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Study of association between polymorphisms in the *PSMB5* (rs11543947) and *PSMA3* (rs2348071) genes and multiple sclerosis in Latvians

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Functional realization of many signalling proteins and transcription factors implicated in the development and progression of multiple sclerosis is mediated by proteasomes. ***Aim*** *of this case-control study was to evaluate genetic variations in the PSMB5 and PSMA3 genes encoding proteasomal subunits on the susceptibility to multiple sclerosis in Latvians.* ***Methods****. The rs11543947 (PSMB5) and rs2348071 (PSMA3) loci were genotyped in 291 multiple sclerosis patients and 305 healthy individuals and analysed general, subtype and sex-specific associations with the disease.* ***Results****. Loci rs11543947 and rs2348071 were identified as disease neutral and susceptible respectively. The rs2348071 heterozygous genotype GA showed strong main effect ($P < 0.001$; OR = 1.891, 95 % CI [1.360–2.628]), and moderate ($P < 0.01$; OR = 1.663, 95 % CI [1.152– 2.402]) and strong ($P < 0.001$; OR = 2.459, 95 % CI [1.534–3.943]) association with relapsing-remitting and secondary progressive phases of disease respectively. No genotype-sex interaction associated with multiple sclerosis has been detected.* ***Conclusions****. Our results suggest susceptibility of the rs2348071 heterozygous genotype to multiple sclerosis in Latvians.*

Keywords: chromosome 14q, proteasomal genes, SNPs, PSMB5, PSMA3, multiple sclerosis.

Introduction. Multiple sclerosis (MS) is the most common, extremely heterogeneous clinically, chronic inflammatory disease of the CNS affecting about 2.5 million people around the world (2500 of them in Latvia), presumably young adults, with onset usually at the second to fourth decade of life and, similarly to other autoimmune diseases, women being affected 3–4 times more frequent than men [1].

About 90 % of MS patients experience the relapsing-remitting MS course (RRMS), the majority of these patients enter a secondary progressive course (SPMS) and about 10 % of MS patients show a primary progressive MS form, characterized by the progression of neurological disability from onset [2]. The cause of MS is not clear. The disease develops in genetically susceptible individuals with contributions of environmental factors, such as infection, sunlight exposure, vitamin D deficiency [3, 4]. The MS susceptible loci had been identified in the regions containing genes with immune, co-stimulatory, signal transduction functions and related to vitamin D function [5–10].

Ubiquitin proteasome system (UPS) plays a crucial role in immunity and its disregulation and/or modulation may influence the MS development and progression. The 20S proteasome had been identified as a target of the humoral autoreactive immune response [11] and a major autoantigen in MS patients [12]. The proteolytic activities of proteasomes are reduced in brain tissue of MS patients [13]. The inhibition of proteasomes and lysosomal proteases involved in major histocompability complex II antigen presentation was shown to improve the MS therapeutic effect [11, 14].

Modulation of UPS efficiency could be influenced by polymorphisms in the genes encoding UPS related proteins. The immunoproteasome *PSMB9* codon 60HH variant was observed to have a reduced risk of developing MS in HLA-A*02 + Italian females [15].

Coexistence of autoimmune diseases recently evaluated statistically [16] suggests a possibility of their common origin. In fact, despite generally heterogeneous genetic architecture of the particular disease, some polymorphic loci were shown to be shared by MS and other autoimmune diseases including rheumatoid arthritis [6, 17, 18] and type 1 diabetes mellitus [19, 20].

Genetic variations in the 14q11-24 genes encoding proteasomal subunits were implicated previously in susceptibility to autoimmunity, type 2 diabetes mellitus, cardio-vascular disorders, and population adaptation to environment [21, 22]. It appears that there is a large potential for some of these mutations also to be associated with multiple sclerosis.

The aim of the current study was to genotype the rs11543947 and rs2348071 polymorphisms of the *PSMB5* and *PSMA3* genes encoding proteasomal proteins on the MS main, subtype and sex specific association in Latvian population.

