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α -Galactosidase A Deficiency Leads to Increased Tissue Fibrin Deposition and Thrombosis in Mice Homozygous for the Factor V Leiden Mutation

Yuechun Shen, Peter F. Bodary, Fernando B. Vargas, Jonathon W. Homeister, David Gordon, Kristen A. Ostenson, James A. Shayman, Daniel T. Eitzman

Background—Factor V Leiden (FVL) is a common genetic risk factor for vascular thrombosis in humans. Fabry disease, an X-linked lysosomal storage disorder attributable to α -galactosidase A (GLA) deficiency, is associated with premature vascular events that may be thrombotic in nature.

Methods and Results—To examine a potential interaction between *FvL* and *Gla* deficiency in vivo, we analyzed tissue fibrin deposition in mice carrying combined mutations in *FvL* and *Gla*. *Gla* deficiency markedly increased tissue fibrin deposition in mice carrying the *FvL* mutation ($0.33 \pm 0.03\%$; $n=7$) compared with *FvL* mutation ($0.14 \pm 0.02\%$; $n=10$; $P<0.0005$).

Conclusions—These observations demonstrate a synergistic interaction between *Gla* deficiency and *FvL* toward tissue fibrin deposition in mice. Concomitant mutations in these genes may increase the penetrance of vascular thrombotic events in humans. (*Stroke*. 2006;37:1106-1108.)

Key Words: fibrin ■ genetics ■ thrombosis

A common point mutation in factor V (factor V Leiden [FVL]; 2% to 7% prevalence for European populations) leads to activated protein C resistance and thrombophilia.¹ Several reports in humans and animals have documented a synergistic risk of thrombosis when FVL is combined with other genetic modifiers of thrombosis.²

Fabry disease is an X-linked disorder that results from deficiency of α -galactosidase A (GLA) enzymatic activity.³ Premature vascular events in Fabry patients suggest a propensity toward vascular thrombosis.⁴ An increased thrombotic response has been shown to occur after arterial injury in *Gla*-deficient mice,⁵ although the effect of *Gla* deficiency in spontaneous thrombosis is unclear.

To determine whether *Gla* deficiency in mice is associated with an increased tendency toward spontaneous thrombosis, compound mutant mice carrying mutations in *Gla* and *FvL* were generated and analyzed for tissue fibrin deposition and thrombosis.

Methods

FvL (*FvL*^{0/0})⁶ mice were crossed to mice deficient in *Gla*⁷ and then genotyped as previously described.⁶⁻⁷ Mice were perfusion fixed with zinc formalin, and sections were stained for fibrin(ogen) as done previously.² Tissue fibrin(ogen) was graded by a blinded observer using Image-Pro Plus software (Media Cybernetics). Each section was quantitated for percentage fibrin staining using automated color detection. Hematoxylin and eosin along with fibrin-stained sections were also reviewed for the presence of thrombus.

Thrombi in each section were counted by an observer blinded to mouse genotype and were defined as organized, fibrin-stained, vascular occlusions.

Values are expressed as mean \pm SEM. The statistical significance of differences between groups was determined by 1-way ANOVA followed by Dunn's post hoc analysis when >2 experimental groups were included. The Student 2-tailed *t* test was performed when only 2 groups were being compared. $P<0.05$ was considered significant.

Results

At 22.3 ± 0.6 months of age, 5 organs from each mouse, including kidney, lung, liver, heart, and brain, were analyzed for fibrin deposition. Mice homozygous for *FvL* (*Gla*⁺⁰ *FvL*^{0/0}) demonstrated increased fibrin deposition (Figure 1A) compared with wild-type mice (*Gla*⁺⁰ *FvL*⁺⁰) as described previously. Fibrin deposition in mice with deficiency of *Gla* (*Gla*⁻⁰ *FvL*⁺⁰) alone was not significantly elevated compared with wild-type (WT) mice (*Gla*⁺⁰ *FvL*⁺⁰). However, in the presence of *FvL*, deficiency of *Gla* (*Gla*⁻⁰ *FvL*^{0/0}) greatly increased tissue fibrin deposition compared with either homozygous *FvL*- or *Gla*-deficient mice (Figure 1A).

