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Reduced mouse allergen is associated with epigenetic changes in regulatory genes, but not mouse sensitization, in asthmatic children

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ABSTRACT

Chronic exposure to mouse allergen may contribute greatly to the inner-city asthma burden. We hypothesized that reducing mouse allergen exposure may modulate the immunopathology underlying symptomatic pediatric allergic asthma, and that this occurs through epigenetic regulation. To test this hypothesis, we studied a cohort of mouse sensitized, persistent asthmatic inner-city children undergoing mouse allergen-targeted integrated pest management (IPM) vs education in a randomized controlled intervention trial. We found that decreasing mouse allergen exposure, but not cockroach, was associated with reduced *FOXP3* buccal DNA promoter methylation, but this was unrelated to mouse specific IgE production. This finding suggests that the environmental epigenetic regulation of an immunomodulatory gene may occur following changing allergen exposures in some highly exposed cohorts. Given the clinical and public health importance of inner-city pediatric asthma and the potential impact of environmental interventions, further studies will be needed to corroborate changes in epigenetic regulation following changing exposures over time, and determine their impact on asthma morbidity in susceptible children.

1. Introduction

As many as 25–50% of inner-city children with asthma have evidence of allergic sensitization to mouse (Matsui et al., 2006; Ahluwalia et al., 2013). This trend suggests that chronic exposure to mouse allergen may contribute greatly to the inner-city asthma burden, particularly in the major metropolitan areas of the Northeastern United States. The multi-faceted environmental intervention by the Inner-City Asthma Study (ICAS) group, as well as others, has shown that successful reduction of indoor allergens can lead to long-lasting decreases in asthma symptoms, asthma exacerbations, missed school and disrupted sleep in children (Morgan et al., 2004; Pongracic et al., 2008; Johnson et al., 2009). Nonetheless, its ability to induce immune modulation is unknown and has implications for understanding the natural course of allergic asthma, identifying those at greater risk, and determining optimal treatment.

Environmental epigenetic regulation may induce immune modulation. This is supported by observations that measures of multiple environmental toxicants, including air pollution and allergens, are associated with altered inflammatory, allergic and regulatory gene methylation (Nadeau et al., 2010; Niedzwiecki et al., 2012; Salam et al., 2012). But far less studied are the impacts of potential changes in

Abbreviations: FOXP3, forkhead box P3 gene; Ig, immunoglobulin; ICAS, Inner-City Asthma Study; IPM, integrated pest management; IFN, interferon; IQR, interquartile range; MAAIT, Mouse Allergen and Asthma Intervention Trial; PASTURE, Protection Against Allergy: Study in Rural Environments

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exposures over time on changes in epigenetic marks, and whether these changes in epigenetic marks are clinically relevant. As an example, human rhinovirus infection changed global methylation in nasal epithelial cells to levels that varied by asthma diagnosis (McErlean et al., 2014). In the Normative Aging Study of elderly men, changes in particle numbers, levels of black carbon, and ozone over the preceding 4 weeks were associated with inflammatory gene specific changes in DNA methylation (Bind et al., 2014). However, comparable studies of changes in pediatric cohorts are scant. One exception is the Protection Against Allergy: Study in Rural Environments (PASTURE) study that observed significant differences in the DNA methylation of several genes assessed in cord blood and then repeated at age 4.5 years. The differences with aging during childhood also varied by whether there was prenatal exposure to a farm environment, and by whether the child was subsequently diagnosed with asthma (Michel et al., 2013).

