/ml increase: 1.08 [95% CI: 1.04 to 1.16], p = 0.039; HR per 100 ng/ml

Table 2 **R nship Between PCSK9 Levels at Randomization* and Risk of MCVE and Major Coronary Events**

	All Patients		Atorvastatin 10 mg		Atorvastatin 80 mg	
	HR (95% CI)†	p Value	HR (95% CI)†	p Value	HR (95% CI)†	p Value
MCVE						
Unadjusted model	1.02 (0.97–1.08)	0.45	1.10 (1.03–1.18)	0.0082	0.92 (0.84–1.01)	0.075
Adjusted model	1.03 (0.97–1.09)	0.39	1.08 (1.04–1.16)	0.039	0.95 (0.86–1.04)	0.24
Major coronary events						
Unadjusted model	1.05 (0.98–1.12)	0.18	1.12 (1.03–1.21)	0.008	0.96 (0.87–1.06)	0.39
Adjusted model	1.05 (0.98–1.12)	0.18	1.09 (1.005–1.19)	0.038	0.99 (0.89–1.10)	0.84

Adjusted model incorporated age, sex, treatment group, smoking status, hypertension, diabetes, body mass index, and low-density lipoprotein cholesterol levels at randomization. p Value for treatment by proprotein convertase subtilisin kexin type 9 (PCSK9) interaction was 0.03 for major cardiovascular events (MCVE) and 0.11 for major coronary events (adjusted model). *At the time of randomization, all participants had been on 10 mg atorvastatin for at least 8 weeks. †Per 100 ng/ml increment in the PCSK9 concentration.

CI = confidence interval; HR = hazard ratio.

JACC Vol. 59, No. 20, 2012
May 15, 2012:1778–84

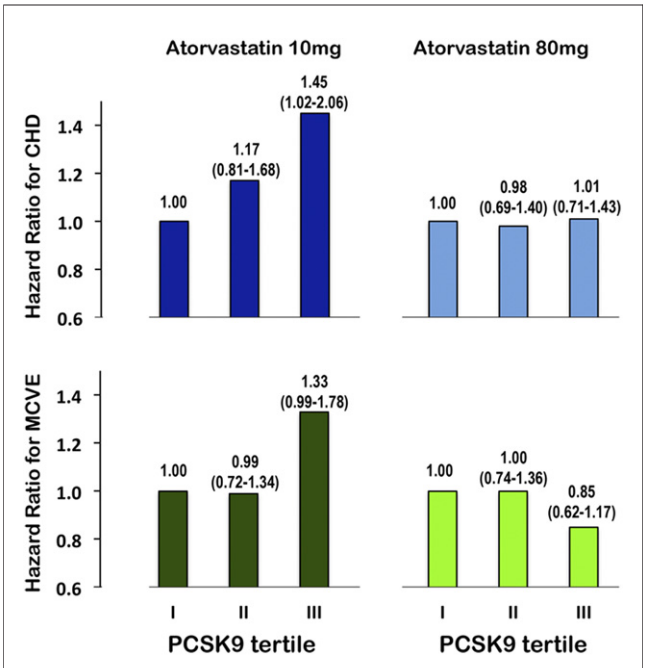

Figure 2 Relationship Between PCSK9 Levels and Cardiovascular Risk in the TNT Study

Relationship between proprotein convertase subtilisin kexin type 9 (PCSK9) levels stratified by tertiles at time of randomization and the risk of coronary heart disease (CHD) **(top)** or major cardiovascular events (MCVE) **(bottom)** in the group randomized to remain on 10 mg atorvastatin and the group up-titrated to 80 mg atorvastatin. Hazard ratios per 100 ng/ml increment in the PCSK9 concentration and 95% confidence intervals are

increase: 1.09 [95% CI: 1.005 to 1.19], p = 0.0 tively). In contrast, in the group randomized to receive atorvastatin 80 mg, PCSK9 levels measured after randomization predicted neither subsequent MCVE nor CHD events (HR per 100 ng/ml increase: 0.95 [95% CI: 0.86 to 1.04], p = 0.24; HR per 100 ng/ml increase: 0.99 [95% CI: 0.89 to 1.10], p = 0.84, respectively) (Table 2). In the atorvastatin 10 mg treatment arm of the study, the predictive value of PCSK9 at randomization for CHD and MCVE was maximal (HR per 100 ng/ml increase: 1.45 [95% CI: 1.02 to 2.06]; HR per 100 ng/ml increase: 1.33 [95% CI: 0.99 to 1.78], respectively) in the group of patients with the highest PCSK9 levels, as shown in Figure 2. In this analysis

