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Risk Factors for Inhibitor Formation in Hemophilia: A Prevalent Case-Control Study

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Abstract

Background—Inhibitor formation is a major complication of hemophilia treatment.

Aim—In a prevalent case-control study, we evaluated blood product exposure, genotype, and HLA type on hemophilia A inhibitor formation.

Methods—Product exposure was extracted from medical records. Genotype was determined on stored DNA samples by detection of virtually all mutations-SSCP (DOVAM-S) and subcycling PCR. HLA typing was performed by PCR amplification and exonuclease-released fluorescence.

Results—Cases experienced higher intensity factor, 455 vs. 200 U per exposure, $p<0.005$, more frequent central nervous system (CNS) bleeding, 7 of 20 (35.0%) vs. 1 of 57 (1.7%), $p=0.001$, and more commonly from inhibitor families, 7 of 20 (35.0%) vs. 0 of 57 (0%), $p<0.001$, and African-American, 12 of 63 (19.0%) vs. 6 of 117 (5.1%), $p=0.015$. Among the latter, CNS bleeding was more commonly the initial bleed, 60% vs. 0%, $p<0.001$, and survival was shorter, 14 vs. 38 yr, $p=0.025$. Inhibitor formation was uncommon in those with missense mutations, 2 of 65 (3.1%) vs. 31 of 119 (26.0%), $p=0.008$, and unrelated to factor VIII immunogenic epitope, $p=0.388$, or HLA type, $p>0.100$. Genotype was not associated with race. Time to immune tolerance was shorter for titers < 120 vs. ≥ 120 BU/ml, 6 vs. 16 months, $p<0.01$, but unaffected by tolerizing dose regimen, $p>0.50$.

Conclusions—Inhibitor formation is associated with high intensity product exposure, CNS bleeding, African-American race, and low frequency of missense mutations. The ideal time to initiate prophylaxis to reduce CNS bleeding and inhibitor formation will require prospective studies.

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The authors declare no competing financial interests.

Keywords

central nervous system bleeding; factor VIII; hemophilia; inhibitor

Introduction

Inhibitor formation is a major complication of hemophilia treatment that interferes with clinical response to factor infusion and results in significant morbidity. Estimated to occur in up to 15–25% of hemophilia A patients treated with factor VIII [1], inhibitor antibody formation is a T cell-dependent immune response directed against infused factor VIII [2–4]. Inhibitors occur early in life, typically after nine exposure days [1], are familial, with racial predilection among African-Americans [5,6]. HLA type and hemophilia genotype are considered weak predictors [7], although disruptive factor VIII gene mutations, e.g. large deletion or nonsense gene mutations, especially those with stop codons, have been identified in individuals with inhibitors [8,9]. Environmental factors associated with inhibitor formation include young age [6,10–14] and high intensity factor exposure [15], potential danger signals at the time of factor infusion [16]. Predictors of inhibitor formation, however, remain elusive, as most studies to date are small and uncontrolled and lack sufficient characterization of environmental and genetic factors. We, therefore, conducted a prevalent case-control study to determine environmental (clotting factor exposure intensity) and genetic (hemophilia A genotype, HLA type) factors predictive of hemophilia A inhibitor formation.

Methods

Study Subjects

A total of 950 patients cared for at 16 U.S. hemophilia treatment centers were enrolled on The Hemophilia Inhibitor Study (HIS), an NHLBI-funded prevalent case-control study to determine predictors of inhibitor formation. Study subjects included 283 hemophilia A inhibitor cases and 667 age-matched hemophilia A controls without inhibitors, all previously enrolled in the Hemophilia Malignancy Study (HMS), an NCI-funded case-control study to evaluate malignancy in over 3,000 hemophilia patients from 18 treatment centers within the U.S. between 1989 and 1993 [17]. Sixteen of the 18 HMS sites agreed to participate in the HIS study. Using ID numbers from the HMS study database, inhibitor cases were defined as those hemophilia A subjects with an inhibitor titer measured in Bethesda units (BU/ml) above the normal range. For each identified case, three age-matched (within 3 years) controls without an inhibitor were identified from the database at the same hemophilia center. Separate data collection forms on cases and controls were distributed to each site, to collect demographic information, blood product exposure, concurrent vaccinations, infections, peak inhibitor titer, immune tolerance dose regimen, and time to tolerance. Peripheral blood mononuclear cells stored at −80°C collected during the HMS study were available for genotype analysis and HLA typing. Analysis was limited to cases and controls with hemophilia A only, and genotyping was limited to factor VIII gene analysis only on cases with at least one matched control.

