Interaction between early-life pet exposure and methylation pattern of ADAM33 on Allergic Rhinitis among a cohort of kindergartens in China

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Research

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Abstract

Background

Recent research has pointed out the important role of epigenetic modifications in the development and persistence of Allergic Rhinitis (AR), especially DNA methylation. We investigated whether AR susceptibility genes are epigenetically regulated, and whether methylation modulation of these genes in response to early-life environment could be a molecular mechanism underlying the risk of AR in a cohort of kindergartens in China.

Methods

Peripheral blood mononuclear cell (PBMCs) samples were collected from 130 kindergarten patients, aged 3–6 years with AR and 154 matched healthy controls to detect promoter methylation in 25 AR susceptibility genes with the MethylTarget approach. Methylation level was compared for each CpG site, each amplified region, and each gene. In addition, the relationship among DNA methylation, early-life environment risk factors and AR were assessed.

Results

Maternal allergic history (P = 0.0390) and exposure to pet (P = 0.0339) were significantly associated with increase AR risk. Differential methylation analyses were successfully performed for 507 CpG sites, 34 amplified regions and 17 genes and significant hypomethylation was observed in the promoter region of ADAM33 in AR patients [multiple test-corrected (FDR) P-value < 0.05]. Spearman correlation analysis revealed that the hypermethylation of ADAM33 was significantly associated with lower serum total eosinophil counts (Spearman's $\rho$: -0.187, P-value = 0.037). According to the results of the multiple regression analysis, after adjusting for cofounders, the interaction of early-life pet exposure and methylation level of ADAM33 increased the risk of AR 1.423 times in kindergartens (95% CI = 0.0290–4.109, P-value = 0.005).

Conclusion

This study provides evidence that early-life pet exposure and low methylation level of ADAM33 increase AR risk in kindergartens, and the interaction between pet exposure and methylation level of ADAM33 may play an important role in the development of AR.

Background

Allergic rhinitis (AR) is an common IgE-mediated disorder involving troublesome symptoms of nasal congestion, nasal itch, sneezing, and associated eye symptoms(1). As AR is a multifactorial disease triggered by genetic and environmental factors, as well as their interaction, classical genetic association studies including genome-wide association studies (GWASs) was unable to explain the missing heritability as well as such high and still increasing prevalence of AR (2–5).

Considering the dramatic increase in the prevalence of AR(6), the epigenetic modification may be important genetic factor to better understand the environmental effects on allergic diseases. DNA methylation refers to the addition of a methyl group to DNA, which plays a crucial role in controlling gene expression in the genome. In recent genome-wide profiling of DNA methylation in T-cells, DNA methylation profiles clearly and robustly distinguished AR patients from controls (7). Zhang et al modeled differences in genome-wide DNA methylation and allergic sensitization during adolescence and found that DNA methylation at cg10159529 was associated with AR and strongly correlated with expression of IL5RA(8). Methylation modulation of several candidate genes were also reported to have important role in AR developing(9).
Like many chronic health conditions, AR are complex and stem from complex gene-environment interactions(10). Recent studies have supported a relationship between external exposome in the prenatal and early-life risk factors and their effects on the development of allergic diseases later in life(11). The association of these risk factors and the subsequent development of AR with focuses on maternal allergic history (12), mode of delivery (13), the microbial exposure (14, 15), indoor allergens (furred pet exposure, for example) (16), and environmental air pollutants (17) during early-life have been previously reported. One mechanism underlying the effect of air pollutants on AR using mice model has been reported recently, showed that PM2.5 exposure exacerbated AR by increasing DNA methylation in the IFN-gamma gene promoter in T cells (18).

Considering the established role of DNA methylation and the important effect of the early-life environment on the development of AR, we conducted a cross-sectional study to explore the association between early-life environment risk factors, methylation of AR susceptibility genes and AR risk in a population of Chinese kindergartens.

