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Risk Factors for Inhibitor Development in Persons with Non-Severe Hemophilia A

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ABSTRACT

Hemophilia A, or factor VIII (FVIII) deficiency, is a congenital bleeding disorder affecting 1/10,000 males. A significant complication of hemophilia A is the formation of antibodies that bind FVIII at the time of treatment and inhibit FVIII activity. These antibodies are termed inhibitors. One-quarter of new inhibitors occur in those with non-severe (FVIII 1-40%) disease. In non-severe hemophilia A, intensive treatment with FVIII has been observed in case series to precede inhibitor formation in a majority of patients.

To estimate the risk of inhibitor formation following intensive exposure to FVIII, defined as 6 or more consecutive days of FVIII, a case control study was performed. Cases were defined as having had an inhibitor titer >1 BU/ml on two occasions. Information on subject characteristics and treatments during the year prior to inhibitor development or enrollment was retrospectively gathered. A blood sample was obtained for FVIII genotyping.

Approximately 55% of case subjects had received intensive FVIII exposure, during the year prior to inhibitor formation compared to 25.5% of controls during the year prior to enrollment [unadjusted OR 4.55 (95% CI 1.78-11.60)]. In subjects 30 years of age or older, intensive exposure had a greater association with inhibitor formation than in those that were less than 30 years of age (OR 13.65 and 1.73 respectively). After adjusting for a baseline FVIII of 1-2%, the odds ratio measuring the association between intensive exposure to FVIII and inhibitor development was increased to 5.61 consistent with a confounding effect. On multivariate analysis, intensive exposure to FVIII and a baseline FVIII of 1-2% were associated with inhibitor formation after adjusting for age <30 years, race, recombinant product use, and having less than 50 lifetime FVIII exposure days. None of the subject or treatment characteristics were clearly associated with inhibitor formation on subset analysis, although surgery as the indication for intensive exposure as well as receiving FVIII by continuous infusion both showed a trend toward an association.

This study confirms that intensive exposure is a strong risk factor for inhibitor formation in non-severe hemophilia A. This association was present after adjustment for the number of prior exposure days to FVIII and severity of disease and was stronger in those over thirty years of age.

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INTRODUCTION

Hemophilia A is an inherited bleeding disorder caused by dysfunctional or deficient production of coagulation factor VIII (FVIII). Treatment of hemophilia A is primarily accomplished by replacement of FVIII via intravenous infusion. Although the availability of FVIII has led to dramatic improvement in the health and well being of those affected by hemophilia, the most significant complication of replacement therapy is the development of an inhibitory antibody (inhibitor). Inhibitors bind to exogenous FVIII rendering clotting factor replacement ineffective. When this occurs, treatment is more difficult and morbidity increases (1).

Although persons with severe hemophilia A (FVIII activity <1%) are at greatest risk, one quarter of new inhibitors develop in patients with mild or moderate hemophilia A (FVIII activity 1-40%) (1). Inhibitor development in mild or moderate hemophilia A has been observed to occur during intensive exposure to FVIII (2, 3). However, most patients with mild or moderate hemophilia who receive FVIII in an intensive fashion do not develop an inhibitor. To date, there are no comparisons between those patients with mild or moderate hemophilia who develop an inhibitor and those who do not. This study was designed to estimate the risk associated with intensive FVIII replacement and to examine the interaction of this exposure with other potential modifiers. This project is an important first step in understanding inhibitor development in persons with mild or moderate hemophilia A.

BACKGROUND

Hemophilia A affects 1 in 10,000 male births. Approximately 50% of those affected will have mild (6-40% FVIII) or moderate (1-5% FVIII) disease. In this milder disease population, typical clinical manifestations include abnormal bleeding with trauma or surgery and infrequent spontaneous joint or muscle bleeding. Thus FVIII infusions in many with non-severe hemophilia A may be limited to management of hemostasis around surgery or trauma requiring multiple days of intensive therapy.

