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Research of HLA II Class DRB1, DQA1, DQB1 Genetic Markers in Patients with HIV Infection and AIDS

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Authors' contributions

This work was carried out in collaboration between all authors. Author JE designed the study, DNA purification from blood samples, HLA and DRB1 typing, wrote the protocol, and wrote the first draft of the manuscript. Author DK performed the HLA and DRB1 typing, managed the literature searches and wrote draft of the manuscript. Author VJ managed the literature searches, performed the statistical analysis. Authors GS and LV gathered and formed a group of patients. Author AS managed the analyses of the study. Authors JG and AK performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To find out the most frequent associations of the HLA II class loci DRB1*, DQA1*, DQB1* with the HIV/AIDS infection.
Place and Duration of Study: The study took place in The Laboratory of Clinical Immunology and Immunogenetics (LCII) of Riga Stradiņš University (RSU), Riga, Latvia, Riga Eastern Clinical University Hospital, "Infectology Centre of Latvia", between May 1991 and December 2004.

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Methodology: We analysed the medical documentation of 2500 patients and included 1180 (888 men, 292 women, 185 of them in AIDS phase) HIV infected patients. Genomic DNA was extracted from the blood with phenol-chloroform extraction method. Low-resolution HLA typing for HLA- DRB1*; DQB1*; DQA1* was performed by polymerise chain reaction (PCR) with amplification with sequence-specific primers (SSP). PCR products were separated on 3% agarose, the amplified bands were visualized, and the HLA-DRB1; DQA1; DQB1 type was deduced.

Results: Genetic markers of immunologic alleles upon development of HIV infection – HLA-DRB1*03(17:01); 05(11:01); 07:01; HLA-DQA1*01:01; 02:01; 03:01; 06:01; HLA-DQB1* 03:02; 05:01; 03:03; 03:04, as well as resistance markers connected with slow development of HIV infection – HLA-DRB1*01:01; 04:01; 06(13:01); HLA-DQA1*01:03; 04:01; 05:01; HLA-DQB1*03:01; 03:03; 04:01-2; 06:01; 06:02-8 are located in different groups of patients. High risk markers in case of HIV infection development belonging to the following groups of alleles: HLA-DRB1*03(17:01), DRB1*05(11:01), DQA1*01:01; 03:01 un DQB1*05:01; 03:02, as well as three-loci haplotypes HLA-DRB1*03(17:01)/ DQB1*05:01/ DQA1*01:01; HLA-DRB1*05(11:01)/DQB1*03:01/ DQA1 *05:01; DRB1*01:01/DQB1*03:02/ DQA1*03:01 and DRB1*01:01/DQB1*05:01/DQA1*01:01 are determined. Resistance to HIV infection development forms in the following groups of alleles: HLA-DRB1*01:01; 06(13:01), HLA-DQB1* 03:01; 06:02-8; HLA-DQA1*01:02; 01:03, as well as in haplotypes HLA- DRB1*01:01/DQB1*06:02-8/DQA1*01:02;HLA-DRB1*06(13:01)/DQB1*06:02-8/DQA1*01:02; HLA-DRB1*01:01/DQB1*03:01/DQA1*01:02; and HLA-DRB1*06(13:01)/DQB1*06:02-8/ DQA1*01:02 in different groups of HIV/AIDS patients.

Conclusion: The prevalence of genes DRB1; DQA1; DQB1 and DRB1-DQA-DQB1 combinations in the five groups of HIV infected patients have been established. Comparative analysis was performed also in the group of healthy donors (control group). The role of the main histocompatibility complex has been established, it enables marker functions and that can be used in the additional prognostic diagnostics in case of HIV infection. The obtained results testify that upon the identification of HIV genes it is possible to understand the molecular mechanisms in case of progression of AIDS syndrome complex; this possibly can be beneficial for the determination of the clinical results of infected patients.

Keywords: HIV; AIDS; DRB1; DQA1; DQB1.

1. INTRODUCTION

One of the greatest health problems of contemporary mankind – Acquired Immune Deficiency Syndrome (AIDS) – appeared at the end of the 20th century. AIDS is a disease engendered by multi factorial aetiology, a virus [1].

This virus is known as the Human Immunodeficiency Virus (HIV) that causes AIDS – a complex of syndromes that causes changes of immunity [1,2]. An acute phase of seizure that leads to a quick drop of viraemia which leads to a response of immunity to the virus infection is an important factor in the slow progression of HIV infection to the AIDS phase. During the maturation of immune competent cells, the identification marker CD4+ forms on the surface of T lymphocytes. It is only possible on the cells that have the II class (HLA II) antigens of the major histocompatibility complex – proteins on the plasma membrane [3].

This direction of human tissue compatibility complex became very topical after Nobel Prize winners Baruj Benacerraf (USA), Jean Dausset (France) and George D. Snell (USA), who won the prize in 1980 for discovering the genetically determined structure on the surface of tissue that regulates the immunologic reactions, acquired evidences for biogenetic human individuality and polymorphism [4,5]. Zinkernagel RM and Doherty PS. discovered that T lymphocytes recognize the immune antigen of viruses through the protein of the major tissue compatibility complex. These and other discoveries enabled them to draw conclusions that genetic differences in a locus that codes HLA protein can influence the intensity of immune response and efficient response of the host body to the infection by determining the result of interaction [6,7].

Different associated versions with the consequent incompatibility of HIV infection and these genes were obtained in the researches with different HLA genes [8-10]. This fact testifies that the information on the influence of genetic factors on the progress of disease, as well as on the limit of molecular genes of genetic polymorphism of HLA II class locus, on encoding process presentation antigen determinants T cells is not sufficient [11-14]. However, practical significance is insufficiently disclosed in the scientific literature so the comparative immunogenetics on HLA II class genes in the group of HIV infected patients is very topical.

