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17q12-21 and asthma: interactions with early-life environmental exposures

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ABSTRACT

Background: 17q12-21 polymorphisms are associated with asthma presence and severity across different populations.

Objective: To extensively investigate the genes in this region among Croatian schoolchildren in a case-control study, taking account of early-life environmental exposures.

Methods: We included 423 children with asthma and 414 controls aged 5 to 18 years. Fifty-one haplotype tagging single-nucleotide polymorphisms (SNPs) were genotyped (GSDMA, GSDMB, ORMDL3, IKZF3, ZBP2, and TOP2). Data on exposure to smoking and furry pet ownership were collected using a validated questionnaire. Information on severe asthma exacerbations with hospital admission were retrieved from hospital notes. All patients underwent spirometry.

Results: We found 2 SNPs (1 novel rs9635726 in IKZF3) to be associated with asthma. Among children with asthma, 4 SNPs (in ZBP2, GSDMB, and GSDMA) were associated with hospital admissions and 8 SNPs with lung function. One SNP (rs9635726) remained significantly associated with a predicted forced expiratory volume in 1 second after false discovery rate correction. Nine markers across 5 genes showed interaction with early-life environmental tobacco smoke (ETS) exposure in relation to asthma and 2 with furry pet ownership. Among children with asthma, we observed significant interactions between early-life ETS exposure and 3 SNPs for lung function and among early-life ETS exposure, 3 SNPs (in ORMDL3 and GSDMA), and hospital admission with asthma exacerbation. Three SNPs (in ORMDL3) interacted with current furry pet ownership in relation to hospital admissions for asthma exacerbation.

Conclusion: Our results indicate that several genes in the 17q12-21 region may be associated with asthma. This study confirms that environmental exposures may need to be included into the genetic association studies.

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Introduction

Asthma is a common chronic illness with moderate prevalence among Croatian schoolchildren.¹ It has an important genetic component confirmed by twin studies but no clear pattern of inheritance.² Hundreds of studies have investigated genetic associations of asthma,³ but few of the reported associations have been successfully replicated in multiple populations.^{3–5}

The rapid increase in asthma prevalence⁶ suggests an important role for environmental exposures in the etiology of the disease.

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Exposure to environmental tobacco smoke (ETS) in early life has been associated with the development of asthma.^{7,8} Maternal smoking during pregnancy affects the immune system development, lung structure, and lung function in offspring,⁹ but the effect of ETS exposure may differ between individuals with different genetic predispositions.¹⁰ Other early life factors that have been associated with the development of asthma include ownership of cats and dogs, but whereas some studies found these exposures to be protective, others have reported that they increase the risk of asthma.¹¹ Reasons for this are unclear but may include reverse causality (ie, where those at highest risk of disease avoid pets) or possibly different genetic susceptibilities.

Genome-wide association studies have identified the 17q21 region to be associated with childhood asthma.^{12,13} Further analyses found that variants in this region determined expression of ORMDL3 in Epstein-Barr virus-transformed lymphoblastoid cell lines from asthmatic children, suggesting ORMDL3 as a potential asthma candidate gene, and the single-nucleotide polymorphism

(SNP) rs7216389 as a major susceptibility locus for childhood asthma.¹² The SNP rs7216389 is found within a linkage disequilibrium (LD) block, which includes not only *ORMDL3* but also *GSDMB*, *ZBP2*, *IKZF3*, *GSDMA*, and *TOP2* genes. *ORMDL* is a gene family comprising 3 genes in humans (*ORMDL1*, *ORMDL2*, and *ORMDL3*), which are expressed ubiquitously in adult and fetal tissues; moderately high in the pancreas, placenta, and brain; and low in skeletal muscle and lung.¹⁴ They encode transmembrane proteins anchored in the endoplasmic reticulum,¹⁴ and their function is unclear. Subsequent candidate gene association studies^{15–23} have confirmed an association between asthma and variants in this region among a variety of ethnically diverse populations. However, results are not always consistent across populations, and it still remains unclear which gene(s) in this region is causally associated with asthma.

We sought to comprehensively genotype tagging SNPs across 6 genes in the 17q12-21 region and investigate their association with asthma. In addition, among children with asthma, we investigated whether genetic variation in this region was associated with hospital admission for asthma exacerbations or lung function (as markers of asthma severity). We then examined whether there were interactions between variants in this region with early-life ETS exposure and pet ownership in relation to asthma presence and severity.

Methods

Study Design

This case-control study was approved by the local institutional review board, and all parents (and children when appropriate) gave written informed consent.

Setting

The study took place between 2006 and 2008 at the Department of Paediatrics, General Hospital “Dr Josip Benčević” Slavonski Brod, Croatia, a stable area of white, mixed urban-rural population.

Participants

Children aged 5 to 18 years with asthma were recruited from the pediatric asthma clinic to make up the case population. All patient had a physician diagnosis of asthma, were currently taking asthma medications, and had had 2 or more asthma symptoms (wheezing, coughing, and both) in the last 12 month. Nonasthmatic controls were participants randomly selected from patients with non-respiratory conditions who sought care from other hospital departments (eg, fracture clinic) or from local schools; absence of

respiratory symptoms or prescription of asthma treatments was confirmed by a validated, interviewer-administered questionnaire.²⁴ Cases and controls were not matched by sex and age (Table 1).

