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Asthma severity, polymorphisms in 20p13 and their interaction with tobacco smoke exposure

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Abstract

Background: We investigated the association between genetic variation in chromosomal region 20p13-p12 (*ADAM33* and flanking genes *ATRN*, *GFRA4*, *SIGLEC1* and *HSPA12B*) and asthma. Amongst asthmatics, we then investigated the association between genetic variants and asthma severity. We evaluated the effect of environmental tobacco smoke (ETS) exposure in the context of genetic variants.

Methods: In a case–control study, we recruited 423 asthmatic children and 414 non-asthmatic controls (age 5–18 yr). Amongst asthmatics, we measured lung function and extracted data on hospitalisation for asthma exacerbation from medical records. Early-life ETS exposure was assessed by questionnaire. We included 85 single-nucleotide polymorphisms (SNPs) in the analysis.

Results: Seventeen SNPs were significantly associated with asthma; one (rs41534847 in *ADAM33*) remained significant after correction for multiple testing. Thirty-six SNPs were significantly associated with lung function, of which 15 (six *ARTN*, three *ADAM33*, five *SIGLEC1* and one *HSPA12B)* remained significant after correction. We observed a significant interaction between 23 SNPs and early-life ETS exposure in relation to lung function measures. For example, for rs512625 in *ADAM33*, there was significant interaction with ETS exposure in relation to hospitalisations (p_{int} = 0.02) and lung function (p_{int} = 0.03); G-allele homozygotes had a 9.15-fold [95% CI 2.28–36.89] higher risk of being hospitalized and had significantly poorer lung function if exposed to ETS, with no effect of ETS exposure amongst A-allele carriers.

Conclusion: We demonstrated several novel significant interactions between polymorphisms in 20p13-p12 and early-life ETS exposure with asthma presence and, amongst asthmatics, a significant association with the severity of their disease.

A disintegrin and metalloprotease 33 (*ADAM33*) was the first gene for which association with asthma was discovered using positional cloning (1). The linkage analysis identified two peaks in the region 20p13; using a more stringent definition of asthma with airway hyper-responsiveness (AHR), the strength of the association increased, with further analysis confirming *ADAM33* as the strongest signal (1). Expression studies confirmed that ADAM33 was expressed in lung fibroblasts and airway smooth muscle cells, providing evidence for its role in the pathogenesis of asthma through airway remodelling and AHR (1).

Results from more than 20 subsequent association studies in ethnically diverse populations have confirmed the association between *ADAM33* and asthma, or related phenotypes (2,3). However, it is of note that there is a considerable heterogeneity between studies, with associations not being reported consistently across all single-nucleotide polymorphisms (SNPs). Furthermore, in different studies, the same genetic variants conferred either an increased risk or were protective (e.g. for SNP S2-rs528557, some studies reported increased risk of asthma with rare C allele (4–8), whilst others found the same association with common G allele (1,9,10)). However, not all studies replicated the associations (11–14), and some failed to demonstrate single SNP associations, but reported a haplotypic association (15,16). Several studies found significant associations between *ADAM33* variants and lung function (7,9,17), whilst one failed to find such association (6).

There are several possible reasons for this heterogeneity (e.g. differences in phenotype definition, sample size, choice of SNPs etc.)(2). It is also plausible that other genes or regulatory

elements in the vicinity of *ADAM33* may be responsible for the observed association; of note, eight of the 24 SNPs associated with asthma in the discovery study were in two of *ADAM33*'s flanking genes, glial cell line-derived neurotrophic factor family receptor alpha 4 (*GFRA4*) and sialic acid-binding Ig-like lectin 1 (*SIGLEC1*)(1).

The modifying effects by gene–gene or gene–environment interactions may also be important (18). For example, tobacco smoke exerts most of its effects locally in the lung and may induce or suppress expression of tissue-specific asthma susceptibility genes. Given the proposed role of *ADAM33* in contributing to airway smooth muscle and vascular modelling and remodelling (19), it is plausible that there may be an interaction between genetic variants and tobacco smoke exposure in modifying asthma risk. Several studies have investigated this interaction; as with genetic studies, the results are heterogenous. For example, one study reported a significant effect modification of *ADAM33* polymorphisms by *in utero* environmental tobacco smoke (ETS) exposure on lung function and AHR in children (6), but another study did not detect any such interactions (7).

We aimed to investigate the association between genetic variation in chromosomal region 20p13-p12 (*ADAM33* and flanking genes) and asthma; amongst asthmatic patients, we then investigated the association between asthma severity (assessed by hospital admissions with acute exacerbation and lung function) and genetic variants. The effect of ETS exposure on the risk of asthma and asthma severity in the context of genetic variants was also evaluated.

