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CLINICAL RESEARCH

Circulating PCSK9 and Risk of Myocardial Infarction

The HUNT Study in Norway

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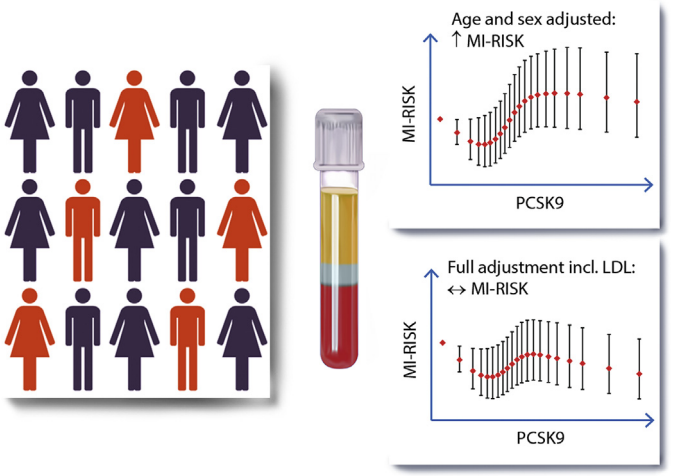
VISUAL ABSTRACT



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HIGHLIGHTS

- **The enzyme PCSK9 binds to the hepatic LDL receptor and targets it for a intracellular degradation, leading to decreased number of LDL receptor on cells and reduced removal of circulating LDL cholesterol.**
- **The usefulness of circulating PCSK9 as a marker for risk of coronary heart disease in the general population remains unclear.**
- **In this large prospective population study, serum levels of PCSK9 were modestly associated with increased risk of myocardial infarction in age- and sex-adjusted analysis. However, after adjustment for LDL-C and other lipids, the strength of the association was largely attenuated.**
- **Our findings suggest that serum levels of PCSK9 do not contribute additional useful information in cardiovascular risk assessment beyond the information provided by lipid measurements.**
- **Moreover, our results are consistent with the biological understanding of PCSK9 and of its effect on atherosclerosis being mainly mediated by changes in LDL-receptor function.**

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SUMMARY

The usefulness of circulating proprotein convertase subtilisin-kexin type 9 (PCSK9) as a risk marker of coronary heart disease in the general population remains unclear. In a nested case-control study in Norway, 1,488 incident myocardial infarctions were registered during 11.3 years of follow-up and compared with 3,819 controls. Compared with participants in the lowest quartile of PCSK9, myocardial infarction risk was 47% higher in the highest quartile after adjustment for age and sex. After additional adjustment for low-density lipoprotein cholesterol, the association was strongly attenuated. Thus, circulating PCSK9 does not contribute useful information in the assessment of myocardial infarction risk in the general population beyond the information provided by lipid measurements. (J Am Coll Cardiol Basic Trans Science 2016;1:568–75)

ABBREVIATIONS AND ACRONYMS

BMI = body mass index
CI = confidence interval
CRP = C-reactive protein
CVD = cardiovascular disease
HDL-C = high-density lipoprotein cholesterol
hsCRP = high-sensitivity C-reactive protein
LDL = low-density lipoprotein
LDL-C = LDL cholesterol
MI = myocardial infarction
OR = odds ratio
PCSK9 = proprotein convertase subtilisin-kexin type 9

The enzyme proprotein convertase subtilisin-kexin type 9 (PCSK9) binds to the hepatic low-density lipoprotein (LDL) receptor and targets it for intracellular degradation, leading to decreased number of LDL receptor on cells and reduced removal of circulating LDL cholesterol (LDL-C) (1). The importance of PCSK9 for LDL-C homeostasis is illustrated in individuals with loss- and gain-of-function mutations in this enzyme, leading to hypo- or hypercholesterolemia, respectively, with dramatic effects on the incidence of atherosclerotic cardiovascular disease (CVD) (2). Anti-PCSK9 therapies have recently been approved for clinical use and seem to reduce LDL-C levels and to lower incidence of CVD in patients with hyperlipidemia. The effect of the medication is currently under investigation in other high-risk populations (1,3,4).

