Genetic landscape of Human neutrophil antigen variants in India from population-scale genomes

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Abstract

Background
Antibodies against human neutrophil antigens (HNAs) play a significant role in various clinical conditions such as neonatal alloimmune neutropenia (NAIN). Transfusion-related acute lung injury (TRALI) and other non-hemolytic transfusion reactions. This study aims to identify the genotype and allele frequencies of HNAs in the healthy Indian population.

Methods
Genetic variants from whole genomes of 1029 healthy Indian individuals were retrieved to accurately perform frequency estimation of HNA-1, HNA-3, HNA-4 and HNA-5 alleles using in-house computational pipeline.

Results
In HNA class I, the genotype frequencies of FCGR3B*01 (HNA1a/a), FCGR3B*02 (HNA1b/b) and FCGR3B*03 (HNA1c/c) were 0.29%, 27.31% and 1.75% respectively. In HNA-3 the frequencies of HNA3a/a (SLC44A2*01), HNA3a/b and HNA3b/b (SLC44A2*02) were found to be 62.0%, 31.7% and 5.8% respectively. Frequency of ITGAM*01 encoding HNA4a/a was 90.1% and that of ITGAM*02 encoding HNA4b/b was 0.3%. Furthermore, HNA5a/a (ITGAL*01) and HNA5b/b (ITGAL*02) were found to have 12.9% and 48.6% genotype frequencies in the Indian population respectively. It was also found that the allele frequency HNA-5 variant, rs2230433 (ITGAL_chr16:30506720G>C) encoding 5b allele was highly prevalent (78.2%) in the Indian population which was comparable to South Asians (65.6%) but differed greatly from East Asians (14.3%), Latino Americans (25.7%), African-Americans (42.2%), European-Finnish (25.4%), European-non-Finnish (29.4%), Greater Middle Easterners (34.5%), Amish (30.2%) and Ashkenazi Jewish (31.4%).

Conclusion
This study presents the first comprehensive report of HNA variant and genotype frequencies using large scale representative whole genome sequencing data of the Indian population. Significant difference was observed in the prevalence of HNA5a and HNA5b in India in comparison to other global populations.

Introduction

Human neutrophil antigens (HNAs), are a group of glycoprotein antigens that are expressed on the surface of human neutrophils[1,2]. These antigens are systematically classified into five major classes from HNA-1 to HNA-5 on the basis of serologically defined epitopes. Molecular characterization revealed that antigens of HNA class 1-5 are encoded by FCGR3B, CD177, CLT2, ITGAM and ITGAL genes respectively[2]. Although HNA-1 and HNA-2 have been found specifically expressed on the neutrophil surfaces,[3] classes HNA-3 to HNA-5 have a broad range of expression in other blood cells[4]. HNAs play a significant clinical role in the field of transfusion and transplantation medicine as antibodies elicited against these antigens (both allo- and auto-) are implicated in the range of disease conditions such as transfusion-related acute lung injury (TRALI), neonatal alloimmune neutropenia (NAIN)[5,6,7,8] autoimmune neutropenia (AIN)[9,10,11] and febrile nonhemolytic transfusion reactions (FNHTR)[12].

Owing to their clinical relevance, variant and genotype frequencies of HNAs have been studied among various global populations including Iranian[13], Japanese[14], Malays, Chinese[15], Hong Kong[16], Thai[17], Turkish, German[18], African American[19,20], Danish and Zambian [21].

While there is a paucity of reports on the genotype frequencies of HNA in the Indian population encompassing a sixth of the world population, the recent availability of population-scale genomic datasets have motivated us to undertake a systematic analysis of allele and genotype frequencies of HNA variants in Indian population. We hope our analysis would also provide insights and also potentially to estimate the risk of HNA alloimmunizations and devisee prevention strategies.

