

# HLA class II DRB1, DQA1, DQB1 loci in patients with HIV infection and tuberculosis in a Latvian cohort group

ALENA SOHA<sup>1</sup>, INGA AZINA<sup>2</sup>, BAIBA ROZENTALE<sup>2</sup>, KSENIJA KRAMICHA<sup>3</sup>, GUNTA STURE<sup>2,4</sup>, OKSANA SAVICKA<sup>5</sup>, GALINA TITOVICA<sup>6</sup>

<sup>1</sup>Doctoral studies, Riga Stradins University, Riga, Latvia

<sup>2</sup>Latvian Centre of Infectious Disease, Riga East University Hospital, Riga, Latvia

<sup>3</sup>Postdoctoral studies, Riga Stradins University, Riga, Latvia

<sup>4</sup>Department of Internal Diseases of Riga East University Hospital, Riga Stradins University, Riga, Latvia

<sup>5</sup>Infectology Centre of Latvia, National Microbiology Reference Laboratory, Riga East Clinical University Hospital, Riga, Latvia

<sup>6</sup>Daugavpils Regional Hospital, Daugavpils, Latvia

## Abstract

**Introduction:** Until the COVID-19 pandemic, tuberculosis (TB) was the leading cause of death from a single infectious agent, ranking above HIV/AIDS. It is also the key cause of death among people infected with HIV. Tuberculosis incidence in Latvia has decreased by 25% during the last 30 years, but the mortality level of TB remains significant. The HLA class II genes are responsible for antigen presentation and regulation of immune responses, which plays an important role in individual susceptibility to infection disease. Whether or not differential HLA polymorphism contributes to TB with HIV infection and TB without HIV infection in Latvian patients is unknown.

**Material and methods:** For the detection of HLA class II DQA1, DQB1, and DRB1 alleles a total of 616 subjects were enrolled, including 80 primary active TB (PATB) patients, 168 HIV-1/TB patients, 168 HIV-1 patients and 200 HC individuals.

**Results:** For immunodeficiency caused by TB, HIV-1 or HIV-1/TB coinfection, alleles DRB1\*12:01, 14:01, 16:01, DQA1\*01:02, 01:03, 02:01, 06:01, DQB1\*03:03, 06:01 are identified as protective, but DRB1\*07:01, 11:01, 15:01, DQA1\*02:01, 03:01, DQB1\*03:01, 05:01 are identified as risk alleles.

**Conclusions:** The results of our experimental pilot studies demonstrated that HLA class II genes may contribute to the genetic risk of TB and HIV-1/TB co-infection, possibly by reducing the presentation of protective *Mycobacterium tuberculosis* antigens to T-helpers. It is necessary to conduct repetitive, multicentre, and large sample studies in order to draw more scientific conclusions and to confirm the relationship between TB, HIV and HIV-1/TB co-infection susceptibility and gene polymorphisms.

**Key words:** tuberculosis, HIV-1, HLA class II, HLA polymorphism.

(Cent Eur J Immunol 2024; 49 (1): 1-8)

## Introduction

Until the COVID-19 pandemic, tuberculosis (TB) was the leading cause of death from a single infectious agent, ranking above human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) [1]. An increasing frequency of multidrug-resistant tuberculosis (MDR TB) and the advance of the HIV epidemic can be mentioned among the reasons of this unfavourable situation [2]. It is also the key cause of death among people infected with human HIV [3].

In 2021, there were an estimated 1.4 million deaths among HIV-negative people and 187,000 deaths among

HIV-positive people, for a combined total of 1.6 million. An estimated 10.6 million people fell ill with TB in 2021, an increase of 4.5% from 10.1 million in 2020. The TB incidence rate rose by 3.6% between 2020 and 2021 [1].

Tuberculosis incidence in Latvia has decreased by 25% during the last 30 years, but the mortality level of TB remains significant. 403 new cases of tuberculosis were recorded only in 2019, 323 new cases in 2020, and 238 new cases in 2021 [4, 5].

Tuberculosis and HIV infection create a synergistic effect on the organism, which impairs the effect of therapy and leads to the patient's death [2].

Correspondence: Alena Soha, Riga Stradins University, Doctoral studies, Latvia, e-mail: [alena.soha@gmail.com](mailto:alena.soha@gmail.com)

Submitted: 25.08.2023, Accepted: 24.02.2024

New cases of HIV-1/TB coinfections increased by 40% in 2018-2019, highlighting the need for testing, counselling and rapid treatment, along with additional assessment of various biomarkers for the patient's condition [2].

Since its discovery by Zinkernagel and Doherty in the 1970s, classical human leukocyte antigen (HLA) has stood out as the leading candidate for infectious disease susceptibility [6]. HLA is an important type of protein for recognition of both foreign and host cells by the immune system and represents the key system by which "self" is distinguished from "non-self" in the human body [7].

