ASSOCIATION STUDY OF GENETIC VARIANTS IN THE 14q11 – 14q13 PROTEASOMAL GENES CLUSTER WITH JUVENILE IDIOPATHIC ARTHRITIS (JIA) IN LATVIAN POPULATION

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The possible role of proteasomes in the development of autoimmune diseases was hypothesised after discovery of the involvement of proteasomal LMP2 and LMP7 subunits in antigen processing. The objective of this study was to determine the association between allelic variants of the genes encoding proteasomal proteins PSME1, PSME2 and PSMA6 and juvenile idiopathic arthritis (JIA) in the Latvian population. One Indel G⁻⁴⁵⁴³CA⁻⁴⁵⁴⁴→GA and four SNPs related to the PSMA6 gene (A⁻²⁴⁸⁶→G and C⁻¹⁹¹⁰→T, upstream promoter, C⁻¹¹⁰→A of promoter, and C⁻⁸→G of 5'UTR), of two cSNP in PSME1 (G¹⁴⁵⁷→A:Val¹⁰⁴, exon 6 and C²⁵³⁶→A: Lys²⁴⁴→Thr, exon 11) and in PSME2 (C¹¹⁵³→G:Arg⁶¹→Gly, exon 4 and A¹⁴⁴⁰→C:His⁸⁹→Pro, exon 6) were genotyped by means of primer-specific PCR, CAPS assay and/or sequencing in case/control study composed from the 156 JIA patients and 214 healthy individuals. Allele frequency and genotype distribution was similar in cases and controls for Indel, and SNPs A⁻²⁴⁸⁶→G, C⁻¹⁹¹⁰→T and C⁻⁸→G of PSMA6, as well as for all studied cSNPs in PSME1 and PSME2 genes. Differences in A⁻¹¹⁰ allele and CG genotype frequencies were close to the statistically significant P level in JIA patients and healthy individuals, however, when an additive model was applied, the difference in the C⁻¹¹⁰→A locus turned out to be statistically significant. The results support the hypothesis of the possible association of PSMA6 gene allelic variants with JIA in the Latvian population.

Key words: PSMA6, chromosome 14, SNP polymorphism, juvenile idiopathic arthritis, association study.

INTRODUCTION

Juvenile idiopathic arthritis (juvenile rheumatoid arthritis, JIA) is the most prevalent paediatric rheumatic diagnosis among children in many countries (Glass and Giannini, 1999). JIA is a clinically heterogeneous group of phenotypes that have in common chronic inflammatory synovitis in children under the age of 16 (Pralhad *et al.*, 2001). Seven subtypes of JIA, based on the clinical characteristics at onset are distinguished; including the more frequent oligo, poly, psoriatic and systemic JIA variants (Petty *et al.*, 2004). The genetic contributions to disease risk have been observed in many case/control studies (Glass and Giannini, 1999; Prahalad *et al.*, 2001). A genome scan revealed linkage of JIA to the HLA region, additional evidence supporting linkage of JIA was observed at 1p36, 19p13, and 20q13 (Thompson *et al.*, 2004). However, the low odds ratios for

most individual “JIA risk alleles” suggest that gene–gene interactions, which are poorly described or understood at present, likely influence whether pathology develops (Glass and Giannini, 1999). This consideration encourages extension of the search for JIA candidate genes.

The chromosome 14q region appears to be prospective for the above goal, as it harbours several genes of interest. Linkage of the locus to autoimmune diseases has been already reported (John and Worthington, 2001). Possible candidate genes include the genes encoding the nuclear factor kappa B inhibitor (NFKBI) on 14q13 (le Beau *et al.*, 1992), the transcription factor FOS on 14q23, TGF-beta3 on 14q24, and immunoglobulin heavy chain locus at 14q32.33 (Kamnasaran and Cox, 2002). Several proteasomal genes are located at 14q including proteasomal activator subunits 1 and 2 (*PSME1* and *PSME2*, respectively) and proteasome

subunit of beta type 5 (*PSMB5*) at 14q11.2, proteasome subunits of alpha type 6 (*PSMA6*) at 14q13, and alpha type 3 (*PSMA3*) at 14q23 (Chistyakov *et al.*, 2000).

The proteasome is a multisubunit enzyme complex which plays a central role in the degradation of proteins controlling transcription rate, cell-cycle progression and apoptosis; therefore, an important role in pathogenesis of human diseases has been ascribed to the structure (Glickman *et al.*, 2001; Almond *et al.*, 2002; Naujokat *et al.*, 2002; Sjakste *et al.*, 2002a; 2002b). To a known extent, proteasomes interfere also with the atherogenesis process. Proteasomes degrade IF- κ B factor with subsequent activation of the atherogenic NF- κ B (Wilson *et al.*, 2001). Oxidised LDL causes activation of the proteasome activity followed by decline of this activity. HDL is an inhibitor of the proteasome activity (Vieira *et al.*, 2000; Robbesyn *et al.*, 2003).

The latter gene group appears to be of special interest as it was shown that the proteasomal protein LMP2 gene polymorphism affects the susceptibility to, and severity of HLA-B27 associated juvenile idiopathic arthritis (Pryhuber *et al.*, 1996).

