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Mathematical model of thrombin generation and bleeding phenotype in Amish carriers of Factor IX:C deficiency vs. controls

S. Gupta[a,*], **M.C. Bravo**[b], **M. Heiman**[a], **C. Nakar**[a], **K. Brummel-Ziedins**[b], **C.H. Miller**[c], **A. Shapiro**[a]

[a]Indiana Hemophilia and Thrombosis Center, Indianapolis, IN, United States of America

[b]University of Vermont, Colchester, VT, United States of America

[c]Division of Blood Disorders, National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA, United States of America

Abstract

Introduction—Factor IX:C (FIX:C) levels vary in hemophilia B carriers even in pedigrees with a unifying genetic defect. Analyzing the balance between pro-and anticoagulants might increase our understanding of carriers' bleeding potential.

Aim—In this research study, we evaluated bleeding scores (BS) and a novel mathematical model of thrombin generation (TG) in Amish FIX:C deficient carriers and controls.

Methods—Blood samples and BS were obtained from post-menarchal females, including 59 carriers and 57 controls from the same extended pedigree. Factors II, V, VII, VIII, IX, X, antithrombin, tissue factor pathway inhibitor and protein C were assayed to generate mathematical models of TG in response to 5pM tissue factor (TF) and for TF + thrombomodulin. BS was based on a modification of the MCMDM-1VWD scoring system.

Results—Carriers had a lower mean FIX:C (68% vs. 119%), von Willebrand factor antigen (108 vs.133) and Tissue activatable fibrinolysis inhibitor (103 vs. 111) compared to controls; both groups had a similar mean BS. Carriers demonstrated significantly lower TG parameters on both mathematical models compared to controls. Carriers with FIX:C ≤ 50% had lower TG curves than those > 50% but similar BS.

Conclusion—Thrombin generation showed significant differences between carriers and controls, between low (≤50%) and high (> 50%) FIX:C carriers, and specifically in the TF + thrombomodulin model, between high FIX:C carriers and controls, although the BS were not different.

*Corresponding author at: Indiana Hemophilia and Thrombosis Center, 8402 Harcourt Rd., Ste. 500, Indianapolis, IN 46260, United States of America, sgupta@ihtc.org (S. Gupta).

Declaration of competing interest

None. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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Keywords

Carriers; Hemophilia B; Bleeding scores; Mathematical model; Thrombin generation

1. Introduction

Hemophilia B (HB) is an X-linked bleeding disorder resulting in a deficiency of FIX:C. Variable FIX:C levels can be seen in carriers within the same pedigree with a unifying mutation [1]. This variation has been attributed to differences in lyonization although there are other possible modifying factors [2–6]. FIX:C level has been utilized to predict bleeding risk and for dictating replacement therapy in both affected HB individuals and carriers; however FIX:C levels in carriers may not have a linear relationship to bleeding risk [7]. Some carriers with normal levels > 50% have increased bleeding tendency [8]. Quantitative assessment of bleeding symptoms can be performed with the use of bleeding scores [9–11]. The bleeding phenotype may be a cumulative effect of several variables of which FIX:C activity, mutation, age, stressors experienced as well as other circulating hemostatic parameters likely play an important role. Thrombin generation potential reflects the interplay between the procoagulant and anticoagulant proteins and may be an important predictor of bleeding phenotype in bleeding disorders [12]. However, these data have been inconsistent across carrier studies [13]. As thrombin is a key enzyme in hemostatic processes [14–16], aberrant thrombin formation can affect the critical balance of these processes yielding inappropriate bleeding or clotting [17–21]. Decades of research describing the reaction network of thrombin generation have enabled the creation of computational models of thrombin generation that are increasingly being used to understand normal hemostasis as well as coagulopathies by identifying underlying mechanisms, accelerating hypothesis testing, and simulating therapeutic interventions. In these models, initial concentrations of reactants will direct outcomes (i.e. thrombin generation) and thus, to the extent that individuals vary in the concentrations of coagulation components, their responses to a given hemostatic challenge will vary. Several groups have used patient factor level profiles to simulate thrombin generation using various models of the coagulation network in trauma [22], HIV and mortality [23], stroke [24], the effects of acidosis [25], and dilutional coagulopathy [26]. In principle, coagulation factor protein levels reflect the sum of developmental, environmental, genetic, nutritional, infectious agent, and pharmacological influences on the liver and other organs that regulate their synthesis and turnover [27]. Thus, computational models of the coagulation system that incorporate individual specific composition data are in principle linking the overall health of the individual and their hemostatic potential.

This research study is the first to evaluate mathematical models of thrombin generation and bleeding scores in a cohort of HB carriers with a unifying genetic mutation vs. an unaffected group from the same population.

2. Methods

2.1. Study design, study setting and patient population

The study was conducted by Indiana Hemophilia & Thrombosis Center (IHTC), the Centers for Disease Control and Prevention (CDC), and the University of Vermont from 2008 to 2013. Three populations were studied in this analysis – Amish FIX:C deficient carriers, Amish controls and non-Amish controls (NAC). The NAC samples were purchased commercially. The IHTC enrolled 170 female Amish individuals aged 1–75 years, including 102 HB carriers and 68 controls from the same extended pedigree. All enrolled individuals or their guardians signed an IRB-approved informed consent or assent based upon age. HB carriers included obligate carriers or were individually genotyped and had the c.1025C > T; p.Thr342Met mutation, which is associated with moderate HB [1]. Amish unaffected controls were recruited by testing family members of the affected who were not at risk and those who had negative carrier testing results. Due to limited resources and difficulty in recruiting, the controls were not age matched but did belong to the same extended pedigree and were found not to have the familial *F9* mutation, c.1025C > T. As the bleeding score is based on symptoms in two categories relevant only to post-pubertal women, post-partum hemorrhage (PPH) and menstrual bleeding, the final analysis was performed on post-menarchal females only, reducing the analyzable numbers to 59 carriers and 57 controls. The Amish studied represent a unifying mutation and limited genetic pool; therefore to ensure that other inheritable factors which could potentially impact coagulation did not affect the TG results, we also included 15 samples of fresh frozen plasma from healthy female non Amish controls (NAC; age = 33 ± 9, range 21–51) purchased from Innovative Research (Novi, MI).

