RĪGAS STRADIŅA UNIVERSITĀTE

Jeļena Eglīte

# Research of HLA II class DRB1, DQA1, DQB1 genetic markers on patients with HIV infection and AIDS

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## LIST OF ABBREVIATIONS

**ABC** – Abacavir
**AIDS** – Acquired Immune Defficiency Syndrome
**APC** – Antigen Presenting Cells

| | | |
|---|---|---|
| **ART** | – | Active Anti-Retroviral Therapy |
| **AZT** | – | Azidothymidine |
| **BLC** | – | State agency "Biomedical Research & Study Centre" |
| **CDC** | – | Centers of Diseases Control |
| **CD4** | – | T helperi jeb līdzētājšūnas |
| **CMV** | – | Citomegalovīruss |
| **DNA** | – | Deoxyribonucleic acid |
| **EFV** | – | Efavirenz |
| **ELISA** | – | Enzyme linked immunosorbent assay |
| **F** | – | Fišera testa vērtība dispersiju analīzē |
| **FGS** | – | Fibrogastroduodenoskopija |
| **gf** | – | Gēna sastopamības biežums |
| **PCR** | – | polymerase chain reaction |
| **HAART** | – | Highly Active Anti-Retroviral Therapy |
| **HIV** | – | Human immunodeficiency virus |
| **HLA** | – | Human Leukocyte Antigens |
| **HTLV** | – | Human T-cell leukaemia virus |
| **KIISL** | – | RSU Interdepartmental Laboratory of Clinical Immunology and Immunogenetics |
| **LIC** | – | State Agency "Infectology Centre of Latvia" |
| **MHC** | – | Major Histocompatibility Complex |
| **OR** | – | Odds Ratio |
| **RSU** | – | Riga Stradiņš University |
| **SD** | – | Standard Deviation |
| **TAP** | – | Transporters Antigen Processing |
| **TNF-α** | – | Tumor necrosis factor α |
| **WB** | – | Western blott |

# MAIN DEFINITIONS

**Allele** – inheritance factor marking a pair alternative version of a specific trait, for example, genetic material inherited from one parent in locus. In the literal sense allele is an alternative form of a gene occupying specific positions (loci) on homologous chromosomes, but just one allele is expressed in haploid organism.

**Allelic gene** – different alternative forms of one and the same gene that are located at identical loci of homologous chromosomes and determine the phenotypic variety of traits. Gene that codes an alternative form/version of one and the same trait.

**Gene** – genes are factors of heredity, stretches of DNA or RNA molecules the function whereof is determining of specific traits of an organism. Each gene determines the synthesis of polypeptide molecule of a functionally active product – RNA or the result of its translation. One gene consists of 500 to 600 pairs of nucleotides. A gene can be established because it has different alternative forms – alleles the existence whereof is discovered with genetic analysis. Allelic and non-allelic genes interact.

**Genotype** – the terms is derived from "haploid genotype", that is commonly used to mark combination of alleles of the major histocompatibility complex of a human on one locus.

**Haplotype** – is the totality of genes located on one chromosome, unique combination of alleles of the particular locus that is completely inherited. The term is derived from "haploid genotype" that is commonly used to mark combination of alleles of the major histocompatibility complex of a human on one chromosome. The term has originated due to producing of HLA specificity, i.e. such specificity that is controlled by connected loci, some HLA haplotypes

are excessively represented in population and this phenomenon is called *linkage disequilibrium* – disturbance of connection balance.

**HLA haplotype –** special combination of alleles of HLA genes on one chromosome that codes such specific traits or functions as HLA markers of immune system.

**Locus –** location of a gene on a chromosome. Location of chromosome where a gene coding a particular trait is mapping. Genetic locus – particular gene that is responsible for synthesis of one protein and that is divided from the neighbouring gene by a recombination.

# INTRODUCTION

## Topicality of the paper

One of the greatest health problems of the contemporary mankind - immune deficiency syndrome (AIDS) – appeared at the end of 20th century. HIV holds the leader position in the group of social infections. AIDS is a disease engendered by multifactorial aetiology, a virus [1].

This virus is known as the human immunodeficiency virus (HIV) that causes AIDS – complex of syndromes that causes great changes of immunity in the result of what death of a patient can be provoked [1,2]. With slow progressing of HIV infection to the AIDS phase an important factor is sharp phase of seizure that leads to quick drop of viraemia and this a response of immunity to the virus infection.

Cell and humoral immune response on HIV is directed to the external protein coat, and also to other viral proteins that are synthesized in the infected cells. Cells of different types are susceptible to infection with HIV in vitro. Coat glycoprotein gp120 HIV-1(in case of HIV-2 gp105) at CD4 cell receptor shows infection of a cell with the virus. When compared with other cells, the largest amount of CD4 receptors is located on the upper cytoplasm coat of T lymphocytes (helpers) [3].

During maturation of immune competent cells the identification marker CD4+ forms on the surface of T lymphocytes. It is possible only on cells that have the II class (HLA – II) antigens of the major histocompatibility complex – proteins on the plasma membrane. Since these cells have also receptors for recognition of HLA – II class proteins, the CD4+ lymphocytes recognizing the antigen identity at the same time both an unknown antigen, and HLA – II class proteins, and only in this case their reactive proliferation and immune response materialize [3].

Human (*Human Leukocyte Antigens* - HLA) tissue gene occurrence system is one of the many polymorphological genetic systems that perform different functions in human body. The most important of them are the response of genetic control immunity and preservation of immunity homeostasis [4].

This direction of human tissue compatibility complex became very topical after in 1980 Nobel Prize winners: *Baruj Benacerraf* (USA), *Jean Dausset* (France) and *George D. Snell* (USA), who won the prize about discovering of genetically determined structure on the surface of tissue that regulate the immunologic reactions, acquired evidences for biogenetic human individuality and polymorphism [5,6]. *Zinkernagel R.M*. and *Doherty P.S*. discovered that T lymphocytes recognize the immune antigen of viruses through the protein of the major tissue compatibility complex. These and other discoveries enable to draw conclusions that genetic differences in a locus that codes HLA protein can influence the intensity of immune response and efficient response of host body to the infection by determining the result of interaction [7, 8].

HLA polymorphism progress in a research with polymerase chain reaction (PCR), as well as application of new methods and technologies in HLA genotyping was discovered by *Kary Mullis* [9, 10].

Molecular technologies enable researching genetic polymorphism within the limits of gene molecular influence.

Different association versions with the consequent incompatibility of HIV infection and these genes were obtained in the researches with different HLA genes [11, 12, 13].

This fact testifies that knowledge on 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 are not sufficient [14–17].