To address this research gap, we measured prospectively and repeatedly promoter DNA methylation and expression of targeted asthma candidate genes associated with regulating allergy (T regulatory gene forkhead box P3 gene (*FOXP3*), and allergy suppressive gene interferon *(IFN)γ)*. Methylation of both genes previously was found to occur in association with ambient environmental exposures (Liu et al., 2008; Brand et al., 2012; Kohli et al., 2012; Niedzwiecki et al., 2012; Runyon et al., 2012; Tang et al., 2012) and with allergy and asthma (Liu et al., 2008; Brand et al., 2012; Runyon et al., 2012). We utilized pyrosequencing to capture and quantify small differences predicted to underlie potential changes in the exposure-outcome relationships (Murphy et al., 2012; Michel et al., 2013; Richmond et al., 2015; Clifford et al., 2017). This observational substudy combined measures from mouse sensitized, moderate to severe asthmatic inner-city children (n = 200; 6–17yr) undergoing mouse allergen-targeted integrated pest management (IPM) vs education in a randomized control intervention. We sampled buccal cells that comprise the aerodigestive track epithelium because they are accessed easily in children (Breton et al., 2011; Kuriakose et al., 2011; Lovinsky-Desir et al., 2014), and undergo molecular alterations following environmental exposures (Bhutani et al., 2008; Salam et al., 2012; Wan et al., 2014), and in association with airway inflammation (Breton et al., 2011; Salam et al., 2012). We postulated that reducing allergen exposure would modulate the immunopathology underlying persistent pediatric allergic asthma through epigenetic regulation. Specifically, we hypothesized that changes in mouse allergen exposure would be associated with changes in buccal cell methylation and expression, and that these would alter mouse-specific immunoglobulin (Ig)E.

2. Materials and methods

Children with persistent asthma and an exacerbation in the previous year underwent measurement of home settled bed dust and bed floor dust for mouse allergen (Mus m 1) and bed dust for cockroach (Bla g2) and dust mite allergen (Der f 1) by ELISA (Indoor Biotechnologies, Charlottesville, VA). Mouse-specific IgE (against mouse urine proteins, e72) was tested by Immunocap (ThermoFisher, Uppsala, Sweden) as described (Matsui et al., 2006; Sedaghat et al., 2016). Mouse exposed (determined by bed dust mouse allergen concentration of ≥0.4 μg/g or a bedroom floor dust mouse allergen concentration of ≥0.5 μg/g) and sensitized children were enrolled in a home-based Mouse Allergen and Asthma Intervention Trial (MAAIT; Fig. 1) (Sedaghat et al., 2016). The pest management education that was delivered as a control included information about setting mouse traps, sealing holes and cracks, and housekeeping practices. The IPM was delivered in treatments. The first treatment included targeted cleaning to remove allergen reservoirs, placement of traps, application of rodenticide, sealing holes and cracks, installation of allergen-proof mattress and pillow encasements (CleanBrands, LLC, Warwick, Rhode Island), and two portable air purifiers (Filtrete™ Room Air Purifier, 3M, St. Paul, Minnesota). This was followed by a visit 1–2 weeks later to reset traps and complete any work remaining from the primary visit. Each treatment was delivered by a licensed pest technician and by study staff.

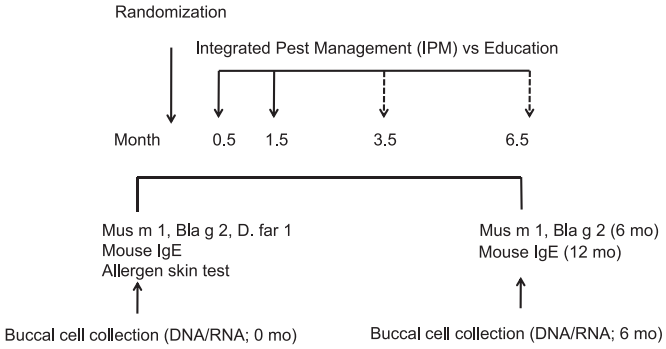

Fig. 1. Study algorithm. The IPM group received two IPM visits at 0.5 and 1.5 months (solid arrows). If there was evidence of mouse infestation at 3, 6, and 9 months, a subsequent IPM visit occurred at 3.5, 6.5 (see dashed arrows) and 9.5 months (not shown). The second mouse IgE level was measured at 12 month time-point, in contrast to the allergen (Mus m 1, Bla g 2) and epigenetic biomarkers, each measured at baseline and 6.0 months.