Methods

Study design and setting

We carried out this case–control study in the Department of Paediatrics, General Hospital Slavonski Brod, Croatia. The study was approved by the local ethics committee, and informed consent was obtained from all parents (and children when appropriate).

Participants

Cases

Children with asthma aged 5–18 yr were recruited from the paediatric asthma clinic if all of the following criteria were met: (i) physician-diagnosed asthma, (ii) asthma symptoms (wheeze, cough, or both) within the previous 12 months, and (iii) current use of antiasthma medication.

Controls

Children in the same age range without respiratory symptoms (confirmed by an interviewer-administered questionnaire) were randomly selected from patients with non-respiratory conditions attending other hospital departments (e.g. fracture clinic); social and environmental variables matched with the general population.

Cases and controls were not matched by gender and age.

Data sources/measurement

Hospital admission with acute asthma

Trained paediatrician extracted all data on hospital admissions due to acute asthma exacerbation from paper-based hospital medical records.

Lung function was assessed in asthmatic children using spirometry according to the ATS/ERS guidelines (20,21) to measure forced expiratory volume in one second (FEV_1) as a percentage of predictive values (22), FEV_1/forced vital capacity (FVC) ratio and forced expiratory flow when 50% of the FVC has been expired (FEF_{50}).

ETS exposure

ETS exposure in early life was ascertained using a validated questionnaire and defined as a positive answer to the question: 'Did mother smoke during pregnancy?'

Genotyping

Gene and tagging SNP Selection

Haplotype-tagging SNPs were selected in the 20p13-p12 region for *ADAM33* and its flanking genes (*attractin* [*ATRN*], *GFRA4*, *SIGLEC1* and *heat shock 70kD protein 12B* [*HSPA12B*]), using GVS (http://gvs.gs.washington.edu/GVS), complemented by NCBI's dbSNP (www.ncbi.nlm.nih.gov/projects/SNP) and Haploview (23), using LD-based tagSNP selection with a pairwise algorithm LDSelect (24), available in GVS and Haploview (Tagger). SNPs identified in populations of European descent with a minor allele frequency (MAF) $\geq 5\%$ were included in the algorithm. We selected an LD threshold of $r^2 = 0.8$. TagSNPs from all generated bins were selected and genotyping performed using Sequenom® MassARRAY® iPLEX™ (25). Three functional SNPs (*ATRN,* rs17782078 and rs3886999 and *SIGLEC1,* rs6037651) were forced included. Details of the 124 genotyped SNPs (46 in *ATRN*, 6 in *GFRA4*, 37 in *ADAM33*, 27 in *SIGLEC1* and 8 in *HSPA12B*) are presented in Tables E1–E5. Quality control (QC) procedures were then applied and redundant SNPs excluded (Online Supplement).

Statistical analysis

Statistical analysis was carried out using SVS 7.2.3 (Golden Helix, Bozeman, MT, USA) and SPSS 19 (SPSS, Chicago, IN, USA). For SNPs showing a trend for association in additive model, dominant and recessive model were tested. For consistency, all analyses were performed using major allele as reference. Correction for multiple comparisons was carried out using the false discovery rate (FDR) method (26). Results are presented as p-values (uncorrected and FDR corrected), and odds ratios (OR) and 95% confidence intervals (95% CI) as a measure of effect size.

We analysed the associations between genetic variants and asthma using χ^2 test and logistic regression. All analyses were adjusted for gender. Analysis of gene–environment interactions was carried out using appropriate multivariate regression models.

For quantitative outcomes (lung function), we used correlation/trend test, univariate linear regression and general linear model univariate one-way independent ANOVA. FEV_1 and FEV_1/FVC followed a normal distribution; results are expressed as mean and 95% CI. FEF_{50} followed a log-normal distribution and hence was subject to a log transformation; the results are expressed as geometric mean (GM). FEV_1/FVC and FEF_{50} analyses were adjusted for age, gender and height.

Results

Participants

We approached 923 children (438 asthmatics, 485 controls), of whom 86 declined and 837 agreed to participate in the study (91%; 423 asthmatics, 414 controls); 824 participants provided blood for DNA (417 asthmatics and 407 controls). Demographic characteristics of the study population are presented in Table 1. There was a significant difference in gender between the groups (males, 58.6% vs. 43.1%, cases vs. controls, $p < 0.001$); subsequent analyses were therefore adjusted for gender. There was a small, but statistically significant difference in age between the groups (11.1 vs. 12.2 yr, cases vs. controls, $p < 0.01$, Table 1), which was deemed unlikely to be relevant for the analysis. There were no differences between the groups in early-life ETS exposure, area of residence or position in sibship. Amongst children with asthma, 150 (36%) were admitted to hospital at least once with asthma exacerbation, and 53 (12.7%) were hospitalized two or more times. There was no association between early-life ETS exposure and hospital admissions for acute asthma.