Methods

Subjects and DNA Specimens

This study was approved by the Ethics Committee of Tongren Hospital Affiliated to Shanghai JiaoTong University, School of Medicine (NO: TR2019.050.01). Written informed consent was obtained from all legal guardians prior to blood collection. A total of 130 kindergarten patients with AR were recruited from the Department of Otorhinolaryngology. The control population comprised 154 healthy children undergoing a regular physical examination. Individuals with history of asthma or atopic dermatitis were excluded. All subjects were born and long-term residents in Shanghai. Genomic DNA extraction was performed on PBMC samples collected and isolated by centrifugation using the QIAmap DNA Blood kit (QIAGEN, German), according to the manufacturer's instructions. Serum total eosinophil counts were measured using an XN-9000 (Sysmex Co., Kobe, Japan).

Clinical Diagnoses

According to the Initiative on Allergic Rhinitis and its Impact on Asthma guidelines, a thorough history that included typical AR symptoms, physical examination and an allergen skin prick test [SPT] were used to establishing the diagnosis of AR(19). SPT was performed by trained practitioners and positivity was defined as described elsewhere(20). Children patients recruited were carrying classic AR symptoms and positive SPT, and patients with comorbid asthma were excluded by lung function and bronchial provocation testing.

Questionnaire survey

A questionnaire was answered by mother of all of the study subjects, and the following variables were recorded: gender, weight, height, history of maternal allergic disease, season of birth (March to August was defined as spring-summer; September to February was defined as autumn-winter), secondhand smoke exposure (yes or no), and pet exposure (yes or no) in the child's home before their kindergarten life.

Selection of AR-associated genes

Top twenty-five AR-susceptibility genes were collected using Phenopedia database (https://phgkb.cdc.gov/PHGKB/startPagePhenoPedia.action). Genes were ranked according to the number of previously published gene–disease association studies, thus providing a disease-centered view of genes involved in AR(21).

DNA methylation analysis

DNA methylation level was analysis by an multiplex PCR and next-generation sequencing-based Targeted CpG methylation analysis method—MethylTarget™ (Genesky Biotechnologies Inc., Shanghai, China). The validity and reliability of this
method has been previously reported (22–24). Specifically, CpG islands located in the promoter of genes of interest were selected according to the following criteria: (1) 200 bp minimum length; (2) above 50% GC content; (3) above 0.6 ratio of observed/expected CpG. Sodium bisulfite conversion of DNA was performed using EZ DNA Methylation™-GOLD Kit (Zymo Research), following the manufacturer’s protocols. Primers were designed and provided by Genesky Company and multiplex PCR was performed. After PCR amplification (HotStarTaq polymerase kit, TAKARA, Tokyo, Japan) and library construction, samples were sequenced (Illumina MiSeq Benchtop Sequencer, CA, USA) using the paired-end sequencing protocol according to the manufacturer’s guidelines.

One gene on X-chromosome and seven genes without CpG islands or failed to be amplified were excluded from following analysis. In total, 34 amplicons of CpG regions in the promoter of 17 genes were sequenced (the detailed information related with gene names, location of the amplicons, amplification primers, and product size can be found in Additional file 1). All samples achieved a mean coverage of > 800 X and no significant difference of bisulfite conversion efficiency was identified between groups (Additional file 2). Methylation level at each CpG site was calculated as the percentage of the methylated cytosines over the total tested cytosines. The average methylation level of all measured CpG sites within the amplified region or the gene was used for identifying differentially methylated amplicons and genes.

**Statistical analysis**

The data were analyzed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). For basic characteristics and potential risk factors, the differences between groups were measured using the χ2 test for categorical variables or t tests for continuous variables. Mann-Whitney U test was used to compare methylation levels of the AR-associated genes between AR patients and normal controls. Spearman correlation test was used to evaluate the relationship among study variables in AR patients. Receiver operating characteristic (ROC) curve and area under curve (AUC) were used to evaluate the predictive power or feasibility of the methylation as a biomarker for AR. False discovery rate (FDR) was applied for the multiple test correction. Associations were considered significant when P values were less than 0.05.