We have previously addressed the possible association of the *PSMA6* gene in the genetic susceptibility to various diseases. The *PSMA6* gene is encoded by a single-copy gene on chromosome 14q13.2 between markers D14S960 and D14S889 (Sjakste *et al.*, 2001). In the initial studies we investigated a microsatellite polymorphism in intron 6 of the gene in several human populations (Sjakste *et al.*, 2002c). Further studies showed that this marker is associated with Graves' disease (Sjakste *et al.*, 2004) and diabetes mellitus type 2 (DM2, Kalis *et al.*, 2002; Sjakste *et al.*, 2007a). Genotyping of the single nucleotide polymorphism (SNP) in the promoter and 5'-UTR region of the *PSMA6* gene, resulted in the discovery of the association of the genotype -8C/G and haplotype C⁻¹¹⁰/G⁻⁸ with a higher risk of DM2 (Sjakste *et al.*, 2007b).

In this study we investigated whether chromosome 14q region SNP is associated with increased risk of juvenile idiopathic arthritis.

MATERIALS AND METHODS

Case/control study description. The polymorphisms were genotyped in a case/control study of 156 juvenile idiopathic arthritis patients and 214 subjects without JIA from the Latvian population. The case group (63% females) consisted of patients attending the outpatient clinic at the Pauls Stradiņš Clinical University Hospital and the Children's Hospital "Gaiļezers". The group represented about 60% of all registered in Latvia JIA patients. Patients (57% females) to the Riga Bīķernieki Hospital specialised in trauma medicine, were recruited to participate in the study control group (without autoimmune diseases). Informed consent was obtained from all patients; the study was approved by the Central Medical Ethics Commission of the Latvian Medical Academy.

DNA extraction was performed using a kit for genomic DNA extraction from nucleated blood cells (Fermentas, Vilnius, Lithuania). DNA quantity and quality was determined by electrophoresis in agarose gel.

Genotyping of 6 SNP including G¹⁴⁵⁷→A and C²⁵³⁶→A of *PSME1*; C¹¹⁵³→G and A¹⁴⁴⁰→C of *PSME2*; and A⁻²⁴⁸⁶→G and C⁻⁸→G of *PSMA6* was conducted by MALDI-TOF Mass Spectrometry approach (Wenzel *et al.*, 2003) on a MALDI TOF Autoflex Bruker Daltonics using genoSNIP specific primers (Table 1). One base pair insertion/deletion polymorphism (InDel) G⁻⁴⁵⁴³CA⁻⁴⁵⁴⁴→GA and SNP C⁻¹⁹¹⁰→T were genotyped by CAPS (cleaved amplified polymorphism) assay using correspondingly *BseMI* and *EcoNI* restriction sites in the intergenic region upstream *PSMA6* gene. SNP C⁻¹¹⁰→A (*PSMA6* promoter) was analysed by allele specific PCR. Information on the primer sequences, amplified and restricted fragment sizes, SNPs GenBank accession numbers, and function of the polymorphism is given in Table 1.

PCR was performed in a final volume of 30 μ l containing around 100 ng genomic DNA, 10 $\times$ DreamTag polymerase buffer (final concentration 1 $\times$), 25 mM MgCl₂ (from 1.5 mM to 3 mM), 10 mM dNTP Mix (0.5mM), 10 pM forward and reversal primers (0.33 pM each) and 0.75 units of DreamTaq polymerase (Fermentas, Vilnius, Lithuania). PCR denaturation at 95 °C 5 min was followed by 35–40 cycles each of 30 sec of denaturation at 95 °C, 30 s of annealing (at 55–63°C depending on the polymorphism analysed) and 60 s of extension at 72 °C, with final extension step of 7 min at 72 °C. PCR products were tested by electrophoresis in 1% agarose gel. Allele specific PCR was performed using allele specific forward primers differing only in the 3'-end polymorphic nucleotide and the reversal primer common for both alleles (Table 1). Presence or absence of the amplified product directly indicated the presence or absence of the corresponding allele. PCR product was digested in case of CAPS assay by the corresponding restriction enzyme. Digestion was performed in final volume of 25 μ l containing about 1,000 ng of DNA in PCR product, 10 $\times$ Buffer R (final concentration 1 $\times$) and 0.5 units of the corresponding restriction enzyme (Fermentas, Vilnius, Lithuania) during 2 hours at 55 °C or 37 °C for *BseMI* or *EcoNI*, correspondingly. Results of the digestion were tested by gel electrophoresis in 1% or 1.5% agarose gel. Fragment sizes of the digested amplicons are indicated in Table 1. Primer design was performed by the Primer 3.0 programme using the 14q11.2–14q13.2 sequence information at the NCBI nucleotide service (GenBank accession NT_026437). Primers were synthesised in "Metabion International AG" (Germany).

SNP functionality was analysed *in silico* for the affinity to transcription factors (TF) using Genomatix software (DiAlign TF, Release 3.1, and MatInspector, Release 7.4 tools, at <http://www.genomatix.de/>) and scored as loss or generation of the transcription factor binding sites (TFBS) (Fig. 1). Functionality of the coding region SNP was evaluated as amino acid substitution (Table 1).