Results of recent studies suggest that circulating PCSK9 is positively associated with established risk factors for CVD, including LDL-C, triglycerides, glucose, prevalent diabetes, body mass index (BMI), carotid intima media thickness, and C-reactive protein (CRP) (5–7) as well as common and low-frequency genetic variants in the PCSK9 locus (8). Furthermore, high PCSK9 levels predict adverse outcomes in patients with established CVD (9,10). To this end, however, only a few studies have examined circulating PCSK9 levels and risk of CVD in the general population, and results have been conflicting. Thus, Leander et al. (11) found a dose-dependent association between PCSK9 and incident events of CVD (n = 491) in the general population (n = 4,232). By contrast, Ridker et al. (12) found no association between PCSK9 and risk of cardiovascular events in a nested case-control study from the Women's Health Study (n = 358 in each group), and Zhu et al. (13) found no association in a follow-up of 1,527 middle-aged men.

Although PCSK9 appears to play a crucial role in atherosclerosis, the usefulness of circulating PCSK9 as a biomarker for risk of CVD in the general population remains unclear. Therefore, we conducted a nested case-control study within the general population in Norway (HUNT [Nord-Trøndelag Health] study), and assessed serum levels of PCSK9 in relation to risk of myocardial infarction (MI) among 1,488 patients with a first incident MI and 3,819 age- and sex-matched controls.

METHODS

STUDY POPULATION. The HUNT Study constitutes a large database of clinical, anthropometric, and socioeconomic information collected during 3 surveys

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of the population in Nord-Trøndelag County in Norway. Nord-Trøndelag is 1 of 19 Norwegian counties and is located in the central part of Norway. The population is stable and ethnically homogenous, with only a small percentage (3%) of people of non-Caucasian origin. The size of the population was stable between the HUNT surveys, with a net emigration out of the county of only 0.3% per year (14).

We conducted a case-control study nested within the second wave (HUNT2) of the HUNT Study. All residents in Nord-Trøndelag aged 20 years or older were invited to attend between August 1995 and June 1997. Among a total of 93,898 invited individuals, 65,215 (69%) provided information in a self-administered questionnaire and attended a clinical examination that included blood sampling (15). Among the 65,215 participants, 5,221 were excluded because they reported a history of MI, angina pectoris, or stroke, and 1,233 participants were excluded because of missing values on relevant covariates, leaving 58,761 participants eligible for follow-up.

Cases. The cohort members were followed up for a first MI, using the unique 11-digit identification number of Norwegian citizens as a linkage tool to clinical information provided by the 2 hospitals that serve Nord-Trøndelag County (Levanger and Namsos hospitals that together make up Nord-Trøndelag Hospital Trust). For the present study, follow-up for MI was complete from baseline (1995 to 1997) through 2008. MI cases were diagnosed according to the European Society of Cardiology/American College of Cardiology consensus guidelines (16), and the diagnostic criteria included symptoms of coronary ischemia, elevated serum levels of troponins and cardiac enzymes, in addition to specified electrocardiography changes.

Controls. Controls were selected from the underlying cohort using incidence density sampling, which is the recommended procedure for nested case-control studies (17). Before control selection, we had no information to indicate whether a sufficient serum volume was available, and we oversampled the controls, but as a minimum, 2 controls with available serum were selected per MI case, matched by age at risk and sex. Use of age at risk as a time dimension allows a very precise adjustment for age and is recommended for epidemiological studies in which the outcome is strongly age-dependent, as is the case for MI (18).

Among the 58,761 participants who were eligible for inclusion at baseline, 1,700 participants were diagnosed in hospital with a first incident MI during a mean follow-up of 11.3 years. A total of 1,587 of these patients had a stored serum volume that was sufficient for PCSK9 analysis. As controls, 3,959 participants with sufficient and available serum volume were matched to the cases by age at risk and by sex.

