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Short-term exposure to PM_{2.5} and vanadium and changes in asthma gene DNA methylation and lung function decrements among urban children

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Abstract

Background: Both short and long-term exposure to traffic-related air pollutants have been associated with asthma and reduced lung function. We hypothesized that short-term indoor exposure to fine particulate matter <2.5 μm (PM_{2.5}) and vanadium (V) would be associated with altered buccal cell DNA methylation of targeted asthma genes and decreased lung function among urban children in a nested subcohort of African American and Dominican children.

Methods: Six day integrated levels of air pollutants were measured from children's homes (age 9–14; *n* = 163), repeated 6 months later (*n* = 98). Buccal samples were collected repeatedly during visits. CpG promoter loci of asthma genes (i.e., interleukin 4 (IL4), interferon gamma (IFNγ), inducible nitric oxide synthase (NOS2A), arginase 2 (ARG2)) were pyrosequenced and lung function was assessed.

Results: Exposure to V, but not PM_{2.5}, was associated with lower DNA methylation of IL4 and IFNγ. In exploratory analyses, V levels were associated with lower methylation of the proinflammatory NOS2A-CpG⁺⁵⁰⁹⁹ among asthmatic overweight or obese children but not nonasthmatics. Short-term exposure to PM_{2.5}, but not V, appeared associated with lower lung function (i.e., reduced z-scores for forced expiratory volume in one second (FEV₁), FEV₁/forced vital capacity [FEV₁/FVC] and forced expiratory flow at 25–75% of FVC [FEF_{25–75}]).

Conclusions: Exposure to V was associated with altered DNA methylation of allergic and proinflammatory asthma genes implicated in air pollution related asthma. However, short-term exposure to PM_{2.5}, but not V, appeared associated with decrements in lung function among urban children.

Keywords: Vanadium, PM_{2.5}, Short-term exposure, DNA methylation, Overweight, Asthmatic children

Background

Both short-term and long-term exposure to fine particulate matter <2.5 μm (PM_{2.5}) have been associated with reduced lung function [1–5]. However, pediatric cohort studies on short-term effects of PM_{2.5} on lung function are relatively scarce. Further, it is far from evident what components of PM_{2.5} cause these adverse health effects,

and their underlying mechanisms. These components may include toxic agents like trace metals.

In the current study, we focused on vanadium (V) as the trace metal PM component because previous studies have shown that ambient levels of V, emitted from the burning of residual oil fuel mainly from residential heating and shipping ports [6] and traffic emissions [7], exhibited marked spatial variability in New York City (NYC) [8]. Measures of V have been associated with increased 1) cellular stress responses (i.e., Nuclear Factor kappa B) in human bronchial epithelial cells [9], 2) risk of PM_{2.5}-related respiratory and cardiovascular

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hospitalizations [10], 3) mortality among elderly individuals [11], and 4) wheeze [12] and decreased lung function (i.e., forced vital capacity (FVC)) among children [13]. In the latter case, the V findings persisted after adjusting for co-pollutants (e.g., $PM_{2.5}$ or elemental carbon (EC)), suggesting that V itself may be an important independent contributor of adverse respiratory effects of $PM_{2.5}$. Moreover, susceptibility to these pollutants may vary by asthma phenotype, including obesity-related asthma [14, 15]. Overweight asthmatics exhibited more asthma-like symptoms in association with exposure to $PM_{2.5}$, nitrogen dioxide (NO_2) and polycyclic aromatic hydrocarbons (PAH) [16, 17], and greater declines in lung function in association with exposure to ozone [14].

Environmental epigenetic regulation also may underlie mechanisms of air pollution-associated asthma [18, 19]. As examples, recent ambient $PM_{2.5}$ levels were associated with lower DNA methylation of the proinflammatory gene inducible nitric oxide synthase (iNOS encoded by NOS2A) [20], and chronic exposure to PAH was associated with methylation of the asthma regulatory gene Forkhead box transcription factor 3 (FOXP3) [21]. In a study of boilermakers, higher occupational levels of $PM_{2.5}$, presumably representing high levels of metals [22], were associated positively with methylation of long interspersed nuclear element-1 (LINE-1) in peripheral blood leukocytes [23], indicating higher global methylation. However, pediatric cohorts have not yet investigated epigenetic changes of asthma genes in response to measures of air pollutants, and specifically for the V component.

