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Genetic variants in the *PSMA6*, *PSMC6* and *PSMA3* genes associated with childhood asthma in Latvian and Taiwanese populations

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Proteasomes mediate functional realization of signaling proteins implicated in asthma pathogenesis. ***Aim****. To evaluate main and sex-specific association between the PSMA6, PSMC6 and PSMA3 proteasomal genes variations and childhood asthma in Latvians and Taiwanese.* ***Methods****. SNPs rs2277460, rs1048990, rs2295826, rs2295827 and rs2348071 were genotyped in 102 Latvian and 159 Taiwanese cases for comparison with genetic diversity in populations (191 and 1097 subjects respectively).* ***Results****. Haplotype CGACG showed strong ($P < 0.0001$) association with asthma risk in both populations. All loci heterozygous genotypes and haplotype CCGTA were identified as asthma risk factors in Latvians; rs1048990 and rs2348071 GG homozygotes and rs2295826 and rs2295827 heterozygotes showed asthma risk and protective effect in Taiwanese females respectively. The multi locus genotypes homozygous for alleles being common in Latvian population were identified as protective in Latvians and disease susceptible in Taiwanese.* ***Conclusions****. Our results suggest an association of the 14q13-23 proteasomal genes polymorphisms with the childhood asthma in Latvians and Taiwanese and highlight risk and/or protective factors being the same or different between the populations.*

Keywords: chromosome 14q13-23, SNPs, PSMA6, PSMC6, PSMA3, childhood asthma.

Introduction. Asthma is a chronic inflammatory disease caused by complex gene–gene and gene–environment interactions with hyper-responsiveness to various nonspecific stimuli [1–3] being to a large extent genetically heterogeneous between human populations [4]. A number of genes implicated in asthma encode various signaling proteins and transcription factors including those driven by NF-κB signaling pathways [5–7].

However, interplay of multiple risk alleles and/or genotypes and primary driver of the disease remains still unclear.

In eukaryotes, processing and degradation of vast majority of regulatory proteins are mediated by ubiquitin-proteasome system (UPS). Proteasomes, key UPS enzymatic complex possess several types of peptidase, endoribonuclease, protein-chaperone and DNA-helicase activities [8–10] allowing strict control and coordination of all steps of gene expression, genes and proteins networks and processes of genome–environment interaction. Insufficient proteasome function was implicated in pathophysiology of various acute and chronic lung diseases and their complications [11–14] and potentially could be a consequence of particular proteasomal genes structural variations.

Multiple studies including several GWAS analyses, indicated the 14q11-24 genome region as susceptible to asthma [15–21]. This genomic region possesses a cluster of proteasomal genes including the *PSMA6*, *PSMC6* and *PSMA3* genes implicated previously in susceptibility to autoimmunity [22–24], type 2 diabetes mellitus [25, 26], cardio-vascular disorders [27] and population adaptation to environment [28]. It appears that there is a large potential for the 14q proteasomal genes association studies to provide novel insights into the bronchial asthma (BA) pathogenesis in particular human populations and in general.

Aim of the current study was to genotype five single nucleotide polymorphisms (SNPs) belonging to the *PSMA6* (rs2277460 and rs1048990), *PSMA3* (rs2348071), and *PSMC6* (rs2295826 and rs2295827) proteasomal genes and evaluate main and sex-specific association between variations of these genes and asthma in Latvians and Taiwanese.

Materials and methods. One hundred two children (28 girls) aged under five and 159 (69 girls) aged under three represented Latvian (LV) and Taiwanese (TW) asthma groups respectively. LV BA patients were enrolled from the outpatient clinic of P. Stradins Clinical University Hospital and Children Clinical University Hospital «Gailezers» in Riga, Latvia.

TW study subjects were enrolled from elementary school for allergy diseases screen Taoyuan General Hospital, Taiwan. All patients were diagnosed with mild or moderate persistent asthma according to the guidelines of the Global Initiative for Asthma (GINA; http://www.ginasthma.org/local/uploads/files/GINA_Under5_Pocket_20091_1.pdf). The studies were approved by the Central Medical Ethics Commission of the Republic of Latvia Ministry of Health and Ethics Committee of Taoyuan General Hospital, Taiwan. Informed consent was obtained from parents of study participants.

Latvian and Taiwanese control groups of 191 (age = = 54.8 ± 18.6; 117 women) and 1097 (aged under five; 558 girls) participants respectively, were described and genetic diversity of SNPs of interest was studied previously [28] providing primary genotyping data to be used in current study to evaluate asthma main effects for each particular SNP, construct multi locus genotypes and stratify controls by sex to reveal asthma sex specific associations in single- and multi-locus models.

DNA extraction and genotyping technologies were the same as in [28].

For quality control, of the 16 randomly chosen samples per each marker were genotyped in duplicate in different experiments for asthma samples from both Latvian and Taiwanese collections. The concordance of the genotyping was 100 %. The chromosome 14 GRCh37.p5 assembly (NCBI reference sequence: NC_000014.8) sequence information was used for loci description.

Personalised genotyping data documentation resulted in knowledge of 5 locus genotype (5-LG: rs2277460/rs1048990/rs2295826/rs2295827/rs2348071) of each individual participant of the study. The 5-LGs, single locus genotypes (SLGs) and alleles frequencies were estimated by direct gene counting. DnaSP version 5 (http://www.ub.es/dnasp/ [29] was used to reconstruct the haplotypes from un-phased genotypes, evaluate the nucleotide and haplotype genetic diversity and pairwise linkage disequilibrium (LD) between the loci (D' and r^2). Both the two-tailed Fisher's exact test and the χ^2 test were applied to evaluate the linkage between the rs2295826 and rs2295827 polymorphic sites at three p-value levels ($p < 0.05$; $p < 0.01$; $p < 0.001$). The Bonferroni correction included in DnaSP analysis was taken into account to support the significance of the revealed disequilibrium ($\alpha' = = 0.05$).