The study was approved by the regional committee for ethics in medical research, by the National Directorate of Health, and by the Norwegian Data Inspectorate. Written informed consent to participate in the study was obtained from each participant.

SERUM PCSK9 AND HIGH-SENSITIVITY C-REACTIVE PROTEIN. Peripheral venous blood was drawn into pyrogen-free tubes without any additives. After clotting the blood at room temperature, the tubes were centrifuged at 1,500*g* for 10 min and serum was stored at -70°C. Serum levels of PCSK9 and high-sensitivity C-reactive protein (hsCRP) were measured in duplicate by enzyme immune-assay with antibodies obtained from R&D Systems (Minneapolis, Minnesota). To evaluate the impact of postprandial variation, we collected serum samples from 15 individuals sampled fasting at 8:00 AM and nonfasting at 8:00 AM the next day. We found no effect of fasting as on PCSK9 (7% difference; $p = 0.51$). The intra- and interassay coefficients of variations were 4.2% and 2.5% for PCSK9 and 2.6% and 9.1% for hsCRP.

CLINICAL INFORMATION AT BASELINE. The clinical examination in HUNT2 was conducted by trained nurses and included standardized measurements of blood pressure, weight, height, and waist and hip circumference. Systolic and diastolic blood pressure were measured with a Dinamap 845XT (Criticon/GE Healthcare, Tampa, Florida) based on oscillometry, and the average of the second and third measurement was used in the analysis. Height and weight were recorded with participants wearing light clothes without shoes, and BMI was computed as weight (in kilograms) divided by the squared value of height (in meters). Information on health was collected by means of a self-administered questionnaire. Responses to questions related to smoking were categorized as current, previous, or never smoking.

Creatinine was measured by the Jaffe method, and the day-to-day coefficient of variation was 3.5%. Nonfasting serum concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured in fresh serum samples at Levanger Hospital using enzymatic colorimetric methods with reagents from Boehringer Mannheim on a Hitachi 911 Autoanalyzer (Roche Diagnostics, Indianapolis, Indiana). The day-to-day coefficients of variation were 1.3% to 1.9%, 2.4%, and 0.7% to 1.3%, respectively. LDL-C was calculated using Friedewald's formula (i.e., LDL-C = total cholesterol − HDL-C − [triglycerides/2.2]). Participants with

triglycerides ≥4.52 mmol/l were excluded from the analyses because Friedewald's formula is invalid in this situation. Thus, the final study population consisted of 1,488 cases and 3,819 controls.

STATISTICAL ANALYSIS. The distribution of baseline characteristics across PCSK9 quartiles was compared using the chi-square test for categorical variables (%) and 1-way analysis of variance for continuous variables (mean ± SD). All covariates were tested for normality and log transformations were performed to normalize hsCRP and creatinine levels. Pearson correlation coefficients were calculated to evaluate correlation between PCSK9 and the other covariates.

We used conditional logistic regression analysis to estimate age- and sex-adjusted odds ratios (ORs, with 95% confidence intervals [CIs]) of MI according to quartile categories of PCSK9 concentrations, based on the quartile distribution of PCSK9 among the controls. Because statistical analyses were stratified on groups of each patient and his or her matched controls using conditional logistic regression analysis, the unequal number of controls for each case did not bias the estimates. For tests of trend of the association across PCSK9 quartiles, we assigned each participant the median PCSK9 level within his or her quartile and treated these median values as a continuous variable in the logistic models. We used a restricted cubic spline analysis with 5 knots to assess linearity of the association of PCSK9 with MI risk. In a separate analysis, we also estimated the risk of MI associated with a 1 SD higher serum PCSK9 level assessed as a continuous variable.

