# Combined effect of hygienic and polygenic risk scores in children with allergic rhinitis

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**Abstract**

**ABSTRACT Background** Although the development of allergic rhinitis (AR) is associated with multiple genetic and hygienic environmental factors, previous studies have focused mostly on the effect of a single factor on the development of AR. This study aimed to investigate the combined effect of multiple genetic and hygienic environmental risk factors on AR development in school children. **Methods** We conducted a cross-sectional study, comprising 1,797 children aged 9–12 years. Weighted environmental risk score (ERS) was calculated by using four hygienic environmental factors, including antibiotic use during infancy, cesarean section delivery, breast milk feeding, and having older siblings. Weighted polygenic risk score (PRS) was calculated by using four single nucleotide polymorphisms (SNPs), including interleukin-13 (rs20541), cluster of differentiation 14 (rs2569190), toll-like receptor 4 (rs1927911), and glutathione S-transferase P1 (rs1695). Multivariable logistic regression analysis was used. **Results** More than three courses of antibiotic use during infancy increased the risk of current AR (adjusted odd ratio [aOR], 2.058; 95% confidence interval [CI]: 1.290–3.284). Having older siblings, especially >2 (aOR, 0.526; 95% Cl: 0.303–0.913) had a protective effect. High ERS (>median; aOR, 2.079; 95% Cl: 1.466–2.947) and PRS (>median; aOR, 1.627; 95% Cl: 1.117–2.370) increased the risk of current AR independently. Furthermore, children who had both high ERS and PRS showed a higher risk of current AR (aOR, 3.176; 95% Cl: 1.787–5.645). **Conclusions** Exposure to multiple hygienic risk factors during early life increases the risk of AR in genetically susceptible children. **Key words**: Allergic rhinitis, Hygiene, Genes, Risk factors, Child, Gene-environment interaction, Anti-bacterial agent

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**Running title: Gene-environment interaction in AR**

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**Conflicts of interest**

The authors declare no conflicts of interest in relation to this study.

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**ABSTRACT**

**Background**

Although the development of allergic rhinitis (AR) is associated with multiple genetic and hygienic environmental factors, previous studies have focused mostly on the effect of a single factor on the development of AR. This study aimed to investigate the combined effect of multiple genetic and hygienic environmental risk factors on AR development in school children.

**Methods**

We conducted a cross-sectional study, comprising 1,797 children aged 9–12 years. Weighted environmental risk score (ERS) was calculated by using four hygienic environmental factors, including antibiotic use during infancy, cesarean section delivery, breast milk feeding, and having older siblings. Weighted polygenic risk score (PRS) was calculated by using four single nucleotide polymorphisms (SNPs), including interleukin-13 (rs20541), cluster of differentiation 14 (rs2569190), toll-like receptor 4 (rs1927911), and glutathione S-transferase P1 (rs1695). Multivariable logistic regression analysis was used.

**Results**

More than three courses of antibiotic use during infancy increased the risk of current AR (adjusted odd ratio [aOR], 2.058; 95% confidence interval [CI]: 1.290–3.284). Having older siblings, especially >2 (aOR, 0.526; 95% Cl: 0.303–0.913) had a protective effect. High ERS (>median; aOR, 2.079; 95% Cl: 1.466–2.947) and PRS (>median; aOR, 1.627; 95% Cl: 1.117–2.370) increased the risk of current AR independently. Furthermore, children who had both high ERS and PRS showed a higher risk of current AR (aOR, 3.176; 95% Cl: 1.787–5.645).

**Conclusions**

Exposure to multiple hygienic risk factors during early life increases the risk of AR in genetically susceptible children.

**Key words** : Allergic rhinitis, Hygiene, Genes, Risk factor\souts, Child, Gene-environment interaction, Anti-bacterial agent

## INTRODUCTION

Allergic rhinitis (AR) is an immunoglobulin E (IgE)-mediated inflammatory condition, which occurs in the nose with symptoms, such as nasal obstruction, watery rhinorrhea, itching, and sneezing. Following a global trend, in Korea, the prevalence of AR among children, adults, and the elderly has also increased rapidly over the past few decades.[1-3] Various environmental factors, such as indoor allergens, air pollution, exposure to certain drugs during pregnancy, and factors affecting the microbiome based on hygiene hypotheses have been mentioned as the causes of increase in AR.[4-7]

The number of siblings may affect the development of hay fever and eczema, which suggests that changes in hygienic environmental factors during early childhood may cause an increase in allergic diseases.[4,6-10]In the past few decades, in Korea, hygienic environmental factors have drastically changed due to rapid westernization and industrialization, and children have been exposed to various environments.[7,11]Therefore, it is appropriate to explain the development of allergic diseases as a result of the combined effects of various environmental factors, rather than one environmental factor.