Deviation from the Hardy-Weinberg equilibrium and differences between case and control groups in al-

lele, genotype and haplotype frequencies as well as permutation test (Monte Carlo method/number of simulations = 10000) were evaluated by χ^2 using XLSTAT 2013 software for Windows. Dominant, recessive, over dominant and multiplicative genetic models for every individual locus were designed according to Lewis [30] and analysed by using 2 × 2 contingency tables. Odds ratio (OR) more than 2 and less than 0.5 was considered to be clinically significant. Stratification was performed by sex.

Results and discussion. In both Latvian and Taiwanese sample collections the genotyping call rate was 100 % for all markers; alleles and genotypes frequencies are given in Tables 1 and 2. The rs2348071 being in Latvian patients in HWE, significantly ($P < 0.001$) deviated from equilibrium in Taiwanese. Other markers were found to be in HWE in both LV and TW patients. The rs2295826 and rs2295827 were observed in complete ($D' = 1$, $r^2 = 1$) and slightly disrupted ($D' = 1$, $r^2 = 0.896$) LD in Latvians and Taiwanese respectively.

The distributions of alleles and genotypes in case groups were compared with those previously identified in the populations [28] and the data on single-locus association are summarized in Table 1 and Table 2. In Latvians all five loci showed the asthma main effect for rare alleles and heterozygous genotypes. The rs1048990 was associated with the disease in both females and males. The asthma susceptibility of resting loci was characterized by nonadditivity; that was, the rs2277460 and rs2348071 were associated with asthma in females, and the rs2295826 and rs2295827 were associated with asthma in males. In Taiwanese, the rs2277460 appears to be asthma neutral and resting loci showed the disease susceptibility only in females. The asthma risk effect was observed for the rs1048990 and rs2348071 GG genotypes and rs2348071 allele G. Rare alleles and heterozygous genotypes of the rs2295826 and rs2295827 showed female-specific asthma protective effect.

The multi-locus genotypes showing asthma risk or protective effect in any of our populations are listed in Table 3 (see suppl.). In Latvians a statistically significant protective effect was observed for all variants of multi locus genotypes being homozygous for the alleles common in the population. The 5LG of CC/CC/AA/CC/GG configuration being protective in Latvians (OR = = 0.322 [0.196 – 0.651]), showed the asthma risk effect in Taiwanese females (OR = 2.911 [1.327–6.387]). Similarly, being protective in Latvians the AA/CC/GG (rs2295826/rs2295827/rs2348071) genotype appears to be the disease susceptible in Taiwanese. In Latvians, the risk effect was observed for the rs2277460/rs2348071, rs1048990/rs2348071, rs1048990/rs2295826/rs2295827 and rs2295826/rs2295827/rs2348071 genotypes being simultaneously heterozygous at all loci involved. All mentioned genotypes were neutral in Taiwanese. In contrast, the rs2295826/rs2295827/rs2348071 genotype of AG/CT/AA configuration being neutral in Latvians, showed the protective effect in Taiwanese females.

The data of haplotype analysis are given in Table 4 (see suppl.). Haplotype diversity was higher in Latvian patients than in the population and did not differ between the cases and population in Taiwanese. The most frequent in Latvians the Hap1 (CCACG) showed male specific asthma protective (OR = 0.633 [0.416–0.963]) effect in this population. The Hap6 (CGACG) showed strong ($P < 0.0001$) association with the asthma risk in both Latvians (OR = 4.525 [2.286–8.958]) and Taiwanese (OR = 2.448 [1.763–3.399]) for both females and males. Minor in both populations and Taiwanese cases the Hap9 (CCGTA) was strongly ($P < 0.0001$) associated with the asthma phenotype in Latvians.

Identification of the genetic risk factors for asthma is complicated by potential interaction of genes and metabolic pathways, genotype with sex and environment; most of the reported asthma genes were not replicated across populations [4, 31]. The 14q11-24 chromosomal region is one of well replicated asthma susceptibility loci [15–21]; the *PSMA6*, *PSMA3* and *PSMC6* proteasomal genes located in the region were implicated earlier in susceptibility to autoimmunity [22–24], inflammation [25–27] and historical and geographical adaptation [28].

In this paper we provide for the first time the evidence that polymorphism in the *PSMA6*, *PSMC6*, and *PSMA3* proteasomal genes may contribute to the risk of childhood asthma in both Latvian and Taiwanese populations. The most remarkable finding of our study is that haplotype CGACG was revealed to be a strong ($P < 0.0001$) asthma risk factor in both Latvians and Taiwanese. Other identified asthma risk and protective single- and multi-locus genetic variants are different between two populations. The difference between human

Table 1
SNP allele and genotype distribution and data on association with paediatric asthma in Latvian population