The HLA gene is located on the short arm of the 6<sup>th</sup> chromosome, is about 4000 kilobases in length, contains 224 loci, and encodes a series of complex markers on the surface of the cell membrane [8].

In this work we consider DQA1, DQB1, and DRB1 subregions of the HLA class II genes. The HLA class II genes are mainly responsible for antigen presentation and regulation of immune responses, which play an important role in individual susceptibility to an infection disease [9].

In this study we review the available information about host genetic factors in TB, HIV-1 infection and HIV-1/TB coinfection that helps in understanding the development of susceptibility to TB and to identify the presence of risk alleles and protective alleles in patients with immunodeficiency states as a result of HIV-1, TB infections and HIV-1/TB co-infection. Defining the host genetic factors is critical to develop host-directed therapies that may enhance host defences and perhaps reduce tissue damage from chronic TB infections [10].

HLA genes involved in the immune response are the most numerous and the most diverse in the human genome, reflecting evolutionary advantages of a diverse immunological response to a wide range of infectious pathogens [11-13].

There are currently many studies reporting positive genetic associations between classical HLA loci, major bacterial infectious diseases (leprosy and tuberculosis) and parasitic infections (malaria, leishmaniasis, echinococcosis, and schistosomiasis), for which a great number of studies indicate that HLA association may be real [14]. A recent review summarized 15 genes with solid replicable associations, although many other variants have been reported [15]. The majority of these 15 gene variants code HLA, where variants in HLA-DRB1 are especially well represented [15].

The role of HLA in the incidence of TB, mainly in the form of a strong association with the HLA class II alleles in a strain-specific manner, has been demonstrated [16]. For example, a study in Thailand showed that HLA-DRB1\*09:01 and HLA-DQB1\*03:03 alleles are highly involved in the susceptibility to modern strains of TB [16]. A recent study in Uganda suggested an important role of the HLA-DQB1\*03:03 allele in the development of TB [18]. Several other studies in South-East Asia have shown

that HLA II alleles are important in terms of susceptibility to TB [13].

In Central Asia, HLA-DRB1\*08:01, HLA-DRB1\*08:03, HLA-DQA1\*03:02, HLA-DQA1\*03:03, and HLADQB1\*06:01 alleles were discovered to be alleles associated with the risk of TB [18]. Among Korean TB patients, HLA-DRB1\*08 and HLA-DQB1\*06 alleles also have demonstrated an increased risk of TB [19]. In the Northwest region of Russia, the HLA-DRB1\*04 allele was identified as a risk allele of TB [20]. In Iran, TB is associated with HLA-DB1\*07 and HLA-DQA1\*01:01 [21]. In Poland, TB developed most often in people with HLA-DRB1\*16:01 and HLA-DQB1\*05:02 alleles [22]. In Italy and in Portugal the risk increased with the presence of the HLA-DRB1\*04 [23] and the HLADRB1\*14 allele [24] respectively.

The results of large-scale genome-wide association studies for TB are pending, and it will be of interest to see whether HLA associations are sufficiently strong to achieve significance in this context [13]. HLA may definitely be among the factors affecting susceptibility to TB, considering that antigen presentation by MHC II molecules is an inductor of a specific immune response [14].

Whether or not differential HLA polymorphism contributes to TB with HIV infection and TB without HIV infection in Latvian patients is unknown. Here, we conducted a cohort study in Latvia of newly diagnosed individuals with active pulmonary TB, pulmonary TB with HIV-1 infection, HIV-1 infection without TB and healthy controls.

This study identified markers of genetic predisposition to TB, HIV-1 and HIV-1/TB coinfection in Latvia.

## Material and methods

### Ethical approval

The study protocol was approved by the Riga Stradiņš University (RSU) Ethics Committee of the Faculty of Medicine on 27 September 2012 and the Ethics Committee of the Riga East Clinical University Hospital (RAKUS) in October 2017 (No. 9-A/17), and the Latvian Central Medical Ethics Committee, Riga, Latvia (approval code No. 01-29.1/14) for the genetic analysis. Written informed consent was obtained from all study participants or their legal representatives before enrolment.

### Study design and procedures

A retrospective study was jointly conducted at the Joint Laboratory of Clinical Immunology and Immunogenetics of RSU (RSU/JLCII), RAKUS, Latvian Centre of Infectious Diseases (LIC), and Daugavpils Regional Hospital (DRH) between 2014 and 2019.

Four groups were included in the study. The first group comprising patients with TB (TB,  $n = 80$ ) was represented

by DRH. The second group included HIV-1/TB patients (HIV-1/TB,  $n = 168$ ) and the third group of subjects with HIV-1, but without TB (HIV-1,  $n = 168$ ), was represented by RAKUS, LIC. For the fourth, control group, data on 200 healthy individuals (HC,  $n = 200$ ) were obtained from the gene bank of RSU/JLCII.

