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Original study article

Human leukocyte antigen class II (DRB1 and DQB1) alleles frequencies in patients with various forms of pemphigus among the Russian population

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ABSTRACT

BACKGROUND: Autoimmune bullous dermatoses are known to be the most severe blistering conditions of skin. *HLA-DRB1* and *DQB1* alleles might play a crucial role in their onset. In pemphigus HLA class II molecules stimulate the division of T helper cells, which in turn stimulate B cells to produce antibodies to epidermal keratinocytes causing acantholysis. The *HLA-DRB1* and *DQB1* alleles' frequencies studied in pemphigus in a vast variety of populations worldwide. However, as of yet, this mechanism was not investigated in Russian population.

AIM: To estimate the prevalence of the *HLA-DRB1* and *DQB1* alleles at a low- and high-resolution levels in patients with various forms of pemphigus. We observed 86 patients with pemphigus vulgaris, 13 — with pemphigus foliaceus, 6 patients with paraneoplastic pemphigus and 92 healthy volunteers.

MATERIALS AND METHODS: HLA typing for *DRB1* and *DQB1* was performed with 50 nanogram DNA extraction and polymerase chain reaction.

RESULTS: At a low-resolution level *HLA-DRB1*4* and *DRB1*14* alleles were statistically significant more frequent in pemphigus vulgaris and pemphigus foliaceus patients compared to those in control subjects, whereas *HLA-DRB1*11*, *DRB*16*, and *DRB1*3* alleles were more frequent in healthy volunteers. At a high-resolution level, *DRB1*04:02* allele was observed to show its statistically significant higher frequency in all variants of pemphigus, including paraneoplastic pemphigus. However, *DRB1*14:05* HLA allele was more frequent in pemphigus vulgaris and pemphigus foliaceus patients, whereas *DRB1*11:04* one was found to be 3.7 times more frequent in healthy controls. Additionally, at a low-resolution level for *HLA-DQB1* alleles no statistically significant results were observed. However, at a high-resolution level the chances for more frequent indication of *DQB1*03:02* allele were 7.09 times higher in pemphigus foliaceus group and 2.49 higher in pemphigus vulgaris patients compared to healthy volunteers. Moreover, *DQB1*05:03* was identified more frequently in pemphigus vulgaris and paraneoplastic pemphigus groups of patients, whereas DQB1*03:01 allele was shown to be increased in the group of healthy donors.

CONCLUSION: *HLA-DRB1*4*, *DRB1*14*, *DRB1*04:02*, *DRB1*14:05*, *DQB1*03:02* and *DQB1*05:03* alleles might be considered as the genetic markers for pemphigus vulgaris susceptibility, while *HLA-DRB1*11*, *DRB*16*, *DRB1*3*, *DRB1*11:04* and *DQB1*03:01* allelic groups appear to be protective for Russian population.

Keywords: HLA DRB1 alleles; HLA DQB1 alleles; pemphigus.

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Оригинальное исследование

Оценка распространённости HLA DRB1 и DRQ1 аллелей у больных разными формами пузырчатки в российской популяции

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АННОТАЦИЯ

Обоснование. Аутоиммунные буллёзные дерматозы представляют собой наиболее тяжёлые заболевания кожи и часто могут заканчиваться летальным исходом. Известно, что генетические факторы, а именно ассоциация с *DRB1* и *DQB1* HLA аллелями II класса играет одну из ключевых ролей в дебюте аутоиммунных буллёзных дерматозов. Так, например, при пузырчатке молекулы HLA II класса посредством Т-лимфоцитов участвуют в стимуляции В-клеток к выработке антител класса IgG к клеткам шиповатого слоя, вызывая акантолиз. Исследование распространённости HLA *DRB1* и *DQB1* аллелей у больных пузырчаткой проводилось во многих популяциях, однако подобных работ в российской популяции ещё не было.

Цель исследования — оценить частоту распространённости HLA *DRB1* и *DQB1* аллелей на уровнях низкого и высокого разрешения у пациентов с различными формами пузырчатки.

Материалы и методы. В исследовании приняли участие 86 больных вульгарной, 13 — листовидной, 6 — паранеопластической пузырчаткой и 92 здоровых донора. HLA-типирование для *DRB1* проводилось с помощью полимеразной цепной реакции с применением специфичных праймеров.