A major complication of treatment is the development of inhibitory antibodies. It is currently the most important problem affecting hemophilia management now that the majority of children with severe hemophilia are on routine prophylaxis to prevent joint bleeding with resultant arthropathy and now that the risk of blood-borne infections has been reduced to essentially zero. Estimates of the cumulative risk of inhibitor formation has varied between 20-33% in patients with severe hemophilia A and 3-13% in those in mild or moderate hemophilia A (4-9). The incidence of inhibitor formation in patients with severe hemophilia A was estimated to be 6.4 per 1000 person years for all age groups with the highest rate occurring in those less than 5 years of age (34.4 per 1000 person years. In patients with mild and moderate hemophilia A, inhibitors occur with approximately one-quarter the frequency of that seen in patients with severe hemophilia A; 1.7 per 1000 person years for all ages and 9.3 per 1000 persons in those less than 5 years of age (1).

The development of an antibody is a complex process but broadly requires: 1) failure of self tolerance, 2) exposure to foreign antigen, and 3) the development of an

immune response upon exposure to the foreign antigen. Therefore, both patient and treatment characteristics which influence tolerance, antigen exposure, and immune responses, may influence the risk of inhibitor development within individuals (see table 1). In patients with severe hemophilia A, patient-related characteristics have been well characterized, but their role in mild or moderate hemophilia A is less clear.

Table 1. Characteristics which may influence inhibitor formation

Patient-related	**Treatment-related**
Severity of disease	Type of FVIII concentrate
FVIII mutation	Age at first product use
Race	Method of FVIII concentrate delivery
Family history	Circumstances of first FVIII exposure
Polymorphisms of IL10, TNF α, and CTLA4	FVIII prophylaxis

Compared to patient-related characteristics, there is less evidence that treatment-related characteristics influence inhibitor formation in severe hemophilia A. Only one cohort has evaluated the effect of intensive FVIII replacement on inhibitor formation in patients with severe hemophilia A. This study found that 5 or more consecutive days of FVIII replacement at the time of first exposure to FVIII was associated with an increased risk for inhibitor formation (RR 3.3, 95% CI 2.1-5.3) and surgery at the time of first factor infusion also increased the risk of inhibitor formation (RR 2.6, 95% CI 1.3-5.1) (10). In contrast, this cohort study also found that receiving at least once weekly regular infusions of FVIII in a preventive way was protective (RR 0.4, 95% CI 0.2-0.8) (10). The apparent discrepancy in the circumstances of FVIII use and inhibitor formation is consistent with the “danger theory”. The danger theory suggests that injured or dying cells activate antigen presenting cells and further amplify immunological responses (11).

Therefore, at a time of surgery, trauma, or during a major bleeding episode, antigen presentation of FVIII may be more likely to be accompanied by co-stimulatory signals that lead to FVIII being perceived as dangerous and in need of an antibody response. Conversely, FVIII given as a prophylactic infusion would be less likely to be accompanied by co-stimulatory signals indicating danger and thus a tolerogenic response would be more likely.

Although much is known about inhibitor development in severe hemophilia A, how these concepts apply to those with non-severe hemophilia A is less clear. One major reason to consider them as potentially distinct is the presence of circulating endogenous FVIII in those with non-severe disease which should facilitate development of self-tolerance to FVIII.

The largest reported cohort of persons with non-severe hemophilia A included 26 subjects. Sixteen of the 26 reported cases (61.5%) had their inhibitor detected following intensive FVIII replacement therapy for surgery, trauma, or muscle bleeding (2). Details regarding the nature of the intensive exposure were lacking. Specifically, there was no reported information regarding: duration of therapy, time between intensive exposure and inhibitor detection, method of delivery of FVIII replacement, the proportion of subjects with each indication for intensive FVIII replacement or the presence of other confounding factors.

Sharathkumar et al. provided the only other clinical investigation of inhibitor formation in persons with mild hemophilia A. In this retrospective study, 29 boys who had been exposed to FVIII were identified. Of these 29, 16 boys had received daily FVIII for at least 6 consecutive days; 7 by continuous infusion and 9 by bolus injection. Four of

