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 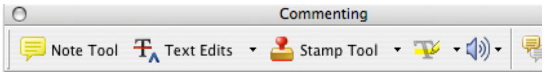

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 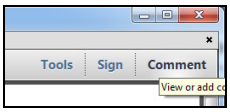

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 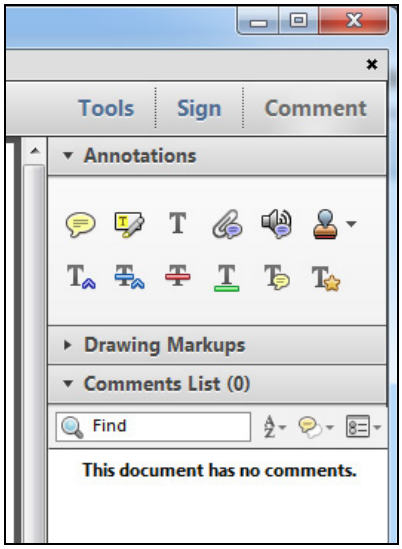
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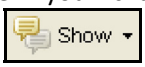
Review	To review your changes, click on the 'Show' button  on the Commenting tool bar. Choose 'Show Comments List'. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.	Your changes will appear automatically in a list below the Comment tool bar. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.
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Letters

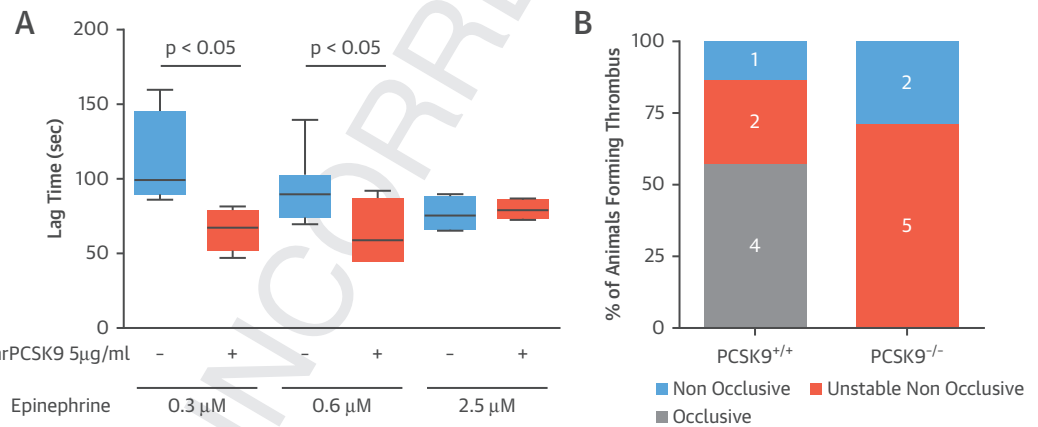
PCSK9 as a Positive Modulator of Platelet Activation

High circulating levels of proprotein convertase subtilisin/kexin 9 (PCSK9) were shown to be predictive of cardiovascular events in patients with atrial fibrillation and in those with acute coronary syndrome (1,2). Because high PCSK9 plasma levels were significantly correlated with 11-dehydro-thromboxane B_2 (11-dh-TxB_2) (1) and with platelet reactivity (2), it is conceivable to hypothesize a direct effect of PCSK9 on platelet activation. In this study, we investigated the effect of PCSK9 on platelet aggregation, activation, and thrombosis in in vitro and in vivo experimental models.

We assessed the effect of human recombinant PCSK9 (hrPCSK9) (5 μg/ml; BPS Bioscience, San Diego, California) on epinephrine-induced platelet aggregation performed with light transmission aggregometry on platelet-rich plasma samples of healthy donors (n = 8; 50% males; mean 35 ± 6 years of age). Platelet activation markers (activated glycoprotein IIbIIIa [GpIIbIIIa], P-selectin, and platelet-leukocyte aggregates) were assessed by using whole-blood flow cytometry with specific monoclonal antibodies. Contribution of PCSK9 to arterial thrombosis was evaluated in a $FeCl_3$-induced carotid artery injury model in adult $PCSK9^{+/+}$ and $PCSK9^{-/-}$ mice (n = 7/group).