Genotyping success rates were generally high (>95%); 26 SNPs with a success rate <90%, three SNPs which were monomorphic in our population and 10 SNPs in high LD with other SNPs ($r^2 > 90\%$) were excluded (Online supplement). We included 85 SNPs in the final analysis (LD plots shown in Fig. E1).

Table 1 Demographic characteristics of the study population

	Cases (N = 423)	Controls (N = 414)	p-value
Age (years): mean (95% CI)	11.1 (10.8–11.4)	12.2 (11.9–12.6)	<0.01
Gender (%)			
Male	248 (58.6)	178 (43.0)	<0.001
Family history (%)			
Asthma			
Maternal	37 (8.7)	4 (1.0)	<0.001
Paternal	27 (6.4)	9 (2.2)	0.003
Rhinitis			
Maternal	32 (7.6)	14 (3.4)	0.009
Paternal	42 (10.0)	8 (1.9)	<0.001
Eczema			
Maternal	10 (2.4)	16 (3.9)	0.20
Paternal	8 (1.9)	7 (1.7)	0.83
Siblings (%)			
Older siblings			
yes	239 (56.5)	242 (58.9)	0.49
Younger siblings			
yes	253 (59.8)	238 (57.9)	0.58
Area of residence (%)			
Current			
Urban	195 (46.1)	186 (45.3)	0.81
Rural	228 (53.9)	225 (54.3)	
At birth			
Urban	196 (46.3)	182 (44.3)	0.55
Rural	227 (53.7)	229 (55.3)	
ETS exposure (%)			
Pregnancy			
Mother	86 (20.3)	85 (20.7)	0.90
Father	228 (54.0)	227 (55.2)	0.73
1st year of life			
Mother	122 (28.8)	133 (32.4)	0.27
Father	234 (55.5)	235 (57.2)	0.62
Current			
Mother	140 (33.1)	158 (38.4)	0.11
Father	187 (44.4)	206 (50.2)	0.10

Association analysis

Asthma presence

Seventeen SNPs were significantly associated with asthma (Table 2, Fig. 1). For one SNP (rs41534847 in *ADAM33*), this association remained significant after correction for multiple testing, and for two *ADAM33* SNPs (rs2485700, rs7354032), there was a strong trend after correction for multiple testing ($p = 0.08$).

Asthma severity

Hospital admission with acute asthma exacerbation. Amongst children with asthma, G-allele homozygotes in rs512625 (*ADAM33*) and T-allele carriers in rs532448 (*SIGLEC1*) had increased risk for two or more hospital admissions compared with major allele homozygotes (OR [95% CI], 2.61 [1.10–6.17], $p = 0.03$ and 1.99 [1.04–3.81], $p = 0.04$, respectively).

Lung function. Thirty-six SNPs were significantly associated with different measures of lung function (Fig. 2, Table E6), of which 15 (six in *ARTN*, three in *ADAM33*, five in *SIGLEC1* and one in *HSPA12B*) remained significant after correction for multiple testing (Table 3). For example, carriers of G allele of *ATRN* rs11696647 had significantly higher FEV_1 and FEF_{50} than A-allele homozygotes (FDR $p = 0.01$). This SNP explained 6% of the variance in FEV_1. Carriers of minor allele on *ADAM33* rs4987246 had significantly lower FEV_1 and FEF_{50} than major allele homozygotes (FDR $p < 0.03$).

Interaction between genetic variants and early-life ETS exposure

Asthma presence

We observed significant interactions between six SNPs and early-life ETS exposure in relation to asthma (Table E7). For example, early-life ETS exposure significantly increased the risk of asthma amongst carriers of G allele on rs3848809 in *ATRN* (OR [95% CI], 2.09 [1.02–4.28], $p = 0.04$), but there was no effect of ETS exposure on asthma risk amongst A-allele homozygotes (0.91 [0.65–1.28], $p = 0.6$; Fig. E2).

