Heterozygous antithrombin deficiency improves in vivo haemostasis in factor VIII-deficient mice

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Summary
Decreased levels of factor VIII (FVIII) limit the amount of thrombin generated at the site of injury, but not the rate that thrombin is neutralised by antithrombin (AT). We hypothesised that FVIII-deficient mice with heterozygous AT deficiency will demonstrate increased thrombin generation and therefore less in vivo bleeding compared to FVIII-deficient mice with normal AT levels. Therefore, we performed tail bleeding experiments in wild-type (WT), heterozygous AT deficient (AT+/-) mice, FVIII-deficient (FVIII-/-) mice, and FVIII-deficient mice with heterozygous AT deficiency (FVIII-/-/AT+/-). Amount of bleeding was assessed by measuring absorbance of haemoglobin released from lysed red blood cells collected after tail transection. In addition, we measured thrombin generation, activated partial thromboplastin time (aPTT), and AT activity in plasma from the different mice groups. Tail bleeding was significantly reduced in FVIII-/-/AT+/- mice compared to FVIII-/- mice. On the other hand, there was no difference in tail bleeding between AT+/+ and wild-type mice. Thrombin generation was dependent on the mice genotype, and increased in the following order: FVIII-/- < FVIII-/-/AT+/- < WT < AT+/+. The aPTT was not influenced by reduced AT activity (i.e. AT+/- genotype), but was significantly prolonged in FVIII-/- and FVIII-/-/AT+/- mice. Using FVIII-deficient mice as an in vivo murine model of reduced thrombin generation, we demonstrated that moderately reduced AT levels increase thrombin generation and decrease bleeding after traumatic tail vessel injury. In agreement with congenital thrombotic conditions, our data elucidate that bleeding phenotypes can be modulated by the balance between procoagulant and anticoagulant proteins.

Keywords
Antithrombin, thrombin generation, coagulation factors, haemophilia

Introduction
After vascular injury, trace quantities of activated factor X (FXa) are rapidly generated by circulating FVIIa which forms a complex with subendothelial tissue factor (TF). This initiation step proceeds to generate trace thrombin (2–5 nM), which amplifies its own generation by feedback activation of cofactors FV, FVIII, and zymogen FXI. In patients with haemophilia A (deficiency of FVIII) and B (deficiency of FIX), the propagation phase of thrombin generation is not sustainable due to the lack of intrinsic tenase (FVIIa-FIXa-Ca++) (1). The limited availability of thrombin via TF-FVIIa pathway is in part due to rapid inhibition of FXa and thrombin by tissue factor pathway inhibitor (TFPI) and antithrombin (AT) (2, 3). In particular, plasma AT levels are normally high (2.6 μM), and AT is capable of inhibiting trace amounts of FXa and thrombin (3, 4). The lack of intrinsic tenase poses bleeding problems particularly in joints and skeletal muscles where TF expression is low (5, 6). We have previously demonstrated in vitro that endogenous thrombin generation is enhanced in FVIII-deficient plasma with exogenous FVIIa when AT is reduced to 50% of normal levels (7). Further, thrombin generation was less decreased than expected in an in vitro haemodilution model due to decreased AT levels (8). In agreement, low TFPI and AT levels in neonates are deemed to be important in augmenting thrombin generation with lower levels of procoagulant factors (9, 10). Taken together, we hypothesised that moderate reduction of AT would increase thrombin formation in an in vivo murine model of reduced thrombin generation, and therefore might confer beneficial haemostatic effects in vivo. Thus, we performed a tail bleeding study using FVIII-deficient mice with heterozygous AT deficiency (FVIII-/-/AT+/-) in comparison to FVIII-deficient mice with normal AT levels (FVIII-/-), wild-type (WT), or heterozygous AT deficient (AT+/-) mice. In addition, we evaluated thrombin generation, and activated partial thrombin time (aPTT).