In the multivariable analyses of PCSK9 levels and MI risk, we adjusted for the following variables: BMI, LDL-C, HDL-C, systolic blood pressure, diastolic blood pressure, log-transformed hsCRP and creatinine, smoking (current, previous, or never smoking), and diabetes mellitus (yes/no). The inclusion of potential confounders to our models was based on previous knowledge concerning their relation to PCSK9 and to risk of MI (5-7,11,17). Established cardiovascular risk factors such as high blood pressure, high BMI, smoking, dyslipidemia, reduced kidney function, diabetes mellitus, and hsCRP may act as both confounding and mediating factors for the association of PCSK9 level with MI risk. We therefore analyzed the data both with and without the factors included in the analysis.

We tested for effect modification by BMI, LDL-C, blood pressure (high blood pressure was defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg), smoking status, diabetes mellitus, creatinine, and hsCRP. Statistical interaction was assessed on the multiplicative scale by deviance tests based on comparisons of likelihoods in nested models with and without cross-product terms.

In a sensitivity analysis, we analyzed the first 5 years and the rest of the follow-up separately to assess whether the associations of PCSK9 and risk of early MI events differed from risk of late MI events. In another sensitivity analysis, we adjusted for the continuous established cardiovascular risk factors expressed as restricted cubic splines to account for potential nonlinear associations of these risk factors with MI risk. In another set of separate analyses, we adjusted for untransformed hsCRP and creatinine instead of the log-transformed variables. Because blood sampling was nonfasting, blood lipid values, especially triglyceride levels, could be influenced by recent food intake. In a sensitivity analysis, we therefore adjusted for time since last meal.

All statistical analyses were conducted in STATA, version 12.2, for Windows (StataCorp LP, College Station, Texas).

RESULTS

BASELINE CHARACTERISTICS. At baseline, mean age was 66.2 ± 12.8 years, and 62.9% of the participants were men. PCSK9 concentrations were slightly skewed to the right, as shown in Supplemental Figure 1. Among MI cases, the median PCSK9 concentration was 128 ng/ml (interquartile range: 76 to 182 ng/ml) compared to 121 ng/ml (interquartile range: 69 to 174 ng/ml) among controls ($p < 0.001$). The median value was higher in women than in men; 136 ng/ml versus 116 ng/ml ($p < 0.001$).

The baseline characteristics of participants within each PCSK9 quartile are shown in **Table 1**. Participants in the higher quartiles of PCSK9 were more likely to be women; current smokers; have higher levels of creatinine, hsCRP, and BMI; and generally a more unfavorable serum lipid profile compared with those in the lower quartiles. However, levels of HDL-C were higher among participants in the higher quartiles of PCSK9. Assessed as a continuous variable, serum PCSK9 concentrations correlated positively with LDL-C ($r = 0.21$; $p < 0.001$), total cholesterol ($r = 0.27$; $p < 0.001$), triglycerides ($r = 0.12$; $p < 0.001$), BMI ($r = 0.12$; $p < 0.001$), and log-transformed (hsCRP) ($r = 0.18$; $p < 0.001$).

PCSK9 IN SERUM AND RISK OF MI. **Table 2** presents the age- and sex-adjusted and several multivariable-adjusted ORs for MI according to PSCK9 quartiles and per SD higher serum PCSK9 level. Compared with

