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Mutations in HLA DRB1*0101 Exon 2 in a Sub-population of Latvia

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Authors' contributions

Author DK designed the study, DNS purification from blood samples, HLA DRB1 typing, wrote the protocol, and wrote the first draft of the manuscript. Author EE performed the statistical analysis and HLA DRB1 TYPING, managed the literature searches, wrote draft of the manuscript. Authors VJ and ED managed the literature searches. Authors GS and LV gathered and formed a group of patients. Author AS managed the analyses of the study. Author LK performed DNS purification from blood samples, managed the literature searches, wrote draft of the manuscript. All authors read and approved the final manuscript.

Case Study

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ABSTRACT

Aims: To find out whether ongoing missense mutations in the exon 2 of DRB1*01:01 affect the operation of this protective allele in HIV patients.
Place and Duration of Study: the Clinical Immunology and Immunogenetics Laboratory of Riga Stradiņš University, Riga Eastern Clinical University Hospital, "Infectology Center of Latvia", between May 2006 and December 2011.
Methodology: The study includes 200 HIV-infected patients. DNA was isolated from venous blood samples using the Qiagen QIAamp DNA kit reagents and the exon 2 nucleotide sequence of HLA was determined by the automatic sequencing – "Big Dye Terminator mix" (*Applied Biosystems*, USA). Statistical analysis was performed using Microsoft Excel, DOS *StatCalc* programs. The significance of the differences in

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indicators was evaluated according to reliability p≤0.05. The odds ratio was calculated according to Wolf's method.
Results: We found missense: at codon 47– in 80% of cases; at codon 67– in 20% of cases; at codon 75 – in 11% of cases; at codon 82– in 10% of cases; at codon 86– in 10% of cases (p<0.05) (See Table 3). One of the HIV patients had a STOP-codon (codon 13). Besides, a balance between nucleotide transversion and transition has been observed, suggesting mutations in the exon 2 (transversion in a human genome is rare) (OR 0.05, 95% CI 0.00-0.053).
Conclusion: The results of the study are not complete in order to be able to say conclusively that the existing mutations in the exon 2 of HLA-DRB1 *01:01 gene cause wrong immune response, thus the protective functions of this allele are not fulfilled. For a fuller understanding of the importance of ongoing mutations in the exon 2 in the development of HIV/AIDS, it is necessary to increase the study group.

Keywords: HLA; DRB1; HIV; AIDS; exon 2.

1. INTRODUCTION

The major histocompatibility complex (MHC) is a term widely used in transplantation [1]. It describes a group of genes that are inherited and in most cases of their incompatibility tissues are rejected. The human leukocyte antigen (HLA) is the histocompatibility complex in humans and it helps to recognize and present antigens [2]. It is located in chromosome 6 and contains most of genes that are responsible for the realization of the immune system functions in a human body [3,4]. HLA molecules are divided into three classes. Classes I and II are responsible for the presentation of antigen, but class III produces some of proteins of the complement system [5,6].

Each person has a unique HLA complex of antigens that is inherited from both parents. Two main classes I and II code receptors on the surface of cells that presents pathogenic – derivate peptides to T-cells. HLA class I antigens are located on almost all nucleated cells [7], while HLA class II molecules are located on antigen-presenting cells [8].

There is a connection between HLA – antigen and its genetic predisposition to various diseases. For example, HLA B27 antigen has been identified in 85% of patients with ankylosing spondylitis and Reiter's syndrome [9]. HLA DR3, DR4 antigens have been identified in more than 95% of patients with Type 1 diabetes [10]. There are other autoimmune diseases that are related to this (Graves syndrome, rheumatoid arthritis, multiple sclerosis, etc.).

