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Full Length Article

Characterization of hemostasis in mice lacking the novel thrombosis susceptibility gene *Slc44a2*

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A B S T R A C T

Introduction: Recent genome wide association studies (GWAS) identified a novel susceptibility locus for thrombosis, harbouring the *SLC44A2* gene which encodes the Solute Carrier Family 44 Member 2 protein (SLC44A2). Thus far, SLC44A2 has not been studied in the context of thrombosis, and may be a unique contributor to thrombotic disease. Here we utilize mice lacking SLC44A2 (*Slc44a2*$^{-/-}$) to evaluate a possible role of SLC44A2 in hemostasis.
Methods: *Slc44a2*$^{-/-}$ mice were evaluated in key aspects of normal hemostasis including a challenge of vascular damage by applying laser induced injury to the cremaster muscle arteriole.
Results: *Slc44a2*$^{-/-}$ mice had comparable levels of thrombin generation and gene expression of coagulation related genes, as compared to littermate wild type controls. Lower levels of circulating plasma Von Willebrand factor (VWF) were measured in *Slc44a2*$^{-/-}$ mice, while no difference in VWF multimerization or vascular localization was detected. Upon *in vivo* laser injury of the cremaster arterioles, we detected an impairment of clot formation for *Slc44a2*$^{-/-}$ mice.
Conclusions: Although mice lacking SLC44A2 are normal for several hemostasis parameters, we do observe a reduction of plasma VWF levels and an altered response upon vascular damage, which suggests that SLC44A2 contributes to hemostasis upon injury. These findings are in line with the reported GWAS data and support further research on SLC44A2 in thrombosis.

1. Introduction

Thrombosis is a major contributor to the global health burden, with genetic predisposition being an important underlying element [1]. Recently, a genome wide association study (GWAS) aimed at identifying novel genetic risk factors for venous thromboembolism (VTE, *i.e.* deep vein thrombosis (DVT) and pulmonary embolism (PE)), identified a susceptibility locus containing *SLC44A2* [2]. Interestingly, unlike the loci previously known to associate with VTE (*ABO, F2, F5, F11, FGG, and PROCR*), the *SLC44A2* locus did not associate with hemostasis phenotypes included in the GWAS, such as enhanced thrombin generation, platelet counts and Von Willebrand factor (VWF) levels [2]. Moreover, a second, independent GWAS detected an association between *SLC44A2* and self-reported blood clotting events (DVT, PE, ischemic stroke), strengthening the implication that *SLC44A2* is linked to thrombosis [3]. Therefore, it is plausible that SLC44A2 plays a role in the pathophysiology of thrombosis.

SLC44A2 encodes Solute Carrier Family 44 Member 2 (SLC44A2) [4], a presumed choline transporter based on its homology to other transport proteins [5]. Notably, an SLC44A2 polymorphic site forms the human neutrophil antigen 3, an epitope for alloantibodies that mediate transfusion related acute lung injury (TRALI) [6,7]. TRALI, like thrombosis, has a central role in its pathogenesis for endothelial cells and neutrophils, both of which express SLC44A2, in addition to several other tissues [4]. Furthermore, a recent TRALI study described SLC44A2 to be a binding partner of VWF, a key molecule in hemostasis [8]. Additionally, SLC44A2 has been associated with autoimmune hearing loss, due to its importance in hair cell viability [9].