TABLE 1 Baseline Characteristics by Quartiles of PCSK9: The HUNT Study

	Total (N = 5,307)	Quartile 1 (n = 1,264)	Quartile 2 (n = 1,281)	Quartile 3 (n = 1,363)	Quartile 4 (n = 1,399)	p Value
PCSK9, ng/ml		<98	98 –<121	121 –<151	>151	
PCSK9, ng/ml	123 (53)	84 (22)	110 (11)	134 (15)	177 (39)	
Age, yrs	66.2 ± 12.8	65.2 ± 13.3	66.2 ± 12.7	67.3 ± 12.4	66.0 ± 12.6	0.08
Male	3,340 (62.9)	1,004 (79.4)	859 (67.1)	786 (57.7)	691 (49.4)	<0.001
Current smoking	1,466 (27.6)	357 (28.2)	329 (25.7)	373 (27.4)	407 (29.1)	0.001
Diabetes mellitus	280 (5.3)	82 (6.5)	70 (5.5)	61 (4.5)	67 (4.8)	0.10
Systolic blood pressure, mm Hg	150.7 ± 23.8	148.1 ± 23.2	148.9 ± 24.2	152.1 ± 23.1	153.2 ± 24.3	0.13
Diastolic blood pressure, mm Hg	85.3 ± 12.1	84.7 ± 12.1	84.5 ± 12.3	86.2 ± 12.0	85.8 ± 11.8	0.45
Total cholesterol, mmol/l	6.4 ± 1.2	5.9 ± 1.0	6.2 ± 1.1	6.5 ± 1.2	6.8 ± 1.3	<0.001
LDL-C, mmol/l	4.2 ± 1.1	3.8 ± 0.9	4.1 ± 1.0	4.3 ± 1.1	4.5 ± 1.2	<0.001
Triglycerides, mmol/l	1.9 ± 0.8	1.7 ± 0.8	1.8 ± 0.8	1.9 ± 0.8	2.0 ± 0.9	0.03
HDL-C, mmol/l	1.35 ± 0.39	1.33 ± 0.3	1.32 ± 0.36	1.37 ± 0.39	1.38 ± 0.40	0.001
BMI, kg/m^2	26.9 ± 3.9	26.3 ± 3.5	26.6 ± 3.7	27.1 ± 4.0	27.5 ± 4.2	<0.001
Creatinine, μmol/l	90.0 ± 18.0	92.0 ± 18.0	91.0 ± 19.0	90.0 ± 17.0	88.0 ± 18.0	<0.001
hsCRP median, mg/l	2.1 (2.4)	1.7 (2.0)	2.0 (2.2)	2.2 (2.6)	2.5 (2.6)	<0.001

Values are range, median (interquartile range), mean ± SD, or n (%).

BMI = body mass index; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; HUNT = the Nord-Trøndelag Health Study; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; PCSK9 = proprotein convertase subtilisin-kexin type 9.

participants in the lowest quartile, MI risk was 47% higher among participants in the highest quartile, after adjustment for age and sex. Across quartiles, the ORs from lowest (reference) to highest serum PCSK9 quartile were 1.16 (95% CI: 0.96 to 1.40), 1.35 (95% CI: 1.13 to 1.63), and 1.47 (95% CI: 1.23 to 1.77), (p for linear trend <0.001). However, after adjustment for established cardiovascular risk factors, including serum lipids, BMI, diabetes, smoking, blood pressure, creatinine, and hsCRP (Table 2, model 4), the association of PSCK9 with MI risk was strongly attenuated, and there was no convincing trend across quartiles (p for linear trend = 0.53). Among factors included in the multivariable analysis, LDL-C appeared to be the single most important factor to attenuate the association of PCSK9 with MI risk, followed by serum triglycerides and HDL (Table 2, models 2 and 3).

Using PCSK9 as a continuous variable, the OR of MI per SD (47 ng/ml) higher serum PCSK9 at baseline was 1.13 (95% CI: 1.07 to 1.20) in the age- and sex-adjusted analysis (Table 2). We also expressed PCSK9 concentrations as restricted cubic splines, and found that the age- and sex-adjusted association of PCSK9 with MI risk displayed a nonlinear shape. Thus, there was a gradual increase in MI risk starting at approximately 100 to 175 ng/ml of PCSK9, followed by a leveling off (Figure 1A). After adjustment for established cardiovascular risk factors, the cubic spline analysis suggested that PCSK9 concentrations were not associated with increased MI risk (Figure 1B).

