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

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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±0.03%; n=7) compared with FvL mutation (0.14±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 (FvL0/FvL0) mice were crossed to mice deficient in Gla0 and then genotyped as previously described.5–7 Mice were perfusion fixed with zinc formalin, and sections were stained for fibrinogen as done previously.5,7 Tissue fibrinogen 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±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 (Gla0/FvL0) demonstrated increased fibrin deposition (Figure 1A) compared with wild-type mice (Gla+/− Fv+/−) as described previously. Fibrin deposition in mice with deficiency of Gla (Gla−/− Fv+/−) alone was not significantly elevated compared with wild-type (WT) mice (Gla+/− Fv+/−). However, in the presence of FvL, deficiency of Gla (Gla−/− Fv00) 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+/− Fv00) showed significantly increased fibrin staining compared with mice homozygous for FvL with WT Gla (Gla+/− Fv00). Gla heterozygous FvL mice (Gla+/− Fv00) were intermediate between Gla+/− Fv00 and
Gla−/FvQ/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−/FvQ/Q; n=7) and were present in veins of the kidneys and lungs. However, with concomitant Gla deficiency (Gla−/FvQ/Q; n=7) and WT Gla mice (Gla+/FvQ/Q; n=4), WT mice (Gla−/FvQ/Q; n=6; Gla+/FvQ/Q; n=4) were used as control. *P<0.05; **P<0.0005. C, Immunohistochemical staining of fibrin(ogen), shown as red. Bar=40 um.

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

Gla−/FvQ/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+/FvQ/Q and Gla+/FvQ/Q, 0.3±0.1 thrombi/mouse) and were present in veins of the kidneys and lungs. However, with concomitant Gla deficiency (Gla−/FvQ/Q and Gla−/FvQ/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−/FvQ/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−/FvQ/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, 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−/FvQ/Q mouse. Thrombus stains red. Bar=60 um.
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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