HLA-DRB1 locus of HLA class II at the moment is considered to be the most polymorphic region of the human genome, because several variations of these alleles have been found. According to the IMGT/HLA database, 2 065 alleles of HLA class II are detected, 1 317 of which are HLA-DRB1 [11]. There are studies that demonstrate the protective activity of the DRB1, thus a disease might develop slower or it does not develop at all [12, 13]. The results of our laboratory studies prove the same, which is — the allele HLA-DRB1 *01:01 has protective qualities for patients infected with HIV. Thus the development of the complex of syndromes lasts longer than in patients who do not have this allele. However in our previous HLA and HIV researches in combinations with several variants of alleles that are located in HLA locus, the allele HLA-DRB1 *01:01 does not perform its protective functions [14]. So it might seem that the allele loses its protective function in combination with other alleles and it

is no longer able to fully carry out its functions, or the problem is in the structural changes in the HLA-DRB1*0101 gene. After several of our researches and taking into account the structure of this gene, it was found that probably the structural changes in the HLA gene promotes an inadequate and incorrect binding of a peptide and presentation to T-cells. It is known that the formation of the molecule of HLA class II α chain and β chain is coded directly due to the HLA-DRB1*, DQA1*, and DQB1* genes of the exon 2. The exon 2 in HLA class II is responsible for the creation of a peptide-binding groove in *β*1 and *α*1 domains. Any mutations that change the sequence of amino acids in the region, thus creating incorrect binding of peptides and their presentation, leads to disorders in the functions of the immune system. Or it is possible that in some variants the peptide binding to the antigen is stronger than in others. In our case, we wanted to clarify the role of the amino acid replacement in the HLA-DRB1*0101 gene and whether it affects a more rapid progression of AIDS in the HIV patients.

2. MATERIAL AND METHODS

This is the pilot study of HLA DRB1*01:01 exon 2 in a sub-population of Latvia. The study includes 200 HIV-infected patients from Riga Eastern Clinical University Hospital, “Infectology Center of Latvia”. The biological material used for the study is stored in the Clinical Immunology and Immunogenetics Laboratory of Riga Stradiņš University. All of the patients chosen for this study are in the AIDS stage of HIV infection.

Patients for the study that have been selected:

1. HIV-1 infected patients – women and men over 18 years of age;
2. HIV-1 infected patients with all stages of HIV-1 infection;
3. HIV-1 infected patients with various sources of infection.

Patient exclusion criteria:

1. Under 18 years of age;
2. Pregnant women;
3. Patients who are in custody or in detention before trial;
4. Patients who are continuously abroad or work abroad;
5. Post-splenectomy patients or patients who use glucocorticoids;
6. Patients who are not inhabitants or citizens of Latvia;
7. Non-compliant patients;
8. Patients that are over 18 years of age, but have acquired HIV infection through vertical transmission;
9. Patients with HIV-2 infection.

Table 1. Description of the population of study and analysable subgroups

Characteristic quantity	Unit of measurement, presentation mode	Value (%)
G-1 group, n=100	patients for whom sustained remission was observed for longer than 6 years	(50%)
G-2 group, n= 65	patients for whom in the result of treatment remission was observed for longer than 6 years	(32.5%)
G-3 group, n= 35	patients who were registered with fulminant AIDS syndrome	(17,5%)
Total AIDS group n=200	All HIV patients in AIDS stage	(100%)

n=number of patients

DNA was isolated from venous blood samples using the QiagenQIAamp DNA kit reagents. Its quality and quantity was tested with Qubit ® fluorometer (Invitrogen, USA). The alignment of nucleotide sequence of the exon 2 of HLA-DRB1*01:01 was determined by the method of sequencing. "Genetic Analyzer 3130 XL", and "GeneAmp® PCR System 9700" (Applied Biosystems, USA) programmable thermostats were used for the system of the sequencing analysis.

The exon 2 nucleotide sequence of HLA was determined by the automatic sequencing – "Big Dye Terminator mix" (Applied Biosystems, USA).

Primers were used in PCR reaction: PCR matrix is human genomic DNA (50ng/µl).

Primers used for the amplification:

RBSeq1 - 5'–tcccagtgcccgcacccc -3' – forward primer (18 nt)

RBSeq2 - 5'–gagctgggaatctgagtgtgt -3' – reverse primer (21 nt)

RBSeq3 -5'–tcagtgtcttctcaggaggc -3' – sequencing primer (20 nt)

Statistical analysis was performed using Microsoft Excel and DOS *StatCalc* programmes. The significance of the differences in indicators was evaluated according to reliability $p \leq 0.05$. The odds ratio was calculated according to Wolf's method. 200 HIV-infected patients was compared with the reference sequence ORIGIN (270 bp) 8157..8426 – the allele of the exon 2 of DRB*01:01 (see Table 2.) was made by using Contig Express (*Invitrogen*, USA) computer programme and IMGT/HLA database (the International ImMunoGeneTics database) [15].