TABLE 2 Association of PCSK9 With Risk of MI: The HUNT Study

	PCSK9 Quartiles (ng/ml)						
	Q1 (<98)	Q2 (98–<121)	Q3 (121–<151)	Q4 (>151)	Linear Trend*	p for Linear Trend*	Per 1 SD† Higher PCSK9
Cases	298 (23.6)	336 (26.2)	407 (29.9)	447 (32.0)			
Controls	966 (76.4)	945 (73.8)	956 (70.1)	952 (68.0)			
Model 1‡	1.00 (ref)	1.16 (0.96-1.40)	1.35 (1.13-1.63)	1.47 (1.23-1.77)	1.04 (1.02-1.06)	<0.001	1.13 (1.07-1.20)
Model 2§	1.00 (ref)	1.08 (0.89-1.30)	1.18 (0.97-1.42)	1.17 (0.97-1.42)	1.02 (1.00-1.04)	0.09	1.04 (0.97-1.10)
Model 3‖	1.00 (ref)	1.04 (0.86-1.26)	1.12 (0.92-1.35)	1.10 (0.90-1.34)	1.01 (0.99-1.03)	0.32	1.03 (0.96-1.09)
Model 4¶	1.00 (ref)	1.06 (0.87-1.29)	1.10 (0.90-1.34)	1.07 (0.88-1.32)	1.01 (0.99-1.03)	0.53	1.02 (0.95-1.09)

Values are n (%) or odds ratio (95% confidence interval). *For tests of trend of the association, we assigned each participant the median PCSK9 level within his or her quartile, and treated these median values as a continuous variable in the logistic models. †1 SD = 47 ng/ml. ‡Adjusted for age and sex. §Adjusted for age, sex, and LDL-C. ‖Adjusted for age, sex, LDL-C, HDL-C, and triglycerides. ¶Adjusted for age, sex, diabetes mellitus, LDL-C, BMI, smoking, systolic blood pressure, diastolic blood pressure, triglycerides, HDL-C, log-transformed creatinine, and log-transformed C-reactive protein.

CI = confidence interval; OR = odds ratio; other abbreviation as in Table 1.

We found no statistical evidence for any effect modification of PCSK9 by LDL-C (p for interaction = 0.51), and no evidence that the association of serum PCSK9 with MI risk could be modified by other cardiovascular risk factors, including BMI, current smoking, hypertension, diabetes mellitus, creatinine, or hsCRP (all p for interaction >0.05).

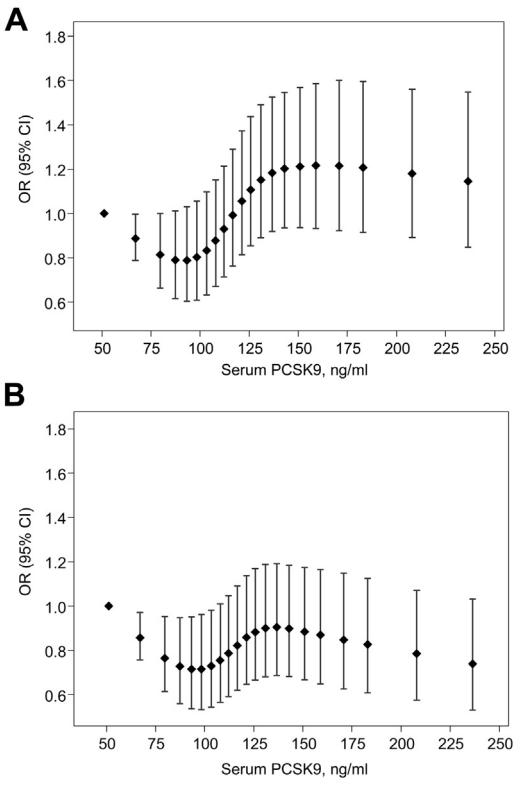
SENSITIVITY ANALYSIS. In sensitivity analyses (Supplemental Tables 1A and 1B), the estimated associations did not differ for MI events (N = 559) that occurred during the first 5 years of follow-up compared with later MI events (N = 929) (p for interaction with early vs. late follow-up = 0.96). Using restricted cubic splines of the continuous established cardiovascular risk factors in the multivariable analysis showed results that were similar to the main findings (Supplemental Table 2). Adjustment for untransformed hsCRP and creatinine instead of the log-transformed variables showed similar results (data not shown). Adjustment for time between last meal and the venipuncture had virtually no effect on the results (data not shown).