Table 1

INFORMATION ON THE SNP, METHOD OF GENOTYPING, SEQUENCES OF THE FORWARD (F) AND REVERSAL (R) PRIMERS, AND EXPECTED FRAGMENT SIZES

Gene / chromosome location	Polymorphism			Genotyping method	Primers	Fragment size (bp)
	Description	ID	Function			
<i>PSME1</i> / 14q11.2	G ¹⁴⁵⁸ →A	rs11574508	Exon 6 Val ¹⁰⁴ →Val	genoSNIP	F: 5'–CACACAGGCTCAATCATGT–3' R: 5'–GACTTGAAGCCTGAGAGTTT–3' 5'–TCCTCCCTGLGGCCAGT–3' 5'–TCCTCCCTGLGGCCAGTG–3' 5'–TCCTCCCTGLGGCCAGTAACTG–3'	179
	C ²⁵³⁶ →A	rs14930	Exon 11 Lys ²⁴⁴ →Thr	genoSNIP	F: 5'–ACGTTGGATGGCCCTGTAGGCTGTGTTAT–3' R: 5'–ACGTTGGATGCCACTCCCTCTACCTCTTCT–3' 5'–biotin-CAAGAAGCCLAGGGGAGAAA-3'	264
<i>PSME2</i> / 14q11.2	C ¹¹⁵³ →G	rs1136581	Exon 4 Gly ⁶¹ →Arg	genoSNIP	F: 5'–AGGCATGAGCCTAGGTTG–3' R: 5'–GAGGTTGAAGGGCTATCTT–3' 5'–GGCTGACTTGACTLCCCTC–3' 5'–GGCTGACTTGACTLCCCTCG–3' 5'–GGCTGACTTGACTLCCCTCCG–3'	263
	A ¹⁴³⁰ →C	rs7146672	Exon 6 Pro ⁸⁹ →His	genoSNIP	F: 5'–GGGTGATGGCACACTGAC–3' R: 5'–AATGCATTTCTCTTTGAGAGT–3' 5'–CAGGGAGAALTCCACACTTA–3' 5'–CAGGGAGAALTCCACACTTAT–3' 5'–CAGGGAGAALTCCACACTTAGGGACT–3'	153
<i>PSMA6</i> / 14q13.2	G ⁻⁴⁵⁴³ CA ⁻⁴⁵⁴⁴ →GA	rs5807825	Intergenic region	CAPS <i>BseMI</i>	F: 5'–GTCCTCAGGGAGGAAGCTCT–3' R: 5'–AGCCTGAGGTAAGCACTCCA–3'	981 (754+227)
	A ⁻²⁴⁸⁶ →G	rs9322944	Intergenic region	genoSNIP	F: 5'–CAGAATAGCAGAGCAGGAAC–3' R: 5'–GCCAATGGGATATTTTTCAC–3' 5'–AGGGGGAGALGTATTAAGAA–3' 5'–AGGGGGAGALGTATTAAGAAA–3' 5'–AGGGGGAGALGTATTAAGAAGTTA–3'	211
	C ⁻¹⁹¹⁰ →T	rs7493194	Intergenic region	CAPS <i>EcoNI</i>	F: 5'–TCCAGAGAAGGGTCCAGCTA–3' R: 5'–CAGGAGATCCAGGTTGCAGT–3'	920 (405+515)
	C ⁻¹¹⁰ →A	rs2277460	Promoter	Allele specific PCR	F: 5'–ATGCAAGAGCGGAAGAAAC–3' F: 5'–ATGCAAGAGCGGAAGAAAA–3' R: 5'–CTGAATTGCCCTGTCATGGTA–3'	256
	C ⁻⁸ →G	rs1048990	5'-UTR	genoSNIP	F: 5'–ACGTTGGATGCTCCAGATGAAAGCCTGA–3' R: 5'–ACGTTGGATGGCCCTATCTTCCTTAACCTCTC–3' 5'–biotin-AGGGATTGTGTTTAAAGTAGTGCTT–3'	899

Amplified and restricted sizes are indicated without and with brackets, correspondingly. Position of the polymorphic loci is numbered from the first coding ATG. MALDI-TOF Mass Spectrometry approach is denoted as genoSNIP. ID indicates GenBank polymorphic loci accession number.

Fig. 1. Functional significance of *PSMA6* SNP⁻¹¹⁰ (rs2277460). Transcription factors NRSF/NRSE.01 and BARB/BARBIE.01 occupy region from -113 to -98 and from -116 to -102 nt, correspondingly. SNP⁻¹¹⁰ is boxed; allelic variants are indicated by a double arrow; 5'UTR is marked in grey. Numbering is given from the first ATG of the gene.

Data analysis. Numbers and frequencies of allele and genotype were estimated by direct gene counting. Expected heterozygosity indexes were estimated as $H_{exp} = 1 - \sum p_i^2$, where p_i is the frequency of each allele at the locus. Population heterozygosity was calculated as the ratio of the number of heterozygous individuals to the total number of sub-

jects. Deviations from the Hardy-Weinberg equilibrium were tested by the χ^2 test. Significance of differences between the groups for whole loci was estimated using the χ^2 test ($P_a < 0.05$) following the additive model. In parallel, differences were also estimated for alleles and genotypes separately. Corrected significance levels (P_b) were calcu-

lated by means of permutation analysis, performed using the PAST (PALaeontological Statistics, ver. 1.63) programme. Odds ratio (OR) and 95% confidence intervals were calculated using the dominant model by the MedCalc 3000 programme (<http://medicalcalc3000.com/OddsRatio.htm>).