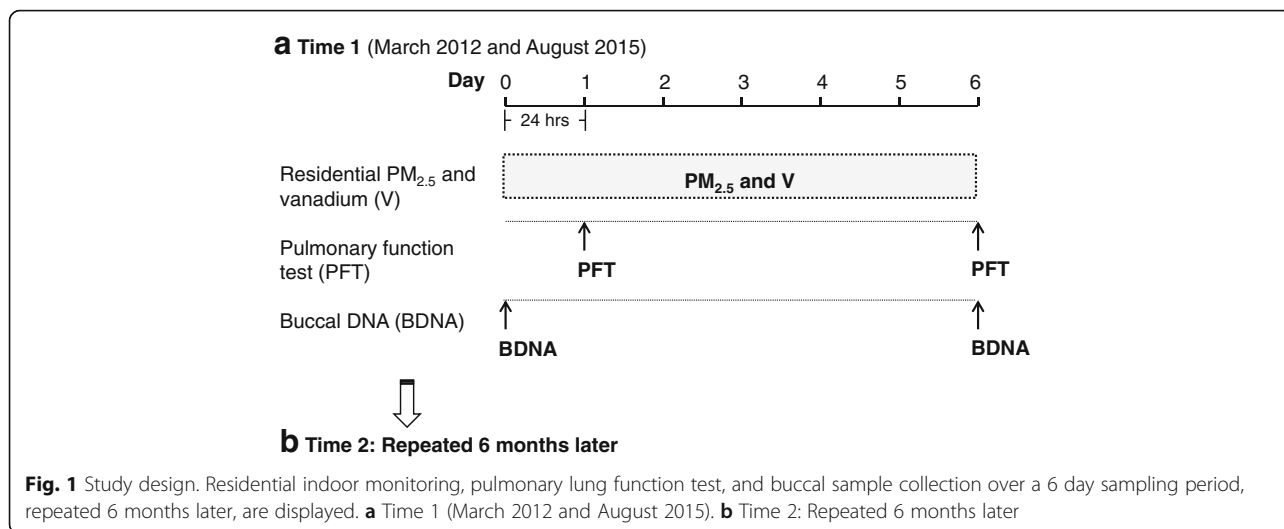
Our objective was to delineate the association between residential exposure to air pollution, including $PM_{2.5}$ and its metal component V, on epigenetic regulation and lung function in a nested cohort of asthmatics and healthy urban children. We specifically targeted epigenetic loci previously implicated in air pollution-related asthma [24–27]. We also quantified DNA methylation levels in buccal cells, aerodigestive track epithelium where air pollution-related molecular changes have been documented [28], via pyrosequencing technology to capture small differences [29–32]. We hypothesized that exposure to $PM_{2.5}$ and V, assessed by residential measures integrated over 6 days, repeated 6 months later, would be associated with changes in buccal cell DNA methylation of targeted CpG loci in the promoter region of several asthma inflammatory genes (e.g., interleukin 4 (IL4), interferon gamma ($IFN\gamma$), NOS2A and arginase2 (ARG2)) among urban African American and Dominican children. Further, we explored whether such methylation would vary by obesity-asthma. We also hypothesized that short-term residential exposure to $PM_{2.5}$ and V would be associated with decreased lung function.

Methods

Study population and residential air monitoring

Seven hundred twenty seven nonsmoking mothers of African-American and Dominican ethnicity living in Northern Manhattan and the South Bronx were recruited during pregnancy as part of the Columbia Center for Children's Environmental Health cohort (CCCEH) birth cohort [33]. For this nested study, participants were recruited in order based on age criteria (9–14 years old still enrolled in CCCEH) and enriched for current asthma status (57% asthmatic vs 43% non-asthmatic). Children were classified as asthmatic if a specialized physician (allergist, pulmonologist) diagnosed them with asthma using study standardized and objective criteria, and if they had symptoms or used asthma medication in the 12 months prior to enrollment in the nested study [34]. Children without any asthma-related symptoms between age 5 and enrollment, or determined not to be asthmatic by our standardized criteria [34], in the nested study were classified as non-asthmatic. Height, weight, and body fat percentages (%BF) were measured at each visit (Time 1 and Time 2, 6 months later) using a portable stadiometer (SECA, Hamburg, Germany) and a segmental body composition monitor (Tanita Corporation, Tokyo, Japan). Children with body mass index (BMI) \geq the age- and sex-specific 85th percentile of the year 2000 CDC growth charts for age and sex were classified as 'overweight' [35]. Waist circumference (WC) was measured twice at a level midway between the top of the hip bone and the lowest rib. High %BF and high WC, classified as the upper 33% of mean whole %BF and mean WC, respectively, were further investigated based on the strong correlations between BMI and %BF or WC in studies with young adolescents [36, 37].