Extracted DNA underwent bisulfite conversion and pyrosequencing of the upstream enhancer (*FOXP3* only) and promoter (both genes) areas (Lovinsky-Desir et al., 2014). The *FOXP3* CpG loci within the gene promoter area were selected based on our previous associations with Treg function and asthma (Runyon et al., 2012) and their relatively large range in methylation levels across individuals (data not shown). The *IFN*γ CpG loci within the gene promoter were selected based on their conservation in mice (Niedzwiecki et al., 2012; Collison et al., 2013), specific roles in regulating gene expression (Gonsky et al., 2009; Brand et al., 2012; Kohli et al., 2012; Belsky et al., 2013), susceptibility to allergen and air pollution (Liu et al., 2008; Brand et al., 2012; Niedzwiecki et al., 2012), and previously implicated role in allergy and asthma (Kohli et al., 2012; Runyon et al., 2012).

Total RNA was extracted using Trizol (Molecular Research Center, Inc., Cincinnati, OH; Supplemental Tables 1A,B). qRT-PCR was carried out using the SuperScript First-Strand Synthesis System and the Applied Biosystems® 7500 Real-Time PCR Systems. The housekeeper gene cystatin A (CSTA) was selected based on its high and specific expression in the buccal mucosa (Magister and Kos, 2013). Mouse and cockroach allergen levels and buccal cells were collected at baseline and 6 months later. Dust mite allergen levels were collected at baseline only. Mouse IgE was assessed at baseline and 12 months later. The study was approved by the Institutional Review Boards of Columbia University Medical Center, Johns Hopkins University, and Harvard University.

The distribution of each variable was examined and an appropriate transformation was applied as necessary to meet parametric model assumptions. Analyses were conducted separately for each CpG gene site. Allergen levels and RNA values were natural log transformed. Values (eg, mouse allergen) below a limit of detection (LOD) were assigned a value at half the LOD. Methylation levels within the *FOXP3* enhancer highly correlated with each other (Spearman correlation coefficients 0.90–0.94) at each visit, and in subsequent analyses the average of the 4 values were used as a single variable.

The signed rank test was used to compare differences in allergen and mouse IgE levels between visits. The Kruskal-Wallis test was to used to detect bivariate associations between the methylation or gene expression levels and categorical variables including sex, site (Boston, Boston Children's Hospital, Harvard Medical School; Baltimore, Johns Hopkins University), race/ethnicity, insurance type, any reported exposure to second hand smoke, maternal allergy status and particulate matter less than 10 μm or not, at each visit. The spearman correlation coefficient was used to indicate bivariate association between quantitative variables, specifically the correlations among individual buccal biomarkers and between the arithmetic change in allergen and change in methylation. To examine concurrent associations between mouse allergen and

Table 1
Participant baseline characteristics (n = 200).

	Number	%
Site Baltimore (vs. Boston)	168	84
Income < $25,000 annual	117	58.5
Male gender	117	58.5
Race/ethnicity		
Black	158	79.0
Non-black	42	21.0
Public medical insurance	178	89.0
Smoke exposure*	98	47.0
Maternal allergy**		
Yes	70	35.0
No	119	59.5
Unknown	11	5.5
Number positive skin tests (in addition to mouse)		
0	9	4.5
1–2	28	14.0
3+	163	81.5

The age of the participants ranged 5.2–17.5 years (mean 9.7 ± 3.4 standard deviation (SD)).
* Defined as any smoker in the home.
** Based on maternal report.