Marker Allele or genotype	Distribution of alleles and genotypes, *n* (%)					
	BA patients			Controls*		
	Total (*n* = 102)	Females (*n* = 28)	Males (*n* = 74)	Total (*n* = 191)	Females (*n* = 117)	Males (*n* = 74)
rs2277460						
C	176 (86.27)	47 (83.93)	129 (87.16)	357 (93.46)	232 (94.87)	135 (91.22)
A	28 (13.73)	9 (16.07)	19 (12.84)	25 (6.54)	12 (5.13)	13 (8.78)
CC	74 (72.55)	19 (67.86)	55 (74.32)	166 (86.91)	105 (89.74)	61 (82.43)
CA	28 (27.45)	9 (32.14)	19 (25.68)	25 (13.09)	12 (10.26)	13 (17.57)
rs1048990						
C	165 (80.88)	44 (78.57)	121 (81.76)	348 (91.10)	211 (90.17)	137 (92.57)
G	39 (19.12)	12 (21.43)	27 (18.24)	34 (8.90)	23 (9.83)	11 (7.43)
	–	–	–	–	–	–
CC	65 (63.73)	16 (57.14)	49 (66.22)	158 (82.72)	95 (81.20)	63 (85.14)
CG	35 (34.31)	12 (42.86)	23 (31.08)	32 (16.75)	21 (17.95)	11 (14.86)
GG	2 (1.96)	–	2 (2.70)	1 (0.52)	1 (0.85)	–
rs2295826						
A	166 (81.37)	47 (83.93)	119 (80.41)	342 (89.53)	204 (87.18)	138 (93.24)
G	38 (18.63)	9 (16.07)	29 (19.59)	40 (10.47)	30 (12.82)	10 (6.76)
AA	66 (64.71)	19 (67.86)	47 (63.51)	155 (81.15)	90 (76.93)	65 (87.84)
AG	34 (33.33)	9 (32.14)	25 (33.78)	32 (16.75)	24 (20.51)	8 (10.81)
GG	2 (1.96)	–	2 (2.70)	4 (2.09)	3 (2.56)	1 (1.35)
rs2295827						
C	166 (81.37)	47 (83.93)	119 (80.41)	342 (89.53)	204 (87.18)	138 (93.24)
T	38 (18.63)	9 (16.07)	29 (19.59)	40 (10.47)	30 (12.82)	10 (6.76)
CC	66 (64.71)	19 (67.86)	47 (63.51)	155 (81.15)	90 (76.93)	65 (87.84)
CT	34 (33.33)	9 (32.14)	25 (33.78)	32 (16.75)	24 (20.51)	8 (10.81)
TT	2 (1.96)	–	2 (2.70)	4 (2.09)	3 (2.56)	1 (1.35)
rs2348071						
G	119 (58.33)	34 (60.71)	85 (57.43)	270 (70.68)	170 (72.65)	100 (67.57)
A	85 (41.67)	22 (39.29)	63 (42.57)	112 (29.32)	64 (27.35)	48 (32.43)
GG	35 (34.31)	8 (28.57)	27 (36.49)	102 (53.40)	65 (55.55)	37 (50.00)
GA	49 (48.04)	18 (64.29)	31 (41.89)	66 (34.56)	40 (34.19)	26 (35.14)
AA	18 (17.65)	2 (7.14)	16 (21.62)	23 (12.04)	12 (10.26)	11 (14.86)

*Data on an allele and genotype presentation in control group are given according to Sjakste *et al*. [24, 28]; *P* - probability calculated by χ^2 test;

Marker ID Allele or genotype	Statistics			
	Genetic model	Group	*P* (P_c)	OR [95 % CI]
rs2277460				
C	A *vs* C	Total	0.0038 (0.0045)	2.272 [1.293–3.933]
A	–	Females	0.0032 (0.0059)	3.702 [1.506–9.101]
CC	CA *vs* CC	Total	0.0023 (0.0029)	2.512 [1.379–4.578]
CA	–	Females	0.0031 (0.0066)	4.145 [1.568–10.957]
rs1048990				
C	G *vs* C	Total	0.0004 (0.0006)	2.419 [1.478–3.960]
G	–	Females	0.0167 (0.0238)	2.502 [1.157–5.342]
	–	Male	0.0054 (0.0085)	2.779 [1.339–5.768]
CC	CG *vs*	Total	0.0007 (0.0011)	2.596 [1.491–4.519]
CG	CC + GG	Females	0.0047 (0.0077)	3.429 [1.435–8.192]
GG	–	Male	0.0190 (0.0308)	2.583 [1.166–5.722]
rs2295826				
A	G *vs* A	Total	0.0056 (0.0064)	1.957 [1.213–3.158]
G	–	Male	0.0011 (0.0022)	3.363 [1.596–7.088]
AA	AG *vs*	Total	0.0012 (0.0016)	2.484 [1.424–4.334]
AG	AA + GG	Male	0.0008 (0.0008)	4.209 [1.783–9.937]
GG	–	–	–	–
rs2295827				
C	G *vs* A	Total	0.0056 (0.0064)	1.957 [1.213–3.158]
T	–	Male	0.0011 (0.0022)	3.363 [1.596–7.088]
CC	AG *vs*	Total	0.0012 (0.0016)	2.484 [1.424–4.334]
CT	AA + GG	Male	0.0008 (0.0008)	4.209 [1.783–9.937]
TT	–	–	–	–
rs2348071				
G	A *vs* G	Total	0.0026 (0.0039)	1.722 [1.208–2.454]
A	–	–	–	–
GG	GA *vs*	Total	0.0244 (0.0343)	1.751 [1.075–2.851]
GA	GG + AA	Females	0.0035 (0.0059)	3.465 [1.485–8.085]
AA	–	–	–	–

P_c – corrected probability calculated by Monte Carlo method with 10000 simulations.