**Results**

Demographic data and clinical manifestations

A total of 130 patients with AR (78 boys, 52 girls) and 154 controls (98 boys, 56 girls) were recruited. No statistically significant differences were found between cases and controls in terms of sex, age, weight, and height (all P-value > 0.05). Maternal allergic history (P = 0.0390) and exposed to pet (P = 0.0339) significantly increased the risk of developing AR. However, no effects were found of season of birth or exposed to second-hand smoke on AR risk. The demographic details of the sample are given in Table 1.
Table 1
Characteristics of study participants

| Variable                        | AR       | control  | P-value |
|---------------------------------|----------|----------|---------|
| N                               | 130      | 154      |         |
| Male (%)                        | 78 (60.00)| 98 (63.64)| 0.532  |
| Age (SD), month                 | 53.72 (11.04)| 52.14 (10.11)| 0.209   |
| Weight (SD), kg                 | 19.27 (1.76)| 18.25 (1.77)| 0.0800  |
| Height (SD), mm                 | 105.81 (4.98)| 106.09 (5.07)| 0.581   |
| Maternal allergic history (%)   | 75 (57.69)| 70 (45.45)| 0.0390  |
| Season of birth                 |          |          |         |
| Spring-summer                   | 62       | 77       | 0.698   |
| Autumn-winter                   | 68       | 77       |         |
| Exposed to pet (%)              | 49 (37.69)| 40 (25.97)| 0.0339  |
| Exposed to second-hand smoke (%)| 59 (45.38)| 66 (42.86)| 0.669   |
| Serum total eosinophil counts (cells/mL³) | 372.35 (108.02) | | |

Differentially Methylated Sites, Amplicons, And Genes

The result showed that 34 amplicons contained 507 CpG sites in promoter region of the 17 AR-susceptibility genes were sequenced (one to three amplicons for each gene, detailed information can be found in Additional file 1). To better characterize the DNA methylation of the 17 AR-susceptibility genes, differential methylation analyses were performed for the 507 CpG sites, 34 regions and 17 genes, respectively. The results showed that 55 of 520 CpG sites, all located on gene ACE or gene ADAM33, were differently methylated in AR patients compared to controls (all P < 0.05) (Additional file 3). However, the CpG site at the position of 24 bp of the first amplicon of ACE (ACE_1) (Fig. 1) was the only CpG site remained significant after correcting for multiple testing (FDR P = 0.0337).

As shown in Table 2, three amplicons of the CpG regions (GSTP1_1, ADAM33_1 and KCNE4_3) were differently methylated in AR patients compared to healthy controls (all P < 0.05). Locations of each amplicon were shown in Fig. 1. The difference of GSTP1_1 and ADAM33_1 was still significant after correcting for multiple testing (FDR P = 0.04833). In addition, we evaluate the differences between AR cases and controls in the DNA methylation levels of genes. The results found that the DNA methylation levels of ADAM33 and GSTP1 genes were significantly different between AR patients and controls (all P < 0.05). The differences were still significant after correcting for multiple testing (P = 0.0483) (Table 3). Since there were no significant methylation difference for all the CpG sites in GSTP1, ADAM33 was selected for following analysis. The methylation levels of promoter regions in ADAM33 in AR group and control group was shown in Fig. 1.
Table 2
Differentially methylated amplicons of CpG region between AR samples and control samples.

| Target/Gene | Mean in AR | Mean in control | MethylDiff | P-value (U test) | FDR P-value (U test) |
|-------------|------------|----------------|------------|-----------------|---------------------|
| GSTP1_1     | 0.30953    | 0.31456        | -0.00502   | 0.00142         | 0.0483              |
| ADAM33_1    | 0.37788    | 0.38639        | -0.00852   | 0.00261         | 0.0483              |
| KCNE4_3     | 0.91735    | 0.90976        | 0.00759    | 0.04671         | 0.3729              |

Target, the name of the amplicon; Mean in AR, average methylation degree of the AR group; Mean in control, average methylation degree of the control group; MethylDiff, average methylation degree of the AR group minus average methylation degree of the control group; P value (U-test): the U-test model is used to calculate the P value.