DISCUSSION

In this large prospective population study of individuals free of clinical CVD at baseline, serum levels of PCSK9 were positively but modestly associated with risk of MI in age- and sex-adjusted analysis. However, after adjustment for established CVD risk factors, the strength of the association was strongly attenuated. Most of the attenuation could be attributed to LDL-C followed by serum triglycerides and HDL-C. Thus, our results suggest that the association of PCSK9 may reflect an effect involving the metabolism of lipids rather than being an independent risk factor for MI. Moreover, our results are consistent with the biological understanding of PCSK9 and of its effect on atherosclerosis being mainly mediated by changes in LDL-receptor function.

The association of PCSK9 with CVD risk has not been well-studied in population studies. A few relatively small studies from the general population have prospectively investigated circulating PCSK9 levels and risk of CVD, and the results have been conflicting (11–13). In a nested case-control study from the Nurses' Health Study, including 358 women in each group, Ridker et al. (12) reported that PCSK9 levels were not associated with increased risk of CVD events during 17 years of follow-up. Similarly, Zhu et al. (13) found no evidence for a strong association between log-transformed serum PCSK9 and CVD events in 1,527 middle-aged men over a 7-year follow-up after adjustment for established cardiovascular risk factors (hazard ratio: 1.16 [95% CI: 0.93 to 1.44]). Moreover, PCSK9 was not associated with measures of vascular structure and function, such as carotid intima media thickness or flow-mediated dilatation. However, direct comparison of these studies is complicated by sex differences because the association between LDL-C and PCSK9 may be sex-dependent (19). Results of a recent study suggested that circulating PCSK9 may be elevated either immediately before or at the time of MI; therefore, time from baseline measurement until event may be too long to capture any effect of PCSK9 (20). We could not address that possibility, but in a sensitivity analysis, we explored whether PCSK9 might reflect preclinical or underlying

FIGURE 1 Sex- and Age- and Multi-Adjusted* Association Between Serum PCSK9 Concentrations (Expressed as a Restricted Cubic Spline) and Risk of Incident MI (ORs; 95% CI)



(A) Sex- and age-adjusted; **(B)** multi-adjusted. *Adjusted for age, sex, diabetes mellitus, LDL-C, BMI, smoking, systolic blood pressure, diastolic blood pressure, triglycerides, HDL-C, log-transformed creatinine, and log-transformed C-reactive protein. †ORs with 95% CIs are displayed at the 2.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 97.5 percentiles of PCSK9, based on the quantile distribution among controls and using the 5th percentile as the reference level. BMI = body mass index; CI = confidence interval; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MI = myocardial infarction; OR = odds ratio; PCSK9 = proprotein convertase subtilisin-kexin type 9.

disease by excluding the first 5 years of follow-up, but the results remained essentially unchanged.