Data Sources and Measurement

Data on ETS exposure and pet ownership in the home (both current and in the first year of life) were collected by questionnaire.²⁴ A trained pediatrician retrieved information on hospital admission for acute asthma exacerbations from paper-based hospital medical records. All asthmatic patients underwent spirometry according to American Thoracic Society/European Respiratory Society specifications to measure forced expiratory volume in 1 second (FEV₁) (expressed as percentage predicted) and forced expiratory flow between 25% and 75% (FEF_{25%–75%}). We performed skin prick tests to a panel of inhalant and food allergens (mite cat, dog, grass pollen, ragweed, birch pollen, peanut, and egg) and defined atopy as a wheal diameter 3 mm greater than negative control to at least one allergen.

Genotyping

To capture most of the common genetic variation within this region, we selected haplotype-tagging SNPs with a minor allele frequency of 5% or greater in the 17q12-21 chromosomal region, including *GSDMA*, *GSDMB*, *ORMDL3*, *IKZF3*, *ZBP2*, and *TOP2A*, using the GVS (<http://gvs.gs.washington.edu/GVS/>), complemented by the National Center for Biotechnology Information's SNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) and Haploview (www.broadinstitute.org/haploview/haploview).^{25–28} We force-included relevant SNPs reported in the literature into the algorithm. TagSNPs from all generated bins and from singleton bins²⁹ were selected for genotyping (Sequenom).^{30–32} eTable 1 summarizes the genotyping information for 51 typed SNPs.

Statistical Analyses

Statistical analyses were performed using SNP & Variation Suite (SVS) 7.2.3 (Golden Helix Inc, Bozeman, Montana) and SPSS statistical software, version 19.1 (SPSS Inc, Chicago, Illinois). The association between 17q12-21 markers and asthma was investigated using χ^2 tests and logistic regression. We tested additive models, and where a trend was seen, dominant and recessive genotype models were tested to find the best fit. False discovery rate (FDR) control was used to correct for multiple comparisons.^{33,34} Results are presented as *P* values (uncorrected and FDR corrected), odds ratios (OR), and 95% confidence intervals (CIs).

Table 1
Demographic characteristics of the study population

Characteristics	No. (%) of participants ^a		<i>P</i> value
	Cases (n = 423)	Controls (n = 414)	
Mean age, y	11.8	12.3	<.001
Male	248 (58.6)	178 (43.0)	<.001
ETS exposure in utero by household member	268 (63.7)	265 (64.5)	.43
ETS exposure in utero by mother	86 (20.3)	85 (20.7)	.48
ETS exposure during first year of life by household member	282 (66.7)	284 (69.1)	.25
ETS exposure during first year of life by mother	122 (28.8)	133 (32.4)	.15
Current ETS exposure by household member	304 (71.9)	311 (75.7)	.12
Atopic	383 (90.5)	73 (17.7)	<.001
Cat or dog in home in first year of life	34 (8.0)	66 (16.1)	<.001
Cat or dog in home currently	47 (11.1)	91 (22.1)	<.001
Admitted to hospital with asthma ever	150 (35.5)	NA	
Admitted to hospital once	97 (22.9)	NA	
Admitted to hospital ≥ 2 times	53 (12.5)	NA	

Abbreviations: ETS, environmental tobacco smoke; NA, not applicable.

^aData are presented as number (percentage) of study participants unless otherwise indicated.

Analysis of gene-environment interactions was performed using appropriate multivariate regression models. The quantitative outcomes (lung function) were analyzed using correlation/trend test, univariate linear regression, and general linear model univariate 1-way independent analysis of variance. The FEF_{25%-75%} analyses were adjusted for age, sex, and height.

Results

Participants

A total of 923 children were randomly selected and approached about recruitment into the study (438 asthmatic patients and 485 controls). The overall response rate was 91% ($n = 837$; 423 asthmatic patients and 414 controls). A total of 824 participants provided blood samples for DNA analysis (417 asthmatic patients and 407 controls).

Descriptive Data

Demographic characteristics of the studied population are presented in Table 1. The proportion of males was higher among case patients (58.6% vs 43.1%, $P < .01$); therefore, all analyses were adjusted for sex. We observed no differences between groups in early-life or current ETS exposure. Pet (cat or dog) ownership was more common in controls than in asthmatic patients, both during the first year of life (8% asthmatic patients and 16.1% controls, $P < .01$) and currently (11.1% asthmatic patients and 22.1% controls, $P < .01$). Only 4 children were sensitized to cat and currently had a cat in their home, and 3 children were sensitized to dog and currently had a dog in their home. Other environmental or socioeconomic factors (eg, area of residence and position in sibship) did not significantly differ between the groups (data not shown).

Among cases, 150 children (35.5%) had at least one hospital admission for an acute exacerbation of asthma. Of those, 53 (12.7%) were hospitalized 2 or more times. No significant association was observed between early-life ETS exposure and hospital admissions for acute asthma exacerbation. In addition, no association was found between current cat and dog ownership and hospital admissions.

Genotyped and Analyzed SNPs

Of the 51 selected SNPs, 3 failed genotyping, 1 was monomorphic, and 1 was not in Hardy-Weinberg equilibrium (eTable 1). For the remaining 46 SNPs, we applied LD filtering algorithm to identify tagging SNPs, while keeping key SNPs reported in other published studies. This resulted in 35 SNPs being used in the analysis; patterns of LD are shown in eFigure 1.