Table 2
SNP allele and genotype distribution and data on association with paediatric asthma in Taiwanese population

Marker Allele or genotype	Distribution of alleles and genotypes, *n* (%)					
	BA patients			Controls*		
	Total (*n* = 159)	Females (*n* = 69)	Males (*n* = 90)	Total (*n* = 1097)	Females (*n* = 558)	Males (*n* = 539)
rs2277460						
C	315 (99.06)	137 (99.28)	179 (99.44)	1081 (99.27)	547 (99.01)	534 (99.54)
A	2 (0.63)	1 (0.72)	1 (0.56)	16 (0.73)	11 (0.99)	5 (0.46)
CC	157 (98.74)	68 (98.55)	89 (98.89)	1081 (98.54)	547 (98.03)	534 (99.07)
CA	2 (1.26)	1 (1.45)	1 (1.11)	16 (1.46)	11 (1.97)	5 (0.93)
rs1048990						
C	209 (65.72)	85 (61.59)	124 (68.89)	1480 (67.46)	738 (66.13)	742 (68.83)
G	109 (34.28)	53 (38.41)	56 (31.11)	714 (32.54)	378 (33.87)	336 (31.17)
CC	66 (41.51)	28 (40.58)	38 (42.22)	462 (42.12)	228 (40.86)	234 (43.42)
CG	77 (48.43)	29 (42.03)	48 (53.33)	556 (50.68)	282 (50.54)	274 (50.83)
GG	16 (10.06)	12 (17.39)	4 (4.44)	79 (7.20)	48 (8.60)	31 (5.75)
rs2295826						
A	286 (89.94)	129 (93.48)	157 (87.22)	1855 (84.55)	948 (84.95)	907 (84.14)
G	32 (10.06)	9 (6.52)	23 (12.78)	339 (15.45)	168 (15.05)	171 (15.86)
AA	129 (81.13)	60 (86.96)	69 (76.67)	778 (70.92)	404 (72.40)	374 (69.39)
AG	28 (17.61)	9 (13.04)	19 (21.11)	299 (27.26)	140 (25.08)	159 (29.05)
GG	2 (1.26)	–	2 (2.22)	20 (1.82)	14 (2.51)	6 (1.11)
rs2295827						
C	289 (90.88)	129 (93.48)	160 (88.89)	1872 (85.32)	962 (86.20)	910 (84.42)
T	29 (9.12)	9 (6.52)	20 (11.11)	322 (14.68)	154 (13.80)	168 (15.58)
CC	130 (81.76)	60 (86.96)	70 (77.78)	775 (70.64)	404 (72.40)	371 (68.83)
CT	29 (18.24)	9 (13.04)	20 (22.22)	322 (29.35)	154 (27.60)	168 (31.17)
TT	–	–	–	–	–	–
rs2348071						
G	126 (39.62)	61 (44.20)	65 (36.11)	759 (34.59)	380 (34.05)	379 (35.16)
A	192 (60.38)	77 (55.80)	115 (63.89)	1435 (65.41)	736 (65.95)	699 (64.84)
GG	40 (25.16)	19 (27.54)	21 (23.33)	204 (18.60)	97 (17.39)	107 (19.85)
GA	46 (28.93)	23 (33.33)	23 (25.56)	351 (31.99)	186 (33.33)	165 (30.61)
AA	73 (45.91)	27 (39.13)	46 (51.11)	542 (49.41)	275 (49.28)	267 (49.54)

*Data on an allele and genotype presentation in control group are given according to Sjakste *et al*. [28]; *P* – probability calculated by χ^2 test;

Marker ID Allele or genotype	Statistics			
	Genetic model	Group	*P* (P_c)	OR [95 % CI]
rs2277460				
C	–	–	–	–
A	–	–	–	–
CC	–	–	–	–
CA	–	–	–	–
rs1048990				
C	–	–	–	–
G	–	–	–	–
CC	GG *vs*	Females	0.0192 (0.0311)	2.237 [1.135–4.410]
CG	CC + CG	–	–	–
GG	–	–	–	–
rs2295826				
A	G *vs* A	Total	0.0114 (0.0117)	0.612 [0.418–0.896]
G	–	Females	0.0066 (0.0094)	0.394 [0.200–0.776]
AA	AG *vs*	Total	0.0094 (0.0091)	0.570 [0.372–0.873]
AG	AA + GG	Females	0.0266 (0.0346)	0.488 [0.220–0.911]
GG	–	–	–	–
rs2295827				
C	T *vs* C	Total	0.0076 (0.0099)	0.583 [0.392–0.868]
T	–	Females	0.0165 (0.0215)	0.436 [0.221-0.861]
CC	CT *vs* CC	Total	0.0023 (0.0024)	0.523 [0.344–0.796]
CT	–	Females	0.0093 (0.0117)	0.394 [0.194–0.799]
TT	–	–	–	–
rs2348071				
G	C *vs* A	Females	0.0185 (0.0228)	1.534 [1.74–2.192]
A	–	–	–	–
GG	GG *vs*	Females	0.0404 (0.0315)	1.806 [1.025–3.182]
GA	GA + AA	–	–	–
AA	–	–	–	–

P_c – corrected probability calculated by Monte Carlo method with 10000 simulation.

populations in asthma genetics is a well-known phenomenon described for a number of asthma susceptible loci having mainly ethnos specific differences in genetic diversity [4]. The Latvian and Taiwanese populations significantly differ in genetic diversity of loci studied here [28]. This suggests involvement of these loci in the processes of evolutional and/or geographical adaptation to environment and a potential for allele substitutions to have different ethnic specific influence on the human health and population morbidity [28].