RESULTS

Functional significance of the polymorphisms analysed is summarised in Table 1. Four SNPs related to *PSME1* and *PSME2* genes were located in the corresponding exons and were nonsynonymous rare mutations. Five SNPs related to *PSMA6* gene were chosen for the analysis including InDel and two SNPs in 5' upstream the gene region, and SNPs at -110 position and at -8 positions from the first ATG. The latter two are of the special interest as they were located in promoter (-1 position from the 5' UTR) and in 5'UTR close to the Kozak sequence correspondingly. *In silico* analysis (www.genomatix.de) revealed that the sequence encom-

passing SNP at -110 position possesses binding capacity to several TFs forming finally the module ETSF_SPIF_02 whose function is regulation of the gene expression by a co-operative interaction between the ubiquitously expressed factor SPI and the lymphoid/myeloid factor PU.1. Moreover, the transition C⁻¹¹⁰→A changes the locus affinity to two other TFs, namely to the simultaneously loss and generation of affinity to neuron-restrictive silencer factor and barbiturate-inducible element, correspondingly (Fig. 1). All SNPs chosen for the analysis were previously identified in the European population (HapMap at <http://www.hapmap.org>). Recently *PSMA6* gene SNPs at positions of -110 and -8 were intensively analysed for association with human disorders (Ozaki *et al.*, 2006; Sjakste *et al.*, 2007b, Takashima *et al.*, 2007; Barbieri *et al.*, 2008; Benett *et al.*, 2008, Freilinger *et al.*, 2009; Lui *et al.*, 2009).

Genotyping results. Table 2 summarises data on the allele frequencies of the nine SNPs related to three proteasomal genes (Table 1). The study consisted of two genotyping

Table 2

ALLELE FREQUENCIES IN JIA CASE/CONTROL STUDY IN LATVIAN POPULATION

Gene	SNP	Sample number case/control	Genotyping results								
			allele	number		frequencies		statistically significant			
				case	control	case	control	P_a	P_b	OR	CI 95%
PSME1	G ¹⁴⁵⁸ →A	50/118	G	100	232	1.00	0.98	0.19	0.32	0.00	—
			A	0	4	0.00	0.02				
			Sum	100	236						
	C ²⁵³⁶ →A	32/41	C	64	82	1.00	1.00	1.00	1.00	—	—
			A	0	0	0.00	0.00				
Sum			64	82							
PSME2	C ¹¹⁵³ →G	23/115	C	46	230	1.00	1.00	1.00	1.00	—	—
			G	0	0	0.00	0.00				
			Sum	46	230						
	A ¹⁴³⁰ →C	52/120	A	103	239	0.99	0.99	0.54	1.00	2.32	0.14 – 37.48
			C	1	1	0.01	0.01				
Sum			104	240							
PSMA6	G ⁻⁴⁵⁴³ CA ⁻⁴⁵⁴⁴ →GA	83/201	-	67	164	0.40	0.41	0.92	0.93	0.96	0.67 – 1.39
			+	99	238	0.60	0.59				
			Sum	166	402						
	A ⁻²⁴⁸⁶ →G	44/142	A	87	276	0.99	0.97	0.37	0.48	0.40	0.05 – 3.21
			G	1	8	0.01	0.03				
			Sum	88	284						
	C ⁻¹⁹¹⁰ →T	156/210	C	156	210	1.00	1.00	1.00	1.00	—	—
			T	0	0	0.00	0.00				
			Sum	156	210						
	C ⁻¹¹⁰ →A	156/214	C	282	404	0.90	0.94	0.039*	0.033*	1.80	1.03 – 3.13
			A*	30	24	0.10	0.06				
			Sum	312	428						
	C ⁻⁸ →G	105/118	C	178	202	0.85	0.86	0.81	0.90	1.07	0.63 – 1.80
			G	32	34	0.15	0.14				
			Sum	210	236						

P_a and P_b indicate P value based on χ^2 method and 10,000 permutations analysis, correspondingly. OR and CI 95% indicate odds ratio and its 95% confidence interval, correspondingly, only the rear allele is taken into account. Statistically significant results are marked by an asterisk. InDel alleles are shown as (+) and (-) for insertion and deletion, correspondingly.

steps including preliminary evaluation of the loci genetic diversity in the Latvian population (case group ranged from 23 to 56 individuals) and polymorphic loci genotyping for association with JIA.

Three loci including $C^{2536} \rightarrow A$ of *PSME1*, $C^{1153} \rightarrow G$ of *PSME2* and $C^{-1910} \rightarrow T$ related to *PSMA6* (5' region upstream the gene) were found to be monomorphic in Latvian population. Another three loci were slightly polymorphic ($G^{1458} \rightarrow A$ of *PSME1*, $A^{1430} \rightarrow C$ of *PSME2*, and $A^{-2486} \rightarrow C$ located in 5' region upstream the *PSMA6* gene) with a very low level of the rare allele presentation close to the accidental mutational rate. Loci identified as monomorphic or rare mutations were not genotyped in full JIA case/control study. Three resting loci showed higher diversity in the preliminary study and were evaluated as potentially prospective to be analysed for disease association.

Indel $G^{-4543}CA^{-4544} \rightarrow GA$ was revealed as the most diverse locus in both groups. However, allelic frequencies did not differ between cases and controls (frequencies of deletion and insertions were about 0.4 and 0.6 in both groups). The locus at position -8 of the *PSMA6* gene was presented by common C and rare G alleles of the same frequency in case and control groups.

The locus at position -110 was found to be the most interesting. Rare allele A presentation in the control group was

close to the mutational rate (frequency 0.06), but 1.7× more frequent in the case group (frequency 0.10). Differences in the A^{-110} allele and CG genotype frequencies were close to the statistically significant P level in JIA patients and healthy individuals ($P_a = 0.055$, $P_b = 0.067$, not shown), however, when an additive model was applied, the difference in $C^{-110} \rightarrow A$ locus was statistically significant ($P_a = 0.039$, $P_b = 0.033$, Table 2).