each of the methylation or gene expression outcome variables with repeated measures over time, we used marginal linear models with mouse allergen as the predictor. The generalized estimating equations (GEE) approach, taking into account within-person correlation in repeated measures, was used to estimate model parameters and make statistical inference. Because a sex difference in the *FOXP3* enhancer and association between age and gene expression was evident, we controlled for sex (age) in the marginal models. To determine whether a decrease in mouse (or cockroach) allergen changed *FOXP3* or *IFN*γ methylation or gene expression, linear regression models were built with the change in the methylation or gene expression as the dependent variables, and the change in mouse (or cockroach) allergen as the primary independent variable. Models were controlled for mouse allergen, CpG methylation or gene expression at baseline. Sex and age were not related to the change in *FOXP3* or *IFN*γ methylation over time, so the models exhibited in Tables 2 and 3 controlled only for baseline levels. Linear models were used to examine whether a change in methylation (over 6 months) was associated with a change in mouse IgE (over 12 months), controlling for mouse–specific IgE and CpG methylation or gene expression at baseline.

Table 3
Change in methylation and expression over 6 month was not associated with change in mouse IgE over 12 month.

Change in *FOXP3*	**Enhancer**	**CpG^{-138}**	**CpG^{-126}**	**Expression**
B	−0.01	−0.01	−0.02	−0.03
SE	0.01	0.004	0.01	0.03
Change in *IFN*γ	**CppG-186**	**CpG-54**	**Expression**	
B	−0.002	0.004	0.02	
SE	0.01	0.01	0.04	

B: Estimated regression coefficient. SE: Standard error
Relative expression of *FOXP3* was measured using log transformation (variable + 0.1)
Relative expression of *IFN*γ was measured using log transformation (variable + 0.5)
Analyses controlled for both mouse allergen at baseline (natural log transformed) and CpG methylation or gene expression at baseline. Sensitivity analyses of gene expression including only those with qRT-PCR cycle length < 37 did not alter the results.
N = 179 for all analyses, with specimens excluded due to lack of 12 month blood specimen (n = 12 samples from 11 individuals), or inadequate or pyrosequencing reactions failed (up to n = 15 for *IFN*γ methylation analyses).

3. Results and discussion

Participant baseline characteristics are displayed in Table 1. The age of the participants ranged 5.2–17.5 years (mean 9.7 ± 3.4 standard deviation (SD)). The average number of positive skin tests in addition to mouse was 4.7 ± 2.8. In our cohort of n = 200, similar to that previously published (Matsui et al., 2017), mouse allergen in the bed (median 0.9, IQR 2.7 to median 0.6, IQR 1.3, $p < 0.0001$) and floor (median 4.2, IQR 16.0 to median 1.8, IQR 6.4, $p < 0.0001$), and cockroach allergen in the bed (median 0, IQR 0.02 to median 0, IQR 14.7, $p = 0.04$), decreased overall between visits. Also, in our subset of n = 187 with paired data for mouse IgE analyses, similar to what we previously published (Matsui et al., 2017), mouse IgE decreased overall (median 10.7 kU/L, IQR 33.9 to median 7.0, IQR 22.6, $p < 0.0001$).

Boys had lower baseline buccal DNA methylation than girls at the *FOXP3* enhancer at both visits (mean ± SD: visit 1-boys: 4.96 ± 5.38% vs. girls: 40.59 ± 7.61%; visit 2-boys 4.39 ± 5.01 vs. girls: 41.02 ± 6.11; $p < 0.0001$ for both visits). Baseline sex effects were apparent in the opposite direction at the two *FOXP3* promoter sites (mean ± SD *FOXP3* CpG^{-138}: visit 1-boys: 66.38 ± 11.28% vs. girls: 46.03 ± 11.03%; visit 2-boys: 69.12 ± 12.57 vs girls: 47.40 ± 10.70; $p < 0.0001$ at both visits; mean ± SD CpG^{-126}: visit 1-boys: 86.15 ± 7.10% vs. girls: 82.67 ± 8.25%, $p = 0.003$; visit 2-boys: 88.28 ± 7.38 vs. girls: 84.23.40 ± 6.60, $p < 0.0001$). Significant sex effects for *IFN*γ were not observed. *FOXP3* and *IFN*γ gene expression were associated inversely with age at visit 1 only (Spearman correlation

Table 2
The association between the change in mouse allergens over time and change in *FOXP3* and *IFN*γ promoter methylation.

