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EDITORIAL COMMENT

The PCSK9 Problem

Is it Predictive, Punitive, or Puzzling?*

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a low-abundance plasma protein that promotes the degradation of the low-density lipoprotein (LDL) receptor (LDLR), thus contributing to elevated LDL cholesterol concentration (1). Two main molecular forms of PCSK9 are found in circulation: a 62-kDa mature form (PCSK9_62) and a 55-kDa furin-cleaved form (PCSK9_55). Commercially available enzyme-linked immunosorbent assays do not discriminate between these 2 forms. Therefore, the relationship between the circulating subpopulations of PCSK9 and coronary heart disease (CHD) outcomes is underexplored. In this issue of *JACC: Asia*, Kataoka et al (2) demonstrate that PCSK9_55 is the only molecular form—among total PCSK9, PCSK9_62, and PCSK9_55—that associates with circulating high-sensitivity C-reactive protein (hsCRP), high diastolic blood pressure, and incident coronary events in 1,436 statin-naive Japanese patients over 13.6 years of follow-up.

The mature and furin-cleaved PCSK9 forms exhibit distinct kinetics and functional properties—the latter displays a shorter half-life, ineffectively degrades the LDLR, and is inefficiently secreted (3), suggesting that their link to disease outcomes may also differ. Kataoka et al (2)—using their molecular form-specific, noncommercial enzyme-linked immunosorbent assay—measured the circulating total, mature, and furin-cleaved PCSK9 levels and assessed their relation to cardiovascular risk factors, such as atherogenic lipid fractions, inflammation, and blood pressure parameters. Although the second and third tertiles of total and mature PCSK9 did not correlate with ischemic stroke, CHD, and a composite of both CHD and stroke events, the second and third tertiles of furin-cleaved PCSK9 associated with CHD and composite events. Furthermore, plasma hsCRP levels and systolic blood pressure were directly associated with only furin-cleaved but not with mature or total PCSK9 levels. These findings highlight the concept that each molecular form displays distinct biology and carries exclusive associations with cardiovascular risk factors and outcomes. As the investigators duly acknowledge, the molecular mechanisms underpinning these observations are currently unknown.

The notion that plasma PCSK9 levels may have utility as a biomarker for risk assessment has thus far been studied mostly in patient cohorts treated with lipid-lowering therapies. Although elevated serum PCSK9 levels seem to associate with future CHD events for statin-treated patients in some reports, the true value for risk assessment in primary prevention lies in statin-naive cohorts. In this group, serum PCSK9 levels were associated with carotid intima-medial wall thickness, a measure of subclinical atherosclerosis and predictor of future coronary events in asymptomatic individuals in Dutch, Korean, and Australian community-based cohorts (4-6). On the other hand, in a statin-naive prospective primary prevention subcohort of the Women's Health Study, plasma PCSK9 levels did not predict future CHD events (7). Accordingly, in the statin-naive Japanese cohort, although total and mature PCSK9 levels did not associate with ischemic stroke, CHD, and composite events, furin-cleaved PCSK9_55 did predict these events. Extending these findings to patients in the clinical setting would, of course, first require analyses that adjust for statin use and the canonical

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cardiovascular risk factors, including some metric of LDL.

In addition to the proteolytic cleavage that leads to 2 major circulating forms, PCSK9 undergoes posttranslational modifications, such as binding to APOB-containing lipoproteins, *N*-glycosylation, and phosphorylation, that affect its biological activity. Using targeted mass spectrometry approaches, Gauthier et al (8) have shown that the serine phosphorylation at the 688 residue protects PCSK9 from furin cleavage and improves its LDLR degradation capacity. Although statin therapy is known to raise plasma PCSK9 levels, 1 year of statin therapy resulted in an increase in 7 measured plasma PCSK9 peptides, with a decrease in the circulating phosphorylated (at serine 688) PCSK9 (8). Because commercial enzyme-linked assays measure total PCSK9 regardless of its cleavage or phosphorylation state, studies with total PCSK9 levels might miss the associations between the PCSK9 forms and disease outcomes. For example, it is yet unknown how its phosphorylation status relates to CHD outcomes. It is possible that the increase in plasma PCSK9 levels in response to statin treatment is mostly accounted for by an inactive form, such as furin-cleaved or unphosphorylated PCSK9. In future studies, in both statin-treated and statin-naive participants, it will be important to determine how circulating post-translationally modified forms of PCSK9 relate to clinical CHD risk factors and CHD outcomes.