Результаты. На уровне низкого разрешения *HLA-DRB1*4* и *DRB1*14* аллели статистически значимо чаще выявлялись у больных вульгарной и листовидной пузырчаткой по сравнению со здоровыми донорами, в то время как *HLA-DRB1*11*, *DRB*16* и *DRB1*3*, наоборот, достоверно чаще встречались в контрольной группе. На уровне высокого разрешения наблюдалось следующее распределение HLA *DRB1* аллелей: *DRB1*04:02* являлись предрасполагающими для всех разновидностей пузырчатки, включая паранеопластическую. *DRB1*14:05* HLA встречался статистически значимо чаще у больных вульгарной и листовидной пузырчаткой, тогда как *DRB1*11:04* в 3,7 раза чаще у здоровых доноров. На уровне низкого разрешения по HLA *DQB1* аллелям статистически значимой разницы не выявлено, однако при высоком разрешении показано, что шансы получить *DQB1*03:02* были в 7,09 раза выше у больных листовидной пузырчаткой и в 2,49 раза выше для группы вульгарной пузырчатки по сравнению со здоровыми донорами. Следует также отметить, что *DQB1*05:03* аллель достоверно часто встречался у больных вульгарной и паранеопластической пузырчаткой, тогда как *DQB1*03:01* — статистически значимо чаще в группе контроля.

Заключение. В нашем исследовании показано, что *HLA-DRB1*4*, *DRB1*14*, *DRB1*04:02*, *DRB1*14:05*, *DQB1*03:02* и *DQB1*05:03* аллели являлись предрасполагающими к развитию пузырчатки, в то время как *HLA-DRB1*11*, *DRB*16*, *DRB1*3*, *DRB1*11:04* и *DQB1*03:01* были протективными к развитию пузырчатки, поскольку статистически значимо чаще встречались в группе контроля.

Ключевые слова: HLA DRB1 аллели; HLA DQB1 аллели; пузырчатка.

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ЭКО•ВЕКТОР

BACKGROUND

Autoimmune bullous dermatoses are severe and often fatal skin diseases. Currently, the mechanisms underlying the pathogenesis of autoimmune bullous dermatoses are unclear. Environmental factors, genetics including *DR* and *DQ* alleles, the use of certain drugs, and viral infections may trigger the onset of these diseases [1]. For instance, pemphigus is an autoimmune bullous skin disease based on genetic mechanisms, wherein immunoglobulins of class G (IgG) are fixed in the spiny epidermis of the skin and/or mucous membranes, which causes acantholysis with subsequent intraepidermal blister formation [2].

The human leukocyte antigens (HLA) is a group of more than 150 histocompatibility antigens. A significant number of immune-related genes are located on chromosome 6. Consequently, HLA class II molecules present antigens from the extracellular space to T-lymphocytes and stimulate the division of T-helper cells, stimulating B-cells to produce antibodies. Owing to its polymorphism, HLA class II encodes different molecules with varying binding affinities, which enables higher binding of a variable antigenic peptide to CD4+ cells. Notably, T cells that were directly regulated by *DRB1*04:02* HLA exhibited higher tropism to desmogleins 3 and 1, which triggered acantholysis [3]. Increased levels of various HLA class II alleles in vesicular patients have been reported in numerous populations [4–6].

DRB1 HLA gene polymorphisms are directly linked to the development of several autoimmune diseases, such as rheumatoid arthritis, type 2 diabetes, and lupus erythematosus. HLA variations determine the autoimmune response through the activation of T-cell immunity and T-regulatory cells, which are responsible for the production of pro-inflammatory cytokines, particularly interleukin-10 (IL-10). IL-10 facilitates IgG4 antibody switch and activates IgG production. Several studies revealed increased levels of this interleukin in the serum and blister/bubble fluid of vesicular patients [7–9].

The frequency of *DRB1* and *DQB1* HLA alleles in patients with pemphigus has been investigated in numerous populations; however, no such studies have been conducted in the Russian population.

This study aimed to assess the prevalence of *DRB1* and *DQB1* HLA alleles at low- and high resolution levels in patients with different forms of pemphigus.

MATERIALS AND METHODS

Study design

A case-control study was conducted.

The primary endpoint was the detection of differences in HLA alleles of the *DRB1* and *DQB1* class II in pemphigus patients and healthy donors. No intermediate endpoints were determined.

Eligibility criteria

Inclusion criteria: Patients with a histologically and immunohistochemically confirmed diagnosis of pemphigus and an age of ⩾18 years.

Exclusion criteria: Patient refusal to participate in the study.

Settings

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Duration of the study

The study was conducted between 2016 and 2023.