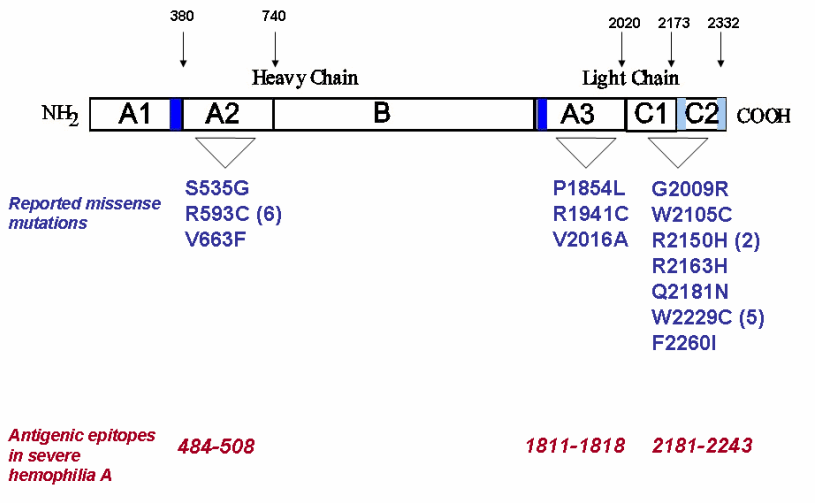
the seven (57%) treated with continuous infusion developed an inhibitor compared to none of the 9 treated with bolus injection (p=0.02) (3). In those 4 that developed an inhibitor, the exposure to 6 consecutive days of factor VIII occurred 4-6 weeks prior to inhibitor detection. The indication for treatment was hemarthrosis (2 subjects), ankle fracture, and neonatal intracranial hemorrhage.

Based upon these observational studies, intensive exposure to FVIII appears to be a risk factor for inhibitor development in those with non-severe hemophilia A. However, given the lack of a control in the study reported by Hay et al, the risk is unconfirmed and the magnitude of risk is unknown. Additionally, it is unknown whether the risk of inhibitor formation associated with intensive exposure in non-severe hemophilia A is the result of the intensity of exposure or that is likely the only exposure these patients might experience. Alternatively, the indication for intensive exposure or the method of delivery of FVIII may be confounding factors.

The FVIII genotype is a major risk factor for inhibitor development in patients with severe hemophilia A. The FVIII genotype has been reported in thirty-four patients with non-severe hemophilia. The mutations in these 34 cases occur in either the A2 or A3 domain or at the junction between the C1 and C2 domain (figure 1) (2, 3, 12-18). The most commonly reported missense mutation in association with inhibitors in non-severe hemophilia is R593C (2, 12, 13, 16, 17). From these reports, it has been hypothesized that there are “hot spots” for mutations that predispose to inhibitor formation in this population. However, in the absence of comparison to unaffected individuals, a reporting bias may exist. Nonetheless, missense mutations in the A2, A3 and C1-C2 domain junction may in fact predispose to inhibitor development since these mutations are in

very close proximity to major antigenic epitopes which have been well defined in persons with severe hemophilia A and inhibitors (aa 484-504, 1811-1818, and 2181-2243) (19).

Figure 1. Domain structure of FVIII, reported missense mutations and antigenic epitopes



METHODS

Null Hypothesis

In patients with non-severe hemophilia A, the proportion of persons that have received 6 or more consecutive days of FVIII is equivalent between those with an inhibitor (case) and those without an inhibitor (control).

Study Design

Retrospective case-control design

Research Subjects

The majority of cases were initially identified from a cohort of persons with hemophilia complied by the Division of Hereditary Blood Disorders of the Centers for Disease Control and Prevention (CDC).

CDC Cohort

Since 1998, using a public health surveillance system, the Universal Data Collection (UDC) Project of the CDC has been assisting the nation's 134 specialized hemophilia treatment centers (HTCs) to monitor the safety of treatment products and the occurrence of complications of bleeding disorders. To date, more than 15,000 people with bleeding disorders have been enrolled in the UDC. Data collected as part of the UDC project makes up the only national database of persons with hemophilia. The UDC cohort served as the source for the majority of cases and controls.

UDC Patient Recruitment. All persons with bleeding disorders who receive care in HTCs are asked to give informed consent to participate in the UDC. The UDC has been approved by the human investigational review boards of CDC and those of each HTC parent institution. An estimated 70% of persons with hemophilia receive their care in an HTC and more than 90% of eligible patients are enrolled (20).

UDC Data collection. Methods of patient recruitment and data collection for this surveillance project have been described elsewhere (21). Briefly, participation in this study involves the annual collection of a standardized set of demographic, clinical and treatment information and donation of a blood specimen for hepatitis and HIV testing by CDC. Month and year of birth, self-reported race and baseline FVIII activity are collected at the time of first enrollment in the surveillance.

