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Interleukin-1R antagonist gene and pre-natal smoke exposure are associated with childhood asthma

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ABSTRACT: The interleukin-1 receptor antagonist (IL1RN) is a potent anti-inflammatory cytokine. In the present study, association of the human *IL1RN* gene polymorphisms with asthma, bronchial hyperresponsiveness and forced expiratory volume in one second/forced vital capacity ratio was tested and the data was stratified by environmental tobacco smoke exposure in order to investigate a gene-smoking interaction.

In an unselected subset (n=921) of the Isle of Wight birth (UK) cohort, which has previously been evaluated for asthma and related manifestations at ages 1, 2, 4 and 10 yrs, three *IL1RN* single nucleotide polymorphisms (SNP) were genotyped. Logistic regression and repeated measurement models for tests of association using a representative SNP rs2234678 were used, as all SNPs tested were in strong linkage disequilibrium.

In the overall analysis, the SNP rs2234678 was not associated with asthma. However, in the stratum with maternal smoking during pregnancy the rs2234678 GG genotype significantly increased the relative risk of asthma in children, both in analyses of repeated asthma occurrences and persistent asthma.

In conclusion, the present results show that in the first decade of life, the gene–environment interaction of the interleukin-1 receptor antagonist gene polymorphism rs2234678 and maternal smoking during pregnancy increased the risk for childhood asthma.

KEYWORDS: Asthma, genetic association, interleukin-1 receptor antagonist, interleukin-1 receptor antagonist gene, maternal smoking, smoking

Asthma is a chronic inflammatory lung disease that affects millions of people all over the world, and its incidence is the highest in the USA and other industrialised countries [1, 2]. It is a multifactorial disorder, resulting from the concerted actions of multiple genes and environmental factors. Genome scans for asthma have identified various chromosomal regions to be linked with asthma and related phenotypes [3–7]. The present authors are particularly interested in linkages to human chromosome 2q12–q14 as this location is orthologous to the murine locus for allergen-induced bronchial hyperresponsiveness *Abhr1,* which has been described previously [8]. The *Abhr1* quantitative trait locus contains the murine interleukin (IL)-1 receptor antagonist gene (*Il1rn*), orthologous to the human interleukin-1 receptor antagonist gene (*IL1RN*).

Members of the IL-1 family are involved in inflammatory processes and have host defence properties. The IL-1 signalling pathway involves the IL-1 receptor type I, through which the products of the genes for IL-1α (*IL1A*) and IL-1β (*IL1B*) induce a pro-inflammatory response. This response is abrogated when the product of the IL-1 receptor antagonist gene, IL-1Ra, competitively binds to the receptor but does not elicit the downstream signal transduction cascade. The pro-inflammatory effect of IL-1 and the anti-inflammatory effect of IL-1Ra in asthma have been documented in a number of human and animal studies [9–13].

The studies that have examined the relationship between *IL1RN* polymorphisms and asthma or related phenotypes [14–17], have been performed on cross-sectional adult case–control populations. In a multifactorial disease like asthma, patients manifest a dynamic combination of multiple phenotypes at various stages of disease during the course of their lifetime [18]. Longitudinal genetic association studies that examine the

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dynamics of both objective and subjective asthma phenotypes over different ages provide additional information valuable for preventive and age-specific asthma management strategies [19, 20]. One objective of the current study was to test the association of the *IL1RN* gene polymorphisms with asthma in a longitudinal cohort of children who were evaluated for asthma and related phenotypes at ages 1, 2, 4 and 10 yrs. Along with asthma, additional phenotypes tested for genetic association were bronchial hyperresponsiveness (BHR) and a standard measure of airway obstruction *i.e.* forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) ratio.

The second objective of the study was to examine gene (*IL1RN*)–environment (smoke exposure) interactions as they relate to asthma. Studies that have previously reported associations of asthma with *IL1RN* did not take covariates or gene–environment interactions into consideration [14, 17]. Maternal smoking during pregnancy and environmental tobacco smoke (ETS) exposure in childhood were assessed in the current study, as they are major environmental risk factors for asthma [21–24]. Moreover, *in utero* exposure to maternal smoking has been shown to be associated with increased risk of asthma and related phenotypes in the offspring of mothers who smoke [25–30]. Thus, to examine this relationship, the impact of ETS on the asthma risk-conferring abilities of the *IL1RN* polymorphism was evaluated.