The number of patients infected with HIV in Latvia increases year by year. According to the information of the AIDS Prevention Centre, the first case of HIV infection in Latvia was registered in 1987 (patient No.1), as to 31 December 2013 according to the information from the register of “State Health Agency” 5836 cases of HIV infection and 1335 cases of AIDS are registered in Latvia, 1066 persons have died. [1,2]

The major histocompatibility complex (MHC) HLA in the human body is to be regarded as the most complicated in the genetic system. This part includes immune response genes and determines the largest part of genetic dispositions (susceptibility) in cases of different diseases that are connected with the immune system [3,15,16]. This is the “starting point” in the development of practical immunogenetics (science about immunogenetic diversity and influence on immunity and nonspecific resistance of the body). Discovery of Polymerase Chain Reaction (PCR) [17,18] and the new HLA genotyping methods developed on the grounds thereof have facilitated the progress of polymorphism research of the human histocompatibility complex HLA system.

2. MATERIALS AND METHODS

2.1 Research Methodology, Selection Principles of the Total Patient Group and Exclusion Criteria

Totally as to 1 February 2013, 5836 HIV infected patients were registered in Latvia, 1335 of them are in AIDS phase (data from ambulatory cards of Riga Eastern Clinical University Hospital, Infectology Centre of Latvia). In the course of the work, medical documentation (hospital and ambulatory cards) of 2500 patients was analysed for the period from 1991 to 2004, and the following actions were performed: examination of patients – inquiry of epidemiological data, clinical – objective examination, primary and approving diagnostics of HIV diagnosis, as well as diagnosis of opportunistic diseases that is based on clinical, serological, bacteriological, radiological, morphological, functional diagnostics examinations. HLA II class DRB1*; DQA1* and DQB1* genes and their combinations (alleles, haplotypes and genotypes) were determined for all patients. Patients of both genders with different ways

of infection both in HIV and AIDS phases were included in the research. Examination of the patients was performed in dynamical periods – both clinical and laboratorial (complete blood count, HIV viral load test – twice a year, determination of number of lymphocytes in subpopulation – once in three months).

2.1.1 Criteria for inclusion of patients

1. HIV I infected patients – women and men aged 18 and older;
2. HIV infected patients in all phases of HIV infection (AI, AII, AIII, BI, BII, BIII, CI, CII, CIII);
3. HIV I infected patients with different ways of infection.

2.1.2 Criteria for exclusion of patients

1. Patients younger than 18 years of age;
2. Pregnant women;
3. Patients being in prison or pre-trial investigation isolator;
4. Patients who permanently stay or work abroad;
5. Patients who have undergone splenectomy or who use glycocorticosteroids;
6. Patients not being citizens or permanent residents of Latvia;
7. Non-compliant patients;
8. Patients older than 18 years, but who were infected by means of vertical transmission;
9. Patients with HIV 2 infection.

On the grounds of the criteria for inclusion and exclusion, having analysed the medical documentation of 2500 patients, data of 1180 sick persons were used in the present paper. All 1180 HIV positive patients included in the research are in the state agency of dynamic observation “Infectology Centre of Latvia” and have been familiarized with the document “Information for patients” and have signed the “Patient Agreement Statement”. 888 (75%) of 1180 patients included in the research are men and 292 (25%) are women. 185 patients who were included in the research were with HIV infection in AIDS phase. The average age of patients is 33.6 years (Table 1). HIV infection for all patients (100%) was approved with the primary test by determining antibodies against HIV and Western Blot tests.

Table 1. Demographical and clinical information on patients of total research group

Characteristic	Unit, form of presentation	Value n (%)
Included patients	HIV positive	1180 (100%)
Gender	Men	888 (75%)
	Women	292(25%)
Age	years, average (± SD)	33.6 (±13,4)

n=number of patients

2.2 Immunological Research Methods

2.2.1 Human DNA extraction from blood

The material belongs to the unique collection of the Joint Laboratory of Immunogenetic and Immunology Laboratory of Riga Stradiņš University and was used for DNA researches.

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) blood. Genomic DNA was extracted from the blood specimen with the phenol-chloroform extraction method.

2.2.2 HLA-DRB1; DQA1; DQB1 typing

HLA typing tests were performed in the Joint Laboratory of Immunogenetic and Immunology of Riga Stradiņš Clinical University and Riga Eastern Clinical University Hospital, Infectology Centre of Latvia. For control group, data of healthy donors (n=173) were used from database of the Joint Laboratory of Immunogenetic and Immunology.

Low-resolution HLA typing for HLA DRB1*; DQB1*; DQA1* was performed by PCR with amplification with sequence-specific primers (SSP). PCR products were separated on 3% agarose, the amplified bands were visualized, and the HLA DRB1; DQA1; DQB1 type was deduced.

2.3 Statistical Data Processing

Statistical analysis of data was performed by means of software: Microsoft Office Excel 2003 and DOS Stat Calc software [19].

Statistical analysis was performed on a computer in Microsoft Excel program. The results were considered as statistically credible if the correction of the Fisher's test with a small number of measurements was $P \leq 0.05$. Chi squared test (x^2) and gene incidence frequency (gf) were used to verify the hypothesis.