Table 2. Reference sequence ORIGIN (270 bp) 8157..8426 – the exon 2 of HLA–DRB1*01:01:01

Bp	Sequence
1	cacgtttcctgtggcagcctaagagggagtgtcatttcttcaatgggacggagcgggtgc
61	ggttcctggacagatacttctataaccaggaggagtccgtgcgcttcgacagcgacgtgg
121	gggagttccgggcggtgacggagctggggcggcctgacgctgagtactggaacagccaga
181	aggacctcctggagcagaggcgggccgcggtggacacctactgcagacacaactacgggg
241	ttggtgagagcttcacagtgcagcggcgag

3. RESULTS AND DISCUSSION

3.1 Results

Taking into account the incidence of nucleotide polymorphism in the exon 2 of HLA-DRB1*01:01 gene, the "hottest spots" of missense were found in 200 patients of the study. Missense: at codon 47– in 80% of cases; at codon 67– in 20% of cases; at codon 75 – in 11% of cases; at codon 82– in 10% of cases; at codon 86– in 10% of cases (p=.05) (See Table 3). One of the HIV patients had a STOP-codon (codon 13). Besides, a balance between nucleotide transversion and transition has been observed, suggesting mutations in the exon 2 (transversion in a human genome is rare) (OR 0.05, 95% CI 0.00-0.053).

Looking at the results it can be noticed that half of all the positions are invariable, but some of the codons of DRB1*01:01 can code as many as 7 amino acids. These changes alter polypeptide to be coded for an HLA molecule that presents it on the surface of antigen-presenting cells.

The table represents only the results and values that are statistically significant.

Table 3. The incidence of amino acid polymorphism in the exon 2 of HLA-DRB1*01:01 gene in HIV patients of various groups

Codon No.	**9**	**10**	**11**	**12**	**13**
Ref.amino acid	Trp	Gln	Leu	Lys	Phe
Positions	UGG	CAG	CUU	AAG	UUU
Missense	Glu	Tyr	*	*	Ser
G-1, n=100	0.52	*	*	*	0.5
G-2, n=65	*	*	*	*	0.71/0,28/p=.03
G-3, n=35	*	*	*	*	0.8
Gn=200	0.72	*	*	*	0.9

Codon No.	**16**	**18**	**23**	**25**	**26**
Ref.amino acid	His	Phe	Arg	Arg	Leu
Positions	CAU	UUC	UGG	CGG	UUG
Missense	*	*	*	*	Tyr
G-1, n=100	*	*	*	*	0.8/0,20/p=.04
G-2, n=65	*	*	*	*	0.71/0,15/p=.01
G-3, n=35	*	*	*	*	0.9
Gn=200	*	*	*	*	0.95

Codon No.	**28**	**30**	**31**	**32**	**34**
Ref.amino acid	Glu	Cys	Ile	Tyr	Gln
Positions	GAA	UGC	AUC	UAU	CAA
Missense	*	Tyr	*	His	*
G-1, n=100	*	0.95/ 0,53/p=.01	*	*	*
G-2, n=65	*	*	*	0.29/,0,32/p=.02	*
G-3, n=35	*	*	*	*	*
Gn=200	*	0.97	*	*	*

Codon No.	**37**	**38**	**40**	**41**	**42**
Ref.amino acid	Ser	Val	Phe	Asp	Ser
Positions	UCC	GUG	UUC	GAC	AGC
Missense	*	Leu	*	*	*
G-1, n=100	*	*	*	*	*
G-2, n=65	*	*	*	*	*
G-3, n=35	*	0.22	*	*	*
Gn=200	*	*	*	*	*

Codon No.	**44**	**47**	**48**	**57**	**58**
Ref.amino acid	Val	Tyr	Arg	Asp	Ala
Positions	GAC	UAC	CGG	GAU	GCC
Missense	*	Phe	*	*	*
G-1, n=100	*	*	*	*	*
G-2, n=65	*	*	*	*	*
G-3, n=35	*	0.8/2,33/p=.04 3	*	*	*
Gn=200	*	*	*	*	*

Codon No.	**60**	**65**	**67**	**69**	**70**
Ref.amino acid	Tyr	Lys	Leu	Glu	Gln
Positions	CGA	GAA	CCU	GAG	CAG