		Outcome: change in *FOXP3*				**Outcome: change in *IFN*γ**		
		Enhancer	**CpG^{-138}**	**CpG^{-126}**	**Expression**	**CpG^{-186}**	**CpG^{-54}**	**Expression**
	n	191	198	198	198	186	186	198
		B (SE)	**B (SE)**	**B (SE)**	**B (SE)**	**B (SE)**	**B (SE)**	**B (SE)**
Mouse allergen predictor	**Bed dust**	−0.15	**1.78***	−0.45	0.16	−0.33	0.30	0.04
		(0.37)	(0.70)	(0.37)	(0.10)	(0.40)	(0.37)	(0.09)
	Bed floor dust	−0.25	0.90	−0.25	0.09	−0.51	0.36	0.05
		(0.32)	(0.60)	(0.31)	(0.08)	(0.33)	(0.31)	(0.07)

B: Estimated regression coefficient
SE: Standard error.
Enhancer defined as average methylation at CpG^{-4506}, CpG^{-4500}, CpG^{-4494}, CpG^{-4484}.
Relative expression of *FOXP3* was measured using log transformation (variable + 0.1).
Relative expression of *IFN*γ was measured using log transformation (variable + 0.5).
Analyses controlled for both mouse allergen at baseline (natural log transformed) and CpG methylation or gene expression at baseline. Sensitivity analyses of gene expression including only those with qRT-PCR cycle length < 37 did not alter the results. Missing samples occurred when DNA or RNA or allergen specimens were inadequate or when pyrosequencing reactions failed.
* $p = 0.01$.

coefficient r = −0.19, r = −0.22 respectively, p < 0.05; Supplemental Table 2). Methylation at *IFN*γ CpG^{-186} also was lower with age ((r = −0.18, p = 0.02) at visit 2, Supplemental Table 2)).

Using marginal models that accounted for within-subject correlation in repeated measures, higher levels of mouse allergen in bed dust were associated with lower methylation in the *FOXP3* promoter at CpG^{-126} (Estimated regression coefficient B = −0.62, p = 0.004), but not buccal DNA methylation at other sites or regulatory gene expression. None of the methylation variables were associated with mouse-specific IgE.

In prospective analyses of the changes in methylation or gene expression levels between study visits, we found the opposite association between allergen and methylation. Decreases in mouse allergen in the bed dust borderline correlated with decreases in methylation at the *FOXP3* enhancer sites (r = 0.14, p = 0.05). Further, to assess whether change in mouse allergen over time predicted change in DNA methylation, regression models were run that controlled for baseline levels of mouse allergen in the bed. We found that decreases in mouse allergen predicted decreases in *FOXP3* promoter methylation, in this case at the CpG^{-138} site (estimated regression coefficient B = 1.78, p = 0.01, Table 2). Controlling for *FOXP3* CpG^{-138} and mouse allergen level at baseline, a 50% reduction of mouse allergen in bed dust was associated with mean reduction of 1.23% methylation in *FOXP3* CpG^{-138}.

In addition, we restricted the analyses to only those with undetectable dust mite allergen at baseline and we found the same association between change in mouse allergen and change in methylation, suggesting that there was not confounding by dust mite allergen. We reran the main models using cockroach allergen (Bla g 2 in bed dust) as the predictor on the same epigenetic outcomes. No significant association between the change in cockroach allergen over time and change in DNA methylation were detected, with and without further control for mouse allergen variable at baseline. Adding the cockroach variable at baseline did not significantly alter the associations between change in mouse allergen over time with the change in DNA methylation. We also did not find any significant changes in gene expression, including during sensitivity analyses that excluded any samples that required a high number of cycles to amplify (i.e, those with presumed very small amounts of RNA) (Table 2).