All subjects enrolled in the study met certain eligibility criteria:

- permanent residence in Latvia,
- no relatives in the group,
- children under the age of 18 were excluded,
- for the control group, healthy donors, third-generation residents of Latvia.

### Study population

- The first group (primary active TB – PATB) included 80 patients with bilateral, bacteriologically confirmed, drug-susceptible primary TB pneumonia with no HIV infection. Individuals newly diagnosed with active TB were recruited prior to the initiation of TB treatment.
- The second group (HIV-1/TB group) was the target sample of 168 HIV-1 positive patients co-infected with TB.
- The third group (HIV-1 group) included 168 patients with HIV-1 infection without TB.

To confirm HIV-1/TB infections and repeatedly diagnosed TB the Abbott RealTime HIV-1 test (Germany) was performed. Diagnosis of pulmonary TB was confirmed by the chest X-ray radiography and positive bacteriological sputum culture (GeneXpert MTB/RIF, Cepheid, France). To diagnose extrapulmonary and miliary tuberculosis chest and abdominal computed tomography, pleural puncture, and bacteriological urine culture were performed. In patients of the HIV-1/TB group, TB was diagnosed within an interval from 0 to 16 years after detection of HIV-1 infection (mean interval =  $6 \pm 5$  years). All patients with HIV-1 infection and TB received antiretroviral therapy and anti-tuberculosis therapy in accordance with the European Guidelines for treatment of HIV infected adults [25].

- The fourth group (healthy control – HC) was represented by samples of 200 healthy individuals, without active TB or HIV. HC group subjects had to be asymptomatic and have a negative QuantiFERON-TB Gold Plus test [26] at the time of the study, as well as a standard chest X-ray Qiagen, Latvia.

### DNA extraction and HLA-II typing

For the detection of HLA class II alleles 5 ml of peripheral blood was collected into EDTA-containing tubes and stored at  $-20^{\circ}\text{C}$  until detection. DNA was extracted from stored blood samples using the QIAamp DNA kit (Qiagen, Germany). The extracted DNA was quantified by spectrophotometry using the Nanophotometer NP80 (Implen GmbH, Germany).

DNA amplification was performed by polymerase chain reaction (“low resolution” PCR-SSP) using sequence-specific primers with a DTLite thermocycler [27]. HLA typing included identification of 13 alleles of HLA-DRB1, 8 alleles of HLA-DQA1, and 12 alleles of HLA-DQB1.

The reaction mixture was then subjected to 35 amplification cycles, each comprising denaturation at  $94^{\circ}\text{C}$  (60 s), followed by one cycle, then annealing the mixture at  $94^{\circ}\text{C}$  (20 s),  $67^{\circ}\text{C}$  (2 s) followed by seven cycles and an extension at  $93^{\circ}\text{C}$  (5 s),  $65^{\circ}\text{C}$  (4 s), with a final extension step consisting of 35 cycles.

Associations of DRB1, DQB1 and DQA1 alleles among patient groups were examined individually using the  $\chi^2$  test ( $p$ -value  $< 0.05$ ). Odds ratios (OR) were calculated using EPI INFO software version 6 with 95% confidence intervals and Fisher correction for small numbers [28].

### Results

A total of 616 subjects were enrolled, including 80 PATB patients, 168 HIV-1/TB patients, 168 HIV-1 patients and 200 HC individuals (Table 1).

Associations were found between gender, mean age, and TB infection. It was observed that males were more exposed to TB than females ( $p < 0.0001$ ). When comparing the mean ages between the four groups (TB, HIV-1/TB, HIV-1 and HC), older people (mean age of 32 years) were found to be infected with TB and HIV-1/TB more frequently than the younger ones (mean age of 27 years) ( $p$ -value = 0.0015 and 0.0003 respectively).

Polymorphism of HLA class II was assessed by the comparison of observed frequencies of alleles in the four study groups.

Protective and risk effects in the HLA-DRB1 locus are presented in the Table 2.

The alleles HLA-DRB1\*07:01, 11:01, 15:01 were identified as risk alleles in PATB, HIV-1/TB, HIV-1

**Table 1.** Clinical and demographic information on patients of total research groups

Parameters	Patient group			
	Primary active TB (PATB)	HIV-1/TB patients	HIV-1 patients	Healthy individuals (HC)
Number of samples analysed per HLA (HLA-DRB1/DQA1/DQB1)	80	168	168	200
Mean age	50 (18-85)	41 (23-59)	41 (18-65)	31 (18-45)
Gender (male/female)	51/29	119/49	117/51	140/60