However practical meaning 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 the information of AIDS Prevention Centre, the first case of HIV infection in Latvia was registered in 1987 (patient No. 1), as to 31 December 2010 according to the information from the register of "State Health Agency" 4901 cases of HIV infection and 928 cases of AIDS are registered in Latvia, 603 persons have died. However, according to the estimates of WHO and UNAIDS the actual number of persons infected with HIV could be twice as big as is shown by the official statistical data (*Figure No. 1.*). [1, 2]

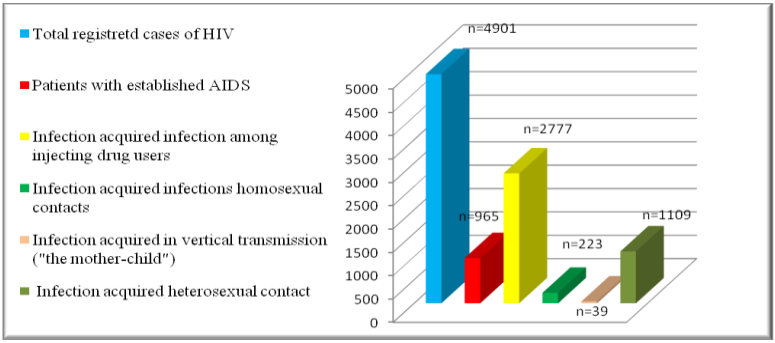



**Fig. 1. Cases of HIV infection totally registered in Latvia to 1 February 2011 according to the information furnished by the Infectology Centre of Latvia**

## HLA system

HLA system was discovered in 1958. [3, 4, 5, 6, 7, 8]

The major histocompatibility complex (MHC) HLA in 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, 19, 20]. This is the "starting point" in development of practical immunogenetics (science about immunogenetic diversity and influence on immunity and nonspecific resistance of body). Discovery of polymerise chain reaction (PCR) [9, 10] and the new HLA genotyping methods developed on the grounds thereof have facilitated the progress of polymorphism research of human histocompatibility complex HLA system. Nowadays an international HLA gene polymorphism research data base (HLA nomenclature 2011) is created.

HLA is located on the short arm of the sixth chromosome, in the region 6p21.31- 6p21.32 (Figure No. 2.1). Its length is 4 million base pairs. HLA region genes and gene products (i.e. specific antigens) are divided in I class, II class and III class genes and their products.

HLA structure complex is regarded as quite compact whereby the rare frequency of recombination is to be explained. HLA complex has ca. 105–106 genes, i.e., ca. 1/1000 of the total human gene pool [11].

Nowadays totally 6074 HLA alleles are discovered:

HLA I class – 4 721 specificity;

HLA II class – 1353 specificity;

HLA-DRB1*– 966;

HLA-DQA1* – 35;

HLA-DQB1* – 144 specificity (Annex No. 1. HLA nomenclature 2011.) [21, 22, 23.]

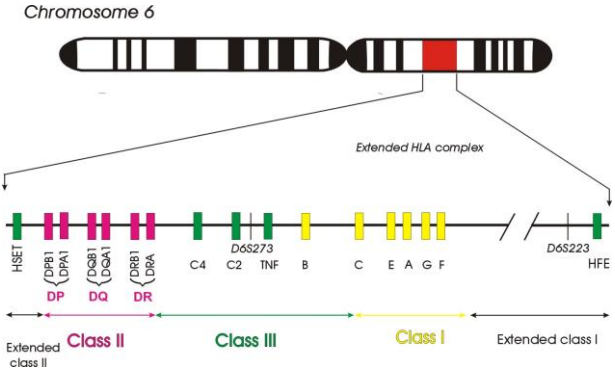



**Fig.2. Structure of the sixth chromosome; genes containing the major histocompatibility complex HLA (Expert Reviews in Molecular Medicine 2003, Cambridge University Press.)**

## Functions of HLA antigens

One of the most important physiological functions of HLA system is provision of immunodominant peptides and presentation (Fig.3 and 4.). Such peptide is a product of proteolysis of a foreign body – antigen occurring in a host cell against that the immune response shall be induced and later formed. This HLA function of antigen system is facilitated by the structure of the molecules that irrespective of differences in structure of I and II class antigen HLA molecules enable forming the so called peptide linking wrinkle on their external ends where the peptide necessary for recognition is also located. HLA-III class histocompatibility complex genes located between the regions of I and II class code the proteins that are not able to recognize (establish) the antigen.

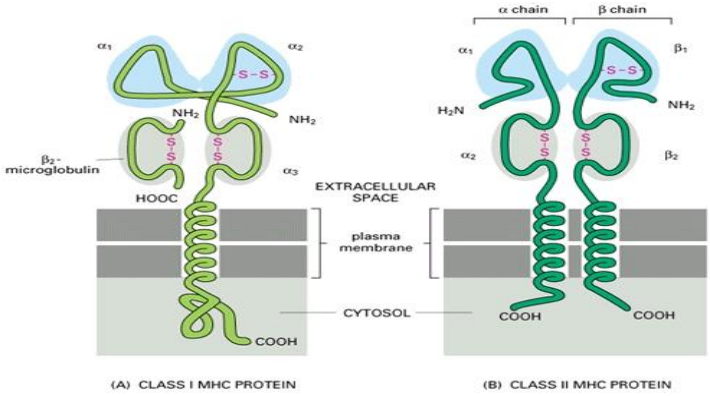



**Fig. 3. Structure of HLA I and II class molecules (HLA molecules link the antigen peptides in a wrinkle that is formed with two α-spirals (HLA I class molecules) and HLA II class molecules consist of α/β – spirals to form (HLA-peptide), complex that is recognized by T cell receptors) (publishing Blackwell 2008.)**

## AIM OF THE PAPER

To appraise association of genetic polymorphism of HLA II class loci DRB1*, DQA1*, DQB1* with the body protection ability during infection.

## TASKS OF THE PAPER

1. To determine HLA II class alleles, genotypes and haplotypes for patients infected with HIV and the control group.
2. To determine and appraise predisposition of genetic markers and resistance in patients infected with HIV/AIDS that shall determine the clinical progress of disease.
3. To determine polymorphism of the gene HLA-DRB1*0101 in the second exon during the development of progress of disease.

## SCIENTIFIC INNOVATION OF THE PAPER

Data on incidence frequency of genes HLA-DRB1*/DQA1*/DQB1* and their connection with HIV/AIDS in different groups of patients within the territory of Latvia is obtained.

Genetic markers for each group, as well as the total genotype of markers that associates with predisposition and resistance in development of infection process for patients infected with HIV virus are determined.

First data on polymorphism of nucleotides in the second exon protective gene DRB1*0101 for the HIV infected patients in AIDS phase are received.

On the grounds of the results of the research new conception for application of gene HLA II class as the forecasting markers in differential analysis in case of HIV virus infection is created. Hypothesis on influence of polymorphism of second exon on HLA II class gene, its influence on the immune response in case of infectious diseases is revised.

## PRACTICAL APPLICATION

Data on gene incidence frequency HLA-DRB*/DQA1*/DQB1* and all haplotypes in different groups of HIV/AIDS infected patients are of genetic significance and can be used as a base in international researches, as well as for qualitative control when researching interaction of HLA and diseases.

Genetic research of infectious disease research by evaluating haplotypes DRB1/DQA1/DQB1 is in the ground of the newest methods. The formulated conception “marker genotype” and hypothesis “functional immune response” can be a great investment in the theory of immunogenetic and immunologic science, in development of immunofarmacogenomic area.

## THE STRUCTURE AND EXTENT OF THE STUDY

The dissertation consists of 10 chapters: Introduction, Review of literature, Materials and methods, Results, Discussion, Conclusions, Clinical implications, References and Appendix. The original version of this thesis is written in Latvian on 181 pages, including 60 tables and 14 figures. The list of references consists of 157 titles. There are 9 publications on the 11 doctoral theses and 10 author's certificate.

## MATERIALS AND METHODS

### Research methodology, selection principles of the total patient group and exclusion criteria

Totally as to 1 February 2011 4901 HIV infected patients were registered in Latvia, 965 of them are in AIDS phase (data from ambulatory cards in the State Agency "Infectology Centre of Latvia"). In course of the work medical documentation (hospital and ambulatory cards) of 2500 patients were analysed for the period from 1991 to 2004, and the following was performed: examination of patients – inquiry of epidemiologic 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. Second exon of gene DRB1*0101 was sequences for 100 patients with HIV/AIDS. 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 period dynamics – both clinically and laboratorial

(complete blood count, HIV viral load test – twice a year, determination of number of lymphocytes in subpopulation – once in three months).