Table 3
Differentially methylated genes between AR samples and control samples.

| Gene      | Mean in AR | Mean in control | MethylDiff | P-value (U-test) | FDR P-value (U-test) |
|-----------|------------|-----------------|------------|-----------------|---------------------|
| ADAM33    | 0.20249    | 0.20783         | -0.00534   | 0.00243         | 0.0483              |
| GSTP1     | 0.10541    | 0.10323         | 0.00218    | 0.00424         | 0.0483              |

Mean in AR, average methylation degree of the AR group; Mean in control, average methylation degree of the control group; MethylDiff, average methylation degree of the AR group minus average methylation degree of the control group; P value (U-test): the U-test model is used to calculate the P value.

Differentially methylated CpG sites in ADAM33

To evaluate the potentiality of the CpG sites as a biomarker for AR, ROC curve analysis was performed on all the CpG sites in ADAM33 gene. Mean methylation level and the AUC of ROC curve of each CpG site was shown in Fig. 3 and Table 4, the highest AUC was 0.6233 for the CG site at position 66 bp of the sequencing region of ADAM33_2. Four CpG sites (CpG sites at position of 45 bp, 85 bp, 87 bp and 89 bp of ADAM33_1 had mean methylation level difference above 0.01 in AR and controls.
Table 4
Methylation sites in promoter region of ADAM33

| Target   | Position | Type | P-value (U-test) | FDR P-value (Utest) | OR(L95–U95) (Logistic) | AUC (Logistic) | MethylDiff | Mean in AR | Mean in control |
|----------|----------|------|-----------------|---------------------|------------------------|------------------|------------|-----------|---------------|
| ADAM33_1 | 45       | CG   | 0.052           | 0.475               | 0.9761(0.9549–0.9978)  | 0.592            | -0.05618   | 0.21179   | 0.26797       |
| ADAM33_1 | 85       | CG   | **0.040**       | 0.456               | 0.9567(0.9024–1.0143)  | 0.598            | -0.01416   | 0.73703   | 0.75119       |
| ADAM33_1 | 87       | CG   | 0.055           | 0.475               | 1.0328(0.995–1.072)    | 0.619            | -0.04202   | 0.77197   | 0.81399       |
| ADAM33_1 | 89       | CG   | **0.049**       | 0.475               | 1.009(0.9988–1.0193)   | 0.593            | -0.09300   | 0.35000   | 0.44300       |
| ADAM33_1 | 109      | CG   | 0.057           | 0.475               | 0.8988(0.8054–1.003)   | 0.590            | -0.00904   | 0.46346   | 0.47250       |
| ADAM33_1 | 131      | CG   | **0.047**       | 0.462               | 0.9215(0.8309–1.022)   | 0.594            | -0.00761   | 0.46077   | 0.46838       |
| ADAM33_1 | 148      | CG   | **0.046**       | 0.462               | 0.9051(0.7932–1.0328)  | 0.594            | -0.00564   | 0.33127   | 0.33692       |
| ADAM33_1 | 171      | CG   | **0.044**       | 0.459               | 0.9589(0.8777–1.0477)  | 0.595            | -0.00518   | 0.33279   | 0.33797       |
| ADAM33_1 | 173      | CG   | **0.042**       | 0.459               | 0.6178(0.4049–0.9426)  | 0.603            | -0.00498   | 0.00858   | 0.01357       |
| ADAM33_1 | 176      | CG   | 0.053           | 0.475               | 0.8126(0.6548–1.0083)  | 0.592            | -0.00456   | 0.05248   | 0.05704       |
| ADAM33_2 | 26       | CG   | **0.045**       | 0.460               | 0.5744(0.3513–0.9392)  | 0.601            | -0.00304   | 0.00577   | 0.00881       |
| ADAM33_2 | 30       | CG   | 0.051           | 0.475               | 0.5615(0.3543–0.8898)  | 0.593            | -0.00301   | 0.01210   | 0.01512       |
| ADAM33_2 | 38       | CG   | **0.040**       | 0.456               | 0.7737(0.5536–1.0813)  | 0.597            | -0.00231   | 0.01141   | 0.01372       |
| ADAM33_2 | 46       | CG   | **0.044**       | 0.459               | 0.4853(0.243–0.9691)   | 0.598            | -0.00222   | 0.00628   | 0.00851       |
| ADAM33_2 | 51       | CG   | 0.053           | 0.475               | 0.5236(0.2937–0.9335)  | 0.592            | -0.00207   | 0.01878   | 0.02086       |
| ADAM33_2 | 56       | CG   | **0.039**       | 0.456               | 0.7242(0.4734–1.108)   | 0.597            | -0.00181   | 0.00791   | 0.00972       |
| ADAM33_2 | 66       | CG   | 0.058           | 0.475               | 0.1676(0.0344–0.8158)  | 0.623            | -0.00162   | 0.00684   | 0.00846       |
| ADAM33_2 | 69       | CG   | 0.053           | 0.475               | 0.8777(0.6093–1.2642)  | 0.592            | -0.00098   | 0.00792   | 0.00889       |

