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Polymorphisms and haplotypes of the chromosome locus 17q12-17q21.1 contribute to adult asthma susceptibility in Slovenian patients

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ABSTRACT

One of the major asthma susceptibility loci is 17q12-17q21.1, but the relationship between this locus and adult asthma is unclear. Association analysis of 13 single nucleotide polymorphisms (SNPs) and haplotypes from 17q12-17q21.1 was performed in 418 adult patients with asthma and 288 controls from Slovenia. Single SNP analysis revealed only marginal associations with adult asthma for SNPs located in *GSDMA*, *GSDMB*, *ORMDL3* and *ZBPB2* genes, and rs7219080 was the most highly associated. Analyses of asthma phenotypes found no association with atopy or lung function, but rs2305480 and rs8066582 were associated with childhood asthma and rs9916279 was associated with asthma in smokers. Notably, haplotypes consisting of rs9916279, rs8066582, rs1042658, and rs2302777 harbouring *PSMD3*, *CSF3* and *MED24* genes were highly associated with asthma. The four most common haplotypes, TCCG, TTGA, CCGA and TTCA, were more frequent in patients with asthma, whereas TCCG, TCCA, TCTA and TTGG were more frequent in controls. Only 3% of asthma patients belonged to haplotypes TCCG, TCCA, TCTA and TTGG compared with nearly one-third (31%) of controls. Associations confirmed that the 17q12-17q21.1 locus harbours a genetic determinant for asthma risk in adults and suggest that in addition to the previously known *ORMDL3-GSDM* locus, *CSF3-PSMD3-MED24* also plays a role in asthma pathogenesis.

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1. Introduction

Asthma is a complex inflammatory airway disease that is evoked by interactions between multiple genes with small effects and environmental triggers [1]. One of the major asthma susceptibility loci is 17q12-17q21.1, which was discovered in a genome-wide association study (GWAS), and this locus harbours several single nucleotide polymorphisms (SNPs) with significant asthma association in diverse ethnic groups [2–5]. Many genes within

the 17q12-17q21.1 locus were not previously considered biologically plausible asthma candidates and the role of these genes in asthma is not yet fully understood. These loci play roles in immune system functioning, the regulation of gene expression, and possibly contribute to asthma development. Genes with at least a partially known function are involved in inflammation via the induction of pro-inflammatory cytokines (e.g., *CSF3*; colony stimulating factor 3) or the triggering of the unfolded protein response (*ORMDL3*; *ORM1*-like 3 (*Saccharomyces cerevisiae*)) [6–7]. Other genes, such as *MED24* (mediator complex subunit 24) and *GSDMA* (gasdermin A), are highly expressed in lungs [8,9]. However, no functions for some of the 17q12-17q21.1 genes, such as *PSMD3* (proteasome non-ATPase regulatory subunit 3), *ZBPB2* (zona pellucida binding protein 2) and *GSDMB* (gasdermin B), have been proposed in asthma. A functional role of the 17q12-17q21.1 locus in immunological processes is further supported by associations with other autoimmune disorders, such as Crohn's disease [10] and biliary cirrhosis [11]. On the molecular level, 17q12-17q21.1 polymorphisms cis-regulate neighbouring genes, which leads to altered gene expression [2,12]. The causative variant for asthma has not been identified, but gene expression results primarily indicate roles for

Abbreviations: GWAS, genome-wide association study; SNP, single nucleotide polymorphism; *CSF3*, colony stimulating factor 3; *ORMDL3*, *ORM1*-like 3 (*S. cerevisiae*); *MED24*, mediator complex subunit 24; *GSDMA*, gasdermin A; *GSDMB*, gasdermin B; *PSMD3*, proteasome non-ATPase regulatory subunit 3; *ZBPB2*, zona pellucida binding protein 2; LD, linkage disequilibrium; FEV1%, percentage of predicted forced expiratory volume in 1 s; VC%, percentage of lung vital capacity; PCR, polymerase chain reaction; ORs, odds ratios; CI, confidence interval; EM algorithm, expectation maximization algorithm; IQR, interquartile range.

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ORMDL3, *GSDMB* and *GSDMA* [2,8]. However, nearby genes should not be neglected because of high linkage disequilibrium (LD) within 17q12-17q21.1. One of the approaches suggests that we could obtain additional information by analysing the haplotypes in these regions instead of assuming a direct relationship between a phenotype and an individual SNP because one or more disease-causing mutations may be in high LD with adjacent SNPs and haplotypes that are formed by these known SNPs, which may be undiscovered markers for disease-causing variants [13].

The current study explored the association of 13 SNPs in the 17q12-17q21.1 region with adult asthma risk and different asthma phenotypes in Slovenian patients. In addition to single SNP association analysis, we also analysed the association between 17q12-17q21.1 haplotypes and asthma susceptibility.

2. Materials and methods

2.1. Patients

A total of 418 unrelated adults with physician-diagnosed asthma following the diagnostic criteria of GINA guidelines [14] were retrospectively analysed in the University Clinic Golnik, Slovenia. Data on asthma patients were obtained from patients' medical records. Asthma was diagnosed based on medical history and physical examination. Lung function parameters, such as the percentage of predicted forced expiratory volume in 1 s (FEV1%) and the percentage of lung vital capacity (VC%), were measured. The criteria for asthma diagnosis were a positive methacholine challenge test, which was defined as a 20% decrease in baseline FEV1 with a cumulative dose of methacholine (PD₂₀) less than 4 mg or an increase in FEV1 of >12% and >200 mL from baseline in bronchodilator reversibility testing after the administration of a bronchodilator. Skin prick tests were performed in atopy-susceptible patients using a standard test panel for Europeans, which includes hazel (*Corylus avellana*), alder (*Alnus incana*), birch (*Betula alba*), plane (*Platanus vulgaris*), cypress (*Cupressus sempervirens*), grass mix (*Poa pratensis*, *Dactylis glomerata*, *Lolium perenne*, *Phleum pratense*, *Festuca pratensis*, *Helictotrichon pretense*), olive (*Olea europaea*), mugwort (*Artemisia vulgaris*), ragweed (*Ambrosia artemisiifolia*), *Alternaria alternata* (*tenuis*), *Cladosporium herbarum*, *Aspergillus fumigatus*, *Parietaria*, cat, dog, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, and cockroach (*Blattella germanica*). Specific IgEs were tested only in patients who were susceptible to specific allergen hypersensitivity. Asthma clinical characteristics that determining asthma phenotypes, such as atopy (defined as at least one positive allergy test, skin-prick test >3 mm wheal diameter or specific IgE >0.35 kU/L), childhood asthma onset (defined by patient self-reported health history) and lung function parameters (FEV1% and VC%) were also investigated. Smoking status was included in the analysis as an important environmental factor. Some patients with asthma did not have complete clinical characteristics (Table 1). The control group consisted of 288 subjects. The following inclusion criteria were used for controls: no symptoms or history of asthma or other pulmonary diseases and no symptoms or history of atopy. The national ethics committee for medical studies approved the study, and written informed consent was obtained from all participants.

2.2. Selection of polymorphisms and genotyping

Genotype data for the CEU population were obtained from HapMap database release #27, phase II + III (NCBI build 36, dbSNP b126) for the asthma susceptibility locus 17q12-17q21.1. A 160-kb region ranging from 35,270 to 35,430 kb was analysed. Pairwise LD was calculated as r^2 using Haploview 4.2 (Broad Institute,

Table 1
Clinical characteristics of asthma patients.