Materials and Methods

Reference and study datasets
A total of ten (10) variants in 4 human genes as approved by the International Society of Blood Transfusion - Granulocyte Immunobiology Working Party (ISBT-GIWP) for encoding alleles of human neutrophil antigens class I - V was used for the analysis. **Table 1** provides a comprehensive summary of these reference variants. The IndiGen dataset of genomic variants from whole genome sequences of 1029 healthy individuals from India was taken up for the analysis. Briefly, this dataset encompassed a total of 55,898,122 variants. The 10

variants associated with HNA were parsed in this dataset to retrieve the genotype and allele frequencies.. A schematic summary of the variant processing is shown in **Figure 1.**

Comparison of frequencies with other global populations

With the view of identifying significant differences in the prevalence of human neutrophil antigen variants in the Indian population, HNA allele frequencies were compared with major global population datasets retrieved from gnomAD database[22]. Fisher's exact test with a p value <= 0.05 was used to estimate the statistical significance.

Results

Frequencies of HNA variants in Indian population

HNA variant frequencies were analyzed in a total of 1029 whole genome sequences. **Table 2** summarizes the allele frequencies of HNA variants in the Indian population. Based on the zygosity information the genotype frequencies were duly estimated for HNA-1, 3, 4 and 5 alleles. In HNA class I, genotype frequencies of HNA1a/a, HNA1b/b and HNA1c/c were observed as 0.29%, 27.31% and 1.75% respectively. Similarly, HNA3a/a, HNA3a/b and HNA3b/b of HNA class 3 were found at 62.00%, 31.68% and 5.83% respectively. In HNA class 4 and 5, the genotype frequencies were as follows, HNA4a/a - 90.18%, HNA4a/b - 9.23%, HNA4b/b - 0.29%, HNA5a/a - 12.93%, HNA5a/b - 38.10% and HNA5b/b - 48.59%.

HNA variant frequencies among various global populations

Minor allele frequencies of HNA variants were fetched from the study dataset and were duly compared with global population frequencies. **Figure 2** provides a schematic overview of the distribution of the frequency distribution HNA variants in various populations. Comprehensive summary of genotype frequency comparison among globally reported populations is summarized in **Table 3.** Of the total, 5 variants were found to have significantly distinct differences (P <0.05) in Indian population in comparison to all the global populations. (FCGR3B_chr1:161629853T>C, FCGR3B_chr1:161629983A>G, FCGR3B_chr1:161629989G>C, ITGAM_chr16:31265490G>A and ITGAL_chr16:30506720G>C). The data is summarized in **Table 4.**

Discussion and Conclusion

Understanding the HNA variants are of clinical relevance due to their importance in allo and auto immunization resulting in diseases like TRALI, NAIN, AIN and graft rejections. It is therefore not surprising that the recent years have witnessed multiple attempts to decipher population specific HNA genotype and allele frequency profiles. While no population-scale datasets for HNA variants have been available for India, encompassing over a sixth of the world population, the recent availability of population-scale genome datasets motivated us to analyse allele and genotype frequencies of HNA-1a, -1b, -3a, -3b, -4a and -5a . Whole genome sequencing data of 1029 unrelated self declared healthy individuals was used to perform the *in-silico* genotyping analysis.

Alloimmunizations against HNA-1 systems has been well studied as a major cause of neonatal neutropenia and TRALI in Caucasian populations. Striking differences in prevalence of HNA-1 alleles have been observed in global populations with HNA-1a allele being the frequent in most Asian populations, whereas HNA-1b was found common in Caucasian[23]. A recent study by Hajar et al in 2019 suggested that unlike other Asian populations, HNA-1b was found common in samples with Indian ancestry[15]. In concordance with the earlier findings, HNA-1b/b homozygous individuals were found to constitute 27.3% of the population.

Allele encoding HNA-3a were found at a frequency of 0.78 in the Indian population which was concordant with the previous observations in Causian and Chinese populations with frequencies of 0.79 and 0.74 respectively [24,20,23]. Antibodies against HNA-3a have been reported as one of the main causes of fatal TRALI in Germany in a few other countries [25,26,27]. On the other hand, clinical consequences of alloantibodies against HNA-3b were found less severe.