2.2. Bleeding score (BS)

BS was calculated for each participant using an in-house developed bleeding questionnaire based on the MCMDM-1 scoring system [28]. The questionnaire (Supplement 1, S1) was administered by trained research personnel to each subject, requiring an average of 15–25 min for completion. The 8 categories queried included bruising, epistaxis, bleeding from minor wounds, post-partum hemorrhage (PPH), menorrhagia, bleeding post dental work, surgical bleeding and hemarthrosis; scoring for each bleeding symptom was 0 to +3, with an increasing score based on severity and need for medical care/intervention; maximum achievable total BS was 24 (Supplement 2, S2, Table S1). The bleeding phenotype of the Amish carriers has been reported previously, using a BS graded using only 7 bleeding symptoms [1]. For this study, carriers and controls were rescored with new category descriptions using all 8 bleeding symptoms, including bleeding from minor cuts and wounds.

2.2.1. Blood collection—Blood samples were drawn into 3.2% sodium citrate tubes and centrifuged within 2 h of sample collection; platelet-poor plasma was immediately frozen at −70 °C and thawed as needed for assay. Mutation analysis was performed on blood collected into EDTA tubes, as previously described [1].

2.2.2. Measurement of procoagulants and anticoagulants—Procoagulant factors (F) II, V, VII, VIII, IX, X, XI, and XII activities were assayed by one-stage methods using appropriate factor-deficient plasmas (Precision Biologic, Dartmouth, Nova Scotia, Canada) and STA PTT-A (Diagnostica Stago, Parsippany, NJ, USA) or STA Neoplastin CI Plus (Diagnostica Stago). Fibrinogen was measured by Clauss method, STA-Fibrinogen (Diagnostica Stago, Parsippany, NJ, USA). VWF:Ag was measured using latex bead immunoassay (LIATest, Diagnostica Stago). Thrombin activatable fibrinolysis inhibitor (TAFI) was measured using a chromogenic substrate method (Pefakit TAFI, Pentapharm Ltd., Basel).

2.2.2.1. Anticoagulant factors: Antithrombin (AT) was measured by chromogenic assay (Stachrom AT III; Diagnostica Stago). Protein C (PC) was measured by clotting assay (Staclot C, Diagnostica Stago). Tissue factor pathway inhibitor (TFPI) was measured using ELISA (Diagnostica Stago).

All factors were expressed as percentages of the mean values in the healthy population. All assays were performed on STA-R Evolution (Diagnostica Stago, Parsipanny, NJ, USA).

Select factor levels (II, V, VII, VIII, IX, X, AT, PC and TFPI) were measured as described above on plasma of the 15 non-Amish controls. FXII and FXI were not measured in this subset as our mathematical model is based on tissue factor (TF) pathway of coagulation and hence measurements of these factors is not needed to run computational models of TF initiated thrombin generation. (Supplement 3, S3, Table S2).

Reference ranges were derived in-house from testing of 123 healthy control subjects and validated with reagent changes.

2.2.3. Simulated thrombin generation—Mathematical simulations of tissue factor (TF) initiated thrombin generation were produced as previously described [29–31]. Two models were used that initiate coagulation simulation with 5 pM tissue factor (TF); one model also includes 1 nM thrombomodulin (TM) to initiate simulation activation of the PC-negative feedback system (TF + TM Model) [32]. The TF + TM model expands on the TF model by including TM binding to thrombin and meizothrombin and the activation of protein C (APC) by these complexes [33,34], AT inhibition of thrombin-soluble TM complexes, APC cleavage of FVa [31], and assembly and function of partially proteolyzed FVa species in prothrombinase [35–38]. For each individual, concentrations of procoagulants FII, V, VII, VIII, IX and X and anticoagulants TFPI, AT and PC were translated into molar concentrations using literature values for the mean plasma concentrations (Supplement3, S3, Table S2); 10% of FVII level is estimated to be in the active form for simulations [29,39]. Simulated reactions were solved for thrombin over a 1200s time frame. Individual profiles were summarized by parameters describing the initiation, propagation and termination phases of thrombin generation: maximum level of thrombin generation (MaxL), maximum rate of thrombin generated (MaxR), time to 10 nM thrombin (clot time, CT), and total thrombin generated (area under the curve, AUC). Results for a particular group of individuals were graphically presented as a curve, which represents the mean thrombin concentration at each second of the reaction. For carriers with low

(≤50%) FIX:C levels, thrombin generation simulations were subsequently conducted with increasing FIX:Clevels to simulate medical supplementation with FIX:C to variable target levels up to 100% keeping other factors constant. Thrombin generation from the non-Amish control (NAC) population was used as a reference point.

2.2.4. Statistical analyses—Data were stored and collated using Microsoft Excel (Microsoft, Redmond, WA). Data are reported as the mean with range and/or standard deviation. Statistical comparisons were made using student's *t*-test, chi-square tests and ANOVA with a statistical significance set at $p < 0.05$ for a two-sided difference. Pearson correlation coefficients were calculated between two parameters. SAS statistical software, SAS 9.4 by SAS Institute, Inc. was used for all determinations of significance. Non-parametric statistical comparison of factor levels (specifically those used for simulating thrombin generation) across BS value for each subject was not reliable due to the low number of subjects with BS > 2. Subjects were subsequently reclassified into one of three BS groups: BS = 0, BS = 1–2, or BS = 3+ and analyzed using ANOVA.

3. Results

3.1. Subject characteristics, FIX:C levels and BS

Among post-menarchal females, including 59 carriers and 57 controls, carriers were significantly younger than controls (mean age 32 vs. 41) and had lower mean FIX:C level at 68 (range 19–172) vs. 119 (range 59–196), but the two groups were comparable in total mean BS (1.7 and 1.6) and reported bleeding symptoms. A sub-analysis was performed on the carriers based on FIX:C level, with 30% (18/59) having factor IX ≤50% (low FIX:C) and 70% (41/59) having FIX:C > 50% (high FIX:C). The low FIX:C group had a mean FIX:C of 40 (range 19–50), compared to 80 (range 52–172) in the high FIX:C group. There was no significant difference in age, 33y vs. 32y, in the two groups. BS was higher in the low FIX:C group, 2.0 vs. 1.6, but the difference was not statistically significant. There was a > 10 fold higher frequency of reported dental and minor wound bleeding in carriers with low FIX:C as compared to those with high FIX:C. Other bleeding symptoms were comparable (Table 1).