**Table 2.** Phenotypic distribution of the DRB1 alleles within different TB groups

HLA-DRB1 Alleles/patients groups	Number of alleles, <i>n</i> (phenotypic frequency %)				<i>p</i> -values	OR-TB	OR-HIV/TB	OR-HIV
	Primary active TB (PATB), <i>n</i> = 80	HIV-1/TB patients, <i>n</i> = 168	HIV-1 patient, <i>n</i> = 168	Healthy individuals (HC), <i>n</i> = 200				
01:01	18 (0.22)	55 (0.33)	59 (0.35)	52 (0.26)	0.299	0.83	1.39	1.54
03:01	24 (0.30)	42 (0.25)	44 (0.26)	46 (0.23)	0.004	1.43	1.12	1.19
04:01	12 (0.15)	21 (0.13)	20 (0.12)	48 (0.24)	0.02	0.56	0.45	0.43
07:01	13 (0.16)	21 (0.13)	14 (0.08)	8 (0.04)	0.02	4.66	3.43	2.18
08:01	4 (0.05)	7 (0.04)	8 (0.05)	24 (0.12)	0.018	0.39	0.32	0.37
09:01	2 (0.02)	4 (0.02)	5 (0.03)	4 (0.02)	0.839	1.26	1.2	1.5
10:01	1 (0.01)	3 (0.02)	6 (0.04)	10 (0.05)	0.138	0.24	0.35	0.7
11:01	35 (0.44)	57 (0.34)	44 (0.26)	30 (0.15)	0.002	4.41	2.91	2.01
12:01	4 (0.05)	6 (0.04)	15 (0.09)	36 (0.18)	0	0.24	0.17	0.45
13:01	6 (0.08)	37 (0.22)	31 (0.18)	22 (0.11)	0.031	0.66	2.29	1.83
14:01	6 (0.08)	0	10 (0.06)	32 (0.16)	0	0.43	0	0.33
15:01	34 (0.42)	81 (0.48)	59 (0.35)	30 (0.15)	0	4.19	5.28	3.07
16:01	1 (0.01)	2 (0.01)	21 (0.13)	58 (0.29)	0	0.03	0.03	0.35

**Table 3.** Phenotypic distribution of the DQA1 alleles within different TB groups

HLA-DQA1 Alleles/patients groups	Number of alleles, <i>n</i> (phenotypic frequency %)				<i>p</i> -values	OR-TB	OR-HIV/TB	OR-HIV
	Primary active TB (PATB), <i>n</i> = 80	HIV-1/TB patients, <i>n</i> = 168	HIV-1 patients, <i>n</i> = 168	Healthy individuals (HC), <i>n</i> = 200				
01:01	31 (0.39)	82 (0.49)	91 (0.54)	58 (0.29)	0.299	1.55	2.33	2.89
01:02	22 (0.27)	59 (0.35)	68 (0.40)	86 (0.43)	0.004	0.5	0.72	0.9
01:03	11 (0.14)	14 (0.08)	8 (0.05)	32 (0.16)	0.02	0.84	0.48	0.26
02:01	20 (0.25)	39 (0.23)	30 (0.18)	48 (0.24)	0.02	1.06	0.96	0.69
03:01	24 (0.30)	43 (0.26)	41 (0.24)	54 (0.27)	0.018	1.16	0.93	0.87
04:01	6 (0.07)	10 (0.06)	7 (0.04)	14 (0.07)	0.839	1.08	0.84	0.58
05:01	44 (0.55)	89 (0.53)	91 (0.54)	96 (0.48)	0.138	1.32	1.22	1.28
06:01	2 (0.02)	0	0	12 (0.06)	0	0.4	0	0

groups compared to HC group, and HLA-DRB1\*12:01, 14:01, 16:01 were identified as protective alleles in these groups. These alleles in our region occur in patients with an immunodeficiency state caused by HIV-1, TB infection, or HIV-1/TB co-infection.

However, the allele HLA-DRB1\*07:01 is overrepresented in the PATB group (16%, OR = 4.66) and HIV-1/TB group (13%, OR = 3.43) as compared to the HIV-1 group (8%, OR = 2.18) and HC group (4%). The allele DRB\*11:01 seems to be more significant in the group PATB since it is present in 44% of the patients in that group (OR = 4.41), as compared to 34% and 26% for HIV-1/TB and HIV-1 groups, respectively.

The allele HLA-DRB1\*14:01 is underrepresented in the HIV-1/TB group (0%) as compared to PATB (8%) and

HIV-1 (6%). The allele HLA-DRB1\*16:01 is overrepresented in the HIV-1 group (21%) as compared to PATB (1%) and HIV-1/TB (1%).

Protective and risk effects in the HLA-DQA1 locus are presented in the Table 3.

The most prevalent was the DQA1\*05:01 allele with a frequency of 52% (320/616), the DQA1\*01:01 allele with a frequency of 42.5% (262/616) and the DQA1\*01:02 allele with a frequency of 38.1% (235/616) (Table 3). The HLA-DQA1\*06:01 allele was underrepresented in the PATB, HIV-1/TB, HIV1 groups compared to HC (*p*-value 0.001).