hrPCSK9 added to platelet-rich plasma samples significantly enhanced platelet aggregation induced by subthreshold concentration of epinephrine (0.3 and 0.6 μM), reducing the lag time (~40%) (Figure 1A) and increasing the area under the curve (~15%). Whole-blood flow cytometry analysis showed that hrPCSK9 significantly increased (+36%) the percentage of platelets expressing activated-GpIIbIIIa (data not shown). When maximal aggregation was induced by the highest concentration of epinephrine used (2.5 μM), no more enhancing effect of the protein was observed.

FIGURE 1 PCSK9, Platelets, and Thrombus Formation



(A) Lag time values of platelet aggregation (Born's method) induced by epinephrine in the absence or presence of human recombinant PCSK9 (hrPCSK9) are shown. The **central line** illustrates median and **box limits** indicate the 25th and 75th percentiles, respectively. **(B)** Type of thrombi formed on carotid arteries of $PCSK9^{+/+}$ and $PCSK9^{-/-}$ mice upon application of $FeCl_3$. **Histograms** report the percentage of occlusive thrombi (flow rate <80% of initial blood flow; **gray box**: number of animals); unstable nonocclusive thrombi with transient and partial occlusion (flow rate <70% of initial blood flow) that resolved **(red boxes)**; and nonocclusive thrombi whose arteries remained patent throughout the period of observation **(blue boxes)**.

The loss of PCSK9 reduced the formation and stability of arterial thrombus and platelet function in mice. The topical application of $FeCl_3$ was unable to induce, in a 30-min observation period, a total occlusion of carotid artery in all $PCSK9^{-/-}$ mice, and ~70% of them formed unstable nonocclusive thrombi. By contrast, 57% of $PCSK9^{+/+}$ mice displayed a total occlusion with stable thrombi within 15.8 ± 7.4 min after application of $FeCl_3$ (**Figure 1B**). Moreover, platelet activation, assessed by flow cytometry in terms of activated GpIIbIIIa and P-selectin expression and of platelet-leukocyte aggregates, was reduced by approximately 60% in the mutant mice compared to the rate in $PCSK9^{+/+}$ mice in response to injury (data not shown).

Overall, these data identify a new actor in the complex scenario of platelet activation. Plasma levels of PCSK9 predict cardiovascular events in coronary artery disease patients. By contrast, carriers of a loss-of-function mutation in the *PCSK9* gene are largely protected from the incidence of coronary heart disease (3). However, the reductions in events associated with PCSK9 sequence variations are larger than those predicted from low-density lipoprotein (LDL)-lowering trials (3). Lifelong reductions in plasma LDL-cholesterol were proposed as the mechanisms behind this finding. This explanation, however, may be reviewed in light of our data. Indeed, platelet activation plays a key role not only in thrombus formation but also in the onset and progression of atherothrombotic disease (4). The genetic variations of a positive modulator of platelet activation may thus contribute, together with the reduced levels of LDL-cholesterol, to the benefit of *PCSK9* loss-of-function mutations in terms of risk of cardiovascular events. Our data showing that $PCSK9^{-/-}$ mice are protected from arterial thrombosis fit well with this hypothesis, further highlighting the possible direct effect of PCSK9 on platelet function.

Taken together, this study adds circulating platelets to the list of targets of PCSK9, thus opening the possibility for this protein to act directly on mechanism(s) not yet suspected. Our hypothesis needs to be confirmed in clinical trials where the monoclonal antibodies to PCSK9 may carry the added value to control platelet reactivity.

*Marina Camera, PhD
Laura Rossetti, PhD
Silvia S. Barbieri, PhD
Ilaria Zanotti, PhD
Barbara Canciani, PhD
Daniela Trabattoni, MD
Massimiliano Ruscica, PhD
Elena Tremoli, PhD
Nicola Ferri, PhD

*Dipartimento di Scienze Farmacologiche e Biomolecolari
Università degli Studi di Milano
Via Balzaretti 9
20133, Milan
Italy
E-mail: marina.camera@unimi.it

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