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F8 haplotype and inhibitor risk: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort

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Abstract

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All authors contributed substantially to this work. The specific contributions are as follows: J. S. designed research, performed research, analyzed and interpreted data, performed statistical analysis, wrote the manuscript; J.A. designed research, performed research, collected data, analyzed and interpreted data, wrote the manuscript; E. D. M. designed research, performed research, analyzed and interpreted data, performed statistical analysis, wrote the manuscript; M. C. performed research, collected data; S.M.D. designed research, performed research, analyzed and interpreted data, wrote the manuscript; E.D.G. designed research, performed research, collected data, provided critical review of the manuscript; G.W.N. designed research; J.O. designed research, performed research, collected data, analyzed and interpreted data, provided critical review of the manuscript; A.P. performed research, analyzed and interpreted data; A.D.S designed research, performed research, collected data, provided critical review of the manuscript; C.A.W. designed research, performed research, collected data, analyzed and interpreted data, provided critical review of the manuscript; E.B. designed research, performed research, collected data, analyzed and interpreted data, wrote the manuscript. All authors approved the final manuscript.

DISCLOSURES

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Background—Ancestral background, specifically African descent, confers higher risk for development of inhibitory antibodies to factor VIII (FVIII) in hemophilia A. It has been suggested that differences in the distribution of factor VIII gene (*F8*) haplotypes, and mismatch between endogenous *F8* haplotypes and those comprising products used for treatment could contribute to risk.

Design and Methods—Data from the HIGS Combined Cohort were used to determine the association between *F8* haplotype 3 (H3) vs. haplotypes 1 and 2 (H1+H2) and inhibitor risk among individuals of genetically-determined African descent. Other variables known to affect inhibitor risk including type of *F8* mutation and HLA were included in the analysis. A second research question regarding risk related to mismatch in endogenous *F8* haplotype and recombinant FVIII products used for treatment was addressed.

Results—H3 was associated with higher inhibitor risk among those genetically-identified (N=49) as of African ancestry, but the association did not remain significant after adjustment for *F8* mutation type and the HLA variables. Among subjects of all racial ancestries enrolled in HIGS who reported early use of recombinant products (N=223), mismatch in endogenous haplotype and the FVIII proteins constituting the products used did not confer greater risk for inhibitor development.

Conclusion—H3 was not an independent predictor of inhibitor risk. Further, our findings did not support a higher risk of inhibitors in the presence of a haplotype mismatch between the FVIII molecule infused and that of the individual.

Keywords

F8 haplotype; FVIII inhibitors; haplotype mismatch

INTRODUCTION

Data from related and unrelated subjects with hemophilia A clearly indicate that the immunological outcome of replacement therapy and the risk of developing neutralizing antibodies (inhibitors) are to a large extent determined by patient-related genetic factors [1, 2]. The most extensively studied genetic risk factor for inhibitors is the causative factor VIII gene (*F8*) mutation. Patients with certain types of mutations are at higher risk for the development of inhibitors than those with others [3]. Other genetic markers of potential importance for the immune response to the deficient factor include the human leukocyte antigen (HLA) class II (i.e. DRB1*15 and DQB1*0602) and immune regulatory genes [4–7]. A two-fold higher incidence of inhibitors in those of African descent compared with Caucasians further supports the importance of genetic factors [2, 8]. It has been suggested that this discrepancy may be due to the different distribution of *F8* haplotypes by race, with a higher risk for inhibitors in the case of a mismatch between the proteins encoded by the endogenous *F8* haplotype and those comprising replacement products used for treatment [9, 10]. The haplotypes consist of four nonsynonymous SNPs located across the gene. Each mutation results in a nonterminating amino acid change in the factor VIII protein construction. The biologic implications of the amino acid changes have not fully been explored, but two of the residues are located in immunodominant epitopes, i.e. R484H and M2238V, whereas R776G and D1241E are located in the B-domain. The haplotypes H3, H4, and H5 have only been found among blacks, while H1 and H2 are found primarily in whites and are most commonly present in infused recombinant products [10]. The Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort was used to further explore the suggested relationship between haplotype and inhibitor status among those of African ancestry, and mismatch of haplotype and product use on inhibitor development by

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adjustment for the type of *F8* mutation and previously described HLA class II risk alleles among the subset of HIGS participants.