Association Analysis

A summary of significant associations in relation to asthma presence and asthma severity (hospital admissions and lung function) is shown (as P values) in eTable 1.

Asthma

Of the 35 analyzed SNPs, only 2 were found to be associated with asthma. Asthma was more common among T allele homozygotes for rs9635726 (*IKZF3*) and G allele carriers in rs12150079 (*ZBP2*) compared with other genotype groups (OR, 1.53; 95% CI, 1.14–2.05; $P < .01$; and OR, 1.46; 95% CI, 1.10–1.94; $P = .01$; respectively). None remained significant after FDR correction (FDR $P > .12$). We observed no association between rs7216389 or rs2305480 and asthma ($P > .2$).

Interaction Between 17q12-21 Polymorphisms and Early-Life ETS Exposure

Nine variants were found to significantly interact with early-life ETS exposure in relation to asthma, but 7 of these were in high LD ($r^2 > 0.9$) so unlikely to represent independent effects (eTable 1 and eFig 1). For example, early-life ETS exposure significantly increased the risk of asthma among T allele homozygotes of rs4795405, with no effect of ETS exposure among C allele carriers (eFig 2).

Interaction Between 17q12-21 Polymorphisms and Pet Ownership

Initially, we performed the analysis for cat and dog ownership separately. However, because the observed interactions were in the same direction, all further analyses were performed on combined pet (cat and dog) ownership. We found a significant interaction between 2 *GSDMA* SNPs (rs921651 and rs8077456) and current pet ownership in relation to asthma presence. For 1 of these SNPs (rs921651), there was also an interaction with pet ownership during the first year of life in that pet ownership in early life was protective among A allele homozygotes, with no effect of pet ownership among G allele carriers (Fig 1A).

Hospital Admissions With Acute Asthma Exacerbation

Among children with asthma, 4 SNPs were associated with hospital admission with acute asthma exacerbation (Table 2 and eTable 1). For example, G allele carriers in rs7212938 had increased risk for hospital admission compared with T allele homozygotes (OR, 1.71; 95% CI, 1.01–2.88; $P = .04$). Two of these SNPs (rs8067378, rs2290400) are in high LD and probably represent the same effect. However, none of these associations passed FDR correction.

Interaction Between Genetic Variants and Early-Life ETS Exposure

We observed significant interactions between 3 SNPs (in *ORMDL3* and *GSDMA*) and early-life ETS exposure in relation to hospital admissions (eTable 1). Two of these SNPs (rs4795408 and rs8079416) are in high LD and probably represent the same effect.

Interaction Between Genetic Variants and Current Pet Ownership

Three SNPs in high LD in *ORMDL3* (rs3744246, rs4795403, rs4795404) interacted with current pet ownership in relation to hospital admissions (eTable 1), probably representing a single effect (eg, among asthmatic patients pet ownership significantly increased the risk of hospital admission with asthma exacerbation among carriers of the T allele of rs4795403, with no effect of pet ownership among C allele homozygotes) (Fig 1B).

Lung Function

Eight SNPs were significantly associated with lung function among asthmatic patients (FEV₁ or FEF_{25%-75%}, Table 3). Six SNPs were associated with FEV₁ (2 in *IKZF3* and *ORMDL3* and 1 in *GSDMA* and *TOP2A*). Five SNPs were associated with FEF_{25%-75%} (1 in *IKZF3*, *ORMDL3*, and *GSDMA* and 2 in *TOP2A*). One SNP (rs9635726 in *IKZF3*) remained significantly associated with FEV₁ after correction for multiple comparisons, with T allele homozygotes having significantly lower FEV₁ compared with C allele carriers (Table 3).

Interaction Between Genetic Variants and Early-Life ETS Exposure

There was an interaction between early-life ETS exposure and 3 variants in relation to FEV₁ ($P < .04$). For example, major allele homozygotes in rs9303277 (*IKZF3*), rs8067378 (*GSDMA*), and rs12603332 (*ORMDL3*) had adverse effect if they were exposed to ETS compared with the minor allele carriers.