Recently, attempts to identify the association between genetic factors and prevalence of AR have increased. In recent studies, the A allele of interleukin-13 (IL-13) single nucleotide polymorphism, rs20541, is associated with the risk of AR in Asians.[1,12]Additionally, we previously reported the association between AR and Toll-like receptor 4 (TLR4) (rs1927911)/cluster of differentiation 14 (CD14) (rs2569190), which are receptors that enable response to microbes or cause tissue damage.[13,14]Glutathione S-transferase P1 (GSTP1) (rs1695) modulates the effect of environment-induced respiratory symptoms in children.[15,16]Studies on various genes have revealed the possibility that the development of AR is affected by multiple genes.[1,13,17,18]However, there are few studies on the combined effect of multiple genes on the development of AR.

Studies on environmental risk score (ERS) have been initiated to identify the effect of various environmental factors on the risk of developing chronic diseases, such as cardiovascular disease and diabetes mellitus.[19,20]The concept of polygenic risk score (PRS) is based on the assumption that even though a single genetic variant has an insignificant effect on the development of chronic disease, a combination of multiple genetic variants may exert a polygenic effect that increases disease risk, including allergic diseases.[18,21,22]

However, there are no studies on gene-environment interactions using ERS and PRS in children with AR. Therefore, we investigated the combined effect of early life hygienic ERS and PRS on the development of AR in school-age children.

## MATERIALS AND METHODS

### Study population

In this study, a total of 1,797 children aged 9–12 years were recruited from Seoul and Jeongeup cities in Korea. Of the total children recruited, 351 who did not answer questions related to AR in the questionnaire were excluded from the analysis. Current AR patients were defined as children who were diagnosed with AR by the physician and had AR symptoms during the last 12 months based on the questionnaire. This study was conducted with approval from the institutional review boards of Hallym University and University of Ulsan College of Medicine and the principals of the children's school (IRB number: 2008-0208).

### Questionnaire data

The questions were excerpted from the International Study of Asthma and Allergies in Childhood (ISAAC) protocol. Demographic information, environmental factors, confounding factors, and the prevalence of diagnosis and symptoms of AR were evaluated by a questionnaire. The presence of AR was determined by answers to the following questions in the questionnaire: "has your child ever been diagnosed with AR by a physician?" and "has your child ever had any symptoms of sneezing, runny nose, or stuffy nose in the last 12 months without a cold or flu?" Antibiotic use during infancy, cesarian section delivery, breast feeding, and having older siblings were selected as environmental factors related to the risk of current AR.

### Single nucleotide polymorphism genotyping

Genomic DNA was obtained from the peripheral blood of the participants using the Gentra Puregene Blood kit (Qiagen Sciences, Germantown, MD, USA) with consent of the participant's parents. The genotyping of IL-13 (rs20541), CD14 (rs2569190), TLR4 (rs1927911), and GSTP1 (rs1695) SNPs was performed by TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA, USA), in accordance with the manufacturer's instructions. Duplicate samples and negative controls were included to ensure genotyping accuracy. Details are described in a previous study.[1,13,23]

### Calculation of ERS and genetic risk score

Environmental risk score was calculated using four environmental factors: antibiotic use during infancy, cesarean section delivery, breast milk feeding, and having older siblings. Weighted ERS was obtained using the following procedure: scores assigned to children according to the absence and presence of each environmental risk factor were multiplied by the crude odds ratio (OR) of each factor and then the sum of these scores was calculated and transformed in the natural log.