Description of the medical intervention

All patients diagnosed with pemphigus received an initial dose of systemic glucocorticoids at 80–100 mg/day. Then, the dosage was gradually decreased according to clinical guidelines. Furthermore, venous blood was analyzed to determine HLA alleles by HLA typing.

Outcomes

The main outcome of the study was the absence of lethal outcomes or severe complications in patients with pemphigus. The primary outcome was the identification of differences in the frequency of *DRB1* and *DQB1* HLA class II alleles in patients with pemphigus and healthy donors through HLA typing at high and low resolutions.

Subgroup analysis

The pemphigus vulgaris group included 56 (65%) female patients and 30 (35%) males, whereas the pemphigus foliaceus group had 7 (53.85%) female patients and 6 (46.15%) males. All 6 (100%) patients in the paraneoplastic pemphigus group were females. Additionally, the control group of healthy donors consisted of 40 (43.47%) males and 52 (56.53%) females (Fig. 1). The mean age of the patients was >40 years. The bimodal age distribution of the pemphigus foliaceus group was 30–44 and 58–72 years. The paraneoplastic pemphigus group included older patients, with a mean age of 63.5 years (median age: 67 years). The median age of healthy donors was 45 years (Table 1).

Methods for recording outcomes

A blood sample was obtained from all patients for HLA typing of *DRB1* and *DQB1* alleles at low and high resolutions. The following *DRB1* HLA alleles were evaluated at the low-resolution level: *DRB1*4, DRB1*14, DRB1*13, DRB1*11,*

*DRB1*1, DRB1*7, DRB1*15, DRB1*3, DRB1*16*, and *DQB1*2*; and the following *DQB1* alleles were evaluated at the high resolution level: *DRB1*04: 02, DRB1*14:05, DRB1*13:01, DRB1*11:04, DRB1*14:04, DRB1*15:01, DRB1*04:03, DRB1*07:01, DRB1*01:02, DRB1*13:02, DRB1*04:04, DRB1*14:01, DRB1*03:01, DRB1*16: 01, DRB1*11:01, DRB1*01:01; DQB1*03:02, DQB1*05:03, DQB1*05:01, DQB1*03:01, DQB1*05:02, DQB1*02:01, DQB1*06:03, DQB1*06:04, DQB1*06:02*, and *DQB1*02:02*. Alleles were recorded using the QIAamp DNAMini Kit (Qiagen, Germany). HLA typing of *DRB1* and *DQB1* alleles was performed by extraction of 50 nanograms of DNA and polymerase chain reaction using specific primers (HISTOTYPE SSP Kits, BAG, Germany; AllSet+TM Gold SSP Typing Kits, Invitrogen Corp., Madison, Wi, USA). Polymerase chain reaction products were separated by electrophoresis on a 2% agarose gel and stained with ethidium bromide. The resulting images were then analyzed using the HISTO MATCH software (Germany).

Ethical review

The study was approved by the Ethical Committee of Sechenov University (protocol no. 03-22, dated February 3, 2022).

Statistical analysis

Modern universal nonparametric (reversal randomization) algorithms were used for determining confidence intervals (CI) and statistical comparisons based on bootstrap and Monte Carlo methods.

A compact recording form was employed for CIs, with the lower and upper interval limits indicated as subscripts to the left and right of the point estimate [10].

For statistical description of quantitative indicators, the mean and median values with 95% CI were estimated, and the agreement of the distribution with the normal law was evaluated. Additionally, the standard deviation and coefficient of variation around the mean value were calculated.

