

# GWAS-identified variants to allergic disease and early environmental exposure in Chinese schoolchildren with allergic rhinitis induced by house dust mite

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## Abstract

**Background:** There is growing evidence that environmental exposure in early life is associated with the development of childhood allergic rhinitis.

**Objective:** To investigate whether polymorphisms in previously published genome wide association studies (GWAS) allergic disease loci are associated with childhood house dust mite-induced allergic rhinitis (HDM-AR) and interaction effects of genetic and environmental factors on it.

**Methods:** 156 cases diagnosed by HDM-AR and 173 controls were enrolled. Potential confounders were analyzed by using Logistic regression. Twenty-one single nucleotide polymorphisms (SNPs) of GWAS-related allergic diseases including *EMSY-LRRC32*, *IL18R1*, *IL18RAP*, *IL13*, *IL4*, HLA region, *KIF3A* were genotyped and analyzed using the improved multiplex ligation detection reaction (imLDR) technique in all the subjects.

**Results:** Only *IL18R1\_rs2287037* was associated with HDM-AR in children. After adjusting for several likely confounders, the protective TT genotype of *IL18R1\_rs2287037* was found in the population analyzed with the fittest recessive model. (adjusted odds ratio [aOR]: 0.44; 95% confidence interval [CI]: 0.21-0.95). The rs2287037\_TT might interact with early-life exclusive breastfeeding in the first 4 months (aOR: 0.33; 95%CI: 0.14-0.97) or full-term birth (aOR: 0.45; 95%CI: 0.19-0.95) exposure to decrease the risk of HDM-AR.

**Conclusions:** These data suggest that *IL18R1* polymorphism may play a role in controlling risk to HDM-AR and underline the importance of early environmental exposure into studies of genetic risk factors.

**Key words:** Children; Allergic rhinitis; house dust mite; variants; early environment

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## Introduction

The prevalence of allergic diseases, including allergic rhinitis (AR), in children is increasing in most countries in the world. AR, a chronic inflammatory disease of the upper airway, also impairs children's quality of life such as decreased school performance, sleep disturbance, and emotional and other psychosocial problems.<sup>1</sup> The reported prevalence of AR varies widely, ranging from 7.83-48% in China, resulted from sampling strategy, the definition of AR, the ages of the children and the study results across cities with different gross domestic product and health-system coverage.<sup>2-4</sup>

In 2015, Li et al.<sup>5</sup> reported that the prevalence of AR was 12.9% in school-aged children in Shanghai, China. The house dust mite (HDM) is the most important source of indoor allergens that cause allergic disease in China.<sup>6</sup>

AR is typically a multifactorial disease caused by the interplay of genetic and environmental factors. Genetic variations are components of genetic factors. Genome-wide association studies (GWAS) have revealed allergic disease susceptibility loci in an unbiased and hypothesis-free manner. Many candidate genes in susceptibility loci suggest roles for innate immunity and immunoregulation including type 2 T helper (Th2) cell differentiation and effectors' functions, epithelial barrier functions, *IL1* family signaling, and regulatory T cells in the pathogenesis of allergic diseases. The first GWAS on allergic sensitization identified putative susceptibility loci including 11q13.5 near *EMSY* (previously known as *C11orf30*) and the HLA region at 6p21.32 in European origins with AR only sensitization to a single allergen (grass),<sup>7</sup> which was previously associated with atopic dermatitis<sup>8</sup> and asthma.<sup>9</sup> Independent replication of genotype-phenotype associations in distinct populations is generally thought to provide the most convincing evidence for the identification of a true disease susceptibility gene.<sup>10</sup> Furthermore, *IL-4* polymorphism<sup>11</sup> and the HLA region alleles<sup>6,12</sup> may contribute to the susceptibility to HDM-AR in a Chinese population.

Despite the numerous efforts to discover genes associated in allergy through various approaches including GWAS, the investigation of gene-environment interactions has been mainly limited to candidate genes, candidate environmental exposures. AR in school-aged children was found to be associated with pre- and postnatal events including breastfeeding mode, delivery method, age of gestation and other early environmental exposure.<sup>5</sup> Sensitization to mite allergens in the first years of life associates in the long term with poorer clinical outcomes in respiratory health.<sup>13</sup> Meantime, the fact that moderation of breastfeeding effects on the IQ by genetic variation was reported in two birth cohort.<sup>14</sup> A genome-wide interaction analysis revealed interaction genes for asthma and atopy in a farming environment.<sup>15</sup> However, little is known about how genetic and environmental factors or their interactions contribute to the development and progression of AR.<sup>16</sup>

In the present study, we conducted a study to test whether genetic loci associated with allergic diseases including *EMSY* and leucine rich repeat containing 32 (*LRRC32*) at 11q13.5, the HLA region at 6p21.32, *IL18R1* and *IL18RAP* at 2q12.1, and *IL13*, *IL4*, and *KIF3A* at 5q31.111 may contribute the susceptibility to HDM-AR. Moreover, we investigated the interaction between candidate genetic and early environmental exposure to learn about the challenges in detecting it. Confirming the association will strengthen the evidence for a causal relationship, and improve gene-environment interaction discovery.

## Methods

### Study subjects

We used a population-based case-control association study design to assess a possible interaction between GWAS-related allergic disease loci and early-life environment on the risk of childhood HDM-AR.

156 patients suffering from AR whose age ranged from 5-10 years were recruited from the outpatient clinic of Otolaryngology Department at Shanghai Children's Medical Center from June 2015 to May 2016. Age and gender matched controls were healthy children undergoing regular physical examination in our hospital. All subjects were ethnic Han Chinese, who had lived in the Shanghai region, China, since birth. The study approved by the ethics committee of Shanghai Children's Medical Center, and written informed consent was obtained from the parents or guardians.

### Clinical evaluation

156 patients were diagnosed as having clinical AR according to ARIA 2008 guidelines in the clinic of Otolaryngology Department at Shanghai Children's Medical Center, with recurrence of symptoms over a year; i.e., two or more persistent symptoms of water-like tears, nasal itching, congestion, or sneezing lasting for more than 1 hour every day.<sup>17</sup> HDM or HDM along with other more serum IgE species (SIgE) were found positive ( $\geq 0.35$  kU/l) by Western blotting using AllergyScreen™ human serum specific IgE allergen detection kit for specific inhalant allergens including HDM (*Dermatophagoides pteronyssinus* [*Der p 1*] and *Dermatophagoides fainae* [*Der f 1*]), cat/dog hair, molds, seasonal grass/tree and pollens, cockroaches, ragweed, *Artemisia argyi*, *Penicillium notatum*, *Aspergillus fumigatus*.

To identify relationships between genetic loci and AR, we set the following exclusion criteria including patients with co-morbid asthma, eczema, or any other allergic disease, or tumor in the nasal cavity or any other inflammatory nasal disease or respiratory issue or autoimmune disease, hematological disease, congenital heart disease or other chronic diseases.