The PRS was calculated using four SNPs: IL-13 (rs20541, risk allele = A), CD14 (rs2569190, T), TLR4 (rs1927911, T), and GSTP1 (rs1695, G). The weighted PRS was calculated by the following procedure: the scores of each SNP were assigned to children according to the number of risk alleles carried by a child multiplied by the crude OR of each SNP for the risk of current AR, summed up, and transformed in a natural log.[24]

### Statistical analysis

All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). The Pearson's chi-square test and independent $t$ -test were used to compare the frequency and mean of categorical and continuous valuables, respectively, between children in current AR and control groups. Multivariate logistic regression analysis was used to calculate adjusted OR (aORs) and 95% confidence interval (CI) after adjusting for age, gender, body mass index (BMI) of children, exposure to tobacco smoke during early life, parental history of allergic disease, maternal education, parental income, and area of residence.

The ERS and PRS were divided into two groups, low and high, by median value, and the low group was used as a reference. Multiplicative interaction between ERS and PRS on current AR in children was calculated in the multivariate logistic regression model.

## RESULTS

### Population characteristics and prevalence of current AR

The prevalence of current AR was about 17% (246/1446 participants) (Table 1). In children with current AR, the proportion of males, urban dwellers, history of parental allergy, income of parents, and educational levels of parents were statistically significantly higher. The children with current AR took antibiotics more frequently during infancy and had fewer siblings.

### Association between hygienic environmental risk factors and current AR

The use of antibiotics during infancy (aOR, 1.569; 95% Cl, 1.108–2.222) and having older siblings (aOR, 0.660; 95% Cl, 0.478–0.910) were significantly associated with the current AR (Table 2). The risk of current AR was more than doubled in children who took antibiotics for [?]3 courses during infancy compared to children who did not (aOR, 2.058; 95% Cl, 1.290–3.284). In contrast, the risk was halved in children with two or more older siblings compared to children without older siblings (aOR, 0.526; 95% Cl, 0.303–0.913). However, cesarean section delivery and breastfeeding did not show statistically significant association with current AR (Table 2).

### Association between genetic risk factors and current AR

We analyzed the association between each polymorphism and development of current AR. However, each IL-13 (rs20541), CD14 (rs2569190), TLR4 (rs1927911), and GSTP1 (rs1695) polymorphism did not increase the risk of current AR (Table 3).

### Association between ERS or PRS and current AR

Children with high ERS had a higher risk of current AR (aOR, 2.079; 95% Cl, 1.466–2.947) compared to children with low ERS (Table 4). Children with high PRS also had a higher risk of current AR (aOR, 1.627; 95% Cl, 1.117–2.370) compared to children with low PRS.

### Combined effect of ERS and PRS in current AR

In the model designed to assess the combined effect of ERS and PRS, children with high ERS and PRS had a higher risk of current AR (aOR, 3.176; 95% Cl, 1.787–5.645) compared to children with low ERS and PRS (Table 5, $p$ for interaction = 0.119).

## DISCUSSION

Our study showed that antibiotic use during infancy increased the risk of AR in childhood, whereas the presence of older siblings had a protective effect on AR. The risk of AR increased more than twice when the ERS was high compared to when it was low. Furthermore, although each SNP, including IL-13 (rs20541), CD14 (rs2569190), TLR4 (rs1927911), and GSTP1 (rs1695) did not increase the risk of AR individually, a high PRS was associated with an increased risk of AR. This result suggests that polygenic effects generated by multiple SNPs may contribute to the development of AR. Above all, high ERS and PRS were not only independently associated with the risk of AR but also showed a combined effect on the risk of AR. These results suggest that the interaction of susceptibility of multiple genes and hygienic environmental risk factors might be associated with current AR, and can be applied to early detection of high-risk groups of school-age AR and prevention of school-age AR.

Antibiotic use in early childhood and the presence of older siblings are associated with allergic diseases. The risk of AR is increased in school children who used antibiotics in infancy and within two years after birth.[25,26]The use of antibiotics during early childhood and presence of older siblings has the potential to affect the distribution of gut bacteria and induce changes in early immune system formation.[9,27]This study

showed that antibiotic use had a dose-effect, an important hygienic environmental factor increased the risk of AR, and that AR risk was known to decrease as the number of older siblings increased.

Several SNPs have been mentioned as genetic factors affecting the development of AR, of which SNP rs20541 located in exon 4 of the IL-13 gene was found to be strongly associated with high levels of plasma IgE and AR development.[1,12,17]In our study, SNP rs20541 had a combined effect with ERS, and SNP rs20541 increased the risk of AR by interaction with mold exposure.[1] These results suggest that the SNP of the IL-13 gene is involved in the risk of AR by gene-environment interaction.