In HNA-4 system, HNA-4a/a allele was found most common in India (90.18%) as seen in most global populations. Genotype frequency of HNA-4a was reported to range from 73%-100% [16,23,28,18,21,15,14,17] in various global population studies. Interestingly, in contrast to most global reports HNA-5b/b homozygous individuals represented 48.59% of the Indian population. HNA-5a/b, which was previously reported to be the most frequent in Zambians (52.5%)[21] and Malaysians (49.5%)[15] was found in about 38.09% of the population.

In conclusion, significant similarities albeit some differences in frequencies of HNA variants were observed in Indian population in comparison to other global populations. We sincerely hope these insights would guide cost-effective strategies for screening of HNAs. To the best of our knowledge, this is also the first and comprehensive study of HNA variants in the Indian population.

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Conflicts of Interest

None declared

Figures and Tables

Figure 1. Schematic representation of the methodical workflow followed in the identification and estimation of HNA frequencies in India.

Figure 2. Schematic comparison of frequency distribution of ISBT approved human neutrophil antigen variants among global populations. Variants whose frequencies were found significantly distinct in India in comparison to all other global populations are marked in blue.

Table 1. List of ISBT approved human neutrophil antigen variants used as reference in the study

Table 2. Frequencies of HNA variants observed in the Indian population

Table 3. HNA genotype frequencies observed among various global populations

Table 4. Summary of blood HNA variants found significantly distinct between India and global population datasets

HNA Class	Chr	Pos (hg38)	RSID	Ref	Alt
HNA-1	chr1	161629781	rs2290834	T	C
HNA-1	chr1	161629853	rs147574249	T	C
HNA-1	chr1	161629864	rs5030738	G	T
HNA-1	chr1	161629903	rs448740	T	C
HNA-1	chr1	161629983	rs527909462	A	G
HNA-1	chr1	161629989	rs200688856	G	C
HNA-3	chr19	10631490	rs147820753	C	T
HNA-3	chr19	10631494	rs2288904	A	G
HNA-4	chr16	31265490	rs1143679	G	A
HNA-5	chr16	30506720	rs2230433	G	C

Table 1. List of ISBT approved human neutrophil antigen variants used as reference in the study

HNA Class	Chr	Pos (hg38)	RSID	Ref	Alt	Allele count	Allele Number	Allele Frequency
HNA-1	chr1	161629781	rs2290834	T	C	AC=39	AN=1070	AF=0.036
HNA-1	chr1	161629853	rs147574249	T	C	AC=89	AN=1186	AF=0.075
HNA-1	chr1	161629864	rs5030738	G	T	AC=158	AN=1210	AF=0.131
HNA-1	chr1	161629903	rs448740	T	C	AC=1172	AN=1402	AF=0.836

HNA-1	chr1	161629983	rs527909462	A	G	AC=192	AN=1662	AF=0.116
HNA-1	chr1	161629989	rs200688856	G	C	AC=198	AN=1670	AF=0.119
HNA-3	chr19	10631490	rs147820753	C	T	NA	NA	NA
HNA-3	chr19	10631494	rs2288904	A	G	AC=1602	AN=2048	AF=0.782
HNA-4	chr16	31265490	rs1143679	G	A	AC=101	AN=2052	AF=0.049
HNA-5	chr16	30506720	rs2230433	G	C	AC=1392	AN=2050	AF=0.679