**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

**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. denormalization 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 familiarized with the document "Information for patients" and have signed the "Patient agreement statement". 898 (75%) of 1180 patients included in the research are men and 302 (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. HIV infection for all patients (100%) was

approved with the primary test by determining antibodies against HIV and *Western Blot* tests.

## Immunological research methods

## Human DNA extraction from blood

The material belongs to the unique collection of RSU KIIS laboratory and was used for DNA researches. Genomic DNS was extracted from the blood specimen with the standard phenol-chloroform extraction method.

### HLA-DRB1; DQA1; DQB1 typing

HLA typing tests were performed in the Immunogenetic and Immunology Interdepartment Laboratory of Riga Stradiņš Clinical University hospital (Head of Laboratory J. Eglīte). For control group, data of healthy donors (n=1173) were used from database of the Clinical Immunogenetic and Immunology Interdepartmental Laboratory.

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) blood. Genomic DNA was extracted from blood sample using standard phenol-chloroform extraction method. HLA typing low-resolution 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.

## Sequencing research methods for determination of second exon gene DRB1*01

Typing of genetic polymorphisms was performed in the Genom Centre laboratories of Latvian Biomedical Research & Study Centre.

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) blood. Genomic DNA was extracted from blood sample using standard phenol-chloroform extraction method. Then region of interest was amplified

using polymerase chain reaction (PCR). Oligonucleotide primers designed and synthesized for amplification and sequencing reaction are given in :

5'- tcccagt gcccgcaccc c -3' – forward primer (18 nt)

5'-gagctggga atctgagtgt gt -3' – reverse primer (21 nt)

5'-tcagtgtc ttctcaggag gc -3' – sequencing primer (20 nt) (*Kotsch et al., 1999 Tissue antigens (53): 486-497 (primers)).*

Then region of interest was amplified using polymerase chain reaction (PCR). Oligonucleotide primers: 8157..8426 – 2. eksona gēna HLA–DRB1*0101 "Big Dye Terminator mix" (*Applied Biosystems*, ASV)

ORIGIN PRIMERS (270 bp) 8157.. 8426 – 2.eksons gēna HLA–DRB1*01

1 cacgtttcct gtggcagcct aagagggagt gtcatttctt caatgggacg gagcgggtgc

61 ggttcctgga cagatacttc tataaccagg aggagtccgt gcgcttcgac agcgacgtgg

121 gggagttccg ggcggtgacg gagctggggc ggcctgacgc tgagtactgg aacagccaga

181 aggacctcct ggagcagagg cgggccgcgg tggacaccta ctgcagacac aactacgggg

241 ttggtgagag cttcacagtg cagcggcgag

The reaction mix for polymerase chain reaction contained 28 ng DNA and 1 Mm oligonucleotides. Reactions were performed in 15 mkL of a 2× PCR solution of MasterMix (*Fermentas*, Lithuania). Amplification was performed with an initial denaturation of 95°C for 5 mins, followed by 32 cycles of 95°C for 15 secs, 56°C for 30 secs, and 72°C for 30 secs; the reaction was completed with a final extension step of 72°C for 10 mins.

PCR products were then purified (Sap-Exol) and further investigated by automated direct sequencing using ABI prism 3100 DNA (*Applied Biosystems)* sequencer according to manufacturer recommendations.

## Statistical data processing

Statistical analysis of data was performed by means of software: *Microsoft Office Excel 2003* and DOS *StatCalc* [126]. ARLEQUIN 3.11 software [127].

Statistical analysis was performed on a computer in program Microsoft Excel. Credibility of result differences was evaluated according to criteria of Student's t-test and Pearson's test. Meaning of differences of parameters was evaluated at credibility $p \leq 0.05$. Odds ratio (OR) was calculated according to Wolf's method using 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 credibility was determined according to Fisher's criteria. 95% credibility interval (95%CI) was determined according to the formula: 95%CI=InOR±l,96.

# RESULTS

## 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 are used in the present paper. All 1180 HIV positive patients included in the research are in the state agency "Infectology Centre of Latvia" and have familiarized with the document "Information for patients" and have signed the "Patient agreement statement".

*Table 2*

**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 n (%)<br>Woman n (%) | 898 (75%)<br>302( 25%) |
| **Age** | years, average (± SD), | 33.6 (±13,4) |

*Table 3*

**Characteristic of total research population and subgroups to be analysed**

| | | |
|---|---|---|
| Total HIV/AIDS group<br>AIDS group | all HIV infected patients<br>infected patients in AIDS phase | 1180 (100%).<br>185 (16%). |
| Heterosexuals (Hetero/sek.) | patients who have infected in result of heterosexual relations | 577 (49%) |
| Homosexuals (Homo/sek.) | patients who have infected in result of homosexual relations | 59 (5%) |
| Intravenous drug users (IVDU group) | patients who have infected using shared syringes and needles, intravenously injecting the drugs and psychotropic substances | 544 (46%) |
| Control group (Control group)<br>Gender | healthy blood donors (residents of Latvia)<br>Men<br>Women | 173 (100%)*<br>137 (79%)<br>36 (21%) |

*The material used in the research was taken from blood bank of the Interdepartmental Laboratory of Clinical Immunology and Immunogenetics of Riga Stradiņš University.

To obtain new data about 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 where OR is less than 1,0 link with the protective gene. The results were considered as statistically credible if Fischer test correction with little measurements was $p \leq 0{,}05$. Chi quadrate test ($x^2$) and gene incidence frequency (gf) was used to verify the hypothesis.

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

When researching HLA II class genes it is begun with analysis of polymorphism HLA-alleles, genotype and haplotype for HIV infected patients and control group.

The characteristic specificity in the total group of HLA II class locus DRB1 where all HIV/AIDS infected patients were included was researched in the initial stage. In the examples to be analysed 14 alleles versions of the gene DRB1 were determined in that different influence stages (both positive and negative) were discovered. Results are shown in the Table *4.*

*Table 4*

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

| HLA-DRB1 | Total HIV/AIDS group | AIDS group | Hetero/sek. group | Homo/sek. group | IVDU group |
|---|---|---|---|---|---|
| **Immunogenetic risk markers (predisposition markers)** | | | | | |
| Alleles *(OR/p)* | 03 (2.18/0.0001) 07 (4.22/0.0001) | 03 (1.93/0.007) 07 (4.22/0.002) | 03 (3.35/0.0001) 05 (1.82/0.001) | 07 (2.65/0.006) | 03 (2.48/0.0001) 07 (6.04/0.0001) |
| Geno Types (*OR/p)* | 02/03 (1.36/0.516) 01/07 (1.07/0.868) 03/06 (2.69/0.116) | 02/03 (1.18/0.755) 02/05 (1.34/0.270) 07/07 (2.26/0.002) | 03/07 05/05 05/07 07/07 | 01/05 (1.86/0.053) 02/03 (3.58/0.036) 02/05 (2.12/0.032) | 01/03 (2.19/0.037) 01/07 (2.48/0.014) 02/03 (2.89/0.020) 03/06 (6.10/0.004) |

*continuation of table 4.*

| Protective markers (resistance markers) | | | | | |
|---|---|---|---|---|---|
| Alleles *(OR/p)* | 01 (0.55/0.001) 06 (0.30/0.0001) | 06 (0.61/0.036) | 01 (0.37/0.0001) 04(0.52/0.00 1) 06 (0.33/0.0001) | 04 (0.67/0.297)* 06 (0.70/0.301)* | 04 (0.59/0.011) 06 (0.34/0.0001) |
| Genotypes *(OR/p)* | 01/02 (0.38/0.0001) 01/04 (0.34/0.001) 01/06 (0.48/0.005) | 01/04 (0.41/0.221) 06/06 (0.54/0.048) | 06/06 (0.65/0.010) | 02/04 (0.36/0.044) 01/06 (0.318) | 05/06 (0.22/0.0001) 06/06 (0.60/0.001) |

When looking for a connection between the associative HLA II class DRB1* genes, it is possible to draw a conclusion on diversity of risk genes. Genes DRB1*03, DRB1*07 are more frequent in the HIV infected patient group. These genes were encountered also in other groups – in AIDS and IVDU HIV infected groups. In the group of homosexuals the gene DRB1*03 had no credible result. Gene DRB1*07 did not appear in the group of homosexuals. Incidence frequency of the gene DRB1* 05 (11; 12) is larger in the DRB1*03 alleles in the group of heterosexuals.