The 173 healthy controls who presented no clinical features related to nasal diseases when they did not have a cold or the flu were recruited. None of these subjects had a history of allergic disease and atopic family history. Moreover, all the controls were examined for serum SIgE test with no positive antigen-specific IgE against any of the common allergens described above.

### Assessment Questionnaires of early environmental exposure

The sociodemographic questionnaire surveyed for factors that might have had a significant impact on AR based on our previous findings. These comprised the following variables: gender, age, parental educational level (middle school and below/high school/college and above), resident area per capita (m<sup>2</sup>), family numbers (< 3 persons/3 persons/ $\geq 4$  persons), gestational age (< 37 weeks/37–41 weeks/42 weeks), model of delivery (vaginal delivery/cesarean section), exclusive breastfeeding (BF) in the first 4 months (yes/no), in addition to passive smoking exposure prior to 1 year-old (home exposure to smoking parents or any other smoking person).

### Selection of twenty three common variants associated with allergic diseases identified by published GWAS

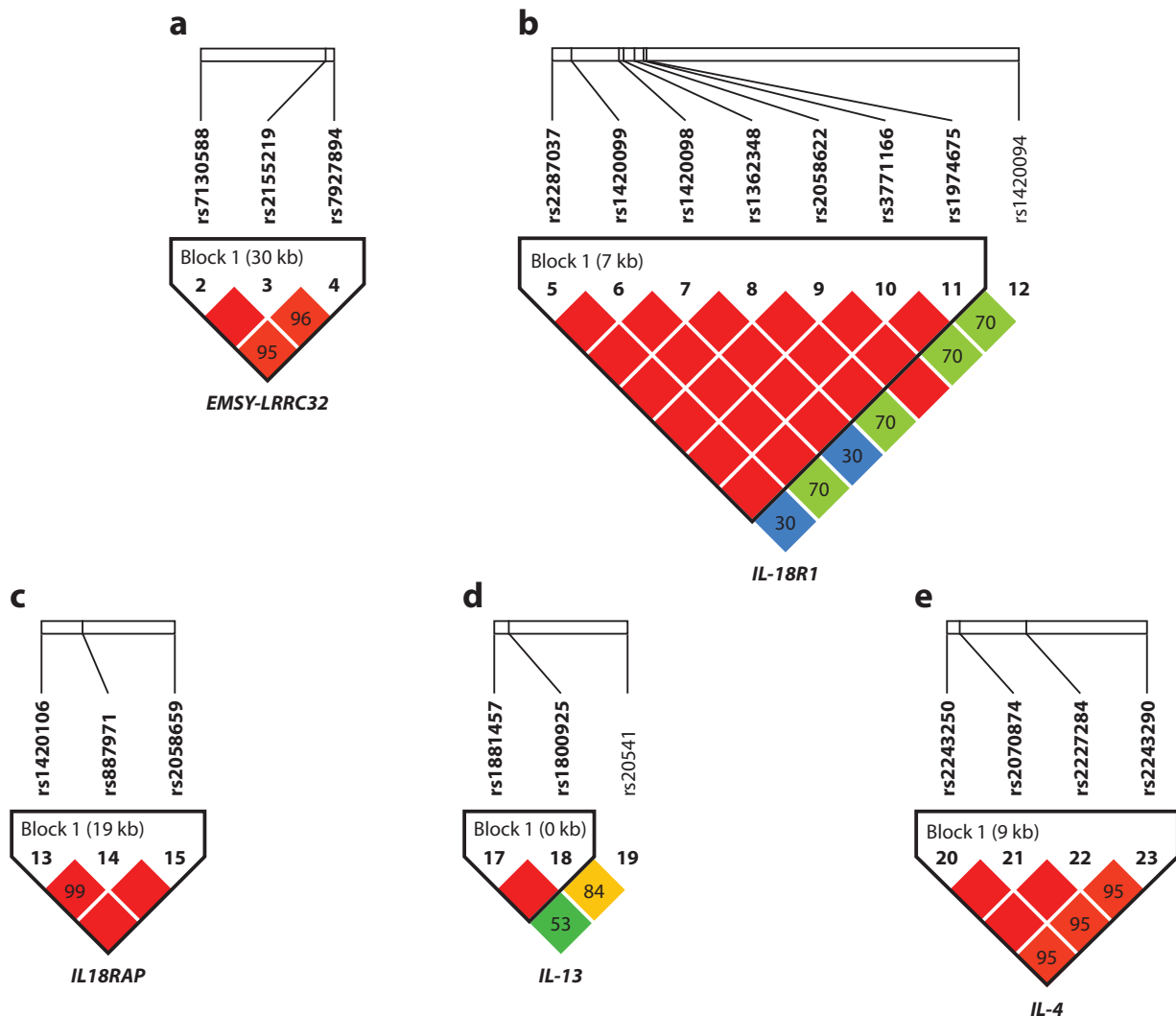
Based on the published literature such as recent GWAS<sup>18</sup> of allergic diseases and public SNP databases provided by the CHB (Han Chinese in Beijing, China) from the HapMap Project (<http://www.hapmap.org>, HapMap data rel 27 Phase

II+III, Feb 2009), SNPs within or near *EMSY* (previously known as *C11orf30*) and *LRRC32* (11q13.5), HLA region (6p21.32), *IL18R1* and *IL18RAP* (2q12.1), and *IL13*, *IL4*, and *KIF3A* (5q31.1) were assessed in our study. After genotyping for 21 SNPs, linkage disequilibrium (LD,  $D'$ ) patterns of these SNPs were assessed to observe the non-random association of alleles at different loci in our dataset. (**Supplemental Table S1**) (**Figure 1**) All  $r^2$  values  $< 0.2$  were set to 0 and each SNP was assigned an  $r^2$  value of 1.0 with itself. (**Figure 2**) Tag SNPs (tSNPs) exhibited strong LD in several groups of SNPs with correlation coefficients ( $r^2$ ) greater than or equal to 0.8, indicating that most common SNPs can be represented by a subset of tSNPs using our dataset. Further selection of

the final tSNPs to be was made setting the Hardy-Weinberg  $p$  value, minor allele frequency (MAF) as  $< 0.01$  and  $< 0.05$ , respectively. We elected total 15 SNPs to represent the entire loci for the research in total samples excluding the deviation from Hardy-Weinberg equilibrium. (**Supplemental Table S2**).