Table 2. Frequencies of HNA variants observed in the Indian population

Genotype	India (N=1029)	Hong Kong (N=300)	Guangzhou (N=493)	British (N=97)	German (N=119)	Turkish (N=118)	Danish (N=200)	Zambians (N=200)	Iranian (N=150)	Japanese (N=570)	Malays (N=97)	Thai (N=300)
HNA-1a/a	3	137	210	11	0.151	0.161	26	39	14/150	193/570	0.186	-
HNA-1b/b	281	28	44	60	0.361	0.305	84	32	43/150	64/570	0.134	-
HNA-1a/b		133	238	65	0.479	0.517	78	49	37/150	266/570	0.68	-
HNA-3a/a	60	154	104	79	0.555	0.559	245	183	38.7/150	238/570	0.041	-
HNA-3a/b	326	118	80	57	0.378	0.356	106	10	48.6/150	269/570	0.918	-
HNA-3b/b	638	28	11	4	0.067	0.085	15	0	12.7/150	63/570	0.041	-
HNA-4a/a	928	297	489	110	0.832	0.763	163	146	73.3/150	570/570	1	284/300
HNA-4a/b	95	3	4	27	0.151	0.237	44	31	24/150	0/570	0	16/300
HNA-4b/b	3	0	0	3/97	0.017	0	3	4	2.7/150	0/570	0	0/300
HNA-5a/a	133	215	361	76/97	0.555	0.585	111	42	51.3/150	364/570	0.443	186/300
HNA-5a/b	392	81	120	54/97	0.353	0.339	82	105	40.7/150	125/570	0.495	86/300
HNA-5b/b	500	4	12	10/97	0.092	0.076	17	42	8/150	19/570	0.062	18/300

Table 3. HNA genotype frequencies observed among various global populations

Variant	SAS_fish	Lat_Adm	EUR_nF_	AFR_AM	ASH_JE	EUR_FIN	AMISH_f	GME_fis	Others_fi

	ers_pval	_AMR_fishers_pval	fishers_pval	R_fishers_pval	W_fishers_pval	_fishers_pval	ishers_pval	hers_pval	shers_pval
FCGR3B_chr1:161629853T>C	0.0000020	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0078481	0.0007960	0.0000001
FCGR3B_chr1:161629983A>G	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
FCGR3B_chr1:161629989G>C	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
ITGAM_chr16:31265490G>A	0.0097256	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
ITGAL_chr16:30506720G>C	0.0000161	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000

Table 4. Summary of blood HNA variants found significantly distinct between India and global population datasets

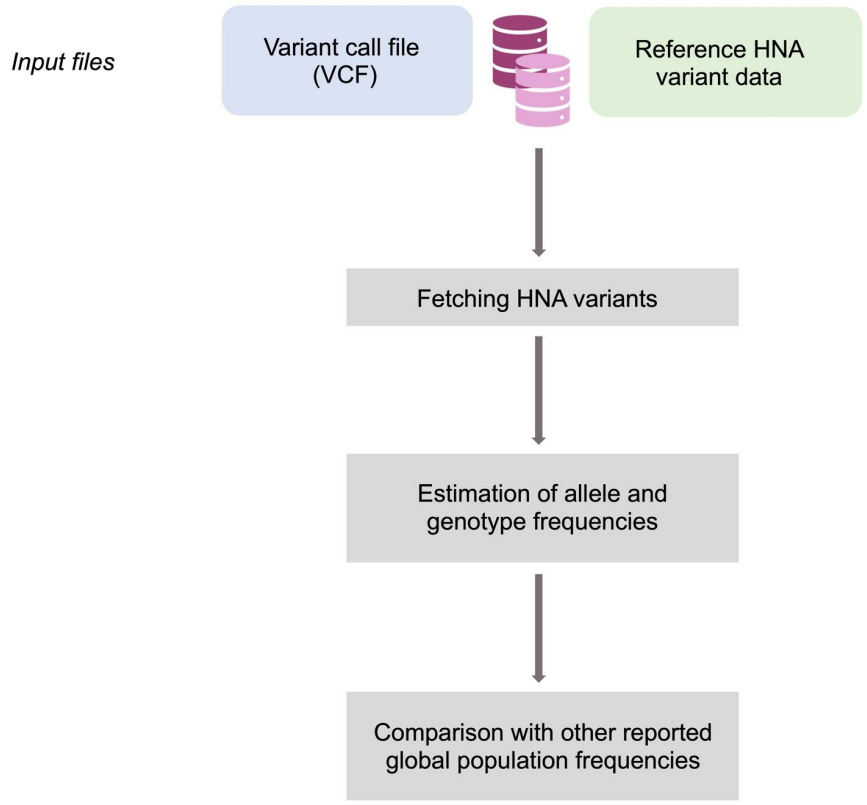

Figure 1. Schematic representation of the methodical workflow followed in the identification and estimation of HNA frequencies in India.