Indoor air monitors collected six-day integrated $PM_{2.5}$ filter samples at each of the 163 homes between March 2012 and August 2015 (Time 1; initial set-up) (Fig. 1). Samplings started on Wednesday or Thursday to minimize variation in air pollution exposure by day of the week [38]. The sampling was repeated approximately six months after the initial sampling period (Time 2; $n = 98$) to capture the seasonal variability in air pollution levels. Residential indoor monitors were placed in a room where the child spent most of his or her time. Data were analyzed for those children ($n = 149$) for whom measures of residential $PM_{2.5}$ and V were available (Fig. 2). In order to perform a sensitivity analysis including chronic exposure to $PM_{2.5}$, we obtained residential indoor $PM_{2.5}$ data that were collected 4–8 years prior to Time 1, using the same sampling methodology. Of 163 children, 106 children had previously available $PM_{2.5}$. The study was approved by the Columbia University Institutional Review Board and written informed consent and assent were obtained.



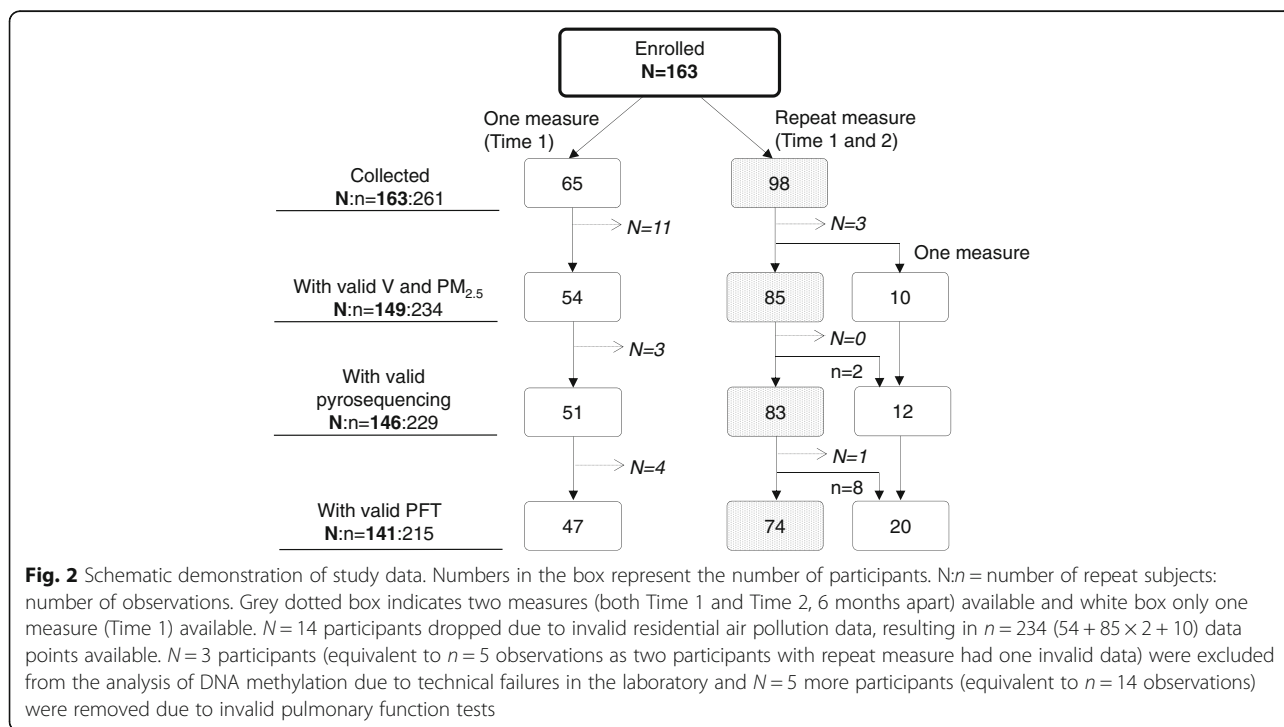
Residential indoor assessment

PM_{2.5} was analyzed by weighing (post-pre weight) Teflon filter samples collected from a cyclone with a 2.5 μm aerodynamic-diameter cut point (model SCC 1.062, BGI, Inc.) that operated at 1.5 L/min (±15% of standard deviation; SD) for six days. V levels on the same filters were analyzed using XEPOS Energy-Dispersive X-Ray Fluorescence Spectrometer (XEPOS 3, Spectro, Kleve, Germany) in specially-designed polytetrafluoroethylene (PTFE) holders to keep the filters in a fixed flat geometry [39]. The XEPOS had been calibrated for PM_{2.5} filters for V and other metals. Filter blanks and an