The alleles HLA-DQA1\*01:02 and 02:01 were identified as protective alleles in PATB, HIV-1/TB, HIV-1 groups compared to the HC group. The allele HLA-

**Table 4.** Phenotypic distribution of the DQB1 alleles within different TB groups

HLA-DQB1 Alleles/patients groups	Number of alleles <i>n</i> (phenotypic frequency %)				<i>p</i> -values	OR-TB	OR-HIV/TB	OR-HIV
	Primary active TB (PATB), <i>n</i> = 80	HIV-1/TB patients, <i>n</i> = 168	HIV-1 patients, <i>n</i> = 168	Healthy individuals (HC), <i>n</i> = 200				
02:01-2	34 (0.43)	54 (0.32)	47 (0.28)	64 (0.32)	0.299	1.57	1.01	0.83
03:01	33 (0.41)	71 (0.42)	68 (0.40)	78 (0.39)	0.004	1.1	1.14	1.06
03:02	11 (0.14)	27 (0.16)	27 (0.16)	26 (0.13)	0.02	1.07	1.28	1.28
03:03	5 (0.06)	8 (0.05)	9 (0.05)	26 (0.13)	0.02	0.45	0.33	0.38
03:04	2 (0.02)	2 (0.01)	2 (0.01)	4 (0.02)	0.018	1.26	0.59	0.59
03:05	0	1 (0.01)	2 (0.01)	0	0.839			
04:01-2	10 (0.13)	14 (0.08)	11 (0.07)	14 (0.07)	0.138	1.9	1.21	0.93
05:01	25 (0.31)	57 (0.34)	76 (0.45)	44 (0.22)	0.002	1.61	1.82	2.93
05:02-4	8 (0.10)	25 (0.15)	28 (0.17)	24 (0.12)	0	0.81	1.28	1.47
06:01	3 (0.04)	4 (0.02)	2 (0.01)	22 (0.11)	0.031	0.32	0.2	0.1
06:02-8	29 (0.36)	73 (0.43)	64 (0.38)	98 (0.49)	0	0.59	0.8	0.64

DQA1\*01:02 is underrepresented in the PATB group (27%) as compared to HIV-1/TB (35%) and HIV-1 (40%).

The allele HLA-DQA1\*03:01 was identified as a risk allele in the PATB group compared to HIV-1/TB and HIV-1 groups. The difference in the distribution of this allele between PATB, HIV-1/TB and HIV-1, HC groups is insignificant, which does not allow us to make any conclusions as to possible associations.

Protective and risk effects were also identified in the HLA-DQB1 locus (Table 4).

In PATB, HIV-1/TB, HIV-1 groups, HLA class II DQB1\*03:01, 05:01 alleles were found more frequently than in the HC group, and these alleles were identified as risk alleles for immunodeficiency, while the DQB1\*03:03

and 06:01 alleles were less frequent, and these alleles were identified as protective for immunodeficiency.

The risk allele DQB1\*05:01 is more prevalent in the HIV-1 group since it is present in 45% of the patients in that group, as compared to 31% and 34% for PATB and HIV-1/TB groups, respectively.

The HLA-DQB1\*03:04 allele was detected less frequently in HIV-1/TB and HIV-1 groups than in the PATB group. This allele is identified as a risk allele for TB infection. The HLA-DQB1\*05:02-4 allele was detected less frequently in the PATB group than in HIV-1/TB and HIV-1 groups, and was identified as protective against TB infection.

As seen in Table 5 on immunodeficiency caused by TB, HIV-1 or HIV-1/TB coinfection, alleles DRB1\*12:01,

**Table 5.** Distribution of protective and risk alleles of HLA class II in PATB, HIV-1/TB, HIV-1 and HC groups

Variable	Risk alleles	Protective alleles
	DRB1	DRB1
PATB vs. HC ( <i>n</i> = 80 vs. <i>n</i> = 200)	07:01, 11:01, 15:01	12:01, 14:01, 16:01
HIV-1/TB vs. HC ( <i>n</i> = 168 vs. <i>n</i> = 200)	07:07, 13:01, 15:01	12:01, 14:01, 16:01
HIV-1 vs. HC ( <i>n</i> = 168 vs. <i>n</i> = 200)	15:01	04:01, 08:01, 12:01, 14:01, 16:01
	DQA1	DQA1
PATB vs. HC ( <i>n</i> = 80 vs. <i>n</i> = 200)	02:01; 03:01	01:02
HIV-1/TB vs. HC ( <i>n</i> = 168 vs. <i>n</i> = 200)		01:03
HIV-1 vs. HC ( <i>n</i> = 168 vs. <i>n</i> = 200)		01:03
	DQB1	DQB1
PATB vs. HC ( <i>n</i> = 80 vs. <i>n</i> = 200)	03:04	03:03, 05:02-4, 06:01, 06:02-8
HIV-1/TB vs. HC ( <i>n</i> = 168 vs. <i>n</i> = 200)	03:01, 05:01	03:03, 03:04, 06:01, 06:02-8
HIV-1 vs. HC ( <i>n</i> = 168 vs. <i>n</i> = 200)	03:01, 05:01	03:03, 03:04, 06:01, 06:02-8