Materials and methods. *Case-control study*. The case group consisted of 281 MS patients referred to the Latvian Maritime Medicine Center, Vecmilgravis Hospital. MS patients were diagnosed according to the revised 2010 McDonald criteria [23] and assigned to the RRMS (188 patients) and SPMS (93 patients) groups. Detailed description of the subject groups is given in Table 1. Total control group of 305 samples were represented by the two independent sample sets including the previously characterized [21, 22] first Latvian population study consisting of 191 samples and the second Latvian population study of 114 newly collected samples. Ethnic origin of the subjects was not recorded; all of them represent very mixed inhabitants of Riga, forming some «average» genotype for North-East Europe. The data on the rs11543947 and rs2348071 allele and genotype frequencies in the first Latvian population sample set were extracted from [21, 22] to be compared with the data on genetic diversity obtained for the second Latvian population study and then to be grouped providing a total control group of 305 healthy individuals. All controls were carefully assessed to exclude the diagnosis of any inflammatory disorders. The studies were approved by the Central Medical Ethics Commission of the Republic of Latvia Ministry of Health and informed consent was obtained from all the studies participants.

Table 1
Description of the sample collections

Group Subgroup	Sex	Number	Age ± SD (years)
Cases			
MS	Total	281	42.77 ± 11.10
	Females	199	43.21 ± 10.87
	Males	82	41.51 ± 11.65
RRMS	Total	188	38.80 ± 9.50
	Females	132	39.42 ± 9.47
	Males	56	37.30 ± 9.50
SPMS	Total	93	50.56 ± 9.88
	Females	67	50.61 ± 9.53
	Males	26	50.42 ± 10.92
Controls			
Common	Total	305	38.80 ± 10.54
	Females	179	38.78 ± 11.52
	Males	126	37.22 ± 9.33
First population	Total	191	54.80 ± 18.60
	Females	117	56.04 ± 19.84
	Males	74	53.56 ± 17.36
Second population	Total	114	21.20 ± 2.47
	Females	62	21.52 ± 3.19
	Males	52	20.88 ± 1.29

DNA extraction and genotyping. Genomic DNA was extracted from nucleated blood cells using a kit for the genomic DNA extraction («Fermentas», Lithuania) and from oral swabs using the salting out method [24]. The rs11543947 and rs2348071 genotyping technologies were the same as published previously [21, 22] including primer sequences, basic PCR procedure and analyses of amplified and digested products. For quality control, the 16 randomly chosen samples per each marker were genotyped in duplicate in different experiments. The concordance of genotyping was 100 %. The genotyping data were verified by direct sequencing of the corresponding DNA fragments in both directions

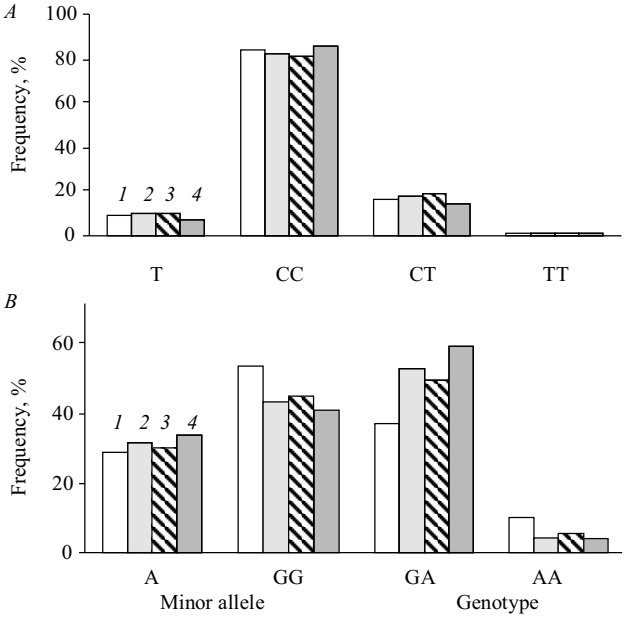
using the Applied Biosystems 3130*xl* Genetic Analyzer. Loci description and nucleotide numbering are given according to the recommended nomenclature system (http://www.genomic.unimelb.edu.au/mdi/mutnomen/recs.html). The chromosome 14 GRCh37.p5 assembly (NCBI reference sequence: NC_000014.8) sequence information was used for loci description.

Data analysis. Single locus genotypes and alleles' frequencies were estimated by direct gene counting. The deviation from the Hardy-Weinberg equilibrium (HWE) and differences between case and control groups in allele, genotype and haplotype frequencies were evaluated by χ^2 using XLSTAT 2013 software for Windows. The dominant, recessive, over dominant and multiplicative genetic models for every individual locus were designed according to [25] and analysed by using 2 × 2 contingency tables. The odds ratio (OR) more than 2 and less than 0.5 was considered to be clinically significant. Stratification was performed by MS courses and sex.