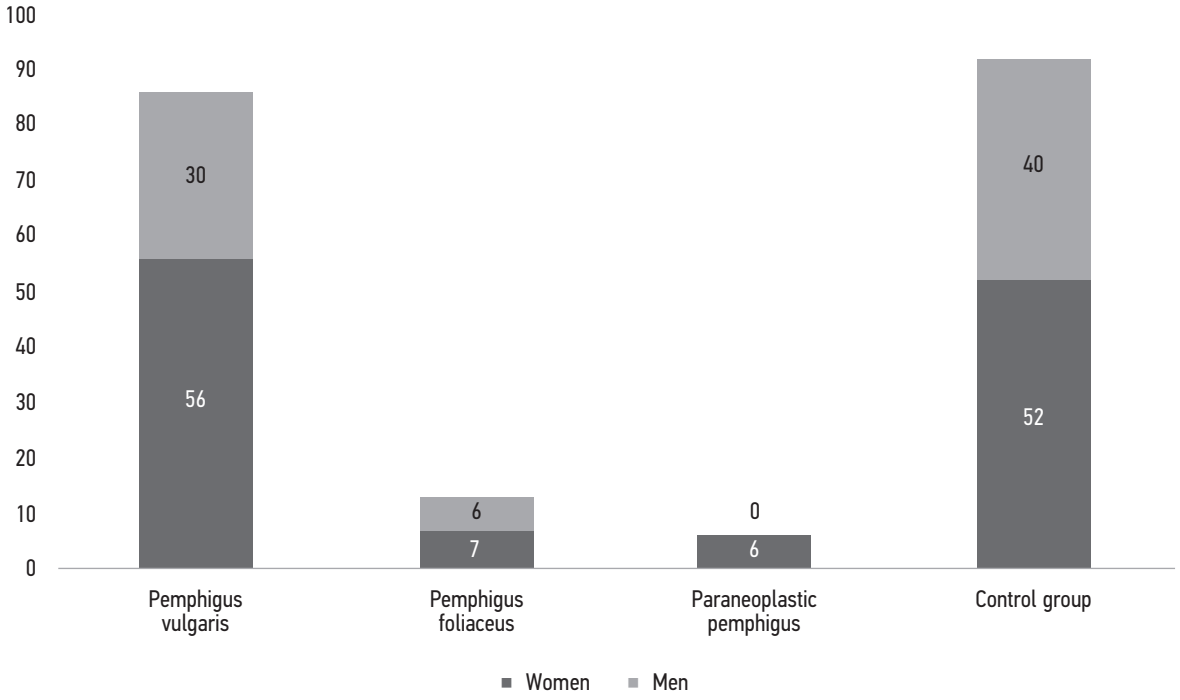

Fig. 1. Distribution of sick and healthy donors by sex, *n*.
Рис. 1. Распределение больных и здоровых доноров по полу, *n*.

Table 1. Main statistical indicators for age relative to diagnoses and control group
Таблица 1. Основные статистические показатели для возраста относительно диагнозов и контрольной группы

Diagnosis	Total	Min	Max	Mean	Me	MSD*
Pemphigus vulgaris	86	25	97	~53	53	~14
Pemphigus foliaceus	13	30	72	~49	44	~14
Paraneoplastic pemphigus	6	32	83	63,5	67	~19
Control group	92	35	75	45	-	-

Note. * Ско — standard deviation.
Примечание. * Ско — среднеквадратичное отклонение.

The Anderson–Darling, Lilliefors, and Jarque–Bera criteria, with Monte Carlo calculation of p-values for all criteria, were employed to assess the concordance between the observed distributions of each indicator and normal (Gaussian) distribution. The null hypothesis in each test of distribution for each indicator was that the distribution of the studied indicator agreed with the normal law. The following alternative hypothesis was generated: the distribution of the studied indicator differed from normal. For the indicators whose distribution in each group agreed with the normal distribution, the parametric criterion was applied to compare the groups. For the indicators whose distribution differed from normal, the nonparametric criterion was additionally calculated.

Two groups were quantitatively compared using two distinct statistical criteria: the parametric Student's t-criterion for independent samples and nonparametric Mann–Whitney U criterion. For the Student's t-criterion, the null hypothesis for each trait was that the mean values of the corresponding trait in the groups of sick and healthy donors did not differ. The alternative hypothesis was that the mean values were different. For the Mann–Whitney U criterion, the null hypothesis was that the distributions of the corresponding attribute — and median values — in the groups with the presence/absence of a certain categorical attribute did not differ. The alternative hypothesis was that the distributions of the attribute — and median values — were different. If the null hypothesis was rejected, it could be concluded that the quantitative factor may be related to the binary indicator (presence/absence of the trait). For the parametric criterion, the difference in mean with 95% CI was shown, whereas for the nonparametric criterion, the difference in Hodges–Lehman medians with 95% CI was revealed. A Cohen's or Hodges standardized effect of differences (for groups of <16) or a biserial correlation coefficient was calculated from the comparison of the two groups. The p-value was dependent on sample size; the standardized effect was not. The effect was interpreted according to the lower bound of the CI.

The four groups were compared by quantitative characteristics using one-factor analysis of variance (ANOVA). This was done using either the F-criterion or Welch test in the case of heterogeneity of dispersions in the group. The homogeneity of dispersions was assessed using the Levene test. The null hypothesis was that the mean values of the quantitative trait in different groups are equal. The alternative hypothesis was that the mean value differs at least in one group. Moreover, the effect of the differences in the second factor, ω^2, was analyzed using ANOVA.

