sciendo

PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B,
Vol. 73 (2019), No. 2 (719), pp. 189–191.

DOI: 10.2478/prolas-2019-0030

Short communication

A PILOT STUDY ON MARKERS OF GENETIC PREDISPOSITION IN TUBERCULOUS PNEUMONIA PATIENTS IN LATGALE

Ksenija Kramiča[1,#], Jeļena Eglīte[2], Aleksandrs Koļesovs[3], Tatjana Kramiča[4], Gaļina Titoviča[5], Diāna Džeriņa[5], Glafira Nikolajeva[5], Ludmila Vīksna[6], and Oksana Koļesova[2]

[1] Rīga Stradiņš University, Doctoral Studies, 16 Dzirciema Str., LV-1007, Rīga, LATVIA

[2] Joint Laboratory of Clinical Immunology and Immunogenetics, Rīga Stradiņš University, 7 Rātsupītes Str., LV-1067, Rīga, LATVIA

[3] Department of Psychology, University of Latvia, 1 Imantas 7th line, Rīga, LATVIA

[4] T. Kramičas family doctor's practice, 31 Ventspils Str., LV-5404, Daugavpils, LATVIA

[5] Daugavpils Regional Hospital, 311 18. Novembra Str., LV-5413, Daugavpils, LATVIA

[6] Department of Infectology and Dermatology, Rīga Stradiņš University, 3 Linezera Str., Rīga, LV-1006, LATVIA

[#] Corresponding author, ksenija.kramica@gmail.com

Contributed by Ludmila Vīksna

Tuberculosis (TB) is still one of the top ten leading causes of death in the world. Compared to other Baltic and Eastern European countries, TB incidence (24.8 new cases per 100 000 people in 2017) in Latvia is relatively high. One of the regions with the highest TB incidence is Latgale (31.1 cases per 100 000 people). The aim of this pilot study was to identify markers of genetic predisposition to TB in Latgale. The study included 26 patients (16 males and 10 females) aged between 18 and 85 with bilateral TB pneumonia and without HIV infection. HLA typing was performed in HLA-DRB1, -DQA1*, and* -DQB1 *loci by a polymerase chain reaction with low resolution sequence-specific primers.* HLA-DRB1*07 *and* HLA-DRB1*11 *alleles were identified as risk alleles for TB.* HLA-DRB1*15 *allele was a protective allele. Due to the limitations of this exploratory study, a broader study needs to be conducted to revealing specific risk and protective HLA Class II alleles for TB in the subpopulation of Latgale.*

Key words: *HLA Class II, tuberculosis, Latgale.*

Despite 53 million saved lives in the last 20 years, tuberculosis (TB) remains one of the top ten leading causes of death in the world. TB incidence in Latvia is one of the highest among Baltic and Eastern European countries (Anonymous, 2017). According to statistical data, 24.8 new cases per 100 000 people were registered in Latvia in 2017. This is the lowest indicator since 1991. However, the mortality of TB has remained significant. One of the regions with the highest TB incidence is Latgale with 31.1 cases per 100 000 people (Anonymous, 2018).

The region of Latgale is located in the southeast part of Latvia. It borders the Russian Federation, Republic of Belarus, and Lithuania. Spanning a few centuries, many different ethnicities have lived in this region — Latvians, Russians, Belarusians, Poles, and other ethnic groups (Anonymous, 2002). The aim of this pilot study was to explore markers of genetic predisposition to TB in the region of Latgale.

Human Leucocyte Antigens (HLA) of the Major Histocompatibility Complex are among factors affecting susceptibility for TB. Findings have demonstrated that 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 (Sveinbjornsson *et al*., 2016).