HLA-DRB1*06 should be stated as the protective allele in all researchable groups. DRB1* 01; DRB1*04; DRB1*06 was also stated in the group of heterosexuals, DRB1*04; DRB1*06 – in the group of homosexuals, DRB1*05, DRB1*06 – in IVDU group (*Tab. 4.*). Incidence of other genes DRB1* among the sick and healthy individuals had little difference or had no statistical credibility.

As to genotypes of locus HLA-DRB1*, the obtained results were divided in five groups and some coincidences were established. Risk genotype usually consists of open genes, as well as risk genes over and over again approve 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; DRB1*05; DRB1*07 are practically proved in all surveyed groups. Greater risk degree exists for the patients infected with the HIV virus. Genotypes DRB1*02/03; DRB1*02/05, DRB1 07/07 consisting of allele risk theoretical proof in all groups showed high risk (*Tab. 4.*).

Protective alleles DRB1*04, 06, in the total genotype group 01, 02 decelerate the process of infection.

In can be concluded that the genotype DRB1*02/03; DRB1*02/05, DRB1 07/07 is a genetic marker with increased risk of chronic infection process appearance. Allele genotype DRB1*01; DRB1*06 is related to the disease by causing minimum risk and it results that it lessens 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 (*Tab. 4.*).

## Analysis of gene polymorphism in the locus HLA–DQA1* in different HIV infected patient groups

Further 8 versions of alleles of gene DQA1 are determined for the HIV infected patients by means of the selection to be analysed (*Tab. 5.*).

*Table 5.*

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

| HLA-DQA1 | Total HIV/ AIDS group | AIDS group | Hetero/sek. group | Homo/sek. group | IVDU group |
|---|---|---|---|---|---|
| **Immunogenetic risk markers (predisposition markers)** | | | | | |
| Alleles *(OR/p)* | 0101 (1.78/0.0001) 0201 (1.42/0.042) 0301 (1.70/0.001) | xxxxxxxxx | 0101 (1.37/0.051) | 0301 (1.49/0.156) | 0601 (2.53/0.018) |

*continuation of table 5.*

| | | | | | |
|---|---|---|---|---|---|
| Genotypes *(OR/p)* | 0101/0501 (2.85/0.004) 0102/0301 (4.23/0.001) | 0101/0501 (1.18/0.755) 0102/0301 (1.34/0.270) | 0101/0501 (5.29/0.0001) 0102/0301 (3.60/0.003) | 0101/0301 (9.37/0.001) 0102/0301 (3.37/0.005) 0201/0501 (2.70/0.042) | 0101/0501 (4.46/0.0001) 0102/0301 (3.30/0.0001) 0103/0301 (4.05/0.044) |
| **Protective markers (resistance markers)** | | | | | |
| Alleles *(OR/p)* | 0401 (0.43/0.002) | 040 1(0.37/0.025) | 0401 (0.62/0.113) | 04 (0.68/0.492) | 0501 (0.74/0.038) |
| Genotypes *(OR/p)* | 0601/0601 (0.24/0.050) | 0101/0401 (0.41/0.221) 0102/0401 (0.54/0.048) | xxxxxxxxx | 0103/0103 (0.76/0.557) | 0102/0103 (0.25/0.083) 0103/0103 (0.46/0.001) 0601/0601 (0.39/0.051) |

When analysing gene polymorphism in genes of locus DQA1* within different groups of HIV infected patients, risk associations were established with particular HLA-DQA1*0101; 0601; 0201; 0301 and protective associations with DQA1*0103; 0401; 0501 (*Tab. 5.*).

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* (*Tab. 5.*), the obtained results are divided in 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 combinations of genotypes (*Tab. 5.*)

## 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 means of the selection to be analysed (*Tab. 6.*)

*Table 6.*

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

| HLA-DQB1 | Total HIV/AIDS group | AIDS group | Hetero/sek. group | Homo/sek. group | IVDU group |
|---|---|---|---|---|---|
| **Immunogenetic risk markers (predisposition markers)** | | | | | |
| Alleles *(OR/p)* | 0302 (4.99/0.0001) 0501 (2.67/0.0001) | 0302 (1.90/0.036) 0304 (10.35/0.001) | 0303 (1.71/0.040) 0304 (9.93/0.001) | 0302 (1.60/0.270)* 0501 (1.31/0.340)* | 0302 (2.20/0.001) 0304 (6.39/0.001) |
| Geno types *(OR/p)* | 0301/0302 (3.51/0.003) 0301/0502-4 (2.81/0.014) 0302/0501 (6.84/0.002) 0302/0602-8 (3.0/0.031) | 0301/0302 (4.97/0.002) 0304/0304 (11.90/0.001) | 0302/0602-8 (3.17/0.025) 0304/0304 (6.90/0.002) | 0201-2/0301 (2.12/0.032) 0301/0602-8 (1.83/0.050) 0401-/0602-8 (5.95/0.020) | 0201-2/0501 (2.03/0.002) 0301/0302 (4.08/0.0005) 0301/0502-4 (2.86/0.013) 0302/0302 (2.32/0.002) 0302/0501 (8.12/0.001) |
| **Protective markers (resistance markers)** | | | | | |
| Alleles *(OR/p)* | 0301 (0.39/0.0001) 0401-2 (0.43/0.003) 0601 (0.21/0.001) 0602-8 (0.50/0.0001) | 0602-8 (0.58/0.005) | 0301 (0.71/0.020) 0602-8 (0.68/0.010) | 0601 (0.36/0.330) 0602-8 (0.76/0.310) | 0602-8 (0.58/0.001) |
| Genotypes *(OR/p)* | 0302/0401-2 (0.13/0.008) 0501/0601 0602-8/0602-8 (0.67/0.003) 0601/0601 (0.29/0.001) | 0301/0602-8 (0.44/0.017) 0601/0601 (0.18/0.042) | 0201-2/0502-4 (0.38/0.029) 0301/0602-8 (0.72/0.059) | xxxxxxxxxx | 0302/0401-2 (0.17/0.019) 0303/0602-8 (0.29/0.002) 0401-2/0401-2 (0.50/0.042) 0601/0601 (0.25/0.0005) 0602-8/0602-8 (0.65/0.003) |

When analysing gene polymorphism in genes of locus DQB1* within different groups of HIV infected patients, positive association was obtained with specificity HLA-DQB1*0302; 0304. The same genes were established in

the particular groups to be researched: AIDS, heterosexuals and UVDU infected patients. In the group of HIV infected homosexual patients no significant (credible) results were obtained, but the gene HLA-DQB1*0302 was not established in the group of HIV infected heterosexual patients. Apart from the allele HLA-DQB1*0304, reliable more frequent incidence of the gene HLA-DQB1* 0303 was discovered in the group of HIV infected heterosexuals patients. Protective associated alleles are DQB1*0301; 0401-2; 0602-8. Frequency of other HLA-DRB1* genes slightly differs between the sick and healthy individuals or the difference is not credible (*Tab. 6*).