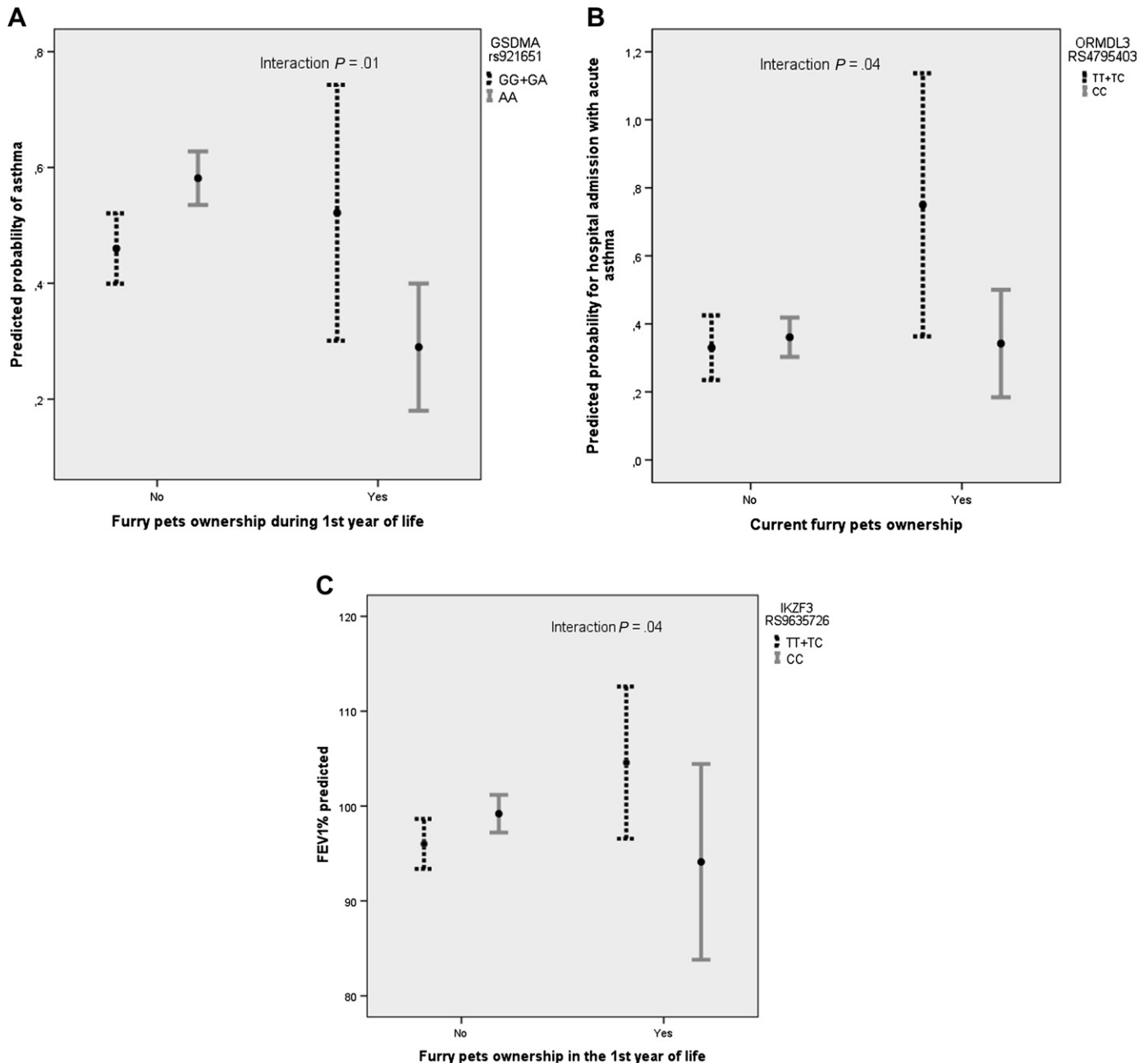


Figure 1. Interaction between single-nucleotide polymorphisms (SNPs) and pet ownership. A, For SNP rs921651 in *GSDMA*, pet ownership during first year of life decreased risk of asthma among A allele homozygotes with no effect among G allele carriers. B, For SNP rs4795403 in *ORMDL3*, current pet ownership was associated with an increase in admission to hospital with acute asthma exacerbation for the T allele carriers, with no effect among C allele homozygotes. C, For SNP rs9635726 in *IKZF3*, pet ownership during the first year of life was associated with higher forced expiratory volume in 1 second (FEV₁) percent predicted for the T allele carriers, with no effect of pet ownership on FEV₁ percent predicted amongst C allele homozygotes.

Interaction Between Genetic Variants and Pet Ownership

We observed significant interaction for 1 SNP (rs9635726 in *IKZF3*) with pet ownership in the first year of life in relation FEV₁. T allele carriers had significantly higher FEV₁, with no effect of pet ownership among C allele homozygotes (Fig 1C).

Discussion

Principal Findings

To the best of our knowledge, our study in which we comprehensively genotyped tagging SNPs across 6 genes in the 17q12-21 is

the first to demonstrate significant interactions between polymorphisms in this region and pet ownership in relation to asthma presence and, among asthmatic patients, to asthma severity. Early-life pet ownership decreased the risk of asthma, but this protective effect was observed only among children homozygous for the common allele of *GSDMA* SNP rs921651. Among asthmatic children, we observed an opposite effect of current pet ownership in relation to asthma exacerbations in that current pet ownership increased the risk for hospital admission but only in children who carried the minor allele in *ORMDL3* SNPs rs3744246, rs4795403, and rs4795404.

We also report for the first time, to our knowledge, an association between SNP rs9635726 in *IKZF3* and asthma. This intronic

Table 2

Association between genetic variants in the region 17q12-21 and admission to hospital with acute asthma exacerbation

SNP	Gene	Allele ^a	P value	FDR	Genetic model	OR (95% CI)
rs12150079	<i>ZBP2</i>	G/A	.02	0.75	AA+AG vs GG	0.61 (0.40-0.93)
rs8067378 ^b	<i>GSDMB</i>	A/G	.04	0.43	GG vs GA+AA	0.56 (0.32-0.99)
rs2290400 ^b	<i>GSDMB</i>	T/C	.03	0.91	CC vs TC+TT	0.52 (0.28-0.95)
rs7212938	<i>GSDMA</i>	T/G	.04	0.69	GG+GT vs TT	1.71 (1.01-2.88)

Abbreviations: CI, confidence interval; FDR, false discovery rate; OR, odds ratio; SNP, single-nucleotide polymorphism.