Material and methods
Animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Emory University (Atlanta, Georgia, USA). The limited availability of thrombin via TF-FVIIa pathway is not sustainable due to the lack of intrinsic tenase (FVIIIa-FIXa-Ca++) (1). The propagation phase of thrombin generation is in part due to rapid inhibition of FXa and thrombin by tissue factor pathway inhibitor (TFPI) and antithrombin (AT) (2, 3). In particular, plasma AT levels are normally high (2.6 μM), and AT is capable of inhibiting trace amounts of FXa and thrombin (3, 4). The lack of intrinsic tenase poses bleeding problems particularly in joints and skeletal muscles where TF expression is low (5, 6). We have previously demonstrated in vitro that endogenous thrombin generation is enhanced in FVIII-deficient plasma with exogenous FVIIa when AT is reduced to 50% of normal levels (7). Further, thrombin generation was less decreased than expected in an in vitro haemodilution model due to decreased AT levels (8). In agreement, low TFPI and AT levels in neonates are deemed to be important in augmenting thrombin generation with lower levels of procoagulant factors (9, 10). Taken together, we hypothesised that moderate reduction of AT would increase thrombin formation in an in vivo murine model of reduced thrombin generation, and therefore might confer beneficial haemostatic effects in vivo. Thus, we performed a tail bleeding study using FVIII-deficient mice with heterozygous AT deficiency (FVIII-/-/AT+/-) in comparison to FVIII-deficient mice with normal AT levels (FVIII-/-), wild-type (WT), or heterozygous AT deficient (AT+/-) mice. In addition, we evaluated thrombin generation, and activated partial thrombin time (aPTT).

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Georgia, USA). We made use of two different mice strains for our animal experiments: B6;129S4-F8tm1Kaz/J (FVIII-/-) mice were obtained from the Jackson Laboratories (Bar Harbor, ME, USA). As previously described, these mice (homozygous females and males) carry less than 1% of normal FVIII activity (11). The heterozygous AT-deficient strain, C57BL/6 AT+- was provided by the Nagoya University School of Medicine (Nagoya, Japan) (12). The FVIII+-/AT+- mice were generated as follows: AT+- female mice were bred with FVIII-/- males resulting in mice, which were genotyped by PCR as AT+++, AT+/-, and also FVIII+/- and FVIII+--. Among these, AT++ and FVIII+/- offspring were backcrossed with FVIII+/- (males and females) to produce FVIII+/-/AT+/- genotyped mice for colony expansion and experimentations. FVIII+/- and AT+/- were bred in parallel colonies to maintain their original backgrounds. Normal C57BL/6 (FVIII+/-/AT+/-) mice, and heterogeneous B6;129S4-F8tm1Kaz/J FVIII-/- served as controls. All mice used in the experiments were bred and managed by the Department of Animal Resources, Emory University (Atlanta, GA, USA). At age 5–6 weeks tails of the mice used for experiments were snapped and genotyping was performed by the Winship Cancer Center Transgenic Mouse Facility (Atlanta, GA) according to Emory University transgenic facility protocols using previously described primers (11, 12) from Integrated DNA Technologies, Inc. (Coralville, IA, USA). Only genotyped mice of both sexes, 6–12 months old, with a median weight of 29 g (interquartile range [IQR] 26 to 32 g) were used in the investigation. Median (IQR) values of haemoglobin, haematocrit, weight of 29 g (interquartile range 26 to 32 g) were used in the investigation. Median (IQR) values of haemoglobin, haematocrit, and platelet count were 15.0 (13.7–16.6) g/l, 40.4 (37.0–44.4) %, and 843 (580 –1123) x 10^9/l, respectively.

**Tail bleeding**

All tail bleeding experiments were performed by the same investigator blinded to the genotype according to a method described previously (13). Briefly, anaesthesia was induced with 4% isoflurane (Isoflurane Novaplus®; Novation LLC, Irving, TX, USA) in oxygen at a flow of 1,000 minute (min) using a RC® Rodent Circuit Controller (Vet-Equip®; Pleasanton, CA, USA), and then decreased to 2% isoflurane in oxygen at a flow rate of 500 ml/min. Tails were amputated 4–5 mm from the tip such that tail artery and veins were all transected. The cut end was immediately immersed in a 15 ml clear conical tube containing 13 ml of normal saline (NS) at 37 °C. Bleeding was allowed to continue for 40 min while mice were kept warm and anaesthetised with 2% isoflurane. At the end of 40 min, mice were immediately euthanised with carbon dioxide. Tail bleeding experiments were performed in 13 normal WT, AT+/-, FVIII-/-, and FVIII-/-/AT+/- groups and from five mice from the FVIII+/- group were run in duplicate according to method of Hemker (14). Briefly, to each well of a 96-well microtiter plate (Microfluor black, Thermolabsystems, Franklin, MA, USA), we added 80 μl of mouse PPP. Calibrator wells, in which 20 μl of thrombin calibrator (Synapse BV) was added to 80 μl of plasma samples, were run in parallel for each mouse plasma. Coagulation was triggered with 20 μl commercially available PPP reagent (1 μM repilidated TF). The reaction was started by adding 20 μl/well of a buffer mixture Z-GGR-AMC (fluorescent substrate for thrombin) and CaCl2 (Fluka substrate buffer, Diagnostica-Stago, Parsippany, NJ, USA). The development of fluorescence was continuously monitored for 70–90 min with the fluorescence reader (Fluoroscan Ascent, 390/460 nm excitation/emission wavelengths, Thermo Labsystems). A dedicated software program (Thrombinscope™, Synapse BV, Maastricht, The Netherlands) measures the onset and the amount of thrombin generation based on the change in fluorescence produced by the hydrolysis of a fluorogenic peptide that acts as substrate of thrombin.

