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Genotype frequency of human neutrophil antigen-3 (HNA-3) polymorphisms in the Yi, Han and Tibetan populations of China

Qing Chen^{1,2,*}, Kshitij Srivastava^{6,*}, Zhong Liu³, Jianyu Xiao¹, Chengyin Huang¹, Jun Sun¹, Min Li^{4,5}, and Willy Albert Flegel⁶

¹Jiangsu Province Blood Center; Nanjing, Jiangsu

²Soochow University, Suzhou, Jiangsu

³Institute of Blood Transfusion, Chinese Academy of Medical Science & Peking Union Medical College, Chengdu, Sichuan

⁴Institute of Dermatology, Chinese Academy of Medical Science & Peking Union Medical College, Nanjing, Jiangsu

⁵Jiangsu Key Laboratory of Molecular Biology for Skin Diseases and STIs, Nanjing, Jiangsu, all in China

⁶Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health, Bethesda, MD, USA

Abstract

Background—Human neutrophil antigen-3 (HNA-3) alloantibodies can cause fatal transfusion-related acute lung injury (TRALI). Most frequencies of *SLC44A2* alleles encoding the HNA-3a/b antigens have been established in Han individuals by PCR-SSP. We sequenced a *SLC44A2* gene fragment and determined its allele frequencies in 3 ethnicities of China.

Study design and methods—Genomic DNA was extracted from 448 samples of 100 blood donors of Yi ethnicity in Xichang, Liangshan; 248 Han in Nanjing, Jiangsu; and 100 Tibetan in Lhasa, Tibet. A PCR-SSP was applied to determine the phase of 2 SNPs; *SLC44A2* haplotypes were constructed.

Results—In the 567 nucleotides of the *SLC44A2* gene covered by our sequencing approach in Han individuals, we detected the known 331-44G>A (rs12972963) and 461G>A (rs2288904)

Address for correspondence: Willy A Flegel MD, Laboratory Services Section, Department of Transfusion Medicine, NIH Clinical Center, National Institutes of Health; Bethesda MD 20892, USA, waf@nih.gov, Phone : (301) 594-7401, FAX : (301) 496-9990.

*Both authors contributed equally to this study and are listed alphabetically.

Supporting Information: Additional Supporting Information may be found in the online version of this article:

Supplementary Materials and Methods. Amplification and sequencing of the *SLC44A2* gene.

Figure S1. Primer locations in the *SLC44A2* gene for amplification and sequencing.

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polymorphisms. In the 243 nucleotides sequenced in Yi and Tibetan populations, we detected the known 461G>A and 503-15T>C (rs1560711) polymorphisms. A PCR-SSP for the common *HNA-3a/b* SNP was 100% concordant. The frequencies of the *HNA-3a* allele were 0.58, 0.66 and 0.69 in Yi, Han (Nanjing) and Tibetan, respectively (0.42, 0.34 and 0.31 for *HNA-3b*).

Conclusions—The Yi population of China had the highest frequency of blood donors at risk of harboring anti-HNA-3a compared to any population studied so far. We confirmed that the underlying *SLC44A2**2 allele is more common in China than in any European or African populations.

Introduction

Alloantibodies against the human neutrophil antigen-3a (HNA-3a) have been frequently implicated in severe and fatal transfusion-related acute lung injury (TRALI)¹⁻⁴ and are occasionally associated with fetal/neonatal alloimmune neutropenia (FNAIN)⁵ or febrile transfusion reactions.⁶ The antithetical antigens HNA-3a (*SLC44A2**1 allele) and HNA-3b (*SLC44A2**2 allele) are defined by a single nucleotide polymorphism (SNP) at position 461G>A (rs2288904).^{7,8}

The *SLC44A2**2 allele frequencies vary among populations⁹⁻²¹ and ranges in the Han population from 38.2% (Nanning)¹⁸ to 26.2% (Guangzhou).²² Although TRALI is much less frequently reported in China^{22,23} than in the US,²⁴ the susceptible allele *SLC44A2**2 is more prevalent in any East Asian population compared to 17% in Caucasians⁹ and 7 % in African Americans.⁹

The Liangshan Yi autonomous prefecture is located in the southwest of Sichuan province; approximately 49% of its population is of Yi ethnicity, representing 2,226,755 people. According to the 2010 Chinese census, 97% of the population in the Tibet (Xizang) autonomous province is comprised of ethnic Tibetan, representing 3,040,400 people. Nanjing is the capital of the Jiangsu province and populated by more than 8 million ethnic Han people. Among the 56 ethnic groups present in China, Han Chinese account for 92% of the overall population;²⁵ the Yi population represents 0.65% (total > 8 million) and the Tibetan 0.47% (total 5.4 million), many of whom live in China outside of Sichuan and Tibet.