Target, the name of the amplicon; POS, the specific location of the methylation site in the amplicon; P value (U-test): the U-test model is used to calculate the P value; OR (L95–U95) (Logistic), AUC (Logistic), odds ratio and area under curve was calculated through the logistic regression model; Mean in AR, average methylation degree of the AR group; Mean in control, average methylation degree of the control group; MethylDiff, average methylation degree of the AR group minus average methylation degree of the control group;
| Target    | Position | Type | P-value (U-test) | FDR P-value (U-test) | OR(L95-U95) (Logistic) | AUC (Logistic) | MethylDiff | Mean in AR | Mean in control |
|-----------|----------|------|-----------------|---------------------|------------------------|-----------------|------------|-----------|----------------|
| ADAM33_2  | 109      | CG   | 0.041           | 0.458               | 0.122(0.0229–0.6488)   | 0.597           | -0.00087   | 0.00635   | 0.00721       |
| ADAM33_2  | 132      | CG   | 0.038           | 0.456               | 0.7905(0.4372–1.4293)  | 0.601           | -0.00071   | 0.00693   | 0.00764       |
| ADAM33_2  | 134      | CG   | 0.058           | 0.475               | 0.4438(0.1491–1.3204)  | 0.590           | -0.00070   | 0.00511   | 0.00582       |
| ADAM33_2  | 137      | CG   | 0.055           | 0.475               | 1.5811(1.081–2.3123)   | 0.597           | 0.00475    | 0.01382   | 0.00908       |

Target, the name of the amplicon; POS, the specific location of the methylation site in the amplicon; P value (U-test): the U-test model is used to calculate the P value; OR (L95–U95) (Logistic), AUC (Logistic), odds ratio and area under curve was calculated through the logistic regression model; Mean in AR, average methylation degree of the AR group; Mean in control, average methylation degree of the control group; MethylDiff, average methylation degree of the AR group minus average methylation degree of the control group;

**Correlation of ADAM33 methylation with clinical manifestations**

The serum eosinophil count (normal range: 50–500 cells/mL) was 372.35 ± 108.02 cells/mL in AR patients. Spearman correlation analysis revealed that the hypermethylation of ADAM33 was significantly associated with lower serum total eosinophil counts (Spearman's $\rho$ = -0.187, $P$ = 0.037; Fig. 4).

**Risk factor on ADAM33 promoter methylation levels in the AR and control groups**

We compared the mean ADAM33 promoter methylation levels between the AR and control groups stratified by maternal allergic history and exposure to pet, two risk factors identified in this study (Table 5). In the AR group, children have a daily life exposure to pet had significantly lower methylation levels compared to those without pet exposure ($P$-value = 0.009). The difference in control group was not significant. Impact of Maternal allergic history on methylation level of ADAM33 was not found in both AR and control group.
Table 5