^aCommon allele is first; risk allele is in bold.

^bSNPs in high linkage disequilibrium ($r^2 > 90$).

SNP has not been genotyped before in other published studies and is not in LD with other SNPs in this gene that have been associated with asthma; therefore, this may represent a novel and independent effect. We confirmed a significant association between SNP in *ZBP2* (rs12150079) and asthma.¹³ However, we did not find associations between asthma and the SNPs most commonly reported in other studies (rs7216389 and rs2305480).

No interactions were found between ETS exposure and SNPs across the region, in keeping with previously published studies.^{17,18,32} However, we acknowledge that none of these results were significant after correction for multiple testing. Eight SNPs were associated with lung function among children with asthma, one of which (rs3169572 in *ORMDL3*) remained significant after correction for multiple comparisons. We also observed significant interactions between early-life ETS exposure and 17q12-21 variants in relation to lung function.

Limitations and Strengths

We acknowledge that cases and controls were not matched by sex and that the proportion of males was higher among cases. To address this, all analyses were adjusted for sex. We also cannot exclude the possibility that the presence of undetected population stratification influenced our results.

Information about ETS exposure and pet ownership was collected retrospectively and may be subject to recall bias. Proportion of pet owners was lower among cases than controls, and it is possible that some families avoided keeping pets because of a family history of allergies.

One of the strengths of our study is that we conducted comprehensive genotyping of the known common variants within

a broad region of 17q12-21, including *GSDMA*, *GSDMB*, *ORMDL3*, *IKZF3*, *ZBP2*, and *TOP2*. To reinforce our findings, we adjusted for multiple comparisons. By taking into account the total number of variants tested, we increased the probability that our results reflect true associations. Both uncorrected and corrected *P* values are shown.

Our case definition was accurate, and asthma diagnosis was confirmed by a pediatric pulmonologist. We used hospital admission with asthma exacerbation as a marker of asthma severity; this information was extracted from medical records and is more reliable than parentally reported questionnaire data.

Interpretation

We have previously reported an association between *GSDMB* SNP rs7216389 and hospital admission for asthma in the adult UK population.³⁵ In the current study of Croatian schoolchildren, we observed associations between SNPs in *GSDMA* and *ZBP2* and hospital admission with asthma exacerbation. Some associations between genetic variants with disease presence and severity were only revealed when relevant environmental exposures (early-life ETS exposure or furry pet ownership) were taken into account. Previous studies^{12,13,17,36} that have investigated the association between SNPs in this region and asthma reported inconsistent results, which may be the consequence of multiple interactions between genetic variants and environmental exposures.

To date, several studies have investigated the interaction between ETS exposure and polymorphisms in the 17q12-21 region in relation to childhood asthma. Bouzigon et al¹⁷ reported associations between 11 SNPs in the 17q12-21 region and early onset of asthma only among children who have been exposed to ETS in early life but not in the unexposed subgroup.¹⁷ Flory et al¹⁸ reported a modifying effect of ETS exposure for 9 17q12-21 SNPs. Associations between these 9 SNPs and asthma were stronger in the group exposed to ETS. Data from 2 independent birth cohorts in the Netherlands reported an association of rs2305480 with asthma-like symptoms in preschool children; however, this association was modified by ETS exposure during fetal life and in infancy.³⁷ A study³² in adults found a significant interaction between active smoking and 17q12-21 polymorphisms. However, in a nested case-control study of 1845 infants, no statistically significant interactions were found between rs7216389 and maternal smoking during pregnancy and infancy in relation to the risk of recurrent wheeze.³⁸ To the best of our knowledge, ours is

Table 3

Genetic variants in the region 17q12-21 and lung function measures

SNP	Gene	Allele ^a	FEV ₁ , % predicted				FEF _{25%-75%}			
			P value	FDR	Genetic model	Mean (95% CI)	P value	FDR	Genetic mModel	Mean (95% CI)
rs9900538	<i>IKZF3</i>	T/C	0.04	0.35	CC+CT TT	102.30 (98.04-106.57) 97.43 (95.77-99.09)	0.01	0.18	CC+CT TT	4.48 (4.24-4.71) 4.11 (4.02-4.20)
rs9635726	<i>IKZF3</i>	C/T	<0.01	0.01	TT CT+CC	86.01 (79.23-92.79) 98.63 (97.06-100.20)				
rs3169572	<i>ORMDL3</i>	G/A	0.02	0.20	AA+AG GG	102.80 (98.74-106.85) 97.41 (95.75-99.06)	0.01	0.10	AA+AG GG	4.46 (4.24-4.69) 4.12 (4.03-4.21)
rs4795403	<i>ORMDL3</i>	C/T	0.04	0.33	TT+TC CC	100.93 (97.84-104.01) 97.27 (95.50-99.04)				
rs4795408	<i>GSDMA</i>	G/A					0.03	0.18	AA+AG GG	4.10 (3.99-4.20) 4.33 (4.15-4.50)
rs921651	<i>GSDMA</i>	A/G	0.04	0.29	GG+GA AA	95.83 (93.11-98.54) 99.20 (97.32-101.09)				
rs471692	<i>TOP2A</i>	G/A	0.04	0.31	AA AG+GG	107.94 (98.66-117.22) 97.84 (96.28-99.41)	0.02	0.18	AA+AG GG	4.32 (4.17-4.48) 4.10 (3.99-4.20)
rs11650680	<i>TOP2A</i>	C/T					0.02	0.20	TT+TC CC	4.02 (3.88-4.16) 4.23 (4.12-4.35)

Abbreviations: CI, confidence interval; FDR, false discovery rate; FEF_{25%-75%}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; SNP, single-nucleotide polymorphism.

