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Human neutrophil antigen allele frequencies and assessment of HNA alloimmunization risk in donors and hematological patients

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ABSTRACT

Human neutrophil antigens (HNAs) are localized on glycoproteins which are positioned on the surface membrane of human neutrophils. Alloantibodies against HNA are implicated in a number of clinical conditions, including immune-mediated neutropenia and transfusion reactions. Genotyping for HNA systems is important in the diagnosis of disorders involving alloimmunization to HNA.

Aim. To assess the risk of HNA alloimmunization in donors and patients with hematological diseases in St. Petersburg based on the study of HNA allele and genotype frequencies.

Materials and methods. DNA samples of 303 blood donors and 302 hematological patients were obtained and typed for HNA-1, -3, -4, -5. Polymerase chain reactions with homemade sequence-specific primers were used for typing. Genomic DNA was isolated from whole blood by a multistage purification method using the CTAB reagent. The results were detected in real time using the EVAGreen intercalating dye. Pearson's chi-squared test was used to compare the HNA genotype frequencies in donors, patients with hematological diseases and in other populations.

Results. In the study, the frequency of HNA-1bd allele was 0.584–0.588, of HNA-1a – 0.376–0.384, of HNA-1bc – 0.032–0.036. HNA-1bc allele was represented in the genotypes HNA-1a/bc/bd (0.023–0.036), HNA-1a/bc (0.020–0.043) and HNA-1bc/bd (0.007–0.010). The genotypes HNA-1bc/bc and HNA-1null were not identified. Allele "a" of HNA-3, -4, -5 systems was found in the majority of studied individuals (0.795–0.804; 0.887–0.898; 0.699–0.708). The highest calculated risk of HNA alloimmunization was noted in the absence of HNA-5b, HNA-1a, HNA-3b, and HNA-4b alleles in the genotype and was 0.250, 0.233, 0.231, and 0.163, respectively.

Conclusions. Our data are consistent with the results of studies on the HNA allele and genotype frequencies in populations of Europeans and are significantly different from those of East and Southeast Asia, Africa and South America. The frequencies of HNA-1, -3, -4, -5 alleles and genotypes among donors in St. Petersburg and patients with hematological diseases did not have statistically significant differences. It was shown that the highest calculated risk of alloimmunization was observed in the absence of HNA-5b, HNA-1a, HNA-3b, and HNA-4b alleles in the genotype. These data are consistent with the results of similar studies on populations of white Europeans conducted by other authors.

Key words: donor, neutrophil antigens, genotyping.

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Частота встречаемости антигенов нейтрофилов человека и риск аллоиммунизации у доноров и больных гематологическими заболеваниями

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РЕЗЮМЕ

Актуальность. Антигены нейтрофилов человека (Human neutrophil antigens, HNA) локализованы на гликопротеинах, расположенных на поверхностной мембране нейтрофилов. Иммунизация к HNA во время беременности или вследствие трансфузий компонентов крови может привести к выработке аллоантител. Одним из факторов развития аллоиммунизации является частота встречаемости HNA. В связи с этим представляется важным изучить особенности распределения аллелей и генотипов HNA у доноров и больных гематологическими заболеваниями г. Санкт-Петербурга для прогнозирования риска аллоиммунизации.

Цель. Оценить риск HNA аллоиммунизации у доноров и больных гематологическими заболеваниями г. Санкт-Петербурга на основании изучения частот встречаемости аллелей и генотипов HNA.

Материалы и методы. Материалом исследования служили образцы периферической крови 303 доноров г. Санкт-Петербурга и 302 больных гематологическими заболеваниями, получавших терапию в Российском научно-исследовательском институте гематологии и трансфузиологии. Геномная ДНК была выделена из цельной крови методом многоступенчатой очистки с использованием реактива *цетилтриметиламмония бромида.* Типирование HNA проводили методом аллель-специфичной полимеразной цепной реакции с использованием разработанных олигонуклеотидных праймеров. Сравнения частот встречаемости генотипов HNA у доноров, больных гематологическими заболеваниями, и представителей других популяций проводили с помощью критерия согласия Пирсона χ^2.