METHODS

Population

Between January 1989 and February 1990 children born on the Isle of Wight (UK) were recruited to participate in a longitudinal study (n=1,456). The local research ethics committee approved the study and informed written parental consent was obtained for all the participants. The population was largely Caucasian (99%), living in a semi-rural environment with no heavy industry.

At birth, data from birth records and extensive questionnaires were collected, including information on asthma and allergy family history, as well as maternal smoking habits. At ages 1, 2, 4 and 10 yrs, the original questionnaire-based information was updated, a study physician performed physical examinations on the children, and symptoms of asthma and allergic diseases were recorded. Additionally, at 10 yrs of age: International Study of Asthma and Allergy in Childhood (ISAAC) written questionnaires were used to assess respiratory, nasal and dermatological symptoms [28]; a subset of the population underwent pulmonary function testing; and anticoagulated blood samples were collected and stored frozen for subsequent DNA analysis (n=921). The characteristics of the study population are shown in table 1.

Outcomes

The following three outcomes were investigated: asthma, airway obstruction and BHR. The analyses were carried out on a representative unselected subset of 921 individuals from the Isle of Wight birth cohort, where DNA was available for genetic studies. To perform spirometry and assessment of BHR, children were required to be free from respiratory infection for 14 days, not taking oral steroids, not have taken a β_2-agonist for 6 h, and abstained from caffeine intake for $\geqslant$4 h.

Asthma

The presence of asthma was determined at ages 1, 2, 4 and 10 yrs. The operational definition of asthma was investigator-diagnosed asthma based on the minimum criteria of a history of physician-diagnosed asthma plus at least one episode of wheezing in the last 12 months. Additionally, during the early childhood period an alternative minimum criterion for asthma diagnosis was a history of three separate episodes of persistent wheezing ($\geqslant$3 days duration) because asthma diagnosis is often not given to infants/young children. Using contingency tables for asthma occurrences at ages 1, 2, 4 and 10 yrs, three longitudinal asthma categories were identified: 1) disease-free children; 2) children with transient asthma; and 3) children with persistent asthma. Category 2 comprises early onset/transient asthma (asthma at 1 and/or 2 yrs only) and single occurrences at other ages. The persistent asthma category (category 3) includes children diagnosed with asthma by the age of 4 yrs (or earlier) and persisting to 10 yrs of age.

Airway obstruction

Baseline spirometry was performed in all children attending the Research Centre (St Mary's Hospital, Newport, Isle of Wight, UK) at 10 years of age (n=981), following the American Thoracic Society guidelines [31], using Koko spirometry software (Pds Instrumentation, Louisville, CO, USA) and values of FEV_1 and FVC were extracted from the forced expiratory manoeuvre. The ratio of FEV_1/FVC is used as a sensitive indicator of airway obstruction in asthma [32].

BHR

All children with past or current wheezing were invited to perform a methacholine bronchial challenge at 10 yrs of age to assess BHR using a Koko dosimeter (Pds Instrumentation; with compressed air source at 8 $L \cdot min^{-1}$ and nebuliser output at 0.8 $L \cdot min^{-1}$). Initial inhalation of 0.9% saline was followed 1 min later by spirometry recording to obtain a baseline value. Subsequently, incremental concentrations from 0.0625 $mg \cdot mL^{-1}$ to 16 $mg \cdot mL^{-1}$ of methacholine were serially administered. The provocation concentration causing a 20% fall in FEV_1 (PC_{20}) from the post-saline value was interpolated and expressed as PC_{20}. BHR was defined as present when the PC_{20} was $<$4.0 $mg \cdot mL^{-1}$.

Risk factors evaluated for the outcomes tested

IL1RN genetic variants were evaluated as risk factors contributing to asthma, BHR and FEV_1/FVC ratios in the study population.

Environmental risk factors

The modifying effects of the various degrees of exposure to ETS on the genetic risk that the children had for asthma, BHR and reduced FEV_1/FVC ratios were evaluated. The levels of childhood ETS exposure were classified into the following three groups. When mothers did not smoke during pregnancy and there was no exposure to household ETS in children up to the age of 10 yrs, children were categorised under the group ''ETS-0'' (n=431). Children, whose mothers did not smoke during pregnancy, that were exposed up to the age of 10 yrs to household members who smoked were categorised as ''ETS-1'' (n=194). When mothers smoked during pregnancy and the children were also exposed to household ETS at some point up