^aCommon allele is listed first; risk allele is in bold.

the first study to report the interaction between SNPs in *ORMDL3* and *GSDMA* and exposure to ETS in early life in relation to the hospital admission with acute asthma. For example, for rs7212938 in *GSDMA*, the highest risk was seen among children carrying the G allele whose mothers smoked during pregnancy. This SNP has been associated with presence of asthma in some^{12,32} but not all¹⁷ previous studies. Others have looked for associations between 17q12-q21 and other indicators of asthma exacerbation or severity. Two studies^{16,21} in children, in which exacerbations were defined as the use of oral corticosteroids, found an association between the T allele of rs7216389 and asthma exacerbations. Two studies performed in adults observed an association between the same allele and greater asthma severity among early-onset asthma cases.^{15,20} However, these studies investigated only this one SNP (rs7216389). In our study we found a trend for association between exacerbations and rs7216389 ($P = .09$, data not shown). We investigated a much broader panel of SNPs and found the strongest associations to be with SNPs rs8067378 and rs2290400, both of which lie within *ORMDL3* in the same LD block as rs7216389.

We also found several associations between 17q12-21 polymorphisms and lung function measures, with a significant effect modification by early-life ETS exposures. Most studies found no association between variants in this region and lung function (except one study¹⁹ in an African American population in relation to bronchodilator reversibility and 1 study³² in adults that reported the association of 17q21 polymorphisms with lung function in the context of smoking).

Only 1 previous study³⁸ investigated a potential interaction between polymorphisms in this region and furry pet ownership and reported a significant interaction between the SNP in *GSDMB* (rs7216389) and furry pets in that pet ownership increased the risk of recurrent infant wheeze among children with CC genotype but reduced the risk among T allele homozygotes. In our study, we found that variants in *GSDMA* decrease the risk for asthma, but only if furry pets were present in the home during the first year of life. Among children with asthma, we observed a significant interaction between pet ownership and variants in *ORMDL3* in relation to asthma exacerbations in that pet ownership increased the risk of hospital admission, but only among children who carried the minor allele in *ORMDL3* SNPs rs3744246, rs4795403, and rs4795404.

Expression studies^{12,22,39} indicated that the SNPs rs7216389, rs4795405, and rs12936231 are most likely to be functional; in our study there was an interaction between these SNPs and early-life ETS in relation to asthma. In accordance with a number of previous studies,^{40,41} our findings support the idea that gene-environment interactions play an important role in determining complex phenotypes.

In conclusion, our study has found significant interactions between polymorphisms in the 17q12-21 region with pet ownership and ETS exposure in relation to asthma presence. Furthermore, among children with asthma, we observed similar interactions with respect to severe asthma exacerbations requiring hospital admission and the level of lung function.

Supplementary Data

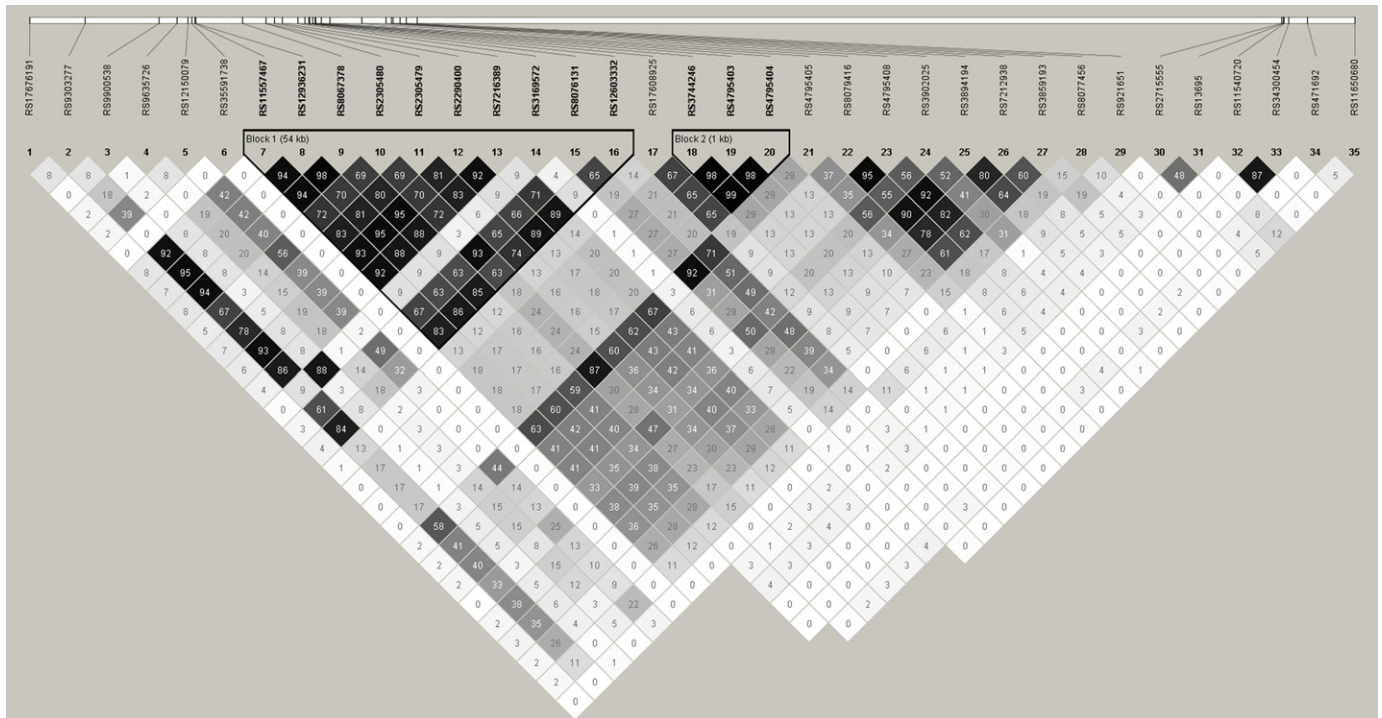
Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2013.01.021>