In Central Asia, *HLA-DRB1*08:01, HLA-DRB1*08:03, HLA-DQA1*03:02, HLA-DQA1*03:03*, and *HLA-DQB1*06:01* alleles were discovered to be TB risk alleles (Kuranov *et al*., 2014). Among Korean TB patients, *HLA-DRB1*08* and *HLA-DQB1*06* alleles demonstrated also in-

Table 1

PROTECTIVE AND RISK ALLELES OF HLA CLASS II IN TB (n = 26) AND CONTROL (n = 100) GROUPS

Alleles	TB f_{rel}	Control f_{rel}	χ^2	p	OR	OR 95% CI	*p*
*HLA-DRB1*07*	0.23	0.04	10.28	0.001	7.20	(1.86; 27.88)	0.004
*HLA-DRB1*11*	0.56	0.12	22.08	0.000	8.56	(3.21; 22.77)	0.000
*HLA-DRB1*15*	0.12	0.39	7.00	0.008	0.20	(0.06; 0.73)	0.014

TB, tuberculosis; f_{rel}, relative frequency; OR, odds ratio; CI, confidence interval.

creased risk of TB (Kim *et al*., 2005). In the Northwest region of Russia, the *HLA-DRB1*04* allele was identified as a risk allele of TB (Starshinova *et al*., 2015). In Iran, TB is associated with *HLA-DB1*07* and *HLA-DQA1*01:01* (Amirzargar *et al*., 2004). In Europe, TB is associated with the presence of different alleles in the genotype. In Poland, TB developed most often in people with *HLA-DRB1*16:01* and *HLA-DQB1*05:02* alleles (Dubaniewicz *et al*., 2005); in Italy with *HLA-DRB1*04* (Ruggiero *et al*., 2004), and in Portugal, risk was increased with presence of the *HLA-DRB1*14* allele (Duarte *et al*., 2011). Therefore, different alleles are associated with TB in different populations. It should be noted that there is no available data regarding TB patients HLA Class II gene profile in Latvia and its regions.

This study started in October of 2017. Permissions of the Ethics Committee of Rīga East Clinical University Hospital and the Central Medical Ethics Committee, Rīga, Latvia, were obtained for the genetic analysis. Patients of the Lung Disease and Tuberculosis Ward of Daugavpils Regional Hospital participated in the study after signing an agreement. These patients formed the target sample of TB patients in the period between October 2017 and September 2018. Inclusion criteria were: 18 years of age or older, not pregnant, not incarcerated, confirmed pulmonary TB, and negative HIV1/2 test result.

The pilot study group was composed of 26 patients (16 males and 10 females) aged between 18 and 85 (mean age was 50). In all patients, bilateral TB pneumonia was confirmed. At the time of the study, 25 patients were undergoing first-line anti-TB therapy and one patient was undergoing second-line anti-TB therapy because of primary resistance of *Mycobacterium tuberculosis*. Among the patients, three afflicted relatives (two siblings and their parent) were detected. This factor was controlled at the step of the data analysis.

HLA typing was performed in *HLA-DRB1, -DQA1*, and *-DQB1* loci by a polymerase chain reaction with low resolution sequence-specific primers (DNA-Technology, Russia) according to the manufacturer instruction. DNA extraction was performed by using the QIAamp® DNA Blood Kit (QIAGEN, Germany) according to the manufacturer instruction. Amplification was performed using a thermocycler "DT-Lite" (DNA-Technology, Russia). For genetic analysis, 5 ml of blood with EDTA was used and stored at –20 °C. To determine risk and protective alleles of the *HLA Class II* gene, HLA-profiles of 100 people (without active TB) were used. This control group included blood donors without HIV infection, viral hepatitis C and cytomegalovirus infection aged from 18 to 65 years. No relatives were involved in this group. In both subsamples — clinical and control — the same protocol for DNA isolation and HLA typing was applied. The analysis was conducted in the Joint Laboratory of Clinical Immunology and Immunogenetics of Rīga Stradiņš University.

IBM SPSS 22.0 was used for the statistical analysis. Frequencies of alleles were compared using the Pearson's chi-square test. The Odds ratio (OR) was calculated with the Cochrane-Mantel-Haenszel test.

The research results are presented in Table 1. The results showed that *HLA-DRB1*07* (OR = 7.20) and *HLA-DRB1*11* (OR = 8.56) were presented more frequently in patients with TB than in the control group, while *HLA-DRB1*15* was presented less frequently in patients with TB than in the control group (OR = 0.20). *HLA-DRB1*07* and *HLA-DRB1*11* alleles were identified as risk alleles for TB. In contrast, the *HLA-DRB1*15* was protective allele. It should be noted that excluding the siblings from the analysis did not change the revealed effects of the alleles.