To determine the effect of heterozygous *Gla* deficiency, female mice were also analyzed. Homozygous *FvL* mice with deficiency of *Gla* (*Gla*^{-/-} *FvL*^{0/0}) showed significantly increased fibrin staining compared with mice homozygous for *FvL* with WT *Gla* (*Gla*^{+/+} *FvL*^{0/0}). *Gla* heterozygous *FvL* mice (*Gla*^{+/-} *FvL*^{0/0}) were intermediate between *Gla*^{+/+} *FvL*^{0/0} and

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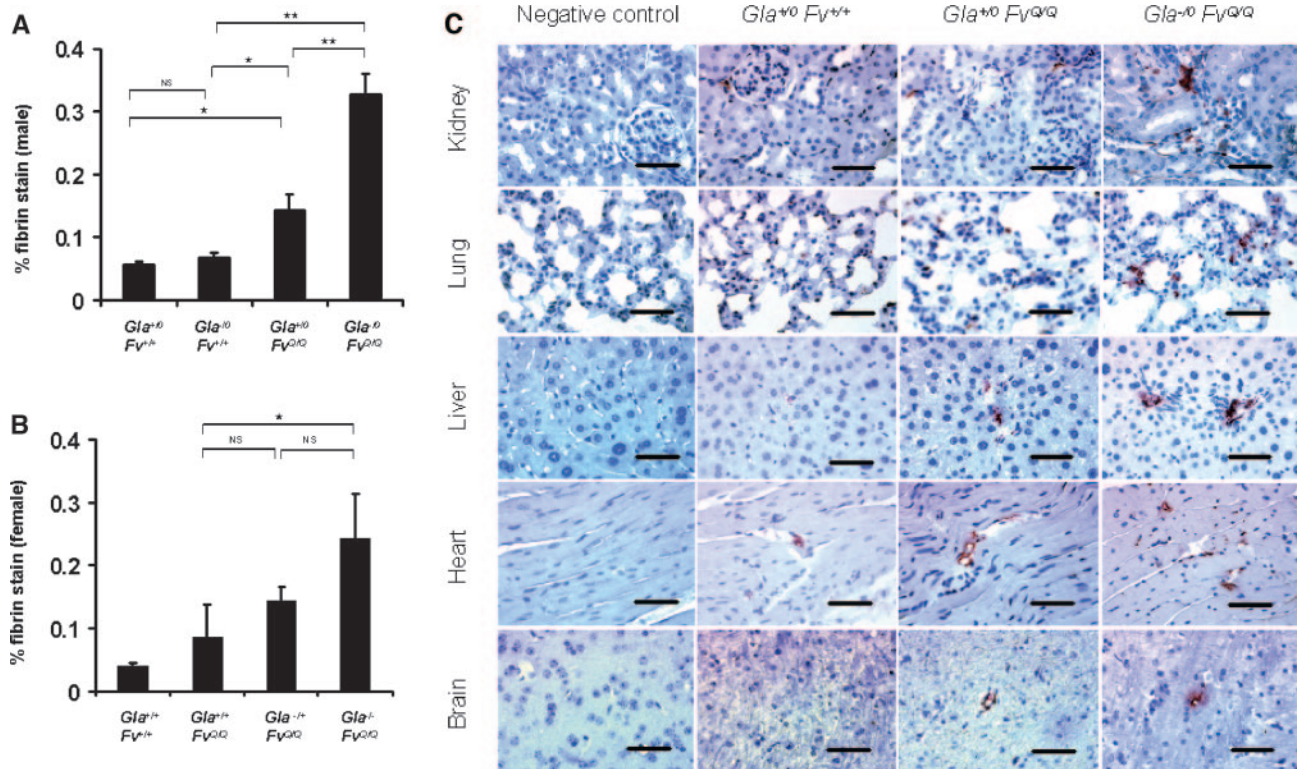