DESIGN AND METHODS

Study Population

Our data comprised three multi-center studies: the Hemophilia Inhibitor Genetics Study, the Malmö International Brother Study (MIBS), and the Hemophilia Growth and Development Study (HGDS) (N=833). The HIGS study group included in the current analysis is composed of brother pairs, one or both of whom has a history of an inhibitor, and singletons with a history of inhibitors, enrolled in Europe, North America, Latin America, and South Africa. The MIBS is composed, almost exclusively, of siblings pairs enrolled in Europe and North America, and the HGDS is a population-based group enrolled in hemophilia treatment centers in the US. Data collection from all cohorts included demographics, severity of hemophilia, history of and current inhibitor status, maximum lifetime Bethesda titer, and type of *F8* mutation. HIGS data collection also included retrospective identification of the type(s) of replacement products used prior to development of the inhibitor. For those not having an inhibitor, i.e., brothers of participants with inhibitors, the type(s) of factor used in the subject's first 25 exposure days, or in as many exposures to FVIII as his brother had when his brother developed an inhibitor, were also collected. For the analysis, an inhibitor was defined as a current or history of an inhibitor 1 Bethesda unit (BU).

The procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation for all three cohorts, and with the Helsinki Declaration. The MIBS and HIGS are registered at ClinicalTrials.gov.

F8 Haplotyping

To determine factor VIII haplotypes, four non-synonymous single nucleotide polymorphisms (SNPs) on the *F8* gene, G1679A, A2554G, C3951G, and A6940G, were genotyped using the Assay-on-Demand from Applied Biosystems standard protocols (www.AppliedBiosystems.com). Haplotypes were constructed using the four markers that were genotyped. Because the population was almost exclusively male (99.9%), all but one individual was hemizygous, as all markers are located on the X chromosome. Typing was completed for all but 7.1% of the markers. An E-M algorithm [11, 12] was used to infer haplotypes for individuals with missing information. Individuals with missing genotypes were assigned the haplotype that demonstrated the highest posterior probability. One of the 833 study participants was not haplotyped, reducing the analysis sample to 832.

F8 Mutation Typing and HLA Class II Typing

Approximately 96% percent of the participants in the HIGS Combined Cohort were *F8* mutation typed. The remaining 4% of subjects were not typed for either technical reasons or lack of sufficient DNA. For HIGS and MIBS, if the *F8* gene mutation was not already documented at enrollment, a blood sample was sent for determination to the Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany. Standard methods for the analyses of the *F8* gene were used [13]. In HGDS, the presence or absence of an inversion mutation in the *F8* gene was determined for 58% of the HGDS cohort [14]. The remaining HGDS samples were mutation typed at the Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany by the methods outlined above. Class II HLA genotyping was performed using high-resolution (4-digit) sequence based typing (SBT) protocols recommended by the 13th International Histocompatibility Workshop [15]. Typing was completed for 99.9% of the Combined Cohort.

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Recombinant Replacement Products

The recombinant FVIII replacement products included in the current analysis were: Recombinate, antihemophilic factor concentrate manufactured by Baxter Healthcare Corporation [16, 17], derived from H2 proteins; Advate, antihemophilic factor produced by a plasma- and albumin-free method, manufactured by Baxter Healthcare Corporation [18], also derived from H2 proteins; and Kogenate, antihemophilic factor manufactured by Bayer HealthCare Pharmaceuticals [19], derived from H1 proteins.