Case Selection

Persons with mild and moderate hemophilia (FVIII $\leq$ 1%) with an inhibitor (Bethesda titer $\geq$1) were identified from the UDC data set. A Bethesda titer is a measure of inhibitor concentration based on the residual FVIII activity that is present after a patient's plasma is mixed with normal plasma. Within the cohort of 4,653 persons with non-severe hemophilia, 110 persons with a Bethesda titer $\geq$ 1 were initially identified at 58 HTCs. Two inhibitor titers $\geq$ 1 BU/ml were required to be a case. Of the 110, 53 cases at 20 HTCs were verified. These 29 HTCs were invited to participate. Ultimately 16 HTCs participated by enrolling identified case subjects.

Control Selection

Persons with mild or moderate hemophilia (FVIII 1-40%) that have had prior exposure to FVIII and no history of an inhibitor (Bethesda titer $\leq$ 0.6 BU/ml) were

invited to participate. Persons who had never received FVIII would not be at risk to develop an inhibitor and thus where not part of the pool from which controls were selected. Blood was collected and a Bethesda titer performed to confirm the lack of inhibitor at the time of enrollment.

Protection of human subjects

Approval to obtain additional data and a blood sample from local institutional review boards affiliated with each HTC parent institution was obtained. Consent from individual subjects was obtained in accordance with requirements for the protection of human subjects and health information.

Data Collection

Individual treatment centers completed a data collection form on each case and control subject. The primary exposure of interest was intensive exposure to FVIII, defined as 6 or more consecutive days of FVIII infusion. This duration of exposure was chosen based on its use in prior literature reviews (3) and it is a duration of therapy that is greater than that used for routine spontaneous bleeds. Information was collected on intensive FVIII exposure during the year preceding inhibitor development in cases and the preceding year for enrollment of controls. Additional data included: 1) the indication for FVIII replacement, 2) the method of FVIII delivery (continuous infusion vs. bolus injection), 3) type of product utilized (plasma derived vs. recombinant), 4) total amount of FVIII used during intensive therapy, 5) estimate of the number of lifetime exposure days to FVIII, and 6) family history of an inhibitor. Where possible, information was based on medical record review, but if not available, patient recall was employed.

A blood sample was obtained for FVIII gene mutation analysis that was performed in the laboratory of Dr. Craig Hooper at the CDC by genetic sequencing.

Data Analysis

The odds ratio was calculated from a 2 x 2 table. Confidence intervals (CI) were calculated using the Cornfield's method. Significance of the odds ratio was determined using the Chi-square test. To adjust for confounding by race, family history of inhibitor, and total number of lifetime exposure days, a stratified analysis was performed. When the odds ratio was relatively constant between subgroups, the odds ratio was combined using the Mantel-Haenszel method to form an adjusted odds ratio. To determine if effect modification was occurring, the Breslow Day test for heterogeneity was performed. In addition to a stratified analysis, multivariate analysis was done using logistic regression. The inclusion of interaction terms in the multivariate model was assessed using the Wald test and Likelihood ratio test.

The sub-group of exposed cases and controls were analyzed according to the type of product used (plasma derived vs. recombinant), the indication for factor infusion (surgical vs. non-surgical), and the method of delivery (bolus injection vs. continuous infusion.

RESULTS

Neither the mean age nor the baseline FVIII was different between cases and controls [30.0 years (95%CI 25.5-35.7) versus 30.1 years (95% CI 21.8-38.1) and 6.5% (95% CI 4.0-9.6) versus 7.3% (95% CI 5.5-9.0)]. The frequency of baseline and exposure characteristics in cases and controls is shown in table 1a. Cases were more likely to have received 6 or more consecutive days of FVIII during the year prior to inhibitor development than controls during the year prior to enrollment. Vaccination status was less likely to be known in case subjects compared to control subjects. Age, race, ethnicity, family history of an inhibitor, baseline FVIII activity, number of lifetime FVIII exposure days, and product use during the previous year were not different between cases and controls. The univariate association between these characteristics and inhibitor formation is shown in table 2a. Having a baseline FVIII activity between 1-2% was associated with inhibitor formation (OR 2.59, 95% CI 1.01-6.65). Additionally, having received 6 or more consecutive days of factor VIII during the prior year was strongly associated with inhibitor development (OR 4.55, 95% CI 1.78-11.60).