Characteristic	Asthma	Missing data	Controls
Number of subjects, n	418		288
Male, n (%)	160 (38.3)	0	155 (53.8)
Age, median (IQR)	45.0 (24.7)	0	44 (29.0)
Atopy, n (%)	239 (59.2)	14	0 (0.0)
VC%, median (IQR)	100 (20)	13	
FEV1%, median (IQR)	86 (20)	6	
Childhood asthma, n (%)	66 (17.0)	30	0 (0.0)
Smoking, n (%)	113 (30.2)	44	
Eosinophil%, median (IQR)	3.0 (3.8)	284	
Neutrophil%, median (IQR)	60.9 (12.8)	284	
Lymphocyte%, median (IQR)	26.6 (11.9)	284	
Monocyte%, median (IQR)	8.1 (3.1)	284	
Basophil%, median (IQR)	0.4 (0.3)	284	

Cambridge, MA, USA) [15]. We selected 13 tag SNPs with a minor allele frequency of >10%, which represents 50 SNPs with an $r^2 > 0.8$. Some of the SNPs that were previously associated with asthma were included as tag SNPs using the forced inclusion option. Information for 13 tag SNPs from HapMap were obtained from dbSNP. Several of these SNPs were quattro allelic, or SNPs that failed in the genotyping assay design were substituted with biallelic SNPs that were in complete LD based on a proxy search in SNAP [16]. Genomic DNA was purified from peripheral blood or buccal swab samples from patients and healthy controls using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Allelic discrimination was performed in 5 µl reaction volumes using approximately 10 ng of DNA as a template, TaqMan® Fast Advanced Master Mix and pre-designed or custom SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). PCR was performed on ABI PRISM 7500 sequence detection system using Applied Biosystems 7500/7500 Fast Real-Time PCR v2.0 software.

2.3. Statistical and bioinformatics analyses

Deviation from the Hardy-Weinberg equilibrium was examined in controls using the chi-square goodness-of-fit test for each analysed SNP. We analysed our data using recessive, dominant and log-additive genetic models. A homozygote with a higher frequency in asthma patients was combined with a heterozygote for a dominant genetic model and compared to other homozygotes. A homozygote with a higher frequency in asthma patients was compared to a combination of two other genotypes for a recessive model.

Allele and genotype frequencies for categorical data were compared on contingency tables between cases and controls and between different asthma phenotypes. Chi-squared or Fisher's exact test were used, and odds ratios (ORs) with 95% confidence intervals (95% CI) were calculated. Parametric statistics (unpaired *t* test) were used on normally distributed data for quantitative comparisons, such as FEV1% and VC%, between different genotypes, and non-parametric statistics (Mann-Whitney) were used if the distribution deviated from normal. We used GraphPad PRISM program (version 5.0 for Windows, GraphPad Software, San Diego, CA). Logistic regression was performed for analyses of single SNPs to adjust for sex and age to investigate the association between genotypes and asthma risk using SPSS software (SPSS, Inc., Chicago, IL). Haplotypes were identified using Haploview 4.2 (Broad Institute, Cambridge, MA, USA) [15], and association analyses adjusted for sex and age were performed using SNPsstats, which is a web-based tool that allows the estimation of haplotypes frequencies using the expectation-maximization (EM) algorithm [17]. *P* values were conservatively Bonferroni-corrected for the total number of SNPs tested (13) and haplotype number from asthma associated haplotype block (8) (0.05/21), and *P* value < 0.002 was accepted as significant. *P* values < 0.05 were considered marginally significant.

Several bioinformatics tools and databases were used to analyse our data and results. HaploReg v4 [18] and RegulomeDB [19] were used for annotations of the analysed SNPs and SNPs in LD with our lead SNPs. We used HaploReg v4 to analyse changes in regulatory motifs, which are based on position-weighted matrices discovered from ENCODE experiments [20], enhancer annotations (addition of 90 cell types from the Roadmap Epigenome Mapping Consortium) and expression quantitative loci (eQTLs from the GTEx eQTL browser) [21].

Statistical power was calculated using a Genetic Power Calculator [22] for single SNPs using case-control data under the assumption of a dominant or recessive genetic model, 9% disease prevalence, 5% type I error rate (α) and a high-risk allele frequency, as found in our study.

3. Results

3.1. 17q12-17q21.1 single polymorphism associations

The case-control study comprised 418 adult patients with asthma and 288 control subjects. Table 2 lists the genotype and allele frequencies of 13 SNPs (rs1042658, rs2302777, rs2305480, rs3744246, rs3893044, rs4795405, rs7216389, rs7219080, rs8066582, rs8069202, rs9916279, rs12936231, and rs8079416) located in asthma susceptibility locus 17q12-17q21.1 that were analysed in the present study. The overall genotyping efficiency was 100%. None of the polymorphisms deviated significantly from Hardy-Weinberg equilibrium.

Single SNP analysis detected no significant associations with asthma. Only a marginally significant association was found, in which rs7219080, located in *GSDMA*, was the most highly associated ($P = 0.004$). Four other marginally associated SNPs, rs12936231, rs2305480, rs7216389 and rs4795405, located in *ZBP2*, *GSDMB* and *ORMDL3* genes, were found when a dominant or an additive genetic model was assigned (Table 2). The only SNP that suited a recessive genetic model for asthma risk was rs7219080, in which the CC genotype was more frequent in asthma patients compared with the control group (43% vs. 33%).

No significant association with analysed SNPs was found when asthma patients were further analysed according to atopy and lung

function parameters (data not shown). Analysis of the group of asthma patients according to asthma onset and smoking status revealed that two of the 13 analysed SNPs were marginally associated with childhood asthma onset, and only one SNP was associated with smoking in asthma patients (Tables 3 and 4). The GG genotype in rs2305480, located in the *GSDMB* gene (45% vs. 32%; $P = 0.023$, OR 1.97, 95% CI 1.10–3.52), and the TT genotype in rs8066582, located in *PSMD3* (29% vs. 16%; $P = 0.032$, OR 2.06, 95% CI 1.08–3.95), were more frequent for childhood asthma onset than for adult-onset asthma. The comparison of asthma onset to control group for two polymorphisms that were associated with childhood asthma onset revealed that only rs2305480 was associated with childhood asthma onset. The GG genotype frequency in controls was similar to patients with adult asthma onset, specifically 28%, and this frequency was marginally different only from the group of patients with childhood asthma onset ($P = 0.039$, OR 1.84, 95% CI 1.04–3.27). Genotype distributions of rs8066582 between childhood and adult asthma onset were not distinct from controls (data not shown).

Ever-smokers with asthma exhibited a significantly higher CC + CT genotype frequency in rs9916279 located in the *PSMD3* gene than non-smokers with asthma (39% vs. 24%; $P = 0.005$; OR 1.98, 95% CI 1.23–3.20) and controls regardless of smoking status. These data were not available in controls (39% vs. 23%, $P = 0.011$, OR 1.83, 95% CI 1.15–2.91) (data not shown).