Odds ratio (OR) was calculated according to the formula (axd)/(bxc), where a – number of patients with the particular allele; b – number of patients not having the particular allele; c – number of healthy persons with the particular allele; d – number of healthy persons not having the particular allele. In case any of the values a, b, c or d is zero, odds ratio is calculated according to Haldane's modified formula that is anticipated for small groups of numbers [(2a+1)(2d+1)]/(2b+1)/ (2c+1)]. Statistical significance was determined according to Fisher's criteria. 95% credibility interval (95%CI) was determined according to the formula: 95%CI=ln OR±l, 96.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Research of HLA II class DRB1, DQA1, DQB1 genetic markers with HIV infected and AIDS patients

On the grounds of criteria for inclusion and exclusion, medical documentation of 2500 patients was analysed, data of 1180 patients is used in the present paper. All 1180 HIV positive patients included in the research are registered at the Riga Eastern Clinical University Hospital, Infectology Centre of Latvia and have been familiarized with the document "Information for Patients" and have signed the "Patient Agreement Statement" (Table 2).

Table 2. Characteristics of total research population and subgroups to be analysed

Characteristics	Unit of measurement, presentation mode	Value n (%)
Total HIV/AIDS group	all HIV infected patients	1180
AIDS group	infected patients in AIDS phase	(100%) 185 (16%)
Heterosexuals (Hetero/sex.)	patients who have been infected as result of heterosexual relations	577 (49%)
Homosexuals (Homo/sex.)	patients who have been infected as result of homosexual relations	59 (5%)
Intravenous drug users (IVDU group)	patients who have been infected using shared syringes and needles, intravenously injecting the drugs and psychotropic substances	544 (46%)
Control group	healthy blood donors (residents of Latvia)	173 (100%)
(Control group) Gender	Men	137 (79%)
	Women	36 (21%)

n=number of patients

To obtain new data about the connection between HLA II class genes and patients infected with HIV virus, coherencies in HIV/AIDS cases were searched between the immunogenetic risk markers and the protective markers in HLA II class loci DR and DQ.

To determine the possible morbidity risk rate (OR), the presence or nonexistence of gene genotype of the particular person is compared to the infected patients and control group. Positive OR associations are such where OR is equal or more than 1.0. Those in which OR is less than 1.0 link with the protective gene. The results were considered as statistically credible if the correction of Fisher's test with a small number of measurements was $P \leq 0.05$. Chi quadrate test (x^2) and gene incidence frequency (gf) were used to verify the hypothesis.

3.1.1.1 Analysis of gene polymorphism in the locus HLA-DRB1 in different HIV infected patient groups

The research of the HLA II class genes was begun with the analysis of polymorphism HLA alleles, genotype and haplotype for HIV infected patients and the control group.

The characteristic specificity in the total group of HLA II class locus DRB1 where all the HIV/AIDS infected patients were included was researched in the initial stage. In the analysed examples, 14 versions of alleles of the gene DRB1* were determined, in which different degrees of impact (both positive and negative) were discovered. Results are shown in (Table 3).

When looking for a connection between the associative HLA II class DRB1* genes, it is possible to draw a conclusion on the diversity of risk genes. The genes DRB1*03 (17:01), DRB1*07:01 are more frequent in the HIV infected patient group. These genes were encountered also in other groups – in AIDS and Intravenous Drug Users (IVDU) HIV infected groups. In the group of homosexuals the gene DRB1*03(17:01) had no significant result. The gene DRB1*07:01 did not appear in the group of homosexuals. Incidence frequency of the gene DRB1* 05(11:01) is larger in the DRB1*03(17:01) alleles in the group of heterosexuals.

HLA-DRB1*06 (13:01) should be stated as the protective allele in all researchable groups. DRB1* 01:01; DRB1*04:01; DRB1*06(13:01) was also stated in the group of heterosexuals, DRB1*04:01; DRB1*06(13:01) – in the group of homosexuals, DRB1*05(12:01), DRB1*06(13:01) – in IVDU group (Table 3). Incidence of other genes DRB1* among the sick and healthy individuals had little difference or had no statistically significant result.

As to genotypes of locus HLA-DRB1*, the obtained results were divided into five groups and some coincidences were established. Risk genotype usually consists of open genes, as well as risk genes over and over again prove the degree of influence of different genes on genetic predisposition of development of different clinical versions in the process of disease procedure. Existence of protective genes influences resistance of particular individuals in the respective pathological processes.

Risk alleles DRB1*03(17:01); DRB1*05(11:01); DRB1*07:01 are practically proved in all surveyed groups. Greater risk degree exists for the patients infected with the HIV virus. The genotypes DRB1*15:01/03(17:01); DRB1*15:01/05(11:01), DRB1 07:01/07:01 showed high risk in progression of the virus (Table 3).

The protective alleles DRB1*04:01, 06(13:01), in the total genotype group 01:01, 15:01 decelerate the process of infection.

It can be concluded that the genotypes DRB1*15:01/03(17:01); DRB1*15:01/05(11:01), DRB1 07:01/07:01 are genetic markers with an increased risk of chronic infection process appearance. The allele genotype DRB1*01:01; DRB1*06(13:01) are related to the disease by causing minimum risk and it results in lessening the process of infection, and also causes no complications after the disease.

It is necessary to research the mutual coherence mechanism of alleles, and also the combinations of haplotypes and genotypes (Table 3).

3.1.1.2 Analysis of gene polymorphism in the locus HLA-DQA1 in different HIV infected patient groups

Further were identified 8 versions of alleles of the gene DQA1 in HIV infected patients (Table 4).

When analysing gene polymorphism in the genes of locus DQA1* within different groups of HIV infected patients, risk associations were established in particular with HLA-DQA1*01:01; 06:01; 02:01; 03:01 and protective associations with DQA1*01:03; 04:01; 05:01 (Table 4).

Incidence of other genes HLA-DQA1* has little or unimportant difference between the sick and healthy individuals.