RESULTS

Participants of the study

The study included 86 patients with pemphigus vulgaris, 13 with pemphigus foliaceus, and 6 with paraneoplastic pemphigus and 92 healthy donors.

Distribution of DRB1 HLA class II alleles at the low-resolution level in patients with different forms of pemphigus: In the pemphigus vulgaris group, *DRB1*4* (41.86%) and *DRB1*14* (24.4%) alleles were significantly more frequent at the low-resolution level compared to the control group (8.7% and 6.52%, respectively) (Table 2). Notably, *DRB1*11* (21.74%), *DRB1*16* (10.87%), and *DRB1*3* (8.70%) alleles were significantly more frequent in the control group than in the pemphigus vulgaris group (6.98, 1.16, and 1.16%, respectively). Additionally, the *DRB1*14* (46.15%) and *DRB1*4* (38.46%) alleles were significantly more frequently detected in patients with pemphigus foliaceus than in the control group (6.52% and 8.70%, respectively). In paraneoplastic pemphigus patients, as in the pemphigus vulgaris group, the *DRB1*4* (50%) allele was significantly more frequently detected than in the control group (8.70%; $p < 0.015$) (Table 2).

Distribution of DRB1 HLA class II alleles at the high resolution level in patients with different forms of pemphigus vulgaris: At the high resolution level, *DRB1*04:02* and *DRB1*14:05* alleles were significantly more frequent in patients with pemphigus vulgaris than in controls (34.88% vs. 5.43%, $p = 2{,}02\times10^{-6}$; 14.86% vs. 3.26%, $p < 0.007$, respectively). However, the odds of having *DRB1*11:04* allele in patients with pemphigus vulgaris were 3.13 (1/0.32) times lower than in controls (Table 3). Furthermore, *DRB1*04:02* and *DRB1*14:05* alleles were significantly more frequent in the pemphigus foliaceus group than in the control group (30.77% vs. 5.43%; $p < 0.01$; 23.08% vs. 3.26%; $p < 0.02$, respectively). The *DRB1*04:02* allele was significantly more frequent in paraneoplastic pemphigus patients than in healthy donors (50% vs. 5.43%, $p = 0.002$) (Table 3).

The distribution of DQB1 HLA class II alleles at the low-resolution level among patients with different forms of pemphigus: For all *DQB1* low-resolution HLA typing values in patients diagnosed with pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus, statistical significance was set at 5%. Based on the confidence interval and p-value calculated using the chi-square test, a statistically significant result could not be obtained (Table 4).

The distribution of DQB1 HLA class II alleles at the high resolution level among patients with different forms of pemphigus: *DQB1*03:02* and *DQB1*05:03* alleles were statistically significantly more frequently detected in patients with pemphigus vulgaris than in healthy donors (29.07% vs. 14.13%; 26.74% vs. 10.87%, respectively), whereas the *DQB1*03:01* allele was significantly more frequent in the control group than in the pemphigus vulgaris group (8.14% vs. 19.57%) (Table 5). The *DQB1*03:02* allele was 7.09 times significantly more frequent in patients with pemphigus foliaceus compared to controls (53.85% vs. 14.13%), whereas the *DQB1*05:03* allele was 8.2 times more frequent in patients with paraneoplastic pemphigus (50.00% vs. 10.87%) (Table 5).

Table 2. Distribution of *DRB1* low-resolution level HLA typing at a low-resolution level for patients with different forms of vesicular vesicles and controls, abs. (%)

Таблица 2. Распределение HLA-типирования *DRB1* low-resolution level на уровне низкого разрешения для пациентов с различными формами пузырчатки и контрольной группы, абс. (%)