Blood Product Exposure and Clinical Data Collection

Blood product exposure, including the number of units per treatment and the number of treatments, was available on 20 hemophilia A cases with inhibitors and 57 age-matched (within 5 years) hemophilia A controls without inhibitors from one HIS site, the Hemophilia Center of Western Pennsylvania (HCWP). Blood product exposure data were collected from the date of initial exposure to the date of first inhibitor detection in the cases, and from the

date of initial exposure to the date of first inhibitor detection in the case for each matching control. Concurrent vaccinations and infections within 4 months of inhibitor detection were also obtained.

Time to Immune Tolerance Inhibition in Inhibitor Cases

The time to immune tolerance was available on 39 hemophilia A inhibitor cases. Immune tolerance was defined as the date when anti-VIII=0 BU/ml was achieved. The time to tolerance was defined as the time from initiation of a tolerizing regimen to the time immune tolerance was achieved.

Hemophilia A Genotype and HLA Analysis

Hemophilia A genotype and HLA class II type analyses were performed on 65 inhibitor cases and 119 matched controls, matched within 3 years of age of cases, from the 16 HIS sites, on whom peripheral blood mononuclear cells were available (see above). Genetic analyses were performed on frozen genomic DNA samples obtained from cases and controls. Intron 1 and intron 22 inversions of the factor VIII gene [18] were assayed by PCR [7,14,19]. Point mutations in exons and splice junctions were performed by DOVAM-S (detection of virtually all mutations-SSCP), a robotically enhanced, high throughput and multiplex form of SSCP (single strand polymorphism) that detects virtually all mutations [20,21]. The exons and splice junctions of the factor VIII gene were amplified and pooled by the ABI PRISM 877 integrated thermal cycler (Applied Biosystem, Inc, Foster City CA), denatured, and electrophoresed under five non-denaturing conditions in which gel matrix, buffer, temperature, and additive were varied as previously described [20,21]. The frequency of mutations associated with immunogenic epitopes in the F.VIII A2 and C2 domains, was also determined.

HLA Typing

Molecular typing of HLA-DQ and HLA-DR was performed by fluorescence-based oligonucleotide probe assay [22,23]. Using sequence-specific priming (SSP) and exonuclease-released fluorescence (SSPERF), with fluorogenic probes for the 5′nuclease assay to detect polymerase chain reaction (PCR)-amplified target DNA, double-labeled fluorescent probes were utilized to detect HLA-DRB [22], HLA-DQB1 [23], HLA-A [24], and HLA-B.

Statistical Analysis

In order to determine risk factors associated with inhibitor formation, demographic characteristics were compared between cases and controls, including race, severity, family history, blood product exposure, concomitant events, and survival. Descriptive statistics, including measures of central tendency, i.e. means, medians, percentiles, and dispersion, i.e. standard deviations, ranges, were computed for continuous data such as age and clotting factor usage at inhibitor detection. Frequency distributions were estimated for categorical data such as race. For discrete data, the chi-square test or Fisher’s exact test was used, and for continuous data, the Student’s t test or Wilcoxon rank sum test was used to compare risk factors between cases and controls. Analysis of genotype and HLA type data was limited to the subset of 65 cases with 1–3 unique age-matched controls. The relationship between inhibitor formation and dichotomous variables, hemophilia A genotype and HLA type, was analyzed by Cochran Mantel Haenszel test to control for matching. Conditional logistic regression was used to determine factors independently associated with inhibitor development.