For thrombin generation experiments, PPP samples from 15 mice from the WT, AT+/-, FVIII-/-, and FVIII-/-/AT+/- groups and from five mice from the FVIII+/- group were run in duplicate according to method of Hemker (14). Briefly, to each well of a 96-well microtiter plate (Microfluor black, Thermolabsystems, Franklin, MA, USA), we added 80 μl of mouse PPP. Calibrator wells, in which 20 μl of thrombin calibrator (Synapse BV) was added to 80 μl of plasma samples, were run in parallel for each mouse plasma. Coagulation was triggered with 20 μl commercially available PPP reagent (1 μM repilidated TF). The reaction was started by adding 20 μl/well of a buffer mixture Z-GGR-AMC (fluorescent substrate for thrombin) and CaCl2 (Fluka substrate buffer, Diagnostica-Stago, Parsippany, NJ, USA). The development of fluorescence was continuously monitored for 70–90 min with the fluorescence reader (Fluoroscan Ascent, 390/460 nm excitation/emission wavelengths, Thermo Labsystems). A dedicated software program (Thrombinscope™, Synapse BV) was used to record the experiment, and for the calculation of the thrombinogram parameters used (lag time, time to peak, peak thrombin level, and endogenous thrombin potential). In addition, we calculated the slope of thrombin generation as follows: slope (nM/ min) = Peak thrombin generation / (time to peak – lag time). As demonstrated previously (7, 15), low AT levels affect the initial rate of thrombin generation (slope) as well as peak thrombin level, and endogenous thrombin potential. In addition, we calculated the slope of thrombin generation as follows: slope (nM/ min) = Peak thrombin generation / (time to peak – lag time). As demonstrated previously (7, 15), low AT levels affect the initial rate of thrombin generation (slope) as well as peak thrombin level, and ETP because plasma AT reacts with FXa and thrombin during and after the propagation of thrombin generation.
Laboratory measurements

AT activity was determined in plasma samples from six WT, AT<sup>+</sup>/-, FVIII<sup>-/-</sup>, and AT<sup>+</sup>/FVIII<sup>-/-</sup> mice and from three FVIII<sup>+</sup>/- mice. AT activity was measured in duplicate in 25 μl of mouse plasma, using Coamatic<sup>®</sup> Antithrombin kit (Chromogenix Instrumentation Laboratory Co., Lexington, MA, USA). The AT determination was performed using microtiter plate format and the absorbance was read on the SPECTRAMax<sup>®</sup> 340 Microplate Spectrophotometer at wavelength 405 nm. The aPTT was determined in the plasma samples from eight WT, AT<sup>+</sup>/-, FVII<sup>-/-</sup>, and AT<sup>+</sup>/FVIII<sup>-/-</sup> mice and in plasma samples from four FVIII<sup>+</sup>/- mice. The aPTT (Diagnostica Stago, Parsippany, NJ, USA) was measured according to the manufacturer’s instruction in duplicate, in 50 μl of plasma, using the STart<sup>®</sup> 4 instrument (Diagnostica Stago, Asnieres, France).