As to the genotypes in locus HLA-DQB1 (*Tab. 6*), some coincidences of the obtained results are established within the five groups. The discovered genes constitute mainly both risk genotype and risk genes that approves over and over again the particular influence of different genes of the process of infectious disease progress. Gene protective existence in the genes influences the resistance of the particular individuals during the respective pathological processes (*Tab. 6*). It is necessary to research the interconnection mechanism of alleles, and also the combinations of haplotypes and genotypes.

## Research gene combination (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 7.)*

*Table 7.*

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

| HLA DQB1/DQB1 /DQA1 | Total HIV/AIDS group | AIDS group | Hetero/ sek. group | Homo/ sek. group | IVDU group |
|---|---|---|---|---|---|
| **Immunogenetic risk haplotypes (predisposition haplotypes)** | | | | | |
| 01/0302/0301 | 6.17/0.027 | | | | 9.52/0.007 |
| 01/0501/0101 | | 2.35/0.009 | | 2.41/0.032 | |
| 02/0302/0102 | 8.34/0.013 | | | | 11.04/0.003 |
| 02/0302/0301 | 8.34/0.013 | | | | 10.53/0.004 |
| 02/0501/0101 | | 3.49/0.039 | | 5.32/0.013 | |
| 03/0501/0101 | 2.66/0.032 | | | | 3.11/0.013 |
| 05/0301/0501 | | 2.03/0.002 | 1.68/0.035 | | |
| | *(OR/p)* | *(OR/p)* | *(OR/p)* | *(OR/p)* | *(OR/p)* |
| **Protective haplotypes (resistance haplotypes)** | | | | | |
| 01/0301/0102 | 0.44/0.054 | | 0.11/0.009 | | |
| 01/0602-8/0102 | 0.27/0.008 | | | | 0.22/0.008 |
| 01/0602-8/0103 | 0.31/0.030 | | | | 0.14/0.008 |
| 06/0602-8/0102 | 0.24/0.0001 | | 0.33/0.005 | | 0.17/0.0001 |

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 syndrome complex AIDS, is located in allele groups DRB1*03(17;18), DRB1*07 and DRB1*05, with specificity DQA1*0101 and DQB1*0501, as well as with three-locus haplotypes HLA-DRB1*01/DQA1*0101/DQB1*0501, DRB1*10/DQA1* 0101/DQB1*0501 and DRB1*04/DQA1*0301/DQB1*0302. Resistance against the syndrome complex AIDS is determined for the phenotype in allele group HLA-DRB1*02, with specificity HLA-DQA1*0102 and haplotypical combination DRB1*02/ DQA1*0102/DQB1*0602 and DRB1*02/DQA1* 0103/DQB1*0601. *(Table 7.)*

## Gene conformation polymorphism researches in second exon HLA-DRB1*0101 HIV infected patients in AIDS phase

From the acquired results it is possible to draw a conclusion that particular gene haplotypes HLA-DRB1*, DQA1*, DQB1* in HIV infected patients are responsible for predisposition and protective functions (*Tab. 8.*).

The acquired results testify that the allele HLA-DRB1*0101 possesses protective characteristics in development of HIV infection. But in combinations with some versions of alleles located in HLA locus, the allele DRB1*0101 does not perform the protective functions anymore.

Considering the structure of genes (*Fig. 5.*), it is regarded that the presenting molecules HLA II –DRB1*0101 class formation is influenced by gene DRB1*0101 exon 2.

It is known that formation of molecule HLA II class $\alpha$ chain and $\beta$ chain is encoded exactly thank to exon 2 HLA-DRB1*, DQA1* and DQB1* genes.

It is considered that exon 2 conformation changes in HLA II class genes can affect the formation of immune response.

To research this phenomenon, sequence of gene HLA-DRB1*0101 exon 2 nucleotides was determined applying the sequencing method. The existing region 290 bp encodes the polymorphism of nucleotides. This nucleotide region is responsible for linking of viral peptide on surface of HLA molecule.

When researching polymorphism of the gene HLA-DRB1* 0101 second exon nucleotides, the patients were divided in three groups (*Table 8.*).

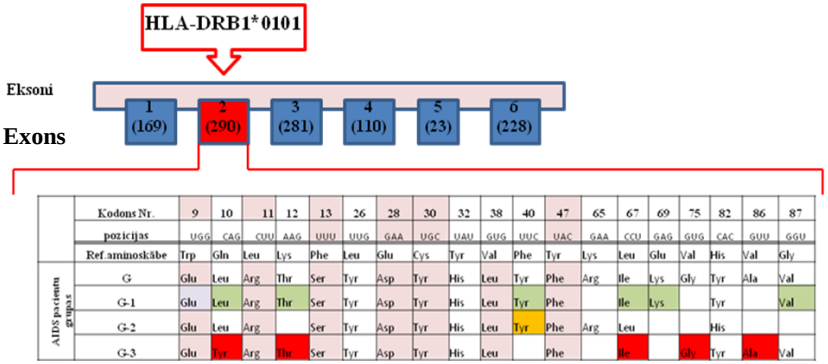
*Table 8.*

**Characteristic of total research population and subgroups to be analysed**

| | | |
|---|---|---|
| Total AIDS group | all HIV infected patients in AIDS phase | 100 (100%). |
| G-1 group | patients to whom lasting remission was observed for more than 6 years | 21 (21%) |
| G-2 group | patients to whom remission was observed for more than 6 years in the result of received treatment | 7 (7%) |
| G-3 group | patients with registered fulminate AIDs syndrome development | 10 (10%) |
| G group (Control group) | healthy blood donors (residents of Latvia) | 23 (100%) |

Analysis of obtained sequences of DRB1 gene exon 2: comparison with reference sequence (DRB*01010101 allele) was performed applying *Contig Express* (*Invitrogen*, USA) software and IMGT/HLA database (*the international ImMunoGeneTics database*).

Considering the incidence of nucleotide polymorphism in exon 2 HLA-DRB1*0101 gene, the "hot points" of mutations of this exon were found: in codon 9, 11, 13, 28, 30, 38, 47 and 82. STOP codon (in codon 13) was observed in the sample of one HIV patient. Besides, balances relation of nucleotide transversion and transition was observed that testifies of mutation in exon 2 (transversion in human body is a phenomenon of rare occurrence) (*Fig. 5.)*




| | Kodons Nr. | 9 | 10 | 11 | 12 | 13 | 26 | 28 | 30 | 32 | 38 | 40 | 47 | 65 | 67 | 69 | 75 | 82 | 86 | 87 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pozicijas | UGG | CAG | CUU | AAG | UUU | UUG | GAA | UGC | UAU | GUG | UUC | UAC | GAA | CCU | GAG | GUG | CAC | GUU | GGU |
| | Ref.aminoskābe | Trp | Gln | Leu | Lys | Phe | Leu | Glu | Cys | Tyr | Val | Phe | Tyr | Lys | Leu | Glu | Val | His | Val | Gly |
| AIDS pacientu grupas | G | Glu | Leu | Arg | Thr | Ser | Tyr | Asp | Tyr | His | Leu | Tyr | Phe | Arg | Ile | Lys | Gly | Tyr | Ala | Val |
| | G-1 | Glu | Leu | Arg | Thr | Ser | Tyr | Asp | Tyr | His | Leu | Tyr | Phe | | Ile | Lys | | Tyr | | Val |
| | G-2 | Glu | Leu | Arg | | Ser | Tyr | Asp | Tyr | His | Leu | Tyr | Phe | Arg | Leu | | | His | | |
| | G-3 | Glu | Tyr | Arg | Thr | Ser | Tyr | Asp | Tyr | His | Leu | | Phe | | Ile | | Gly | Tyr | Ala | Val |