The association between 6 or more consecutive days of FVIII and inhibitor formation was further explored utilizing a stratified analysis (see table 3a). Age (less or more than 30 years) showed effect modification on the association between 6 or more consecutive days of FVIII and inhibitor formation. The stratum specific odds ratio in those less than 30 years of age was 1.73 compared with 13.65 in those 30 years of age or greater (Breslow Day p=0.04). After adjustment for a baseline FVIII activity of 1-2%, the odds ratio for the association between 6 or more consecutive days of FVIII and inhibitor

formation was 5.61, suggesting a confounding effect. After adjustment for other variables (race, lifetime exposure, age at first factor exposure, recombinant product use, or vaccination) no confounding or effect modification was seen.

On multivariate analysis, baseline FVIII between 1-2% and 6 or more consecutive days of FVIII remained significantly associated with inhibitor formation (OR 4.56, 95% CI 1.46-14.26 and OR 6.44, 95% CI 2.19-18.89) (see table 4a). To further evaluate the confounding effect of a baseline FVIII between 1-2%, logistic regression models with and without the variable FVIII between 1-2% were performed. When FVIII level of 1-2% were not included in the model, the point estimate of the odds ratio for the association between 6 or more consecutive days of FVIII and inhibitor formation was 4.89 (95% CI 1.83-13.08). However, when FVIII level 1-2% was included in the model, the OR increased to 6.44 (95% CI 2.20-18.88), confirming the effect of confounding by baseline FVIII between 1-2% on the association between 6 or more consecutive days of FVIII and inhibitor formation. The variables of age at first factor infusion and vaccination during the prior year were not included in the final model. The independent impact of each of these variables on the model was assessed. Only those 75 subjects for which vaccination status was known were used when comparing the final model with and without vaccination. The point estimate of the odds ratio of the final model excluding vaccination (n=75) was 7.3 and was reduced by less than 10% to 6.80 when vaccination was included. This confirms that vaccination does not confound the association between 6 or more consecutive days of FVIII and inhibitor formation. Additionally, since vaccination had no association with inhibitor formation on univariate analysis nor does any prior literature clearly support its association, this variable was not included in the final model.

Age at first factor infusion ($\leq$ 5 years) was evaluated in a similar fashion, however, the entire sample population was utilized (n=87). The point estimate of the odds ratio for the association between 6 or more consecutive days of FVIII and inhibitor formation was 6.40 when the variable age of first factor exposure was included in the model. This calculation was not different from the odds ratio point estimate when the variable age at first factor exposure was excluded (6.44). Thus, age at first FVIII infusion was not included in the final model.

Interaction between age less than 30 years and 6 or more consecutive days of FVIII and age less than 30 years and a baseline FVIII of 1-2% were assessed by adding the two interaction terms to the final model (see table 5a). The model with the two interaction terms was compared using the Likelihood ratio test (LRT) and Wald test. The model with the two interaction terms was not significantly different than the model without the interaction terms (LRT=0.059 Wald=0.071). The interaction terms were then examined independently. Models with and without the interaction term age <30 years and baseline FVIII 1-2% were not statistically significantly different (LRT=0.341 and Wald =0.343). However, models with and without the interaction term, age <30 years and 6 or more consecutive days of FVIII were statistically significantly different (LRT=0.019 and Wald =0.024).

An analysis of the subgroup of patients that were exposed to 6 or more consecutive days of FVIII concentrates was performed. The frequency of characteristics in cases and controls is shown in table 6a. The distribution of the age at first factor exposure was different between exposed cases compared to exposed controls. The majority of controls received their FVIII at 3-10 years compared with the majority of

cases that received their first FVIII after 10 years. Other variables were not different between cases and controls. On univariate analysis of variables in the subgroup that had received 6 or more consecutive days of FVIII there were no odds ratios that had 95% confidence intervals that did not include one. However, a baseline FVIII of 1-2%, less than 50 lifetime FVIII exposure days, continuous infusion as the method of delivery, and surgery as the indication for treatment had an OR point estimate >3 and the lower end of the 95% CI >0.5 (see table 7a). Since no single variable was associated with inhibitor formation in this subgroup, a multivariate analysis was not performed.

FVIII genotype results are available on 41 of the 87 subjects. Of those for which results are available, there was no difference in the proportion of cases or controls that had mutations in the proposed “hot spots”(see table 8a).