Результаты. Частота встречаемости аллеля HNA-1bd составила 0,584–0,588, а HNA-1a – 0,376–0,384. Частота встречаемости аллеля HNA-1bc составила 0,032–0,036, и данный аллель был представлен в генотипах HNA-1a/bc/bd (0,023–0,036)00, HNA-1a/bc (0,020–0,043) и HNA-1bc/bd (0,007–0,010). Генотипы HNA-1bc/bc и HNA-1null выявлены не были. Аллель «а» систем HNA-3, -4, -5 встречался у большинства исследуемых в каждой группе (0,795–0,804; 0,887–0,898; 0,699–0,708 соответственно). На основании полученных частот встречаемости аллелей и генотипов рассчитали вероятность аллоиммунизации к HNA. Наибольшая величина расчетного риска аллоиммунизации при трансфузиях компонентов крови отмечена при отсутствии в генотипе аллелей HNA-5b, HNA-1a, HNA-3b, HNA-4b и составляет 0,250; 0,233; 0,231 и 0,163 соответственно, что подтверждает результаты аналогичных исследований.

Заключение. Статистически значимых различий в частоте встречаемости аллелей и генотипов HNA-1, -3, -4, -5 у доноров г. Санкт-Петербурга и больных гематологическими заболеваниями не установлено. Наибольшая величина расчетного риска аллоиммунизации при трансфузиях компонентов крови, полученная на основании частот встречаемости аллелей и генотипов, отмечена при отсутствии в генотипе аллелей HNA-5b, HNA-1a, HNA-3b, HNA-4b. Полученные данные согласуются с результатами исследований распределения аллелей и генотипов систем HNA в популяции европейцев и значимо отличаются от популяций Восточной и Юго-Восточной Азии, Африки и Южной Америки. Предложенный метод типирования HNA может быть использован для создания клеточной панели, типированной по антигенам нейтрофилов, с целью определения специфичности аллоантител у доноров, и для диагностики аллоиммунных конфликтов в педиатрии, трансфузиологии и трансплантологии.

Ключевые слова: антигены нейтрофилов, генотипирование, аллель-специфичная полимеразная цепная реакция.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведения исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании и согласие на забор крови. Протокол исследования одобрен локальным этическим комитетом ФГБУ РосНИИГТ ФМБА России (протокол № 56 от 26.12.2018).

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INTRODUCTION

Human neutrophil antigens (HNAs) are localized on glycoproteins, which are positioned on the surface membrane of neutrophils. Immunization against HNA during pregnancy or as a result of blood component transfusion can lead to the production of alloantibodies to neutrophil antigens. HNA antibodies can cause development of such clinical conditions as neonatal alloimmune neutropenia (NAN), autoimmune neutropenia (AIN), transfusion-related acute lung injury (TRALI), febrile non-hemolytic transfusion reactions, immune neutropenia after bone marrow transplantation, and drug-induced immune neutropenia [12]. In addition, according to the literature, HNA polymorphism is a risk factor not only for the above mentioned conditions, but also for other diseases, including bacterial infections (periodontitis), chronic inflammatory diseases (vasculitis, systemic lupus erythematosus, rheumatoid arthritis), and susceptibility to malaria [3]. There is no information on the distribution of HNA among donors in the Russian Federation.

To date, 5 HNA systems (HNA-1, -2, -3, -4, -5) have been described [1].

The HNA-1 system includes HNA-1a, HNA-1b, HNA-1c, and HNA-1d antigens located on the Fc-γ receptor IIIb (FcγRIIIb, CD16b). The expression of HNA-1b is always accompanied by the expression of HNA-1d or HNA-1c. FcγRIIIb is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein expressed on the neutrophil surface and encoded by the *FCGRIIIB* gene [4].

The HNA-2a antigen is located on the GPI-anchored protein CD177 encoded by the *CD177* gene [5]. HNA-2a is found in most individuals; the absence of HNA-2a is determined by a defect in the transcription of the CD177 gene [6].

HNA-3a and HNA-3b are located on the choline transporter-like protein 2 (CTL 2) encoded by the *SLC44A2* gene [7].

The antigens of the HNA-4 and HNA-5 systems are localized on the αM (CD11b) and αL (CD11a) integrin subunits and encoded by the *ITGAM* and *ITGAL* genes, respectively [8].

The presence of each allele of the HNA-1 system is determined by a combination of 6 single nucleotide polymorphisms (SNPs) in the *FCGRIIIB* gene, located close to one other. The HNA-3, -4, -5 systems include two alleles each, the differences between which are determined by replacement of one nucleotide in the DNA sequence of the corresponding genes [1].