internal standard consisting of a NYC PM_{2.5} filter were counted with each analysis batch of 10 filters.

Buccal sample collection, DNA extraction and methylation

Buccal samples were collected during in-home visits on Day 0 (set-up day) and Day 6 (take-down day; Fig. 1) to capture changes in DNA methylation over 6 days. The participants were asked to rinse out their mouths with water before buccal swabs sampling to avoid contamination from food and then brush the inside of each cheek for fifteen seconds with a CytoSoft cytology brush (Fischer Scientific, Pittsburgh, Pennsylvania). The



brushes were placed immediately in cell lysis solution (Qiagen Sciences, Germantown, Maryland) and stored at room temperature until processing. To ensure a homogenous cell population, brush smears were generated from twenty-five participants selected at random and stained using hematoxylin and eosin. Eight fields were randomly selected and imaged at 10X using a Nikon Eclipse TS100 (Tokyo, Japan) brightfield microscope. Two individuals independently counted and determined that 94% percent of the total cells were squamous epithelial cells among the 24 participants (kappa agreement score 0.934).

DNA was extracted and isolated using the Gentra Puregene Buccal Cell kit (Qiagen), qualified and quantified using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA). Extracted DNA was bisulfite converted with the EZ-96 DNA Methylation-Lightning Kit (Zymo Research, Irvine, CA). Targeted promoter region CpG loci were selected based on their known importance to allergy and to NOS2A-related inflammation [25, 40], and published epigenetic links to asthma-related environmental exposures and/or asthma outcomes (Additional file 1: Figure S1 and Table S1). PCR primers were designed for the targeted regions using Pyromark Assay Design SW 2.0 Software (Qiagen) (Additional file 1: Table S2). PCR was performed using Qiagen HotStarTaq DNA Polymerase (Qiagen), and methylation levels for each of the targeted CpGs was measured using the Pyromark Q96 MD pyrosequencing instrument (Qiagen).

Quality assurance measures included: 1) methylated and unmethylated DNA (Qiagen) was added to each PCR plate as a control, and 2) the methylation level of a duplicate buccal sample collected at the same time was compared for 13% of the cohort ($n = 23$ -29). The average absolute percent differences for the IL4, IFN γ , and NOS2A measures between the primary and duplicate buccal sample ranged between 2% and 7%, indicating a good agreement. The ARG2 loci exhibited greater average percent differences, ranging from 54% to 76% for CpG⁻³⁰ and CpG⁻³², respectively. One additional data point was excluded due to PCR contamination.

Pulmonary function tests (PFTs)

PFTs were conducted during in-home visits on Day 1 and Day 6 using a portable spirometer (Koko, nSpire Health, Longmont, Colorado), in accordance with ATS and ERS guidelines [34] and repeated 6 months later (Fig. 1). Tests were considered acceptable if they met the following criteria: 1) rapid upstroke, 2) volume extrapolated <5% of FVC, 3) minimal premature termination of exhalation (premature termination = termination at >15% of peak flow), and 4) smooth exhalatory limb [41], as determined by two pulmonologists. PFTs that did not

meet the acceptability criteria were excluded ($n = 14$ out of 229; Fig 2). Four spirometry outcome measures were included for analysis: FVC, forced expiratory volume in one second (FEV₁), FEV₁/FVC, and forced expiratory flow at 25–75% of forced vital capacity (FEF_{25–75}).

Statistical analyses

Chi-square and Mann–Whitney tests were used to detect the difference in demographic characteristics between groups and air pollutant levels by heating season (i.e. October–April), respectively. Consideration of heating season was to assess meteorological conditions such as temperature, humidity, and cold/flu season which could confound measures of air pollution and respiratory morbidity [12, 42, 43]. A spearman correlation coefficient was computed for correlations between PM_{2.5}, and V while the intraclass correlation coefficient (ICC) was calculated for correlations among repeated measures of percent methylations. Air pollutant concentrations were log-transformed to assume normal distribution for subsequent analyses.