TABLE 1 Population characteristics

Variable	Initial sample	Used in analysis
Subjects n	1491	921
Asthma at 1 yr of age		
Yes	133 (8.9)	95 (10.3)
No	1241 (83.2)	776 (84.3)
Missing	117 (7.9)	50 (5.4)
Asthma at 2 yrs of age		
Yes	132 (8.9)	105 (11.4)
No	1099 (73.7)	707 (76.8)
Missing	260 (17.4)	109 (11.8)
Asthma at 4 yrs of age		
Yes	181 (12.1)	133 (14.4)
No	1033 (69.3)	698 (75.8)
Missing	277 (18.6)	90 (9.8)
Asthma at 10 yrs of age		
Yes	178 (11.9)	134 (14.6)
No	1192 (80.0)	786 (85.3)
Missing	121 (8.1)	1 (0.1)
BHR		
Yes	169 (11.3)	157 (17.0)
No	614 (41.2)	542 (58.8)
Missing	708 (47.5)	222 (24.1)
FEV_1/FVC		
With lung function	1033 (69.3)	912 (99)
Without lung function	458 (30.7)	9 (1.0)
Mean (5th–95th percentile)	0.89 (0.78–0.97)	0.89 (0.79–0.97)
Smoke exposure		
ETS-0	647 (43.4)	431 (46.8)
ETS-1	464 (31.1)	194 (21.1)
ETS-2	370 (24.8)	293 (31.8)
Missing	10 (0.7)	3 (0.3)
Asthma trajectories		
Disease-free	1051 (68.42)	631 (68.5)
Transient	280 (18.2)	204 (22.2)
Persistent	93 (6.1)	73 (7.9)
Missing/not defined	112 (7.3)	13 (1.4)

Data are presented as n or n (%), unless otherwise stated. BHR: bronchial hyperresponsiveness; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; ETS: environmental smoke exposure. ETS-0: mothers did not smoke during pregnancy and children were not exposed to ETS in the household; ETS-1: mothers did not smoke during pregnancy but children were exposed to environmental tobacco smoke in the household; ETS-2: mothers smoked during pregnancy and children were exposed to ETS in the household.

to the age of 10 yrs, the exposure was categorised as ''ETS-2'' (n=293). No children had mothers who smoked during pregnancy but no exposure to household tobacco smoke after birth.

Confounders

Maternal smoking during pregnancy, post-natal ETS exposure, low birth weight (<2,500 g), male sex and breastfeeding for at least the first 3 months of life were used as confounders in the tests for genetic association with asthma, BHR and FEV_1/FVC ratios. Low birth weight (<2,500 g), male sex and breastfeeding for at least 3 months of life were used as confounders in the tests used to determine the effect of maternal smoking and ETS exposure on asthma based on *IL1RN* single nucleotide polymorphism (SNP) genotype.

Genotyping

The SNPper [33] and dbSNP [34] databases were checked for SNPs in the *IL1RN* gene. None of the SNPs found in the databases resulted in an amino acid change. The *IL1RN* SNP information available from Hapmap [35] was analysed and found strong linkage disequilibrium (LD) across the *IL1RN* gene. GOHLKE *et al.* [14] also reported the *IL1RN* SNPs they examined to be in strong LD. In line with GOHLKE *et al.* [14], the current study population was primarily Caucasian and as the SNPs within *IL1RN* were in strong LD, the present investigation focused on the SNPs that demonstrated significant association with asthma in the study by GOLKE *et al.* [14]: rs2234678, rs878972 and rs454078 (table 2). Genomic DNA was isolated from blood samples using QIAamp DNA Blood Kits (Qiagen, Valencia, CA, USA) or the ABI PRISM 6100 Nucleic Acid PrepStation (Applied Biosystems, Foster City, CA, USA). DNA yields were quantified by spectrophotometry (NanoDrop Technologies, Wilmington, DE, USA) or by measuring the quantity of the single copy gene, *RNAseP*, using 5′nuclease fluorescent chemistry PCR normalised to known genomic DNA standards by cycle threshold. Genotyping was performed by Pyrosequencing [36]. To avoid background signals, a blocking primer was required for rs2234678 and rs878972 [37]. Primers were designed using pyrosequencing primer design web resources [38, 39]. The SNPs were genotyped in all children with available DNA (n=921).