Results

Clinical Data, Blood Product Exposure, and Inhibitor Formation

A significantly greater proportion of hemophilia A inhibitor cases were African-American, 5 of 20 (25.0%) vs. 2 of 57 (3.5%), p=0.010, had a family history of inhibitors, 7 of 20 (35.0%) vs. 0 of 57 (0%), p<0.001, and received greater intensity blood product exposure than controls, 455 U vs. 200 U per exposure, p<0.005 (Table 1). Central nervous system (CNS) bleeding was significantly more common in inhibitor cases, 35.0% vs.1.7%, p<0.001, accounting for more deaths in cases than controls, 37.5% vs. 4.5%, p=0.045, and occurred more frequently as the first bleeding event in African-American cases, p<0.001, among whom it occurred within the first six months of life and preceded inhibitor formation. Overall, inhibitors contributed to a high proportion of deaths in both inhibitor groups, p=0.536 (Table 2).

Initial blood product exposure in the majority of cases and controls was predominantly factor VIII concentrate, p<0.001, and occurred prior to the availability of hepatitis B (HBV) vaccine (1980), p=0.192. Acute hepatitis (defined as jaundice and/or acute transaminitis), p=0.001, and detectable hepatitis B surface antigen ($HBsAg^{+}$), p=0.023, however, was significantly more common in inhibitor cases than controls (Table 1). These findings were less common in African-American cases, 20.0% vs. 53.3%, p=0.030, in whom initial blood produce exposure occurred earlier than in Caucasian cases, 6 vs. 24 months, p<0.010, although similarly before the availability of HBV vaccine, p=0.469. No other concomitant infections or vaccinations were reported in cases or controls.

The single individual with anaphylaxis occurred in an African-American case following early factor VIII concentrate. Finally, although there were no differences in causes of death, the age at death was significantly younger in African-American than Caucasian cases, 14 vs. 38 years, p=0.025.

Immune Tolerance and Inhibitor Titer

The overall time to tolerance induction in the 39 patients, on whom this information was available, was 8 months. Those with higher titer inhibitors, >120 BU/ml, however, required a longer median time to achieve tolerance, 16 months, than those with lower titer inhibitors, ≤120 BU/ml, 8 months, p<0.01 (Table 3). There was a trend toward achieving tolerance more quickly among Caucasian than African-American cases, 6 vs. 12 months, but this did not reach significance, p=0.09. The tolerizing dose did not appear to have any impact on median time to tolerance, 8 months in each of the groups receiving <100U/kg/day and ≥100 U/kg/day, p>0.50.

Hemophilia A Genotype and Inhibitor Formation

Analysis of hemophilia A genotype in 65 inhibitor cases and 119 age-matched controls by logistic regression revealed that a significantly lower proportion of hemophilia inhibitor cases had missense mutations, 2/65 (3.1%), than did non-inhibitor controls, 31/119 (26.0%), p=0.009 (Table 4). The most common mutation associated with hemophilia, the intron 22 inversion mutation, was also the most common mutation in cases, 34/65 (52.3%), and controls, 49/119 (41.2%), but was not associated with inhibitor formation. There appeared to be no relationship between inhibitor formation and site of the mutation, either in the immunogenic factor VIII A2 or C2 domain, p=0.388. Hemophilia A genotype was unaffected by race (Table 5). The small number of moderate and mild cases precluded analysis of the relationship between genotype and hemophilia severity.

HLA Typing and Inhibitor Formation

The relationship between each of 18 HLA class II alleles and the odds ratio for presence of an inhibitor was assessed by conditional logistic regression (Table 6). Alleles 17, 18, 41 and 1406 were excluded from the analysis because each was found in none of the matched cases and controls. None of the odds ratios differed significantly from 1.0, confirming that there were no associations between HLA class II alleles and inhibitor development.

Discussion and Conclusion

The findings of this prevalent case-control study not only confirm the role of high intensity blood product exposure [15,25,26] on inhibitor formation in hemophilia A, but also provide new information on the impact of race and central nervous system (CNS) bleeding on clinical outcome in those with inhibitors. We found that CNS bleeding is more common and more frequently a cause of death among hemophilia cases with inhibitors, as compared with age-matched non-inhibitor controls. Further, among African-American inhibitor cases, CNS bleeding was the single most common first bleeding event, and preceded inhibitor formation. These findings underscore the importance of early treatment as a risk factor for inhibitor development [12], as well as underscore the benefit of early prophylaxis, now the standard of care, in reducing CNS bleeding and improving life expectancy, as previously shown [28]. Although these findings differ from a previous study [27], CNS bleeding is uncommon in hemophilia [29], and, thus, the prevalence of inhibitor formation among those with CNS bleeding in hemophilia will require large prospective studies.