3.2. 17q12-17q21.1 haplotype associations

We identified 3 haplotype blocks from the 13 analysed SNPs located in 17q12-17q21.1 based on the solid spine of LD using Haploview software (Fig. 1). Haplotypes in block 1 (rs12936231, rs2305480, rs7216389, rs3744246 and rs4795405, harbouring *ZBP2*, *GSDMB*, and *ORMDL3*) and 2 (rs8079416, rs3893044, rs7219080 and rs8069202, harbouring *ORMDL3* and *GSDMA*) were not associated with asthma. Four SNPs, rs9916279, rs8066582, rs1042658 and rs2302777, in haplotype block 3 were not associated with asthma in single-marker analysis, but haplotype analysis detected highly significant differences between asthma and controls (Table 5). This haplotype spans 33 kb and harbours the *PSMD3*, *CSF3* and *MED24* genes. The major haplotype TCCG was

Table 2
Association of 17q12-17q21.1 polymorphisms with asthma risk.

dbSNP	Gene	Allele 1/2	All asthma patients, genotype n (%)				Controls, genotype n (%)				Adjusted P value, ^a OR (95% CI) ^b
			MAF ^d	1/1	1/2	2/2	MAF	1/1	1/2	2/2	
rs12936231	<i>ZBP2</i>	C/G	0.46	118 (28)	212 (51)	88 (21)	0.48	69 (24)	140 (49)	79 (27)	0.019, 1.54 (1.07–2.19) ^D
rs2305480	<i>GSDMB</i>	A/G	0.42	72 (17)	205 (49)	141 (34)	0.47	65 (23)	141 (49)	82 (28)	0.015, 1.31 (1.05–1.63) ^A
rs7216389	<i>GSDMB</i>	C/T	0.46	90 (22)	206 (49)	122 (29)	0.48	80 (28)	137 (48)	71 (25)	0.036, 1.11 (1.00–1.22) ^D
rs3744246	<i>ORMDL3</i>	C/T	0.17	294 (70)	109 (26)	15 (4)	0.16	205 (71)	74 (26)	9 (3)	0.016, 1.31 (1.05–1.63) ^A
rs4795405	<i>LRR3C3</i>	C/T	0.41	144 (34)	204 (49)	70 (17)		86 (30)	137 (48)	65 (23)	0.024, 1.12 (1.01–1.21) ^D
											0.016, 1.30 (1.05–1.61) ^A
rs8079416	<i>ORMDL3</i>	C/T	0.45	81 (19)	216 (52)	121 (29)	0.46	51 (18)	143 (50)	94 (33)	NS ^c
rs3893044	<i>intergenic</i>	C/T	0.44	131 (31)	206 (49)	81 (19)	0.43	78 (27)	156 (54)	53 (18)	NS
rs7219080	<i>GSDMA</i>	A/C	0.35	57 (14)	181 (43)	180 (43)	0.41	42 (15)	151 (52)	95 (33)	0.0041.60 (1.17–2.21) ^R
											0.018, 1.31 (1.05–1.64) ^A
rs8069202	<i>GSDMA</i>	A/G	0.46	89 (21)	209 (50)	120 (29)	0.43	52 (18)	144 (50)	92 (32)	NS
rs8066582	<i>PSMD3</i>	C/T	0.43	131 (31)	212 (51)	75 (18)	0.43	94 (33)	141 (49)	53 (18)	NS
rs9916279	<i>PSMD3</i>	C/T	0.16	12 (3)	108 (26)	298 (71)	0.14	6 (2)	70 (24)	212 (74)	NS
rs1042658	<i>CSF3</i>	C/T	0.35	173 (41)	194 (46)	51 (12)	0.32	134 (47)	121 (42)	33 (11)	NS
rs2302777	<i>MED24</i>	A/G	0.42	145 (35)	197 (47)	76 (18)	0.43	94 (33)	143 (50)	51 (18)	NS

^a P value adjusted for sex and age.

^b D – dominant genetic model, R – recessive genetic model, A – log-additive genetic model

^c NS – non-significant

^d MAF – minor allele frequency

Table 3
Associations between 17q12–17q21.1 SNPs and asthma onset.

dbSNP	Gene	Allele 1/2	Childhood asthma onset, genotype n (%)			Adult asthma onset, genotype n (%)			Adjusted <i>P</i> value ^a , OR (95% CI) ^b
			1/1	1/2	2/2	1/1	1/2	2/2	
rs2305480	<i>GSDMB</i>	A/G	8 (12)	28 (42)	30 (45)	59 (18)	161 (50)	102 (32)	0.023, 1.97 (1.10–3.52) ^R
rs8066582	<i>PSMD3</i>	C/T	20 (30)	27 (41)	19 (29)	101 (31)	168 (52)	52 (16)	0.022, 1.63 (1.06–2.50) ^A 0.032, 2.06 (1.08–3.95) ^R

A – log-additive genetic model

Only significant associations are shown.

^a *P* value adjusted for sex and age.^b R – recessive genetic model**Table 4**
Association between 17q12–17q21.1 SNP and smoking.

dbSNP	Gene	Allele 1/2	Asthma ever-smokers, genotype n (%)			Asthma non-smokers, genotype n (%)			Adjusted <i>P</i> value ^a , OR (95% CI) ^b
			1/1	1/2	2/2	1/1	1/2	2/2	
rs9916279	<i>PSMD3</i>	C/T	6 (5)	38 (34)	68 (61)	6 (2)	58 (22)	198 (76)	0.005, 1.98 (1.23–3.20) ^D 0.004, 1.82 (1.21–2.73) ^A

A – log-additive genetic model

Only significant associations are shown.

^a *P* value adjusted for sex and age.^b D – dominant genetic model

more frequent in patients with asthma than in the controls. The four most common haplotypes, TCCG, TTTA, CCGA and TTCA, were more frequent in patients with asthma, whereas the remaining haplotypes, TTCC, TCCA, TCTA and TTTG, were more frequent in the control group (Table 5). Notably, a clear haplotype pattern that discriminated between asthmatics and controls was observed. Only 3% of asthma patients belonged to haplotypes TTCC, TCCA, TCTA and TTTG compared with nearly one-third (31%) of control subjects. The most redundant haplotype was used as a reference and revealed that three of eight haplotypes exhibited a considerably reduced risk for asthma. Haplotype TTCC was found in only 0.6% of asthma patients, compared with 12.2% of controls ($P < 0.001$, OR 12.32, 95% CI 4.39–34.58), and TCCA was found in only 0.3% of asthma patients, compared with 9.5% of controls ($P = 0.001$, OR 11.58, 95% CI 3.43–39.12). Haplotype TCTA was found in only 0.6% of asthma patients and 7.1% of controls ($P = 0.001$, OR 8.15 95% CI 2.87–23.15). Rare haplotypes with <1% of frequency in both groups were excluded from the analysis.

3.3. Statistical power

We performed power calculations using the Genetic Power Calculator (http://pengu.mgh.harvard.edu/_purcell/gpc/). We determined that the case-control sample had a 97% power of detecting a high-risk allele with 51% frequency (which represents a minimal high-risk allele frequency; power increases for higher allele frequencies) under the dominant genetic model and an OR of 2.0 if the disease prevalence is 9%. The power decreases to 65% for dominant genetic model and OR of 1.5. Our study of 418 patients could detect an OR of 1.6 with 80% power with a dominant genetic model. The power decreased to 9% for detecting an OR of 1.1. The power of 13% for OR 1.6 is relatively modest with our sample size and recessive genetic model.