14:01, 16:01, DQA1\*01:02, 01:03, 02:01, 06:01, DQB1\*03:03, and 06:01 are identified as protective, but DRB1\*07:01, 11:01, 15:01, DQA1\*02:01, 03:01, DQB1\*03:01, and 05:01 are identified as risk alleles.

## Discussion

The HLA system plays a critical role in regulating the immune response, tissue or organ transplantation, autoimmunity, vaccine development, susceptibility or resistance disease, and pharmacogenomics [29].

The HLA alleles are variable and polymorphic, and individuals with different HLA genotypes may trigger different immune responses against pathogens [30].

Different HLA antigens seem to influence the variability of disease progression in patients from different ethnic backgrounds [12].

Studies show that the risk of developing TB in patients with HIV-1 depends to a great extent on HLA class II genes regulating the process of antigen presentation to CD4<sup>+</sup> T lymphocytes [6-8, 31-33]. For example, in patients with HIV-1, TB was associated with the HLADRB1\*10 allele in Brazil [31], the 11 allele in Mexico [6], 13, 15, and 16 alleles in India [8, 32], and 13 and 15 alleles in Ukraine [33]. The HLA-DQB1\*05 allele is associated with a higher risk of TB in patients with HIV-1 in Brazil [31] and the 06 allele in India [32]. In addition, protective alleles in the genotype are associated with a decreased risk of HIV-associated TB. In the Ukrainian patients' group, protection against TB was related to HLADRB1\*01, 04, 07, and 11 alleles [34]. Considering that antigen presentation by MHC II molecules is an inductor of the specific immune response [35], it would be valuable to assess the relationship between HLA class II alleles and the immune system reaction of patients with immunodeficiency caused by TB, HIV-1 or HIV-1/TB coinfection.

The HLA class II region contributes to the genetic risk of TB, possibly through the reduced presentation of protective *Mycobacterium tuberculosis* antigens to T helper cells [14].

The comparison of the results with other studies [6, 7, 31-33] shows the protective effect of HLA-DRB1\*01 revealed in Ukraine [34]. Considering the geographical closeness of Latvia and Ukraine, this allele can be considered as a factor of resistance against tuberculosis in patients in Eastern Europe. HLA-DQB1\*05:02 and HLADQB1\*06:02 alleles are risk factors for developing tuberculosis coinfection in Brasilia [31] and North India [32]. But in our study the HLADQB1\*06:02 allele is identified as a protective allele for TB, HIV-1 and HIV-1/TB co-infection. The observed incompatibility of the results can be explained by more pronounced differences between the populations.

In the long run, we expect that this knowledge will constitute part of a medical future where detailed genet-

ic information can contribute to targeted prognostics and therapeutics [36].

To minimize the differences in ethnic and geographic characteristics we have selected for our research groups the population of one and the same region that does not have any family ties. For the control group we used a group of healthy subjects living in this region for the third generation. Accordingly, the polymorphism of the distribution of alleles of the studied genes is typical for our region. The accumulation of certain genetic variants found in TB studies is likely the result of evolutionary selection, contributing to the resistance to TB in our region.

According to Kasjko *et al.* research 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; and 06\*02-8 are considered to be "protective" against HIV infection. These alleles provide a more effective presentation of the HIV epitope to CD4<sup>+</sup> T lymphocytes. As a result, the body's immune system fights the HIV infection more effectively [37].

In our study these alleles, HLA-DRB1\*04:01; HLA-DQA1\*01:03; HLA-DQB1\*03:04; 06:01; and 06:02-8, also are identified as protective alleles against HIV infection in HIV-1 and HIV-1/TB groups.

The conducted study indicated that the efficiency of the immune response depends on a particular HLA II class haplotype, and that also supported the hypothesis of the influence of a haplotype marker on immune response function [37]. In immunodeficiency states resulting from HIV-1 infection, TB infection, or HIV-1/TB co-infection, we found common genetic mechanisms in the form of common risk alleles and protective alleles. This indicates that HIV-1, TB and HIV-1/TB co-infection affect the immune response in a similar way.