This study also confirms the importance of other risk factors, including young age at exposure [10,11,12,27], African-American race [5,6,30], and a family history of inhibitors [25,26,31]. The fact that not all cases are familial is consistent with the existence of other genetic factors, such as polymorphisms in and near the IL-10 gene in animals and humans with hemophilia A inhibitors [32,33], and/or other environmental factors.

We also found that peak inhibitor titer, but not the tolerizing dose regimen, is a significant predictor of time to tolerance, as previously reported [34]. Further, the observation that those receiving a lower tolerizing dose regimen, <100 U/kg/day, achieved tolerance as quickly as those receiving higher doses suggests lower dose tolerization may be preferable, a question to be resolved in an ongoing prospective trial [35].

The risk of inhibitor development was also found to be significantly lower among those with missense mutations, consistent with published findings [9], but there was no association between inhibitor formation and genotype by race, or between inhibitor formation and immunodominant A2 and C2 domains [36]. As inhibitor antibodies typically bind to multiple critical binding sites in F.VIII, associated with slow kinetics, steric hindrance, and interference with factor IX or VWF binding [37], factors other than the specific mutation site may be critical in predicting inhibitor formation. Finally, consistent with the findings of others [38,39], we found no association between inhibitor formation and HLA type. Given the well-recognized inhibitor discordance in monozygotic twins, it is likely that other immune response genes may contribute to factor VIII inhibitor antibody response.

There are several limitations with this study. It is a small series, and thus sub-analyses involve relatively small groups, which could account for differences with previous published studies. Further, while 16 centers participated in this study, only one had sufficient data to evaluate clinical product use, and the study was retrospective.

In summary, the findings of this study confirm that inhibitor formation is associated with high intensity blood product exposure, African-American race, lack of missense F.VIII

mutations, and for the first time, the high frequency of CNS bleeding. Given the increased early mortality in those with inhibitors, and the potential benefits of prophylaxis in reducing inhibitor formation and CNS bleeding [28], it is critical to direct efforts at early prevention through early institution of prophylaxis. Urgent questions remain, including when to start propylaxis to produce as few inhibitors as possible, and whether to start prophylaxis after the first severe bleed. Answers to these questions will require prospective clinical trials.

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M.V. Ragni and O. Ojeifo designed the research, analyzed data, and wrote the paper. M. V. Ragni and O. Ojeifo performed research and collected data. J. Feng, J. Yan, K. Hill, S. Sommer, and M. Trucco performed research assays and analyzed data. M. Ragni and D. Brambilla analyzed data and performed statistical analyses.

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Table 1

Blood Product Exposure and Hemophilia A Inhibitor Formation

Characteristic	Cases	Controls	p value
Severity of Hemophilia	N = 20	N = 57	
Severe	18/20 (90.0%)	46/57 (80.7%)	0.140
Moderate	1/20 (5.0%)	4/57 (7.0%)	
Mild	1/20 (5.0%)	7/57 (12.3%)	
Race			
Caucasian	15/20 (75.0%)	55/57 (96.5%)	0.010
African-American	5/20 (25.0%)	2/57 (3.5%)	
Genetic Predisposition			
Family History of Hemophilia	10/20 (50.0%)	29/57 (50.9%)	0.545
Family History of Inhibitors	7/20 (35.0%)	0/57 (0%)	<0.001
Blood Product Exposure *			
Median Age at 1st Exposure (mos)	11	26	>0.500
Time 1st Exposure to Inhibitor (mos)	8	-	
Mean No. Exposures	9 ± 1	17 ± 4	0.052
Median No. Units	4,100	2,400	0.125
Median Units/Exposure	455	200	<0.005
Anaphylaxis	1/20 (5.0%)	0/57 (0%)	0.260
Initial Blood Product			
Factor VIII Concentrate	10/20 (50.0%)	5/57 (8.6%)	<0.001
Non-Concentrate Products	8/20 (40.0%)	50/57 (87.7%)	<0.001
Cryoprecipitate	6/20 (30.0%)	30/57 (62.6%)	0.155
Fresh Frozen Plasma	2/20 (10.0%)	17/57 (31.6%)	0.522
Whole Blood	0/20 (0%)	3/57 (5.3%)	0.400
None	0/20 (0.0%)	2/57 (3.5%)	0.545
Year of Initial Blood Product Exposure			
Before 1980	13/20 (80.0%)	50/57 (87.7%)	0.192
Concomitant Events †			
Acute Hepatitis	8/20 (40.0%)	0/57 (0.0%)	0.001
HBsAg+	6/19 (31.6%)	3/39 (7.7%)	0.023
Breastfed	4/20 (20.0%)	16/57 (28.1%)	0.225
Complications			
CNS Bleeding, Ever	7/20 (35.0%)	1/57 (1.7%)	<0.001
Survival			
Mortality (%)	8/20 (40.0%)	22/57 (38.6%)	0.255
Median Age at Death (yr)	32	31	0.936
Cause of Death			
CNS Bleed	3/8 (37.5%)	1/22 (4.5%)	0.045
Endstage Liver Disease	2/8 (25.0%)	1/22 (4.5%)	0.152
AIDS	1/8 (12.5%)	18/22 (81.8%)	0.001