3.1.1. Comparison of other plasma coagulation factor levels—When compared to Amish controls, carriers had significantly lower VWF:Ag 108 vs.133 (range 58–219 vs 59–264) and TAFI, 103 vs. 111 (range 63–117 vs. 69–169).

Fibrinogen, FII, VII, IX, X, XII and PC were significantly lower in the carriers with ≤50% vs. > 50% (Table 2). Except for FIX:C other coagulation factors were in the normal reported hemostatic range.

3.1.2. BS and factor levels—Subjects were classified into three bleeding score categories BS = 0, BS = 1–2, or BS ≥ 3 with an almost equal number of controls and carriers in each category (Table 3). An almost equal percentage of carriers (27%) and controls (24%) had BS ≥ 3, although the FIX:C level was twofold higher among controls, 129% vs. 54%, in this score category. Carriers with BS ≥ 3 had the lowest mean factor IX level 54% compared to those with a BS = 0, who had a mean FIX:C of 78%. The only other differences were in

PC, which was lower in carriers compared to controls in the BS ≥ 3 category (126% vs 150%), and FII levels, which were variable in the controls across increasing BS (Table 3).

3.1.3. Thrombin generation kinetics from computational simulations—Plots of the mean thrombin generation curves for the carriers and controls are shown in TF Model (Fig. 1A) and TF + TM Model (Fig. 1B); mean simulations for the NAC are provided as a reference. Carriers were characterized by significantly slower max rates (MaxR) and lower max levels (MaxL) of thrombin generation, irrespective of model (Table 4, Fig. 1A, B).

All TG parameters for both models were significantly lower for carriers with low FIX:C levels than those with higher (> 50%) FIX:C. The majority of parameters were lower for higher FIX:C carriers compared to controls (Table 4). TG curves in the TF model were lowest for low FIX:C carriers, while those for higher FIX:CFIX:C:C carriers, controls, and NAC almost overlapped (Fig. 1C). TG curves in the TF + TM model were lowest for FIX:C carriers ≤ 50%, and successively higher for FIX:CFIX:C:C carriers > 50%, controls, and the NAC group (Fig. 1D). MaxL and Max R of TG correlated with FIX:C levels in both carriers and controls in both models, with slightly higher correlation coefficients for the TF model (Fig. 1E, F,G, H).

The FIX:C carriers with levels > 50% had lower thrombin generation for both models when compared to controls, more pronounced for TF + TM model (Fig. 1D).

Correlation plots of thrombin MaxL vs. Factor IX levels for the different populations (Carriers – black triangles, Controls – open circles, Non-Amish Controls – grey squares) in the (E) TF model, Carriers $r = 0.77$, Controls $r = 0.79$; or (F) TF + TM models, Carriers $r = 0.66$, Controls $r = 0.59$. Correlation plots of thrombin MaxR vs. Factor IX levels for the different populations (Carriers – black triangles, Controls – open circles, Non-Amish Controls – grey squares) in the (G) TF model, Carriers $r = 0.77$, Controls $r = 0.79$; or (H) TF + TM models, Carriers $r = 0.72$, Controls $r = 0.81$.

3.1.4. Thrombin generation simulation of factor IX replacement—Thrombin simulations on the low FIX:C carriers were conducted with a titration of increasing FIX:C levels to simulate potential medical intervention, changing only FIX:C levels (60, 80, or 100%), while keeping all other factor levels of the individual constant. In the TF model, these carriers showed a 26–47% increase in MaxL as a group; a full restoration of FIX:C levels to mean physiological levels (100%) achieved only 90% of the MaxL achieved in the carriers with high FIX:C levels (Fig. 2A). In the TF + TM model, the low FIX:C carriers experienced a 41–130% increase based on the FIX:C titration. A restoration of FIX:C levels to 80% of mean physiological achieved nearly 85% of the MaxL achieved by the carriers with high FIX:C levels. (Fig. 2B).

4. Discussion

Factor levels in hemophilia carriers have been documented to be quite variable within a specific hemophilia type and even in those with a unifying pathologic variant [1], largely attributed to individual differences in lyonization. Our data in HB carriers show similarly

variable FIX:C levels ranging from 19 to 172% with a mean of 68%. Of our postmenarche carriers, 30% had levels ≤50%. Historically it is known that females in the mild deficiency range, factor levels of 5–50%, are symptomatic with bleeding episodes similar to males with mild hemophilia; however, females are also at increased risk of gender-specific bleeding events related to the reproductive system. The decision to provide clotting factor replacement/hemostatic therapy to a carrier based upon a specific level is not entirely elucidated, and real world practice varies widely. The prediction of bleeding risk in carriers based upon levels alone has been confounded by reports of abnormal bleeding symptoms reported in carrier females of FVIII deficiency whose levels are above 40% or within the normal range [10]. Bleeding scores like MCMDM-1, Self-BAT (bleeding assessment tool), and ISTH BAT have been evaluated as a surrogate marker for prediction of bleeding events in a variety of populations including hemophilia [9–11].

In this report, an in-house bleeding questionnaire and scoring system were utilized. The standard BAT underestimates the bleeding potential in this population, because higher scores are assigned for medical care and interventions, which the Amish are less likely to seek [40]. Our finding of BS < 2 in all participants is in contrast to other studies which have shown a higher BS ≥ 3 using different BATs in HB carriers [7,11]. This is possibly due to the lack of reporting bleeding symptoms in the Amish owing to their stoic nature and hesitation in seeking medical care compared to other populations [40]. The only significant finding was in BS categories and FIX:C levels, showing an inverse relation with BS ≥ 3 having the lowest FIX:C levels, although correlation between factor IX:C levels and BS has not been consistently seen in other studies [7].