Figure 1. Analysis of fibrin(ogen) in organs. A, Quantification of fibrin(ogen). In the presence of *FvL* homozygosity, deficiency of *Gla* (*Gla*⁻⁰ *FvL*^{Q/Q}; n=7) greatly increased tissue fibrin deposition compared with homozygous *FvL* (*Gla*⁺⁰ *FvL*^{Q/Q}; n=10) or deficiency of *Gla* mice alone (*Gla*⁻⁰ *FvL*^{+/+}; n=4). B, Heterozygous deficiency of *Gla* mice (*Gla*^{+/-} *FvL*^{Q/Q}; n=7) were intermediate between homozygous (*Gla*^{-/-} *FvL*^{Q/Q}; n=7) and WT *Gla* mice (*Gla*^{+/+} *FvL*^{Q/Q}; n=4). WT mice (*Gla*^{+/+} *FvL*^{+/+}, n=4) were used as control. **P*<0.05; ***P*<0.0005. C, Immunohistochemical staining of fibrin(ogen), shown as red. Bar=40 μm.

Gla^{-/-} *FvL*^{Q/Q} mice for tissue fibrin deposition (Figure 1B and 1C) but not significantly different from either group.

Thrombi from each of 5 organs were counted from all mice included in the fibrin analysis. No thrombi were observed in WT mice or mice with *Gla* deficiency. Thrombi were only identified in mice homozygous for *FvL* (*Gla*^{+/+} *FvL*^{Q/Q} and *Gla*⁺⁰ *FvL*^{Q/Q}; 0.3±0.1 thrombi/mouse) and were present in veins of the kidneys and lungs. However, with concomitant *Gla* deficiency (*Gla*^{-/-} *FvL*^{Q/Q} and *Gla*⁻⁰ *FvL*^{Q/Q}), the mean number of thrombi was significantly increased (1.9±0.7 thrombi/mouse; *P*<0.04). Thrombi were observed in veins of the kidney, lung, and liver and 2 *Gla*⁻⁰ *FvL*^{Q/Q} mice exhibited organized thrombi in major coronary arteries (Figure 2). During the 22-month observation period, we observed 1 mouse with an apparent spontaneous stroke at 18 months of age that was of the *Gla*^{-/-} *FvL*^{Q/Q} genotype. Histologic analysis demonstrated cerebral arterial thrombosis with perivascular inflammation.

Discussion

The *FvL* mouse is a useful model to uncover relevant genetic modifiers of thrombosis. Mice homozygous for the murine *FvL* mutation display activated protein C resistance and spontaneously deposit fibrin in their tissues,⁶ suggesting chronic low-grade thrombin generation. This model has been used previously to unmask the phenotype of antithrombotic genes.²

In the current study, we used the *FvL* mouse model to determine the effect of *Gla* deficiency on spontaneous thrombosis in mice. In the presence of *FvL* homozygosity, deficiency of *Gla* greatly increased fibrin deposition and occlusive thrombus formation compared with mice homozygous for *FvL* or with deficiency of *Gla* alone. This observation suggests that under certain circumstances, *Gla* deficiency leads to increased propensity toward spontaneous thrombosis. Although the mechanism is unclear, a vascular wall defect

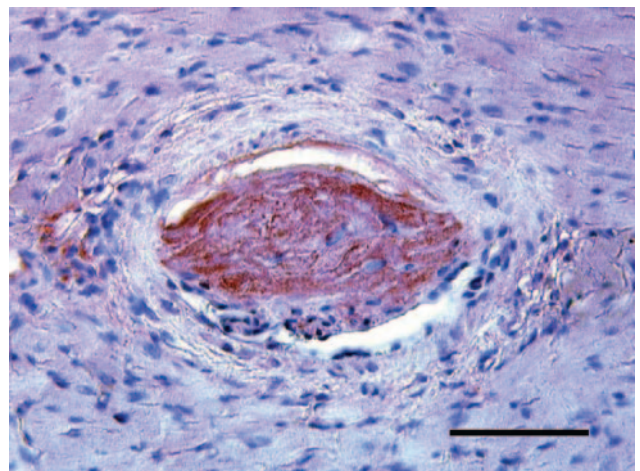


Figure 2. Organized occlusive thrombus in a coronary artery. Fibrin(ogen) staining of heart from *Gla*^{-/-} *FvL*^{Q/Q} mouse. Thrombus stains red. Bar=60 μm.

leading to dysregulation of NO with resultant oxidative stress may play a role.⁸⁻⁹

The findings in this study are consistent with a recent human clinical study, which found increased ischemic cerebral lesions in Fabry patients carrying the FVL mutation.¹⁰

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