4. Discussion

The results of our study support the hypothesis of the involvement of 17q12–17q21.1 in asthma pathogenesis [2–5]. Single SNP analysis identified five SNPs that were marginally associated with

asthma risk: rs12936231, rs2305480, rs7216389, rs4795405 and rs7219080. The effect sizes of the associations ranged from OR 1.11 to 2.03, which is consistent with our expectations because the minor allele frequencies of the analysed SNPs are relatively high in general population. Analysis of asthma clinical characteristics revealed associations with childhood asthma onset and smoking, but no associations were found for lung functions and atopy. Notably, we found a highly statistically significant association of haplotypes composed of rs9916279, rs8066582, rs1042658 and rs2302777 with asthma risk, and ORs were stronger, up to 12.32, and a global haplotype association with a $P < 0.0001$. Haplotype analysis is a promising approach for the detection of genetic variations that are responsible for complex human diseases [23].

Single SNP analysis revealed that the SNP that was most associated with asthma risk was rs7219080, located in the 5' region of the *GSDMA* gene, and CC was the risk genotype for asthma. Haploreg and RegulomeDB revealed that rs7219080 interrupts TATA and TEF-1 motifs. There are also several SNPs in high LD with rs7219080 and rs62068174 located in the binding site of GATA3, which is a protein that is involved in T cell development and T helper 2 (Th2) cell differentiation [24]. There is limited evidence on the role of *GSDMA* in asthma despite reproducible results of asthma association studies. However, 17q12–17q21.1 SNPs influence the expression levels of *GSDMA* [8,25]. There were also two SNPs in tight LD ($r^2 = 0.70$) located in the *GSDMB* gene that were marginally associated with asthma, rs2305480 and rs7216389, which are two of the most known 17q12–17q21.1 asthma susceptibility SNPs. The AA genotype in rs2305480 was less frequent in asthma. This SNP is a missense mutation that causes an amino acid change from proline to serine that is associated with *ORMDL3* expression levels [26]. SNP rs8069176, which is in complete LD with rs2305480, was marginally associated with FeNO levels when 2.5 million SNPs were analysed in children [27]. The CC genotype in rs7216389 was protective for asthma. Allele T in this intronic SNP was positively associated with *ORMDL3* and *GSDMB* expression, but it was negatively with *GSDMA* expression [8], which is a risk allele for asthma [2]. We also found a marginal association between rs12936231 located in *ZPBP2* and asthma, in which the GG genotype was protective. It has been suggested that rs12936231 is a leading functional candidate that explains the

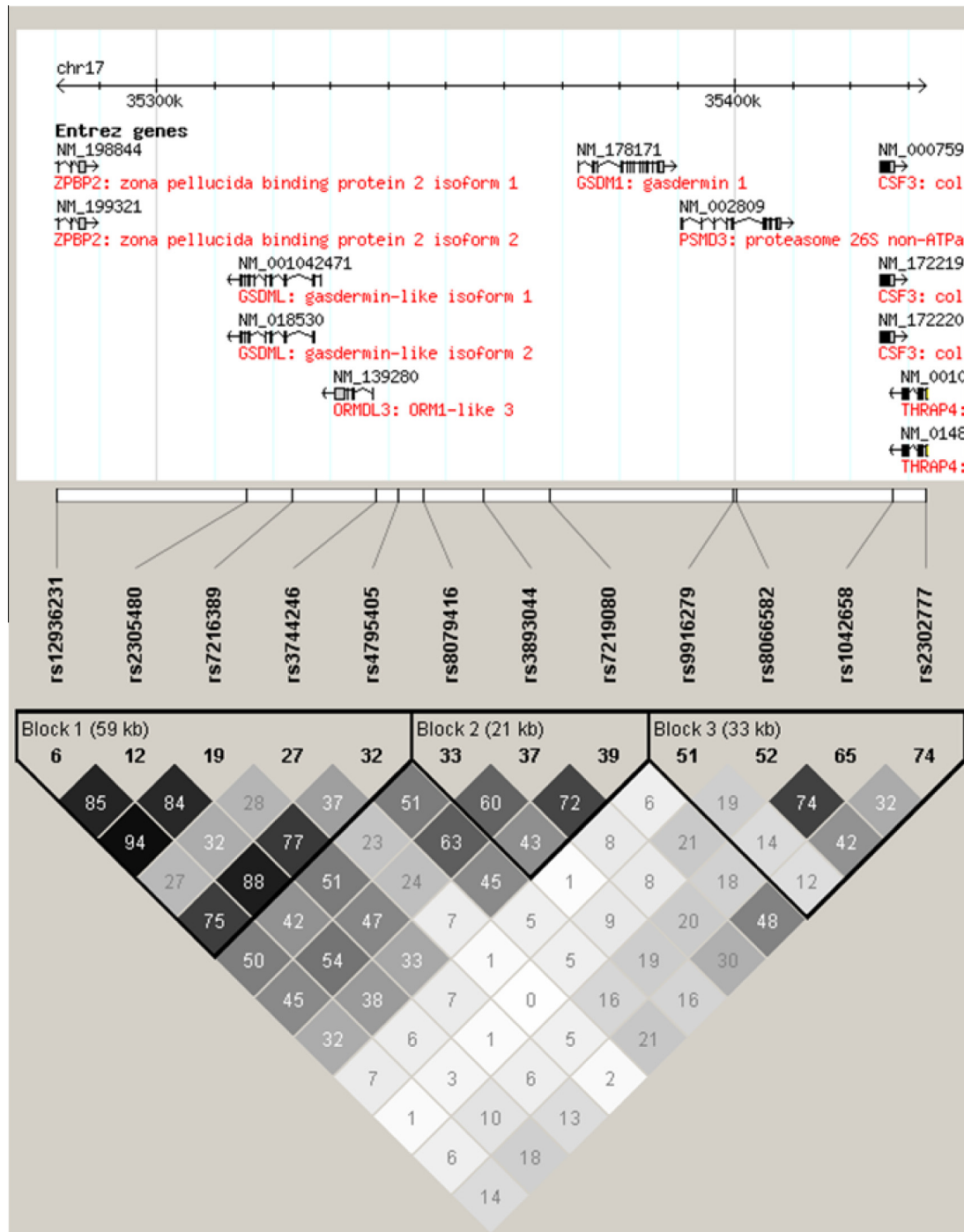


Fig. 1. The pairwise linkage disequilibrium (LD) expressed as r^2 between the investigated 17q12-17q21.1 SNPs in the Haploview software setting. Shading represents magnitude of the pairwise r^2 , which decreases with lighter colour. One of the analysed SNPs, rs8069202, is not shown. Gene names are written in old nomenclature (according to HUGO nomenclature *GSDML* is *GSDMB*, *GSDM1* is *GSDMA* and *THR4P4* is *MED24*).

phenotypic association with asthma because it is part of the regulatory region that governs the expression of several genes (*ZPBP2*, *GSDMB* and *ORMDL3*). This SNP is located in a conserved region that is a binding site for several proteins, and it causes a change in four regulatory motifs, e.g., CTCF-binding, and it influences nucleosome occupancy [28]. This polymorphism is also associated with ankylosing spondylitis [29], which is a chronic inflammatory disease. However, rs4795405 was marginally associated with asthma, and the TT genotype played a protective role, which is consistent with our previous results that demonstrated that the CC

genotype was more frequent in adult and childhood asthma without rhinitis and chronic obstructive pulmonary disease than in healthy controls [30]. Bioinformatic analysis of single SNPs associated with adult asthma in our study revealed that rs4795405 and rs7219080 were eQTLs for *GSDMA* expression in lungs. Several SNPs that were associated with asthma were eQTLs for the same genes, *GSDMB*, *ORMDL3* and *IKZF3*, in whole blood, which suggest that associated SNPs play a functional role in asthma pathology.