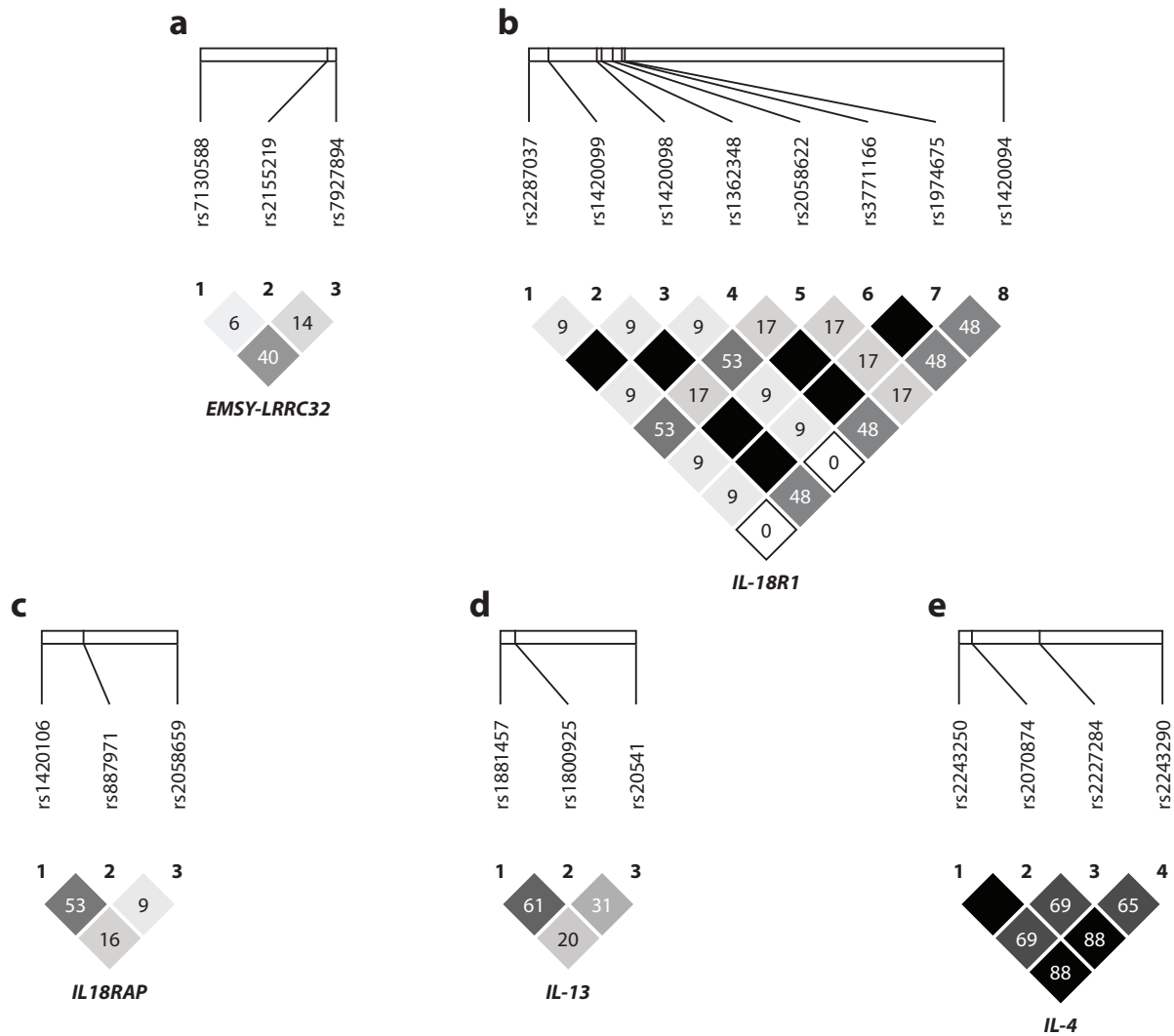
**Specimen collection and DNA extraction**

DNA was isolated from peripheral blood leukocytes using the QIAamp DNA Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions, and stored at 4°C prior to further investigation within 2 days. DNA concentrations were measured with the NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).



**Figure 1. Values of  $D'$  ( $D' \times 100$ ) in Pairwise Linkage disequilibrium (LD) between the *EMSY-LRRC32*, *IL18R1*, *IL18RAP*, *IL13*, *IL4* gene region in AR.**

The measure of LD ( $D'$ ) among all possible pairs of SNPs is shown graphically according to the depth of red color. The numbers in squares are  $D'$  values ( $D' \times 100$ ). Haplotype block structure was estimated with the Haploview program.



**Figure 2. Values of  $r^2$  ( $r^2 \times 100$ ) in pairwise Linkage disequilibrium (LD) between the EMSY-LRRC32, IL18R1, IL18RAP, IL13, IL4 gene region in AR.**

The measure of LD ( $r^2$ ) among all possible pairs of SNPs is shown graphically according to shades of color in those squares shown, where white represents very low  $r^2$  and scarlet represents very high  $r^2$ .

**SNP genotyping and quality control**

The SNP genotyping work was performed using an improved multiplex ligation detection reaction (imLDR) technique developed by Genesky Biotechnologies Inc. (Shanghai, China). A multiplex PCR-ligase detection reaction method was used in the imLDR. For each SNP, the alleles were distinguished by different fluorescent labels of allele-specific oligonucleotide probe pairs. Different SNPs were further distinguished by different extended lengths at the 3'end. Two negative controls were set: one with double-distilled water as the template and the other with the DNA template but without primers while all other conditions are kept the same in one plate. A 5% random sample was tested in duplicate by different persons, and the reproducibility was 100%.

**Statistical analyses**

The values of continuous data were expressed as mean  $\pm$  SD. The differences between groups for basic characteristics were measured with chi-square ( $\chi^2$ ) test for categorical variables or  $t$ -test for continuous variables. The frequency

genotypes were expressed as the number or percentage of total number. Deviation from the Hardy-Weinberg equilibrium (HWE) for each SNP was tested using the Pearson's chi-square test with 1 degree of freedom in control subjects. Pair-wise LD measures were performed using Haploview version 4.0 with default settings. The  $r^2$  for each pair of SNPs was calculated, and haplotype blocks were defined by the method of Gabriel et al.<sup>19</sup> Haplotype blocks are defined based on the pairwise  $D'$  values  $> 0.9$ .

The comparison between genotype and estimated haplotype frequencies of AR and controls was tested by  $\chi^2$ . Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression with adjustment for age and gender in full genotype and common genetic models including co-dominant, dominant, recessive, and log-additive models, and haplotype analysis. The results were also performed after stratification according to different early environmental exposure. The genetic model with the lowest Akaike's information criterion (AIC) and Bays information criterion (BIC) was accepted as the best-fitting genetic model.

In all analyses, the lower frequency allele was considered to be the “risk” allele.

The interaction between rs2287037 genotype and early environmental exposure was tested by multiple logistic regression analysis.

We did not correct for multiple testing, as LD suggested these were not independent tests.<sup>20</sup>  $p < 0.05$  is considered of suggestive significance. No corrections were used to adjust for type I error. Statistical analyses were performed using SPSS 17.0 (Statistical Package for the Social Sciences Inc, Chicago, IL, USA) and PLINK 1.07 software.

The statistical power for the study was calculated using G\*Power 2 software (<http://www.psych.uni-duesseldorf.de/aap/projects/gpower/>). The statistical power for comparison of

AR vs. control was 97.9% with the current sample size; the  $\alpha$  was 0.05 and the  $\beta$  was 0.2.