HLA alleles, which are critical components of all foreign antigen presentation pathways, have been shown in previous studies to confer differential infection susceptibility and severity of the disease. For instance, the associations between HLA genotype and disease severity extend broadly to several viruses. For example, in HIV-1, certain HLA types (e.g. HLA-A\*02:05) may reduce the risk of seroconversion [38]. The influence of HLA supertypes on susceptibility and resistance to HIV-1 infection [38], and in dengue virus, certain HLA alleles (e.g. HLA-A\*02:07, HLA-B\*51), are associated with the increased secondary disease severity among ethnic Thais [39].

## Conclusions

Taken together, this research supports the idea that host genes play an important role for editing host regulation of TB, HIV and HIV/TB co-infection.

The results of our experimental pilot study demonstrated that HLA class II genes may contribute to the genetic risk of TB and HIV-1/TB co-infection, possibly by reduc-

ing the presentation of protective *Mycobacterium tuberculosis* antigens to T-helpers.

The susceptibility of TB and HIV-1 is correlated with various genes in multiple loci, and every single gene plays a certain unique role. But most of the effects in different subjects are inconsistent, which may be influenced by a variety of factors, the diverse source of cases, for example, and the different criteria for inclusion in the study group and the control group. What is more, a small sample size will also have a nonnegligible impact on the results. Therefore, it is necessary to conduct repetitive, multicentre, and large sample research to draw more scientific conclusions, to confirm the relationships between TB, HIV and HIV/TB co-infection susceptibility and gene polymorphisms, and much better clarify the immunopathological mechanisms of TB for a theoretical basis for prevention and treatment of TB in the context of HIV.

---

*The authors declare no conflicts of interest.*

## References

- World Health Organization (2022): Health Topics: Global Tuberculosis Report 2022. <https://www.who.int/publications/m/item/global-tb-report-2022-advocacy-toolkit>.
- ECDC (2020): Tuberculosis – Annual Epidemiological Report for 2020. European Centre for Disease Prevention and Control. <https://www.ecdc.europa.eu/en/publications-data/tuberculosis-annual-epidemiological-report-2020>
- Bell LCK, Noursadeghi M (2018): Pathogenesis of HIV-1 and Mycobacterium tuberculosis co-infection. *Nat Rev Microbiol* 16: 80-90.
- SPKC (2020): Infekcijas slimības – statistika un pētījumi: Pārskati par atsevišķām infekcijas un parazitārajām slimībām. <https://www.spkc.gov.lv/lv/media/15144/download?attachment>
- SPKC (2021): Infekcijas slimības – statistika un pētījumi: Pārskati par atsevišķām infekcijas un parazitārajām slimībām. <https://www.spkc.gov.lv/lv/media/17441/download?attachment>
- Parslow TG, Stites DP, Terr AI, Imboden JB (2001): *Medical Immunology*. McGraw-Hill/Appleton & Lange.
- Bodis G, Toth V, Schwarting A (2018): Role of human leucocyte antigens (HLA) in autoimmune diseases. *Rheumatol Ther* 5: 5-20.
- Cai L, Li Z, Guan X, Cai K, et al. (2019): The Research Progress of Host Genes and Tuberculosis Susceptibility. *Oxid Med Cell Longev* 2019: 9273056.
- Anaya J, Shoenfeld Y, Rojas-Villarraga A, et al. (2013): Autoimmunity from Bench to Bedside. El Rosario University Press, Bogota.
- Tobin DM (2015): Host-directed therapies for tuberculosis. *Cold Spring Harb Perspect Med* 5: a021196.
- Walsh E, Sabeti P, Hutcheson H, et al. (2006): Searching for signals of evolutionary selection in 168 genes related to immune function. *Hum Genet* 119: 92-102.
- Apanius V, Penn D, Slev RP, et al. (2017): The nature of selection on the major histocompatibility complex. *Crit Rev Immunol* 37: 75-125.
- Blackwell JM, Jamieson S, Burgner D (2009): HLA and infectious diseases. *Clin Microbiol Rev* 22: 370-385.
- Laivacuma S, Eglīte J, Derovs A, Viksna L (2019): Distribution of HLA allele frequencies in patients with cystic and alveolar echinococcosis in Latvia. *Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact, and Applied Sciences*. Riga; 296-303. doi: <https://doi.org/10.2478/prolas-2019-0047>
- Stein MC, Sausville L, Wejse C, et al. (2017): Genomics of human pulmonary tuberculosis: from genes to pathways. *Curr Genet Med Rep* 5: 149-166.
- Kone A, Diarra B, Cohen K, et al. (2019): Differential HLA allele frequency in *M. africanum* vs. *M. tuberculosis* in Mali. *HLA* 93: 24-31.
- Murugesan H, Paramasivam S, Bethunaickan R (2018): Influence of genetic polymorphism towards pulmonary tuberculosis susceptibility. *Front Med* 5: 213.
- Kuranov A, Kozhamkulov U, Vavilov M, et al. (2014): HLA-class II alleles in patients with drug-resistant pulmonary tuberculosis in Kazakhstan. *Tissue Antigens* 83: 106-112.
- Kim H, Park M, Song E, et al. (2005): Association of HLA-DR and HLA-DQ genes with susceptibility to pulmonary tuberculosis in Koreans: Preliminary evidence of associations with drug resistance, disease severity, and disease recurrence. *Hum Immunol* 66: 1074-1081.
- Starshinova A, Korneva N, Dovgalyuk I, et al. (2015): Influence of HLA-DRB1\* allelic sets on the development of tuberculosis in children. *Med Immunol (Russia)* 275. doi: 10.15789/1563-0625-2015-3-275-280
- Amirzargar A, Yalda A, Hajabolbaghi M, et al. (2004): The association of HLA-DRB, DQA1, DQB1 alleles and haplotype frequency in Iranian patients with pulmonary tuberculosis. *Int J Tuberc Lung Dis* 8: 1017-1021.
- Dubaniewicz A, Moszkowska G, Szezerkowska Z (2005): Frequency of DRB1-DQB1 two-locus haplotypes in tuberculosis: preliminary report. *Tuberculosis (Edinb)* 85: 259-267.
- Ruggiero G, Cosentini E, Zanzi D, et al. (2004): Allelic distribution of human leucocyte antigen in historical and recently diagnosed tuberculosis patients in Southern Italy. *Immunology* 111: 318-322.
- Duarte R, Carvalho C, Pereira C, et al. (2011): HLA class II alleles as markers of tuberculosis susceptibility and resistance. *Rev Port Pneumol* 17: 15-19.
- Ryom L, Cotter A, Miguel R, et al. (2020): European AIDS Clinical Society (EACS) guideline. 2019 update of the European AIDS Clinical Society Guidelines for treatment of people living with HIV version 10.0. doi: <https://doi.org/10.1111/hiv.12878>
- Products: TB testing with QFT-Plus (2023): <https://www.qiagen.com>.
- Equipment: DTLite Operation Manual. [https://dna-technology.com/dnaproducts/equipments/rtamplificators/dt\\_lite](https://dna-technology.com/dnaproducts/equipments/rtamplificators/dt_lite)
- Harbage B, Dean AG. (1999): Distribution of Epi Info software: An evaluation using the internet. *Am J Prev Med* 16: 314-317.
- Satapornpong P, Jinda P, Jantararoungtong T, et al. (2020): Genetic diversity of HLA class I and class II alleles in Thai populations: Contribution to genotype-guided therapeutics. *Front Pharmacol* 11: 78.