The statistical power of our genetic analysis was adequate for the dominant genetic model, but the power was relatively modest

Table 5
Association of 17q12-17q21.1 haplotypes with asthma risk.

rs9916279	rs8066582	rs1042658	rs2302777	All asthma patients n (%)	Controls n (%)	OR (95% CI)	Adjusted <i>P</i> value ^a
T	C	C	G	167 (40.1)	79 (27.6)	1.00	–
T	T	T	A	141 (33.8)	65 (22.7)	0.92 (0.68–1.24)	NS ^b
C	C	C	A	66 (15.7)	38 (13.0)	0.88 (0.61–1.27)	NS
T	T	C	A	33 (7.8)	12 (4.1)	0.66 (0.38–1.16)	NS
T	T	C	G	2 (0.6)	35 (12.2)	12.32 (4.39–34.58)	<0.0001
T	C	C	A	1 (0.3)	27 (9.5)	11.58 (3.43–39.12)	0.0001
T	C	T	A	3 (0.6)	20 (7.1)	8.15 (2.87–23.15)	0.0001
T	T	T	G	4 (1.0)	8 (2.7)	1.98 (0.89–4.40)	NS

Rare haplotypes with frequency <1% in both groups were excluded from the analysis (1 patient in asthma group and 3 patients in control group).

^a *P* value adjusted for sex and age.

^b NS non-significant

for the recessive genetic model because of the small sample size and modest ORs found. Despite the limited power of our study to detect statistically significant associations under the recessive genetic model, the directions of the associations were consistent with studies in other populations [2–5,26]. This result suggests that the variants identified in other populations, are likely relevant in Slovenian population.

Genetic studies provide important insight into asthma complexity, which is likely comprised of distinct disease phenotypes. Therefore, genetic associations of asthma risk variants with specific phenotypes provide supportive evidence of biological differences between phenotypes [31]. For example, a substantially stronger association of 17q12-17q21.1 for early onset asthma was detected [26]. Our study analysed whether differences in asthma phenotypes, specifically between atopic and non-atopic asthma, lung function in asthma patients, asthma onset and smoking impact 17q12-17q21.1 SNP associations. Asthma can appear in childhood and persist until adulthood, but sometimes asthma is transient. Sometimes asthma occurs late in adulthood [32]. Therefore, we included asthma onset in our association analysis. The association results demonstrated that 2 of the 13 analysed SNPs were marginally associated with childhood asthma that persists in adulthood compared with asthma that occurs in adulthood, specifically rs2305480 in *GSDMB* and rs8066582 in *PSMD3* gene. These results suggest that adult onset asthma have different genetic backgrounds, but the SNPs located in 17q12-17q21.1 contribute to asthma regardless of onset, which is consistent with previous studies [3,5]. The reasons for differences in 17q12-17q21.1 association studies are likely due to differences in asthma pathology because the heritability of asthma (approximately 62%) is independent of the age of onset, except for patients older than 50 years, in whom genetic factors do not increase disease risk [33]. The combination of 17q12-17q21.1 SNPs and environmental effects may lead to childhood or adult onset asthma, but this hypothesis must be investigated.

Tobacco smoke exposure is an important asthma modifier, and 17q12-17q21.1 variants are associated with an increased risk for asthma in patients exposed to tobacco smoke [34]. Genotypes CC + CT in rs9916279 located in *PSMD3* were more frequent in patients with asthma who ever smoked in the present study. Previously, *PSMD3* SNPs were associated with phosphatidylcholine, which is a major component of lung surfactant [35]. Smoking reduces the synthesis and secretion of surfactant, which leads to breathing problems [36]. rs9916279 is an eQTL for *PSMD3*, and it also causes changes in the regulatory motif, which may lead to altered *PSMD3* gene expression. Smoking in patients who have reduced levels of lung surfactant because of genetic background may exhibit an increased risk for asthma development. We propose that the *PSMD3* gene should be further investigated in asthma according to smoking and chronic obstructive pulmonary disease, which is frequent in smokers.

We identified several associations between the analysed 17q12-17q21.1 SNPs and asthma/asthma phenotypes, but the associations were relatively weak. Our 17q12-17q21.1 SNP associations results for asthma onset and smoking must be confirmed in a larger group of asthma patients because of the small sample size in asthma phenotype subanalyses.

The most significant result of our study was an association of haplotypes from a block consisting of rs9916279, rs8066582, rs1042658 and rs2302777 with asthma risk. These polymorphisms are located in a region harbouring *PSMD3*, *CSF3* and *MED24* genes, which are involved in white blood cell differentiation, especially neutrophils [37]. The actual linkage phase of SNPs was unknown in our study because we analysed unrelated subjects, but haplotypes were constructed using the expectation maximization (EM) algorithm, which is coded into SNPstats software that predicts the most possible associations. The four major haplotypes (TCCG, TTCA, CCCA and TTCA) were more frequent in asthma patients in contrast to all remaining haplotypes, with >1% frequency in both groups (TTCG, TCCA, TCTA and TTG), which were more frequent in the control group. Only 3% of asthma patients belonged to haplotypes TTCG, TCCA, TCTA and TTG, compared with nearly one-third (31%) of the control subjects. The haplotype TTCG was found in 12.2% of controls but in only 0.6% of asthma patients, which exhibits a significantly protective role. In contrast to single SNP analysis, haplotypes revealed considerably higher asthma risks. Notably, the haplotypes that most significantly influenced asthma risk did not consist of SNPs that were marginally associated in our single polymorphism analysis. Genetic variation in populations is organised in haplotypes, and haplotypes, rather than single SNPs, are often the principal determinant of phenotypic consequences. Haplotype-based association methods are generally regarded more powerful than methods based on single markers because they allow us to capture additional information, such as epistatic interactions [38–39]. The results from Morris et al. [40] suggest that an analysis based on haplotypes is advantageous over an analysis based on individual SNPs in the presence of multiple susceptibility alleles, such as the 17q12-17q21.1 locus, particularly when the linkage disequilibria between SNPs is weak, such as the SNPs that were highly associated with asthma in our analysis. SNPs that were associated with asthma in only haplotype analysis in our study were located in intronic or non-coding regions, whereas SNP haplotypes with window sizes of 2–15 SNPs cover regions of DNA that are approximately 20–300 kb and generally include (within their span) exons from one or more genes, which may be the reason that the associations found in our analysis were significant in only haplotype analysis. SNPs that form an asthma-associated haplotypes, rs1042658 and rs2302777, were eQTLs for *GSDMA* expression in lungs, and haplotype SNPs were also eQTLs for *GSDMB*, *IKZF3*, *ORMDL3* and *PSMD3* gene expression in whole blood. However, no haplotypes were eQTL for *GSDMA* in whole blood. SNPs that comprised our asthma-associated haplotypes and SNPs in LD

($r^2 > 0.8$) with these haplotypes are binding sites for GATA-1, GATA-2 and STAT3 proteins, and they cause changes in GATA motifs. GATA-1 and GATA-2 bind and inhibit STAT3, which are also located on 17q12-17q21.1.31 and required for asthma airway inflammation, eosinophilia and Th2 cell lung recruitment [41–42]. These data suggest that the 17q12-17q21.1 polymorphisms do not necessarily affect only the genes that are located relatively close, such as *ORMDL3*. However, further functional studies are needed. This study demonstrates the importance of analysing haplotypes in addition to single SNPs in complex disease-risk association studies.