The frequency of HNA is one of the factors of immune response development [9]. In this regard, it seems important to study the distribution of HNA alleles and genotypes in the population. Serological and molecular genetic methods are used to study the frequency of HNA. However, serological typing of neutrophil antigens can be complicated due to the absence of some typing reagents, high cost and short lifetime of neutrophils. Polymerase chain reaction (PCR)-based methods are the most optimal for HNA-1, -3, -4, -5 typing [10], but are often not used due to a lack of regulatory documents and test systems for HNA typing from domestic manufacturers.

The aim of the study was to assess the risk of HNA alloimmunization in donors and patients with hematological diseases in St.-Petersburg based on the study of HNA genotype and allele frequencies.

MATERIALS AND METHODS

Peripheral blood samples were obtained from 303 donors from St.-Petersburg and 302 patients with hematological diseases, who received therapy at Russian Research Institute of Hematology and Transfusiology.

HNA typing was performed by allele-specific PCR (AS-PCR) using homemade oligonucleotide primers. Genomic DNA was isolated from whole blood by the multistage purification method using etyltrimethylammonium–bromide (CTAB) reagent. The results were detected in real time using the EVAGreen intercalating dye.

The results were statistically processed using the Statistica 7 software. Comparison of allele frequencies and HNA genotypes in donors, patients with hematological diseases, and in other populations, as well as check of the correspondence of the observed distributions to the Hardy – Weinberg equilibrium were performed using the Pearson's chi-squared test (χ^2). The differences with $p < 0.05$ were considered statistically significant. The critical χ2 value for alleles of

the HNA-3, -4, -5 systems with *p*-value of 0.05 was 3.84. The critical χ2 value for the alleles of the HNA-1 system with *p*-value of 0.05 was 5.991.

The following formulas were used to estimate the probability of HNA alloimmunization:

– the probability of alloimmunization to the allele "a" = (aa + ab) × bb,

– the probability of alloimmunization to the allele "b" = (bb + ab) × aa,

– where aa, ab, bb are the frequencies of the corresponding genotypes.

RESULTS

Oligonucleotide primers for HNA typing by AS-PCR method were designed using Primer 3.0 and Primer-BLAST. The sequences of oligonucleotide primers for typing antigens of the HNA-1, -3, -4, -5 systems are shown in Table 1.

Table 1

DNA sequences of oligonucleotide primers for HNA-1,-3,-4,-5 genotyping

Antigen	Gene	Forward primer (5'– 3')	Reverse primer (5'– 3')
HNA-1a	*FCGR3B*	CCTCAATGGTACAG**G**GTGCT**C**	GCCTGGCTTGAGATGAGG**T**T
HNA-1b/c	*FCGR3B*	CCTCAATGGTACAG**C**GTGCT**T**	CACTGTCGTTGACTGTGGCA**T**
HNA-1b/d	*FCGR3B*	CCTCAATGGTACAG**C**GTGCT**T**	ACTGTCGTTGACTGTGGCA**G**
HNA-3a	*SLC44A2*	CTACCTCACGTACCTGAATGCT	GCAGGGCAGTCACCATCT**C**
HNA-3b	*SLC44A2*	CTACCTCACGTACCTGAATGCT	GCAGGGCAGTCACCATCT**T**
HNA-4a	*ITGAM*	CTCATGCGAGCCCATCC**G**	ACAAGGAGGTCTGACGGTGA
HNA-4b	*ITGAM*	CTCATGCGAGCCCATCC**A**	ACAAGGAGGTCTGACGGTGA
HNA-5a	*ITGAL*	ATCATCCCCCACAGATCCA**G**	AGCTGGACCCAGTAAGCATC
HNA-5b	*ITGAL*	ATCATCCCCCACAGATCCA**C**	AGCTGGACCCAGTAAGCATC

Note. nucleotides complementary to SNPs that determine the antigen presence are shown in bold.

To analyze the specificity of the primers, DNA samples from 20 donors were used the sequences of the HNA-1, -3, -4, -5 alleles in which were determined by sequencing. The correspondence of the results of sequencing and AS-PCR with the real-time results and agarose gel electrophoresis was 100%. No nonspecific PCR products were identified by gel electrophoresis.