As to the genotype of locus HLA-DQA1* (Table 4), the obtained results are divided into five groups and point at some coincidences. Existence of the protective genes in the genotype influences the resistance of respective individuals against the respective pathological processes. The mechanisms interconnect the alleles, but further research is required for the determination of the combinations of genotypes (Table 4).

3.1.1.3 Analysis of gene polymorphism in the locus HLA-DQB1 in different HIV infected patient groups

Further 10 versions of alleles of gene DQA1 are determined for the HIV infected patients by the means of the selection to be analysed (Table 5).

When analysing gene polymorphism in the genes of locus DQB1* within different groups of HIV infected patients, positive association was obtained with specificity HLA-DQB1*03:02; 03:04. The same genes were established in the particular groups to be researched: AIDS, heterosexuals and IVDU infected patients. In the group of HIV infected homosexual patients no significant results were obtained, but the gene HLA-DQB1*03:02 was not established in the group of HIV infected heterosexual patients. Apart from the allele HLA-DQB1*03:04, reliably more frequent incidence of the gene HLA-DQB1* 03:03 was discovered in the group of HIV infected heterosexual patients. Protective associated alleles are DQB1*03:01;04:01-2;06:02-8. Frequency of other HLA-DQB1* genes slightly differs between the sick and healthy individuals or the difference is not significant (Table 5).

As to the genotypes in locus HLA-DQB1 (Table 5), some coincidences of the obtained results are established within the five groups. The discovered genes constitute mainly both risk genotype and risk genes; this proves over and over again the particular influence of different genes in the process of infectious disease progress. The existence of the protective genes influences the resistance of the particular individuals during the respective pathological processes (Table 5). It is necessary to research the interconnection mechanism of alleles, and also the combinations of haplotypes and genotypes.

3.1.2 Analysis of gene combinations (haplotypes) in different groups of HIV/AIDS infected patients

The next immunogenetic research shall be performed to find out the possible associations between HIV/AIDS development risk and the particular HLA II class gene genotypes – DRB1*/DQA1*/DQB1*. For this purpose, an analysis was made to compare frequency of HLA haplotype incidence for HIV infected patients in different groups and in the control group (healthy residents of Latvia) (Table 6).

Thus research of association coherence between particular gene combination HLA II class DRB1*, DQA1*, DQB1* and HIV determined that high risk immunogenetic markers that develop AIDS-related syndrome complex, is located in the allele groups DRB1*03(17:01), DRB1*07:01 and DRB1*05(11:01), with specificity DQB1*05:01, and DQA1*01:01 as well as in three-loci haplotypes HLA-DRB1*01:01/DQB1*05:01/DQA1*01:01; *15:01/03:02/01:02; *15:01/03:02/03:01; *15:01/05:01/01:01; *03(17:01)/05:01/01:01 and 05(11:01)/03:01/05:01.

Resistance against the AIDS-related syndrome complex is determined for the phenotype in the allele group HLA-DRB1*06(13:01), with specificity HLA-DQA1*01:02 and haplotypical combination DRB1*01:01/DQB1*06:02-8/DQA1*01:02 and DRB1*01:01/DQB1*06:02-8/DQA1* 01:03; DRB1*06(13:01)/DQB1*06:02-8/DQA1*01:02 (Table 6).

Table 3. Incidence frequency in HLA II class locus HLA-DRB1* in different clinical groups for HIV infected patients

HLA-DRB1	Total HIV/AIDS group (OR/P)	AIDS group (OR/P)	Hetero/sex. group (OR/P)	Homo/sex. group (OR/P)	IVDU group (OR/P)	Control group (Gf)
Immunogenetic risk markers (predisposition markers)						
Alleles	03(17:01) (2.18/0.0001)	03(17:01) (1.93/0.007)	03(17:01) (3.35/0.0001)	07:01 (2.65/0.006)	03(17:01) (2.48/0.0001)	0.08
	07:01 (4.22/0.0001)	07:01 (4.22/0.002)	05(11:01) (1.82/0.001)		07:01 (6.04/0.0001)	0.20 0.05
Genotypes	01:01/07:01 (1.07/0.868)	15:01/03(17:01) (1.18/0.755)	05(11:)/05(11:01) (1.34/0.034)	01:01/05(11:01) (1.86/0.053)	01:01/03(17:01) (2.19/0.037)	0.01 0.04
	15:01/03(17:01) (1.36/0.516)	15:01/05(11:01) (1.34/0.270)	05(11;01)/07:01 (1.86/0.039)	15:01/03(17:01) (3.58/0.036)	01:01/07:01 (2.48/0.014)	0.01 0.01
	03(17:01)/06(14:01) (2.69/0.116)	07:01/07:01 (2.26/0.002)	07:01/07:01 (2.26/0.002)	15:01/05(11:01) (2.12/0.032)	15:01/03(17:01) (2.89/0.020)	0.12 0.02
					03(17:01)/06(14:01) (6.10/0.004)	0.001 0.03
Protective markers (resistance markers)						
Alleles	01:01 (0.55/0.001)	06(13:01) (0.61/0.036)	01:01 (0.37/0.0001)	04:01 (0.67/0.297)	04:01 (0.59/0.011)	0.16 0.12
	06(13:01) (0.30/0.0001)		04(0.52/0.001)	06(13:01) (0.70/0.301)	06(13:01) (0.34/0.0001)	0.16
			06(13:01) (0.33/0.0001)			
Genotypes	01:01/15:01 (0.38/0.0001)	01:01/04:01 (0.41/0.221)	06(13:01)/06(13:01) (0.65/0.010)	01:01/06(13:01) (0.318/0.005)	05(12:)/06(13:01) (0.22/0.0001)	0.03 0.02
	01:01/04:01 (0.34/0.001)	06(13:01)/06(13:01) (0.54/0.048)		15:01/04:01 (0.36/0.044)	06(13:01)/06(13:01) (0.60/0.001)	0.03 0.02
	01:01/06(13:01) (0.48/0.005)					0.09 0.05