Table 2 Association between genetic variants in 20p13-p12 and asthma

SNP rs number	Gene	Alleles*	p-Value	FDR	Model	aOR	95% CI Lower	95% CI Upper
RS151518	*ATRN*	A/**G**	0.01	0.16	GG vs. GA+AA	2.22	1.21	4.07
RS2853224	*ATRN*	A/C	0.03	0.32	Additive	1.24	1.02	1.51
RS3848809	*ATRN*	**A**/G	0.02	0.27	GG vs. GA+AA	0.37	0.15	0.91
RS678963	*ATRN*	C/**T**	0.03	0.22	TT vs.TC +CC	1.76	1.06	2.93
RS677421	*ATRN*	A/**T**	0.02	0.47	TT+TA vs. AA	1.70	1.06	2.71
RS583688	*ATRN*	C/**T**	0.04	0.29	TT vs. TC +CC	1.49	1.01	2.19
RS235558	*ATRN*	A/**G**	0.02	0.21	GG vs. GA+AA	2.94	1.15	7.52
RS630909	*GFRA4*	**A**/G	0.03	0.23	GG vs. GA+AA	0.40	0.17	0.95
RS44707	*ADAM33*	A/**C**	0.02	0.22	CC vs. CA+ AA	1.56	1.06	2.31
RS41534847	*ADAM33*	**C**/T	<0.01	**0.04**	TT+TC vs. CC	0.08	0.01	0.61
RS2485700	*ADAM33*	C/**T**	<0.01	**0.08**	TT+TC vs. CC	1.68	1.19	2.36
RS511898	*ADAM33*	C/**T**	0.03	0.37	TT+TC vs. CC	1.37	1.04	1.82
RS3918392	*ADAM33*	A/**G**	0.03	0.41	GG+GA vs. AA	2.03	1.07	3.85
RS2787095	*ADAM33*	C/**G**	0.01	0.14	GG+GC vs. CC	1.50	1.12	2.01
RS487377	*ADAM33*	A/**G**	0.02	0.24	GG vs. GA+AA	2.19	1.09	4.43
RS7354032	*ADAM33*	C/**T**	<0.01	**0.08**	TT+TC vs.CC	2.84	1.48	5.45
RS4813639	*SIGLEC1*	A/**G**	0.04	0.48	GG+GA vs. AA	1.46	1.02	2.11

All analyses are adjusted for gender.
Risk allele in bold type.
FDR, false discovery rate.
*Common allele is listed first/rare allele second.

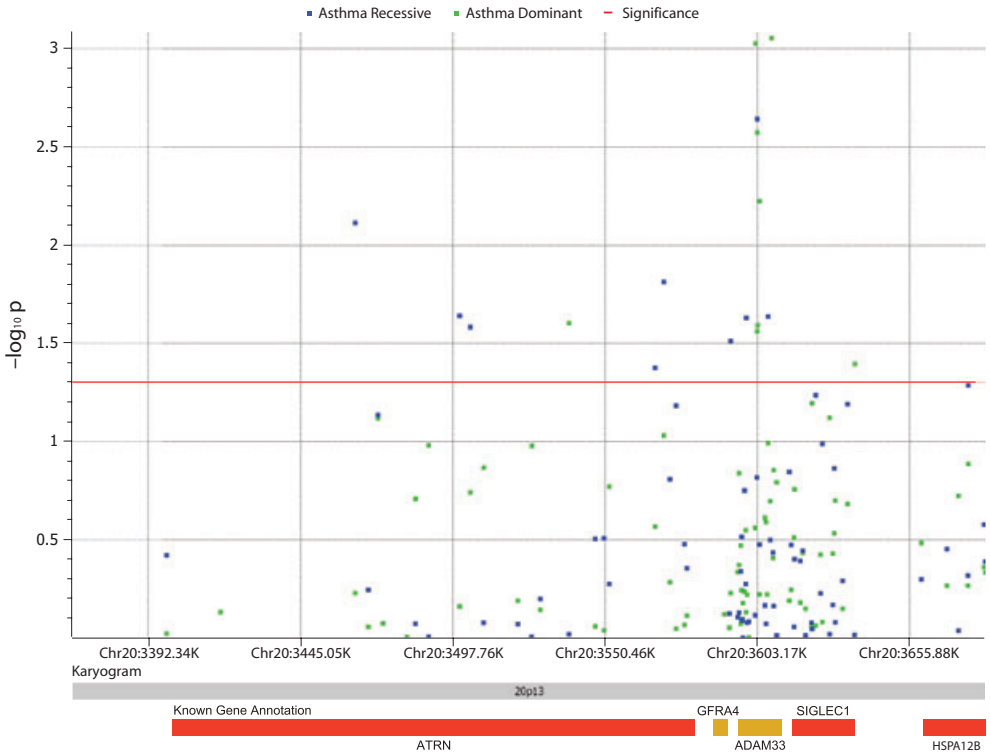

Figure 1 Association between genetic variants in the region 20p13-p12 and asthma using the recessive (blue) and dominant (green) genetic models. The red line indicates significance.