## Results

### Characteristics of study subjects

Basic characteristics and family features associated with allergic disease of our current subjects are shown in **Table 1**. All groups were comparable with respect to gender, age, weight and height, of which were not significantly different for case group vs. control group ( $P > 0.05$ ). Most of the cases (76.9%) have only HDM allergen and 23.1% have other allergen along with HDM. The risk factor of AR including parental education level and family numbers were significantly distributed in AR group as expected.<sup>5</sup>

**Table 1. Demographic characteristics in the study subjects**

Variables	HDM-AR n = 156	Control n = 173	Total n = 329	$P^a$
<b>Demographics</b>				
Gender, n (%)				0.21
Male	106 (67.9)	105 (60.7)	211 (64.1)	
Female	50 (32.1)	68 (39.3)	118 (35.9)	
Mean age (years) $\pm$ SD	6.26 $\pm$ 2.93	6.18 $\pm$ 1.10		0.16
Mean weight (kg) $\pm$ SD	25.58 $\pm$ 10.53	24.31 $\pm$ 4.17		0.12
Mean height (cm) $\pm$ SD	121.48 $\pm$ 9.95	114.51 $\pm$ 9.03		0.07
<b>HDM with other allergen, n (%)</b>				
Yes	39 (25.0)			
No	117 (75.0)			
<b>Early environmental exposures</b>				
Exclusive breastfeeding in the first 4 months				0.203
Yes	48 (30.8)	65 (37.6)	113 (34.3)	
No	108 (69.2)	108 (62.4)	216 (65.7)	
Age of gestation (weeks)				0.09
Term	131 (84.0)	144 (83.2)	275 (83.6)	
Preterm	10 (6.4)	4 (2.3)	14 (4.3)	
Post-term	15 (9.6)	25 (14.5)	40 (12.2)	
Mode of delivery				0.258
Vaginal	66 (42.3)	62 (35.8)	128 (38.9)	
Cesarean	90 (57.7)	111 (64.2)	201 (61.1)	
Maternal education, n (%)				0.001*
Below-high school	3 (1.0)	23 (13.3)	26 (7.9)	
High school	22 (14.1)	31 (17.9)	53 (16.1)	
College	110 (70.5)	96 (55.5)	206 (62.6)	
Above-College	21 (13.5)	23 (13.3)	44 (13.4)	

**Table 1. (Continued)**

Variables	HDM-AR n = 156	Control n = 173	Total n = 329	<i>P</i> <sup>a</sup>
Resident area per capita (m <sup>2</sup> )				0.334
< 15	9 (6.8)	14 ( 8.1)	23 (7.0)	
15-25	42 (31.6)	48 (27.7)	90 (27.4)	
25-35	54 (31.2)	54 (31.2)	108 (32.8)	
> 35	57 (32.9)	57 (32.9)	114 (34.7)	
Family numbers				0.04*
< 3 persons	1 (0.8)	0 (0)	4 (1.2)	
3 persons	54 (42.5)	96 (55.5)	150 (45.6)	
≥ 4 persons	72 (56.7)	77 (44.5)	149 (45.3)	

<sup>a</sup> *P* value for the comparison between mild and controls calculated by  $\chi^2$  test for categorical variables or *t*-test for continuous variables

\* indicates OR within 95%CI and statistically significant at *p* value less than 0.05

### Hardy-Weinberg equilibrium

All the SNPs analyzed were in Hardy-Weinberg equilibrium in HDM-AR and controls, which indicated that 21 SNPs were representative in the field without any deviation of genotype frequencies (*P* > 0.05). (**Supplemental Table S1**)

### Individual SNP association analysis

The results of genotypic and allelic frequency analysis for each tSNPs selected are as shown in **Supplemental Table S1**. Only the TT allele of rs2287037 in *IL18R1* were less prevalent in the patients with HDM-AR compared with controls subjects (aOR: 0.44, 95%CI: 0.21-0.95) after adjusted by age,

gender, parental education level, resident area per capita, family numbers. (**Supplemental Table S2**).

At the study of model fit statistics in rs2287037, recessive model showed that the TT genotype was significant protective effect on HDM-AR (*p* = 0.039, after adjusting age, gender, parental education level, resident area per capita, family numbers, gestation age, model of delivery, BF in the first 4 months and passive smoke exposure. (OR: 0.49, 95%CI: 0.24-0.96) compared with the CC&CT homozygote. The recessive model with the lowest Akaike's information criterion (AIC) and Bays information criterion (BIC) was considered as the best-fitting genetic model regardless of significant *p* value observed in model of codominant. (**Table 2**)

**Table 2. Model fit statistics for all univariate models**

SNP ID	Minor Allele	Model	Genotype	AR (156)		Control (173)		Adjusted <sup>a</sup> OR (95%CI)	<i>P</i> <sup>a</sup>	AIC	BIC	
				No.	%	No.	%					
rs2287037	T	Codominant	CC	67	42.9	64	37	1		457	468.4	
			CT	75	48.1	81	46.8	0.88 (0.56-1.37)	0.493			
			TT	14	9	28	16.2	0.44 (0.21-0.94)	0.035*			
			Dominant	CC	67	42.9	64	37	1	0.202	458	465.6
				CT+TT	89	57.1	109	63	0.74 (0.47-1.18)			
				Recessive	CC+CT	142	91	145	83.8	1	0.039*	455.3
		TT	14		9	28	16.2	0.49 (0.24-0.96)				
		Overdominant	CC+TT		81	51.9	92	53.2	1			
			CT	75	48.1	81	46.8	1.05 (0.68-1.62)	0.82	459.2	466.8	
			Log-additive	-	-	-	-	0.75 (0.54-1.03)	0.076	456.1	463.6	

<sup>a</sup> *P* value from unconditional logistic regression analyses, adjusted for age, gender, parental education level, resident area per capita, family numbers

AIC: Akaike's information criterion

BIC: Bays information criterion

\* indicates a statistically significant difference with a *p* value less than 0.05

### LD analysis and Haplotype analysis

To understand better the relationship between 15 tSNPs within and near relevant genes, we performed LD analysis and identified blocks by confidence intervals. (Figure 1) Four haplotype blocks were found in the subjects. **Supplemental Table S3** summarizes associations between frequencies of haplotypes in blocks and risk of HDM-AR. However, no haplotype was found to be associated with either risk or protection against HDM-AR.

### Early environmental exposure and rs2287037

We also investigated interactions between the *IL18*\_rs2287037 polymorphism and early environmental factors in the risk for HDM-AR, which conducted in 133 HDM-AR and 176 controls with intact information about gestational age, mode of delivery, BF in the first 4 months, in addition to passive smoking exposure.