The numbers in brackets have been indicated in a successive order OR/ P<0.05; OR=odds ratio; Gf= incidence of allele

Table 4. Incidence frequency in HLA II class locus HLA-DQA1* in different clinical groups for HIV infected patients

HLA-DQA1	Total HIV/AIDS group (OR/P)	AIDS group (OR/P)	Hetero/sex. group (OR/P)	Homo/sex. group (OR/P)	IVDU group (OR/P)	Control group (Gf)
Immunogenetic risk markers (predisposition markers)						
Alleles	01:01		01:01	03:01	06:01	0.15
	(1.78/0.0001)		(1.37/0.051)	(1.49/0.156)	(2.53/0.018)	0.12
	02:01					0.13
	(1.42/0.042)					0.02
	03:01					
	(1.70/0.001)					
Genotypes	01:01/05:01	01:01/05:01	01:01/05:01	01:01/03:01	01:01/05:01	0.02
	(2.85/0.004)	(1.18/0.755)	(5.29/0.0001)	(9.37/0.001)	(4.46/0.0001)	0.01
	01:02/03:01	01:02/03:01	01:02/03:01	01:02/03:01	01:02/03:01	0.02
	(4.23/0.001)	(1.34/0.270)	(3.60/0.003)	(3.37/0.005)	(3.30/0.0001)	0.003
				02:01/05:01	01:03/03:01	0.02
				(2.70/0.042)	(4.05/0.044)	
Protective markers (resistance markers)						
Alleles	04:01	04:01	04:01	04:01	05:01	0.05
	(0.43/0.002)	(0.37/0.025)	(0.62/0.113)	(0.68/0.492)	(0.74/0.038)	0.24
Genotypes	06:01/06:01	01:01/04:01		01:03/01:03	01:02/01:03	0.05
	(0.24/0.050)	(0.41/0.221)		(0.76/0.557)	(0.25/0.083)	0.01
		01:02/04:01			01:03/01:03	0.01
		(0.54/0.048)			(0.46/0.001)	0.01
					06:01/06:01	0.01
					(0.39/0.051)	

The numbers in brackets have been indicated in a successive order OR/ P<0.05; OR=odds ratio; Gf= incidence of allele

Table 5. Incidence frequency in HLA II class locus HLA-DQB1* in different clinical groups for HIV infected patients

HLA-DQB1	Total HIV/AIDS group (OR/P)	AIDS group (OR/P)	Hetero/sex. group (OR/P)	Homo/sex. group (OR/P)	IVDU group (OR/P)	Control group (Gf)
Immunogenetic risk markers (predisposition markers)						
Alleles	03:02 (4.99/0.0001)	03:02 (1.90/0.036)	03:03 (1.71/0.040)	03:02 (1.60/0.270)	03:02 (2.20/0.001)	0.05
	05:01 (2.67/0.0001)	03:04 (10.35/0.001)	03:04 (9.93/0.001)	05:01 (1.31/0.340)	03:04 (6.39/0.001)	0.05 0.01 0.14
Genotypes	03:01/03:02 (3.51/0.003)	03:01/03:02 (4.97/0.002)	03:02/06:02-8 (3.17/0.025)	02:01-2/03:01 (2.12/0.032)	02:01-2/05:01 (2.03/0.002)	0.03
	03:01/05:02-4 (2.81/0.014)	03:04/03:04 (11.90/0.001)	03:04/03:04 (6.90/0.002)	03:01/06:02-8 (1.83/0.050)	03:01/03:02 (4.08/0.0005)	0.04 0.01 0.01
	03:02/05:01 (6.84/0.002)			04:01-/06:02-8 (5.95/0.020)	03:01/05:02-4 (2.86/0.013)	0.02 0.003
	03:02/06:02-8 (3.0/0.031)				03:02/03:02 (2.32/0.002)	0.01 0.003
					03:02/05:01 (8.12/0.001)	0.003
Protective markers (resistance markers)						
Alleles	03:01 (0.39/0.0001)	06:02-8 (0.58/0.005)	03:01 (0.71/0.020)	06:01 (0.36/0.330)	06:02-8 (0.58/0.001)	0.22
	04:01-2 (0.43/0.003)		06:02-8 (0.68/0.010)	06:02-8 (0.76/0.310)		0.05 0.05 0.23
	06:01 (0.21/0.001)					
	06:02-8 (0.50/0.0001)					
Genotypes	03:02/04:01-2 (0.13/0.008)	03:01/06:02-8 (0.44/0.017)	02:01-2/05:02-4 (0.38/0.029)		03:02/04:01-2 (0.17/0.019)	0.02 0.09
	06:01/06:01 (0.29/0.001)	06:01/06:01 (0.18/0.042)	03:01/06:02-8 (0.72/0.059)		03:03/06:02-8 (0.29/0.002)	0.01 0.02
	06:02-8/06:02-8 (0.67/0.003)				04:01-2/04:01-2 (0.50/0.042)	0.02 0.02
					06:01/06:01 (0.25/0.0005)	0.14
					06:02-8/06:02-8 (0.65/0.003)	

The numbers in brackets have been indicated in a successive order OR/ P<0.05; OR=odds ratio; Gf= incidence of allele

Table 6. Incidence frequency in HLA II class locus in different clinical groups for HIV infected patients