Although it was expected that reproductive tract bleeding would be higher in carriers as compared to controls, this was not seen in our cohort (menstrual bleeding 5% vs 12%, $p = 0.17$; PPH 32% vs 30%, $p = 0.78$ respectively). Previously, a significantly higher frequency of these bleeding symptoms was reported for HB carriers compared to controls (menstrual bleeding 47% vs 15%, $p < 0.01$; PPH 47% vs 9%, $p < 0.01$) although this report included a smaller sample size of 19 women with a lower range of FIX:C at 27–81%, mean of 54% [7]. Only 3% (2/59, with 54% and 79% FIX:C) of our carriers reported joint bleeds (one knee and 3 ankle), which was not significantly higher than controls. Details about the mechanism of hemarthrosis were not captured through our questionnaire. In other studies of HB carriers, slightly higher hemarthrosis frequencies have been observed at 5–8% [7,8]. Even in the subgroup analysis of carriers by FIX:C level, reproductive bleeding symptoms and hemarthrosis were not significantly higher in the low FIX:C level group; however they had a > 10 fold higher frequency of reported dental and minor wound bleeding. This finding should be interpreted with caution as the Amish have a higher prevalence of poor oral hygiene, which could increase the rate of oral bleeding observed when FIX:C levels are decreased and gingivitis is present. In addition, women actively participate in physically demanding chores which could increase the incidence of minor wounds [40]. The similarity in BS between carriers and controls could also be due to the age difference between the groups, with the younger carriers less likely to have experienced some types of bleeding, such as post-partum hemorrhage, despite having lower FIX:C levels.

This study presents the first report of an extensive coagulation profile in HB carriers. This was done with the two-fold intention of assessing the impact of all procoagulants and anticoagulants on bleeding scores and to create mathematical thrombin generation models for carriers and controls. There was no significant impact of these coagulation factor levels on the total BS. Apart from significant differences in FIX:C only levels of VWF:Ag and TAFI were lower in carriers which did not translate into a higher mean BS in carriers. The subgroup analysis of carriers above and below 50% demonstrated several factors which were lower in the FIX:C > 50% group (I, II, VII, IX, X, XII and PC), although this difference did not impact the BS between the two groups possibly because both anticoagulants and procoagulants were lower balancing out the effect. Although extended coagulograms have been reported in FVIII carriers [10,13], this is the first report, to our knowledge, in HB carriers. There was a significant lower FIX:C in carriers with BS ≥ 3 vs. those with BS = 0, indicating that FIX:C levels do have a negative correlation with bleeding symptoms. Also of note, was the unexpected lower PC in carriers vs. controls in the BS ≥ 3 category. PC levels needs to be further studied in a larger sample size of FIX:C carriers to see if the difference persists. Vitamin K deficiency fails to explain the difference in the PC levels in carriers and controls as other Vitamin K dependent proteins FII and FVII were not significantly different.

Global hemostatic assays, including thrombin generation assay (TGA) and thromboelastogram (TEG), have gained increased attention for their use in evaluation of patients' bleeding disorders and response to a variety of therapies. TGA is performed in plasma samples, which has practical application for use in stored samples. TGA assays may have utility in hemophilia carriers with a range of FVIII or IX levels and variation in bleeding symptoms to increase understanding of the impact of other procoagulants and/or anticoagulants on the bleeding phenotype [12]. Although TGA has several advantages including easy to perform, commercially available, widely accepted by hematologists and lastly easy interpretation; a disadvantage of TGA is the lack of standardization. In our research study, the complex reaction pathway of thrombin formation/inhibition is approximated by the theoretical curves generated by computer modeling which has the capacity to anticipate the presence of minute concentrations of reactants and enzymes. Carriers with ≤50% FIX:C had the lowest TG parameters and curves; and, interestingly, carriers with levels > 50% had significantly lower TG parameters and curves than all controls (Amish and non-Amish) in the TF + TM model, implying that the carriers > 50% might have a higher tendency to bleed as compared to the normal population; this should be further substantiated with real-time global hemostatic assays in a larger set of cases and controls. Using our mathematical models we were able to simulate the effect of factor replacement therapy in those individuals with a mild FIX:C deficiency. The TF + TM model was most responsive to increasing FIX:C levels, an increase to 80% of mean physiological was sufficient to generate an average profile similar to the HB carriers with levels of FIX:C > 50%. This model captured more of the differences between the carriers based on FIX:C levels, most notably the lower levels of PC in those with FIX:C ≤ 50%. Whether these lower PC levels concurrent with lower FIX:C levels are the result of a compensatory mechanism is unknown and may be worthy of future investigation.

4.1. Limitations

We used an in-house bleeding questionnaire only applied in this population, without a validated cut off for a significant bleeding score in women. It is possible that a low score of 1 could be significant in this population; this requires further evaluation with a larger number of subjects in this population for validation. Our Amish controls were not age matched which could result in differences in some coagulation factor levels such as FVIII and VWF:Ag which are known to vary with age; however FIX:C is not known to be affected. The age difference might also affect BS. A cross sectional survey for bleeding symptoms with recall bias in a stoic population could have influenced the reported bleeding symptoms in our study. The results of this study are not generalizable to other FIX:C carriers with other genetic defects as we were limited by a homogenous population with a unifying mutation. Additionally, there are no other reports of the bleeding phenotype in female heterozygous carriers of this specific variant for comparison to our cohort. Although an extensive coagulogram was performed, VWF Ristocetin, blood group, platelet count and platelet function, which could influence bleeding scores, could not be performed due to lack of funding. The mathematical modeling for thrombin generation does not replace a commercially available thrombin generation assay which is a validated and universal standardized tool for evaluating the coagulation system. The mathematical models are a unique way of analyzing thrombin generation in a research setting and allow us to simulate different scenarios (as was performed with FIX:C levels restoration to physiological values, Fig. 2A, B) to look at possible outcomes. It would be ideal to look at actual thrombin generation assay along with the mathematical model for correlation and this is the next step of our project for the 99 FIX:C carrier samples at our center.