In conclusion, we report only a marginal association between 17q12-17q21.1 single SNPs and adult asthma risk and asthma phenotypes. However, we found a highly significant association between 17q21.1 haplotypes spanning through the *PSMD3-CSF3-MED24* gene region and adult asthma risk, which supports the hypothesis that this locus harbours an important genetic determinant for asthma and suggests a role of novel genes in asthma pathogenesis and demonstrates the importance of analysing haplotypes in addition to single SNPs.

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References

- [1] J. Zhang, P.D. Paré, A.J. Sandford, Recent advances in asthma genetics, *Respir. Res.* 9 (2008) 4, <http://dx.doi.org/10.1186/1465-9921-9-4>.
- [2] M.F. Moffatt, M. Kabisch, L. Liang, A.L. Dixon, D. Strachan, S. Heath, M. Depner, A. von Berg, A. Bufer, E. Rietschel, A. Heinzmann, B. Simma, T. Frischer, S.A. Willis-Owen, K.C. Wong, T. Illig, C. Vogelberg, S.K. Weiland, E. von Mutius, G.R. Abecasis, M. Farrall, I.G. Gut, G.M. Lathrop, W.O. Cookson, Genetic variants regulating *ORMDL3* expression contribute to the risk of childhood asthma, *Nature* 448 (2007) 470–473, <http://dx.doi.org/10.1038/nature06014>.
- [3] P.M. Sleiman, K. Annaiah, M. Imielinski, J.P. Bradfield, C.E. Kim, E.C. Frackelton, J.T. Glessner, A.W. Eckert, F.G. Otieno, E. Santa, K. Thomas, R.M. Smith, W. Glaberson, M. Garriss, S. Gunnlaugsson, R.M. Chiavacci, J. Allen, J. Spengel, R. Grundmeier, M.M. Grunstein, M. Magnusson, H. Bisgaard, S.F. Grant, H. Hakonarson, *ORMDL3* variants associated with asthma susceptibility in North Americans of European ancestry, *J. Allergy Clin. Immunol.* 122 (2008) 1225–1227, <http://dx.doi.org/10.1016/j.jaci.2008.06.041>.
- [4] J. Galanter, S. Choudhry, C. Eng, S. Nazario, J.R. Rodríguez-Santana, J. Casal, A. Torres-Palacios, J. Salas, R. Chapela, H.G. Watson, K. Meade, M. LeNoir, W. Rodríguez-Cintrón, P.C. Avila, E.G. Burchard, *ORMDL3* gene is associated with asthma in three ethnically diverse populations, *Am. J. Respir. Crit. Care Med.* 177 (2008) 1194–1200, <http://dx.doi.org/10.1164/rccm.200711-1644OC>.
- [5] F.X. Li, J.Y. Tan, X.X. Yang, Y.S. Wu, D. Wu, M. Li, Genetic variants on 17q21 are associated with asthma in a Han Chinese population, *Genet. Mol. Res.* 11 (2012) 340–347, <http://dx.doi.org/10.4238/2012>.
- [6] T. Queto, Z.F. Vasconcelos, R.A. Luz, C. Anselmo, A.A. Guiné, P.M. e Silva, J. Farache, J.M. Cunha, A.C. Bonomo, M.I. Gaspar-Elsas, P. Xavier-Elsas, G-CSF suppresses allergic pulmonary inflammation, downmodulating cytokine, chemokine and eosinophil production, *Life Sci.* 88 (2011) 830–838, <http://dx.doi.org/10.1016/j.lfs.2011.03.001>.
- [7] K.J. Hsu, S.E. Turvey, Functional analysis of the impact of *ORMDL3* expression on inflammation and activation of the unfolded protein response in human airway epithelial cells, *Allergy Asthma Clin. Immunol.* 9 (2013) 4, <http://dx.doi.org/10.1186/1710-1492-9-4>.
- [8] K. Hao, Y. Bossé, D.C. Nickle, P.D. Paré, D.S. Postma, M. Laviolette, A. Sandford, T. L. Hackett, D. Daley, J.C. Hogg, W.M. Elliott, C. Couture, M. Lamontagne, C.A. Brandsma, M. van den Berge, G. Koppelman, A.S. Reicin, D.W. Nicholson, V. Malkov, J.M. Derry, C. Suver, J.A. Tsou, A. Kulkarni, C. Zhang, R. Vessey, G.J. Opiteck, S.P. Curtis, W. Timens, D.D. Sin, Lung eQTLs to help reveal the molecular underpinnings of asthma, *PLoS Genet.* 8 (2012) e1003029, <http://dx.doi.org/10.1371/journal.pgen.1003029>.
- [9] D. Thierry-Mieg, J. Thierry-Mieg, AceView: a comprehensive cDNA-supported gene and transcripts annotation, *Genome Biol.* 7 (2006), <http://dx.doi.org/10.1186/gb-2006-7-s1-s12>. S121–S14.
- [10] J.C. Barrett, S. Hansoul, D.L. Nicolae, J.H. Cho, R.H. Duerr, J.D. Rioux, S.R. Brant, M.S. Silverberg, K.D. Taylor, M.M. Barmada, A. Bitton, T. Dassopoulos, L.W. Datta, T. Green, A.M. Griffiths, E.O. Kistner, M.T. Murtha, M.D. Regueiro, J.I. Rotter, L.P. Schumm, A.H. Steinhardt, S.R. Targan, R.J. Xavier, NIDDK IBD Genetics Consortium, C. Libioulle, C. Sandor, M. Lathrop, J. Belaiche, O. Dewit, I. Gut, S. Heath, D. Laukens, M. Mni, P. Rutgeerts, A. Van Gossum, D. Zelenika, D. Franchimont, J.P. Hugot, M. de Vos, S. Vermeire, E. Louis, Belgian-French IBD Consortium, Wellcome Trust Case Control Consortium, L.R. Cardon, C.A. Anderson, H. Drummond, E. Nimmo, T. Ahmad, N.J. Prescott, C.M. Onnie, S.A. Fisher, J. Marchini, J. Ghorri, S. Bumpstead, R. Gwilliam, M. Tremelling, P. Deloukas, J. Mansfield, D. Jewell, J. Satsangi, C.G. Mathew, M. Parkes, M. Georges, M.J. Daly, Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease, *Nat. Genet.* 40 (2008) 955–962, <http://dx.doi.org/10.1038/ng.175>.
- [11] G.M. Hirschfield, L. Liu, C. Xu, Y. Lu, G. Xie, Y. Lu, X. Gu, E.J. Walker, B.D. Juran, A.L. Mason, R.P. Myers, K.M. Peltekian, C.N. Ghent, C. Coltescu, E.J. Atkinson, E.J. Heathcote, K.N. Lazaridis, C.I. Amos, K.A. Siminovitch, Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants, *N. Engl. J. Med.* 2360 (2009) 2544–2555, <http://dx.doi.org/10.1056/NEJMoa0810440>.