Overall, results of logistic regression models of early environmental exposure stratified by genotype of rs2287037 in full genotype and recessive model show similar protective effects. Individuals carrying the rs2287037\_TT have a significant decreased HDM-AR risk who are BF in the first 4 months (aOR: 0.33, 95%CI: 0.11-0.97) or born full-term (aOR: 0.44; 95%CI: 0.19-0.98) after adjusting for age, gender, parental education level, resident area per capita, family numbers. (Table 3) Furthermore, the risk for HDM-AR was smaller among subjects

with rs2287037\_TT who had BF in the first 4 months (aOR: 0.33 95%CI: 0.14-0.97) than among subjects with the CC+CT genotype who had not BF in the first 4 months (aOR: 0.7, 95%CI: 0.42-1.16) or those with the CC+CT genotype who had BF in the first 4 months (aOR: 1). (Figure 3a). Similarly, the risk for HDM-AR was smaller among subjects with rs2287037\_TT who were full-term born (aOR: 0.45 95%CI: 0.19-0.95) than among subjects with the CC+CT genotype who were pre/post-term born (aOR: 0.65, 95%CI: 0.26-1.56) or those with the CC+CT genotype who were full-term born (aOR: 1). (Figure 3b). Genetic factors might therefore interact with BF in the first 4 months or full-term birth exposure to decrease the risk of HDM-AR.

Overall, the rs2287037\_TT also modulated the effect of cesarean delivery (aOR: 0.35; 95%CI: 0.13-0.95) or passive smoking exposure in early life on childhood HDM-AR (aOR: 0.32; 95%CI: 0.12-0.90) after adjustment. (Supplemental Table S4, S5) Though the risk for HDM-AR was greater among subjects with rs2287037\_CC+CT who were delivered by cesarean (aOR: 0.66, 95%CI: 0.19-2.28) than among subjects with rs2287037\_TT who were delivered by cesarean (aOR: 0.39, 95%CI: 0.14-1.10) (Figure 3c), there were no interactions between rs2287037\_TT and the risk of cesarean. The same phenomena was also found no relationship of interaction with rs2287037\_TT and passive smoking exposure in early life. (Figure 3d)

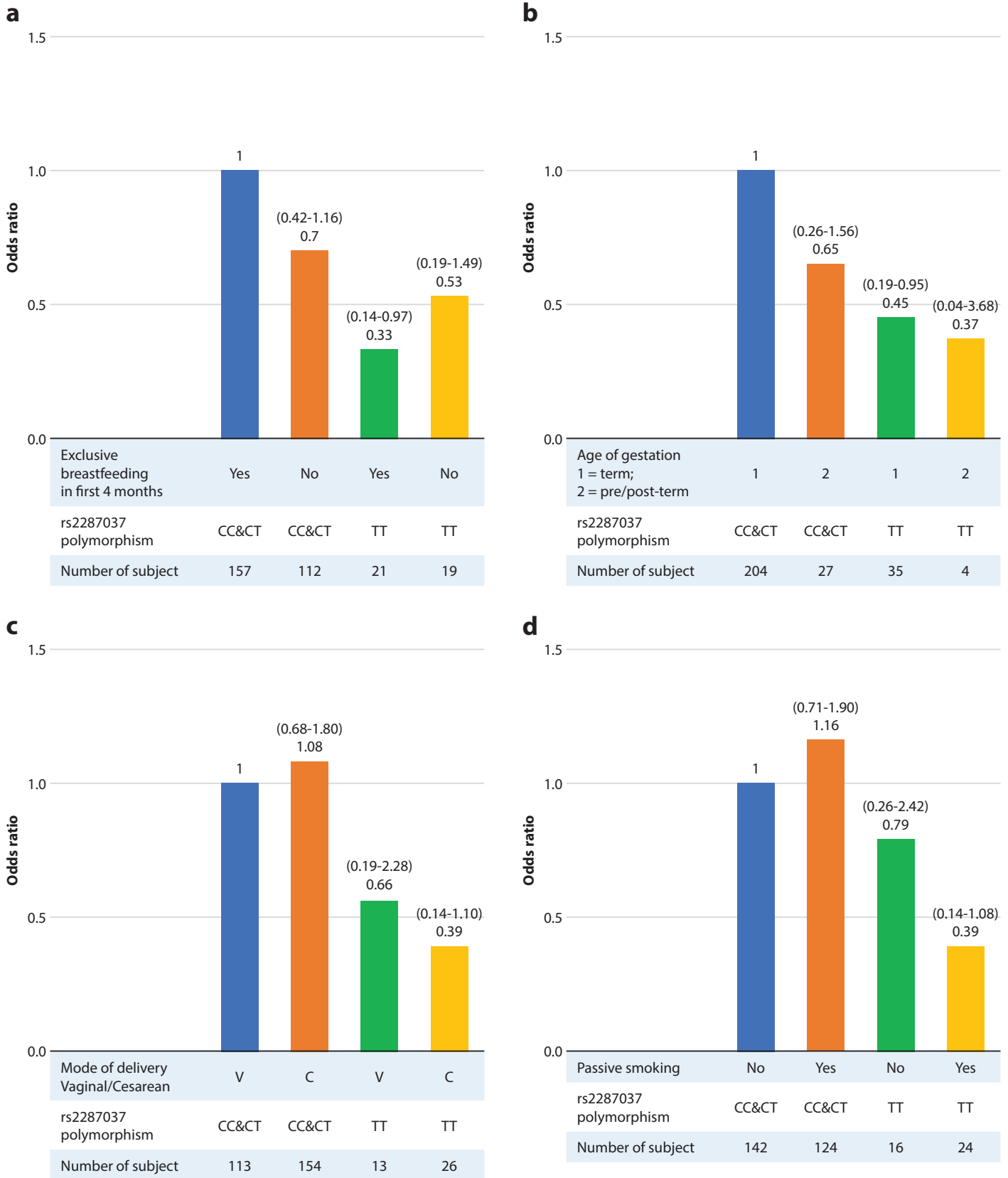
**Table 3. Results of logistic regression models of exclusive breastfeeding in the first 4 months and model of delivery on HDM-AR stratified by genotype in genetic models.**

SNP ID	Genotype	Exclusive breastfeeding in first 4 moths				Age of gestation			
		Yes		No		Term		Preterm/Post-term	
		No. cases/ controls	Adjusted <sup>1</sup> OR (95%CI)	No. cases/ controls	Adjusted <sup>1</sup> OR (95%CI)	No. cases/ controls	Adjusted <sup>1</sup> OR (95%CI)	No. cases/ controls	Adjusted <sup>1</sup> OR (95%CI)
rs2287037									
Model 1	CC	42/37 (50.6/38.9)	1	19/27 (35.8/34.6)	1	54/56 (43.9/36.8)	1	6/8 (60.0/38.1)	1
	CT	36/42 (43.4/44.2)	3.2 (0.99-11.42)	27/39 (50.9/50.0)	1.03 (0.47-2.27)	59/71 (48.0/46.7)	0.80 (0.47-1.36)	3/10 (30/47.6)	0.41 (0.08-2.19)
	TT	5/16 (6.0/16.8)	2.4 (0.78-7.43)	7/12 (13.2/15.4)	0.78 (0.25-2.45)	10/25 (8.1/16.4)	0.39 (0.16-0.91)*	1/3 (10.0/14.3)	0.52 (0.40-6.66)
Model 2	CC+CT	78/79 (93.9/83.2)	1	46/66 (86.8/84.6)	1	113/127 (91.9/83.6)	1	9/18 (90.0/85.7)	1
	TT	5/16 (6.2/16.8)	0.33 (0.11-0.97)*	7/12 (13.2/15.4)	0.76 (0.27-2.19)	10/25 (8.1/16.4)	0.44 (0.19-0.98)*	1/3 (10.0/14.3)	0.77 (0.67-9.0)