Due to non-normal distribution of the log-transformed methylation data, percent methylation of IL4, IFN γ , and NOS2A were dichotomized at the upper tertile of each individual CpG site [44]. Percent methylation of ARG2 were averaged across 3 selected CpG sites then further dichotomized as ‘unmethylated (0)’ vs ‘methylated (1)’ if the average percent methylation was zero or > 0, as described [25]. The associations among residential levels of PM_{2.5}, V, and changes in DNA methylation were analyzed using a modified Poisson regression in generalized estimating equations (GEE) models to estimate relative risks (RR) [45]. The analyses were conducted using PM_{2.5} and V (two-pollutant models), and DNA methylation on Day 6 with adjustment of Day 0 DNA methylation to assess the independent effects of each pollutant on changes in DNA methylation. Final models were further adjusted for common covariates, including race/ethnicity, sex, age, heating season, asthma diagnosis, and overweight. Exploratory analyses were conducted to examine differences in air pollution-related DNA methylation by overweight asthmatic phenotype, suggested in previous in cross-sectional studies [26, 46]. Adjusted models stratified by overweight and asthma (i.e., 4 groups: overweight asthmatics, non-overweight asthmatics, overweight non-asthmatics, and non-overweight non-asthmatics) were run. A similar analysis was performed after replacing overweight with obesity (BMI \geq the age- and sex-specific 95th percentile), high BF%, or high WC.

Spirometric variables were converted to ethnic-specific z-scores, which were adjusted for sex, age, and height, according to the Global Lung Initiative (GLI-2012) equations for African American and Dominican (mixed ethnic origin treated as ‘other’) children using the GLI-2012

software for subsequent analyses (<http://www.ers-education.org/guidelines/global-lung-function-initiative/tools.aspx>) [47]. Multivariable linear regression analyses in two-pollutant models were used to examine the observed effects of $PM_{2.5}$ on each lung function outcome on Day 6, after controlling for V, Day 1 lung function outcome, heating season, asthma diagnosis, and overweight. Of the 149 children with valid air monitoring data, 3 and 8 children, respectively, were excluded due to invalid methylation (i.e. failed pyrosequencing run) and lung function data, resulting in a final sample of 146, and of 141 for the DNA methylation and lung function analysis, respectively (Fig. 2).

Sensitivity analyses were conducted as follow: (1) reanalysis after controlling for time-spent at home to address the impact of residential exposure vs other microenvironments (e.g., school and outdoors), (2) reanalysis after replacing concurrent $PM_{2.5}$ levels with those measured 4–8 years prior to Time 1, as a surrogate for

chronic exposure to $PM_{2.5}$ in methylation analysis, (3) reanalysis after controlling for food intake, by asking the question “In the past two hours, have you had anything to eat or drink? (Yes/No)”, and (4) reanalysis after replacing heating season with four seasons (i.e., spring, summer, fall and winter), given the reported seasonal variation in lung function outcomes [48]. All analyses were performed using SPSS Statistic version 23.0 (SPSS Inc., Chicago, IL, USA) where $p < 0.05$ was considered statistically significant.

Results

Subject characteristics and residential exposure

There were no significant differences in demographic variables by enrollment into the nested cohort, except for a higher proportion of asthma and seroatopy, consistent with our recruitment strategy (Table 1). On daily average, children spent 68% (16.3 h) of their time at home. At Time 1, children were exposed to the median

Table 1 Cohort characteristics

Characteristic	Participants included ^a (<i>n</i> = 149)	CCCEH cohort not included (<i>n</i> = 578)	<i>P</i> -value ^h
Maternal ethnicity			0.57
Dominican	94/149 (63%)	379/578 (66%)	
African American	55/149 (37%)	199/578 (34%)	
Age mean [min-max], yrs	12.5 (9.2–14.3)	-	
Girls	76/149 (51%)	300/578 (52%)	0.85
≥Maternal high school degree	82/144 (57%)	374/569 (66%)	0.05
Maternal asthma (+)	42/149 (28%)	121/578 (21%)	0.06
Prenatal ETS exposure ^b (+)	48/147 (33%)	198/570 (35%)	0.64
Current ETS exposure ^c (+)	12/121 (10%)	-	
Daily time spent home ^d hrs, mean ± SD	16.3 ± 4.8		
BMI ^e z score, mean ± SD	0.86 ± 1.11	-	
Underweight (<5 th %ile)	5/149 (3%)	-	
Normal weight (5 th –85 th %ile)	66/149 (44%)	-	
Overweight (85 th –95 th %ile)	40/149 (27%)	-	
Obesity (≥95 th %ile)	38/149 (26%)	-	
% Body fat, mean ± SD	26.7 ± 8.4	-	
Waist circumference cm, mean ± SD	71.5 ± 11.5	-	
Asthma ^f	87/149 (58%)	83/360 (23%)	<0.001
Seroatopy ^g	74/134 (55%)	113/269 (42%)	0.01