- [12] S. Berlivet, S. Moussette, M. Ouimet, D.J. Verlaan, V. Koka, A. Al Tuwaijri, T. Kwan, D. Sinnett, T. Pastinen, A.K. Naumova, Interaction between genetic and epigenetic variation defines gene expression patterns at the asthma-associated locus 17q12-q21 in lymphoblastoid cell lines, *Hum. Genet.* 131 (2012) 1161–1171, <http://dx.doi.org/10.1007/s00439-012-1142-x>.
- [13] S. Lin, A. Chakravarti, D.J. Cutler, Exhaustive allelic transmission disequilibrium tests as a new approach to genome-wide association studies, *Nat. Genet.* 36 (2004) 1181–1188, <http://dx.doi.org/10.1038/ng1457>.
- [14] Global Initiative for Asthma, Global Strategy for Asthma Management and Prevention NIH Pub. Updated 2006., 2006. Available from: <www.ginasthma.org> [cited 10.10.15].
- [15] J.C. Barrett, Haploview: visualization and analysis of SNP genotype data, *Cold Spring Harbor Protoc.* 10 (2009), <http://dx.doi.org/10.1101/pdb.ip71>.
- [16] A.D. Johnson, R.E. Handsaker, S. Pulit, M.M. Nizzari, C.J. O'Donnell, P.I.W. de Bakker, SNP: A web-based tool for identification and annotation of proxy SNPs using HapMap, *Bioinformatics* 24 (2008) 2938–2939, <http://dx.doi.org/10.1093/bioinformatics/btn564>.
- [17] X. Solé, E. Guinó, J. Valls, R. Iñiesta, V. Moreno, SNPStats: a web tool for the analysis of association studies, *Bioinformatics* 22 (2006) 1928–1929, <http://dx.doi.org/10.1093/bioinformatics/btl268>.
- [18] L.D. Ward, M. Kellis, HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants, *Nucleic Acids Res.* 40 (2012) D930–D934, <http://dx.doi.org/10.1093/nar/gkr917> (Database issue).
- [19] A.P. Boyle, E.L. Hong, M. Hariharan, Y. Cheng, M.A. Schaub, M. Kasowski, K.J. Karczewski, J. Park, B.C. Hitz, S. Weng, J.M. Cherry, M. Snyder, Annotation of functional variation in personal genomes using RegulomeDB, *Genome Res.* 22 (2012) 1790–1797, <http://dx.doi.org/10.1101/gr.146175.112>.
- [20] ENCODE Project Consortium, An integrated encyclopedia of DNA elements in the human genome, *Nature* 489 (2012) 57–74, <http://dx.doi.org/10.1038/nature11247>.
- [21] GTEx Consortium. Human genomics, The genotype-tissue expression (GTEx) pilot analysis: multitissue gene regulation in humans, *Science* 348 (2015) 648–660, <http://dx.doi.org/10.1126/science.1262110>.
- [22] S.I. Purcell, S.S. Cherny, P.C. Sham, Genetic power calculator: design of linkage and association genetic mapping studies of complex traits, *Bioinformatics* 1 (2003) 149–150, <http://dx.doi.org/10.1093/bioinformatics/19.1.149>.
- [23] J. Akey, L. Jin, M. Xiong, Haplotypes vs single marker linkage disequilibrium tests: what do we gain?, *Eur. J. Hum. Genet.* 9 (2001) 291–300, <http://dx.doi.org/10.1186/1471-2156-10-56>.
- [24] G. Wei, B.J. Abraham, R. Yagi, R. Jothi, K. Cui, S. Sharma, L. Narlikar, D.L. Northrup, Q. Tang, W.E. Paul, J. Zhu, K. Zhao, Genome-wide analyses of transcription factor GATA3-mediated gene regulation in distinct T cell types, *Immunity* 3 (2011) 299–311, <http://dx.doi.org/10.1016/j.immuni.2011.08.007>.
- [25] A. Lluís, M. Schedel, J. Liu, S. Illi, M. Depner, E. von Mutius, M. Kabisch, B. Schaub, Asthma-associated polymorphisms in 17q21 influence cord blood *ORMDL3* and *GSDMA* gene expression and IL-17 secretion, *J. Allergy Clin. Immunol.* 127 (1587–94) (2011) e6, <http://dx.doi.org/10.1016/j.jaci.2011.03.015>.
- [26] E. Halapi, D.F. Gudbjartsson, G.M. Jonsdottir, U.S. Bjornsdottir, G. Thorleifsson, H. Helgadóttir, C. Williams, G.H. Koppelman, A. Heinzmann, H.M. Boezen, A. Jonasdottir, T. Blondal, S.A. Gudjonsson, A. Jonasdottir, T. Thorlacius, A.P. Henry, J. Altmueller, M. Krueger, H.D. Shin, S.T. Uh, H.S. Cheong, B. Jonsdottir, B. R. Ludviksson, D. Ludviksdottir, D. Gislason, C.S. Park, K. Deichmann, P.J. Thompson, M. Wjst, I.P. Hall, D.S. Postma, T. Gislason, A. Kong, I. Jonsdottir, U. Thorsteinsdottir, K. Stefansson, A sequence variant on 17q21 is associated with age at onset and severity of asthma, *Eur. J. Hum. Genet.* 18 (2010) 902–908, <http://dx.doi.org/10.1038/ejhg.2010.38>.
- [27] R.J. van der Valk, L. Duijts, N.J. Timponson, M.T. Salam, M. Standl, J.A. Curtin, J. Genuneit, M. Kerhof, E. Kreiner-Møller, A. Cáceres, A. Gref, L.L. Liang, H.R. Taal, E. Bouzigon, F. Demeis, R. Nadif, C. Ober, E.E. Thompson, K. Estrada, E. Hofman, A.G. Uitterlinden, C. van Duijn, F. Rivadeneira, X. Li, S.P. Eckel, A. Berhane, W.J. Gauderman, R. Granell, D.M. Evans, B. St Pourcain, W. McArdle, J. P. Kemp, G.D. Smith, C.M. Tiesler, C. Flexeder, A. Simpson, C.S. Murray, O. Fuchs, D.S. Postma, K. Bonnelykke, M. Torrent, M. Andersson, P. Sleiman, H. Hakonarson, W.O. Cookson, M.F. Moffatt, L. Paternoster, E. Melén, J. Sunyer, H. Bisgaard, G.H. Koppelman, M. Ege, A. Custovic, J. Heinrich, F.D. Gilliland, A.J.