The real-time PCR conditions for the HNA-1, -3, -4, -5 allele typing were the same. For the analysis we used a 2.5x PCR reaction mix in the presence of EVA-Green (SINTOL, Moscow), which includes a 2.5x PCR buffer B (6.25 mmol $MgCL_2$, KCl, TrisHCl (pH 8.8)), SynTaq DNA polymerase, deoxynucleoside triphosphates, glycerol, and Tween 20. For PCR, a mixture containing 50–100 ng of genomic DNA, 1x PCR reaction mix, and 0.2 μmol of forward and reverse primers was added to each tube. The final volume of the mixture was adjusted to 25 μl with double-distilled water. The following protocol was used for PCR: 95° for 5 min, then 33 cycles: 95° – 20 sec, 68° – 30 sec.

HNA-1, -3, -4, -5 allele and genotype frequencies in donors of St.-Petersburg and patients with hematological diseases are shown in Table 2. The deviation of the observed genotype distribution from the one expected in patients with hematological diseases and donors was not statistically significant.

Table 2

Comparison of frequencies of genotypes and alleles of HNA systems in donors and patients with hematological diseases

System	Genotype	Patients, *n* = 302	Donors, *n* = 303	χ^2	Allele	Patients	Donors
HNA-1	a/a	0.142	0.142	$\chi^2 = 0.172$, $p = 0.918$	a	0.376	0.384
	a/bc/bd	0.023	0.036		bd	0.588	0.584
	a/bc	0.043	0.020		bc	0.036	0.032
	a/bd	0.411	0.442		–	–	–
	bc/bd	0.007	0.010		–	–	–
	bd/bd	0.374	0.350		–	–	–
HNA-3	a/a	0.623	0.650	$\chi^2 = 0.150$, $p = 0.699$	a	0.795	0.804
	a/b	0.343	0.307		b	0.205	0.196
	b/b	0.033	0.043		–	–	–

Table 2 (continued)

HNA-4	a/a	0.788	0.802	$\chi^2 = 0.321$, $p = 0.571$	a	0.887	0.898
	a/b	0.199	0.191		b	0.113	0.102
	b/b	0.013	0.007		–	–	–
HNA-5	a/a	0.500	0.488	$\chi^2 = 0.124$, $p = 0.725$	a	0.699	0.708
	a/b	0.397	0.439		b	0.301	0.292
	b/b	0.103	0.073		–	–	–

As can be seen from Table 2, HNA-1, -3, -4, -5 genotype frequencies in patients with hematological diseases who received therapy at Russian Research Institute of Hematology and Transfusiology had no statistically significant differences from those in donors of St. Petersburg. In the examined groups, the frequency of HNA-1bd allele was higher (0.584–0.588) than HNA-1a (0.376–0.384). The frequency of HNA-1bc was 0.032–0.036, and this allele was represented in the genotypes HNA-1a/bc/bd (0.023–0.036), HNA-1a/bc (0.020–0.043), and HNA-1bc/bd (0.007–0.010). The genotypes HNA-1bc/bc and HNA-1null were not identified.

The allele "a" of the HNA-3, -4, -5 systems was found in the majority of individuals in each group (0.795–0.804; 0.887–0.898; 0.699–0.708, respectively). The prevalence of HNA-5a was 0.699–0.708.

Since HNA-1, -3, -4, -5 genotype frequencies in patients with hematological diseases and donors of St. Petersburg did not have statistically significant differences, an assessment of the possible risk of HNA alloimmunization was calculated based on the data on the HNA allele and genotype frequencies in the group that included both donors and patients with hematological diseases (Table 3).

Table 3

Assessment of the possible risk of alloimmunization against HNA-1, 3-5 during transfusions of blood components

Risk of HNA alloimmunization								
HNA 1a	HNA 1bd	HNA 1bc	HNA 3a	HNA 3b	HNA 4a	HNA 4b	HNA 5a	HNA 5b
0.233	0.143	0.064	0.037	0.231	0.01	0.163	0.080	0.250

As can be seen from the presented data, the highest calculated risk of alloimmunization during blood component transfusion was observed in the absence of HNA-5b, HNA-1a, HNA-3b, and HNA-4b alleles in the genotype and was 0.250, 0.233, 0.231, and 0.163, respectively.