5. Conclusion

This research study is the first to use BS, extensive coagulation factor testing, and detailed mathematical models for thrombin generation in genetically similar population of HB carriers and controls. These data support the concept that, overall, carriers of hemophilia B with either low or normal/high FIX:C levels have a significant difference in thrombin generation compared to controls; whether or not this translates into an actual difference in bleeding phenotype will need to be analyzed in a larger sample size across variable FIX:C levels. Mathematical models of thrombin generation are a novel way of analyzing the hemostatic milieu and would need further validation in hemophilia carriers to assess their clinical utility along with extensive correlation with actual thrombin generation assays.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

[1]. Sharathkumar A, Hardesty B, Greist A, et al., Variability in bleeding phenotype in Amish carriers of haemophilia B with the 31008 C→T mutation, Haemophilia. 15 (1) (2009) 91–100. [PubMed: 18721150]

[2]. Mannucci PM, Coppola R, Lombardi R, Papa M, de Biasi R, Direct proof of extreme lyonization as a cause of low factor VIII levels in females, Thromb. Haemost 39 (2) (1978) 544–545. [PubMed: 581005]

[3]. Nisen P, Stamberg J, Ehrenpreis R, et al., The molecular basis of severe hemophilia B in a girl, N. Engl. J. Med 315 (18) (1986) 1139–1142. [PubMed: 3093864]

[4]. Taylor SA, Deugau KV, Lillicrap DP, Somatic mosaicism and female-to-female transmission in a kindred with hemophilia B (factor IX deficiency), Proc. Natl. Acad. Sci. U. S. A 88 (1) (1991) 39–42. [PubMed: 1986380]

[5]. Lyon MF, Sex chromatin and gene action in the mammalian X-chromosome, Am. J. Hum. Genet 14 (1962) 135–148. [PubMed: 14467629]

[6]. Orstavik KH, Scheibel E, Ingerslev J, Schwartz M, Absence of correlation between X chromosome inactivation pattern and plasma concentration of factor VIII and factor IX in carriers of haemophilia A and B, Thromb. Haemost 83 (3) (2000) 433–437. [PubMed: 10744150]

[7]. Olsson A, Hellgren M, Berntorp E, Ljung R, Baghaei F, Clotting factor level is not a good predictor of bleeding in carriers of haemophilia A and B, Blood Coagul. Fibrinolysis 25 (5) (2014) 471–475. [PubMed: 24509327]

[8]. Plug I, Mauser-Bunschoten EP, Brocker-Vriends AH, et al., Bleeding in carriers of hemophilia, Blood. 108 (1) (2006) 52–56. [PubMed: 16551972]

[9]. James PD, Mahlangu J, Bidlingmaier C, et al., Evaluation of the utility of the ISTH-BAT in haemophilia carriers: a multinational study, Haemophilia. 22 (6) (2016) 912–918. [PubMed: 27868369]

[10]. Paroskie A, Gailani D, DeBaun MR, Sidonio RF Jr., A cross-sectional study of bleeding phenotype in haemophilia A carriers, Br. J. Haematol 170 (2) (2015) 223–228. [PubMed: 25832012]

[11]. Young JE, Grabell J, Tuttle A, et al., Evaluation of the self-administered bleeding assessment tool (Self-BAT) in haemophilia carriers and correlations with quality of life, Haemophilia. 23 (6) (2017) e536–e538. [PubMed: 28949433]

[12]. Chitlur M, Young G, Global assays in hemophilia, Semin. Hematol 53 (1) (2016) 40–45. [PubMed: 26805906]

[13]. Olsson A, Hellgren M, Berntorp E, Holmstrom M, Baghaei F, Bleeding phenotype in carriers of haemophilia A does not correlate with thrombin generation, Haemophilia. 21 (1) (2015) e111–e113. [PubMed: 25422177]

[14]. Crawley JT, Zanardelli S, Chion CK, Lane DA, The central role of thrombin in hemostasis, J. Thromb. Haemost 5 (Suppl. 1) (2007) 95–101. [PubMed: 17635715]

[15]. Mann KG, Orfeo T, Butenas S, Undas A, Brummel-Ziedins K, Blood coagulation dynamics in haemostasis, Hamostaseologie. 29 (1) (2009) 7–16. [PubMed: 19151839]

[16]. Hemker HC, Beguin S, Phenotyping the clotting system, Thromb. Haemost 84 (5) (2000) 747–751. [PubMed: 11127849]

[17]. Butenas S, Brummel KE, Branda RF, Paradis SG, Mann KG, Mechanism of factor VIIa-dependent coagulation in hemophilia blood, Blood. 99 (3) (2002) 923–930. [PubMed: 11806995]

[18]. Hemker HC, Giesen P, Al Dieri R, et al., Calibrated automated thrombin generation measurement in clotting plasma, Pathophysiol. Haemost. Thromb 33 (1) (2003) 4–15. [PubMed: 12853707]

[19]. Nair SC, Dargaud Y, Chitlur M, Srivastava A, Tests of global haemostasis and their applications in bleeding disorders, Haemophilia. 16 (Suppl. 5) (2010) 85–92. [PubMed: 20590862]

[20]. Ten Cate H, Thrombin generation in clinical conditions, Thromb. Res 129 (3) (2012) 367–370. [PubMed: 22079443]

[21]. Young G, Sorensen B, Dargaud Y, Negrier C, Brummel-Ziedins K, Key NS, Thrombin generation and whole blood viscoelastic assays in the management of hemophilia: current state of art and future perspectives, Blood. 121 (11) (2013) 1944–1950. [PubMed: 23319573]

[22]. Menezes AA, Vilardi RF, Arkin AP, Cohen MJ, Targeted clinical control of trauma patient coagulation through a thrombin dynamics model, Sci. Transl. Med 9 (371) (2017).

[23]. Brummel-Ziedins KE, Gissel M, Neuhaus J, et al., In silico thrombin generation: plasma composition imbalance and mortality in human immunodeficiency virus, Res. Pract. Thromb. Haemost 2 (4) (2018) 708–717. [PubMed: 30349890]

[24]. Gissel M, Undas A, Slowik A, Mann KG, Brummel-Ziedins KE, Plasma factor and inhibitor composition contributes to thrombin generation dynamics in patients with acute or previous cerebrovascular events, Thromb. Res 126 (4) (2010) 262–269. [PubMed: 20709367]

[25]. Mitrophanov AY, Rosendaal FR, Reifman J, Mechanistic modeling of the effects of acidosis on thrombin generation, Anesth. Analg 121 (2) (2015) 278–288. [PubMed: 25839182]

[26]. Mitrophanov AY, Rosendaal FR, Reifman J, Computational analysis of inter-subject variability and thrombin generation in dilutional coagulopathy, Transfusion. 52 (11) (2012) 2475–2486. [PubMed: 22429019]