Missense	*	Agr	Ile	*	*
G-1, n=100	*	*	*	*	*
G-2, n=65	*	*	*	*	*
G-3, n=35	*	*	0.2/1,44/p=.02	*	*
Gn=200	*	*	*	*	*

Codon No.	**71**	**72**	**73**	**74**	**75**
Ref.amino acid	Arg	Arg	Ala	Ala	Val
Positions	AGG	CGG	GCC	CGC	GUG
Missense	*	*	*	*	Gly
G-1, n=100	*	*	*	*	*
G-2, n=65	*	*	*	*	*
G-3, n=35	*	*			0.11/4,53/p=.05
Gn=200	*	*	*	*	*

Codon No.	**77**	**78**	**82**	**86**
Ref.amino acid	Thr	Tyr	His	Val
Positions	ACC	UAC	CAC	GUU
Missense	Asn	*	Tyr	Ala
G-1, n=100	*	*	*	*
G-2, n=65	0.43/0,74/p=.01	*	*	*
G-3, n=35	*	*	0.1/1,39/p=.02	0.1/2,65/p=.04
Gn=200	*	*	*	*

**does not show statistically significant results, due to the small number of patients*
The numbers have been indicated in a successive order gf/OR/ p<0.05
gf=incidence of allele
OR=odds ratio

This is a preliminary study, for more reliable results it is necessary to compare HIV-positive patients with HIV-negative patients.

3.2 Discussion

The immune system plays a vital role in full viability and interaction with people. The exon 2 of HLA system, which clearly has a critical function in the realization of the immune response, has not yet been fully studied and understood. The studies on the exon 2 of HLA-DRB1 gene, which is responsible for the creation of a peptide-binding groove, binding of the peptide to an antigen and its presentation to T-lymphocytes, could form a "base" for the explanations of the formation mechanism of various diseases. As known, various mutations in these genes caused wrong immune response; in some cases it may even be absent [16].

In the study by *Puerto et al*. in which groups of patients with chronic Chagas disease, caused by a protozoan parasite (*Trypanosomacruzi*), were studied, it was concluded that the patients with the HLA-DRB1*01, HLA-B14 alleles have a lower risk of infection with this disease. As well as, they have a smaller chance of developing a megacolon (toxic megacolon) [17]. The study by *Motta et al*. in which 54 HIV-1 positive patients and 57 healthy patients were studied showed that HLA-DRB1*01 allele was most commonly found in the group of healthy people, but it was rare in HIV positive patients [18]. Also the study that was carried out in Finland and in which 98 patients with sarcoidoses were surveyed showed that HLA-DRB1*01:01 has protective functions [19]. Our studies that were carried out in the Clinical Immunology and Immunogenetics Laboratory of Riga Stradiņš University showed that in HIV positive patients HLA-DRB1*06 (13;14) (0,0.05/0,014, OR=0,30, p= .000); DRB1*04 (0,05/0,11, OR=0,46, p= .000); DRB1*01 (0,09/0,16, OR=0,55, p= .000) DRB1*08 (0,02/0,05, OR=0,44, p= .003)

alleles are less common and are protective in this group of patients. These patients had a longer period of the development immunodeficiency and transition into fulminant form [14].
A recent study of the exon 2 of DRB1 gene that was carried out in cooperation between the Swedish and Finnish scientists showed an increased frequency of transition (T <-> C, A <-> G) and transversion (T <-> A, T <-> G C <-> A, C <-> G) [20].

Our study shows that missense mutations in most cases are found at codon 47 – in 80% of cases; at codon 67 – in 20% of cases; at codon 75 – in 11% of cases; at codon 82 – in 10% of cases; at codon 86 – in 10% of cases (p= .05). This kind of study cannot be found in other literature data and is at its very beginning.

4. CONCLUSION

It has been found that amino acid replacement could increase the risk of faster development of HIV than AIDS. After the assessment of the risk and ratio of the protective alleles, it could be possible to determine for each individual which patient is predisposed to faster development of AIDS and this in turn would determine which patient needs to begin the treatment sooner, thus improving the quality of life and prolong survival.

The results of the study are not complete in order to be able to say conclusively that the existing mutations in the exon 2 of HLA-DRB1 *01:01 gene cause wrong immune response, thus the protective functions of this allele are not fulfilled.

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CONSENT

All authors declare that ‘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 No. A-14 and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist

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