DRB1	Patients	Control group	Odds ratio	Confidence interval	*p*
Pemphigus vulgaris					
4	36 (41.86)	8 (8.70)	7.56	3.25–17.55	7.35×10^{-7}
14	21 (24.42)	6 (6.52)	4.63	1.77–12.13	0.002
13	8 (9.30)	11 (11.96)	0.75	0.29–1.97	0.74
11	6 (6.98)	20 (21.74)	0.27	0.10–0.71	0.01
1	5 (5.82)	9 (9.78)	0.57	0.18–1.77	0.48
7	4 (4.65)	6 (6.52)	0.70	0.19–2.57	0.83
15	4 (4.65)	8 (8.70)	0.51	0.15–1.77	0.44
3	1 (1.16)	8 (8.70)	0.12	0.02–1.01	0.05
16	1 (1.16)	10 (10.87)	0.10	0.01–0.77	0.017
Pemphigus foliaceus					
14	6 (46.15)	6 (6.52)	12.29	3.12–48.30	0.0002
4	5 (38.46)	8 (8.70)	6.56	1.73–24.86	0.009
13	1 (7.69)	11 (11.96)	0.61	0.07–5.18	1
1	1 (7.69)	9 (9.78)	0.77	0.09–6.62	1
Paraneoplastic pemphigus					
4	3 (50.00)	8 (8.70)	10.5	1.81–60.85	0.015
14	2 (33.33)	6 (6.52)	7.17	1.08–47.36	0.12
13	1 (16.67)	11 (11.96)	1.47	0.16–13.80	1

DISCUSSION

Currently, there are approximately 145 studies on the prevalence of *DRB1* and *DQB1* HLA alleles in different populations. An increase in their frequency was found in Jewish, Turkish, Italian, Slovak, Tunisian, Japanese, Moroccan, Iranian, Indo-Asian, Indian, Vietnamese, Brazilian, Pakistani, Korean, Spanish, Argentinean, Syrian, German, English, Dutch, Mexican, and Egyptian populations [11–13]. Interestingly, according to these studies, some similarities and differences in alleles were detected. For example, in the Egyptian population, the *DRB1*08* HLA allele was found to be significantly more common at the low-resolution level. Additionally, in Germany, a higher frequency of the *DRB1*14:01:01/15:54* haplotype was observed in vesicular patients [4]. In a case-control study conducted among the Slovak population and a sample of 113 patients, a significantly higher frequency of the *DRB1*14:54* allele was observed in patients with pemphigus vulgaris than in controls [14]. Similarly, in the French population, the *DRB1*01:02* and *DRB1*04:06* alleles were found to be significantly more frequent in patients with pemphigus vulgaris and pemphigus foliaceus, respectively [15, 16]. Additionally, a study conducted in a Japanese population consisting of 525 patients with pemphigus vulgaris revealed that the *DRB1*04:06* allele was significantly more prevalent in this group [17].

Multiple large studies are underway to investigate the biology of HLA antigens, particularly all sorts of interactions between their molecules, thereby highlighting their possible key role in the development and progression of various autoimmune diseases. Interestingly, those multiple HLA alleles and their multiple molecules and receptors that bind different antigens could be used as therapeutic targets in the future. Antigen-presenting cells present over 10^{12} different proteins. The specific identification or recognition of HLA-peptide complexes is typically driven by αβ-T-cell receptors (αβ-TCRs). For example, a meta-analysis [18] showed that the mechanisms of interaction between HLA, peptides, and αβ-TCRs expressed by CD8+ T cells bound to HLA class I molecules, and CD4+ T cells specific for HLA class II molecules, may differ in various autoimmune diseases. An in-depth study of this interaction will reveal other key links in the pathogenesis of pemphigus and other autoimmune diseases based on association with HLA alleles.

Table 3. Distribution of *DRB1* high-resolution level HLA typing at a high-resolution level for patients with different forms of vesicular vesicles and controls, abs. (%)

Таблица 3. Распределение HLA-типирования *DRB1* high-resolution level на уровне высокого разрешения для пациентов с различными формами пузырчатки и контрольной группы, абс. (%)