[27]. Tripodi A, Mannucci PM, The coagulopathy of chronic liver disease, N. Engl. J. Med 365 (2) (2011) 147–156. [PubMed: 21751907]

[28]. Tosetto A, Rodeghiero F, Castaman G, et al., A quantitative analysis of bleeding symptoms in type 1 von Willebrand disease: results from a multicenter European study (MCMDM-1 VWD), J. Thromb. Haemost 4 (4) (2006) 766–773. [PubMed: 16634745]

[29]. Hockin MF, Jones KC, Everse SJ, Mann KG, A model for the stoichiometric regulation of blood coagulation, J. Biol. Chem 277 (21) (2002) 18322–18333. [PubMed: 11893748]

[30]. Butenas S, Orfeo T, Gissel MT, Brummel KE, Mann KG, The significance of circulating factor IXa in blood, J. Biol. Chem 279 (22) (2004) 22875–22882. [PubMed: 15039440]

[31]. Hockin MF, Cawthern KM, Kalafatis M, Mann KG, A model describing the inactivation of factor Va by APC: bond cleavage, fragment dissociation, and product inhibition, Biochemistry. 38 (21) (1999) 6918–6934. [PubMed: 10346914]

[32]. Brummel-Ziedins KE, Orfeo T, Callas PW, Gissel M, Mann KG, Bovill EG, The prothrombotic phenotypes in familial protein C deficiency are differentiated by computational modeling of thrombin generation, PLoS One 7 (9) (2012) e44378. [PubMed: 22984498]

[33]. Doyle MF, Mann KG, Multiple active forms of thrombin. IV. Relative activities of meizothrombins, J. Biol. Chem 265 (18) (1990) 10693–10701. [PubMed: 2355015]

[34]. Cote HC, Bajzar L, Stevens WK, et al., Functional characterization of recombinant human meizothrombin and Meizothrombin(desF1). Thrombomodulin-dependent activation of protein C and thrombin-activatable fibrinolysis inhibitor (TAFI), platelet aggregation, antithrombin-III inhibition, J. Biol. Chem 272 (10) (1997) 6194–6200. [PubMed: 9045633]

[35]. Egan JO, Kalafatis M, Mann KG, The effect of Arg306→Ala and Arg506→Gln substitutions in the inactivation of recombinant human factor Va by activated protein C and protein S, Protein Sci. 6 (9) (1997) 2016–2027. [PubMed: 9300501]

[36]. Kalafatis M, Mann KG, Role of the membrane in the inactivation of factor Va by activated protein C, J. Biol. Chem 268 (36) (1993) 27246–27257. [PubMed: 8262965]

[37]. Nicolaes GA, Tans G, Thomassen MC, et al., Peptide bond cleavages and loss of functional activity during inactivation of factor Va and factor VaR506Q by activated protein C, J. Biol. Chem 270 (36) (1995) 21158–21166. [PubMed: 7673148]

[38]. Bravo MC, Orfeo T, Mann KG, Everse SJ, Modeling of human factor Va inactivation by activated protein C, BMC Syst. Biol 6 (2012) 45. [PubMed: 22607732]

[39]. Brummel-Ziedins K, Vossen CY, Rosendaal FR, Umezaki K, Mann KG, The plasma hemostatic proteome: thrombin generation in healthy individuals, J. Thromb. Haemost 3 (7) (2005) 1472–1481. [PubMed: 15978105]

[40]. Gupta S, Heiman M, Duncan N, Hinckley J, Di Paola J, Shapiro AD, Variable bleeding phenotype in an Amish pedigree with von Willebrand disease, Am. J. Hematol 91 (10) (2016) E431–E435. [PubMed: 27414491]