Results and discussion. In both MS and the two Latvian population cohorts, the genotyping call rate was 100 %. Both markers were found to be in HWE in controls ($c^2 = 2.72$; $p > 0.05$). Allele and genotype spectrums and distributions were found to be similar ($P > 0.05$) for the first (191 samples, [21, 22]) and the second (114 newly collected samples) Latvian population studies for each marker. The data of both population studies were grouped and total control group of 305 samples was used for further analysis. In cases the genotype distribution corresponded to HWE ($c^2 = 2.72$; $P > 0.05$) for rs11543947, however a strong deviation from HWE was observed for rs2348071 ($c^2 = 14.14$; $P < 0.01$). Figure illustrates SNPs the genetic diversity in cases and controls.

Depending on the transcript variant, the rs11543947 locus belongs to the *PSMB5* gene exon 1 (c. 70 C > T) or intron 1 (c. 112 + 300 C > T). The allele and genotype frequencies of this SNP were found to be similar for cases and controls, RRMS and SPMS cohorts (Figure, *A*) as well as for sexes (not shown). Similarly to current data, in Latvians this SNP did not show any association with juvenile idiopathic arthritis [22] and bronchial asthma in children (own unpublished data). However, this locus appears to be susceptible to familial obesity in Latvian children (own unpublished data).

The rs2348071 SNP is located in the intron 7 of the *PSMA3* gene (c. 543 + 138 G > A or c. 522 + 138 G > A



Allele and genotype presentation in MS patients and controls: *A* – rs11543947; *B* – rs2348071; (*1* – control; *2* – MS (multiple sclerosis); *3*, *4* – RRMS and SPMS (relapsing remitting and secondary progressive MS course respectively))

depending on the transcript variant) and strongly discriminates Asians having as major an ancestral allele A (about 70 %) and other ethnics having as major an allele G (about 70 %). Transition A → G happened in Caucasians about 15,000 years ago and was supported by positive selection in Caucasians over the world [21]. The allele frequencies observed in current study were similar for MS patients and controls, however genotype distribution was found to be significantly different (Figure, *B*). Both common and rare allele homozygotes appear to be MS protective, however, heterozygous GA genotype showed strong ($p < 0.001$) MS main effect, and strong and moderate association with the SPMS and RRMS respectively (Table 2).

Previously we have shown [21, 22] that nucleotide substitution at the rs2348071 may significantly change patterns of local targets for many regulatory molecules including proteins of CART, MEF2 and HBPX families known to mediate transcriptional control of neuronal differentiation and nucleo- and cytoplasmic ribonucleoprotein hnRNP A1 implicated in pathogenesis of many neurodegenerative diseases including multiple sclerosis [26–28]. Multiple sclerosis patients were shown to generate antibodies to hnRNP A1 [26]. This splicing signal affecting splicing and post-transcriptional modifi-

Table 2
Statistical result on the rs2348071 association with multiple sclerosis

Genetic model	Control	MS	RRMS	SPMS	P	OR [95 % CI]
	n = 305	*n* = 281	*n* = 188	*n* = 93		
GG *vs* AA + GA	161 *vs* 144	119 *vs* 162	85 *vs* 103	34 vs 59	$P_{MS} < 0.05$; $P_{SPMS} < 0.01$	$OR_{MS} = 0.657$ [0.474– 0.910]; $OR_{SPMS} = 0.515$ [0.320–0.829]
AA *vs* GG + GA	31 *vs* 274	14 *vs* 267	10 *vs* 178	4 *vs* 89	$P_{MS} < 0.05$; $P_{MS} < 0.001$	$OR_{MS} = 0.463$ [0.243–0.883]; $OR_{MS} = 1.891$ [1.360–2.628]
GA *vs* GG + AA	113 *vs* 192	148 *vs* 133	93 *vs* 95	55 *vs* 38	$P_{RRMS} < 0.01$; $P_{SPMS} < 0.001$	$OR_{RRMS} = 1.663$ [1.152–2.402]; $OR_{SPMS} = 2.459$ [1.534–3.943]

cation of majority of expressed genes in mammals, was shown to interact directly with PSMA3 proteins [29] and may be potentially involved in regulation of the *PSMA3* gene expression through the rs2348071 allele-specific targeting. In our previous studies, the rs2348071 heterozygotes were identified also as risk factor in Latvians for juvenile idiopathic arthritis [22], obesity in children with family history [30] and children bronchial asthma (own unpublished observation). In this study we have tried to analyse separately the groups of RRMS and SPMS patients, although the latter is considered to be the result of progression of the former. It should be mentioned that in some studies [31] the two forms appear to differ genetically. This provided background for the design of the study; in fact in our case the rs2348071 heterozygote genotype was stronger associated with SPMS than with RRMS (Table 2).