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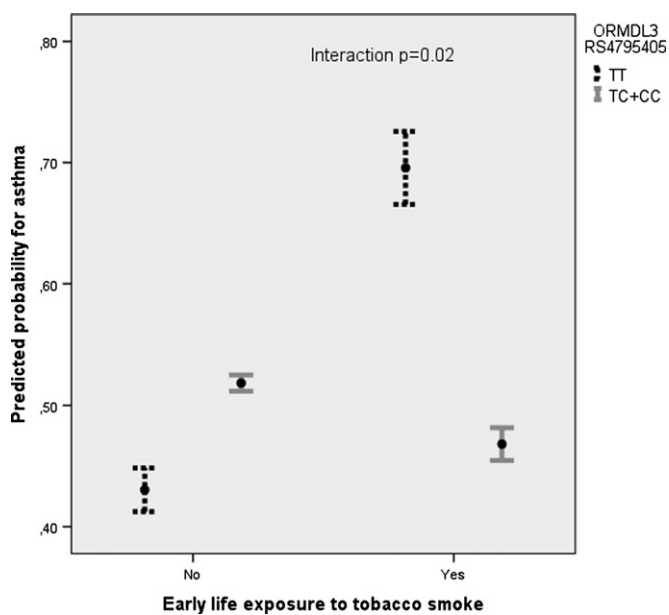
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eFigure 1. The linkage disequilibrium seen among the analyzed SNPs.



eFigure 2. Interaction between genetic variant in *ORMDL3* and early-life ETS exposure in relation to asthma: early-life ETS exposure significantly increases the risk of asthma amongst homozygotes of T allele on rs4795405, with no effect of ETS exposure on asthma risk amongst C allele carriers.