Table 4 Relationship of MCVE to Plasma Biomarker Levels at Randomization for Individuals Randomized to Remain on 10 mg Atorvastatin*

	Unadjusted Model		Adjusted Model	
Biomarker	HR (95% CI)†	p Value	HR (95% CI)†	p Value
PCSK9	1.191 (1.035–1.371)	0.015	1.155 (1.003–1.330)	0.047
LDL-C	1.163 (1.034–1.309)	0.012	1.179 (1.044–1.333)	0.0082
HDL-C	0.769 (0.676–0.874)	<0.0001	0.778 (0.679–0.894)	0.0004
Triglycerides	1.170 (1.013–1.351)	0.033	(…266)	0.27
CRP	1.163 (1.034–1.308)	0.012	1.085 (0.963–…3)	0.18

Adjustment was done for age, sex, smoking status, hypertension, diabetes, and body mass index. *At the time of randomization, all participants had been on 10 mg atorvastatin for at least 8 weeks. †HR (95% CI) per unit increment in the natural logarithm of der Waerden normal scores of the biomarker concentration at randomization in a Cox proportional hazards model.

CI = confidence interval; CRP = C-reactive protein; HDL-C = high-density lipoprotein cholesterol; HR = hazard ratio; LDL-C = low-density lipoprotein cholesterol; MCVE= major cardiovascular event; PCSK9 = proprotein convertase subtilisin kexin type 9.

stratified by tertiles, we observed a dose-effect relationship between plasma PCSK9 levels at randomization and their predictive value for CHD. Levels of PCSK9 measured after 1 year of treatment with atorvastatin were not predictive of subsequent MCV or CHD events in either the combined groups or in the separate treatment groups (Table 3). We also assessed the prognostic value of PCSK9 levels measured at randomization for MCVE after year 1 by excluding the 107 patients who had an event during year 1. Baseline PCSK9 was no longer significant in predicting events post-year 1 in either treatment arm of the study.

Relationship of PCSK9 with MCVE compared with other biomarkers. Table 4 compares the strength of association between PCSK9 and MCVE with the strength of association between other biomarkers and MCVE in the atorvastatin 10 mg arm of the study. The association between circulating PCSK9 levels and clinical outcomes in this group was smaller than that observed between either LDL-C or HDL-C and MCVE, but larger than that observed between either triglycerides or C-reactive protein and MCVE.

Discussion

This nested case-control substudy of the TNT trial was designed to investigate the association between lipid and nonlipid biomarkers and the risk of MCVE in statin-treated

Table 3 Relationships of MCVE and Major Coronary Events to PCSK9 Levels 1 Year After Randomization

	All Patients		Atorvastatin 10 mg		Atorvastatin 80 mg	
	HR (95% CI)*	p Value	HR (95% CI)*	p Value	HR (95% CI)*	p Value
MCVE						
Unadjusted model	0.97 (0.90–1.04)	0.41	0.96 (0.86–1.07)	0.48	0.97 (0.88–1.08)	0.62
Adjusted model	0.98 (0.91–1.06)	0.65	0.99 (0.89–1.12)	0.91	0.97 (0.87–1.09)	0.64
Major coronary events						
Unadjusted model	1.002 (0.92–1.09)	0.097	1.02 (0.90–1.15)	0.84	0.99 (0.88–1.11)	0.86
Adjusted model	1.02 (0.93–1.11)	0.69	1.06 (0.92–1.21)	0.46	0.99 (0.87–1.12)	0.83

p value for treatment by PCSK9 interaction between treatment and clinical outcomes was 0.76 for MCVE and 0.49 for major coronary events (adjusted model). *Per 100 ng/ml increment in the PCSK9 concentration. Adjusted model incorporated age, sex, treatment group, smoking status, hypertension, diabetes, body mass index and low-density lipoprotein cholesterol levels at randomization.

Abbreviations as in Table 2.

stable CHD patients (12). Using the same approach and the same subpopulation, we have tested the possibility that plasma PCSK9, a functional inhibitor of the LDL receptor, is associated with the incidence of cardiovascular events. Circulating PCSK9 levels measured at randomization were predictive of clinical outcomes in the group randomized to remain on atorvastatin 10 mg, but not in the group that intensified atorvastatin treatment to 80 mg. After 1 year of treatment, PCSK9 levels did not change significantly and were no longer predictive of clinical events in both groups.