We suggest that the rs2348071 heterozygote genotype is a case of heterozygote disadvantage resulted from the imbalance of the locus functional capacity affecting human health possibly through the modulation and/or deregulation of the *PSMA3* gene expression, UPS functionality and network of different genes and proteins involved in pathogenesis of multiple sclerosis and other neurodegenerative and autoimmune diseases.

Conclusions. Our results suggest susceptibility of the rs2348071 heterozygous genotype to multiple sclerosis in Latvians.

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Оцінка асоціації поліморфізму генів *PSMB5* (rs11543947) і *PSMA3* (rs2348071) з розсіяним склерозом з-поміж жителів Латвії

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Резюме

Протеасоми опосередковують виконання функцій багатьох сигнальних білків і факторів транскрипції, залучених до розвитку розсіяного склерозу. ***Мета****. Оцінити можливу асоціацію генетичних варіантів генів PSMB5 і PSMA3 зі схильністю до захворювання на розсіяний склероз з-поміж жителів Латвії.* ***Методи****. Локуси rs11543947 (PSMB5) і rs2348071 (PSMA3) генотипували у 291 хворого на розсіяний склероз та у 305 здорових індивідів і оцінювали за асоціацією із захворюваністю на розсіяний склероз як такий, із підтипами хвороби і пов'язаною зі статтю асоціацією.* ***Результати****. Локус rs11543947виявився не пов'язаним з хворобою, а локус rs2348071 – асоційованим з нею. Гетерозиготний генотип ГА локуса rs2348071 тісно асоційований із захворюванням як таким (P < 0,001; співвідношення шансів (СШ) = 1,891; 95 % ДІ [1.360–2.628]), помірно асоційований (P < 0,01; СШ = 1,663; 95 % ДІ [1,152–2,402]) з ремітуючо-рецидивуючою формою захворювання та сильно (P < 0,001; СШ = 2,459; 95 % ДІ [1,534–3,943]) – зі вторинно прогресуючою формою. Пов'язаної з хворобою взаємодії між статтю і генотипом суб'єкта не відмічено.* ***Висновки****. Наші результати вказують на те, що жителі Латвії з гетерозиготним генотипом rs2348071 схильні до захворювання на розсіяний склероз.*

Ключові слова: хромосома 14q, протеасомні гени, однонуклеотидний поліморфізм, PSMB5, PSMA3, розсіяний склероз.

Оценка ассоциации полиморфизма генов *PSMB5* (rs11543947) и *PSMA3* (rs2348071) с рассеянным склерозом среди жителей Латвии

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Резюме

Протеасомы опосредуют выполнение функций многих сигнальных белков и факторов транскрипции, вовлеченных в развитие рассеянного склероза. ***Цель****. Оценить возможную ассоциацию генетических вариантов генов PSMB5 и PSMA3 с подверженностью заболеванию рассеянным склерозом среди жителей Латвии.* ***Методы****. Локусы rs11543947 (PSMB5) и rs2348071 (PSMA3) генотипи-*

ровали у 291 больного рассеянным склерозом и у 305 здоровых индивидов и оценивали по ассоциации с заболеваемостью рассеянным склерозом как таковым, подтипами болезни и связанной с полом ассоциации. ***Результаты****. Локус rs11543947 оказался не связанным с болезнью, а локус rs2348071 – ассоциированным с заболеванием. Гетерозиготный генотип ГА локуса rs2348071 тесно ассоциирован с заболеванием как таковым (P < 0,001; отношение шансов (ОШ) = 1,891, 95 % ДИ [1.360–2.628]), умеренно (P < 0,01; ОШ = 1,663, 95 % ДИ [1,152–2,402]) – с ремиттирующе-рецидивирующей формой заболевания и сильно (P < 0,001; ОШ = 2,459, 95 % ДИ [1,534–3,943]) – со вторично прогрессирущей формой. Связанного с болезнью взаимодействия между полом и генотипом субъекта не отмечено.* ***Выводы****. Наши результаты указывают на подверженность жителей Латвии с гетерозиготным генотипом rs2348071 заболеваемости рассеянным склерозом.*

Ключевые слова: хромосома 14q, протеасомные гены, однонуклеотидный полиморфизм, PSMB5, PSMA3, рассеянный склероз.

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