eTable 1

SNPs selected for genotyping and summary of analysis results

Marker	Gene	Alleles	SNP type/function	Call rate	MAF	HWE	SNP inclusion	Asthma			Exacerbations		
								SNP	SNP ETS ^a	SNP pets ^a	SNP	SNP ETS ^a	SNP pets ^a
								P value	P value	P value	P value	P value	P value
rs2313430	IKZF3	C/T	Intron	0.91	.43	.69	LD excluded	NA	NA	NA	NA	NA	NA
rs2313429	IKZF3	A/G	Intron	1	.06	.48	LD excluded	NA	NA	NA	NA	NA	NA
rs9911688	IKZF3	C/T	Intron	0.98	.06	.45	LD excluded	NA	NA	NA	NA	NA	NA
rs17676191	IKZF3	A/G	Intron	0.96	.1	.3	Analyzed	NS	NS	NS	NS	NS	NS
rs12943633	IKZF3	C/T	Intron	0.95	.17	0	Not in HWE	NA	NA	NA	NA	NA	NA
rs9303277 ^b	IKZF3	C/T	Intron	0.96	.43	.78	Analyzed	NS	.01	NS	NS	NS	NS
rs2060941	IKZF3	G/T	Intron	0.91	.12	.15	LD excluded	NA	NA	NA	NA	NA	NA
rs9900538	IKZF3	T/C	Intron	0.98	.06	.45	Analyzed	NS	NS	NS	NS	NS	NS
rs9635726	IKZF3	C/T	Intron	0.97	.21	.69	Analyzed	<.01	NS	NS	NS	NS	NS
rs1453559	IKZF3	T/C	5' UTR	0.99	.43	.91	LD excluded	NA	NA	NA	NA	NA	NA
rs1453560	ZBP2	T/G	5' intergenic	0.97	.06	.45	LD excluded	NA	NA	NA	NA	NA	NA
rs12150079	ZBP2	G/A	Intron	0.98	.24	.43	Analyzed	.01	NS	NS	.02	NS	NS
rs35591738	ZBP2	C/G	Missense	0.98	0	.92	Analyzed	NS	NS	NS	NS	NS	NS
rs11557467 ^b	ZBP2	G/T	Missense	0.98	.43	.95	Analyzed	NS	.01	NS	NS	NS	NS
rs12936231 ^b	ZBP2	C/G	Intron	0.98	.43	.84	Analyzed	NS	.02	NS	NS	NS	NS
rs11870965	ZBP2	T/A	Intron	0.07	.37	.22	Failed	NA	NA	NA	NA	NA	NA
rs1054609	ZBP2	A/C	3' UTR	0.96	.35	.26	LD excluded	NA	NA	NA	NA	NA	NA
rs8067378 ^b	GSDMB	A/G	Intergenic	0.97	.43	.45	Analyzed	NS	.02	NS	.04	NS	NS
rs2305480	GSDMB	G/A	Missense	0.99	.35	.85	Analyzed	NS	NS	NS	NS	NS	NS
rs2305479	GSDMB	C/T	Missense	0.98	.41	.8	Analyzed	NS	NS	NS	NS	NS	NS
rs2290400 ^b	GSDMB	T/C	Intron	0.96	0.43	.89	analyzed	NS	.01	NS	.03	NS	NS
rs1008723	GSDMB	G/T	Intron	0.96	0.42	.97	LD excluded	NA	NA	NA	NA	NA	NA
rs869402	GSDMB	C/T	Intron	0.98	.43	.54	LD excluded	NA	NA	NA	NA	NA	NA
rs7216389 ^b	GSDMB	T/C	Intron	0.98	.42	.73	Analyzed	NS	.02	NS	NS	NS	NS
rs3169572	ORMDL3	G/A	3' UTR	0.99	.07	.84	Analyzed	NS	NS	NS	NS	NS	NS
rs7942	ORMDL3	T/C	3' UTR	0.91	.01	.84	LD excluded	NA	NA	NA	NA	NA	NA
rs11545311	ORMDL3	A	Missense	0.98	0	1	Monomorphic	NA	NA	NA	NA	NA	NA
rs8076131	ORMDL3	A/G	Intron	0.96	.36	.32	Analyzed	NS	NS	NS	NS	NS	NS
rs12603332 ^b	ORMDL3	C/T	Intron	0.98	.42	.61	Analyzed	NS	.01	NS	NS	NS	NS
rs17608925	ORMDL3	T/C	Intron	0.98	.1	.05	Analyzed	NS	NS	NS	NS	NS	NS
rs3744246 ^b	ORMDL3i	C/T	5' intergenic	.96	.13	.84	Analyzed	NS	NS	NS	NS	NS	.04
rs4795403 ^b	ORMDL3i	C/T	5' intergenic	0.99	.13	.88	Analyzed	NS	NS	NS	NS	NS	.04
rs4795404 ^b	ORMDL3i	C/A	5' intergenic	1	.14	.67	Analyzed	NS	NS	NS	NS	NS	.04
rs4795405	ORMDL3i	C/T	5' intergenic	0.99	.35	.72	Analyzed	NS	.02	NS	NS	NS	NS
rs8079416 ^b	ORMDL3i	T/C	5' intergenic	0.99	.47	.34	Analyzed	NS	NS	NS	NS	.04	NS
rs4795408 ^b	ORMDL3i	G/A	5' intergenic	0.96	.47	.42	Analyzed	NS	NS	NS	NS	.03	NS
rs3902025	GSDMA	T/G	5' UTR	0.99	.39	.71	Analyzed	NS	NS	NS	NS	NS	NS
rs3894194 ^b	GSDMA	G/A	Missense	0.98	.47	.68	Analyzed	NS	.04	NS	NS	NS	NS
rs7212938	GSDMA	T/G	Missense	0.95	.49	.14	Analyzed	NS	NS	NS	.04	.03	NS
rs3859193	GSDMA	T/A	Intron	0.98	.48	.14	Analyzed	NS	NS	NS	NS	NS	NS
rs3859192	GSDMA	C/T	Intron	0.92	.46	.8	LD excluded	NA	NA	NA	NA	NA	NA
rs8077456	GSDMA	G/C	Intron	0.99	.34	.64	Analyzed	NS	NS	.02	NS	NS	NS
rs10852937	GSDMA	G/C	Intron	0.83	.2	.31	Failed	NA	NA	NA	NA	NA	NA
rs921651	GSDMA	A/G	3' UTR	0.97	.2	.77	Analyzed	NS	NS	.02	NS	NS	NS
rs2715555	TOP2A	G/A	3' intergenic	1	.18	.46	Analyzed	NS	NS	NS	NS	NS	NS
rs13695	TOP2A	C/T	3' UTR	0.99	.31	.67	Analyzed	NS	NS	NS	NS	NS	NS
rs11540720	TOP2A	C/A	Missense	0.98	.01	.67	Analyzed	NS	NS	NS	NS	NS	NS
rs34300454	TOP2A	C/T	Missense	0.98	.01	.71	Analyzed	NS	NS	NS	NS	NS	NS
rs471692	TOP2A	G/A	Intron	0.99	.16	.27	Analyzed	NS	NS	NS	NS	NS	NS
rs558068	TOP2A	C	Intron	0	0	0	Failed	NA	NA	NA	NA	NA	NA
rs11650680	TOP2A	C/T	5' intergenic	0.95	.23	.89	Analyzed	NS	NS	NS	NS	NS	NS

Abbreviation: MAF, minor allele frequency; NA, not applicable; NS, not significant. LD excluded means that the SNP was excluded because it was in high linkage disequilibrium with another SNP. HWE is Hardy Weinberg Equilibrium.

^aImplies interaction.

^bSNPs are in LD $r^2 > 0.85$.