Several associations revealed in our study showed non-additivity between sexes. In Latvians the rs2277460 and rs2348071 were associated with asthma in females, and the rs2295826 and rs2295827 were disease susceptible in males; in Taiwanese all asthma susceptible loci were limited to females. Sex specific differences in incidence, prevalence, and severity are also well known features of asthma epidemiology. Sex-specific associations with the disease have been recently reported for SNPs of several genes-candidates including the *IFNG* [31], *IL17F* [32], *TSLP* [33], *VDR* [34], and *KCNB1* [35] genes. Our analysis of the BA main effect in Latvian population is a subject to some limitation as sexes were not equally presented in both BA and control groups. Although a significant asthma main effect was detected for all five loci studied, only the rs1048990 showed an additive effect that was an association in both females and males. The replication study in additional larger cohorts represented by sexes equally is required to validate the results found in the current study for Latvian population.

Due to the pleiotropic effect, a frequent phenomenon in human complex traits and diseases [36], some loci of susceptibility may be shared among many autoimmune and other immune-mediated diseases [37, 38]. Earlier the genetic pleiotropic effect has been reported for asthma and obesity [39, 40] and for asthma and juvenile rheumatoid arthritis [41, 42]. Similarly, SNPs associated with asthma in our current study, previously have been found to be susceptible in Latvians to other immune-mediated pathologies including juvenile idiopathic arthritis [24, 43], children obesity [44] and multiple sclerosis [45]. The rs1048990 was widely genotyped in many human populations and reported as an ethnic specific risk factor for inflammation within the cardio-vascular system [27, 28].

All loci we have studied here belong to the non-coding regions of corresponding genes and nucleotide substitutions potentially may influence the gene expression through allele specific targeting of different regulatory elements. Among the allele-specific targets described by Sjakste with co-authors earlier [24, 28], several sites showed affinity to transcription factors and splicing signals implicated previously in immunity, lung function and lung pathology. The targeting of these regulatory proteins may influence asthma pathogenesis and needs to be mentioned in respect of current study. The rs2277460 ancestral allele C, the major allele in human populations over the world, appears to be functionally neutral. Substitution to A generates a target to hnRNP A1, a multifunctional protein implicated in the association with multiple promoter sequences and modulation of a number of transcriptional events [46]. The hnRNP A1 has been shown to play a key role in many human pathologies including lung cancer and response to viral pathogens [46, 47]. It may influence protein-protein interactions including those with participation of NF-κB [46] playing in turn a significant role in the asthma development and progression [5–7]. Additionally, it is involved in crosstalk with ubiquitin proteasome system at different levels of NF-κB and other regulatory proteins signaling pathways [48]. Allele A also assists to sequence affinity to the BARBIE box proteins found to be involved in inflammatory response of alveolar macrophages [49]. Substitution C → G at the rs1048990 was shown to influence the gene expression *in vivo* and *in vitro* [27, 50] and significantly change the sequence capacity to bind a number of splicing signals and transcription factors [28]. Rare allele G generates binding sites for the multifunctional proteins of p53 and DMRT families implicated in the processes of climatic [51] and evolutional [52] adaptation. The targeting of these proteins potentially could be involved in the mechanisms of natural selection and ethnos specific susceptibility to inflammation [27, 28].

Common allele A of the rs2295826 (first intron of the *PSMC6* gene) generates the targets for the mentioned above hnRNP A1 regulatory protein and for the transcription factor of CREB family involved in transcriptional control of many pro-inflammatory genes [53, 54] and implicated in asthma pathogenesis [55], asthma phenotypes and response to therapy [56].

The rs2348071 SNP strongly discriminates Latvians having a major allele G (about 70 %) and Taiwanese having a major ancestral allele A (about 70 %). Previously we have suggested [28] that transition A → G happened in Caucasians about 15,000 years ago was supported by positive selection. This mutation eliminates potential targets for hnRNP A1 and the transcription factors of CART family shown to be an essential participant of signaling respiratory network [57] and the MEF2 family implicated in transcriptional switch between metabolism and immunity [58].

Summarizing mentioned results we suggest that the nucleotide substitutions we have studied may significantly modulate the transcription of related genes and gene network in response to the inflammation and other environmental stimuli and influence the asthma susceptibility.

Conclusions. Our findings provide an evidence that single- and multi locus variations in the 14q13-23 *PSMA6*/*PSMC6*/*PSMA3* proteasomal genes cluster are associated with childhood asthma in Latvian and Taiwanese populations and could play an important role in asthma and other immune-mediated pathologies in both Caucasians and Asians, as either the risk or protective ethnic- and sex-specific genetic factors.

Identification of genetic variants susceptible to asthma and other immune-mediated pathologies, both common and different across populations, is important in understanding pathogenesis and phenotype variability of these multifactorial diseases. It might be a subject of thorough investigation in the nearest future.