HLA DQB1/DQB1 /DQA1	Total HIV/AIDS group (OR/P)	AIDS group (OR/P)	Hetero/sex. group (OR/P)	Homo/sex. group (OR/P)	IVDU group (OR/P)	Control group (Gf)
Immunogenetic risk haplotypes (predisposition haplotypes)						
01:01/03(17:01)/03:02/03:01	6.17/0.027				9.52/0.007	0.07
01:01/05:01/01:01		2.35/0.009		2.41/0.032		0.12
15:01/03:02/01:02	8.34/0.013				11.04/0.003	0.07
15:01/03:02/03:01	8.34/0.013				10.53/0.004	0.07
15:01/05:01/01:01		3.49/0.039		5.32/0.013		0.36
03(17:01)/05:01/01:01	2.66/0.032				3.11/0.013	0.36
05(11:01)/03:01/05:01		2.03/0.002	1.68/0.035			0.02
Protective haplotypes (resistance haplotypes)						
01:01/03:01/01:02	0.44/0.054		0.11/0.009			0.65
01:01/06:02-8/01:02	0.27/0.008				0.22/0.008	0.65
01:01/06:02-8/01:03	0.31/0.030				0.14/0.008	0.51
06(13:01)/06:02-8/01:02	0.24/0.0001		0.33/0.005		0.17/0.0001	0.02

The numbers in brackets have been indicated in a successive order OR/ P<0.05; OR=odds ratio; Gf=incidence of allele

3.2 Discussion

The body's immune response to viral agents is genetically controlled and is dependent on the major histocompatibility complex – MHC, HLA – in humans. The most active genes of the immune response are HLA II class loci DRB1; DQA1; DQB1. Due to their function, the HLA gene products provide intercellular connection and participate in the realization of pathological processes. By their nature, HLA molecules are not only immunogenetic markers of various diseases, but also the dominant chain of the pathogenic mechanisms of immune response [3,12,13].

Earlier studies show various differences in the distribution of HLA DRB1; DQA1; DQB1 genes. Statistically valid data has been received on the increased amount of HLA-DRB1*05(11:01/12:01); DQA1* 04:01; DQB1*03:01; 03:02 genes in humans infected with tick-borne encephalitis [19]. Patients with the following haplotypes: HLA DRB1*05(11:01/12:01)/DQA1*03:01, HLA-DQB1*03:02/DQA1*03:01, DRB1*03 (17:01)/DQB1*05:01, HLA-DRB1*04:01/DQB1*03:02 have genetically determined predisposition to tick-borne encephalitis. Patients with the following haplotypes: DRB1*03(17:01)/DQB1*03:01/DQA1*01:02 have genetically determined predisposition to severe course of tick-borne encephalitis, but patients with these haplotypes: DRB1*01:01/DQB1*03:03/DQA1*02:01 have a mild course of the disease [20]. It has been found that the formation mechanism of the viral infection that causes liver diseases is controlled genetically with the help of the immune system and is coded with certain genes of the HLA complex. In addition, individual HLA gene complex can not only show that a person is at increased risk of becoming infected with hepatitis, but it can also develop a chronic form, to show how fast the disease will develop possible complications and consequences. On the other hand, if a patient has a chronic HCV infection, scientists have observed a connection of certain HLA alleles with other parameters – a milder course of infection, lower HCV load and predictively effective IFN therapy [21]. It should be noted that the increased correlation of the HLA II class genes was observed in hepatitis C studies. Increased incidence has been found for the following genes: DRB1*05(11:01;12:01); DQB1*03:02; DQA1*01:02; 05:01. The genes HLA DRB1*01:01; DQB1*05:01; DQA1*01:01 were found in people with a milder course of disease, followed by a quick recovery. The correlation with a high probability between the genes of the HLA II class and the progress of the HCV infection in haemophiliacs that was found in the study is likely to be the determining factor of the progress of chronic hepatitis C [21-24].

The data obtained coincided with the conclusions of foreign authors, which stated that haplotypes A*03-B*07-DRB1*15-DQB1*0602 and A*02-B*27-Cw*01-DRB1*0101-DQB1*0501 are connected with the clearance of the virus [25]. Singh R et al. carried out a comparison of hepatitis B and C HLA of the world's population and proved that HLA-DRB1*06(13:01) is connected to a natural release of hepatitis B, while HLA-DRB1* 05(11:01;12:01) and DQB1*03:01 are connected to chronic hepatitis B [26,27]. The research results of HLA I and II classes show that they can influence susceptibility and development of a fulminant disease. For example, HLA–B35 phenotype occurs more often in HIV-infected persons than in other persons. There is evidence that the same phenotype which is the major risk factor for infection is in addicts who are infected with the HIV virus and who inject the drugs intravenously [28,29].

According to the aforementioned information, it can be logically concluded that the HIV infection, which is based on the suppression of the immune system, can be associated with HLA genes, which may be the markers of the susceptibility or independent development of

the AIDS-related syndrome complex, as well as the markers of the progress of the disease (form, latency period, complications, etc.). After being infected with HIV, bodies of individuals react in various ways. Monitoring of 2500 HIV-infected patients conducted in Latvia showed that about 10% of individuals after infection with HIV become sick with AIDS in the first 2-3 years (rapid development), for 5-10% of HIV-infected during 7-10 years have no clinical symptoms and have stable CD4+ cell levels in peripheral blood (do not develop AIDS), and finally there are those HIV-infected patients who become ill with AIDS 10 years after becoming infected with the HIV virus (the typical morbidity). Statistical data shows that 20 years after infection, AIDS does not develop in 10-17% of HIV-infected patients [30,31].