It is well established in humans and animal models that statin treatment increases plasma PCSK9 levels and conversely that attenuation of PCSK9 function enhances the hypolipemic effects of statins (13–16). For example, high doses of atorvastatin have been reported to increase circulating PCSK9 levels by 34% in 12 dyslipemic patients treated with 40 mg daily for 16 weeks (15), and by 45% in 74 normolipemic non-CHD individuals treated with 80 mg for 4 weeks (17). It was therefore expected that up-titration from 10 to 80 mg atorvastatin would increase plasma PCSK9 levels in the present study, as mentioned in a recent report indicating that when atorvastatin dose was increased from 5 to 80 mg daily, plasma PCSK9 levels increased on average by 30% in 53 dyslipidemic patients (18). To our surprise, we found that plasma PCSK9 levels were similar at randomization and 1 year later after up-titration to 80 mg. Major differences in study design could explain these apparent discrepancies. The TNT substudy has potential limitations in terms of design, methodological approach (12). For example, all patients enrolled in the TNT trial, including those in the biobank, had a history of CHD. It is therefore likely that their statin-naive plasma PCSK9 levels, a parameter that was not measured here, were on average higher than those of the normolipemic individuals or of the asymptomatic dyslipemic patients included in the studies mentioned previously (17,18). In addition, inclusion in the TNT study was limited to those who achieved LDL-C levels below 130 mg/dl with atorvastatin 10 mg treatment for 4 weeks, to exclude patients poor responders. This has led to an underrepresentation of individuals who respond poorly to atorvastatin, likely those with a high PCSK9 activity. As a result, LDL-C levels were sharply reduced (by 40% on average) and similar in patients randomized in the TNT cohort. Because statins up-regulate PCSK9 expression, it is likely that 10 mg atorvastatin daily for 8 weeks was sufficient to maximally increase circulating PCSK9 levels to a plateau in this cohort.

It is also well established that as a functional inhibitor of the LDL receptor, plasma PCSK9 is positively correlated with LDL-C in humans (18–21). Because statins simultaneously up-regulate LDL receptor and PCSK9 expression, it is not surprising that statin treatment disrupts the positive correlation between serum PCSK9 and LDL-C (17). In line with this, we did not observe any significant correlation between circulating PCSK9 and plasma lipids measured at randomization, or changes in plasma lipids upon up-titration to 80 mg. Recent reports have also indicated that there is no significant inverse correlation between baseline PCSK9 and changes in LDL-C in response to statin in 2 large cohorts (17,21). The absence of correlation between LDL-C and plasma PCSK9 upon increasing statin dose can also be explained by the opposite regulatory patterns that statins exert: 1) on PCSK9 gene expression; and 2) on the expression of a novel physiological inhibitor of the LDL receptor, the E3 ubiquitine ligase Idol in vivo (22,23). Indeed, both PCSK9 and Idol regulate cellular LDL receptor protein levels by promoting its degradation via distinct molecular pathways that are independent and additive in embryonic stem cells (23). Whether the Idol pathway is also altered upon statin treatment remains to be demonstrated in vivo (24).

The observation that plasma levels of PCSK9 measured at randomization appears to be predictive of future cardiovascular outcomes in the group maintained on 10 mg atorvastatin, but not in patients up-titrated to receive 80 mg, further extends the observation that genetic variation in PCSK9 associates with statin-induced LDL-C lowering in the entire TNT cohort (25). Together, these findings have potential implications for future strategies in secondary prevention of CHD: 1) PCSK9 inhibitors could reveal an interesting therapeutic approach on top of statins to reduce CHD risk in particular for patients in whom increase of statin dose is not feasible; and 2) it can be argued that high-dose atorvastatin eliminates the increased risk associated with high plasma PCSK9 levels, but this does not rule out the possibility that adding a PCSK9 inhibitor on top of high-dose atorvastatin may eventually provide incremental clinical benefit. It can be hypothesized that because the amount of circulating PCSK9 is not fully reflective of PCSK9 activity in vivo (21,26,27), inhibition of PCSK9 may have more beneficial effects than expected from the predictive value of PCSK9 plasma levels.

Considerable progress has been made in the development of PCSK9 inhibitors in the last few years (28–31). In this regard, our results raise the possibility that PCSK9 inhibition may further decrease cardiovascular risk in statin-treated patients. Ultimately, however, this remains to be tested and randomized trials with PCSK9 inhibition in statin-treated patients are eagerly awaited.

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