ADAM33 promoter methylation levels between the AR and control groups stratified by environmental risk factors

| Variable                   | AR       | Mean ADAM33 promoter methylation levels | Control  | Mean ADAM33 promoter methylation levels |
|----------------------------|----------|----------------------------------------|----------|----------------------------------------|
|                            | N        |                                        |          |                                        |
| Maternal allergic history  | 130      | 0.2018 ± 0.008417                       | 154      | 0.2073 ± 0.01042                       |
| YES                       | 75 (57.69) | 0.2018 ± 0.008417                       | 70 (45.45)| 0.2073 ± 0.01042                       |
| NO                        | 55 (42.31)| 0.2029 ± 0.007894                       | 84 (54.55)| 0.2081 ± 0.01130                       |
| P-value                   | 0.346    |                                        | 0.897    |                                        |
| Exposed to pet            |          |                                        |          |                                        |
| YES                       | 49 (37.69)| 0.2012 ± 0.008346                       | 40 (25.97)| 0.2098 ± 0.01172                       |
| NO                        | 81 (62.31)| 0.2044 ± 0.007349                       | 114 (74.03)| 0.2062 ± 0.01015                       |
| P-value                   | 0.009    |                                        | 0.290    |                                        |

Values are presented as means ± SD or numbers. a Comparison between AR and control groups by Mann–Whitney U test. Significant results are in bold.

According to the results of the multiple regression analysis, adjusting for gender, age, height, weight, season of birth and exposed to second-hand smoke, exposed to pet was significantly related to higher risk of developing AR. Furthermore, the interaction of exposed to pet and methylation level of ADAM33 was significantly related to AR risk (OR = 1.423, 95% CI = 0.0290–4.109, P-value = 0.005) (Table 6).

Table 6

Multiple regression analysis for analyzing the relationship among risk factors, the methylation level of ADAM33 and AR, AR as the dependent variable

| Risk factor                        | β       | OR (95% CI)               | P-value |
|------------------------------------|---------|---------------------------|---------|
| Maternal allergic history a        | 0.561   | 1.752(0.817–3.761)        | 0.150   |
| Exposed to pet a                   | 0.721   | 2.057 (1.029–4.109)       | 0.001   |
| Methylation level b                | -0.225  | 0.799 (0.645–1.006)       | 0.114   |
| Methylation level × Exposed to pet | 0.353   | 1.423 (1.007–1.639)       | 0.005   |
| Methylation level × Maternal allergic history | -0.212 | 0.809 (0.699–1.105) | 0.169 |

Note: a 0 = without, 1 = with. B methylation levels were rescaled to rank.

OR: odds ratio; CI: confidence interval.

Adjusted for gender, age, height, weight, season of birth and exposure to second-hand smoke.

Discussion

The aim of the study was to investigate the relationship among environmental risk factors, the methylation level of AR candidate genes reported from polymorphism association studies and AR risk in a cohort of kindergartens in China. We
found that among 17 investigated genes, the DNA methylation levels of ADAM33 was significantly lower in the AR group than controls and the difference was still significant after correcting for multiple testing. Furthermore, we showed that exposed to pet was related to higher risk of AR interacting with DNA methylation level in promoter region of ADAM33.

In our study, maternal allergic history was a strong risk factor of AD among a cohort of Chinese kindergartens. This result is consistent with that of a previous study involving children at 6 year-old, reporting that maternal allergic history was associated with higher risk of AR development (25). The biological mechanism proposed was that childhood allergy development was impaired by maternal allergic disease history through impairment of neonatal regulatory T-cells (12). In contrast, a plenty of contradictory associations exists as to whether furred pet exposure (cats and dogs) may be a risk or a protective factor for the development of AR (16, 26, 27). We also found exposed to pet was another risk factor for AR, which was consistent with a recent study from Finland reported that dog and cat exposure in early life increased risk of developing pet allergies (28). However, the cumulative evidence from several systematic reviews suggests pet allergen exposure has not increased the risk of developing allergic disease(16, 29, 30). The discrepancies are likely due to the ubiquitous nature of pet allergens, pet owners are more concerned about sanitation and many other reasons.

