Genomic ancestry, *F8* variants, and immune tolerance in hemophilia A patients with inhibitors: exome sequencing insights

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Table S1. Overview of Statistical Analyses for Population Structure and Genetic Ancestry.

Analyses	Statistical Analyses and Software	Observation
Autosomal population structure and continental ancestry. We included 2,199 reference individuals for autosomes	We used 42,542 unlinked ($r^2 < 0.4$) autosomal SNVs derived from exome sequencing, and inferred population structure and genomic ancestry using Principal Component Analysis (Patterson et al. 2006), and ADMIXTURE (Alexander et al. 2009, with K=3 European, African, and Native-American parental populations)	-
Ancestry of the X-chromosome in the 192 males of BrazIT	We used ADMIXTURE (K=3) and 654 unlinked ($r^2 < 0.4$) non-pseudoautosomal SNVs on the X-chromosome (Table S2, Supplemental Section 12 for a discussion). We used as reference 1,555 males for the X-chromosome, representing European, African, and Native-American populations (Table S3)	-
Nested analysis of variance (nested-ANOVA) to estimate the apportionment of European, African, and Native-American ancestries among geographic regions and states (within those regions), including kinship coefficients as a covariate	'aov' function in R	We could not quantify the distribution of ancestry among individuals within states due to insufficient degrees of freedom

Inbreeding coefficient estimation for each individual	We estimated inbreeding coefficients for each individual using VCFtools (Danecek et al. 2011)	We estimated as F_{ind} *= (O-E)/(L-E)*, where, for each biallelic locus, *O* is the observed number of loci in homozygosity in an individual, $E = \sum 1 - 2p_i q_i$ is the expected number of loci in homozygosity based on allele frequencies p_i and q_i, and *L* is the number of valid loci for that individual. Considering the admixture of the BrazIT cohort, we introduced a novelty in the estimation of the individual inbreeding coefficients F_{ind}. For a homogeneous population, $2p_i q_i$ is expected to be the same for all individuals. However, populations that are a product of admixture between populations with different levels of diversity $2p_i q_i$ are expected to be higher for those individuals with higher ancestry of the more diverse population (i.e., Africans, Campbell et al. 2014). In Brazilians, using the same $2p_i q_i$ values for all individuals underestimates F_{ind} for individuals with more African ancestry and overestimates F_{ind} for individuals with less African ancestry. To avoid this artifact, we stratified the estimation of F_{ind} by considering six non-overlapping bins of African ancestry of 32-33 individuals each
Association between genetic ancestry (proportions of European, African, and Native-American ancestries) and F8 variant types: large deletions, frameshifts, inversions, nonsense, missense, and splice donor mutations	Generalized linear regression, 'glm' function in R	Covariates: the kinship matrix, Brazilian geographic regions as categorical variables, and historical peak inhibitor titer
Association between genetic ancestry (proportions of European, African, and Native-American ancestries) and inhibitor titer, considering separately the highest inhibitor titer before ITI (historical peak), immediately before ITI starts, and the highest inhibitor titer during ITI	Generalized linear regression, 'glm' function in R	Covariates: kinship matrix, Brazilian geographic regions, and F8 variant types as categorical variables, including large deletions, frameshifts, inversions, nonsense, missense, and splice donor variants
Association between response to ITI (failure, partial success, and complete success) and genomic ancestry (European, African, and Native-American)	Ordinal logistic regression ('polr' function in R and MASS v. 7.3.51.6, Venables and Ripley 2002)	Covariates: the kinship matrix, Brazilian geographical regions, F8 variants (large deletions, frameshifts, inversions, nonsense, missense, and splice donor variants), and a historical inhibitor peak
Association between F8 haplotypes and genomic ancestry (proportions of European, African, and Native-American ancestries), using ancestry estimates from X-chromosome and autosomal variants	Binomial logistic regression model, 'glm' function in R	Covariates: the kinship matrix, Brazilian geographical regions, F8 variants (large deletions, frameshifts, inversions, nonsense, missense, and splice donor variants), inhibitor titer, considering separately the highest inhibitor titer before ITI (historical peak), immediately before ITI starts, and the highest inhibitor titer during ITI

Table S2. The proportion of individual continental ancestry between Brazilian geographic regions and states was estimated by nested-ANOVA (n=193).

Ancestry	Genetic Marker	Source	Degrees of freedom	Sum of squares	Mean squares	F value	P-value
European	**Autosomal**	Between regions	4	0.889	0.22231	9.692	4.03e-07
		Between states within regions	8	0.463	0.05781	2.520	0.013
		Residuals	179	4.129	0.02294	-	-
	X-Chromosome	Between regions	4	1.161	0.29025	4.065	0.003
		Between states within regions	8	1.311	0.16385	2.295	0.023
		Residuals	179	12.781	0.07140	-	-
African	**Autosomal**	Between regions	4	1.213	0.30318	18.856	5.77e-13
		Between states within regions	8	0.159	0.0199	1.238	0.279
		Residuals	179	2.894	0.01608	-	-
	X-Chromosome	Between regions	4	1.163	0.29065	5.713	2.38e-04
		Between states within regions	8	0.240	0.03006	0.591	0.785
		Residuals	179	9.106	0.05087	-	-
Native-American	**Autosomal**	Between regions	4	0.363	0.09072	15.539	6.14e-11
		Between states within regions	8	0.271	0.03385	5.798	1.39e-06
		Residuals	179	1.051	0.00584	-	-
	X-Chromosome	Between regions	4	0.899	0.22479	5.616	2.79e-04
		Between states within regions	8	0.836	0.10456	2.612	0.010
		Residuals	179	7.165	0.04003	-	-

Table S3. Cited studies on Hemophilia A with racial/ethnic classification and association with hemophilia-related traits.

Study	Sample Size	Population Description	Main Results	Data Type	Genomic Ancestry Analysis	Association Tested	Association Result
Kempton et al. 2023 (Cross-Sectional Study)	614	Severe hemophilia A patients in the U.S., focusing on ITI practices and outcomes with an analysis of racial/ethnic disparities.	No significant racial disparities in ITI outcomes were found.	Clinical	No	Race/Ethnicity vs. ITI outcome	No significant association found
Fedewa and Kempton 2024 (Observational Study)	559	Hemophilia A patients (White, Black, Hispanic, Asian) analyzing ITI success rates.	Found comparable ITI success rates across racial/ethnic groups, contradicting the hypothesis that race influences ITI response.	Clinical	No	Race/Ethnicity vs. ITI outcomes	No significant association found
Sant'Anna et al. 2024 BrazIT Cohort (Cross-Sectional Study)	193	Brazil cohort of hemophilia A patients with inhibitor history undergoing ITI, analyzing genomic ancestry for its association with *F8* mutations and inhibitors.	Demonstrated a link between genomic ancestry and *F8* mutation patterns.	Clinical & Genetic (whole exome sequencing)	Yes	Race/Ethnicity vs. ITI outcomes	No significant association found
						Race/Ethnicity vs. inhibitor titers	No significant association found
						Race/Ethnicity vs. *F8* large deletions	No significant association found
						Genomic ancestry vs. inhibitor titers	No significant association found
						Genomic ancestry vs. ITI outcomes	No significant association found
						Genomic ancestry vs. *F8* mutation type	Significant association found (Native-American ancestry and F8 Large Deletion: β=-0.081, 95% CI -0.144,-0.018; p=0.011)
						Genomic ancestry vs. *F8* haplotype	Significant association found (*F8* H2 and H3 haplotypes and African ancestry, X-chromosome: β=2.95-2.96, p always <0.001; Autosomes: β=3.02-3.66, p always <0.04)