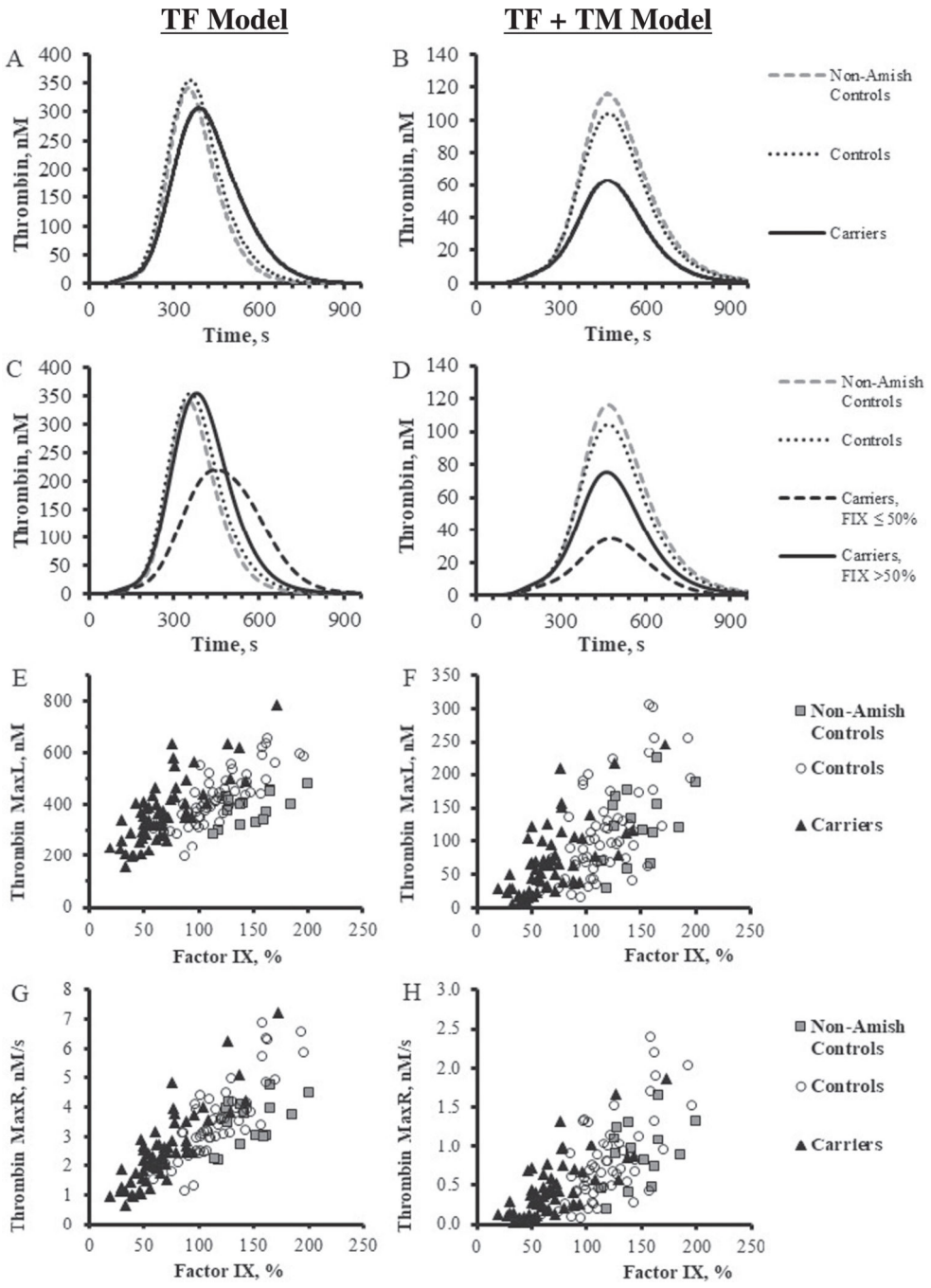

Fig. 1.

Thrombin simulation of Amish cohorts. Computational simulations of carriers (solid black line) and controls (dotted black line) in response to (A) 5 pM TF or (B) 5 pM TF and 1 nM soluble Thrombomodulin. Curves are the Mean + SEM of all individual curves. Simulation of NAC (Non-Amish Controls) (dashed grey line) is provided as a reference. Carriers are then divided based on Factor IX Level ≤50% (dashed black) or > 50% (solid black) and thrombin generation curves in response to (C) 5 pM TF or (D) 5 pM TF and 1 nM soluble Thrombomodulin; Controls and NAC are shown as reference.

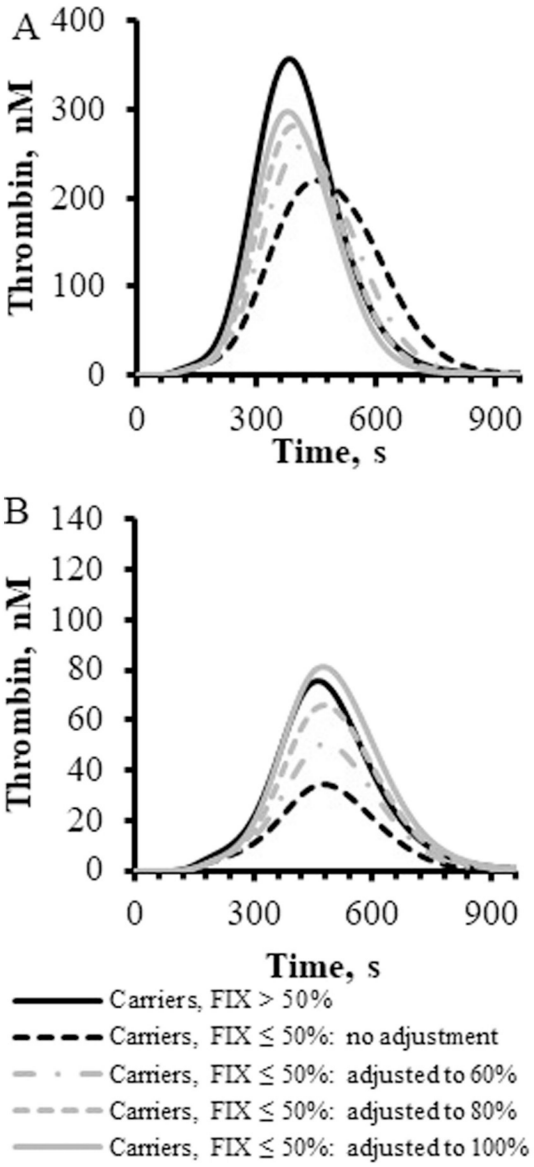

Fig. 2.

Simulated effects of factor IX supplementation. Thrombin simulations using the (A) TF only or (B) TF + TM model were conducted on the post-menarchal Carriers, mean curves were generated based on high (> 50%, solid black line) or low (≤50%, dashed black line) FIX:Clevels. Simulations on low FIX:C Carriers were conducted with a titration of increasing FIX:C levels: 60% (dot+dash grey line), 80% (dash grey line) and 100% (solid grey line).

Table 1

Subject characterization by FIX:C level, bleeding score (BS) and bleeding symptoms in Amish hemophilia B carriers and controls.

	Carriers (N = 59) Mean (SD)	Controls (N = 57) Mean (SD)	p	Carriers FIX:C ≤50% (N = 18/59) Mean (SD)	Carriers FIX:C > 50% (N = 41/59) Mean (SD)	p
Age in years	32.3 (14.5)	40.7 (16.2)	< 0.05	33.2 (14.0)	31.9 (14.9)	0.76
Total BS	1.71 (2.04)	1.61 (2.09)	0.80	2.06 (1.98)	1.56 (2.07)	0.40
FIX:C[a] %	67.9 (30.0)	118.8 (29.3)	< 0.05	40.0 (9.3)	80.1 (27.6)	< 0.05
	%[b]	%[b]	p	%[b]	%[b]	p
Bruising	30.5	31.6	0.90	22.2	34.2	0.36
Epistaxis	8.5	8.8	0.95	5.6	9.8	0.59
Dental bleeding	8.5	5.3	0.50	22.2	2.4	< 0.05
Surgical bleeding	6.8	10.5	0.47	11.1	4.9	0.38
Menorrhagia	5.1	12.3	0.17	5.6	4.9	0.91
PPH[c]	32.2	29.8	0.78	33.3	31.7	0.90
Hemarthrosis	3.4	0.0	0.16	0.0	4.9	0.34
Wound bleeding	3.4	1.8	0.58	11.1	0.0	< 0.05

[a] FIX:C level carriers vs. controls-range 19–172 vs. 59–196.

[b] % of subjects from the group with BS of 1 or above.