**Figure 5. Second exon gene DRB1*0101 polymorphism in HIV infected persons in AIDS phase**

From the obtained results it can be concluded that nucleotide sequence conformation in exon 2 causes changes of aminoacids in HLA molecules. These changes can affect the main function of molecule – affixation and presentation of viral peptide. It is regarded that fulminant development of syndrome complex AIDS can be connected with change of aminoacids in codon 10 (Gln→Tyr), 12 (Lys→Thr), 67 (Leu → Ile), 75 (Val → Gly), 86 (Val →Ala). (Annex No. 31 *Tab. 5.44);* in patients to whom lasting remission was observed for more than 6 years (group G-1), that is connected with change of aminoacids in codon 10 (Gln→Tyr), 12 (Lys→Thr), 40 (Phe → Tyr), 69, (Leu→ Lys), 87 (Gly →Val); in patients to whom remission was observed for more than 6 years in the result of received treatment (group G-2), that is connected with change of aminoacids in codon 40 (Phe → Tyr), 65, (Lys→ Arg) (*Fig. 5.)*

## Researches of correlation among the number of HIV virus RNA copies in plasma (HIV viral loads), number of CD4+ lymphocytes in subpopulation peripheral blood and haplotypes in HLA II class HIV/AIDS patients

The main criterion of clinical course is the number of CD4+ cells and the viral load (HIV viral load test) in patient's blood [1, 2]. However, the particular laboratorial parameters do not show the direct clinical picture. Number of CD4+ cell that is the opportunistic infection AIDS risk indicator, in free form is incident in small amount in blood so the amount of cells infected by virus that is located in the lymph tissues encumbers evaluation of the clinical picture [9]. Distribution parameters of laboratorial dynamic analysis have proven (number of HIV virus RNA copies in plasma and number of CD4+ lymphocytes in subpopulation peripheral blood) that viral load drop correlates with the increased number of CD4+ subpopulation cells in peripheral blood that corresponds to the parameters stated in the literature [5, 8]. Therapy that affects (decreases) HIV virus replication gives great clinic advantages. Clinic forecast requires additional criterion (markers) that enable determining explicit and active infections, as well as the stage of process dynamics in each particular case. Dynamics of the number of HIV virus RNA copies in plasma and the number of CD4+ lymphocytes in subpopulation peripheral blood of HIV/AIDS infected patients was also researched for evaluation of additional criterion. Research was performed in the group of HIV infected persons (N=360) whose disease period lasted from 8 to 10 years. Patients with different HLA II class haplotypes were selected and researched in the particular group *(Tab. 9, Fig. 6. and Tab. 10, Fig 7.)*

In the first group (N=314) haplotypes associated with the infection process development risk: HLA-DRB1*/DQB1*/DQA1*01/0302/0301;

01/0501/0101; 02/0302/0102; 02/0302/0301; 02/0501/0101; 03/0501/0101; 05/0301/0501 (*Tab. 9, Fig. 6*).

*Table 9.*

**Dynamics of the number of HIV virus RNA copies in plasma and the number of CD4+ lymphocytes in subpopulation peripheral blood of high risk HIV/AIDS infected patients' group**

| Haplotypes with the highest risk degree in the group of HIV/ AIDS infected patients HLA-DRB1*/DQB1 */DQA1* | Total HIV/ AIDS group N=314 | Number of CD4 cells c/mm³ | | | | | HIV virus RNA load copies /ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12 w | 4 y | 8 y | 10 y | 12 y | 12 w | 4 y | 8 y | 10 y | 12 y |
| *01/0302/0301 | 20 | 450 | 340 | 500 | 200 | 140 | 40000 | 9000 | $10^4$ | $10^8$ | $10^8$ |
| *01/0501/0101 | 67 | | | | | | | | | | |
| *02/0302/0102 | 27 | | | | | | | | | | |
| *02/0302/0301 | 27 | | | | | | | | | | |
| *02/0501/0101 | 37 | | | | | | | | | | |
| *03/0501/0101 | 43 | | | | | | | | | | |
| *05/0301/0501 | 93 | | | | | | | | | | |

*N- number of patients*

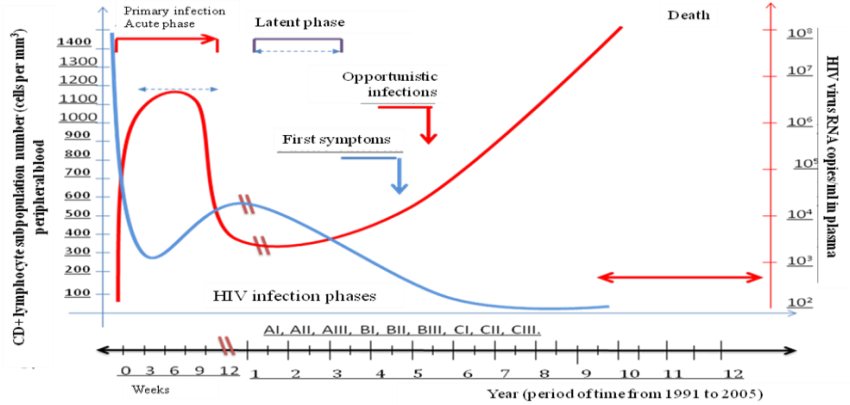



**Figure 6. Dynamics of the number of HIV virus RNA copies in plasma and the number of CD4+ lymphocytes in subpopulation peripheral blood of high risk HIV/AIDS infected patients' group**

Second group (N=46) haplotypes associate with protective abilities of infection process – resistance group HLA-DRB1*/DQB1*/DQA1*01/0301/ 0102; 06/0602-8/0102; 01/0602-8/0102; 01/0602-8/0103. *(Tab. 10, Fig. 7.)*

*Table 10.*

**Dynamics of the number of HIV virus RNA copies in plasma and the number of CD4+ lymphocytes in subpopulation peripheral blood of HIV/AIDS infected patients' group**

| Haplotypes with resistance risk degree in the group of HIV /AIDS infected patients HLA-DRB1*/ DQB1*/DQA1* | Total HIV/AIDS group N=46 | Number of CD4 cells c/mm³ | | | | | HIV virus RNA load copies/ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 12 w | 4 y | 8 y | 10 y | 12 y | 12 w | 4 y | 8 y | 10 y | 12 y |
| *01/0301/0102 | 13 | 700 | 940 | 760 | 300 | 200 | 5000 | 300 | $10^3$ | $10^4$ | $10^8$ |
| *01/0602-8/0102 | 8 | | | | | | | | | | |
| *01/0602-8/0103 | 7 | | | | | | | | | | |
| *06/0602-8/0102 | 18 | | | | | | | | | | |