In the present study, we established the HNA-3a/b antigen frequencies in the three distinct Yi, Han (Nanjing) and Tibetan populations of China using a combination of DNA sequencing and PCR-SSP methods.

Materials and Methods

Blood samples

Blood samples were collected at random from unrelated voluntary blood donors in Xichang, Liangshan Yi autonomous prefecture (Yi ethnicity); Nanjing, Jiangsu province (Han); and Lhasa, Tibet autonomous province (Tibetan). We obtained 100 samples of Yi donors at the Luongson State Center Blood Bank, 248 samples of Han donors at the Jiangsu Province Blood Center and 100 samples of Tibetan donors at the Tibet Autonomous Region Blood Center. Han donors were approximately 70% at the Luongson State Center Blood Bank and

80% at the Tibet Autonomous Region Blood Bank Center, respectively; and, if they self-identified as Han, were excluded from the study. Any donor at the Jiangsu Province Blood Center was considered Han and included in the study. Genomic DNA was extracted from EDTA anticoagulated whole blood (TIANamp Blood DNA kit; Tiangen Biotech, Beijing, China). All human subjects gave written informed consent for their blood donations.

Amplification and sequencing of *SLC44A2* gene

Amplification and sequencing of the *SLC44A2* gene covering the *HNA-3a/b* polymorphism (461G>A) was carried out using a set of novel and published primers (Supplementary Material and Methods).

DNA of the Han samples was sequenced in Nanjing while DNA of the Yi and Tibetan samples was sequenced in Chengdu, Sichuan. Nucleotide sequences were aligned (BioEdit software v.7.1.8)²⁶ to NCBI RefSeq NC_000019.9 (range 10,713,121..10,755,235) and nucleotide positions defined using the first nucleotide of the coding sequence (CDS) of NM_020428.3.

Haplotype confirmation by PCR-SSP

A PCR-SSP was developed to determine the phase of the 2 SNPs identified in the Han population. The 4 novel allele specific primers used were 5'-CGACCTCAGTCCTGAGCA-3', 5'-CAGGGCAGTCACCATCTT-3', 5'-CGACCTCAGTCCTGAGCG-3' and 5'-CAGGGCAGTCACCATCTC-3'. Amplification of a 298 base pair (bp) product was carried out in a final volume of 25 µl containing 50 ng of genomic DNA (OneTaq Hot Start 2× Master Mix with standard buffer; New England Biolabs, Ipswich, MA, USA). The reaction conditions were as follows: Initial denaturation at 94 °C for 45 sec; 35 cycles of 94 °C for 30 sec, 71 °C for 1 min, 68 °C for 30 sec; and final elongation at 68 °C for 5 min. The amplicons were analyzed on a 1% agarose gel.

HNA-3 genotyping by PCR-SSP

HNA-3a/b genotyping was performed with PCR-SSP as previously published.⁹

Statistical description

The χ^2 goodness of fit test was used to detect any deviation from the Hardy-Weinberg equilibrium. 95% confidence intervals (CI) for allele frequencies were calculated using the Poisson distribution.²⁷ The Fisher's exact test was used to compare *HNA-3a* expression between populations; $p < 0.05$ was considered statistically significant.

Results

Genomic DNA from 448 samples was analyzed. They represented 100 blood donors of Yi ethnicity in Xichang, Liangshan; 248 Han in Nanjing, Jiangsu; and 100 Tibetan in Lhasa, Tibet, all in China.

Sequencing

DNA stretches were sequenced spanning from 50 nucleotides 3' of *SLC44A2* intron 4 to 38 nucleotides 5' of intron 7 (567 nucleotides) or spanning from 52 nucleotides 3' of intron 6 to 84 nucleotides 5' of exon 8 (287 nucleotides). The *HNA-3a/b* SNP (461G>A) was detected by both sequencing approaches (Table 1). In addition, we detected the 2 known SNPs 503-15T>C (rs1560711) located in intron 7 in Yi and Tibetan populations and 331-44G>A (rs12972963) located in intron 5 in the Han population (Table 1).²¹ The only other known SNP in these DNA stretches, 457C>T (rs147820753),²¹ was not found in the 3 populations. The SNPs detected in the study were in a Hardy-Weinberg equilibrium ($p>0.05$), with the exception of one SNP pair (503-15T>C and 461G>A) in the Yi population ($p = 0.049$).