Genotypes. Taking into account the allele presentation, we can summarise that the loci at 2536 of *PSME1*, at 1153 of *PSME2* and at -1910 of *PSMA6* gene exist in a homozygous state in the Latvian population. All possible three genotypes were revealed only for InDel and SNP at -8 position of *PSMA6* gene. Other loci were presented by the homozygotes on the common allele and heterozygotes (Table 3). The heterozygote CA locus at position -110 was found to be more frequent than it was reported in HapMap (0.12 and 0.02, correspondingly, for the Latvian population in average and HapMap, www.hapmap.org). It is of interest that the CA genotype at position -110 was more than twice more frequent in the JIA case group compared to healthy individuals (frequency 0.19 and 0.08, correspondingly) with a P value close to the statistically significant level ($P_a = 0.064$, $P_b = 0.077$; not shown). Application of the additive model for the locus $C^{-110} \rightarrow A$ genotypes revealed statistically significant differences between the groups ($P_a =$

Table 3

GENOTYPE FREQUENCIES IN JIA CASE/CONTROL STUDY IN LATVIAN POPULATION

Gene	SNP	Genotyping results								
		genotype	number		frequencies		statistically significant			
			case	control	case	control	P_a	P_b	OR	CI 95 %
<i>PSME1</i>	G ¹⁴⁵⁸ →A	GG	51	114	1.00	0.97	0.18	0.31	0,00	—
		GA	0	4	0.00	0.03				
		Sum	51	118						
<i>PSME2</i>	A ¹⁴³⁰ →C	CC	52	119	0.99	0.98	0.55	1.00	2.28	0.14 – 37.29
		CA	1	1	0.01	0.02				
		Sum	53	120						
<i>PSMA6</i>	G ⁻⁴⁵⁴³ CA ⁻⁴⁵⁴⁴ →GA	- -	11	30	0.13	0.15	0.91	0.92	1.04	0.60 – 1.79
		- +	45	104	0.54	0.52				
		++	27	67	0.33	0.33				
		Sum	83	201						
	A ⁻²⁴⁸⁶ →G	AA	43	134	0.98	0.94	0.36	0.45	0,39	0.05 – 3.20
		AG	1	8	0.02	0.06				
		Sum	44	142						
	C ⁻¹¹⁰ →A	CC	126	190	0.81	0.92	0.031*	0.023*	1,88	1.05 – 3.37
		CA*	30	24	0.19	0.08				
		Sum	156	214						
	C ⁻⁸ →G	CC	75	87	0.71	0.73	0.85	0.88	1.16	0.64 – 2.10
		CG	28	28	0.27	0.24				
		GG	2	3	0.02	0.03				
		Sum	105	118						

P_a and P_b indicate P value based on χ^2 method and 10000 permutations analysis, correspondingly. OR and CI 95% indicate odds ratio and its 95% confidence interval, correspondingly, only genotypes having at least one rare allele are taken into account. Statistically significant results are marked by an asterisk. InDel alleles are shown as (+) and (-) for insertion and deletion, correspondingly.

0.031, $P_b = 0.028$, OR = 1.88 with 95% confidence interval ranging from 1.05 to 3.37).

DISCUSSION

We present here the results of an association study between proteasomal gene polymorphism and JIA in the Latvian population. Proteasomal genes were chosen as gene candidates, taking into account the hypothetical importance of defective proteasomal protein degradation in the pathogenesis of the disease (Deng *et al.*, 1995; Maksymowicz and Russel, 1995; Pryhuber *et al.*, 1996; Heward *et al.*, 1999; Sjakste *et al.*, 2004). We have focussed our attention on three highly evolutionary conserved genes located in the long arm of chromosome 14 including *PSME1*, *PSME2* and *PSMA6* encoding proteasomal activator proteins PSME1 and PSME2 and the proteasomal alpha subunit PSMA6, correspondingly. Nine SNPs were genotyped, including four of *PSME1* and *PSME2* coding regions and five of *PSMA6* non-coding region variations. High conservatism of the *PSMA6* coding gene region was proved in our previous study (Sjakste *et al.*, 2007a). We confirmed here low genetic diversity of the proteasomal genes as coding as well noncoding gene regions. Two *PSMA6* gene SNPs, including those at positions -110 and -8 from the first ATG located in gene promoter and 5'UTR, correspondingly, were of special interest because of their higher level of polymorphism and previously postulated and suggested association to several human disorders (Ozaki *et al.*, 2006; Sjakste *et al.*, 2007b; Takashima *et al.*, 2007; Barbieri *et al.*, 2008; Benett *et al.*, 2008; Freilinger *et al.*, 2009; Lui *et al.*, 2009). *PSMA6* SNP at position -8 is interesting because of its suggested functionality due to location close to the Kozak sequence and possible interference with the initiation of the translation. Some diseases are caused by mutations that perturb the initiation step of translation by changing the context around the AUG^{start} codon or introducing upstream AUG codons (Kozak, 2002; Kozak, 1997). The context around the initiation codon of the *PSMA6* gene (ACCAACaugT) does not represent classical "Kozak" consensus in the +4 position, where G is preferred (Kozak, 1997). Occurrence of the "good" (ANNaugN) but not "perfect" (ANNaugG) context