*N- number of patients*

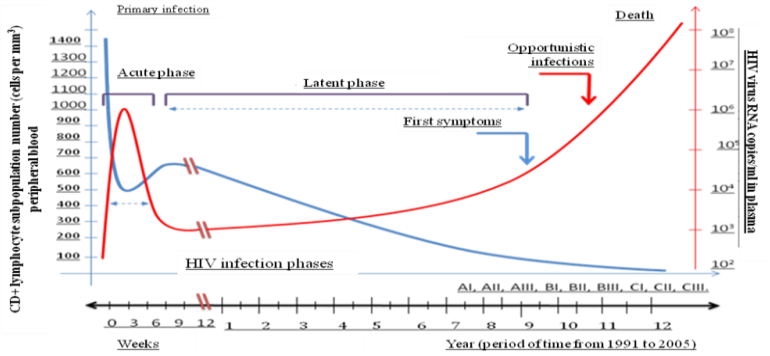



**Figure 7. Dynamics of the number of HIV virus RNA copies in plasma and the number of CD4+ lymphocytes in subpopulation peripheral blood of HIV/AIDS resistance patients' group**

According to the results of analysis in the group of high risk HIV/AIDS patient group (*Figure 6.*) certain decrease of latent period and slight extension of acute phase can be stated when compared with HIV/AIDS patients from resistance group (*Figure 7.)* So the particular researches approve number of HIV virus RNA copies in plasma (HIV viral loads), number of CD4+ lymphocytes in subpopulation peripheral blood and HLA II class haplotype correlation in HIV/AIDS patients.

Results of analysis conform also to information from literature attesting that decrease of viraemia correlates to greater amount of CD4+ cells. In the research coherences between HLA II class genes of particular associated markers show direct coherence between the haplotypes with CD4+ cell dynamics and HIV RNA level in plasma in HIV/AIDS infected patients. So haplotype HLA II class genes can be used also as additionally forecasting criterion who unlike the CD4+ cells and HIV RNA level parameters does not change for all life of an individual and is not dynamic.

## Comparison of antiretroviral therapy (ART) efficiency with different HLA II class haplotypes

HLA II class haplotype distribution analysis was made to HIV/AIDS group patients in whose treatment ART basic scheme was applied.

In the total group to be researched HIV/AIDS infected patients were included:

1. to whom ART was prescribed;
2. who had not received ART previously;
3. who have maximally observed the regimen;
4. who for 24-48 weeks were treated applying ART basic scheme;
5. from 2003 to 2009 415 persons received ART (information from Infectology Centre of Latvia).

254 HIV infected patients were included in the research, 195 of them were men and 59 – women (average age of patients - 34,7 years). When comparing different clinical classification of HIV infection, it must be concluded that HIV infection signs B(II) (no-symptom infection) or B(II) (generalized lymphadenopathy), 40 B(III) are diagnosed to 15 patients, but in 62 cases repeated phase of affection A(III). 63 of 254 got infected by injecting narcotics intravenously. 132 were heterosexuals patients and got infected through intercourse, but 59 had homosexual contacts with HIV infected partners.

1. The basic scheme ART is included in the therapy scheme: NNRTI +2NRTI-EFV+3TC/AZT (*Efavirenz+Lamivudine*/*Azidothymidine*) - EFV+ ABC/ 3TC (*Efavirenz +Abacavir*/*Lamivudine*)
2. Or PI/+2NRTI-SQV+RTV+3TC/AZT (*Saquinavir*/*Ritonavir+ Lamivudine*/ *Azidothymidine*)
3. CD4+ lymphocytes and HIV virus load immunologic parameters that were obtained were used for monitoring when observing the patients for 24-48 weeks.

**Therapy efficiency criterion:**

1. HIV virus RNA load <400 kop/ml – in 16–24 weeks;
2. CD4+ cell increase by 30–70 š/mm³ during the first three months, by 100-150 š/mm³ within a year;
3. After three months therapy there were no ne opportunistic diseases.

Before the therapy CD4+ cell number median for all patients was 155 c/mm³, but HIV virus RNA load median – 55 thousand copies/ml.

HLA II class haplotype distribution analysis was performed in the group to be researched (Annex No. 31 *Tab. 5.45*). When researching HLA II class haplotypes, it was concluded that the greatest credible association with high immunologic efficiency is peculiar to haplotypes HLA-DRB1*/DQB1*/ DQA1*01/0602-8/0103; *01/0301/0102; *06/0602-8/0102, incidence

frequency (gf=0,36/0,09). After 12 therapy weeks amount of CD4+ lymphocytes in the particular group increased to 600-700 cells in one μl – HIV virus RNA load decreased by 5 thousand copies per ml, after 24–48 therapy weeks – lymphocyte CD4+ increased to 806-900 c/mm$^3$, (by 450–500 c/mm³) and HIV virus load RNA decreased <400 copies per ml (decrease by 20–30 thousand copies per ml). These data testifies of efficient ART. HIV infection clinic progression (aggravation of latent opportunistic infection) was not observed with any patient during the treatment in the groups to be researched with the existing haplotypes.

Association with low immunological efficiency was registered to haplotypes: HLA- DRB1*/DQB1*/DQA1*02/0301/0301; *03/0501/0201; *03/0301/0501; *07/0301/0201; *05/0301/0501; *02/0302/0102, incidence frequency (gf=0,03/ 0,04/ 0,05).

Treatment of patients with the particular haplotype facilitates also gradually increase of CD 4+ cell amount in blood and decreases HIV RNA in the HIV/AIDS infected patient group to be researched. After 12 week treatment a trend of increase of CD 4+ cell amount was formed, however the increase was slight, at average 50-100 cells per 1 μl, but HIV virus RNA decreased at average by 2000 copies per ml. Besides, high enough parameters remained also after 24-48 therapy weeks (55 thousand copies per ml). Aggravation of latent opportunistic infections were registered in the groups to be researched with the particular haplotypes, but after 12 weeks after the beginning of ART – side effects (hypersensitivity, diarrhoea, vomiting, etc.) (*Tab. 11, figure 8.)* For 55 (21%) of 254 patients to whom monitoring was applied, treatment was ineffective. Besides, for 29 (11%) patients cause of ineffective treatment was bad susceptibility of the particular therapy, in the result – drug dependence. 11 patients interrupted the treatment in a month, but 15 patients gad no noticeable

positive dynamics during three months that is mainly connected with non-observance of medicine usage regimen.

*Table 11.*

**Connection between the more efficient therapy and HLA-DRB1*/DQB1*/DQA1* haplotypes in the group of HIV/AIDS infected patients**

| DRB1* & DQB1* & DQA1 | Patients together N = 254 | Start of therapy | | in 12 weeks | | in 24-48 weeks | |
|---|---|---|---|---|---|---|---|
| | | Number of CD4 cells c/mm³ | HIV virus RNA load copies/ml | Num. of CD4 cells c/mm³ | HIV virus RNA load copies/ml | Num. of CD4 cells c/mm³ | HIV virus RNA load copies/ml |
| *01/0602-8/ 0103 *03/0301/0201 | 51/0,36 | 140 | 46000 | 961 | 5000 | 598 | <400 |
| *01/0301/0102 *06/0602-8 /0102 | 13/0,09 | 234 | 38000 | 940 | 2000 | 809 | < 40-100 |
| *02/0301/0301 *03/0501/0201 | 7/0,03 | 210 | 37000 | 270 | 32000 | 298 | >5000 |
| *03/0301/0501 *07/0301/0201 | 8/0,04 | 80 | 70000 | 120 | 64000 | 155 | 56000 |
| *05/0301/0501 *02/0302/0102 | 10/0,05 | 110 | 66000 | 99 | 60000 | 104 | 50000 |