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Генетичні варіанти генів *PSMA6*, *PSMC6* і *PSMA3*, асоційовані з бронхіальною астмою у дітей латвійської і тайваньської популяцій

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Резюме

Протеасоми опосередковують реалізацію функцій сигнальних білків, залучених до патогенезу бронхіальної астми. ***Мета****. Оцінити загальну і залежну від статі асоціацію варіацій протеасомних генів PSMA6, PSMC6 і PSMA3 з бронхіальною астмою у дітей із Латвії і Тайваня.* ***Методи****. Однонуклеотидні поліморфізми rs2277460, rs1048990, rs2295826, rs2295827 і rs2348071 генотиповано у 102 хворих з Латвії і 159 – з Тайваня. Для порівняння взято контрольні групи, які представляють генетичне різноманіття в популяціях: 191 латвійських і 1097 тайваньських зразків.* ***Результати****. Гаплотип CGACG виявився тісно (P < 0.0001) асоційованим з ризиком розвитку астми в обох популяціях. Гетерозиготні генотипи за всіма локусами і гаплотип CCGTA ідентифіковано як фактор ризику для розвитку астми у жителів Латвії. Гомозиготи GG по rs1048990 і rs2348071 пов'язані з ризиком, а гетерозиготи по rs2295826 і rs2295827 проявляють захисний ефект з-поміж тайваньських жінок. Багатолокусні генотипи, гомозиготні за розповсюдженими в Латвії алелями, виявилися захисними для жителів Латвії, але пов'язаними з ризиком захворювання серед тайваньців.* ***Висновки****. Наші результати вказують на асоціацію поліморфізмів протеасомних генів локусу 14q13-23 з бронхіальною астмою серед дітей у латвійській і тайваньській популяціях, асоціація може бути пов'язана як з ризиком захворювання, так і з захисним ефектом. За даною ознакою популяції можуть різнитися або не різнитися.*

Ключові слова: хромосома 14q13-23, однонуклеотидні поліморфізми, PSMA6, PSMC6, PSMA3, бронхіальна астма у дітей.

Генетические варианты генов *PSMA6*, *PSMC6* и *PSMA3*, ассоциированные с бронхиальной астмой у детей в латвийской и тайваньской популяциях

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Резюме

Протеасомы опосредуют реализацию функций сигнальных белков, вовлеченных в патогенез бронхиальной астмы. ***Цель****. Оценить общую и зависимую от пола ассоциацию вариаций протеасомных генов PSMA6, PSMC6 и PSMA3 с бронхиальной астмой у детей из Латвии и Тайваня.* ***Методы****. Однонуклеотидные полиморфизмы rs2277460, rs1048990, rs2295826, rs2295827 и rs2348071 генотипированы у 102 больных из Латвии и 159 – из Тайваня. Для сравнения взяты контрольные группы, представляющие генетическое разнообразие в популяциях: 191 латвийских и 1097 тайваньских образцов.* ***Результаты****. Гаплотип CGACG оказался тесно (P < 0.0001) ассоциированным с риском развития астмы в обеих популяциях. Гетерозиготные генотипы по всем локусам и гаплотип CCGTA идентифицированы как фактор риска для развития астмы у жителей Латвии. Гомозиготы GG по rs1048990 и rs2348071 связаны с риском, а гетерозиготы по rs2295826 и rs2295827 проявляют защитный эффект среди тайваньских женщин. Многолокусные генотипы, гомозиготные по распространенным в Латвии аллелям, оказались защитными для жителей Латвии, но связанными с риском заболевания среди тайваньцев.* ***Выводы****. Наши результаты указывают на ассоциацию полиморфизмов протеасомных генов локуса 14q13-23 с бронхиальной астмой среди детей в латвийской и тайваньской популяциях, ассоциация может быть связана как с риском заболевания, так и с защитным эффектом. По данному признаку популяции могут отличаться или не отличаться.*

Ключевые слова: хромосома 14q13-23, однонуклеотидные полиморфизмы, PSMA6, PSMC6, PSMA3, бронхиальная астма у детей.