Statistical analysis

The SNPs were tested for Hardy-Weinberg equilibrium and linkage disequilibrium. As the SNPs genotyped were also in strong LD in the present study population, one representative SNP (rs2234678) was chosen for further analysis, based on its location in the 5′ upstream region of the *IL1RN* gene and thus its potential role in gene regulation. Using logistic regression, the current authors tested the association of rs2234678 with asthma at each individual age as well as with FEV_1/FVC and BHR assessed at 10 yrs of age. Repeated measures methodology (generalised estimating equation (GEE), GENMOD procedure) was used to test for associations of rs2234678 with asthma at 1, 2, 4 and 10 yrs of age. The rationale for using GEE analysis was that the dichotomous outcome variable (asthma)

TABLE 2 Interleukin-1 receptor antagonist gene polymorphisms tested

SNP	Alleles	Allele frequency	Genotype	Genotype frequency
rs2234678	A/G	0.75/0.25	AA/GA/GG	0.56/0.37/0.07
rs878972	A/C	0.75/0.25	AA/AC/CC	0.56/0.38/0.06
rs454078	A/T	0.74/0.26	AA/AT/TT	0.55/0.38/0.07[#]

A total number of 921 individuals were genotyped. SNP: single nucleotide polymorphism. [#]: n=918.

was repeatedly measured over time and the goal was to estimate marginal probabilities [40]. Multinomial logistic regression categorical modelling (CATMOD) was used to handle the case of the three dependent asthma trajectories: no asthma, transient asthma and persistent asthma. The CATMOD procedure uses maximum likelihood estimation of generalised logits [41]. In this analysis, logits are defined as the logarithm of the frequencies ratio of three different categorical and mutually exclusive outcomes (no asthma, transient asthma, persistent asthma). The association of the genotype to FEV1/FVC ratio was tested using a Kruskall–Wallis test.

To test whether the association of the rs2234678 genotypes with asthma was modified by tobacco smoke exposure in different periods (pregnancy and childhood), the analysis of repeated measurement of asthma and the analysis of the longitudinal asthma categories were stratified by ETS exposure (ETS-0, -1 and -2). The comparisons of these different strata provide an assessment of interaction on an additive scale.

The statistical significance threshold was $p \leqslant 0.05$. The significance values for the associations of asthma at each individual age, BHR, FEV1/FVC and asthma trajectories (table 3) were adjusted for the false discovery rate using the Benjamini–Hochberg algorithm [42]. The repeated measurement models were analysed separately as they used correlated outcomes (asthma at 1, 2, 4 and 10 yrs) and thus were not adjusted for multiple testing [43].

RESULTS

All SNPs were in Hardy–Weinberg equilibrium and were in a single haplotype block based on D′ and pairwise r^2 values [44]. Of the children genotyped, 54.25% were homozygous for the major allele in all three loci (n=498), 35.51% were heterozygous at all loci (n=326), and 5.88% were homozygous for the minor allele at all three loci (n=54; table 2).

SNP rs2234678: association with asthma, BHR and FEV1/FVC

No association of *IL1RN* genotypes with repeated measurement of asthma or asthma trajectories was found that classified the longitudinal dataset into disease-free samples, transient asthmatics and persistent asthmatics. There was also no association of *IL1RN* genotypes with BHR or FEV1/FVC ratios (table 3). Along with the longitudinal analyses, cross-sectional analyses were conducted to assess the impact of *IL1RN* genotypes on asthma at individual ages. Asthma is a dynamic disease with characteristics that may change over time, making the analyses at individual ages important. In the cross-sectional analysis the rs2234678 genotype was not significantly associated with asthma at any single age. A trend toward association with asthma at 2 yrs of age was observed (odds ratio (OR) 2.99, 95% confidence interval (CI) 1.38–6.46, p=0.01; table 3) but was not significant after adjusting for false discovery rates (p=0.08).

Effect of smoke exposure on asthma based on IL1RN genotype

In the longitudinal assessments, the rs2234678 genotype AA was used as the reference genotype in the tests for the effect of smoke exposure on asthma based on *IL1RN* genotypes. The rs2234678 genotype GG was significantly associated with persistent asthma in the longitudinal trajectory analysis (ETS-2 group: OR 4.53, CI 1.69–12.1, p=0.0025; table 4) but not with

TABLE 3 Association of the interleukin-1 receptor antagonist gene polymorphism with asthma, bronchial hyperresponsiveness (BHR) and forced expiratory volume in one second (FEV1)/forced vital capacity (FVC)