Adjusted<sup>1</sup> for age, gender, parental education level, resident area per capita, family numbers

Model<sup>1</sup>: full genotype; Model<sup>2</sup>: recessive model

\*  $P < 0.05$



**Figure 3. aORs and 95% CIs for HDM-AR according to early environmental exposure and *IL18R1\_rs2287037* polymorphism in school children.**

After adjusting for age, gender, parental education level, resident area per capita and family numbers. Figure 3a-b. shows the lowest ORs for childhood HDM-AR who had exclusive BF in the first 4 months (a) or full-term birth (b), and the *IL18R1\_TT* significantly. There was no significant interaction in childhood HDM-AR with mode delivery (c) or passive smoking exposure in early life (d), and the *IL18R1\_TT* after adjusting for age, gender, parental education level, resident area per capita and family numbers in Figure 3c-d.



## Discussion

AR and other allergic diseases are complex immunological diseases caused by a combination of environmental and genetic factors. Recent GWAS have implicated that genetic components of allergic diseases involved in underlying immunological pathways.<sup>18,21</sup> Given the HDMs are the most important inhalant aeroallergens for perennial AR patients,<sup>22</sup> the candidate genes such as *EMSY-LRRC32*, HLA region (6p21), *IL18R1* and *IL18RAP*, *IL4* and *IL13*, and *KIF3A* (5q31) have been performed in patients with HDM-AR in our study. We found, for the first time, that rs2287037 (C.-93C>T) in the *IL18R1* promoter region was significantly associated with childhood AR induced by house dust mites. In addition, a validation study investigated the IL-18 pathway in HDM-AR.

So far, it is still unclear that specific or multiple genetic risk factor is responsible for the development of AR. Susceptibility genes for allergic disease have now been identified, suggesting there are common and distinct genetic loci associated with these diseases, which provides new insights into potential disease pathways and mechanisms including cytokines and their receptors.<sup>23</sup> Cytokines play a critical role in the widely used immunological model that explain the increasing prevalence of atopic diseases by an deviated balance of Th1/Th2 immune responses triggered by lifestyle-determined changes in environment.<sup>24</sup> IL-18 receptor itself is formed by a dimer of two subunits, IL-18R $\alpha$  and IL-18R $\beta$ , with the ligand-binding IL-18R $\alpha$  subunit being encoded by the *IL18R1* gene. IL18R $\alpha$  can bind *IL-18* with a high affinity by recruiting IL-18R $\beta$ . IL-18R $\alpha$  and IL-18R $\beta$  are expressed by a variety of cells including macrophages, T cells and natural killer (NK) cells and thought to be essential for IL-18 mediated signaling that results in the production of IFN- $\gamma$ .<sup>25</sup> IFN- $\gamma$  is a typical Th1 cytokine of decisive significance in regulation Th1/Th2 balance. SNPs have been identified in their potentially functional such as the coding sequences, exon-intron junctions, promoter, and untranslated regions. Recently, a three-base deletion (950del CAG) as one polymorphism has been reported in the promoter of the IL-18R $\alpha$  chain gene, which is generated by alternative splicing and is associated with reduced production of IFN- $\gamma$  in the Japanese population.<sup>26</sup> Various SNPs in *IL18R1* were associated with asthma and related traits.<sup>27-29</sup> In the present study, we found that children with TT genotype in *IL18R1\_rs2287037* had significantly lower prevalence of HDM-AR than those with the CT/CC genotypes. Our results suggested that individuals with the TT genotype has a protective role for HDM-AR, which was in accordance with the previous findings that rs2287037\*G were significantly associated as part of a haplotype block with bowel inflammation disease.<sup>30</sup> We postulate the rs2287037 which is located in the promoter region may modify *IL18R1* gene transcription and its functional effects relevant to HDM-AR. However, in haplotype analysis, the A allele of rs2287037 was shown to have significant association with bronchial hyperreactivity in European population.<sup>31</sup> Conversely, other genetic association studies have been reported not to detect significant association between *IL18R1* and different disease including atopic dermatitis,<sup>32</sup> coal workers' pneumoconiosis<sup>33</sup> and cardiovascular disease.<sup>34</sup> Inconsistent results of association studies may arise due to population stratification or true variability in genetic determinants

among different populations.<sup>31</sup> Additional molecular functional studies in our study should be performed to investigate the role of variants in *IL18R1* in the pathophysiology of HDM-AR. The SNP effect on gene expression may be due to decreased RNA stability by affecting microRNA-binding sites. The HDM-AR associated rs2287037 allele T permits hyper-responsiveness to IL-18 may be involved and, to a lesser extent, IL18 pathway in the development of HDM-AR, providing a novel target for therapeutic intervention in HDM-AR.

Furthermore, we further investigated the modulating effect of the *IL18R1\_rs2287037* on the association between early environmental exposure and the development of HDM-AR at school age. Previous studies have examined the effect of early-life environmental exposure on the development of allergic disease. Epidemiologic study has suggested that BF in the first 4 months, full-term birth may reduce the risk of development of AR.<sup>5</sup> In our study, BF in the first 4 months or full-term birth was found to significantly reduce the risk of HDM-AR in homozygous carriers of the minor allele, but there is no effect of exposure in heterozygous carriers of the minor allele or homozygous major allele carriers. The early-life exposure to BF in the first 4 months or full-term modulates the protective effect of the *IL18R1\_rs2287037* polymorphism on development of HDM-AR in children. The results of a stratified analysis for mode of delivery and passive smoking exposure did not change substantially. Allergy is believed to result from the combined effect of genes and environmental factors and their interactions. Similarly, the situation in which both the gene and the environment are each known to have a marginal effect on disease is illustrated by the interaction between the 17q21 locus and early-life exposure to environmental tobacco smoke in subjects with early-onset asthma.<sup>35</sup>

The present study has limitation of several analyses are based on relatively small number of subjects, which should be validated by additional studies using larger sample sizes. Given the limited statistical power of our study, these findings should be interpreted with caution before being replicated in independent HDM-AR.