DRB1	Patients	Control group	Odds ratio	Confidence interval	*p*
Pemphigus vulgaris					
DRB1*04:02	30 (34.88)	5 (5.43)	9.32	3.41–25.45	2.02×10^{-6}
DRB1*14:05	14 (14.86)	3 (3.26)	5.77	1.60–20.85	0.007
DRB1*13:01	6 (6.98)	11 (11.96)	0.55	0.19–1.57	0.38
DRB1*11:04	5 (5.81)	15 (16.30)	0.32	0.11–0.91	0.048
DRB1*14:04	5 (5.81)	0 (0)	-	-	-
DRB1*15:01	4 (4.65)	8 (8.70)	0.51	0.15–1.77	0.44
DRB1*04:03	4 (4.65)	0 (0)	-	-	-
DRB1*07:01	4 (4.65)	6 (6.52)	0.70	0.19–2.57	0.83
DRB1*01:02	4 (4.65)	4 (4.35)	1.07	0.26–4.43	1
DRB1*13:02	2 (2.33)	0 (0)	-	-	-
DRB1*04:04	2 (2.33)	3 (3.26)	0.71	0.12–4.33	1
DRB1*14:01	2 (2.33)	3 (3.26)	0.71	0.12–4.33	1
DRB1*03:01	1 (1.16)	8 (8.70)	0.12	0.015–1.01	0.051
DRB1*16:01	1 (1.16)	6 (6.52)	0.17	0.020–1.43	0.15
DRB1*11:01	1 (1.16)	5 (5.43)	0.20	0.023–1.79	0.24
DRB1*01:01	1 (1.16)	6 (6.52)	0.17	0.020–1.43	0.15
Pemphigus foliaceus					
DRB1*04:02	4 (30.77)	5 (5.43)	7.73	1.75–34.08	0.01
DRB1*14:04	3 (23.08)	0	-	-	-
DRB1*14:05	3 (23.08)	3 (3.26)	8.9	1.58–50.14	0.02
DRB1*13:01	1 (7.69)	11 (11.96)	0.57	0.07–4.76	0.94
DRB1*01:02	1 (7.69)	4 (4.35)	1.69	0.18–16.34	1
DRB1*04:03	1 (7.69)	0	-	-	-
Paraneoplastic pemphigus					
DRB1*04:02	3 (50.00)	5 (5.43)	17.4	2.78–109.20	0.002
DRB1*14:04	2 (33.33)	0 (0)	-	-	-
DRB1*13:01	1 (16.67)	11 (11.96)	1.47	0.16–13.80	1

According to our study, *DRB1*4* and *DRB1*14* HLA alleles were more frequently detected at the low-resolution level in patients with pemphigus vulgaris and pemphigus foliaceus, compared to healthy donors. These alleles could be considered potential genetic biomarkers for these conditions. In contrast, *HLA-DRB1*11*, *DRB1*16,* and *DRB*3* alleles were found to be more frequent in healthy donors and, therefore, may be protective against pemphigus in the Russian population.

At the high resolution level, the following distribution of *DRB1* HLA alleles was observed. The *DRB1*04:02* allele was found to be predisposing to all forms of pemphigus, including paraneoplastic pemphigus. The *DRB1*14:05* allele was significantly more frequent in patients with pemphigus vulgaris and pemphigus foliaceus. Conversely, the *DRB1*11:04* allele was protective, as it occurred 3.7 times more frequently in healthy donors.

At the low-resolution level, no significant difference was found for *DQB1* HLA alleles. However, at high resolution, the odds of having the *DQB1*03:02* allele were 7.09 times higher in patients with pemphigus foliaceus and 2.49 times higher for the pemphigus vulgaris group compared to the control group. Additionally, the *DQB1*05:03* allele

Table 4. Distribution of *DQB1* high-resolution level HLA typing at a low-resolution level for patients with different types of vesicular vesicles and controls, abs. (%)

Таблица 4. Распределение видов HLA-типирования *DQB1* high-resolution level на уровне низкого разрешения для пациентов с различными видами пузырчатки и контрольной группы, абс. (%)

DQB1	Patients	Control group	Odds ratio	Confidence interval	*p*
Pemphigus vulgaris					
5	39 (45.35)	31 (33.37)	1.63	0.89–2.99	0.15
3	33 (38.37)	36 (39.13)	0.97	0.53–1.77	1
6	8 (9.30)	14 (15.22)	0.57	0.23–1.44	0.33
2	6 (6.98)	9 (9.78)	0.69	0.24–2.03	0.69
Pemphigus foliaceus					
3	9 (23.08)	36 (39.13)	2.8	0.87–9.03	0.14
5	3 (69.23)	31 (33.37)	0.54	0.14–2.07	0.54
6	1 (7.69)	14 (15.22)	0.43	0.05–3.54	0.69
Paraneoplastic pemphigus					
5	4 (66.67)	31 (33.37)	3.94	0.68–22.68	0.23
3	2 (33.33)	36 (39.13)	0.78	0.14–4.47	1

Table 5. Distribution of *DQB1* high-resolution level HLA typing at a high-resolution level for patients diagnosed with vesicular vesicular vulgaris and controls, abs. (%)

Таблица 5. Распределение видов HLA-типирования *DQB1* high-resolution level на уровне высокого разрешения для пациентов с диагнозом вульгарной пузырчатки и контрольной группы, абс. (%)