(Kozak, 2002) might increase the possible influence of mutations in the 5'- and 3'-areas from the consensus on the perturbation of the translation initiation. Using several models and approaches, Ozaki and colleagues (2006) reported that the allele G showed approximately 1.5- to 1.8-fold higher gene expression compared to the C allele and that the G allele could bind unknown nuclear factor(s) to exon 1 of *PSMA6*. Taken this into account, this variation was genotyped very intensively in different populations, especially in respect to the association with cardiovascular disorders (Ozaki *et al.*, 2006; Sjakste *et al.*, 2007b; Takashima *et al.*, 2007; Barbieri *et al.*, 2008; Benett *et al.*, 2008; Freilinger *et al.*, 2009; Lui *et al.*, 2009) and DM2 (Sjakste *et al.*, 2007b; Barbieri *et al.*, 2008). Results on the rare allele association with myocardial infarction (MI) are still controversial (Fig. 2), however, statistically significant data were obtained in favour of its association with DM2 (Sjakste *et al.*, 2007b; Barbieri *et al.*, 2008; Fig. 2). Data summarised in Figure 2 clearly demonstrate that, firstly, the SNP⁸ allelic distribution is similar between the Latvian and other European and Asian populations and secondly, that there is no association between locus variation and JIA in the Latvian population.

The most prospective result was obtained for *PSMA6* SNP at position -110. We have shown significant differences for locus C⁻¹¹⁰→A and a borderline association between its rare allele and heterozygous genotype frequencies and JIA in the Latvian population. Previously, a borderline association between the rare A⁻¹¹⁰ allele and DM2 was revealed in the Latvian population (Sjakste *et al.*, 2007b). It should be stressed that in current and in previous studies (Sjakste *et al.*, 2007b) in both Latvian and UK populations we did not reveal homozygotes for the rare allele, this is an additional indication on the negative role of this allele. *In silico* analysis revealed that the SNP C⁻¹¹⁰→A transition is followed by the loss of locus affinity to TF belonging to neuron-restrictive silencer factor family and simultaneously to the generation of binding capacity to barbiturate-inducible element. Both allelic variants of the sequence encompassing SNP at -110 position possess binding capacity to several TFs that form finally the module ETSF_SP1F_02, whose function is regulation of the gene expression by a cooperative interac-

Fig. 2. Data summary on the SNP⁸ (rs1048990) genotyping. JIA, juvenile idiopathic arthritis; DM2, diabetes mellitus type 2; MI, myocardial infarction; IS, ischemic stroke. Abbreviations for populations include: LV, Latvia, JP, Japan, UK, United Kingdom, CH, China, DE, Germany. Scale indicates odd ratio.

tion between the ubiquitously expressed factor SP1 and the lymphoid/myeloid factor PU.1. Further progress in our knowledge of the molecular processes of the genome regulation could reveal more consequences of this variation.

The results obtained in the study are in agreement with hypothesis on the association of *PSMA6* gene polymorphism and JIA and need further increased case/control study.