30. Calliope A, Petersen J, Rossjohn J, Fugger L (2018): HLA variation and disease. *Nat Rev Immunol* 18: 325-339.
31. Stanevicha V, Eglite J, Sochnevs A, et al. (2003): HLA class II associations with rheumatic heart disease among clinically homogeneous patients in children in Latvia. *Arthritis Res Ther* 5: 340-346.
32. Visnevskā M, Stanevica V, Eglite J, et al. (2018): Molecular genetic studies of human leukocyte antigen (HLA) class II alleles and clinical profile of acute rheumatic fever in Latvia. *Pediatr Rheumatol* 16. doi: <https://doi.org/10.1186/s12969-018-0265-6>.
33. Davidsons Z, Eglite J, Lazareva A, et al. (2016): HLA II class alleles in juvenile idiopathic arthritis patients with and without temporomandibular joint arthritis. *Pediatr Rheumatol Online J* 14: 24.
34. Volikova O. (2015): Correlation of locus alleles of DRB1 II class of histocompatibility complex with clinical, immunological and virological parameters in HIV infection. *Eur Sci Rev* 9-10: 48-51.
35. National Library of Medicine (2020): HLA-DRB1 – major histocompatibility complex, class II, DR beta 1. <https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=Details-Search&Term=3123>
36. Sia JK, Rengarajan J (2019): Immunology of Mycobacterium tuberculosis infections. *Microbiol Spectr* 7: 10.1128/microbiolspec.GPP3-0022-2018.
37. Kasjko D, Jasinskis V, Eglite J, et al. (2014): Research of HLA II class DRB1, DQA1, DQB1 genetic markers in patients with HIV infection and AIDS. *Br J Med Med Res* 4: 4482-4500.
38. MacDonald KS, Fowke K, Kimani J, et al. (2000): Influence of HLA supertypes on susceptibility and resistance to human immunodeficiency virus type 1 infection. *J Infect Dis* 181: 1581-1589.
39. Stephens HAF, Klaythong R, Sirikong M, et al. (2002): HLA-A and -B allele associations with secondary dengue virus infections correlate with disease severity and the infecting viral serotype in ethnic Thais. *Tissue Antigens* 60: 309-318.