Hypertension is a risk factor for stroke, CHD, coronary heart disease, and peripheral arterial disease. Kataoka et al (2) report that although total PCSK9 levels were associated with systolic blood pressure, furin-cleaved PCSK9 levels were associated only with diastolic blood pressure. This observation layers yet another filter on the already complex picture that links PCSK9 to blood pressure biology. The epithelial sodium channel (ENaC) regulates sodium homeostasis and plays a central role in sodium-sensitive hypertension. In in vitro studies, PCSK9 has been shown to interact with all ENaC subunits to limit their translocation to the membrane. However, PCSK9-deficient mice did not have increased ENaC expression and alterations in their blood pressure—the lack of effect of PCSK9 on blood pressure was observed in both salt-insensitive and salt-sensitive models. Despite the lack of in vivo supportive evidence, rare and common variants in *PCSK9* are linked to blood pressure, although the variants are not reproducible across studies. The variant rs505151 (E670G) was correlated with diastolic blood pressure in both male and female hypertensive Chinese participants. In the Hypertension Genetic Epidemiology Network Genome-Wide Association Study, cumulatively, all rare *PCSK9* variants were associated with diastolic blood pressure, whereas the Chinese variant did not show any effect. Validation studies were conducted in the Reasons for Geographic and Racial Differences in Stroke cohort, where rare variant cumulative effects were associated with systolic blood pressure but not with diastolic pressure. In both study populations, the median blood pressure was higher by rare-variant status, suggesting that rare variants in *PCSK9* may be important for blood pressure regulation. Overall, although genetic studies suggest a cause-and-effect relationship, the lack of clinical studies combined with the limited understanding of the pathophysiology behind PCSK9, its molecular forms, and their contribution to blood pressure regulation portrays a complex picture that requires further research.

Atherosclerosis is an inflammatory disease, and measuring and addressing subclinical inflammation has emerged as an important component of cardiovascular disease prevention. Although inflammation induces marked changes in lipid and lipoprotein metabolism, evidence linking PCSK9 to inflammation is still being assembled. The strongest evidence to suggest a link between PCSK9 and inflammation is derived from in vivo mouse studies. Livers of mice treated with lipopolysaccharides to induce inflammation show a 25-fold increase in *Pcsk9* expression. In this study, plasma PCSK9 levels were not measured; therefore, it is unknown if the increase in hepatic gene expression translates to an increase in its plasma concentration. In a single-center, statin-naive Chinese cohort, white blood cell counts positively but modestly correlated with plasma PCSK9 levels. In cross-sectional and prospective studies, plasma PCSK9 levels were moderately associated with plasma hsCRP levels in participants not on lipid-lowering therapy. In the Dallas Heart Study, the association between PCSK9 and hsCRP was observed only in women. More recently, higher baseline plasma PCSK9 levels were associated with higher hsCRP levels in Chinese patients presenting with acute myocardial infarction, suggesting that PCSK9 might play a role in the acute phase response. Most recently, Kataoka et al (2) found an association between furin-cleaved, but not mature or total, PCSK9and hsCRP levels. Most of the aforementioned studies used univariable models and reported statistically significant but modest associations ($r = \sim 0.10$) between plasma PCSK9 and hsCRP. Moreover, patients treated with PCSK9 inhibitors or, more recently, a small interfering RNA against PCSK9, do not demonstrate reductions in plasma hsCRP levels.

Therefore, the clinical relevance of the observations linking plasma PCSK9 levels to hsCRP levels is difficult to establish.

The current study by Kataoka et al (2), in concert with many other recent reports, challenges the notion of viewing PCSK9 as a single protein entity. PCSK9 is not a monolithic unit, as is commonly conceived. Different forms of PCSK9 seem to impart different physiologic functions and prognostic significance. As such, looking exclusively at total plasma PCSK9 levels may have limited value and may even be misleading in defining its role in risk assessment. Like any good study, the current evaluation provides more questions than answers. This analysis should serve as a rallying cry for investigators to determine the underlying pathophysiology that can illuminate these interesting findings.

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