Statistical Analysis

Association tests, including Fisher's exact test, were carried out using two by two tables to evaluate the probability of inhibitor occurrence. GEE models were used to account for the relatedness of participants. However, for the recombinant treatment analysis, in which there were 43 family groups consisting of brother pairs or trios among the 223 recombinant product users, GEE models could not be used due to sample size limitations. For this analysis only, the model was reduced to a logistic regression. The outcome variable in the analysis was the presence or absence of a history of inhibitors to FVIII, and the effect of interest was the endogenous *F8* haplotype. The use of genetic information allows for a more powerful method of classifying the ancestry of an individual than data obtained by self-reported cultural identity or race. Principal components were constructed with EIGENSOFT [20], using an additional 13,331 SNPs spaced across the genome to describe population structure. *F8* gene mutations were categorized as high risk: inversions, large deletions, nonsense, small deletions/insertions (outside A-runs), missense (Arg593Cys, Tyr2105Cys, Arg2150His, Arg2163His, Trp2229Cys, Pro2300Leu, and Asn2286Lys), and splice site (at conserved nucleotides at position + or − 1 and 2); or low risk: small deletions/insertions (within A-runs), splice site (at position + or − 3 or more remote), missense (other regions), or other mutation types. A third category contained those for whom no mutation was found. A multiple imputation (MI) procedure [21] was carried out using SAS PROC MI [22] to identify risk category for two genetically-black individuals missing this variable. The MI procedure allows imputation of categorical and ordinal variables through the use of logistic regression models. The variables used to predict mutation risk were haplotype, HLA allele count, severity of hemophilia, principal components, year of birth and family relatedness. Imputations were performed by inhibitor status to allow for possible distributional differences in mutation risk for those with and without inhibitors. For the HLA class II alleles of interest, DRB1*15 and DQB1*0602, analysis was performed based on the number of copies of the allele (i.e., 0, 1 or 2 alleles). Models were analyzed using SAS 9.2.

RESULTS

Descriptors of the population are shown in Table 1. The H1, H2 and H3 haplotypes were observed in all four racial categories (Table 2), with the highest prevalence of H3 occurring in the genetically-determined black population (28.6%). The H4 and H5 haplotypes were not observed in any participants. The haplotype distribution by race in the HIGS Combined Cohort was generally similar to that reported by Viel [10]. A consideration when using the EM algorithm to impute missing genotypes is to establish that the missingness is at random, and not confounded with other predictors such as *F8* mutation. To ensure that the imputation of missing genotypes using the EM algorithm was appropriate, the missing genotypes were compared to *F8* mutation type. None of the marker positions showed an association with mutation type, indicating that the missingness was not likely due to a particular type of mutation. Additionally, none of the haplotypes showed associations with the mutation types (Table 3).

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Haplotype, Race, and Inhibitors

We evaluated the effect of the H3 haplotype on inhibitor status. The white, Hispanic and other populations contained fewer than 3 copies of H3 each, therefore the effect was examined only for the 49 genetically-determined black individuals, 14 of whom had the H3 haplotype. Testing the prevalence of H3 haplotype compared to the H1 and H2 haplotypes on inhibitor status in the group, adjusted for family, the OR was 2.10, p=0.009. Mutation risk category and HLA allele counts were introduced to the model. The effect of haplotype on inhibitor development with mutation risk included in the model was a reduction in risk for H3 haplotype (OR 1.37, p=0.31), and a significant effect of high risk mutations (OR 4.95, p=0.0046). Adjustment for HLA allele count covariates resulted in an OR for H3 of 1.69, p=0.33. When all variables were considered together (H3 haplotype, mutation risk category, and HLA), only mutation was a significant predictor of inhibitor status (OR 8.17, p=0.0032). Although our sample size was small (n=49 in all models), it provided sufficient coverage for the parameters entered into the model.

Haplotype, Recombinant Product Use, and Inhibitors

The most commonly used recombinant products for the treatment of FVIII deficiency are derived from H1 (Kogenate) and H2 (Recombinate, Advate) proteins. Early treatment with a recombinant product was reported for 224 participants in HIGS, 91 (40.6%) using H1 products and 87 (38.8%) using H2 products. The remaining participants used a b-domain deleted product or were on multiple or unknown recombinant products (20.6%), and were therefore ineligible for the analyses.