AIDS is a complex, multifactorial disease with a complex pathogenesis, which is largely based on a genetic component. The genes of the HLA II class include 50% of the total genetic risk in AIDS patients. The largest association with AIDS is registered with the alleles of HLA-DRB1*03(17:01;18:01), DRB1*07:01; DRB1*05 (11:01;12:01), DQA1*02:01; DQA1*03:01; DQB1*05:01, their incidence in the study group is about 80%. At heterozygous (DRB1*03(17:01)/DRB1*07:01) and homozygous (DRB1*11:01/12:01 and DRB1* 17:01/18:01) the specific genes of the combination create a great risk of developing the infection process [32,33].

In this paper, the analysis of the distribution of genes has been carried out for HLA II class loci DRB1, DQA1, DQB1 and their combinations with different groups of HIV-infected people, by using PCR genotyping and sequencing methods. The genes presented in the analyses HLA-DRB1*01:01;*06(13:01);*04:01;*08:01; DQA1*01:03;*04:01;*05:01; DQB1*03:01;*06:02-8 appeared to be the markers for fighting the infection [34]. The variants of genes DRB1*03(17:01;18:01); *05(11:01;12:01); *07:01; DQA1*01:01;*02:01; *03:01;*06:01; DQB1*03:02; *03:04;*05:01; *03:03 at the same time are the risk markers for HIV. The correlations of haplotypes: HLA-DRB1*/DQB1*/DQA1 *03(17:01)/*05:01/*01:01; *05(11:01)/*03:01/*05:01; *01:01/*03:02/*03:01 are considered to be the risk haplotypes, HLA-DRB1*/DQB1*/DQA1*01:01/*03:01/*01:02; *01:01/*06:02–8/*01:02; *01:01/*06:02–8/*01:03; *06/*06:02–8/*01:02 are considered to be the protective haplotypes.

The formation of the gene haplotypes of the HLA II class, which are susceptible to HIV/AIDS, and also prevent the disease, can be seen in Fig. 1.

It can be concluded from the results that the development of HIV infection in an individual haplotype is formed by the largest number of markers and not from the missing risk markers, which also create a favourable impact on the development of HIV infection. When comparing the acquired data with the literature data, it was found that haplotypes of the protective genes of the HLA are associated with a severe infection process.

The study showed that the presence of only one of the protective alleles (DRB1*01:01;*06(13:01;14:01); DQA1*01:03; 04:01; 05:01 and DQB1*03:01, DQB1*06:02-8) is enough in order for a haplotype to be protective. At the same time, the maximum predisposition occurs when the haplotype is composed of three susceptible genes of the HLA alleles – DRB1/DQB1/DQA1: *15:01/03:02/01:02 (OR = 8,34, p=0,013), *01:01/03 (17:01)/03:02/03:01(OR = 9.52, p=0.007) and *05(11:01)/03:01/05:01 (OR = 2.03, p=0.002).

By knowing certain HLA haplotypes, it is possible to predict the evolution of HIV infection.

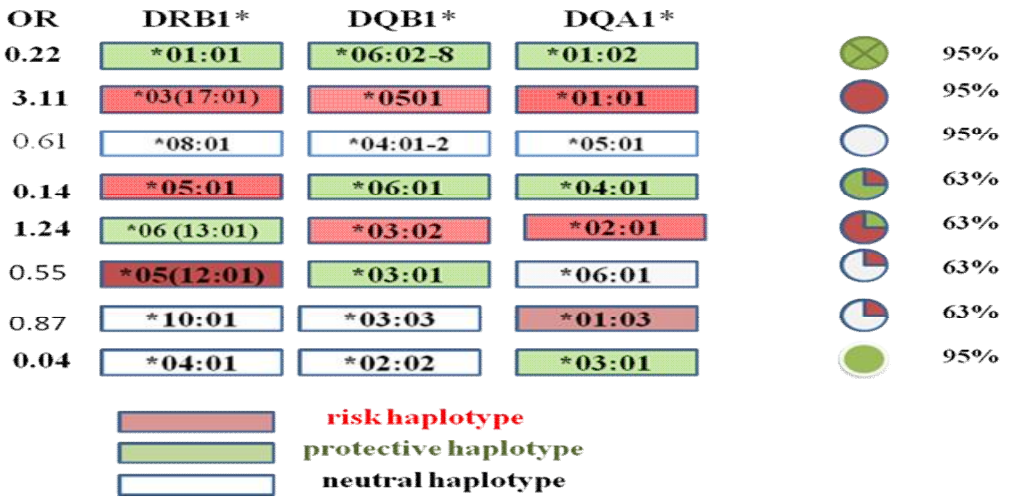

Fig. 1. Theory of haplotype formation

HIV /AIDS-infected patient groups – the analyses of genotype and haplotype suggest that the same variants of genes of the HLA alleles are associated with HIV infection, and therefore it can be concluded that the DRB1, DQA1, DQB1 variations of the genetic markers in certain circumstances determine the susceptibility in the development of the immune response (e.g., genotype/haplotype three options can become pathological)

Thus, it can be assumed that in the development of the disease the main predisposition is formed by the gene variants HLA DRB1, DQA1, DQB1 of alleles [35,36]. However, many studies give conflicting results on the association between HLA genes and various infectious diseases [37,38]. It is considered that the current difference is not only dependent on the allele specificity, but also from the molecular structure of its own immune response gene (HLA). The structure of the HLA gene already includes impact of the specific mechanism. Thanks to the development of the molecular genetics and immunology, the opportunity not only to study in detail the analyses of the HLA antigen, but also to explore the molecular structures of HLA genes has occurred.

