Circ Cardiovasc Genet
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DOI: 10.1161/CIRCGENETICS.116.001479.
DOI: 10.1161/CIRCGENETICS.116.001479.)
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Circ Cardiovasc Genet is available at http://circgenetics.ahajournals.org
DOI: 10.1161/CIRCGENETICS.116.001479

Editorial

Cdkn2a Orchestrates Platelet Production and Reactivity in Atherosclerosis
Hansjörg Schwertz, MD, PhD; Matthew T. Rondina, MD

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 detailed mode(s) of disease development and implementing appropriate prevention and treatment strategies. A widely used model system for the induction and development of atherosclerotic lesions, which augments studies in the Apoe−/− mouse, model systems for the induction and development of atherosclerotic lesions 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 Cdkn2a/Cdkn2b genes. Therefore, both models are deficient in p16ink4a, p19ARF, p14ARF, and P14ARF, 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 strength 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.16 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,17 lineage-restricted gene knockouts (eg, PF4-Cre murine models18), and engineered genetic models using the CRISPR/Cas system19 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 sequencing20,21 may guide us toward new unrecognized targets for future athero- sclerotic research projects.

Sources of Funding
This work was supported by the National Heart Lung and Blood Institute (grant HL126547 to Dr Schwert) and grants HL126547 and HL112311 to Dr Rondina) and the National Institute of Aging (grant AG048222 to Dr Rondina).

Disclosures
None.

References


**Key Words:** Editorials • blood platelets • genetics • megakaryocytes • mice • mice, knockout
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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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http://circgenetics.ahajournals.org/content/9/3/203

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