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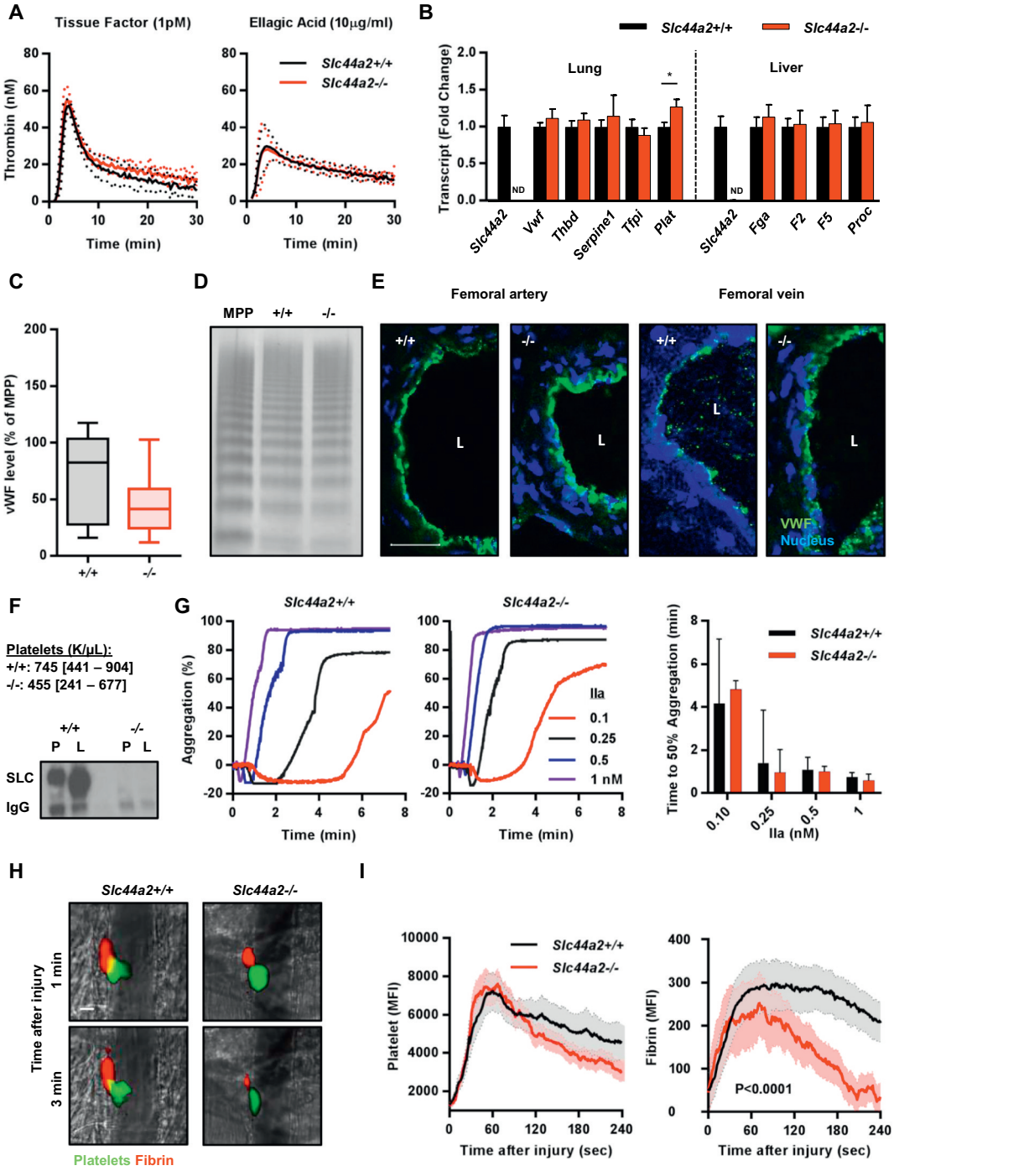
As the GWAS show an association between SLC44A2 and thrombosis, but not a mechanistic insight, further investigation of SLC44A2 with respect to its role in thrombosis is warranted. Here we utilize mice lacking SLC44A2 (*Slc44a2*$^{-/-}$) to gain insight into the role of SLC44A2, if any, in hemostasis as a precursor to further thrombosis studies. To this end we characterized *Slc44a2*$^{-/-}$ mice for several parameters of hemostasis under normal conditions including a challenge of vascular injury.

2. Material and methods

Slc44a2$^{-/-}$ mice were previously generated from mice with *Slc44a2* exons 3–10 flanked with LoxP sites (*Slc44a2*$^{fl/fl}$ mice) and deleter EIIa-Cre transgenic mice [9,10]. The resulting *Slc44a2*$^{-/-}$ mice animals were subsequently backcrossed to FVB/NJ (FVB) (7 generations) to form a FVB line at University of Michigan, USA. FVB; *Slc44a2*$^{+/-}$ mice were used to generate *Slc44a2*$^{-/-}$ mice used for experiments, and *Slc44a2*$^{+/+}$ littermates were used as controls. At Leiden University Medical Center (LUMC), In the Netherlands, a colony of *Slc44a2*$^{-/-}$ mice on an C57BL/6 J (B6) background was established starting from frozen B6;129Sv-*Slc44a2*$^{fl/+F2\text{-}N2}$ embryos obtained from the University of Michigan. After cryorecovery, genotyping [9] and backcross with B6 mice (Charles River, The Netherlands), *Slc44a2*$^{fl/+}$ mice were again combined with in house B6;EIIa-Cre transgenic mice ([10], originally obtained from Jackson Laboratories, Bar Harbor, USA). The resulting EIIa-Cre positive *Slc44a2*$^{+/-}$ mice were used to



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