REFERENCES

1. *Broide DH*. Molecular and cellular mechanisms of allergic disease. *J Allergy Clin Immunol*. 2001;**108**(2 Suppl):S65–71.
2. *Galli SJ, Tsai M, Piliponsky AM.* The development of allergic inflammation. *Nature*. 2008;**454**(7203):445–54.
3. *Wu LS, Sjakste T, Sakalauskas R, et al.* The burden of allergic asthma in children: a landscape comparison based on data from Lithuanian, Latvian, and Taiwanese populations. *Pediatr Neonatol*. 2012;**53**(5):276–82.
4. *Leung TF, Ko FW, Sy HY, et al.* Differences in asthma genetics between Chinese and other populations. *J Allergy Clin Immunol.* 2014;**133**(1):42–8.
5. *Birrell MA, Hardaker E, Wong S, et al.* Ikappa-B kinase-2 inhibitor blocks inflammation in human airway smooth muscle and a rat model of asthma. *Am J Respir Crit Care Med*. 2005;**172**(8):962–71.
6. *Edwards MR, Bartlett NW, Clarke D, et al.* Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. *Pharmacol Ther*. 2009;**121**(1):1–13.
7. *Gagliardo R, Chanez P, Mathieu M, et al.* Persistent activation of nuclear factor-kappaB signaling pathway in severe uncontrolled asthma. *Am J Respir Crit Care Med*. 2003;**168**(10):1190–8.
8. *Konstantinova IM, Tsimokha AS, Mittenberg AG.* Role of proteasomes in cellular regulation. *Int Rev Cell Mol Biol*. 2008; **267**:59–124.
9. *Wang X, Guerrero C, Kaiser P, Huang L*. Proteomics of proteasome complexes and ubiquitinated proteins. *Expert Rev Proteomics*. 2007;**4**(5):649–65.
10. *Yano M, Kanesaki Y, Koumoto Y, et al.* Chaperone activities of the 26S and 20S proteasome. *Curr Protein Pept Sci*. 2005;**6**(2): 197–203.
11. *Albright JM, Romero J, Saini V, et al.* Proteasomes in human bronchoalveolar lavage fluid after burn and inhalation injury. *J Burn Care Res*. 2009;**30**(6):948–56.
12. *Majetschak M, Sorell LT, Patricelli T, Seitz DH, Knoferl MW*. Detection and possible role of proteasomes in the bronchoalveolar space of the injured lung. *Physiol Res*. 2009;**58**(3):363–72.
13. *Sixt SU, Peters J*. Extracellular alveolar proteasome: possible role in lung injury and repair. *Proc Am Thorac Soc.* 2010;**7**(1):91–6.
14. *Zemeckiene Z, Vitkauskiene A, Sjakste T, Sitkauskiene B, Sakalauskas R*. Proteasomes and proteasomal gene polymorphism in association with inflammation and various diseases. *Medicina (Kaunas)*. 2013;**49**(5):207–13.
15. *Hakonarson H, Bjornsdottir US, Halapi E, et al.* A major susceptibility gene for asthma maps to chromosome 14q24. *Am J Hum Genet*. 2002;**71**(3):483–91.
16. *Malerba G, Patuzzo C, Trabetti E, et al.* Chromosome 14 linkage analysis and mutation study of 2 serpin genes in allergic asthmatic families. *J Allergy Clin Immunol*. 2001;**107**(4):654–8.
17. *Mansur AH, Bishop DT, Holgate ST, Markham AF, Morrison JF*. Linkage/association study of a locus modulating total serum IgE on chromosome 14q13-24 in families with asthma. *Thorax*. 2004;**59**(10):876–82.
18. *Munthe-Kaas MC, Gerritsen J, Carlsen KH, et al.* Eosinophil cationic protein (ECP) polymorphisms and association with asthma, s-ECP levels and related phenotypes. *Allergy*. 2007;**62** (4):429–36.
19. *Tulah AS, Beghe B, Barton SJ, Holloway JW, Sayers I*. Leukotriene B4 receptor locus gene characterisation and association studies in asthma. *BMC Med Genet*. 2012;**13**:110.
20. *Ungvari I, Hullam G, Antal P, et al.* Evaluation of a partial genome screening of two asthma susceptibility regions using bayesian network based bayesian multilevel analysis of relevance. *PLoS One*. 2012;**7**(3):e33573.
21. *Zhang Y, Moffatt MF, Cookson WO*. Genetic and genomic approaches to asthma: new insights for the origins. *Curr Opin Pulm Med*. 2012;**18**(1):6–13.
22. *Sjakste T, Eglite J, Sochnevs A, et al.* Microsatellite genotyping of chromosome 14q13.2-14q13 in the vicinity of proteasomal gene PSMA6 and association with Graves' disease in the Latvian population. *Immunogenetics*. 2004;**56**(4):238–43.
23. *Sjakste T, Trapina I, Rumba-Rozenfelde I, Lunin R, Sugoka O, Sjakste N*. Identification of a novel candidate locus for juvenile idiopathic arthritis at 14q13.2 in the Latvian population by association analysis with microsatellite markers. *DNA Cell Biol.* 2010;**29**(9):543–51.
24. *Sjakste T, Paramonova N, Rumba-Rozenfelde I, Trapina I, Sugoka O, Sjakste N*. Juvenile idiopathic arthritis subtype- and sex-specific associations with genetic variants in the *PSMA6*/*PSMC6*/*PSMA3* gene cluster. *Pediatr Neonatol.* 2014;**55**(5): 393–403.
25. *Sjakste T, Kalis M, Poudziunas I, et al.* Association of microsatellite polymorphisms of the human 14q13.2 region with type 2 diabetes mellitus in Latvian and Finnish populations. *Ann Hum Genet.* 2007;**71**(Pt 6):772–6.
26. *Sjakste T, Poudziunas I, Ninio E, et al.* SNPs of PSMA6 gene – investigation of possible association with myocardial infarction and type 2 diabetes mellitus. *Genetika*. 2007;**43**(4):553–9.
27. *Wang H, Jiang M, Zhu H, et al.* Quantitative assessment of the influence of PSMA6 variant (rs1048990) on coronary artery disease risk. *Mol Biol Rep*. 2013;**40**(2):1035–41.
28. *Sjakste T, Paramonova N, Wu L, et al*. *PSMA6* (rs2277460, rs1048990), *PSMC6* (rs2295826, rs2295827) and *PSMA3* (rs2348071) genetic diversity in Latvians, Lithuanians and Taiwanese. Meta Gene. 2014; **2**:283–98.
29. *Rozas J.* DNA sequence polymorphism analysis using DnaSP. *Methods Mol Biol*. 2009;**537**:337–50.
30. *Lewis CM*. Genetic association studies: design, analysis and interpretation. *Brief Bioinform*. 2002;**3**(2):146–53.
31. *Loisel DA, Tan Z, Tisler CJ, et al.* IFNG genotype and sex interact to influence the risk of childhood asthma. *J Allergy Clin Immunol*. 2011;**128**(3):524–31.
32. *Qian F, Zhang Q, Zhou L, et al.* Association between polymorphisms in IL17F and male asthma in a Chinese population. *J Investig Allergol Clin Immunol*. 2012;**22**(4):257–63.
33. *Hunninghake GM, Soto-Quiros ME, Avila L, et al.* TSLP polymorphisms are associated with asthma in a sex-specific fashion. *Allergy*. 2010;**65**(12):1566–75.
34. *Raby BA, Lazarus R, Silverman EK, et al.* Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med*. 2004;**170**(10):1057–65.
35. *Seibold MA, Wang B, Eng C, et al.* An african-specific functional polymorphism in KCNMB1 shows sex-specific association with asthma severity. *Hum Mol Genet*. 2008;**17**(17):2681–90.
36. *Sivakumaran S, Agakov F, Theodoratou E, et al.* Abundant pleiotropy in human complex diseases and traits. *Am J Hum Genet*. 2011;**89**(5):607–18.
37. *Lee SH, Park JS, Park CS*. The search for genetic variants and epigenetics related to asthma. *Allergy Asthma Immunol Res*. 2011;**3**(4):236–44.
38. *Thompson SD, Barnes MG, Griffin TA, Grom AA, Glass DN*. Heterogeneity in juvenile idiopathic arthritis: impact of molecular profiling based on DNA polymorphism and gene expression patterns. *Arthritis Rheum*. 2010;**62**(9):2611–5.
39. *Hallstrand TS, Fischer ME, Wurfel MM, Afari N, Buchwald D, Goldberg J.* Genetic pleiotropy between asthma and obesity in a