Characteristic	Cases	Controls	p value
Other Bleed	1/8 (12.5%)	0/22 (0%)	0.267
Suicide	1/8 (12.5%)	1/22 (4.5%)	0.404
Cancer	0/8 (0%)	1/22 (4.5%)	0.733

*Blood product exposure was blood product use from the time of initial exposure to the date of inhibitor detection in the case for each case-control group.

†Concomitant events were those occurring within 4 months of inhibitor development (see text). CNS is central nervous system. Acute hepatitis was defined in this study as jaundice or elevated AST or ALT; HBsAg+ is hepatitis B surface antigen positive.

Table 2

Race and Hemophilia A Inhibitor Formation

Characteristic	African-Americans	Caucasians	p value
Severity of Hemophilia	N = 5	N = 15	
Severe	5/5 (100.0%)	13/15 (86.7%)	0.553
Moderate	0/5 (0.0%)	1/15 (6.7%)	
Mild	0/5 (0.0%)	1/15 (6.7%)	
Genetic Predisposition			
Family History of Hemophilia	5/5 (100.0%)	6/15 (40.0%)	0.030
Family History of Inhibitors	5/5 (100.0%)	2/15 (13.3%)	0.001
Blood Product Exposure *			
Median Age at 1st Exposure (mos)	6	24	<0.010
Time Exposure to Inhibitor (mos)	6	9	0.582
Median No. Exposures	8	9	0.841
Median No. Units	3,800	4,600	0.053
Median Unit/Exposure	125	400	0.161
Anaphylaxis	1/5 (20.0%)	0/15 (0%)	0.250
Initial Blood Product			
Factor VIII Concentrate	3/5 (60.0%)	9/15 (60.0%)	0.397
Non-Concentrate Product	2/5 (40.0%)	6/15 (40.0%)	0.352
Cryoprecipitate	2/5 (40.0%)	4/15 (26.7%)	0.553
Fresh Frozen Plasma	0/5 (0%)	2/15 (13.3%)	0.522
Year of Initial Blood Product Exposure			
Before 1980	4/5 (80.0%)	12/15 (80.0%)	0.469
First Bleeding Event			
CNS Bleeding	3/5 (60.0%)	0/15 (0.0%)	<0.001
Circumcision	2/5 (40.0%)	5/15 (33.3%)	0.387
Hematoma	0/5 (0.0%)	4/15 (26.7%)	0.282
Mouth, Dental Bleed	0/5 (0.0%)	3/15 (20.0%)	0.399
Trauma (Fracture)	0/5 (0.0%)	1/15 (6.7%)	0.750
Unknown	0/5 (0.0%)	2/15 (13.3%)	0.553
Concomitant Events †			
Acute Hepatitis	1/5 (20.0%)	8/15 (53.3%)	0.030
Vaccination	0/5 (0.0%)	1/14 (7.1%)	0.737
Breastfed	2/4 (50.0%)	3/8 (37.5%)	0.424
Inhibitor Characteristics			
High Titer, Responding ‡	3/5 (60.0%)	10/15 (66.7%)	0.387
Median Peak Titer (B.U./ml)	15.2 32.0	0.091	
Complications			
CNS Bleeding, Ever	3/5 (60.0%)	4/15 (26.7%)	0.176
Survival			
Mortality	2/5 (40.0%)	6/15 (40.0%)	0.397