[c] PPH: post-partum hemorrhage.

Table 2

Mean coagulation factor levels of Amish hemophilia B carriers and controls.

Measure	Carriers[a] (N-59) Mean (SD)	Controls (N-57) Mean (SD)	*P*	Carriers[b] IX:C ≤ 50% (N-18/59) Mean (SD)	Carriers IX:C > 50% (N-41/59) Mean (SD)	*p*
Factor XI, %	126.0 (29.2)	129.8 (31.8)	0.51	124.3 (26.2)	126.7 (30.7)	0.77
Factor XII, %	154.3 (51.4)	148.5 (44.6)	0.52	125.4 (44.9)	166.9 (49.3)	< 0.05
Fibrinogen, g/L	3.44 (0.75)	3.65 (0.7)	0.13	3.10 (0.72)	3.60 (0.71)	< 0.05
VWF:Ag, %	107.8 (30.8)	132.9 (49.2)	< 0.05	108.2 (26.5)	107.7 (32.8)	0.95
TAFI, %	102.6 (19.7)	111.1 (24.8)	< 0.05	96.4 (12.2)	105.2 (21.8)	0.12
Factors (%) used for modeling thrombin kinetics						
Factor II	121.2 (21.4)	119.3 (16.5)	0.59	112.9 (18.7)	124.8 (21.7)	< 0.05
Factor V	104.1 (22.3)	107.6 (20.1)	0.37	105.4 (32.7)	103.5 (16.3)	0.76
Factor VII	115.1 (28.0)	124.9 (34.4)	0.09	103.3 (24.0)	120.2 (28.3)	< 0.05
Factor VIII	142.3 (47.3)	157.5 (52.7)	0.11	137.3 (43.1)	144.6 (49.3)	0.59
Factor IX:C	67.9 (30.0)	118.8 (29.3)	< 0.05	40.0 (9.3)	80.1 (27.6)	< 0.05
Factor X	108.9 (21.0)	110.4 (22.4)	0.71	100.7 (18.9)	112.6 (21.0)	< 0.05
AT	117.0 (15.9)	117.7 (14.6)	0.81	120.8 (16.6)	115.3 (15.5)	0.22
Protein C	132.9 (27.9)	137.2 (31.0)	0.44	121.4 (27.1)	137.9 (27.1)	< 0.05
$TFPI_{Total}$	64.6 (18.1)	68.6 (17.5)	0.23	68.8 (19.6)	62.8 (17.4)	0.24

[a] HB carriers vs. controls-range 19–172 vs. 59–196.

[b] HB carriers ≤50% vs. > 50%-range 19–50 vs. 52–172.

Table 3

Mean factor levels by bleeding score category.

Measure	Cohort	BS = 0	BS = 1–2	BS ≥ 3	*p*
N	**Carrier**	**22**	**21**	**16**	
	Control	**22**	**21**	**14**	
Factor II	Carrier	126.0 ± 24.9	114.2 ± 11.8	123.8 ± 24.6	0.17
	Control	120.3 ± 14.4	112.4 ± 13.8	127.9 ± 19.8	< 0.05
Factor V	Carrier	99.3 ± 14.7	103.8 ± 29.5	110.9 ± 19.5	0.29
	Control	102.8 ± 19.4	108.6 ± 17.3	113.6 ± 24.5	0.29
Factor VII	Carrier	117.3 ± 24.6	108.8 ± 34.5	120.3 ± 22.2	0.43
	Control	123.4 ± 43.4	121.9 ± 30.1	131.6 ± 24.2	0.70
Factor VIII	Carrier	148.5 ± 39.7	141.5 ± 57.4	135.0 ± 43.8	0.69
	Control	157.7 ± 56.7	166.0 ± 53.0	144.3 ± 46.1	0.50
Factor IX:C	Carrier	78.5 ± 32.9 *	67.0 ± 32.0 *	54.4 ± 15.6 *, +	< 0.05
	Control	120.2 ± 28.3	110.9 ± 28.8	128.7 ± 30.2	0.20
Factor X	Carrier	109.7 ± 20.2	112.5 ± 23.3	103.2 ± 18.8	0.40
	Control	115.7 ± 25.1	101.6 ± 20.3	115.4 ± 17.5	0.07
AT	Carrier	112.9 ± 17.4	118.3 ± 15.0	120.9 ± 14.4	0.28
	Control	117.8 ± 14.9	113.8 ± 13.2	123.2 ± 15.3	0.18
Protein C	Carrier	141.4 ± 28.9	129.4 ± 27.5	125.8 ± 25.8 *	0.18
	Control	136.5 ± 34.2	129.3 ± 31.2	150.0 ± 21.5	0.15
$TFPI_{Total}$	Carrier	60.6 ± 13.6	63.1 ± 16.0	72.1 ± 24.2	0.14
	Control	67.8 ± 14.6	71.5 ± 20.6	65.5 ± 17.1	0.60

* Indicates $p < 0.05$ for comparison of carrier and control in the same BS category.

+ Indicates $p < 0.05$ in comparison of BS = 0 vs. BS ≥3 for Carriers.

Table 4

Thrombin generation parameters in hemophilia B carriers and controls.

Model	Measure	Carriers	Controls	Carriers FIX:C ≤ 50%	Carriers FIX:C > 50%
	N	**59**	**57**	**18**	**41**
TF model mean	CT (s)	146.3	146.3	169.0 **	136.4
	MaxL,(nM)	372.2 *	426.5	278.7 **	413.2
	MaxR (nM/s)	2.5 *	3.6	1.56 **	2.96 t
	AUC (nM•s)	85,785	81,498	74,787 **	90,613 t
TF + TM model mean	CT(s)	291.8	284.8	331.8 **	277.2
	MaxL (nM)	69.4 *	115.3	38.6 **	82.9 t
	MaxR (nM/s)	0.44 *	0.81	0.21 **	0.54 t
	AUC (nM•s)	18,862 *	30,713	10,758 **	22,420 t

* Indicates $p < 0.05$ between Carriers and Controls.

** Indicates $p < 0.05$ between Carriers with FIX:C ≤ 50% and FIX:C > 50%.

t Indicates $p < 0.05$ between Controls and Carriers with FIX:C > 50%.