community-based sample of twins. *J Allergy Clin Immunol*. 2005;**116**(6):1235–41.
40. *Murphy A, Tantisira KG, Soto-Quiros ME, et al.* PRKCA: a positional candidate gene for body mass index and asthma. *Am J Hum Genet*. 2009;**85**(1):87–96.
41. *Lee SH, Lee EB, Shin ES, et al.* The interaction between allelic variants of CD86 and CD40LG: a common risk factor of allergic asthma and rheumatoid arthritis. *Allergy Asthma Immunol Res*. 2014;**6**(2):137–41.
42. *Ramirez-Bello J, Jimenez-Morales S, Espinosa-Rosales F, et al.* Juvenile rheumatoid arthritis and asthma, but not childhood-onset systemic lupus erythematosus are associated with FCRL3 polymorphisms in Mexicans. *Mol Immunol.* 2013;**53**(4):374–8.
43. *Trapina I, Rumba-Rozenfelde I, Sjakste N, Sokolovska J, Sugoka O, Sjakste T*. Association study of genetic variants in the 14q11–14q13 proteasomal genes cluster with juvenile idiopathic arthritis (JIA) in Latvian population. Proceedings of the Latvian Academy of Sciences. Section B. 2009; **63**(4–5):214–31.
44. *Kupca S, Sjakste T, Paramonova N, et al.* Association of obesity with proteasomal gene polymorphisms in children. *J Obes.* 2013; **2013**:638154.
45. *Kalnina J, Paramonova N, Sjakste N, Sjakste T.* Study of association between polymorphisms in the PSMB5 (rs11543947) and PSMA3 (rs2348071) genes and multiple sclerosis in Latvians. *Biopolym Cell*. 2014; **30**(4):305–9.
46. *Jean-Philippe J, Paz S, Caputi M*. hnRNP A1: the Swiss army knife of gene expression. *Int J Mol Sci*. 2013;**14**(9):18999– 9024.
47. *Guo R, Li Y, Ning J, Sun D, Lin L, Liu X*. HnRNP A1/A2 and SF2/ASF regulate alternative splicing of interferon regulatory factor-3 and affect immunomodulatory functions in human non-small cell lung cancer cells. *PLoS One*. 2013;**8**(4):e62729.
48. *Wu ZH, Shi Y.* When ubiquitin meets NF-κB: a trove for anti-cancer drug development. *Curr Pharm Des*. 2013;**19**(18):3263–75.
49. *Dozmorov M, Wu W, Chakrabarty K, et al*. Gene expression profiling of human alveolar macrophages infected by *B. anthracis* spores demonstrates TNF-alpha and NF-kappab are key components of the innate immune response to the pathogen. *BMC Infect Dis*. 2009**;9**:152.
50. *Ozaki K, Sato H, Iida A, et al.* A functional SNP in PSMA6 confers risk of myocardial infarction in the Japanese population. *Nat Genet*. 2006; (8):921–5.
51. *Sjalander A, Birgander R, Saha N, Beckman L, Beckman G*. p53 polymorphisms and haplotypes show distinct differences between major ethnic groups. *Hum Hered*. 1996;**46**(1):41–8.
52. *Kopp A.* Dmrt genes in the development and evolution of sexual dimorphism. *Trends Genet*. 2012;**28**(4):175–84.
53. *Imhof A, Wolffe AP.* Transcription: gene control by targeted histone acetylation. *Curr Biol.* 1998;**8**(12):R422–4.
54. *Johnson CA, Turner BM.* Histone deacetylases: complex transducers of nuclear signals. *Semin Cell Dev Biol.* 1999;**10**(2):179–88.
55. *Adcock IM, Ito K, Barnes PJ.* Histone deacetylation: an important mechanism in inflammatory lung diseases. *COPD*. 2005;**2**(4):445–55.
56. *Chiappara G, Chanez P, Bruno A, et al.* Variable p-CREB expression depicts different asthma phenotypes. *Allergy*. 2007;**62**(7):787–94.
57. *Viemari JC.* Noradrenergic modulation of the respiratory neural network. *Respir Physiol Neurobiol*. 2008;**164**(1–2):123–30.
58. *Clark RI, Tan SW, Pean CB, et al.* MEF2 is an *in vivo* immune-metabolic switch. *Cell*. 2013;**155**(2):435–47.

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