*N – number of patients; Only credible results are shown p<0,005*

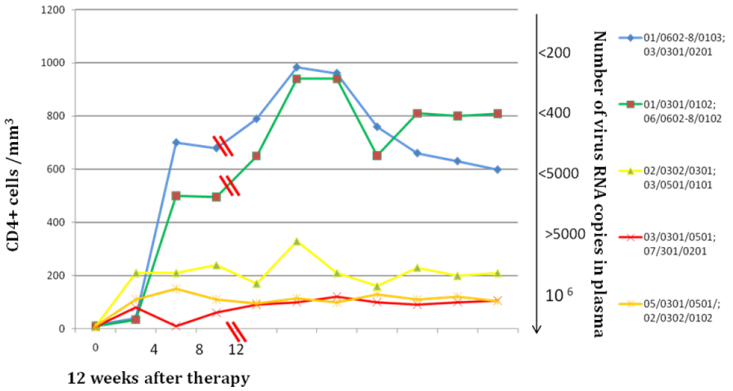



**Figure 8. Monitoring. Immunologic efficiency curve of antiretroviral therapy for the group of HIV infected patients (N=254)**

In general from the obtained data it is to be concluded that correlation between HLA DRB1*/DQB1*/DQA1 *01/0628/0103; *01/0301/0102; *06/0602-8/0102 haplotypes and an efficient basic scheme (HIV RNA level decreased and is less than 400 copies per 1 ml, but the number of median in CD4+ lymphocytes increased by 600 cells per 1 ml).

Meaning of haplotype HLA DRB1*/DQB1*/DQA1*02/0301/0301; *03/0501/0201; *03/0301/0501; *07/0301/0201; *05/0301/0501; *02/0302 /0102 forecast testifies of less efficient response to ART basic scheme. Side effects (digestive tract), aggravation of latent opportunistic infection were established that are connected with low immunologic parameters (CD4+ lymphocytes 250-300 c/mm³; HIV virus RNA load 55 thousand copies per ml).

These results are very important when researching polymorphism of HLA II genes and characterize them as the main “fighters” with infectious agents.

When determining HLA II class haplotypes, a successful therapy can be achieved only partially – the results can be evaluated in combination other successful solutions. Perhaps in future before prescription of different therapies it shall be possible to introduce compulsory determination of HLA types.

## Conclusions

1. 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).
2. Genetic markers of immunologic alleles upon development of HIV infection – HLA DRB1*03; 05; 07; HLA- DQA1*0101;0201;0301; 0601; HLA-DQB1* 0302; 0501; 0303; 0304, as well as resistance markers connected with slow development of HIV infection – HLA DRB1*01; 04;06; HLA- DQA1*0103;0401;0501; HLA-DQB1*0301; 0303;0401-2; 0601; 0602-8 are determined in different groups of patients.
3. High risk markers in case of HIV infection development belonging to the following groups of alleles: HLA-DRB1*03, DRB1*05, DQA1*0101; 0301 un DQB1*0501; 0302, as well as three-loci haplotypes HLA-DRB1*03/DQB1*0501/DQA1*0101; HLA-DRB1*05/DQB1*0301/ DQA1 *0501, DRB1*01 DQB1*0302/ DQA1*0301 un DRB1*01/DQB1*0501/ DQA1*0101 are determined.
4. Resistance to HIV infection development forms in the following groups of alleles: HLA-DRB1*01; 06, HLA-DQB1* 0301; 0602-8; HLA-DQA1*0102; 0103, as well as in haplotypes HLA- DRB1*01/ DQB1*0602-8/ DQA1*0102; HLA- DRB1*06/DQB1*0602-8/ DQA1*0102; HLA-DRB1*01/DQB1*0301/ DQA1*0102; and HLA- DRB1*06/DQB1*0602-8/ DQA1*0102 in different groups of HIV/AIDS patients.
5. According to the obtained results it can be concluded that that nucleotide sequence conformation in exon 2 causes changes of aminoacids in HLA molecules. These changes can affect the main function of molecule – affixation and presentation of viral peptide. It is regarded that fulminant development of syndrome complex AIDS can be connected with the change

of aminoacids in codon 10 (Gln→Tyr), 12 (Lys→Thr), 26 (Leu→Tyr), 32 (Tyr →His), 47 (Tyr →Phe), 87 (Gly →Val).

6. The results of many years long observations approve the correlation between the number of HIV virus RNA copies in plasma (HIV virus load), number of CD4+ lymphocytes in subpopulation peripheral blood and HLA II class haplotypes in HIV/AIDS patients.

   Fulminant development and its quickly progressing process associates with the following haplotypes: HLA – DRB1*01 DQB1*0302/ DQA1*0301; HLA–DRB1*01 DQB1*0501/ /DQA1*0101;HLA-DRB1*02/DQB1*0302/ DQA1*0102;HLA-DRB1*02/DQB1*0302/ DQA1*0301; HLA-DRB1*02/ DQB1*0501/DQA1*0101; HLA-DRB1*03/DQB1*0501/ DQA1*0101; HLA-DRB1*05/DQB1*0301/ DQA1*0501. Moderate HI virus activity and its favourable progress of disease associates with the following haplotypes: HLA- DRB1*01/ DQB1*0602-8/DQA1*0102; HLA-DRB1*06/DQB1* 0602-8/DQA1*0102; HLA-DRB1*01/DQB1*0301/ DQA1*0102; and HLA- DRB1*06/DQB1*0602-8/ DQA1*0102 in different groups of HIV/AIDS patients.

7. In general, from the obtained data correlation between the following haplotypes can be concluded: HLA DRB1*/DQB1*/DQA1 *01/0602-8/0103; *01/0301/0102; *06/0602-8/0102 haplotypes and an efficient basic scheme (HIV RNA level decreased and is less than 400 copies per 1 ml, but the number of median in CD4+ lymphocytes increased by 600 cells per 1 ml). Meaning of haplotype HLA DRB1*/ DQB1*/DQA1*02/0301/0301; *03/0501/0201; *03/0301/0501; *07/ 0301/0201; *05/0301/0501; *02/0302/0102 forecast testifies of less efficient response to ART basic scheme.

8. The role of the main histocompatibility complex is clarified that enables marker functions and that can be used in the additional prognostic

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

9. The produced paper proved that the efficiency of immune response depend on particular HLA II class haplotype that also approves the hypothesis about influence of haplotype marker on the immune response function.

## Practical recommendations

- To apply DNA typing and sequencing method as the latest instrument in diagnostics of diseases.
- Determination of HLA haplotypes is recommended before different forms of therapy are prescribed.
- Research of HLA immunogenetic markers shall ensure an option to order an appropriate and efficient basic scheme of ART and to take preventive measures. They shall have great social significance and apparent economical effect.

## List of abstracts and conference reports

1. Eglīte J, Kovalčuka L, Kasjko D, Stūre G, Bekmane U, Sočņevs A, Vīksna L. HLA-DRB1*0101 exon 2 structure polymorphism's study in-patients with HIV/AIDS" Journal of Antivirals & Antiretrovirals, Journal of AIDS & Clinical Research USA.2011,3:4; ISSN:1948-5964, 227 p.
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1. Eglīte J, Kovalčuka L, Kasjko D, Stūre G, Bekmane U, Sočņevs A, Vīksna L. Antiretrovīrusu terapijas (ART) efektivitātes salīdzinājums ar dažādiem HLA II klases haplotipiem. RSU Zinātniskās konferences tēzes, 2011. 183. lpp.
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1. Stenda referāts „Second exon gene DRB1*0101 polymorphism in HIV infected persons in AIDS or AIDS-Related Complex (ARC).” International conference on Virolory " Novel Therapeutic Strategies inVirology".05-07.09. 2011 g Baltimora (ASV).
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