Of the participants with early recombinant product use, 223 also had haplotype information. In this subset, 72 (79.1%) of the 91 individuals using the H1 product had an inhibitor. The association between haplotype (H2 + H3 vs. H1) and inhibitor status among the participants who received H1 products was tested. The results (Table 5) showed no significant association between haplotype and inhibitor status (OR 0.76 of H2 or H3 having an inhibitor, p=0.71). Among the group of 86 participants receiving the H2 products, 69 (80%) individuals had an inhibitor. No significant effect was found (OR 1.18 of those with H1 or H3 having an inhibitor, p=1.0) when comparing the occurrence of inhibitors in the H1+H3 vs. H2 haplotype groups among those who used an H2 product.

DISCUSSION

The frequency of haplotypes observed was consistent with those previously reported by Viel [10], indicating that our population is similar in genetic *F8* composition to that previously analyzed. We had fewer individuals of African ancestry and the magnitudes of our estimates of risk for inhibitors among those with the H3 haplotype were somewhat lower, but our data support the findings by Viel et al. prior to adjustment for other factors. After adjustment for covariates including the *F8* gene mutation and HLA class II alleles DRB1*15 and DQB1*0602, the association between H3 haplotype and inhibitor risk is no longer significant. Having a high risk *F8* gene mutation remains a significant risk factor. This indicates that the effect previously described by Viel, et al. is, in our cohort, largely explained by other genetic factors, primarily the *F8* mutation. The genetically-determined racial classification used in our investigation may be more accurate than self-report because it is based on differences in allele frequencies that occur among distinct human populations rather than on cultural identity. Use of genetic data to classify ancestry complements the hypothesis that additional, so far unknown, genetic markers will likely explain the higher inhibitor risk in blacks, as has been the case for other immune-mediated disorders that are more prevalent in this population.

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As noted above, our study used principal components to genetically determine ancestry. This method requires a set of genome-wide markers to capture the allele frequency differences between ancestral backgrounds. In instances where a whole genome wide panel of genetic markers is not available, other methods can be used. Use of pattern mixture models based on a specific category of markers was developed by Pritchard, et al. [23]. The pattern mixture models require markers that are known to exhibit polymorphism between racial groups. The set generally consists of, at most, several hundred markers and are selected based on the different racial groups believed to be in the population of interest.

The analysis performed on the type of recombinant FVIII products used for early treatment addresses a different research question. It supports the hypothesis of no association between haplotype and current or history of an inhibitor. Neither of the two recombinant products examined were associated with a greater proportion of inhibitors for mismatched haplotypes. The size of our study group was sufficient to detect any large effect, but with an observed OR of only 0.76 (risk of H2 or H3 developing an inhibitor after exposure to an H1 product) and 80% power, it would take 2,518 participants to see a significant result. With an OR of only 1.18 (risk of H1 or H3 developing an inhibitor after exposure to an H2 product) and 80% power, 7,030 participants would be required to see a significant result.

In conclusion, our findings do not support a substantially higher risk of inhibitors in the presence of a haplotype mismatch between the FVIII molecule infused and that of the individual.