Outcome	rs2234678			p-value	FDR adjusted	GG *versus* AA	GA *versus* AA
	GG	GA	AA			OR (95% CI)	OR (95% CI)
Asthma at age yrs							
1	6/52 (11.5)	32/325 (9.8)	57/494 (11.5)	0.74	0.91	1.34 (0.51–3.49)	0.912 (0.54–1.53)
2	13/49 (26.5)	33/297 (11.1)	59/466 (12.7)	0.01	0.08	2.99 (1.38–6.46)	0.93 (0.57–1.51)
4	10/52 (19.2)	46/311 (14.8)	77/468 (16.5)	0.67	0.91	1.58 (0.74–3.38)	0.97 (0.63–1.47)
10	8/59 (13.6)	47/343 (13.7)	79/518 (15.2)	0.80	0.91	0.97 (0.41–2.30)	0.85 (0.56–1.32)
Repeated measurement of asthma at ages 1, 2, 4 and 10 yrs#	242/1721 (14.1)	142/1150 (12.4)	35/186 (18.8)	0.10		1.58 (0.93–2.70)	0.92 (0.67–1.25)
BHR	22/47 (46.8)	109/265 (41.1)	168/388 (43.3)	0.97	0.97		
Asthma trajectories							
Disease-free	34/59 (57.6)	239/343 (69.9)	358/519 (70.0)	Ref.		1	1
Transient	14/59(23.7)	76/343 (22.2)	114/519 (22.0)	0.51	0.91	1.13 (0.82–1.60)	0.99 (0.85–1.18)
Persistent	6/59 (10.2)	24/343 (7.0)	43/519 (8.3)	0.74	0.91	1.21 (0.76–1.92)	0.91 (0.70–1.19)
Missing/not defined	5/59 (8.5)	4/343 (1.2)	4/519 (0.8)				
FEV1/FVC¶	0.88 (0.79–0.97)	0.88 (0.78–0.98)	0.88 (0.82–0.97)	0.31	0.91		

Data are presented as n/n (%) or mean (5th–95th percentile), unless otherwise stated. Numbers of observations are presented instead of number of children. False discovery rate (FDR) adjusted p-values were calculated using the Benjamini–Hochberg algorithm. OR: odds ratio; 95% CI: 95% confidence interval; Ref.: reference value. #: no FDR adjustment was performed for this variable; ¶: as the FEV1/FVC ratio is a continuous outcome variable, the Kruskal–Wallis Test was used; Chi-squared tests were performed for all other outcomes.

transient asthma. These results, which rely on pattern assessment for assignment of children into transient or persistent asthma groups, were confirmed by repeated measures analysis, which has the advantage of being informative but is independent of pattern assessment. Similar results were seen as the rs2234678 genotype GG was significantly associated with repeated measurements of asthma in children of mothers who smoked during pregnancy (ETS-2 group: OR 4.43, CI 1.62–12.1, p=0.0037; table 4) but not in children without maternal smoking exposure during pregnancy (ETS-0 or ETS-1). These results also demonstrate that repeated measurement analysis of asthma (aged 1, 2, 4 and 10 yrs) estimates the risk for persistent asthma since both outcomes measure the frequency (persistency) of occurrence. The smoking rs2234678 genotype interaction was not tested for asthma occurrence at individual ages due to the resulting small sample sizes following stratification for smoking exposure.

DISCUSSION

The current authors found that *IL1RN* was associated with persistent asthma in Isle of Wight children exposed to maternal smoking during pregnancy. The current analyses showed that *IL1RN* did not have an overall association with asthma at ages 1, 2, 4 or 10 yrs using individual logistic regression and within repeated measurement analysis of asthma (table 3). However, when stratified by ETS exposure levels, rs2234678 genotype GG was associated with both repeated measurements of asthma and with the persistent asthma phenotype (table 4). The adverse effect was due to the homozygous minor allele genotype GG. The relative risk of the heterozygous genotype (AG) was not different from the major allele homozygote, suggesting a recessive model.

IL1RN was selected for this study based on genetic location as a positional candidate gene derived from linkage studies in the current murine model of allergic asthma [8], as well as potential mechanistic relevance to asthma. Mechanistically, *IL1RN* functions to restrain the inflammatory response; thus, dysfunction of this gene could result in the unchecked inflammation that is characteristic of asthma. For example, specific *IL1RN* alleles were associated with lower serum IL-1Ra levels in atopic and nonatopic asthmatics [45]. Previous evidence, while limited, supports a role for genes in the IL-1 cascade in determining the susceptibility or resistance to chronic inflammatory diseases, yet only a few studies have tested *IL1RN* for genetic associations with asthma phenotypes. GOHLKE *et al.* [14] and PATTARO *et al.* [17] found significant association of *IL1RN* polymorphisms with asthma in a German population; they confirmed their results in an independent Italian population and replicated the association of *IL1RN* with asthma in another German population. The polymorphisms that reached significance for asthma in the original study by GOHLKE *et al.* [14] were examined in the current study. In this population of children from the Isle of Wight, the strong LD across these SNPs (table 2) resulted in essentially identical information, thus the results of a single SNP to reduce multiple testing are reported here.