DISCUSSION

Inhibitor formation in non-severe hemophilia A, although rare, is a major complication transforming a manageable disease to one with substantial morbidity. Although much has been learned over the past decade regarding risk factors for inhibitor formation in those with severe hemophilia A, very little is known about risk factors for inhibitor formation in non-severe hemophilia A. In this study, intensive exposure to factor VIII has been confirmed as a risk factor. It appears to be a strong risk factor with an odds ratio of 6.44 (95% CI, 2.19-18.89) after adjustment for age < 30 years, baseline factor VIII 1-2%, < 50 lifetime days of FVIII exposure, race, and the use of recombinant products. Age was an effect modifier of the association between 6 or more consecutive days of FVIII and inhibitor formation. The association was stronger in those over 30 years of age. This may reflect differences in the indication for treatment between those less than 30 years and those older than 30 years. Interestingly, race, family history, and the number of lifetime days of exposure to FVIII were not different between cases and controls. Although the study population is small and only 11 black subjects were enrolled, black subjects represented 12.6% of the study population; a proportion similar to the proportion of blacks in the United States population in 2006 (22). The lack of influence of lifetime exposure days on inhibitor formation may be in part because of the relatively small sample size. However, the point estimate was consistent with, at most, a relatively weak effect (OR 2.40). In contrast, in severe hemophilia A, the first 50 days of exposure to FVIII is considered a high risk period. The risk of inhibitor formation decreases after the first 50 occurring in approximately 2.14 per 1,000 person years (23). Furthermore, in patients with severe hemophilia A, intensive exposure was less of a risk

factor for inhibitor formation when the intensive exposure occurred after the first but within the first 50 days of FVIII exposure (adjusted RR 1.5 (95% CI 0.9-2.5) (10).

The subset analysis suggested, but did not confirm, that in those that received 6 or more consecutive days of factor, surgery was associated with inhibitor formation. Furthermore, surgery as the indication for why intensive FVIII was utilized (6 or more consecutive days) was associated with age over 30 years (p=0.004). Continuous infusion and surgery as an indication for exposure were also potentially correlated in this small sample (p=0.066). This result is in contrast to the study by Sharathkumar where continuous infusion was associated with inhibitor formation, but none of the 4 that developed an inhibitor while receiving a continuous infusion had undergone surgery (3).

Overall, this study has demonstrated that intensive exposure of at least 6 days of FVIII is strongly associated with inhibitor development in patients with non-severe hemophilia A and the risk appears greatest in those 30 years of age or older. Further investigation is required to determine if the indication for exposure or the method of FVIII delivery is influential.

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Table 1a. Frequency of characteristics in groups

Characteristics	Cases N (%)	Controls N (%)	Chi-square	P-value
Age < 30 years	18 (50.0)	25 (49.0)	0.008	0.93
Race				
White	30 (83.3)	44 (86.3)	0.16	0.94
Black	5 (13.9)	6 (11.8)		
Other	1 (2.8)	1 (2.0)		
Hispanic ethnicity	3 (8.3)	6 (11.8)	0.25*	0.73
Family history of inhibitor	4 (11.1)	4 (7.8)	0.25*	0.71
Baseline factor VIII				
≤ 2%	15 (41.7)	11 (21.6)	4.11	0.13
2.1-5%	9 (21.2)	16 (31.4)		
>5%	12 (25.0)	24 (47.1)		
Age at first factor infusion				
≤ 2 years	12 (33.3)	18 (35.2)	2.28	0.32
3-10 years	9 (25.0)	19 (37.2)		
>10 years	15 (41.7)	14 (27.5)		
Lifetime FVIII exposure				
<50 days	20 (55.6)	21 (41.2)	1.78	0.41
50-100 days	6 (16.7)	12 (23.5)		
>100 days	10 (27.8)	18 (35.3)		
Product during prior year				
Plasma-derived	10 (28.6)	9 (17.7)	2.89	0.23
Recombinant	23 (65.7)	34 (66.7)		
None	2 (5.7)	8 (15.7)		
≥ 6 consecutive days of FVIII	20 (55.6)	13 (25.5)	8.10	0.004
HIV Infection				
Negative	30 (83.3)	47 (92.2)	1.89	0.39
Positive	2 (5.6)	2 (3.9)		
Unknown	4 (11.1)	2 (3.9)		
HCV Infection				
Negative	17 (47.2)	28 (54.9)	5.49	0.06
Positive	12 (13.3)	21 (41.2)		
Unknown	7 (19.4)	2 (3.9)		
Vaccination			13.68	0.001
No	11 (34.4)	35 (79.9)		
Yes	11 (34.4)	10 (20.8)		
Unknown	10 (31.3)	3 (6.3)		