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APPENDIX

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Table 1

Summary of variables by cohort

	HGDS (n=265)	HIGS (n=448)	MIBS (n=120)
With Inhibitor 1 BU (n, %)	52 (20%)	360 (80%)	45 (38%)
Race (n, %)	**(n=265)**	**(n=448)**	**(n=119)**
Asian	0 (0%)	10 (2%)	0 (0%)
Black	24 (9%)	23 (5%)	2 (2%)
Hispanic	42 (16%)	50 (11%)	0 (0%)
White	195 (74%)	346 (77%)	115 (97%)
Other	4 (2%)	19 (4%)	2 (2%)
Hemophilia Severity	**HGDS (n=265)**	**HIGS (n=448)**	**MIBS (n=120)**
Mild	16 (6%)	0 (0%)	14 (12%)
Moderate	50 (19%)	0 (0%)	19 (16%)
Severe	199 (75%)	448 (100%)	87 (73%)
Haplotype (n, %)	**(n=265)**	**(n=447)**	**(n=120)**
H1	202 (76%)	377 (84%)	98 (82%)
H2	56 (21%)	61 (14%)	21 (18%)
H3	7 (3%)	9 (2%)	1 (1%)
Treatment with a full-length recombinant product (n, %)			
Kogenate	-	91 (51%)	-
Recombinate/Advate	-	87 (49%)	-

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Table 2

Haplotype by race and inhibitor history

	Black	Hispanic	White	Other	No Inhibitor	Inhibitor	Total
H1	19 (38.8%)	66 (71.7%)	559 (85.3%)	33 (91.7%)	296 (78.7%)	381 (83.6%)	677 (81.4%)
H2	16 (32.7%)	25 (27.2%)	95 (14.5%)	2 (5.6%)	71 (18.9%)	67 (14.7%)	138 (16.6%)
H3	14 (28.6%)	1 (1.1%)	1 (0.2%)	1 (2.8%)	9 (2.4%)	8 (1.8%)	17 (2.0%)
H4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
H5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

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Table 3

F8 mutation type* by haplotype

Mutation Type	H1 (n=654)	H2 (n=131)	H3 (n=16)
Inversion	330 (50.5%)	65 (49.6%)	8 (50.0%)
Large Deletion	36 (5.5%)	3 (2.3%)	0 (0%)
Missense Mutation	82 (12.5%)	16 (12.2%)	4 (25.0%)
Nonsense Mutation	47 (7.2%)	13 (10.0%)	1 (6.3%)
Small Deletion/Insertion	68 (10.4%)	16 (12.2%)	3 (18.8%)
Splice Site Mutation	11 (1.7%)	2 (1.5%)	0 (0%)
No Inversion, or otherwise not determined	76 (11.6%)	15 (11.4%)	0 (0%)
Other	4 (0.6%)	1 (0.8%)	0 (0%)

* *F8* mutation type was missing for N=31 (3.7%)

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Table 4

The effect of *F8* haplotype 3 on inhibitor development in genetically-identified black participants.

	Genetically-identified Black Participants (N=49)		
Model Covariate	**OR**	**95% CI**	***P*-value**
1. Haplotype Only			
Haplotype (H3 vs. H1+H2)	2.10	(1.20, 3.68)	0.0092
2. Haplotype + Mutation			
Haplotype (H3 vs. H1+H2)	1.37	(0.74, 2.55)	0.3143
Mutation Risk (High vs. Low)	4.95	(1.64, 14.93)	0.0046
3. Haplotype + HLA			
Haplotype (H3 vs. H1+H2)	1.69	(0.59, 4.86)	0.3265
DQB 0602	1.39	(0.31, 6.25)	0.6692
DR 15	1.22	(0.41, 3.63)	0.7246
4. Haplotype + HLA + Mutation			
Haplotype (H3 vs. H1+H2)	0.90	(0.34, 2.41)	0.8387
DQB 0602	4.44	(0.71, 27.61)	0.1097
DR 15	0.73	(0.21, 2.52)	0.6223
Mutation Risk (High vs. Low)	8.17	(2.02, 32.96)	0.0032

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Table 5

Haplotype frequencies for each treatment by inhibitor status

Treatment	Haplotype	Inhibitor Status		Odds Ratio	*p*-value
		No	**Yes**		
Kogenate	**H2+H3**	3 (25%)	9 (75%)	0.76	0.71
	H1	16 (20%)	63 (80%)		
Recombinate/Advate	**H1 + H3**	15 (19%)	62 (81%)	1.18	1.00
	H2	2 (22%)	7 (78%)		