In further analyses, the changes in *FOXP3* and *IFN*γ methylation and expression over the 6 month sampling period were not associated with a change in mouse IgE during these same 6 months plus 6 months afterwards (i.e. by the 12 month period; Table 3). These results suggest that these examples of DNA methylation did not induce sustained effects on mouse sensitization. In addition, the change in the mouse allergen in the bed was not associated directly with a change in mouse IgE levels over time. This latter finding contrasts with that from the parent study (Matsui et. al., 2017) that used random effects model and repeat measures of IgE, a different period for change in mouse allergen (6 vs 12 mo), and a larger sample.

Quantifying small differences in specific CpG targets allows us to compare methylation levels across sites. Of the two neighboring *FOXP3* promoter sites (CpG^{-126}, CpG^{-138}), while positively correlated with each other, correlated negatively with CpG methylation levels in the *FOXP3* enhancer (Supplemental Table 3). Buccal methylation levels at the *FOXP3* CpG^{-126} site correlated negatively and weakly with *IFN*γ gene expression at visit 2 only. Buccal methylation at *IFN*γ CpG^{-54} and *IFN*γ CpG^{-186} correlated positively and weakly with *IFN*γ relative gene expression, also only at visit 2.

Overall, these findings suggest that a reduction in mouse allergen, but not cockroach, observed in the setting of IPM vs education intervention trial may have reduced *FOXP3* promoter DNA methylation. The decrease in *FOXP3* methylation in buccal cells may be consistent with upregulated T suppressive activity, presumably following activation of the *FOXP3* gene; although, this was not detected in the buccal RNA. These findings with respect to methylation point to the gene regulatory effects of reducing allergen levels, albeit small, even if there was not a statistically significant effect on mouse specific IgE.

We also identified a strong effect of sex on buccal methylation level in the *FOXP3* enhancer. The levels of *FOXP3* methylation in the enhancer region in males were markedly lower than in females. In contrast, the sex-related differences were less marked in the *FOXP3* promoter, and in the opposite direction. The significance of these differences is uncertain, but one could speculate that it relates to epigenetic regulation of X-chromosome inactivation (XCI). For example, female cells undergo XCI, and DNA methylation of the inactive X is known to contribute to the maintenance of its inactive state; levels of methylation within a gene silenced by XCI can vary by region. Specifically, levels of promoter methylation of genes silenced by XCI have been correlated with susceptibility to XCI, and CpG islands tend to be sites where there is greater methylation on the inactivated X chromosome compared to other areas (Sharp et al., 2011). Because the *FOXP3* gene is subject to XCI (Tommasini et al., 2002), one could speculate that the higher methylation levels measured in the *FOXP3* enhancer of females in this cohort reflects regulation by DNA methylation of XCI to some extent. Although this speculation would not explain the lower methylation levels found in females in the *FOXP3* promoter region, other than demonstrating that variation in methylation levels across genes applies to genes silenced by XCI (Sharp et al., 2011).

In comparison, we previously found that methylation at the *IFN*γ promoter varied by sex in CD4+ lymphocyte but not buccal cell DNA (Lovinsky-Desir et al., 2014). Here too the underlying mechanism still needs to be elucidated. For both genes, differences in hormone levels or unmeasured environmental exposures that vary by behaviors among boys and girls also may explain differential induction of DNA methylation. The sex differences in *FOXP3* we observed suggest that sex effects on methylation, including its variation by CpG region, should be considered when designing studies of epigenetic biomarkers in asthma.

These findings support the premise that during an intervention against mouse allergen, the changing environment over time may drive some plasticity in the level of the DNA methylation, as reported in other studies (Bind et al., 2014; Borsch-Haubold et al., 2014; McErlean et al., 2014). The direction of these results appear different than observed in one report in pollen-allergic patients where local challenge of the nasal mucosal with pollen extract led to an increase in the *FOXP3* expressing T cells (Skrindo et al., 2011). But other studies have not examined epigenetic regulation in symptomatic children following chronic, daily allergen exposure. Further, it could be consistent with other environmental exposures like respiratory syncytial virus infection that reduced T reg function, thereby promoting susceptibility to allergic asthma (Krishnamoorthy et al., 2012). We believe these associations are gene specific as there is substantial evidence that epigenetic regulation of both *FOXP3* and *IFN*γ is associated with clinically relevant outcomes (Liu et al., 2008; Brand et al., 2012; Kohli et al., 2012; Niedzwiecki et al., 2012; Runyon et al., 2012; Tang et al., 2012). There is more limited evidence to suggest that differences in global methylation instead may explain outcomes related to exposure to cigarettes and air pollution (Breton et al., 2009, 2016; Lee et al., 2015) and allergic sensitization (Sordillo et al., 2013) in some, but not other (Chi et al., 2016), studies.