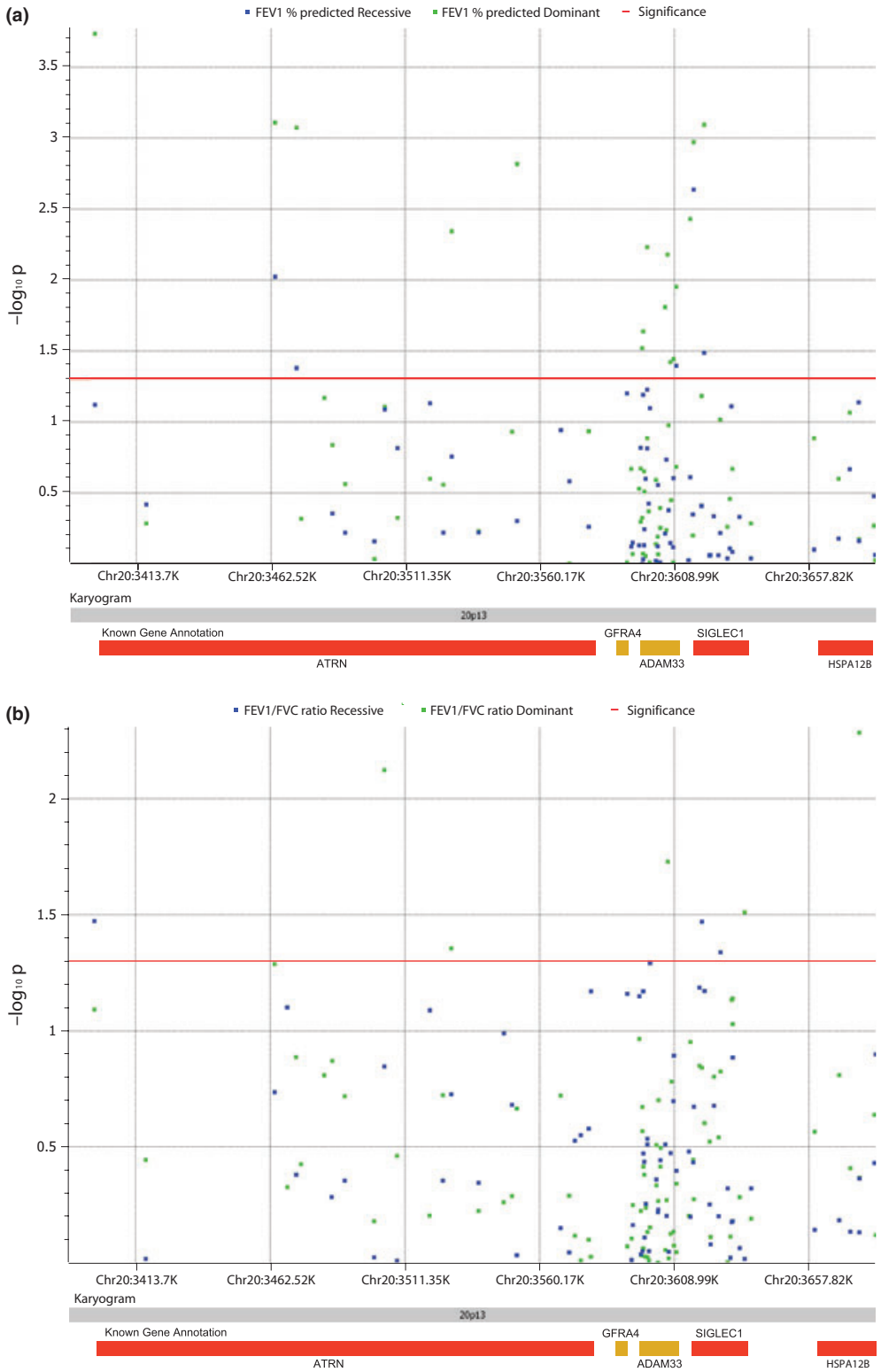

Figure 2 Association between genetic variants in the region 20p13-p12 and lung function measures amongst asthmatic children; results of both dominant (blue) and recessive (green) models are shown. (a) FEV_1% predicted. (b) FEV_1/FVC ratio. (c) FEF_{50}.