The present results differ from those of GOHLKE *et al.* [14] in that the association of rs2234678 with asthma was not confirmed in nonstratified analyses. It is possible that environmental factors contributed to this difference as the risk conferred by specific *IL1RN* genotypes to the incidence of asthma is accentuated by exposure to maternal smoking during pregnancy and ETS exposure after birth (table 4). The present authors have previously reported the association of

TABLE 4 Effect of smoke exposure on asthma, repeated measurement at 1, 2, 4 and 10 yrs of age

IL1RN rs2234678	ETS-0[#]		ETS-1[¶]		ETS-2[+]	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
AA	1.00		1.00		1.00	
GA	0.81 (0.49–1.30)	0.38	0.85 (0.51–1.42)	0.54	1.17 (0.61–2.21)	0.63
GG	1.20 (0.46–3.12)	0.71	0.70 (0.25–2.01)	0.51	4.43 (1.62–12.1)	0.0037
Trajectory analysis[§]						
Transient asthma trajectory						
AA	1.00		1.00		1.00	
GA	0.89 (0.67–1.17)	0.39	1.22 (0.87–1.72)	0.24	0.99 (0.63–1.55)	0.98
GG	1.31 (0.74–2.31)	0.35	1.30 (0.73–2.33)	0.37	1.17 (0.61–2.21)	0.63
Persistent asthma trajectory						
AA	1.00		1.00		1.00	
GA	0.78 (0.34–2.96)	0.31	0.88 (0.56–1.39)	0.59	1.32 (0.76–2.30)	0.33
GG	1.01 (0.49–1.25)	0.98	NE	NE	4.53 (1.69–12.1)	0.0025

Data were adjusted for sex, environmental tobacco smoke (ETS) exposure, breastfeeding ⩾3 months, low birth weight (<2,500 g) and birth order. IL1RN: interleukin-1 receptor antagonist receptor gene; NE: not estimated (due to small numbers). ETS-0: mothers did not smoke during pregnancy and children were not exposed to household ETS; ETS-1: mothers did not smoke during pregnancy but children were exposed to household ETS; ETS-2: mothers smoked during pregnancy and children were exposed to household ETS. [#]: 1,301 observations, 151 subjects; [¶]: 782 observations, 141 subjects; [+]: 453 observations, 119 subjects; [§]: assessed with categorical modelling with all trajectory phenotypes in one model.

maternal smoking during and after pregnancy on the development of asthma at age 1 and 2 yrs [46, 47]. It was not surprising that smoke exposure during gestation and early childhood was an important driver for asthma in this study, as maternal smoking has been shown to be associated with increased risk of asthma in the offspring of mothers who smoke [25–30, 48]. This suggests that the effect of *IL1RN* on asthma could be influenced by maternal smoking during pregnancy and post-natal ETS exposure levels. Indeed, the importance of ETS exposure on genetic linkage for asthma and BHR has been demonstrated by MEYERS *et al.* [49] and COLILLA *et al.* [50]. The present results similarly suggest an important role of ETS exposure in identifying susceptibility loci for asthma. To the best of the current authors' knowledge, this study is the first to report the association of *IL1RN* genotype with asthma influenced by maternal smoking during pregnancy.

A limitation of the present study was that the results were based on the subset of children who donated blood for DNA collection during the 10-yr follow-up. The percentage of children who had asthma and those who had ETS-0 was higher in the samples used for analyses compared with the original sample (table 1). However, tests for associations between ETS and repeated measurements of asthma in the whole population and in the subset of samples used in the analyses yielded nearly identical ORs (data not shown). Additionally, spirometry and BHR were not performed in early childhood, so those children with transient bronchial obstruction at an early age whose lung function returned to normal by 10 yrs of age would have been missed.

In conclusion, the present study provides evidence that maternal smoking during pregnancy modifies the role of the interleukin-1 receptor antagonist gene in asthma. The current findings indicate that gene association studies can lead to false-negative results if the environment is not taken into consideration. Regarding public health, females should be further discouraged from smoking, especially during pregnancy, to protect their children from becoming persistent asthmatics. Regarding future research, there is a need to take gene–environment interaction into account when associating genes and asthma.

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