Two alleles concurred with the results of other studies. *HLA-DRB1*15* was a protective allele for the development of pulmonary TB among children in Northwest Russia (Starshinova *et al*., 2015). HLA-DRB1*07 was identified as a risk allele in a study on Iranian TB patients (Amirzargar *et al*., 2004). The *HLA-DRB1*07* allele was also among the risk alleles for Latvian Lyme disease patients and was strongly associated with the development of severe forms of neuroborreliosis (Kovalchuka *et al.*, 2016). Therefore, this allele is not specific for TB.

We did not find a negative effect of other alleles (*HLA-DRB1*08, *14, *16, HLA-DQA1*03, HLA-DQB1*05,* and **06*), which were described in other populations (Dubaniewicz *et al*., 2005; Kim *et al*., 2005; Duarte *et al*., 2011; Kuranov *et al*., 2014).

The effects of the alleles cannot be generalised to any population. In contrast to our study, *HLA-DRB1*07:01* was associated with a reduced incidence of pulmonary TB in Asian populations (Li *et al*., 2015), and *HLA-DRB1*15* was an allele responsible for susceptibility to TB in China (Wang *et al*., 2005), and in Central India (Mishra *et al*., 2014).

Despite a relatively high statistical significance, the exploratory study had an important limitation associated with the size of the clinical sample. Therefore, the results show only possible risk or protective alleles. Further study is needed for more precise assessment of the role of alleles, associated odds ratio, and confidence intervals. A region-specific control group should be included in the study assessing region-specific risk or protective alleles.

REFERENCES

Amirzargar, A. A., Yalda, A., Hajabolbaghi, M., Khosravi, F., Jabbari, H., Rezaei, N., Niknam, M. H., Ansari, B., Moradi, B., Nikbin, B. (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.

Anonymous (2002). Latgales Reģiona Attīstības Aģentūra. Par Latgali. Vispārīga informācija [Latgale Region Development Agency. About Latgale. General Information] (in Latvian). Available from: http://www.latgale.lv/lv/about/summary (accessed 17 October 2018).

Anonymous (2017). World Health Organization. Tuberculosis surveillance and monitoring in Europe 2017. Available from: https://ecdc.europa.eu/sites/portal/files/documents/ecdc-tuberculosis-surveillance-monitoring-Europe-2017-WEB.pdf (accessed 17 October 2018).

Anonymous (2018). Slimību profilakses un kontroles centrs. Statistika un pētījumi. Tuberkuloze [Center for Disease Prevention and Control. Statistics and studies. Tuberculosis] (in Latvian). Available from: https://www.spkc.gov.lv/lv/statistika-un-petijumi/infekcijas-slimibas/datu-vizualizacija/tuberkuloze (accessed 17 October 2018).

Duarte, R., Carvalho, C., Pereira, C., Bettencourt, A., Carvalho, A., Villar, M., Domingos, A., Barrosb, H., Marques, J., Pinho Costa, P., Mendonça, D., Martins, B. (2011). HLA class II alleles as markers of tuberculosis susceptibility and resistance. *Rev. Port. Pneumol.*, **17** (1), 15–19.

Dubaniewicz, A., Moszkowska, G., Szczerkowska, Z. (2005). Frequency of *DRB1–DQB1* two-locus haplotypes in tuberculosis: Preliminary report. *Tuberculosis,* **85**, 259–267.

Kim, H. S., Park, M. H., Song, E. Y., Park, H., Kwon, S. Y., Han, S. K., Shimet, Y.-S. (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.

Kovalchuka, L., Cvetkova, S., Trofimova, J., Eglite, J., Gintere, S., Lucenko, I., Oczko-Grzesik, B., Viksna, L., Krumina, A. (2016). Immunogenetic markers definition in Latvian patients with lyme borreliosis and lyme neuroborreliosis. *Int. J. Environ. Res. Public Health,* **13** (12), 1194.

Kuranov, A., Kozhamkulov, U., Vavilov, M., Belova, E., Bismilda, V., Alenova, A., Ismailov, S., Momynaliev, K. (2014). *HLA-class II* alleles in patients with drug-resistant pulmonar tuberculosis in Kazakhstan. *Tissue Antigens,* **83**, 106–112.