In conclusion, the results of this study suggest that *IL18R1\_rs2287037*, may protect the schoolchildren from HDM-AR, and this effect may be modulated by early environmental exposure. This study supports the hypothesis that genes and environment may interact to influence the development of AR. Further studies are needed to confirm our findings and to elucidate the biological pathways influenced by *IL18R1* and early environmental exposure.

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## Competing interests

The authors have no financial relationships relevant to this article to disclose. The authors declare that they have no conflict of interest.

## Authors' contributions

Conceived and designed the experiments: YL, YJ, YL, XR, BM, JL. Performed the experiments: YL, YJ, XR, YL, BM. Analyzed the data: YL, YJ, JL, YL. Contributed reagents/materials/analysis tools: YL, YJ, XR, BM, JL. Wrote the paper: YL, YJ, XR, JL.

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**Table S1. Characteristics of 21 GWAS-related SNPs for association with HDM-AR**

Gene (Accession NO.) and Locus	SNP_ID (risk allele)	Chromosome position <sup>a</sup> (hg19)	Alleles A/B	Minor allele frequencies, %			P value for HWE test <sup>b</sup>			
				NCBI	HDM-AR	Control	ALL	HDM-AR	Control	
<i>EMSY-LRRC32</i> 11q13.5	rs2155219	76299194	T/G	0.438	0.409	0.437	0.3932	0.9511	0.3491	
	rs7130588	76270683	A/G	0.102	0.1	0.071	0.4233	0.3817	1	
	rs7927894	76301316	C/T	0.21	0.191	1	0.3256	0.1619	0.154	
<i>HLA region</i> 6p21.32	rs7775228	32658079	T/C	0.259	0.197	0.223	0.8756	1	0.7889	
<i>IL18R1</i> 2q12	rs2287037	102979028	C/T	0.404	0.316	0.393	1	0.3939	0.6327	
	rs3771166	102986222	G/A	0.135	0.169	0.135	0.7088	1	0.4068	
	rs1420099	102980543	C/G	0.150	0.169	0.135	0.7088	1	0.4068	
	rs1420098	102984279	T/C	0.271	0.316	0.393	0.357	0.3939	0.6327	
	rs1362348	102984624	C/G	0.135	0.169	0.135	0.7088	1	0.4068	
	rs1974675	102986375	G/A	0.107	0.169	0.135	0.7088	1	0.4068	
	rs1420094	103015687	C/T	0.148	0.169	0.132	0.2115	1	0.0589	
	<i>IL18RAP</i> 2q12	rs887971	103041167	T/C	0.344	0.316	0.393	0.334	0.8001	0.4305
		rs2058659	103054556	G/A	0.154	0.166	0.129	0.0746	1	0.0066
	<i>IL13</i> 5q31	rs20541	131995964	G/A	0.347	0.3	0.308	0.4415	0.502	0.8344
rs1800925		131992809	C/T	0.180	0.153	0.17	1	0.95	0.8484	
rs1881457		131992409	A/C	0.274	0.225	0.253	0.3629	0.8334	0.4276	
<i>KIF3A</i> 5q31	rs2897442	132049027	C/T	0.182	0.103	0.143	1	1	0.9691	
<i>IL4</i> 5q31	rs2227284	132012725	T/G	0.182	0.103	0.143	1	1	0.9691	
	rs2243290	132018169	A/C	0.261	0.153	0.176	0.3754	0.0969	1	
	rs2243250	132009154	T/C	0.261	0.153	0.184	0.7578	0.2679	0.7943	
	rs2070874	132009710	T/C	0.263	0.153	0.184	0.7578	0.2679	0.7943	

<sup>a</sup> SNP position in chromosome in the hg19 version of the UCSC Genome Browser database

<sup>b</sup> P values were calculated using Pearson's chi-square test

HDM-AR: house dust mite-induced allergic rhinitis; Alleles A/B: Minor/major alleles; HWE: Hardy-Weinberg equilibrium

**Table S2. Association between the genotype of 15 tSNPs identified in GWAS related to allergic disease and HDM-AR risk.**

SNP ID	Genotype	AR subjects (156)		Control subjects (173)		Crude OR (95%CI)	Adjusted OR <sup>a</sup> (95%CI)
		No.	%	No.	%		
rs2155219	TT	107	68.6	122	70.5	1.00	1.00
	TG	40	25.6	47	47.0	1.03 (0.63-1.69)	1.16 (0.69-1.95)
	GG	9	5.8	4	4.0	0.39 (0.12-1.30)	0.43 (0.12-1.50)
rs7130588	AA	56	35.9	54	33.4	1.00	1.00
	AG	79	50.6	91	52.6	1.20 (0.74-1.93)	1.19 (0.72-1.97)
	GG	21	13.5	28	16.2	1.38 (0.72-2.72)	0.24 (0.17-3.33)
rs7927894	CC	107	68.6	122	70.5	1.00	1.00
	CT	40	25.6	47	27.2	1.03 (0.63-1.69)	1.06 (0.63-1.78)
	TT	9	5.8	4	2.3	0.39 (0.12-1.30)	0.34 (0.10-1.18)

Table S2. (Continued)

SNP ID	Genotype	AR subjects (156)		Control subjects (173)		Crude OR (95%CI)	Adjusted OR* (95%CI)
		No.	%	No.	%		
rs7775228	TT	129	82.7	148	85.5	1.00	1.00
	TC	24	15.4	24	13.9	0.87 (0.47-1.61)	0.87 (0.46-1.65)
	CC	3	1.9	1	0.6	0.29 (0.03-2.83)	0.21 (0.02-2.17)
rs2287037	CC	67	42.9	64	37.0	1.00	1.000
	CT	75	48.1	81	46.8	1.13 (0.71-1.8)	1.18 (0.73-1.92)
	TT	14	9	28	16.2	0.49 (0.23-0.96)*	<b>0.44 (0.21-0.95)*</b>
rs1362348	CC	110	70.5	132	76.3	1.00	1.00
	CG	43	27.6	37	21.4	0.72 (0.43-1.19)	0.69 (0.41-1.17)
	GG	3	1.9	4	2.3	1.11 (0.24-5.07)	0.90 (0.19-4.25)
rs1420094	CC	75	48.1	66	38.2	1.00	1.00
	CT	64	41	80	46.2	1.42 (0.89-2.26)	1.48 (0.91-2.40)
	TT	17	10.9	27	15.6	1.81 (0.90-3.60)	1.78 (0.87-3.66)
rs887971	TT	75	48.1	66	38.2	1.00	1.00
	TC	64	41.0	80	46.2	1.42 (0.89-2.26)	1.48 (0.91-2.40)
	CC	17	10.9	27	15.6	1.81 (0.90-3.60)	1.78 (0.87-3.66)
rs20541	GG	75	48.1	81	46.8	1.00	1.00
	GA	68	43.6	77	44.5	1.05 (0.68-1.65)	0.99 (0.62-1.58)
	AA	13	8.3	15	8.7	1.07 (0.48-2.39)	0.77 (0.33-1.78)
rs1800925	CC	75	48.1	81	46.8	1.00	1.00
	CT	68	43.6	77	44.5	1.05 (0.67-1.65)	0.99 (0.62-1.58)
	TT	13	8.3	15	8.7	1.07 (0.48-2.39)	0.77 (0.33-1.78)
rs1881457	AA	110	70.5	119	68.8	1.00	1.00
	AC	41	26.3	48	27.7	1.08 (0.66-1.77)	1.00 (0.60-1.66)
	CC	5	3.2	6	3.5	1.11 (0.33-3.74)	0.79 (0.23-2.75)
rs2897442	CC	105	67.3	107	61.8	1.00	1.00
	CT	46	29.5	57	32.9	1.22 (0.76-1.95)	1.23 (0.76-2.01)
	TT	5	3.2	9	5.2	1.77 (0.57-5.45)	1.58 (0.50-5.00)
rs2227284	TT	127	81.1	127	73.4	1.00	1.00
	TG	27	17.3	43	24.9	1.59 (0.93-2.73)	1.66 (0.95-2.92)
	GG	2	1.3	3	1.7	1.50 (0.25-9.13)	1.64 (0.26-8.26)
s2243290	AA	116	74.4	116	67.1	1.00	1.00
	AC	33	21.2	52	30.1	1.58 (0.95-2.62)	1.63 (0.96-2.77)
	CC	7	4.5	5	2.9	0.71 (0.22-2.32)	0.67 (0.20-2.23)
rs2243250	TT	116	74.4	114	65.9	1.00	1.00
	TC	34	21.8	54	31.2	1.62 (0.99-2.67)	1.65 (0.98-2.78)
	CC	6	3.8	5	2.9	0.85 (0.25-2.86)	0.77 (0.22-2.66)