However, contradictory to these previous findings, the recent prospective study of Leander et al. (11) reported a positive dose-dependent association between PCSK9 and risk of CVD events. During 15 years of follow-up, 491 of 4,232 men and women 60 years of age at baseline had an incident CVD event. In that study, the strength of the crude association of PCSK9 with CVD risk was roughly comparable to the strength of the age- and sex-adjusted result in the present study. Whereas adjustment for LDL-C attenuated the association in our study, serum PCSK9 was positively associated with CVD risk also after extensive covariate adjustment, including LDL-C, in the study by Leander et al. (11) Thus, the adjusted hazard ratios across PSCK9 quartiles were 1.15 (95% CI: 0.88 to 1.50), 1.24 (95% CI: 0.94 to 1.63), and 1.48 (95% CI: 1.12 to 1.95), p for trend 0.0063. Although our outcomes were based on incident hospitalizations because of MI, the study by Leander et al. evaluated a composite endpoint of ischemic CVD events including both fatal and nonfatal MI and stroke, as well as chronic ischemic heart disease and sudden cardiac death. Moreover, because of a limited number of CVD events, the authors did not report subgroup analyses for MI and stroke; thus, it is unknown whether the stronger association with CVD events in their study could partly be driven by cause-specific events. Although the association between lipids and coronary heart disease is firmly established, the association between lipids and ischemic stroke is not equally robust (21). The stronger association observed in the study of Leander et al. (11) could therefore reflect lipid independent cardiometabolic (e.g., inflammation, apoptosis) effects of PCSK9 with a stronger impact on non-MI CVD (22).

Strengths of the present study include the relatively large sample size and the selection of a minimum of 2 controls for each of the 1,488 MI cases, preserving two-thirds of the statistical power that would be achieved by using the whole cohort. Other strengths are the prospective recording of incident MIs, and that biases resulting from recall or self-selection are avoided. Also, the incidence density sampling secures unbiased OR estimates of the incidence rate ratios that would be calculated in the underlying cohort. Thus, this type of nested case-control study is prospective in design and considered to be equivalent to cohort studies in terms of validity (17). Moreover, all MIs were verified in the hospitals, and the specificity of the diagnosis is likely to be very high (17). It is a limitation of the study that we cannot exclude that serum concentrations of PCSK9 and hsCRP may have changed during the approximately 15 years of storage before analysis. However, storage time did not differ between cases and controls, and it seems unlikely that any changes during storage time would systematically differ between cases and controls. Furthermore, we cannot exclude the possibility of confounding by unmeasured factors behind our observations, including lipoprotein(a), which was not measured in the HUNT study. However, to influence our results substantially, any remaining confounder would need to be strongly associated with both PCSK9 and MI and generally be unrelated to the other factors we included in our multivariable models. Moreover, we had no information on the use of statins, and statins may increase PCSK9 levels (23) and are also beneficial for the prevention of MI; thus, the use of statins may potentially confound the association of PCSK9 with MI risk. However, the use of statins as primary cardiovascular prevention was uncommon in Norway at the time of the HUNT survey in 1995 to 1997 (24), and in our study, we only included individuals with no history of CVD (MI, angina, or stroke). Thus, confounding from use of statins should be limited in our study. PCSK9 has been reported to display considerable diurnal variation (25,26). In the HUNT study, all blood samples were drawn between 8:00 AM and 4 PM, but we had no exact information on time during the day the blood sampling was done. However, adjustment for time between last meal and blood sampling had no effect on the estimated results. Nevertheless, a standardized sampling at 1 time would have been the best, and this represent a limitation in our study.

CONCLUSIONS

High levels of PCSK9 were positively but modestly associated with risk of MI in age- and sex-adjusted analysis, but the association was largely attenuated after adjustment for LDL-C. Our results are consistent with the biological understanding of PCSK9 and of its effect on atherosclerosis being mainly mediated by changes in LDL-receptor function. Whereas PCSK9 has emerged as a novel target for preventing and limiting the development of coronary artery disease, our findings suggest that blood levels of PCSK9 do not contribute additional useful information in cardiovascular risk assessment beyond the information provided by lipid measurements.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Anti-PCSK9 therapies seem to reduce LDL-C levels and to lower incidence of CVD. The biological effect of PCSK9 on atherosclerosis is mainly mediated by changes in LDL-receptor function.

TRANSLATIONAL OUTLOOK: Measuring serum levels of PCSK9 does not contribute useful information in the assessment of risk of MI in the general population beyond the information provided by lipid measurements.

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KEY WORDS PCSK9 inflammation, epidemiology, myocardial infarction, prospective study, risk factors

APPENDIX For a supplemental figure and tables, please see the online version of this article.