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REFERENCES

- Almond, J.B., Cohen, G.M. (2002). The proteasome: A novel target for cancer chemotherapy. *Leukemia*, **18**, 433–443.
- Barbieri, M., Marfella, R., Rizzo, M.R., Boccardi, V., Siniscalchi, M., Schiattarella, C., Siciliano, S., Lemme, P., Paolisso, G. (2008). The -8 UTR C/G polymorphism of *PSMA6* gene is associated with susceptibility to myocardial infarction in type 2 diabetic patients. *Atherosclerosis*, **201**(1), 117–123.
- le Beau, M.M., Ito, C., Cogswell, P., Espinosa, R. 3d., Fernald, A.A., Baldwin, A.S. (1992). Chromosomal localization of the genes encoding the p50/p105 subunits of NF-kappa B (NFkB2) and the I kappaB/MAD-3 (NFkB1) inhibitor of NF-kappa B to 4q24 and 14q13, respectively. *Genomics*, **14**, 529–531.
- Bennett, D.A., Xu, P., Clarke, R., Zondervan, K., Parish, S., Palmer, A., Cardon, L., Peto, R., Lathrop, M., Collins, R. (2008). The exon 1–8C/G SNP in the *PSMA6* gene contributes only a small amount to the burden of myocardial infarction in 6946 cases and 2720 controls from a United Kingdom population. *Eur. J. Hum. Genet.*, **16**, 480–486.
- Chistyakov, D.A., Savostanov, K.V., Turakulov, R.I., Nosikov, V.V. (2000). Genetic determinants of Graves disease. *Mol. Genet. Metab.*, **71**, 66–69.
- Deng, G.Y., Muir, A., Maclaren, N.K., She, J.X. (1995). Association of LMP2 and LMP7 genes within the major histocompatibility complex with insulin-dependent diabetes mellitus: Population and family studies. *Amer. J. Hum. Genet.*, **56**, 528–534.
- Freilinger, T., Bevan, S., Ripke, S., Gschwendtner, A., Lichtner, P., Müller-Myhsok, B., Wichmann H.-E., Markus, H.S., Meitinger, T., Dichgans, M. (2009). Genetic variation in the lymphotoxin-alpha pathway and the risk of ischemic stroke in European populations. *Stroke*, **40**, 970–972.
- Glass, D.N., Giannini, E.H. (1999). Juvenile rheumatoid arthritis as a complex genetic trait. *Arthritis Rheum.*, **42**(11), 2261–2268.
- Glickman, M., Ciechanover, A. (2001). The ubiquitin-proteasome proteolytic pathway: Destruction for the sake of construction. *Physiol. Rev.*, **82**, 373–428.
- Heward, J.M., Allhabadia, A., Sheppard, M.C., Barnett, A.H., Franklyn, J.A., Gough, S.C. (1999). Association of the large multifunctional proteasome (LMP2) gene with Graves disease is a result of linkage disequilibrium with the HLA haplotype DRB1*034-DQB1*02-DQA1*0501. *Clin. Endocrinol. (Oxf)*, **51**, 115–118.
- John, S., Worthington, J. (2001). Genetic epidemiology. Approaches to the genetic analysis of rheumatoid arthritis. *Arthritis Res.*, **3**, 216–220.
- Kalis, M., Sjakste, T., Sjakste, N., Luthman, H., Groop, L. (2002). Association study between (TG) repeat polymorphism in *PSMA6* gene and type II diabetes mellitus in Botnia. *Biologija*, **2**, 12–14.
- Kamnasaran, D., Cox, D.W. (2002). Current Status of Human Chromosome 14. *J. Med. Genet.*, **39**, 81–91.
- Kozak, M. (1997). Recognition of AUG and alternative initiator codons is augmented by G in position +4 but is not generally affected by the nucleotides in positions +5 and +6. *EMBO J.*, **16**, 2482–2492.
- Kozak, M. (2002). Pushing the limits of the scanning mechanism for initiation of translation. *Gene*, **299**, 1–34.
- Liu, X., Wang, X., Shen, Y., Wu, L., Ruan, X., Lindpaintner, K., Yusuf, S., Engert, J. C., Anand, S., Tan, X., Liu, L. (2009). The functional variant rs1048990 in *PSMA6* is associated with susceptibility to myocardial infarction in a Chinese population. *Atherosclerosis*, **206**(1), 199–203.
- Maksymowich, W.P., Russel, A.S. (1995). Polymorphism in the LMP2 gene influences the relative risk for acute anterior uveitis in unselected patients with ankylosing spondylitis. *J. Clin. Invest.*, **18**, 42–46.
- Maksymowich, W.P., Tao, S., Luong, M., Suarez-Almazor, M., Nelson, R., Pazderka, F., Russell, A.S. (1995). Polymorphism in the LMP2 and LMP7 genes and adult rheumatoid arthritis: No relationship with disease susceptibility or outcome. *Tissue Antigens*, **46**, 136–139.
- Naujokat, C., Hofman, S. (2002). Role and function of the 26S proteasome in proliferation and apoptosis. *Lab. Invest.*, **82**, 965–980.
- Ozaki, K., Sato, H., Iida, A., Mizuno, H., Nakamura, T., Miyamoto, Y., Takahashi, A., Tsunoda, T., Ikegawa, S., Kamatani, N., Hori, M., Nakamura, Y., Tanaka, T. (2006). A functional SNP in *PSMA6* confers risk of myocardial infarction in the Japanese population. *Nat. Genet.*, **38**, 921–925.
- Petty, R.E., Southwood, T.R., Manners, P., Baum, J., Glass, D.N., Goldenberg, J., Xiaohu, H., Maldonado-Cocco, J., Orozco-Alcala, J., Prieur, A.M., Suarez-Almazor, M.E., Woo, P. (2001). International League of Association for Rheumatology Classification of Juvenile Idiopathic Arthritis: Second Revision, Edmonton. *J. Rheumatol.*, **31**, 2–3.
- Prahalad, S., Kingsbury, D.J., Griffin, T.A., Cooper, B.L., Glass, D.N., Maksymowich, W.P., Colbert, R.A. (2001). Polymorphism in the MHC-encoded LMP7 gene: Association with JRA without functional significance for immunoproteasome assembly. *J. Rheumatol.*, **28**, 2320–2325.
- Pryhuber, K.G., Murray, K.J., Donnelly, P., Passo, M.H., Maksymowich, W.P., Glass, D.N., Giannini, E.H., Colbert, R.A. (1996). Polymorphism in the LMP2 gene influences disease susceptibility and severity in HLA-B27 associated juvenile rheumatoid arthritis. *J. Rheumatol.*, **23**, 747–752.
- Robbesyn, F., Garcia, V., Auge, N., Vieira, O., Frisach, M.F., Salvayre, R., Negre-Salvayre, A. (2003). HDL counterbalance the proinflammatory effect of oxidized LDL by inhibiting intracellular reactive oxygen species rise, proteasome activation, and subsequent NF-kappaB activation in smooth muscle cells. *FASEB J.*, **17**(6), 743–745.
- Sjakste, N., Sjakste, T., Rumba, I., Vikmanis, U. (2002a). Proteasomes in pathology. *Proc. Latv. Acad. Sci. B*, **56**(1/2), 7–16.
- Sjakste, N., Sjakste, T., Vikmanis, U. (2002b). Role of the ubiquitin-proteasome protein degradation pathway in carcinogenesis, tumor progression and susceptibility to tumor treatment. *Exp. Oncol.*, **24**, 243–248.
- Sjakste, T., Eglite, J., Sochnev, A., Marga, M., Pirags, V., Collan, Y., Sjakste, N. (2004). Microsatellite genotyping of chromosome 14q13.2–14q13 in the vicinity of proteasomal gene *PSMA6* and association with Graves' disease in the Latvian population. *Immunogenetics*, **56**, 238–243.
- Sjakste, T., Kalis, M., Poudziunas, I., Pirags, V., Lazdins, M., Groop, L. & Sjakste, N. (2007a). Association of microsatellite polymorphisms of the human 14q13.2 region with type2 diabetes mellitus in Latvian and Finnish populations. *Ann. Hum. Genet.*, **71**, 772–776.
- Sjakste, T., Poudziunas, I., Ninio, E., Perret, C., Pirags, V., Nicaud, V., Lazdins, M., Evans, A., Morrison, C., Cambien, F., Sjakste N. (2007b).