Characteristic	African-Americans	Caucasians	p value
Median Age at Death (yr)	14	38	0.025
Causes of Death			
CNS Bleed	1/2 (50.0%)	2/6 (33.3%)	0.536
Endstage Liver Disease	0/2 (0%)	2/6 (33.3%)	0.536
AIDS	0/2 (0%)	1/6 (6.7%)	0.750
Other Bleed	1/2 (20.0%)	0/6 (0%)	0.250
Suicide	0/2 (0%)	1/6 (6.7%)	0.750

*Blood product exposure was blood product use from the time of initial exposure to the date of inhibitor detection.

†Concomitant events were those occurring within 4 months of inhibitor development.

‡High titer responding inhibitor was defined in this study as > 5 (Bethesda Units/ml (BU/ml). CNS is central nervous system. Acute hepatitis was defined in this study as jaundice or elevated AST or ALT. HBsAg+ is hepatitis B surface antigen positive;

Table 3

Time to Immune Tolerance: Influence of Peak Titer, Race, Tolerance Dose

Variable	Inhibitor Cases (No.)	Median Time to Tolerance* (Months)	p value
Inhibitor Titer			
< 120 BU/ml	25	8 months	
≥ 120 BU/ml	14	16 months	<0.01
Race			
Caucasian	31	6 months	
African-American	8	12 months	0.09
Tolerance Dose			
< 100 U/kg/day	15	8 months	
≥ 100 U/kg/day	22	8 months	>0.50

* Tolerance was defined in this study as the time to anti-VIII = 0 BU/ml.

Table 4

Hemophilia A Genotype and Inhibitor Formation in Cases and Controls

Characteric	Case	Control	p value
Severity of Hemophilia	N = 65	N = 119	
Severe (F.VIII < 0.01 U/ml)	63/65 (96.9%)	111/119 (93.2%)	p = 0.300
Moderate (F.VIII 0.01–0.05 U/ml)	2/65 (3.1%)	6/119 (5.1%)	
Mild (F.VIII > 0.05 U/ml)	0/65 (0%)	2/119 (1.7%)	
Race			
Caucasian	48/63 (76.2%)	98/117 (83.8%)	p = 0.015
African-American	12/63 (19.0%)	6/117 (5.1%)	
Other	3/63 (4.8%)	13/117 (11.1%)	
Factor VIII Genotype			
Intron 22 Inversion	34/65 (52.3%)	49/119 (41.2%)	p = 0.009
Deletion	12/65 (18.5%)	17/119 (14.3%)	
Nonsense	10/65 (15.4%)	8/119 (6.7%)	
Insertion	3/65 (4.6%)	4/119 (3.4%)	
Missense	2/65 (3.1%)	31/119 (26.0%)	
Intron 1 Inversion	2/65 (3.1%)	6/119 (5.0%)	
Splicing	2/65 (3.1%)	4/119 (3.4%)	
Factor VIII Mutation Site			
Microinsertions			
A2 Domain	0/4 (0%)	1/17 (5.9%)	p = 0.388
C2 Domain	0/4 (0%)	0/17 (0%)	
Microdeletion			
A2 Domain	0/7 (0%)	0/31 (0%)	
C2 Domain	2/7 (28.6%)	3/31 (9.7%)	
Missense Mutations			
A2 Domain	2/5 (40.0%)	21/89 (23.6%)	
C2 Domain	0/5 (0%)	14/89 (15.7%)	
Nonsense Mutations			
A2 Domain	1/13 (7.7%)	2/23 (8.7%)	
C2 Domain	3/13 (23.1%)	5/23 (21.7%)	
Deletion Mutations			
A2 Domain	6/11 (54.5%)	3/17 (17.6%)	
C2 Domain	1/11 (9.1%)	6/17 (35.3%)	
All Mutations			
A2 Domain	9/40 (22.5%)	27/177 (15.2%)	
C2 Domain	6/40 (15.0%)	28/177 (15.8%)	