Haplotype confirmation

Haplotype confirmation. There were 103 Han ethnicity individuals who were heterozygous at both 331-44G>A and 461G>A (Table 1). Using PCR-SSP, we determined the haplotypes to be GG and AA in 99 individuals (DNA from 4 samples was unavailable), as known for the previously described alleles KM024996 and KM024997.²¹

HNA-3 genotyping

There was 100% concordance between the DNA sequencing and PCR-SSP results (Table 2). Our data was used to predict the HNA-3 antigen expression and the fraction of HNA-3a-negative individuals (Table 3) who are at risk of developing anti-HNA-3a in the 3 populations.

Novel *HNA-3b* allele

We found an allele (GenBank accession KT361918) probably encoding HNA-3b (Table 1, genotype number 4), which differed from the 2 previously described alleles (KM024997 and KM024998) and also from 2 putative haplotypes encoding HNA-3b.²¹

Discussion

A total of 243 nucleotides harboring the *HNA-3a/b* SNP (461G>A) were sequenced in samples from the Yi and Tibetan populations and 567 nucleotides in samples from the Han (Nanjing) population. The 2 stretches of DNA sequence overlapped at the *HNA-3a/b* SNP and covered a 703 bp DNA segment, when combined. We determined the *SLC44A2* allele frequencies in the Yi and Tibetan populations as well as a representative Han (Nanjing) population for a total of 448 individuals. The HNA-3b antigen frequency was shown to be highest in the Yi population compared to any other population studied so far (Table 3 and 4). We demonstrated that a previously published PCR-SSP⁹ can reliably be applied in China to infer HNA-3a/b antigen status. We found the novel allele KT361918 whose likely HNA-3b expression remains to be confirmed by serology.

Four studies have previously established the HNA-3a/b frequency using PCR-SSP or similar techniques in a total of 768 Han individuals (Table 4). While these studies^{18,22,33,34} cannot detect any SNP that is potentially occurring in the vicinity of the *HNA-3a/b* SNP (461G>A), one study¹¹ established a 521 bp nucleotide sequence without detecting any unknown SNP.

We confirmed this study¹¹ and excluded the possibility of any common hitherto unknown SNP at a position -487 nucleotides to +216 nucleotides relative to the *HNA-3a/b* SNP (461G>A) as well as the known interference of the SNP 457C>T with the SNP 461G>A.^{9,28}

TRALI is reportedly the leading cause of transfusion-related mortality in Western healthcare systems, and anti-HNA-3a is more often associated with severe and fatal TRALI reactions than any other distinct HNA or HLA antibody.^{21,29-32} Donors and patients in China have a higher propensity of expressing only HNA-3b and potentially forming anti-HNA-3a (Table 4). However, TRALI is rarely reported in China. This may be explained by incomplete epidemiological data or missed diagnosis, because of unwarranted concerns about the reporting process or the lack of related knowledge. It is important to establish a better hemovigilance system in China for adverse transfusion reactions and also increase awareness of TRALI among physicians and other health care professionals.

Plasma-containing blood products derived from whole blood are prepared from both male and female blood donors in China. Women who are homozygous for *SLC44A2**2 can develop HNA-3a antibodies during pregnancy and are expected to be more common among Yi than Han (Nanjing) individuals (Table 3). It is time to consider excluding female blood donors in China from plasma and platelet donations. Identifying blood donors who are homozygous for the *SLC44A2**2 allele and excluding such donors would also reduce the risk of TRALI caused by HNA-3a antibodies.

Web resources

Hardy-Weinberg equilibrium calculator

(<http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls>)

Liangshan Yi autonomous prefecture (<http://2014.lsz.gov.cn/publish/portal13>)

Luongson State Center Blood Bank (<http://ls.newssc.org/system/20110928/001362714.html>)

Primer3 software, version 4.0.0 (<http://bioinfo.ut.ee/primer3-0.4.0/>)

Tabulation on the 2010 population census of the People's Republic of China.