Statistics

Data are expressed as mean ± standard deviation (SD) or as median (IQR) where appropriate. Laboratory measurements, weight, and blood count data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni’s post-hoc test. Blood loss and thrombin generation data were logarithmically transformed before analysis in order to avoid variance of heterogeneity. Thereafter, these data were compared by ANOVA. A p-value of less than 0.05 was considered significant. All analyses were performed using SPSS<sup>®</sup> Version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Tail bleeding

Blood loss from tail bleeding as indicated by higher absorbance was significantly increased in the FVIII<sup>-/-</sup> mice group compared with all other groups (all p < 0.001), whereas there was no significant difference in blood loss between the other groups (Fig. 1). Median optical density (range) was 0.95 (0.04–18.08) for WT mice, 17.77 (3.25–145.53) for FVIII<sup>-/-</sup> mice and 1.51 (0.07–23.24) for FVIII<sup>+</sup>/AT<sup>-/-</sup> mice. AT levels had no influence on blood loss in AT<sup>+</sup>/ mice compared to normal mice, but significantly reduced blood loss in FVIII<sup>-/-</sup>/AT<sup>-/-</sup> mice compared to FVIII<sup>-/-</sup> mice. Blood loss in FVIII<sup>+</sup>/ mice was comparable to WT mice (p = 1.000).

Thrombin generation

Table 1 shows the data obtained by thrombin generation experiments. The peak thrombin, ETP, and slope of thrombin generation increased two to three-folds in the FVIII<sup>-/-</sup>/AT<sup>-/-</sup> group in comparison to the FVIII<sup>-/-</sup> group, but statistical analysis showed no significance due to the large variability of tested parameters. Further, peak thrombin and ETP in the FVIII<sup>-/-</sup>/AT<sup>-/-</sup> group were not significantly different from the WT mice group, whereas FVIII<sup>-/-</sup> had significantly lower peak and slope of thrombin generation and ETP. Significance levels did also not change after data were logarithmically transformed. Figure 2 shows typical thrombin generation curves in the different mice groups.

Laboratory measurements

The AT activity was 92 ± 18 % in WT mice, 62 ± 18 % in the AT<sup>+</sup>/- group (p = 0.019 vs. WT), 105 ± 21 % in the FVIII<sup>-/-</sup> group (p = 1.000 vs. WT), 52 ± 9 % in the FVIII<sup>-/-</sup>/AT<sup>-/-</sup> group (p = 0.001 vs. WT), and 120 ± 2% in the FVIII<sup>+</sup> group (p = 0.113 vs. WT).

The aPTT was 28.4 ± 3.3 seconds (sec) in WT mice, 28.1 ± 4.3 sec in the AT<sup>+</sup>/ mice (p = 1.000 vs. WT mice), 42.2 ± 4.0 sec in the FVIII<sup>-/-</sup> group (p < 0.001 vs. WT mice), 39.9 ± 3.5 sec in the FVIII<sup>-/-</sup>/AT<sup>-/-</sup> group (p < 0.001 vs. WT), and 31.9 ± 2.7 sec in the FVIII<sup>+</sup> group (p = 1.000 vs. WT mice). AT levels had no influence on aPTT measurement, as there was no difference in aPTT between WT mice and AT<sup>+</sup> mice (p = 1.000) as well as between FVIII<sup>-/-</sup> mice and FVIII<sup>+</sup>/AT<sup>-/-</sup> mice (p = 1.000).

Discussion

In the present study, we demonstrate the impact of decreased plasma AT level on the murine FVIII-deficient phenotype as an in vivo model of reduced thrombin generation. As shown by the results (higher thrombin generation and decreased tail bleeding volume), low AT levels improved haemostatic function in FVIII-deficient mice with heterozygous AT deficiency. These data are in agreement...
with our previous in vitro study, which demonstrated that endogenous thrombin generation, and the response to recombinant activated FVII (rFVIIa) are improved in human FVIII-deficient plasma with low AT levels (20–50%) (7). Because AT is an important regulator of FXa and thrombin that are initially generated by TFPVIIa complex, lower levels of AT are expected to change the threshold of procoagulant activation (3, 4). We suggest that the mechanism of improved haemostasis in our murine model is similar to the one described in previous works in neonatal plasma in which lower TFPI and AT improve thrombin generation (9, 10).