DQB1	Patients	Control group	Odds ratio	Confidence interval	*p*
Pemphigus vulgaris					
DQB1*03:02	25 (29.07)	13 (14.13)	2.49	1.18–5.27	0.03
DQB1*05:03	23 (26.74)	10 (10.87)	2.99	1.33–6.74	0.01
DQB1*05:01	9 (10.47)	9 (9.78)	1.08	0.41–2.86	1
DQB1*03:01	7 (8.14)	18 (19.57)	0.36	0.14–0.92	0.048
DQB1*05:02	7 (8.14)	12 (13.04)	0.59	0.22–1.57	0.41
DQB1*02:01	3 (3.49)	4 (4.35)	0.80	0.17–3.66	1
DQB1*06:03	3 (3.49)	7 (7.60)	0.44	0.11–1.75	0.39
DQB1*06:04	3 (3.49)	7 (7.60)	0.44	0.11–1.75	0.39
DQB1*06:02	2 (2.33)	0 (0)	-	-	-
DQB1*02:02	2 (2.33)	0 (0)	-	-	-
Pemphigus foliaceus					
DQB1*03:02	7 (53.85)	13 (14.13)	7.09	2.06–24.46	0.002
DQB1*05:03	3 (23.08)	10 (10.87)	2.46	0.58–10.46	0.42
DQB1*03:03	1 (7.69)	5 (5.43)	1.45	0.16–13.49	1
DQB1*03:01	1 (7.69)	18 (19.57)	0.34	0.04–2.81	0.51
DQB1*06:03	1 (7.69)	7 (7.60)	1.01	0.11–8.96	1
Paraneoplastic pemphigus					
DQB1*05:03	3 (50.00)	10 (10.87)	8.2	1.45–46.24	0.034
DQB1*03:01	1 (16.67)	18 (19.57)	0.82	0.09–7.48	1
DQB1*05:01	1 (16.67)	9 (9.78)	1.84	0.19–17.58	1
DQB1*03:02	1 (16.67)	13 (14.13)	1.22	0.13–11.26	1

predisposes to pemphigus vulgaris and paraneoplastic pemphigus, whereas the *DQB1*03:01* allele is protective against the development of pemphigus, particularly pemphigus vulgaris.

Study limitations

Owing to the rarity of the dermatoses presented, a relatively small number of patients were enrolled in our study, which may have led to a systematic selection bias. Therefore, to avoid potential errors, it is recommended that multicenter studies with a larger sample size (≥1,000 patients) be conducted to confirm our findings. The case-control design of the study may also be considered a limitation.

CONCLUSIONS

DRB1 and *DQB1* HLA alleles characteristic of various forms of pemphigus were identified at both high- and low-resolution levels. Moreover, we detected protective alleles against pemphigus. However, further studies on a larger sample of patients are crucial to estimate the frequency of other HLA alleles, particularly those of both class I and II HLA, because of to the high variability of DR molecules.

ADDITIONAL INFORMATION

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Authors' contribution. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work. O.Y. Olisova — search and analytical work, observation of patients with pemphigus, approval of the direction of the manuscript for publication, critical analysis of the study; A.A. Lepekhova — writing an article, literature review, statistical processing, analysis of study groups; N.L. Shimanovsky — conducting HLA research methods in the laboratory (sequencing, polymerase chain reaction), critical analysis of the study; A.S. Dukhanin — conducting HLA research methods in the laboratory (sequencing, polymerase chain reaction), critical analysis of the study, interpretation and evaluation of the results; N.P. Teplyuk — critical analysis of the study, observation of patients with pemphigus, evaluation of the results obtained.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при подготовке рукописи.

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

Вклад авторов. Все авторы подтверждают соответствие своего авторства международным критериям ICMJE (все авторы внесли существенный вклад в разработку концепции, проведение поисково-аналитической работы и подготовку статьи, прочли и одобрили финальную версию перед публикацией). Наибольший вклад распределён следующим образом: О.Ю. Олисова — поисково-аналитическая работа, наблюдение за больными пузырчаткой, одобрение направления рукописи на публикацию, критический анализ исследования; А.А. Лепехова — написание статьи, обзор литературы, статистическая обработка, анализ групп исследования; Н.Л. Шимановский — проведение методик исследования HLA в лаборатории (секвенирование, полимеразная цепная реакция), критический анализ исследования; А.С. Духанин — проведение методик исследования HLA в лаборатории (секвенирование, полимеразная цепная реакция), критический анализ исследования, интерпретация и оценка полученных результатов; Н.П. Теплюк — критический анализ исследования, наблюдение за больными пузырчаткой, оценка полученных результатов.

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