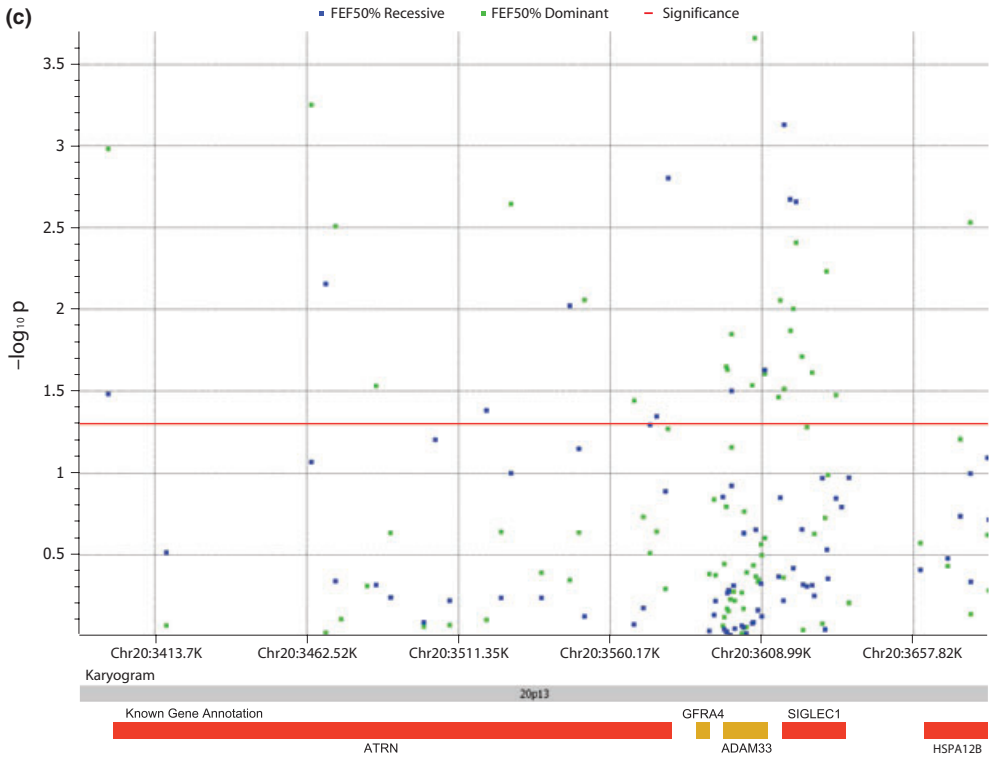

Figure 2 (contiued)

Hospital admission with acute asthma exacerbation

Amongst asthmatic children, we observed a significant interaction between rs512625 in *ADAM33* and early-life ETS exposure in relation to hospital admissions (p_{int} = 0.02), in that homozygotes for G allele had higher risk of being hospitalized two or more time if they were exposed to ETS (OR [95% CI], 9.15 [2.28–36.89], p = 0.002), with no effect of ETS exposure amongst other genotype groups (p = 0.8).

Lung function

We observed a significant interaction between 23 SNPs in this region with early-life ETS exposure in relation to different lung function measures (Table E8). For example, the effect of early-life ETS exposure on FEF_{50} differed between children with different genotypes in *ADAM33* SNP rs512625, in that G-allele homozygotes had lower FEF_{50} if they were exposed to ETS (GM [95% CI], 2.33 [1.79–2.51] vs. 3.16 [2.56–3.89], p = 0.04), with no effect of ETS exposure amongst carriers of A allele (2.82 [2.57–3.09] vs. 2.69 [2.60–2.82], exposed vs. non-exposed, p = 0.52; p_{int} = 0.03) (Fig. E3).

Discussion

Principal findings

To the best of our knowledge, our study is the first to demonstrate amongst asthmatic patients the association between polymorphisms in the 20p13-p12 region (*ADAM33* and flanking genes) with different markers of asthma severity (hospital admissions with acute exacerbations and level of lung function) and a significant effect modification by early-life ETS exposure. We also confirmed the association between asthma presence and genetic variants in this region and a significant interaction with early-life ETS exposure in modifying asthma risk. One of these associations is novel (rs41534847 in *ADAM33*) and remained significant after correction for multiple testing.

We found strong significant associations between 36 SNPs and lung function amongst asthmatics, 15 of which were still significantly associated after FDR correction. Most of these associations are novel (e.g. rs11696647, rs151518, rs2853224, rs678963, rs235515, rs618047, rs2853213, rs532448, rs656635, rs609203, rs3859664, rs4813636 and rs910652), whilst some had previously been reported (e.g. rs597980(17) and rs612709 (10)). The effect sizes appear higher than those reported by the majority of previous studies (e.g. SNP rs11696647 in *ATRN* explained 6% of the variance in FEV_1). Amongst asthmatic patients, we identified genotypes which increased the risk of hospital admission due to acute exacerbations, which was further augmented by ETS exposure. For example, the risk of multiple hospitalisations was doubled amongst homozygotes for G allele in rs512625 (*ADAM33*), with the magnitude of risk increasing further if they were exposed to ETS in early life (9-fold). Similar interaction between this genetic variant and ETS exposure was observed in relation to lung function.