<http://www.stats.gov.cn/english/Statisticaldata/CensusData/rkpc2010/indexch.htm> (accessed 23 July 2015)

Tibet autonomous province (<http://www.xizang.gov.cn/rkmz/51886.jhtml>)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1
SLC44A2 genotypes observed in Yi, Han (Nanjing) and Tibetan samples

Genotype number	SNP position and genotype				Donors observed (n)		
	331-44G>A rs12972963	457C>T rs147820753	461G>A rs2288904	503-15T>C rs1560711	Yi	Han	Tibetan
1	GG	CC	GG	n.t.	0	112	0
2	GA	CC	GA	n.t.	0	103	0
3	AA	CC	AA	n.t.	0	32	0
4	GG	CC	GA	n.t.	0	1	0
5	n.t.	CC	GG	TT	39	0	50
6	n.t.	CC	GA	TC	39	0	38
7	n.t.	CC	AA	CC	22	0	12
Total					100	248	100

n.t. – not tested

Table 2
***SLC44A2* allele frequencies in Yi, Han and Tibetan populations**

Allele	Yi			Han (Nanjing)			Tibetan		
	n	Population frequency (%) [*]	n	Population frequency (%) [*]	n	Population frequency (%) [*]			
<i>SLC44A2*1</i>	117	58 (48 – 69)	328	66 (59 – 74)	138	69 (57 – 81)			
<i>SLC44A2*2</i>	83	42 (33 – 51)	168	34 (29 – 39)	62	31 (24 – 39)			
Total	200		496		200				

^{*} Data are reported as mean (95% CI). CI (confidence interval) calculated by Poisson distribution.

Table 3

HNA-3a expression

Predicted HNA-3a expression	Yi		Han		Tibetan	
	n	%	n	%	n	%
Positive	78	78%	216	87%	88	88%
Negative	22 [†]	22%	32 [†]	13%	12	12%
Total	100		248		100	
p [*]	0.048 [†]				NS	

* Fishers exact test, two sided.

[†] Significant difference between Yi and Han (Nanjing).

NS – not significant

Table 4

***SLC44A2* allele frequencies in various populations**

Population	Country	Samples tested (n)	<i>SLC44A2</i> allele frequency		PCR	Method		Reference
			<i>SLC44A2*01</i>	<i>SLC44A2*02</i>		Nucleotide sequencing (bp)		
Yi (Liangshan)	China	100	0.590	0.410	n.a.	243		Present study
Han (Nanning)	China	119	0.618	0.382	SSO	n.a.		18
Han (Beijing)	China	43	0.650	0.350	2 platforms*	n.a.		33
Japanese	Japan	570	0.654	0.346	SSP	n.a.		12
Han (Zhejiang)	China	400	0.654	0.346	n.a.	521		11
Han (Nanjing)	China	248	0.660	0.340	SSP	567		Present study
Tibetan (Lhasa)	China	100	0.690	0.310	n.a.	243		Present study
Thai (central)	Thailand	500	0.718	0.282	SSP	n.a.		16
Han (Hong Kong)	China	113	0.735	0.265	SSP	n.a.		34
Han (Guangzhou)	China	493	0.738	0.262	SSP	n.a.		22
Caucasian	Germany	67	0.761	0.239	n.a.	2115		28
Caucasian	England	140	0.768	0.232	ASPE	n.a.		10
Thai (northern)	Thailand	300	0.775	0.225	SSP	n.a.		16
Caucasian	USA	4300	0.787	0.213	n.a.	2115 [†]		35
Caucasian	Denmark	366	0.814	0.186	SSO	n.a.		19
Caucasian	USA	93 ⁹ /96 ²¹	0.830 ⁹ /0.807 ²¹	0.170 ⁹ /0.193 ²¹	RT-ASP ⁹	440 ⁹ /8192 ²¹		9,21
African American	USA	241 ⁹ /200 ²¹	0.930 ⁹ /0.962 ²¹	0.070 ⁹ /0.038 ²¹	RT-SSP ⁹	440 ⁹ /8192 ²¹		9,21
African American	USA	2203	0.934	0.066	n.a.	2115 [†]		35
African (Bantu)	Zambia	183	0.974	0.026	SSO	n.a.		19

* Two genotyping platforms (MassEXTEND; Sequenom, San Diego, CA and BeadArray; Illumina, San Diego, CA)

[†] NHLBI Exome Sequencing Project³⁵

n.a. - not applicable, SSO - sequence-specific oligonucleotide probe (TaqMan; Applied Biosystems, Life Technologies, Carlsbad, CA), SSP - sequence-specific primers, RT-ASP - real-time allele-specific primer, ASPE - allele-specific primer extension.