Genetic association studies have advanced our understanding of genetic risk factors for allergic diseases. In the latest GWAS of AR, 41 risk loci related with AR have been reported, including 20 loci that had not previously been related to the disease (2–5), however, none of them have been confirmed to be a hub gene in the development or persistent of allergic diseases. In this study, 17 candidate genes for association with RA were identified using Human Genome Epidemiology (HuGE) Navigator(21) and methylation level of promoter regions were compared in PBMCs of RA cases and control individuals. However, disintegrin and metalloproteinase 33 (ADAM33), the first asthma-susceptible gene identified by positional cloning, was the only gene identified with significant methylation level differences between groups on CpG site level, amplicon level and gene level. ADAM33 has been extensively reported as a susceptibility gene in bronchial hyperresponsiveness, asthma and AR(21, 31–33). ADAM33 is expressed in the smooth muscle, myofibroblasts, and fibroblasts of asthmatic airways, thus the function of this protein might be involved in the airway remodeling (34). Various lines of evidence from previous human and animal studies indicated that the expression level of ADAM33 was upregulated during acute or chronic lung inflammation(35). Even though this functional link between ADAM33 and allergic airway inflammation, its role in the pathophysiology of AR is still to be clarified.

The dramatic increase in the prevalence of allergic disease during the past decades is more likely to be the result of changes in environmental factors, accompanied by epigenetic changes in the human genome. By using Adam33 knock out mouse, a recent report have reported a substantial interaction between ADAM33-mediated airway remodeling and sensitivity to allergen exposure, leading to allergic inflammation and bronchial hyperresponsiveness in early life (36). Since the present work was the first study to report the association between methylation level of ADAM33 in AR and found the interaction between pet-exposure and ADAM33 gene promoter methylation with the AR risk, the mechanisms underlying this effect remain unknown. However, this study suggests that it is important to examine not only the effect of early-life risk factors, but also the interaction effect between early-life risk factors on the DNA methylation level of candidate AR genes.

There are several limitations to our study. First, we used a relatively small sample size, there is a possibility of overestimating the significance of the association of ADMA33 methylation with AR. However, we speculate that the relationship between ADMA33 methylation and pet exposure is involved in AR onset. Second, there were several risk factors that could confound the interaction between pet exposure and the DNA methylation levels of ADMA33 in children with AR, including disinfection habits of pet owners, mode of delivery, etc. Furthermore, since RNA quality was not good enough for measuring expression level of ADAM33, further studies are needed to investigate the potential differential expression pattern of ADAM33 in AR. To overcome these limitations, a prospective cohort study with bigger sample size will be conducted in the future.

Conclusions
In conclusion, the present findings suggest early-life pet exposure is related with high risk of developing AR interacting with the methylation level of \textit{ADAM33} in a cohort of kindergartens. We provide evidence for the important role of gene-environment interaction in the development of AR.

**List Of Abbreviations**

- Allergic Rhinitis, AR
- Peripheral blood mononuclear cell, PBMC
- genome-wide association studies, GWAS
- skin prick test, SPT
- Receiver operating characteristic curve, ROC
- and area under curve, AUC
- False discovery rate, FDR
- disintegrin and metalloproteinase 33, \textit{ADAM33}

**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Tongren Hospital Affiliated to Shanghai JiaoTong University, School of Medicine (NO: TR2019.050.01).

**Consent for publication**

Written informed consent for publication was obtained from all legal guardians prior to blood collection.

**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests

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**Authors’ contributions**

ZY: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. TMY: Investigation, Methodology. LC and LYH: Investigation, Methodology. QXQI: Investigation, Data curation. YL: Data curation. SS: Investigation, Project administration, Supervision.

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Figure 1

CpG regions sequenced around promoter of ACE, ADAM33, GSTP1 and KCNE4. Short lines with arrows indicate amplicons of CpG region analyzed in this study, all of which locate in CpG islands around gene promoters. Range of each region is indicated by its relative distance (in bp) to TSS.
Figure 2

The methylation levels of promoter region in ADAM33 gene in PBMC of paired AR samples and control samples.

Figure 3

The methylation levels of each CpG site in ADAM33 genes in PBMC of paired AR samples and control samples and AUC value of each CpG site in ADAM33 gene showing the potentiality of the CpG sites as a biomarker for AR.
Correlations of the mean methylation statuses in the promoter CpG islands of ADAM33 with serum total eosinophil counts (Spearman's rank correlation).

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