Table 3 Genetic variants in the region 20p13-p12 and lung function measures: significant associations which survived correction for multiple testing, FDR ($p < 0.05$)

			FEV_1 (% predicted)					FEF_{50}				
						95% CI					95% CI	
	Gene	Allele*	FDR	Genotype	Mean	Lower	Upper	FDR	Genotype	GM	Lower	Upper
RS11696647	*ATRN*	A/G	0.01	GG+GA	102.27	99.76	104.78	0.01	GG+GA	3.06	2.87	3.26
				AA	96.13	94.21	98.94		AA	2.59	2.46	2.72
RS151518	*ATRN*	A/G	0.02	GG+GA	101.45	99.16	103.75	0.01	GG+GA	2.78	2.56	3.03
				AA	95.84	93.74	97.94		AA	2.74	2.6	2.89
RS2853224	*ATRN*	A/C	0.01	CC+CA	100.26	98.43	102.1	0.02	CC+CA	2.87	2.74	3.01
				AA	93.52	90.55	96.49		AA	2.42	2.25	2.61
RS678963	*ATRN*	C/T						0.02	TT+TC	2.98	2.82	3.14
									CC	2.49	2.35	2.64
RS235515	*ATRN*	C/G	0.03	GG+GC	101.59	98.98	104.2	0.02	GG+GC	3.08	2.88	3.29
				CC	96.54	94.59	98.48		CC	2.57	2.45	2.7
RS618047	*ATRN*	A/G	0.02	GG+GA	100.32	98.35	102.3	0.05	GG+GA	2.88	2.74	3.03
				AA	95.14	92.62	97.66		AA	2.53	2.37	2.7
RS44707(ST+4)	*ADAM33*	A/C	0.04	CC+CA	100.45	98.47	102.42					
				AA	95.25	92.76	97.74					
RS4987246	*ADAM33*	C/T	0.03	TT+TC	92.5	86.16	98.84	0.02	TT+TC	2.43	2.07	2.86
				CC	98.98	97.37	100.6		CC	2.77	2.66	2.89
RS2853213	*ADAM33*	C/G	0.05	GG+GC	96.96	94.96	98.95					
				CC	100.71	98.27	103.14					
RS532448	*SIGLEC1*	A/T	0.03	TT+TA	96.55	94.55	98.55	0.05	TT+TA	2.63	2.5	2.77
				AA	101.06	98.58	103.54		AA	2.91	2.73	3.1
RS656635	*SIGLEC1*	G/T	0.01	TT+TG	99.53	97.74	101.31					
				GG	94.44	91.33	97.54					
RS609203	*SIGLEC1*	A/C						0.05	CC+CA	2.64	2.48	2.8
									AA	2.83	2.68	2.98
RS3859664	*SIGLEC1*	C/T	0.02	TT+TC	100.28	98.36	102.21	0.03	TT+TC	2.81	2.68	2.96
				CC	95.13	92.56	97.71		CC	2.64	2.47	2.82
RS4813636	*SIGLEC1*	A/G						0.04	GG+GA	2.67	2.54	2.8
									AA	2.86	2.68	3.06
RS910652	*HSPA12B*	A/G						0.02	GG+GA	2.65	2.51	2.81
									AA	2.89	2.72	3.06

FDR, false discovery rate; GM, geometric mean.
*Common allele is listed first.

Limitations and strengths

One of the limitations of the current study is that cases and controls were not matched by gender, and there was a preponderance of boys amongst cases. To address this, we adjusted all case–control analyses for gender. Lung function measurements were not available in the control children; lung function data amongst cases were used as a surrogate marker of asthma severity. We also acknowledge that ETS exposure was assessed by questionnaire rather than objective measurement (e.g. cotinine), which may underestimate the real exposure. We do not have data on the number of cigarettes smoked and could not assess whether there was any dose–response relationship with respect to ETS exposure.

Whilst the majority of previous replication studies focused on a limited number of selected polymorphisms in the region, we carried out a comprehensive investigation of known common variation within all five genes of interest (*ADAM33, ATRN*, *GFRA4*, *SIGLEC1* and *HSPA12B*). Although we utilized all available data on common variation and LD within each gene to identify the lowest number of markers to capture most of the variation, we acknowledge the possibility that additional, as yet unknown, polymorphisms were not included. In addition, it is possible that SNPs other than those that we have identified may be the truly associated variants because of LD within the region.

Our study used a candidate gene approach, with *a priori* hypotheses on plausible associations between selected SNPs with asthma and asthma severity, and the interaction between genetic variants and ETS exposure. Although adjustment for multiple comparisons is therefore not essential, we used the FDR method (26) to adjust for total number of variants tested to increase the confidence that the results reflect true associations. For completeness, we present both uncorrected and corrected p-values, highlighting those that remained significant after correction as the most likely relevant associations.

However, we acknowledge that we cannot completely eliminate the potential impact of multiple testing on the reliability of our conclusions.

One strength of our study is that we have not used questionnaire to ascertain asthma severity, but the information from medical records detailing each asthmatic child's hospital admission due to acute asthma exacerbation. We capitalized on the fact that in this part of Croatia, all asthmatic participants are regularly followed in our asthma clinic. This is the only hospital in the region, and we maintain accurate records of all hospital admission, outpatient appointments and all prescriptions. Such source of information allowed more accurate and precise definition of hospitalisation than studies which relied only on questionnaires.