Tail bleeding and other induced vascular injuries have been often used to demonstrate increased bleeding tendency in previous murine models, although spontaneous bleeding rarely occurs in FVIII-deficient mice (16–18). In human haemophilic patients, skin template bleeding time is only modestly prolonged (19), and recurrent haemarthroses and tissue haematomas are major clinical manifestations. The latter is attributed to low TF expression in joints and skeletal muscles (5). The impact of vascular injury is affected by its size, location, platelet activity, and endogenous thrombin generation; thus findings in murine haemostasis models cannot be simply inferred to clinical phenotypes in humans (19). Nonetheless, FVIII-deficient mice are invaluable in evaluating a certain aspect of pathological bleeding related to haemophilia in human. In the absence of FVIII, intrinsic tenase (FIXa-FVIIa-Ca2+) cannot be formed, and TF-FVIIa complex is the only source for FXa generation. However, FXa cannot sustain thrombin generation because FXa and thrombin are rapidly quenched by two separate protease inhibitors, TFPI and AT (3, 4). Thus, normal regulatory mechanisms for FXa and thrombin are not advantageous for haemostasis in haemophilia (10). Accordingly, the improvement of haemophilia phenotype has been previously suggested in coexisting thrombophilic FVLeiden mutation (20, 21). The slower inactivation of FVLeiden by activated protein C (APC) is presumed to support increased and longer thrombin formation by maintaining prothrombinase (Xa-Va-Ca2+). However, these in vitro findings (21) and murine models (18) had not been consistently validated in clinical patients (22, 23). Schlachterman et al. demonstrated that phenotypic improvement in haemophilia due to FVLeiden differs between haemophilia A and B using transgenic mice (18). Notably, they observed improved clot formation in both haemophilia A and B mice with FVLeiden after the laser-induced injury in the arteriole. However, they observed no change in tail bleeding experiments in FVLeiden/haemophilia A mice, but only modest improvement in FVLeiden/haemophilia B mice. Contrary to AT, a serine protease inhibitor, APC is a serine protease, which requires thrombin-mediated activation. The latter step is inefficient in the absence of excess thrombin or endothelial thrombomodulin (24), and therefore inefficient APC formation in the larger tail vessels seems to explain the lack of improved haemostasis in FVLeiden after a traumatic injury (18). Conversely, we were able to demonstrate improved haemostasis after tail injury in haemophilia A mice with heterozygous AT deficiency (Fig. 1). We were not able to show the difference in aPTT values between FVIII+/ (normal AT activity) and FVIII+/+AT+ mice, but we observed a better correlation between the bleeding phenotype and the peak thrombin generation (Table 1, Fig. 2). The limitation of aPTT in the bleeding evaluation was clearly demonstrated in other studies (25). The time interval from FXII activation to thrombin generation in aPTT is not a direct reflection of thrombin-mediated feedback activation of FX, FVIII and FXI in vivo (1, 5). Furthermore, aPTT only reflects the initial phase of thrombin generation because its end-point (fibrin gel) is reached when about 10 nM thrombin (<5% of normal thrombin generation) is available (26, 27). In contrast, the calibrated thrombin generation assay shows the entire process of thrombin generation and inhibition (15), and therefore it is increasingly becoming the preferred tool in evaluating haemorrhagic and thrombotic conditions (28–30).

Table 1: Thrombin generation data.

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>AT+/-</th>
<th>FVIII+</th>
<th>FVIII+/-AT+/-</th>
<th>FVIII+/-</th>
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<td>(n=15)</td>
<td>(n=15)</td>
<td>(n=15)</td>
<td>(n=5)</td>
<td></td>
</tr>
<tr>
<td>Lag time (min)</td>
<td>2.3 ± 0.9</td>
<td>2.8 ± 1.1</td>
<td>1.9 ± 4.0</td>
<td>1.2 ± 0.7</td>
<td>2.1 ± 2.1</td>
</tr>
<tr>
<td>Thrombin peak (nM)</td>
<td>45 ± 22</td>
<td>58 ± 22</td>
<td>10 ± 11</td>
<td>29 ± 17</td>
<td>37 ± 26</td>
</tr>
<tr>
<td>ETP (nM)</td>
<td>600 ± 497</td>
<td>831 ± 291</td>
<td>94 ± 237</td>
<td>303 ± 258</td>
<td>390 ± 291</td>
</tr>
<tr>
<td>Slope (nM/min)</td>
<td>10.5 ± 6.7</td>
<td>15.1 ± 4.1</td>
<td>2.0 ± 2.1*</td>
<td>4.1 ± 3.1*</td>
<td>8.3 ± 6.1</td>
</tr>
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Values are mean ± SD. Intergroup differences were evaluated by ANOVA followed by Bonferroni’s posthoc test. * = p < 0.01 vs. WT mice. § = p < 0.01 vs. FVIII-/- mice.