- Henderson, V.W. Jaddoe, J.C. de Jongste, EARly genetics & lifecourse epidemiology (EAGLE) consortium. Fraction of exhaled nitric oxide values in childhood are associated with 17q11.2–q12 and 17q12–q21 variants, *J. Allergy Clin. Immunol.* 134 (2014) 46–55, <http://dx.doi.org/10.1016/j.jaci.2013.08.053>.
- [28] D.J. Verlaan, S. Berlivet, G.M. Hunninghake, A.M. Madore, M. Larivière, S. Moussette, E. Grundberg, T. Kwan, M. Ouimet, B. Ge, R. Hoberman, M. Swiatek, J. Dias, K.C. Lam, V. Koka, E. Harmsen, M. Soto-Quiros, L. Avila, J.C. Celedón, S.T. Weiss, K. Dewar, D. Sinnett, C. Laprise, B.A. Raby, T. Pastinen, A.K. Naumova, Allele-specific chromatin remodeling in the ZBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease, *Am. J. Hum. Genet.* 85 (2011) 377–393, <http://dx.doi.org/10.1016/j.ajhg.2009.08.007>.
- [29] R. Qiu, H. Zhang, H. Zhao, J. Li, C. Guo, Y. Gong, Q. Liu, Genetic variants on 17q21 are associated with ankylosing spondylitis susceptibility and severity in a Chinese Han population, *Scand. J. Rheumatol.* 42 (2013) 469–472, <http://dx.doi.org/10.3109/03009742.2013.786755>.
- [30] M. Balantic, M. Rijavec, M. Flezar, T. Camlek, I. Hudoklin, M. Kosnik, P. Korosec, S. Suskovic, A polymorphism in ORM DL3 is associated not only with asthma without rhinitis but also with chronic obstructive pulmonary disease, *J. Investig. Allergol. Clin. Immunol.* 23 (2013) 256–261, <http://dx.doi.org/10.3109/03009742.2013.786755>.
- [31] B. Hesselmar, A.C. Enelund, B. Eriksson, L. Padyukov, L.Å. Hanson, N. Aberg, The heterogeneity of asthma phenotypes in children and young adults, *J. Allergy (Cairo)* (2012) 163089, <http://dx.doi.org/10.1155/2012/163089>.
- [32] E.M. Paaso, M.S. Jaakkola, A.K. Rantala, T.T. Hugg, J.J. Jaakkola, Allergic diseases and asthma in the family predict the persistence and onset-age of asthma: a prospective cohort study, *Respir. Res.* 15 (2014) 152, <http://dx.doi.org/10.1186/s12931-014-0152-8>.
- [33] S.F. Thomsen, S. van der Sluis, K.O. Kyvik, A. Skytthe, V. Backer, Estimates of asthma heritability in a large twin sample, *Clin. Exp. Allergy* 40 (2010) 1054–1061, <http://dx.doi.org/10.1111/j.1365-2222.2010.03525.x>.
- [34] E. Bouzigon, E. Corda, H. Aschard, M.H. Dizier, A. Boland, J. Bousquet, N. Chateigner, F. Gormand, J. Just, N. Le Moual, P. Scheinmann, V. Siroux, D. Vervloet, D. Zelenika, I. Pin, F. Kauffmann, M. Lathrop, F. Demenais, Effect of 17q21 variants and smoking exposure in early-onset asthma, *N. Engl. J. Med.* 359 (2008) 1985–1994, <http://dx.doi.org/10.1056/NEJMoa0806604>.
- [35] J.S. Ried, H. Baurecht, F. Stücker, J. Krumsiek, C. Gieger, J. Heinrich, M. Kabesch, C. Prehn, A. Peters, E. Rodriguez, H. Schulz, K. Strauch, K. Suhre, R. Wang-Sattler, H.E. Wichmann, F.J. Theis, T. Illig, J. Adamski, S. Weidinger, Integrative genetic and metabolite profiling analysis suggests altered phosphatidylcholine metabolism in asthma, *Allergy* 68 (2013) 629–636, <http://dx.doi.org/10.1111/all.12110>.
- [36] J.E. Scott, The pulmonary surfactant: impact of tobacco smoke and related compounds on surfactant and lung development, *Tob. Induc. Dis.* 2 (2004) 3–25, <http://dx.doi.org/10.1186/1617-9625-2-1-3>.
- [37] M.A. Nalls, D.J. Couper, T. Tanaka, F.J. van Rooij, M.H. Chen, A.V. Smith, D. Toniolo, N.A. Zakai, Q. Yang, A. Greinacher, A.R. Wood, M. Garcia, P. Gasparini, Y. Liu, T. Lumley, A.R. Folsom, A.P. Reiner, C. Gieger, V. Lagou, J.F. Felix, H. Völzke, N.A. Guskova, A. Biffi, A. Döring, U. Völker, S. Chong, K.L. Wiggins, A. Rendon, A. Dehghan, M. Moore, K. Taylor, J.G. Wilson, G. Lettre, A. Hofman, J.C. Bis, N. Pirastu, C.S. Fox, C. Meisinger, J. Sambrook, S. Arepalli, M. Nauck, H. Prokisch, J. Stephens, N.L. Glazer, L.A. Cupples, Y. Okada, A. Takahashi, Y. Kamatani, K. Matsuda, T. Tsunoda, T. Tanaka, M. Kubo, Y. Nakamura, K. Yamamoto, N. Kamatani, M. Stumvoll, A. Tönjes, I. Prokopenko, T. Illig, K.V. Patel, S.F. Garner, B. Kuhnle, M. Mangino, B.A. Oostra, S.L. Thein, J. Coresh, H.E. Wichmann, S. Menzel, J. Lin, G. Pistis, A.G. Uitterlinden, T.D. Spector, A. Teumer, G. Eiriksdottir, V. Gudnason, S. Bandinelli, T.M. Frayling, A. Chakravarti, C.M. van Duijn, D. Melzer, W.H. Ouwehand, D. Levy, E. Boerwinkle, A.B. Singleton, D.G. Hernandez, D.L. Longo, N. Soranzo, J.C. Witteman, B.M. Psaty, L. Ferrucci, T.B. Harris, C.J. O'Donnell, S.K. Ganesh, Multiple loci are associated with white blood cell phenotypes, *PLoS Genet.* 7 (2011) e1002113, <http://dx.doi.org/10.1371/journal.pgen.1002113>.
- [38] E.K. Silverman, Haplotype thinking in lung disease, *Proc. Am. Thorac. Soc.* 4 (2007) 4–8, <http://dx.doi.org/10.1513/pats.200607-145JG>.
- [39] N. Liu, K. Zhang, H. Zhao, Haplotype-association Analysis, *Adv. Genet.* 60 (2008) 335–405, [http://dx.doi.org/10.1016/S0065-2660\(07\)00414-2](http://dx.doi.org/10.1016/S0065-2660(07)00414-2).
- [40] R.W. Morris, N.L. Kaplan, On the advantage of haplotype analysis in the presence of multiple disease susceptibility alleles, *Genet. Epidemiol.* 23 (2002) 221–233.
- [41] S. Ezoe, I. Matsumura, K. Gale, Y. Satoh, J. Ishikawa, M. Mizuki, S. Takahashi, N. Minegishi, K. Nakajima, M. Yamamoto, T. Enver, Y. Kanakura, GATA transcription factors inhibit cytokine-dependent growth and survival of a hematopoietic cell line through the inhibition of STAT3 activity, *J. Biol. Chem.* 280 (2005) 13163–13170, <http://dx.doi.org/10.1074/jbc.M413461200>.
- [42] M.C. Simeone-Penney, M. Severgnini, P. Tu, R.J. Homer, T.J. Mariani, L. Cohn, A. R. Simon, Airway epithelial STAT3 is required for allergic inflammation in a murine model of asthma, *J. Immunol.* 178 (2007) 6191–6199, <http://dx.doi.org/10.1371/journal.pone.0011348>.