Hence, with this prospective and repeat sampling design, we may have identified novel conditions for which a change in the indoor environment altered the promoter methylation profile of an immunoregulatory gene, in this case suggesting that the maintenance of T regulatory function may have become further upregulated. The results may reveal that highly exposed, allergic and asthmatic children could be resistant to usual allergen-induced regulatory mechanisms due to undefined molecular impairments (Yamamoto et al., 2011), or the contribution of other genes or CpG sites within the same genes, or even other epigenetic pathways like histone acetylation or hydroxymethylation (Planell-Saguer et al., 2014). However, the results also do not support the paradigm that decreased *FOXP3* methylation is part of the causal pathway leading to changes in mouse IgE, at least according to this timeline for a clinical allergic response. Rather, *FOXP3* methylation

may act more as a marker of effective changes in allergen exposure, or potentially of functional changes in other immune cells.

The relevance of the expression of *FOXP3* and *IFN*γ in the buccal mucosa described here and elsewhere (Scadding et al., 2010), and their exact roles following allergen remediation, are still not elucidated from these results. *FOXP3* methylation, perhaps because it may capture changes over a longer time period than buccal gene expression, may contribute more to the immune response to changed allergen exposure. Indeed, altered buccal DNA methylation has been associated with airway inflammation in previous pediatric cohorts (Breton et al., 2011). Striking variation by CpG site within the promoter was detected and may suggest that individual CpG site may function independently, as reported (Clifford et al., 2017). The marginal models with repeated measures that demonstrated patterns opposite to our prospective findings may have been susceptible to bias due to unmeasured confounding factors. Nonetheless, we also acknowledge that the effect sizes were generally small and we were working with a mixed population of cells. Buccal epithelial cells were used in large part because the process of obtaining them is noninvasive, which is critical in pediatric cohorts studies. We do not yet know whether the changes are universal or similar across tissues, as several asthma studies have shown differences in some gene methylation patterns by cell type (Stefanowicz et al., 2012; Lovinsky-Desir et al., 2014). But buccal cells have revealed epigenetic patterns relevant to allergy, when collected using comparable techniques (Breton et al., 2011; Kuriakose et al., 2011; Salam et al., 2012; Lovinsky-Desir et al., 2014). Our focus on the cytokine profile in buccal epithelial cells is novel, so additional studies would be beneficial to understand more fully their translation. Moreover, cockroach allergen was detected only in 25% of the homes at baseline, with borderline significant decreases over time. This observation, plus our analyses suggesting that this relatively infrequent exposure did not predict epigenetic outcomes, makes confounding by cockroach allergen less likely.

In conclusion, the longitudinal assessment of DNA methylation in this unique pediatric cohort suggests that changing environmental exposure can induce epigenetic regulation, and T suppressive activity may even increase when high levels of mouse allergen are diminished. They also support the epidemiological paradigm that findings in cross-sectional analyses may not replicate under prospective evaluations. Our results place us one step further towards understanding immunoregulation and its susceptibility to change following changes in environmental exposures. Given the high public health burden associated with frequent sensitization to mouse in inner city asthma (Matsui et al., 2006; Ahluwalia et al., 2013), these results may provide a foundation for pursuing the hypothesis further that allergen interventions have the potential to modify the natural course of disease among susceptible children through epigenetic mechanisms.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.04.025.

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