The CD14 gene, which is associated with the innate immune response and located on chromosome 5q31.3, encodes a protein that functions as a co-receptor for TLR and releases pro-inflammatory cytokines.[28] The effect of CD14 rs2569190 is conflicting and influenced by lipopolysaccharides- related factors and interactions with environmental microorganisms.[28] In our study, the TT genotype has a protective effect on the development of AR. A previous meta-analysis reported that CD14 SNP rs2569190 did not affect AR risk in Asians.[12] These results suggest that the CD14 gene alone does not increase the risk of AR, but has different effects on the risk of AR through interaction with various environmental factors.

Toll-like receptor 4 initiates the innate immune system when exposed to environmental factors, and antagonists of TLR4 have been shown to aggravate the symptoms of AR.[14] The CC genotype of TLR4 rs1927911 was associated with a higher risk of AR.[13] Our results are consistent with the results of previous studies in that the CC genotype of TLR4 rs1927911 alone did not increase the risk of AR.

Glutathione S-transferase P1 is the most common form of GST found in the respiratory tract lining fluid, and the GSTP1 genotype is known to be associated with the severity of airway dysfunction.[15,29]Although there have been no previous studies on the association between GSTP1 genotype and AR risk, several studies showed the association between GSTP1 and development of asthma.[15,16]In our study, the GSTP1 rs1695 SNP showed a weak association with increase in AR risk, but did not increase the AR risk alone.

Several studies have introduced the concept of ERS to identify the effects of multiple environmental factors on the development of allergic diseases in children. In a previous study, ERS was calculated with risk factors, such as cesarean delivery and antibiotics use during infancy, which showed that children with higher scores had a higher incidence of atopic AR at school age.[13] A recent study in Lebanon showed that children with higher scores based on risk factors, including environmental factors showed a higher frequency of allergic diseases, which is consistent with our study.[5]

Following studies on ERS, recent studies have been conducted on the effects of multiple genetic factors on the development of allergic diseases. A cohort study from Netherlands calculated a weighted PRS based on 10 SNPs associated with allergies in adults and showed that high PRS increased parental-reported allergy at 5 years of age and diagnosis of allergies in childhood by a physician.[18] From two birth cohorts with 135 SNPs, PRS was associated with an increased risk of atopic march, but having a weak association with allergic diseases characterized by the presence of a single symptom.[21] This suggests that multiple SNPs may influence multiple allergic comorbidities by unknown interactions. In our study, weighted PRS was calculated with four SNPs, which were related to the hygiene hypothesis. Children with high-weighted PRS had an increased risk of developing AR, suggesting that multiple SNPs may be linked to the development of allergic disease.

Our study has some limitations. First, information on antibiotic use in infancy may be biased because it is based on parents' memories after several years have passed. However, our study was designed to study the hygiene hypothesis, and several hygiene related environmental factors were investigated in detail. Second, AR was defined in the questionnaire without laboratory tests or skin prick tests. To supplement this, we used the definition of current AR, which reflects not only the symptoms but also the history of AR diagnosis by a doctor. Third, target SNP was selected with only four hygiene related candidate SNPs.[1,13,14]However, the selected SNPs have been associated with allergic diseases in our previous studies.[1,9,13,14]Further studies using genome-wide association study or prospective birth cohort will be needed. Lastly, this study had a relatively limited number of children in Korea; however, it is a general population-based study.

Our study has the following strengths. First, our study is meaningful in that it is a general population-based study. Finally, the ISAAC questionnaire was verified in many previous studies in Korea, and the response rate to the questionnaire was high at over 95% in this study.

In conclusion, polygenic susceptibility and exposure to multiple hygienic environmental risk factors during infancy increase the risk of AR at school age, which suggests gene-environment interaction. Therefore, it is necessary to decrease exposure to unhygienic environmental risk factors in infancy to prevent AR in school children, especially in susceptible children. Further studies are needed to elucidate the mechanism for this interaction between PRS and ERS that contributes to the development of AR

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**Key message:**

Avoiding exposure to multiple hygienic risk factors during early life might be helpful to prevent the development of allergic rhinitis in children with genetic susceptibility.

