Coronary artery disease (CAD), including its acute manifestations such as myocardial infarction (MI), is one of the leading causes of morbidity and mortality worldwide, especially in countries where the development of atherosclerotic plaques is closely linked to certain dietary habits (ie, Western diet). For decades, research has focused on deciphering the detailed mode(s) of disease development and implementing appropriate prevention and treatment strategies. A widely used model systems for the induction and development of atherosclerotic lesions, which augments studies in the Apoe−/− mouse, is the Ldlr−/− mouse strain. This genetically manipulated murine model is characterized by elevated plasma cholesterol levels with the development of atherosclerotic plaques under specific dietary conditions. However, these animals do not develop spontaneous atherosclerotic lesions or plaques.

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In addition to these murine model systems, in recent years, human genome-wide association studies for CAD/MI have identified associations between increased CAD disease prevalence and distinct gene loci. Among others, a 58-kb risk locus on chromosome 9p21.3 was identified and further characterized as being associated with the severity of CAD, but not of MI. The locus was linked to primary effects on atherogenesis, rather than on acute plaque rupture causing MI. Furthermore, investigations of an expanded 500-kb region, which confirmed the 58-kb CAD core block, also revealed a unique rs518394-tagged block at chromosome 9q21.3, which was highly associated with prevalent MI. Interestingly, this rs518394-tagged block has also been linked to platelet reactivity9 and the formation of platelet–leukocyte aggregates, a sensitive marker of in vivo platelet activation that also regulates inflammatory pathways. In addition, the cell proliferation inhibitor

locus, CDKN2A, is located just upstream of the rs518394-tagged block and has been connected to the regulation of platelet counts in humans.12

Platelet inhibitors (ie, ADP-receptor inhibitors, αIbb3 inhibitors, and others) are routinely used in the care of patients with CAD. Therefore, the identification of a locus involved in platelet activation, regulation of platelet counts, and atherosclerosis builds and extends on emerging and established studies. Moreover, these and other discoveries may lead to potentially exciting new insights into CAD disease development, progression, and therapeutic targets.

In this issue of Circulation: Cardiovascular Genetics, Wang et al describe their elegant studies using murine models to drill down on the functional connection of the 58-kb risk locus on chromosome 9p21.3, the CDKN2A locus, and the status of platelet production and activity in the setting of hypercholesterolemia. Using 2 unique mouse models of Cdkn2a deficiency, both on a B6-Ldlr−/− background, these investigators determined whether the deletion of ≥1 transcripts of the CDKN2A locus would predispose to increased platelet activation and production in mice under hypercholesteremic conditions. The first model uses a heterozygous Cdkn2a knockout that mimics natural gene expression variants in humans, while at the same time avoiding the complications of spontaneous tumorigenesis, which are frequently observed in homozygous knockouts. The second, and even more humanized murine model, introduces MOLFchr4subD into the mouse genome. Introduction of this region into the murine genome confers increased susceptibility to atherosclerosis and also contains a region of homology with the human chromosome 9p21.3 locus, which has been linked to CAD/MI severity and the CDKN2/CDKN2B genes. Therefore, both models are deficient in p16INK4a and p19ARF, but not in other Cdk inhibitor-related transcripts. Notably, to interpret the result of this study, it is important and helpful to note that the MOLFchr4subD strain exhibits a more pronounced deficiency of Cdkn2a transcripts, giving the investigators the opportunity to examine a type of genetic dose–response curve.

The authors used several different methods, spanning from histological to functional platelet assays, to analyze their models. Platelet counts in both models were significantly elevated, suggesting increased megakaryopoiesis and thrombopoiesis. This was also reflected by an increase in the reticulated platelet count (a marker of newly released platelets). By analyzing bone marrow progenitor subpopulations, the authors identified an almost exclusive increase in the megakaryocyte-forming lineage. Uninhibited cell proliferation was also seen in mice deficient in Cdk-inhibitor–related transcripts. Treatment with
the cyclin-dependent kinase 4/6 inhibitor, PD0332991 / palbociclib, reversed this platelet overproduction phenotype. An in vivo marker of thrombin generation, thrombin/antithrombin complexes, also normalized after CDK4/6 inhibition. Platelet functional studies (ie, P-selectin surface expression, JON-A binding of the activated integrin αIIbβ3, platelet–neutrophil aggregate, and platelet–monocyte aggregate) and tail bleeding indicated increased activation of circulating platelets in both murine models, and this hyper-reactivity was further pronounced by introducing a Western-type diet.

What are the most important conclusions one can draw from this exciting study? First, Cdkn2a seems to be an important determinant of platelet production in hypercholesterolemic heterozygous Cdkn2a−/− deficient mice on a B6-Ldlr−/− background. As the investigators utilized 2 different genetically manipulated animal models (resulting in the same downstream consequences) with consistent findings, this approach substantially strengthens the study’s conclusions. In addition, the use of a well-defined pharmacological rescue approach targeting p16INK4a, one of the affected targets, demonstrates a transcript-specific effect. The combination of genetic and pharmacological manipulation is also a strong warranting additional studies in cell models of thrombopoiesis. Second, this study links an MI risk locus on chromosome 9q21.3 to abnormal hematopoiesis/thrombopoiesis. Genetic manipulation of this locus results in an increased number of circulating and hyper-reactive platelets that may be more susceptible to the proinflammatory effects of hypercholesterolemia. Finally, these findings also suggest the intriguing possibility that therapies targeting dysregulated megakaryopoiesis and thrombopoiesis may also modulate the risk of atherothrombosis and MI.

The exact molecular mechanisms regulating the described observations are yet not entirely deciphered. Therefore, these discoveries also highlight the need for additional investigations into the pathways regulating platelet production, atherosclerosis, and CAD risk and the interconnecting pathways. Emerging tools, technologies, and model systems may facilitate future studies. For example, genetically manipulated human CD34+ derived megakaryocytes that form proplatelets, lineage-restricted gene knockouts (eg, PF4-Cre murine models), and engineered genetic models using the CRISPR/Cas system are just some of the exciting tools now available to dissect these mechanisms. Furthermore, transcriptome and proteome analysis of these targeted murine models using RNA deep sequencing may guide us toward new unrecognized targets for future atherosclerosis research projects.

Sources of Funding

This work was supported by the National Heart Lung and Blood Institute (grant HL.126547 to Dr Schwertz, and grants HL.126547 and HL.112311 to Dr Rondina) and the National Institute of Aging (grant AG048022 to Dr Rondina).

Disclosures

None.

References


Key Words: Editorials ■ blood platelets ■ genetics ■ megakaryocytes ■ mice ■ mice, knockout
Cdkn2a Orchestrates Platelet Production and Reactivity in Atherosclerosis
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doi: 10.1161/CIRCGENETICS.116.001479
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

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