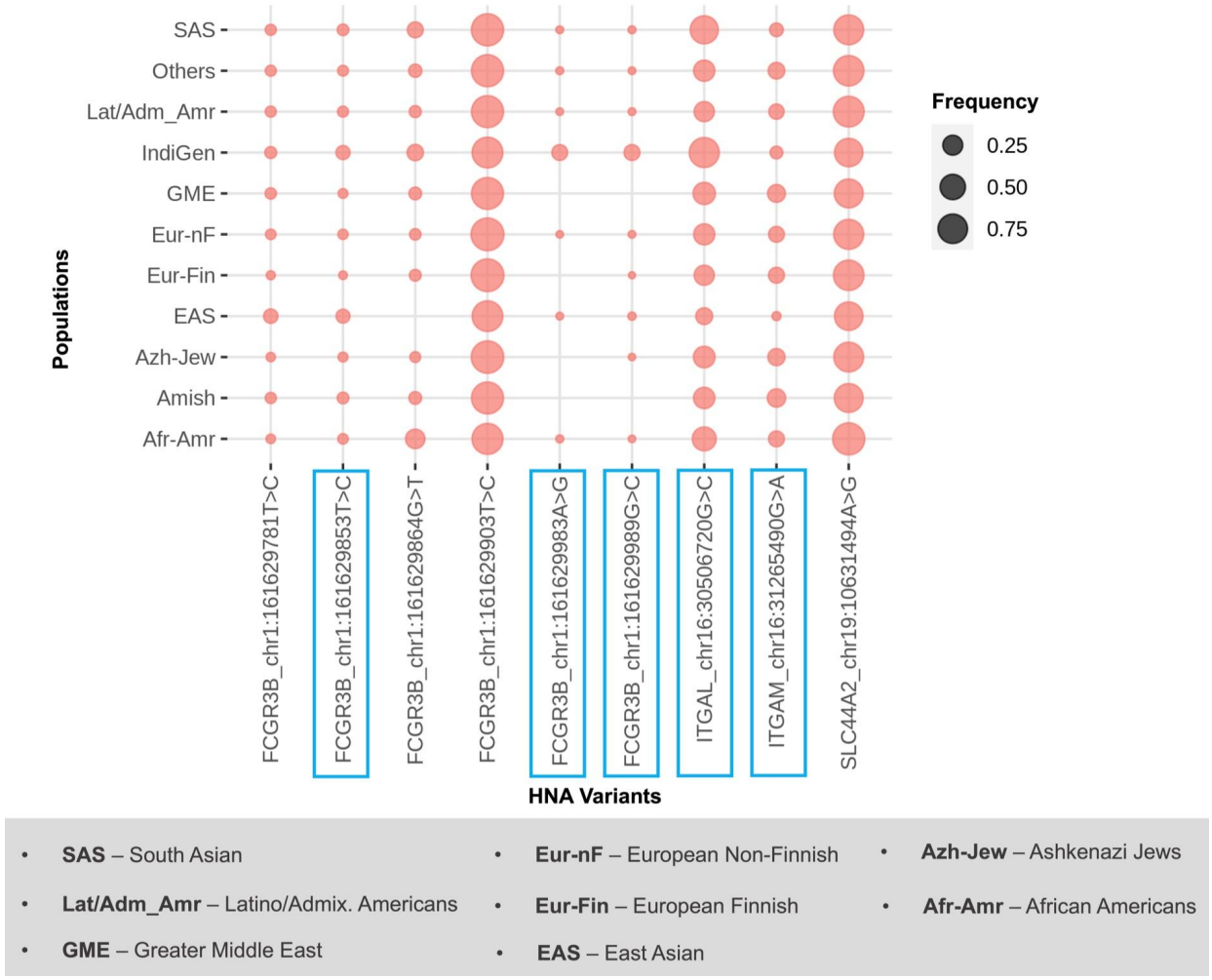

Figure 2. Schematic comparison of frequency distribution of ISBT approved human neutrophil antigen variants among global populations. Variants whose frequencies were found significantly distinct in India in comparison to all other global populations are marked in blue.

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