**Ethical approval**

This study was conducted with approval from the institutional review boards of Hallym University and the University of Ulsan College of Medicine and the principals of the children's school (IRB number: 2008-0208). Written informed consent was obtained from the parents of all the children prior to study initiation. The obtainment of consent was confirmed by the IRB.

**Authorship:** Choi EJ, Lee SY, and Hong SJ designed and wrote the manuscript and performed the analyses. Kun Baek Song, Eun Young Baek, Min Ji Park, Jisun Yoon, Sungsu Jung, Si Hyeon Lee, Mi-Jin Kang, Hea Young Oh, and Kang Seo Park participated in the collection, analysis, and interpretation of the data. Hong SJ and Lee SY supervised the execution of the study.

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| | Current AR (n=246) | Controls (n=1200) | *P*- value |
|---|---|---|---|
| Age (year) | 11.10 ± 0.89 | 11.10 ± 0.87 | 0.942 |
| Male | 140/246 (56.9%) | 584/1198 (48.7%) | 0.020 |
| BMI (kg/m$^2$) | 19.41 ± 3.50 | 19.01 ± 3.23 | 0.084 |
| Urban dweller (Seoul) | 141/246 (57.3%) | 438/1200 (36.5%) | 0.000 |
| Parental history of allergic diseases | 117/239 (49.0%) | 240/1168 (20.5%) | 0.000 |
| Parental income (Korean Won/month) | | | 0.000 |
| Low ([?]2,900,000) | 39/230 (17.0%) | 307/1106 (27.8%) | |
| Middle (3,000,000-4,900,000) | 163/230 (70.9%) | 725/1106 (65.6%) | |
| High ([?]5,000,000) | 28/230 (12.2%) | 74/1106 (6.7%) | |
| Highly educated mother (college graduates) | 145/241 (60.2%) | 526/1124 (46.8%) | 0.000 |
| Exposure to tobacco smoke | 101/241 (41.9%) | 541/1168 (46.3%) | 0.211 |
| Antibiotic use during infancy | | | 0.000 |
| No use | 157/246 (63.8%) | 924/1166 (79.2%) | |
| Use | 89/246 (36.2%) | 242/1166 (20.8%) | |
| 1–2 course(s) | 41/244 (16.8%) | 146/1159 (12.6%) | ?¿? |
| 3 courses | 46/244 (18.9%) | 89/1159 (7.7%) | |
| Cesarean section delivery | 84/244 (34.4%) | 393/1171 (33.6%) | 0.795 |
| Breast milk feeding | | | 0.567 |
| No | 105/245 (42.9%) | 484/1184 (40.9%) | |
| Yes | 140/245 (57.1%) | 700/1184 (59.1%) | |
| During <6 months | 74/234 (31.6%) | 311/1117 (27.8%) | |
| During [?]6 months | 64/234 (27.4%) | 370/1117 (33.1%) | |
| Having older siblings | | | 0.000 |
| No siblings | 124/242 (51.2%) | 439/1182 (37.1%) | |
| Yes | 118/242 (48.8%) | 743/1182 (62.9%) | |
| Only one | 92/242 (38.0%) | 476/1182 (40.3%) | |
| Two or more | 26/242 (10.7%) | 267/1182 (22.6%) | |

Table 1. Demographics of the current AR and control groups

Abbreviations: AR, Allergic rhinitis; BMI, Body mass index; SD, standard deviation

Data presented as mean ± SD (range) or number (%).

*P* -values for comparing the current AR and control groups were calculated using the Pearson's chi-square test or independent$t$ -test, as appropriate.

Table 2. Effect of hygienic environmental risk factors on current AR

| Risk factors | Current AR | Current AR | Current AR |
|---|---|---|---|
| | aOR | 95% Cl | *P*-value |
| Antibiotic use during infancy (>3days) | | | |
| Use (n=273/1158) | 1.569 | 1.108–2.222 | 0.011 |
| No use (n=885/1152) | Reference | Reference | Reference |
| 1–2 course(s) (n=151/1152) | 1.181 | 0.741–1.881 | 0.484?¿? |
| 3 course (n=116/1152) | 2.058 | 1.290–3.284 | 0.002 |
| Cesarean section delivery (n=391/1163) | 0.974 | 0.692–1.370 | 0.878 |