The researched loci HLA DRB1, DQA1, DQB1 also showed alleles that are associated with a rapid development of the disease (DRB1*05(11:01);*03(17:01);*15:01; DQA1*01:01; *02:01; *03:01;*06:01; DQB1*03:02; *05:01; *03:03), as well as with a slower development of the disease (DRB1*01:01; 06(13:01); 04:01; DQA1*01:03; 04:01; 05:01; DQB1*03:01; 06:02-8). However, the combination of these alleles can significantly slow down or speed up the development of AIDS. It has been mentioned in the literature that any combination of alleles that are heterozygous, in the HLA I – A, B, C or HLA II – DQ, DR loci might significantly slow down the development of AIDS, compared to the homozygous allele at one or more loci [39].

The correlation between HIV virus and RNA number of copies in plasma (HIV viral load), CD4 + lymphocyte subpopulations in peripheral blood and HLA II class haplotype in HIV/ AIDS patients has been analysed. It was found that HLA 01:01/03:02/03:01; 01:01/05:01/01:01; 15:01/03:02/01:02; 15:01/03:02/03:01; 15:01/05:01/01:01; 03/05:01/01:01; 05/03:01/05:01 haplotypes are associated with a rapid development of infection. It has been observed that the acute phase for the patients with these haplotypes lasts up to 12 weeks, and the latency period does not exceed 3 to 5 years. For the infected individuals with haplotypes 01:01/03:01/01:02; 06/06:02-8/01:02, 01:01/06:02-8/01:02;

01:01/06:02-8/01:03 the acute phase is short and the latency period lasts 10 or more years. During the analysis that was performed in an elevated risk group of HIV/AIDS patients, it was concluded that the latency time decreased, however, in comparison to the resistance group of the HIV/AIDS patients there was a slight prolongation of the acute stage. Thus, these studies confirm the correlation between HIV virus and RNA number of copies in plasma (HIV viral load), CD4+ lymphocyte subpopulations in peripheral blood and HLA II class haplotype in HIV/ AIDS patients. The results of the analyses which show that the reduction in viraemia correlates with a higher CD4+ cell amount are in line with the literature data. The correlations of the study between certain associated gene markers of the HLA II show a direct correlation between haplotypes with CD4+ cell dynamics and HIV RNA levels in plasma in HIV/AIDS-infected patients. Thus, haplotypes of the of the HLA II class genes can also be used as an additional predictive criteria which, unlike CD4 + cells and HIV RNA levels, remain the same throughout life and are not dynamic [39].

The results of the analyses that have been made in the framework of this study partially confirmed the hypothesis published in the scientific literature about the possible effects of the specific HLA II class haplotypes on the body's ability to resist HIV infection. The alleles of genes HLA-DRB1*01:01; 04:01; 06; HLA-DQA1*01:03; 04:01; 05:01; HLA-DQB1*03:01; 03:03; 04:01-2; 06:01; 06"02-8 are considered to be "protective" against HIV infection. These alleles provide a more effective presentation of the HIV epitope to CD4+ T lymphocytes. As a result, the body's immune system fights the HIV infection more effectively. Epitopes are antigenic determinants – certain HIV areas that are chemically detected by antibodies.

With the determination of the HLA II class haplotypes, successful therapy can be achieved only partially – the results can be considered in combination with other successful solutions. In the future, it might be possible to introduce a mandatory identification of the HLA types prior to prescribing various types of therapy.

4. CONCLUSION

Incidence frequency of genes DRB1; DQA1; DQB1 and DRB1-DQA-DQB1combinations in five groups of HIV infected patients is clarified. Comparative analysis was performed also in the group of healthy donors (control group).

The genetic markers of immunologic alleles upon development of HIV infection – HLA-DRB1*03(17:01); 05(11:01); 07:01; HLA-DQA1*01:01; 02:01; 03:01; 06:01; HLA-DQB1* 03:02; 05:01; 03:03; 03:04, as well as resistance markers connected with slow development of HIV infection – HLA-DRB1*01:01; 04:01; 06(13:01); HLA-DQA1*01:03; 04:01; 05:01; HLA-DQB1*03:01; 03:03; 04:01-2; 06:01; 06:02-8 are determined in different groups of patients.

High risk markers in case of HIV infection development belonging to the following groups of alleles: HLA-DRB1*03(17:01), DRB1*05(11:01), DQA1*01:01; 03:01 and DQB1*05:01; 03:02, as well as three-loci haplotypes HLA-DRB1*03(17:01)/DQB1*05:01/DQA1*01:01; HLA-DRB1*05(11:01)/DQB1*03:01/ DQA1*05:01, DRB1*01:01/DQB1*03:02/DQA1*03:01 and DRB1*01:01/DQB1*05:01/ DQA1*01:01 are determined.

Resistance to HIV infection development forms in the following groups of alleles: HLA DRB1*01:01; 06(13:01), HLA-DQB1* 03:01; 06:02-8; HLA-DQA1*01:02; 01:03, as well as in haplotypes HLA-DRB1*01:01/ DQB1*06:02-8/ DQA1*01:02; HLA-DRB1*06(13:01)/DQB1*06:02-8/ DQA1*01:02; HLA DRB1*01:01/DQB1*03:01/DQA1*01:02;

and HLA-DRB1*06(13:01)/DQB1*06:02-8/DQA1*01:02 in different groups of HIV/AIDS patients.

The obtained results testify that upon identification of HLA genes it is possible to understand the molecular mechanisms in case of progression of an AIDS syndrome complex that possibly can serve in determination of clinical results of infected patients.

The conducted study proved that the efficiency of immune response depends on a particular HLA II class haplotype, and that also proves the hypothesis about the influence of haplotype marker on the immune response function.

CONSENT

All authors declare that a written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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