*Fishers' Exact test
FVIII= Factor VIII
HIV= Human immunodeficiency virus
HCV= Hepatitis C virus

Table 2a. Univariate association of characteristics with inhibitor development

Characteristics	Odds Ratio	95% CI
Age < 30 years	0.96	0.41-2.26
Race: White*	0.80	0.24-2.60
Baseline FVIII 1-2%	2.59	1.01-6.65
<50 Lifetime exposure days to fVIII	1.78	0.75-4.23
Vaccination during the prior year**	2.37	0.87-6.44
6 consecutive days of factor	4.55	1.78-11.60
Recombinant product during the prior year#	0.88	0.36-2.17
Age ≤ 5 years at first factor exposure	0.99	0.42-2.34

*White race versus the combination of Black and Other race
** Included only 75 subjects for which vaccination status was known, 26 cases and 49 controls.
# Use of a recombinant product versus the combination plasma-derived and no product use

Table 3a. Association of 6 or more consecutive days of factor VIII after adjustment for other characteristics

Characteristics	Stratum Specific OR		BD P-value	Adjusted OR	95% CI
	Yes	No			
Age <30 years	1.73	13.65	0.04	NA	NA
Race White	5.88	1.33	0.22	4.38	1.72-11.16
Baseline FVIII 1-2%	8.75	4.88	0.65	5.61	2.04-15.42
<50 Lifetime exposure days to FVIII	11.14	2.14	0.10	4.36	1.71-11.09
Vaccination**	4.67	4.14	0.91	4.34	1.51-12.47
Recombinant product	6.09	2.92	0.48	4.77	1.84-12.37
Age $\leq$ 5 years at first factor exposure	2.57	9.75	0.18	4.79	1.83-12.52

Crude OR 4.55 (95% CI 1.78-11.60)
OR=odds ratio
BD=Breslow Day Test
CI=confidence interval
FVIII=factor VIII
** Used data only from subjects in which the vaccination status was known

Table 4a. Multivariate association of characteristics with inhibitor development

Characteristics	Parameter Estimate	SE	OR	CI
Intercept	-1.74	0.89		
6 consecutive days of factor	1.86	0.55	6.44	2.19-18.89
Age < 30 years	0.18	0.53	1.19	0.42-3.37
Baseline FVIII ≤2%	1.52	0.58	4.56	1.46-14.26
< 50 Lifetime exposure days	0.88	0.52	2.40	0.87-6.64
White race	0.11	0.69	1.11	0.28-4.29
Recombinant product	-0.59	0.54	0.55	0.19-1.59

Hosmer and Lemeshow Goodness of Fit = 6.50, DF=6, p=0.37
Likelihood ratio = 20.49, DF=6, p=0.002
-2 Log Likelihood=118.01 for intercept only and 97.52 for intercept and covariates
FVIII = factor VIII

Table 5a. Multivariate analysis of characteristics and their association with inhibitor formation including interaction

Characteristics	Parameter Estimate	SE	Chi-square	P value
≥ 6 or more consecutive days of FVIII	3.11	0.87	12.88	<0.001
Age < 30 years	1.02	0.86	1.41	0.235
Baseline FVIII 1-2%	1.31	0.93	1.98	0.160
<50 Lifetime exposure days to FVIII	0.82	0.54	2.34	0.126
White race	0.11	0.71	0.03	0.872
Recombinant product	-0.84	0.58	2.01	0.148
Age < 30 years x ≥ 6 consecutive days of FVIII	-2.37	1.12	4.46	0.035
Age < 30 years X Baseline FVIII 1-2%	0.48	1.17	0.17	0.680

Likelihood Ratio= 26.13, DF=8, p=0.001
-2 Log Likelihood= 118.01 for intercept only and 91.88 for intercept and covariates
FVIII = factor VIII
SE= Standard error

Table 6a. Frequency of characteristics in sub-group of subjects who received 6 or more consecutive days of factor VIII