| Risk factors | Current AR | Current AR | Current AR |
|---|---|---|---|
| Breast milk feeding | | | |
| Yes (n=696/1168) | 0.976 | 0.704-1.353 | 0.883 |
| No (n=423/1105) | Reference | Reference | Reference |
| During <6 months (n=326/1105) | 1.095 | 0.741–1.618 | 0.648 |
| During [?]6 months (n=356/1105) | 0.855 | 0.569–1.283 | 0.448 |
| Having older siblings | | | |
| Yes (n=684/1159) | 0.660 | 0.478–0.910 | 0.011 |
| None (n=475/1159) | Reference | Reference | Reference |
| Only one (n=461/1159) | 0.713 | 0.501–1.013 | 0.059 |
| Two or more (n=223/1159) | 0.526 | 0.303–0.913 | 0.022 |

Abbreviations: AR, Allergic rhinitis; BMI, Body mass index; aOR, adjusted odds ratio; CI, confidence interval.

Adjusted odds ratios and 95% CIs obtained from multivariate logistic regression analysis adjusted for sex, BMI, environmental tobacco smoke history of children, parental history of allergy, education of mother, parental income, and region.

Table 3. Effect of genetic risk factors on current AR

| Risk genes | Genotypes | Current AR | Current AR | Current AR |
|---|---|---|---|---|
| | | Number (+/-) | aOR (95% Cl) | *P*-value |
| IL13 | GG | 82/371 | 1 | |
| | AA, AG | 88/373 | 1.254 (0.862–1.824) | 0.237 |
| CD14 | CC, CT | 106/446 | 1 | |
| | TT | 66/298 | 0.938 (0.639–1.378) | 0.744 |
| TLR4 | CC | 54/277 | 1 | |
| | TT, CT | 107/460 | 1.261 (0.852–1.866) | 0.247 |
| GSTP1 | AA | 111/546 | 1 | |
| | GG, AG | 62/248 | 1.427 (0.966–2.106) | 0.074 |

Abbreviations: AR, Allergic rhinitis; BMI, Body mass index; aOR, adjusted odds ratio; CI, confidence interval; IL13, Interleukin-13; CD14, cluster of differentiation 14; TLR4, Toll-like receptor 4; GSTP1, Glutathione S-transferase P1.

Adjusted odds ratios and 95% CIs obtained from multivariate logistic regression analysis adjusted for sex, BMI, environmental tobacco smoke history of children, parental history of allergy, education of mother, parental income, and region.

Table 4. Risk of current AR, depending on weighted ERS and PRS

| Score | Score | Current AR | Current AR | Current AR |
|---|---|---|---|---|
| | | Number (+/-) | aOR (95% Cl) | *P*-value |
| Weighted ERS | Low | 57/460 | 1 | |
| | High | 150/467 | 2.079 (1.466–2.947) | 0.000 |
| Weighted PRS | Low | 69/364 | 1 | |
| | High | 83/329 | 1.627 (1.117–2.370) | 0.011 |

Abbreviations: AR, Allergic rhinitis; aOR, adjusted odds ratio; CI, confidence interval; ERS, environmental risk score; PRS, Polygenic risk score.

Adjusted odds ratios and 95% CIs obtained from multivariate logistic regression analysis adjusted for sex, BMI, environmental tobacco smoke history of children, parental history of allergy, education of mother, parental income, and region.

Table 5. Combined effect of weighted ERS and PRS on the risk of current AR

| Weighted ERS | Weighted PRS | Current AR | Current AR | Current AR |
|---|---|---|---|---|
| | | Number (+/-) | aOR (95% Cl) | *P*-value |
| Low | Low | 20/168 | 1 | |
| Low | High | 21/171 | 1.227 (0.629–2.394) | 0.548 |
| High | Low | 48/183 | 1.704 (0.942–3.085) | 0.078 |
| High | High | 62/149 | 3.176 (1.787–5.645) | 0.000 |

Interaction $P$ :0.119

Abbreviations: AR, Allergic rhinitis; aOR, adjusted odds ratio; CI, confidence interval; ERS, environmental risk score; PRS, Polygenic risk score.

Adjusted odds ratios and 95% CIs obtained from multivariate logistic regression analysis adjusted for sex, BMI, environmental tobacco smoke history of children, parental history of allergy, education of mother, parental income, and region.