OR = odds ratio, CI = confidence interval

Bold\* indicates OR within 95%CI and statistically significant at P value less than 0.05

Adjusted for age, gender, parental education level, resident area per capita, family numbers

Table S3. Associations between common haplotypes and AR risk

Haplo-type <sup>a</sup>	Cases		Controls		Crude OR	P <sup>b</sup>	Adjusted OR	P <sup>c</sup>
	No.	Frequency (%)	No.	Frequency (%)	OR (95% CI)		OR (95% CI)	
Block1: rs7130588-rs2155219-rs7927894 at <i>EMSY-LRRC32</i>								
ATC	132	42.31	144	41.62	1.00 (referent)	-	1.00 (referent)	-
AGC	121	38.78	146	42.20	0.904 (0.645-1.267)	0.558	0.866 (0.610-1.228)	0.419
ATT	29	9.29	29	8.38	1.091 (0.619-1.922)	0.763	0.844 (0.459-1.553)	0.586
GTT	29	9.29	25	7.23	1.265 (0.705-2.271)	0.430	1.281 (0.70-2.345)	0.422
Block2: rs2287037-rs1420098-rs1362348-rs3771166-rs1974675 at <i>IL-18R1</i>								
CCTCGG	160	51.28	164	47.40	1.00 (referent)	-	-	-
TCCCGG	103	33.01	137	39.60	0.771 (0.551-1.078)	0.128	0.750 (0.529-1.063)	0.106
CGTGAA	49	15.71	45	13.01	1.116 (0.705-1.767)	0.639	1.171 (0.727-1.886)	0.517
Block4: rs1881457-rs1800925 at <i>IL-13</i>								
AC	238	76.28	258	74.57	1.00 (referent)	-	-	-
CT	51	16.35	60	17.34	0.921 (0.610-1.392)	0.698	1.029 (0.671-1.579)	0.895
CC	23	7.37	28	8.09	0.890 (0.449-1.589)	0.695	0.802 (0.433-1.486)	0.483
Block5: rs2243250-rs2070874-rs2227284-rs2243290 at <i>IL-4</i>								
TTTA	263	84.29	281	81.21	1.00 (referent)	-	1.00 (referent)	-
CCGC	31	9.94	47	13.58	0.705 (0.434-1.143)	0.156	0.682 (0.413-1.127)	0.135
CCTC	13	4.17	14	4.05	0.992 (0.458-2.150)	0.984	1.100 (0.497-2.435)	0.815

<sup>a</sup> Not available for the rarity of haplotype less than 0.05 for all the subjects

<sup>b</sup> P value from unconditional logistic regression analyses

<sup>c</sup> P value from unconditional logistic regression analyses, adjusted for age, gender, parental education level, resident area per capita, family numbers

Table S4. Results of logistic regression models of mode of delivery on HDM-AR stratified by genotype in genetic models.

SNP ID	Genotype	Mode of delivery			
		Vaginal		Cesarean	
		No. cases/controls	Adjusted <sup>1</sup> OR (95%CI)	No. cases/controls	Adjusted <sup>1</sup> OR (95%CI)
rs2287037					
Model <sup>1</sup>	CC	23/28 (40.4/40.6)	1	37/36 (48.7/34.6)	1
	CT	29/33 (50.9/47.8)	1.12 (0.51-2.47)	33/48 (43.4/46.2)	0.62 (0.32-1.20)
	TT	5/8 (8.8/11.6)	0.67 (0.18-2.51)	6/20 (7.9/19.2)	0.27 (0.10-0.78)*
Model <sup>2</sup>	CC+CT	52/61 (91.2/88.4)	1	70/84 (92.1/80.8)	
	TT	5/8 (8.8/11.6)	0.63 (0.18-2.21)	6/20 (7.9/19.2)	0.35 (0.13-0.95)*

Adjusted<sup>1</sup> for age, gender, parental education level, resident area per capita, family numbers

Model<sup>1</sup>: full genotype; Model<sup>2</sup>: recessive model

\* p < 0.05

**Table S5. Results of logistic regression models of mode of delivery on HDM-AR stratified by genotype in genetic models.**

SNP ID	Genotype	Secondhand smoke exposure			
		No		Yes	
		No. cases/controls	Adjusted <sup>1</sup> OR (95%CI)	No. cases/controls	Adjusted <sup>1</sup> OR (95%CI)
rs2287037					
Model <sup>1</sup>	CC	32/32 (47.1/35.6)	1	29/32 (44.6/38.6)	1
	CT	30/48 (44.1/53.3)	1.76 (0.88-3.55)	30/33 (46.2/39.8)	1.02 (0.49-2.11)
	TT	6/10 (8.8/11.1)	1.74 (0.53-5.73)	6/18 (9.2/21.7)	0.33 (0.11-0.97)*
Model <sup>2</sup>	CC+CT	62/80 (91.2/88.9)	1	59/65 (90.8/78.3)	1
	TT	6/10 (8.8/11.1)	0.78 (0.25-2.41)	6/18 (9.2/21.7)	0.32 (0.12-0.90)*

Adjusted<sup>1</sup> for age, gender, parental education level, resident area per capita, family numbers

Model<sup>1</sup>: full genotype; Model<sup>2</sup>: recessive model

\*  $p < 0.05$