Figure 2: Thrombin generation curves in platelet-poor plasma from wild-type (WT), heterozygous antithrombin (AT)-deficient (AT+/-), FVIII-deficient mice without (FVIII-/-) and with heterozygous AT deficiency (FVIII+/-/AT+/-). Moderately reduced AT levels improve the rate of thrombin generation, peak thrombin generation, and endogenous thrombin potential.
Congenital and acquired AT deficiencies are associated with increased thromboembolism in humans (31, 32). Given the incidence of less than 1 in 5,000 for congenital deficiency, coexistence of haemophilia A and AT deficiency is highly unlikely (33, 34). However, reduced AT activity (≤ 51%) was found in two out of 35 (6%) ‘clinically mild’ haemophilic patients with severe FVIII and FIX deficiency (<0.01 U/ml) but in none out of 33 ‘clinically severe’ haemophilic patients (35). Further, near normal thrombin generation and lower incidences of bleeding in haemophilic neonates despite of low FVIII activity are attributed to lower TFPI and AT levels (approximately 50% of adult levels) (9, 10). Taken together, our results provide further evidence that regulatory mechanisms of thrombin generation including TFPI, AT, and APC can modulate the bleeding phenotype of haemophilia despite the similar plasma FVIII levels (36).

Although homozygous AT deficiency causes embryonic lethality, heterozygous AT-deficient mice rarely develop spontaneous thrombosis unless being challenged with stress or infection (12, 37). No increases in morbidity and mortality were observed in haemophilic AT-deficient (FVIII+/−/AT−/−) mice. Further, major intravascular consumptions of prothrombin and fibrinogen in rabbits challenged with Russell’s viper venom (FX activator) were only observed after immunodepletion of AT to about 10–20%, but not when AT was maintained at above 40% (38).

Our study has several limitations. First, murine knockout models only reflect limited aspects of clinical bleeding (19), and thus our findings may not necessarily parallel changes in bleeding phenotypes of haemophilia. Secondly, tail bleeding tests are known to have large variability, and prolonged bleeding may be found even in normal mice (39). Both platelet activity and endogenous thrombin generation can affect bleeding time, and it is also known that murine platelets differ from human platelets, especially platelet-surface thrombin receptors (40). Instead of measuring the initial arrest of bleeding, we quantified the blood loss over 40 min, which might better reflect the haemophilia-related cycle of temporary haemostasis and rebleeding (16). Third, two different strains of mice, C57BL/6 and B6;129S4 mice were included in the experiments. Although both strains have been used in the similar murine bleeding study (17), some strain differences may influence tail bleeding (39). However, we found that tail bleeding, endogenous thrombin generation, aPTT, and AT activity were similar between heterozygous FVIII+/− mice and C57BL/6 mice. Lastly, the correlation between thrombin generation parameters and the bleeding phenotype (27–29) is promising, but further clinical validation is necessary.

In summary, we demonstrated in an in vivo murine model that moderately reduced AT activity (about 50% of normal) alters bleeding phenotype of FVIII-deficient mice using the tail transection model and endogenous thrombin generation. Heterogeneous bleeding phenotypes in severe haemophilia may be in part explained by the change in regulatory proteins of thrombin generation. Additional studies are needed to validate this model and help further elucidate the fine interplay of AT, TFPI and other coagulation factors in determining the bleeding phenotype of haemophilic patient.

What is known about this topic?
- The bleeding phenotype in severe factor VIII (FVIII) deficiency can be modulated by procoagulant state (e.g. FVIII-depleted).
- Endogenous thrombin potential is increased in vitro in FVIII-deficient plasma with reduced antithrombin (AT) levels.

What does this paper add?
- Moderately reduced AT activity (about 50%) increased thrombin generation in FVIII-deficient mice (haemophilia model).
- Bleeding tendency is improved in FVIII-deficient mice by lowering AT activity.
- Heterogeneous bleeding phenotypes in severe haemophilia may be in part explained by the change in regulatory proteins of thrombin generation.

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References

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