Li, C.-P., Zhou, Y., Xiang, X., Zhou, Y., He, M. (2015). Relationship of *HLA-DRB1* gene polymorphism with susceptibility to pulmonary tuberculosis: Updated meta-analysis. *Int. J. Tuberc. Lung Dis.,* **19** (7), 841–849.

Mishra, G., Kumar, N., Kaur, G., Jain, S., Tiwari, P. K., Mehra, N. K. (2014). Distribution of *HLA-A, B* and *DRB1* alleles in Sahariya tribe of North Central India: An association with pulmonary tuberculosis. *Infection, Genetics and Evolution*, **22**, 175–182.

Ruggiero, G., Cosentini, E., Zanzi, D., Sanna, V., Terrazzano, G., Matarese, G., Sanduzzi, A., Perna, F., Zappacosta, S. (2004). Allelic distribution of human leucocyte antigen in historical and recently diagnosed tuberculosis patients in Southern Italy. *Immunology*, **111**, 318–322.

Starshinova A. A., Korneva N. V., Dovgalyuk I. F., Pavlova M. V., Pavlova I. E., Bubnova L. N. (2015). Influence of *HLA-DRB1** allelic sets on the development of tuberculosis in children [Старшинова А. А., Корнева Н. В., Довгалюк И. Ф., Павлова М. В., Павлова И. Е., Бубнова Л. Н. Влияние аллелей генотипа *HLA-DRB1** на развитие туберкулёза у детей]. *Medical Immunology* [*Медицинская иммунология*], **17** (3), 275–280 (in Russian).

Sveinbjornsson, G., Gudbjartsson, D. F., Halldorsson, B. V., Kristinsson, K. G., Gottfredsson, M., Barrett, J. C., Gudmundsson, L. J., Blonda, K., Gylfason, A., Gudjonsson, S. A., Helgadottir, H. T., Jonasdottir, A., Jonasdottir, A., Karason, A., Kardum, L. B., Knežević, J., Kristjansson, H., Kristjansson, M., Love, A., Luo, Y., Magnusson, O. T., Sulem, P., Kong, A., Masson, G., Thorsteinsdottir, U., Dembic, Z., Nejentsev, S., Blonda, T., Jonsdottir, I., Stefansson, K. (2016). *HLA class II* sequence variants influence tuberculosis risk in populations of European ancestry. *Nat. Genet.*, **48** (3), 318–322.

Wang, J., Song, C., Wang, S. (2001). Association of *HLA-DRB1* genes with pulmonary tuberculosis. *Chinese J. Tuberc. Respir. Dis.* [*Zhonghua jie he he hu xi za zhi*], **24**, 302–305 (in Chinese).

Received 29 October 2018
Accepted in the final form 4 March 2019

ĢENĒTISKĀS PREDISPOZĪCIJAS MARĶIERU PILOTPĒTĪJUMS PACIENTIEM AR TUBERKULOZES PNEIMONIJU LATGALĒ

Tuberkuloze (TB) joprojām ir viens no desmit galvenajiem nāves cēloņiem pasaulē. Salīdzinot ar citām Baltijas un Austrumeiropas valstīm, TB incidence Latvijā ir samēra augsta (24,8 jauni gadījumi uz 100 000 iedzīvotājiem 2017. gadā). Savukārt, Latvijas reģions ar augstāku TB incidenci ir Latgale (31,1 jauni gadījumi uz 100 000 iedzīvotājiem). Pilotpētījuma mērķis bija izpētī TB ģenētiskās predispozīcijas marķierus Latgalē. Pētījuma izlasi veidoja 26 pacienti (16 vīrieši un 10 sievietes) vecumā no 18 līdz 85 gadiem ar abpusēju tuberkulozes pneimoniju un bez HIV infekcijas. HLA tipēšana *HLA-DRB1, -DQA1* un *-DQB* lokusos bija veikta ar polimerāzes ķēdes reakciju ar zemas rezolūcijas sekvences specifiskajiem praimeriem. Alēles *HLA-DRB1*07* un *HLA-DRB1*11* bija identificētas kā TB riska alēles, *HLA-DRB1*15* bija protektīva alēle. Pētījuma ierobežojumi rāda, ka ir jāveic plašāks pētījums, lai atklātu specifiskās TB riska un protektīvās HLA II klases alēles Latgales reģionā.