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Table 5

Hemophilia A Genotype and Race and Hemophilia Severity

	Race			Hemophilia Severity		
	Caucasian	**African-American**	**Other**	**Severe**	**Moderate**	**Mild**
Intron 22 Inversion						
Cases	25/48 (52.1%)	6/12 (50.0%)	3/5 (60.0%)	32/63 (50.8%)	2/2 (100%)	-
Control	42/98 (42.9%)	1/6 (16.7%)	6/15 (40.0%)	47/111 (42.3%)	2/6 (33.3%)	0/2 (0%)
Deletion						
Cases	9/48 (18.8%)	2/12 (16.7%)	1/5 (20.0%)	12/63 (19.0%)	0/2 (0%)	-
Controls	14/98 (14.3%)	1/6 (16.7%)	2/15 (13.3%)	17/111 (15.3%)	0/6 (0%)	0/2 (0%)
Nonsense						
Cases	6/48 (12.5%)	3/12 (25.0%)	1/5 (20.0%)	10/63 (15.9%)	0/2 (0%)	-
Controls	6/98 (6.1%)	1/6 (16.7%)	1/15 (6.7%)	8/111 (7.2%)	0/6 (0%)	0/2 (0%)
Insertion						
Cases	3/48 (6.3%)	0/12 (0%)	0/5 (0%)	3/63 (4.8%)	0/2 (0%)	-
Controls	3/98 (3.1%)	1/6 (16.7%)	0/15 (0%)	4/111 (3.6%)	0/6 (0%)	0/2 (0%)
Missense						
Cases	1/48 (2.1%)	1/12 (8.3%)	0/5 (0%)	2/63 (3.2%)	0/2 (0%)	-
Controls	25/98 (25.5%)	2/6 (33.3%)	4/15 (26.7%)	27/111 (24.3%)	2/6 (33.3%)	2/2 (100%)
Intron 1 Inversion						
Cases	2/48 (4.2%)	0/12 (0%)	0/5 (0.0%)	2/63 (3.2%)	0/2 (0%)	-
Controls	4/98 (4.1%)	0/6 (0%)	2/15 (13.3%)	6/111 (5.4%)	0/6 (0%)	0/2 (0%)
Splicing						
Cases	2/48 (4.2%)	0/12 (0%)	0/5 (0%)	2/63 (3.2%)	0/2 (0%)	-
Controls	4/98 (4.1%)	0/6 (0%)	0/15 (0%)	2/111 (1.8%)	0/6 (0%)	0/2 (0%)
* p value	p=0.009	p=0.335	p=0.658	p=0.017	-	-

* The p values indicate genotype differences by race, in cases and controls. No p value is provided for genotype by severity, in cases and controls, as the number of moderate and mild cases is too small to justify an analysis stratified by severity.

Table 6

HLA Class II Alleles and Inhibitor Formation in Cases and Controls

Class II Allele	Cases	Controls	Odds Ratio	p value
1	10/65 (15.4%)	33/119 (27.7%)	1.92	p = 0.11
2	15/65 (23.1%)	30/119 (25.2%)	1.32	p = 0.48
3	10/65 (15.4%)	24/119 (20.2%)	1.35	p = 0.50
4	17/65 (26.1%)	33/119 (27.7%)	1.14	p = 0.71
7	17/65 (26.1%)	31/119 (26.0%)	0.92	p = 0.81
8	10/65 (15.4%)	10/119 (8.4%)	0.47	p = 0.15
9	2/65 (3.1%)	1/119 (0.8%)	0.27	p = 0.29
10	3/65 (4.6%)	1/119 (0.8%)	0.19	p = 0.16
11	7/65 (10.7%)	21/119 (17.6%)	1.99	p = 0.15
12	6/65 (9.2%)	5/119 (4.2%)	0.37	p = 0.13
13	18/65 (27.7%)	26/119 (21.8%)	0.76	p = 0.44
14	9/65 (13.8%)	10/119 (8.4%)	0.59	p = 0.29
17	0/65 (0%)	0/119 (0%)	-	
18	0/65 (0%)	0/119 (0%)	-	
41	0/65 (0%)	0/119 (0%)	-	
51	16/65 (24.6%)	31/119 (26.0%)	1.22	p = 0.59
52	34/65 (52.3%)	70/119 (58.8%)	1.43	p = 0.26
1406	0/65 (0%)	0/119 (0%)	-	