DISCUSSION

The data obtained are consistent with the results of studies on the distribution of HNA alleles and genotypes in European populations [9] and significantly differ from other populations. Thus, in the populations of South – East Asia, China, and Japan, the HNA-1a allele frequency was significantly higher and was 0.696, 0.667, and 0.623, respectively ($p < 0.001$) [11]. The frequency of HNA-3a was 0.795 – 0.804, which was significantly higher than that in the Japanese population (0.654) [12], and significantly lower than in the populations of Zambia (0.974) and Brazil (1.0) ($p < 0.001$) [13, 14]. The frequency of HNA-4a was 0.887–0.898, and this was significantly lower than in the populations of China and Brazil – 0.995 and 1.0, respectively ($p < 0.001$) [15, 14]. The obtained frequency of HNA-5a was significantly higher than in the Zambian population (0.500) [13], but significantly lower than in the populations of China (0.852), Japan (0.840), and Brazil (0.855) ($p < 0.001$) [15, 12, 14]. The probability of HNA alloimmunization was calculated based on the obtained allele and genotype frequencies. The highest calculated risk of alloimmunization during blood component transfusion was observed in the absence of HNA-5b, HNA-1a, HNA-3b, and HNA-4b alleles in the genotype and was 0.250, 0.233, 0.231, and 0.163, respectively, which confirms the results of similar studies conducted by other authors in the population of white Europeans [10]. The obtained data may be useful for predicting clinical conditions associated with alloimmunization. However, the probability of immune response development is highly dependent on the immunogenicity of the antigen and other factors, which may explain the discrepancy between the calculated risk of alloimmunization and the existing data on the specificity of detected HNA alloantibodies. Thus, for example, at low calculated risk of alloimmunization to HNA-3a (0.37), such antibodies lead to the development of TRALI. It is known that anti-HNA-3a alloantibodies contained in donor plasma are one of the reasons for the development of severe TRALI with fatal outcome [12]. Despite high calculated risk of HNA-3b alloimmunization, rare cases of NAN caused by anti-HNA-3b alloantibodies have been described, and there are no data on the cases of TRALI caused by such antibodies in the literature.

The most common causes of NAN development in the population of white Europeans are alloantibodies to HNA-1a, -1b, -1c and -2a [16]. However, there are cases of NAN caused by alloantibodies to HNA-1d, -3a, -3b, -4a , -4b, -5a that are described in the literature [17, 18].

In clinical practice, transfusions of granulocyte concentrate are carried out for patients with a significant decrease in the absolute number of granulocytes in the blood in the presence of infection, uncontrolled antibiotic therapy, and in sepsis of newborns. In case of transfusion of HNA incompatible blood component, the patient has an increased risk of alloimmunization and, as a result, there is a lack of clinical effect of transfusion [19]. Individual selection of HNA and HLA-compatible blood components is required for such patients. Building of a HNA-typed donor base will help prevent alloimmunization. The development of a HNA-typed cell panel will help solve the problem of diagnosing alloimmune conflicts in pediatrics, transfusiology and transplantology.

CONCLUSION

The conducted research allowed to study the distribution patterns of HNA alleles and genotypes in donors of St.-Petersburg and patients with hematological diseases and to assess the possible risk of HNA alloimmunization. No statistically significant differences in the frequencies of HNA-1, -3, -4, -5 alleles and genotypes were found in donors of St.-Petersburg and patients with hematological diseases. The highest estimated risk of alloimmunization during transfusion of blood components was observed in the absence of HNA-5b, HNA-1a, HNA-3b, an HNA-4b alleles in the genotype, which confirms the results of similar studies conducted by other authors in th population of white Europeans. The data obtained may be useful for predicting clinical conditions associated with alloimmunization. However, the immunization process after transfusion and / or during pregnancy is associated not only with antigenic incompatibility, but also depends on the immunogenicity of the antigen, genetic, epigenetic and environmental factors, which may explain the discrepancy between the calculated risk of alloimmunization and the existing data on the specificity of detected HNA alloantibodies.

The presented data may be useful for prevention of alloimmunization, as well as for population studies. The proposed HNA typing method can be used to develop a HNA-typed cell panel in order to determine the specificity of alloantibodies in donors and to diagnose alloimmune conflicts in pediatrics, transfusiology and transplantology.

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