Characteristics	Cases N=20	Controls N=11	Chi-square	P-value
	N (%)	N (%)		
Age < 30 years	7 (35.0)	7 (63.6)	1.65	0.20
Race				
White	17 (85.0)	9 (72.7)	1.99	0.37
Black	3 (15.0)	2 (18.2)		
Other	0	1 (9.1)		
Hispanic ethnicity	0	1 (9.1)		
HIV Infection				
Negative	18 (90.0)	10 (81.8)	0.42	0.81
Positive	1 (5.0)	1 (9.1)		
Unknown	1	1 (9.1)		
HCV Infection				
Negative	11 (55.0)	8 (63.6)	0.23	0.89
Positive	7 (35.0)	1 (27.3)		
Unknown	2 (10.0)	1 (9.1)		
Age at first factor infusion				
≤ 2 years	4 (20.0)	2 (18.2)	6.57	0.04
3-10 years	4 (20.0)	7 (63.6)		
≥11 years	12 (60.0)	2 (18.2)		
Baseline factor VIII				
≤2.0%	7 (35.0)	1 (9.1)	2.82	0.24
2.1-5.0%	5 (25.0)	5 (45.5)		
>5.1%	8 (40.0)	5 (45.5)		
Family history of inhibitor	1 (5.0)	1 (9.1)	NA	NA
Recombinant product during prior year	15 (75.0)	8 (72.7)	0.32	1.00
Lifetime exposure days				
<50	13 (65.0)	3 (27.3)	4.09	0.13
50-100	3 (15.0)	3 (27.3)		
>100	4 (20.0)	5 (45.5)		
Vaccination				
No	6 (30.0)	7 (63.6)	3.89	0.14
Yes	7 (35.0)	3 (27.3)		
Unknown	7 (35.0)	1 (9.1)		
Continuous infusion	9 (45.0)	2 (18.2)	0.11	0.24
Highest Daily Dose				
<50 U/kg	9 (45.0)	8 (63.6)	2.28	0.32
50-100 U/kg	9 (45.0)	2 (18.2)		
>100 U/kg	2 (10.0)	2 (18.2)		
Indication for 6 or more days of FVIII				
Joint Bleed	3 (15.0)	1 (9.1)	NA	1.0*
Muscle Bleed	5 (15.0)	3 (27.3)	NA	1.0*
Surgery	14 (70.0)	4 (36.4)	NA	0.12*
ICH	2 (10.0)	0	NA	0.52*
Other	0	5 (45.5)	NA	0.002*

Table 6a cont.

Characteristics	Cases N=20	Controls N=11	Chi-square	P-value
Infection during 6 or more days of FVIII	2 (10.0)	4 (45.5)	0.085	0.15
Number of exposures				
1	17 (85.0)	9 (81.8)	0.93	0.63
2	2 (10.0)	2 (18.2)		
3	1 (5.0)	0		
Recombinant product during 6 day exposure	15 (75.0)	8 (72.7)	0.32	1.00

*Fishers exact test
ICH=intracranial hemorrhage
HIV= human immunodeficiency virus
HCV=hepatitis C virus

Table 7a. Univariate association of subject characteristics with inhibitor formation in the subgroup of subjects exposed to 6 or more consecutive days of factor VIII

Characteristics	**Odds Ratio**	**95% CI**
Age < 30 years	0.30	0.07-1.43
White race	0.79	0.12-5.66
Baseline FVIII 1-2%	5.38	0.56-51.17
< 50 FVIII exposure days	3.33	0.67-16.76
≤ 5 years of age at first factor infusion	0.65	0.14-2.89
Vaccination*	2.72	0.47-15.47
Recombinant product	1.12	0.21-5.97
Daily dose >50 U/kg	2.14	0.47-9.70
Continuous infusion	3.68	0.62-21.55
Surgery as indication	4.08	0.86-19.37

* n=75 and includes only those subjects for whom vaccination status was known

Table 8a. Frequency of factor VIII mutations within reported “hot spots”

LOCATION OF MUTATION	CASE N=19	CONTROL N=22	Chi-square	P-Value
	N (%)	N (%)		
Mutation in a “hot spot”	8 (42.1)	8 (36.4)	0.14	0.71
535-663	3 (15.7)	3 (13.6)	-	-
1854-2016	2 (10.5)	2 (9.1)	-	-
2009-2286	3 (15.7)	3 (13.6)	-	-