- SNPs of PSMA6 gene – investigation of possible association with myocardial infarction and type 2 diabetes mellitus. *Russ. J. Genet.*, **43**, 442–448.
- Sjakste, T., Lauberte, L., Collan, Y., Savontaus, M.L., Bajare, A., Scherrer, K., Sjakste, N. (2002c). Identification of an intronic TG repeat polymorphism in the human proteasome core particle PROS-27K gene. *DNA Seq.*, **13**(3), 139–144.
- Sjakste, T.G., Sjakste, N.I., Scherrer, K. (2001). Exon/Intron Organisation of Pros-27 K Gene. *DNA Seq.*, **12**(4), 261–265.
- Takashima, N., Shioji, K., Kokubo, Y., Okayama, A., Goto, Y., Nonogi, H., Iwai, N. (2007). Validation of the Association Between the Gene Encoding Proteasome Subunit α Type 6 and Myocardial Infarction in a Japanese Population. *Circ. J.*, **71**, 495–498.
- Thompson, S.D., Moroldo, M.B., Guyer, L., Ryan, M., Tombragel, E.M., Shear, E.S., Prahalad, S., Sudman, M., Keddache, M.A., Brown, W.M., Giannini, E.H., Langefeld, C.D., Rich, S.S., Nichols, W.C., Glass, D.N. (2004). A genome-wide scan for juvenile rheumatoid arthritis in affected sibpair families provides evidence of linkage. *Arthritis Rheum.*, **50**, 2920–2930.
- Vieira, O., Escargueil-Blanc, I., Jürgens, G., Borner, C., Almeida, L., Salavayre, R., Salvayre, A.N. (2000). LDLs alter the activity of the ubiquitin-proteasome pathway: Potential role in oxidized LDL-induced apoptosis. *FASEB J.*, **14**(3), 532–542.
- Wenzel, T., Elssner, T., Fahr, K., Bimmler, J., Richter, S., Thomas, I., Kostrzewa, M. (2003) Genosnip: SNP genotyping by MALDI-TOF MS using photocleavable oligonucleotides. *Nucleosides Nucleotides Nucleic Acids*, **22**(5–8), 1579–1581.
- Wilson, S.H., Simari, R.D., Lerman, A. (2001) The effect of endothelin-1 on nuclear factor kappa B in macrophages. *Biochem. Biophys. Res. Commun.*, **286**, 968–972.

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ASOCIĀCIJU PĒTĪJUMS STARP 14q11 – 14q13 PROTEASOMU GĒNU KLASTERA ĢENĒTISKIEM VARIANTIEM UN JUVENĪLO IDIOPĀTISKO ARTRĪTU (JIA) LATVIJAS POPULĀCIJĀ.

Hipotēze par iespējamo proteasomu lomu autoimūno slimību attīstībā tika izvirzīta pēc protasomālo subvienību LMP2 un LMP7 līdzdalības atklāšanas antigēnu prezentācijā. Pētījuma mērķis bija pārbaudīt iespējamo asociāciju starp proteasomālo proteīnu gēnu *PSME1*, *PSME2* un *PSMA6* alēļu variantiem un juvenilo idiopātisko artrītu (JIA) Latvijas populācijā. Viens insercijas/delēcijas polimorfisms $G^{-4543}CA^{-4544} \rightarrow GA$ un četri viena nukleotīda polimorfismi *PSMA6* gēnā un tā apkārtnē ($A^{-2486} \rightarrow G$ un $C^{-1910} \rightarrow T$ augšup no promotera, $C^{-110} \rightarrow A$ promotērā, un $C^{-8} \rightarrow G$ 5' netranslējamā apgabalā), divi cSNP *PSME1* ($G^{1457} \rightarrow A$: Val¹⁰⁴, 6. ekzonā un $C^{2536} \rightarrow A$: Lys^{244} \rightarrow Thr}, 11.ekzonā) un *PSME2* ($C^{1153} \rightarrow G$: Arg^{61} \rightarrow Gly}, 4. ekzonā un $A^{1440} \rightarrow C$: His^{89} \rightarrow Pro}, 6. ekzonā) tika genotipēti, izmantojot specifisku praimeru PCR, restrikcijas vietas polimorfismu un/vai sekvencēšanu gadījumu/kontroles pētījumā 156 JIA slimniekos un 214 veselos indivīdos. Alēļu biežums un genotipu sadale bija vienādas kontroles un slimnieku grupās *PSMA6* insercijas/delēcijas polimorfisma un viena nukleotīda polimorfismu: $A^{-2486} \rightarrow G$, $C^{-1910} \rightarrow T$ un $C^{-8} \rightarrow G$, gadījumos, kā arī visu pētīto *PSME1* un *PSME2* gēnu cSNP gadījumos. Atsevišķi analizējot atšķirības A^{-110} alēles un CG genotipu biežumos starp slimnieku un kontroles grupām, tās bija tuvas statistiskai ticamībai, *P* vērtība nedaudz pārsniedza 0.05, aditīvā modeļa izmantošana pierādīja, ka visam $C^{-110} \rightarrow G$ lokusam šīs atšķirības ir statistiski ticamas. Mūsu rezultāti atbalsta hipotēzi par iespējamo *PSMA6* gēna alēļu asociāciju ar JIA Latvijas populācijā.