Interpretation

Several SNPs in *ADAM33*, *ATRN*, *GFRA4*, *SIGLEC1* and *HSPA12B* were associated with asthma and, amongst asthmatics, with severity of their disease, a number of these only in the context of ETS exposure. *ADAM33* encodes a protein which is a member of the transmembrane zinc-dependent matrix metalloproteinases and is involved in cell adhesion, cell fusion, cell signalling and proteolysis (19). Genes in the vicinity of *ADAM33* have a role in inflammation (*ATRN* has several isoforms, some of which are involved in the initial immune cell clustering during inflammatory responses and may regulate chemotactic activity of chemokines (27); *SIGLEC1* encodes a member of the immunoglobulin superfamily expressed by macrophages and is involved in mediating cell-to-cell interactions (28); and variants in *HSPA12B* may confer susceptibility to atherosclerosis (29)). However, it remains plausible that it is other SNPs in LD with the identified SNPs which are causally associated with asthma and abnormalities in lung function. In our study, most SNPs associated with asthma and lung function were found in the introns at the 5′ end of the gene and may be involved in the control of RNA stability and alternative splicing. Studies of alternative splicing of *ADAM33* transcripts suggest that the variability in exon usage occurs at the 5′ end of the gene (the metalloprotease and prodomains, corresponding to SNPs rs487377 through rs3918395)(30). Another proposed functional mechanism suggests that variants on the 5′ end of the gene may directly affect catalytic activity and promote angiogenesis and thus be important in airway wall remodelling (31). Although functional assessments were not within the remit of this study, our results are consistent with findings that soluble ADAM33 in bronchial alveolar lavage is increased in asthma and correlates with disease severity and reduced lung function (32). Further studies are needed to elucidate the functional role of these SNPs and molecular mechanisms that may confer asthma susceptibility.

Maternal smoking is a risk factor for reduced lung function and wheezing (33). However, not all exposed children are affected to a similar degree. In our study population, 1/5 of children were exposed to ETS during early life; ETS exposure *per se* was not a risk factor for asthma, and certain genetic variants in the region were not associated with asthma or lung function. However, potential importance of these variants was uncovered when the early-life ETS exposure was taken into account (for example, carriers of G allele on *ATRN* SNP rs3848809 had two times higher risk for asthma, but only if they were exposed to ETS in early life, with no effect of genotype in the absence of ETS exposure and no effect of ETS exposure amongst A-allele homozygotes). The first reported interaction between *in utero* ETS exposure and *ADAM33* variants with respect to lung function suggested that ETS exposure was associated with lower lung functions amongst carriers of minor allele in *ADAM33* SNPs rs528557 and rs3918396(6). However, two further studies failed to demonstrate any such interactions (7,10). In the current study, the most striking and consistent interaction between genetic variants and ETS exposure was for *ADAM33* SNP rs512625 in relation to asthma severity (G-allele homozygotes who were exposed to ETS had markedly higher risk of multiple hospitalisations and had significantly lower FEF_{50} compared with those not exposed, with no effect of ETS exposure amongst carriers of A allele).

In conclusion, we have demonstrated several novel significant interactions between polymorphisms in 20p13-p12 region and early-life ETS exposure with asthma presence and, amongst patients with asthma, a significant association with the severity of their disease, confirming that 20p13-p12 is as an important asthma susceptibility locus.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table E1. SNPs selected for genotypingon *ATRN* gene.

Table E2. SNPsselected for genotypingon *GFR4* gene.

Table E3. SNPsselected for genotypingon *ADAM33* gene.

Table E4. SNPs selected for genotypingon *SIGLEC1* gene.

Table E5. SNPs selected for genotypingon *HSPA12B* gene.

Table E6. Association between genetic variants in the region 20p13-p12 and lung function measures amongst asthmatic children.

Table E7. Significant interactions between genetic variants in 20p13-p12 and early-life environmental tobacco smoke exposure in relation to asthma.

Table E8. Significant interactions between genetic variants in 20p13-p12 and early-life ETS exposure in relation to lung function amongst children with asthma.

Figure E1. Linkage disequilibrium plot for analysed SNPs in region 20p13-12p showing pairwise r^2 values.

Figure E2. Interaction between genetic variant in *ATRN*and early-life ETS exposure in relation to asthma:Early-life ETS exposure significantly increases the risk of asthma amongst carriers of G allele on rs3848809, with no effect of ETS exposure on asthma risk amongst A allele homozygotes.

Figure E3. Interaction between genetic variant in *ADAM33* and early-life ETS exposure in relation to FEF_{50}: Homozygotes for G allele on rs512625 have lower FEF_{50} if they are exposed to ETS, with no effect of ETS exposure amongst carriers of A allele.