CCCEH Columbia Center for Children’s Environmental Health, BMI Body mass index, ETS: Environmental tobacco smoke, IgE: Immunoglobulin E, SD: standard deviation

The total number of CCCEH cohort participants enrolled at birth = 727; ^aIncludes only the children in nested study that had complete data available for current analysis. Participants excluded if residential indoor sampling data not collected due to entry criteria (*n* = 564) and invalid air pollution data (*n* = 14)

^bReport of any smoker in the house during pregnancy. All mothers were nonsmokers during pregnancy

^cReport of any smoker during 1-week sampling period

^dDetermined by 24-hour questionnaire by answering “Between the time we dropped off the monitor and when you went to bed, did you leave home (Yes/No); then “What time did you leave home?”; “What time did you get home?”

^eWeight (kg)/height (m)², SD, standard deviation

^fDetermined by a specialist physician using standardized criteria at age 5–12 year [34]

^gTotal IgE ≥ 80 IU/mL

^hChi-tests performed

levels (Interquartile range IQR) of 11.9 (9.1) $\mu\text{g}/\text{m}^3$ and 1.44 (1.34) ng/m^3 of residential $\text{PM}_{2.5}$ and V, respectively. A significant seasonal pattern was detected for V. Levels of V, but not $\text{PM}_{2.5}$, were higher during the heating compared to the nonheating season (Additional file 1: Figure S2). While residential levels of $\text{PM}_{2.5}$ at Time 1 moderately correlated with those at Time 2, 6 months later, V weakly correlated with repeated measures, possibly due to substantial seasonal variations (Additional file 1: Figure S3). $\text{PM}_{2.5}$ levels weakly correlated with V levels (Fig. 3). Furthermore, indoor levels of $\text{PM}_{2.5}$ at Time 1 weakly correlated with those measured 4–8 years prior to Time 1, on the same children (Fig. 3), suggesting common chronic sources of $\text{PM}_{2.5}$ air pollution.

Buccal cell asthma gene methylation

In general, the targeted CpG sites in the IL4 promoter were heavily methylated, while the CpG sites in ARG2 were largely unmethylated; the latter is consistent with previous findings in buccal cells (Additional file 1: Figure S4) [25]. IFN γ methylation levels also were comparable to those reported in other urban cohorts [49]. Within each gene loci, two targeted CpG sites weakly correlated (Additional file 1: Figure S5). In general, repeated measures (Day 0 and Day 6) of percent methylation showed low values of ICCs (Additional file 1: Table S3), indicating substantial within-subject variability in methylation levels over the short-term, as previously described [50].

Associations among $\text{PM}_{2.5}$, V exposures and DNA methylation

Overall, $\text{PM}_{2.5}$ levels were not associated with DNA methylation at the CpG loci in IL4, IFN γ , NOS2A, and ARG2 gene in two-pollutant models (Table 2). In comparison, the relative risk of higher (i.e. upper tertile) IL4

CpG⁻³²⁶ and IFN γ CpG⁻⁵⁴ methylation decreased with higher residential V exposure in adjusted models (Table 2). Associations between V exposure and DNA methylation in NOS2A and ARG2 loci were not significant.

In exploratory analyses that stratified by overweight and asthma (i.e., four groups), there were still no significant associations between $\text{PM}_{2.5}$ and DNA methylations in the targeted asthma genes (Additional file 1: Table S4). In comparison, a distinct methylation pattern following V exposure was observed among the 4 groups. A significant association between V levels and lower methylation of NOS2A CpG⁺⁵⁰⁹⁹ was observed among overweight asthmatic children (Additional file 1: Table S5). Interestingly, the opposite pattern (higher methylation) was observed among the overweight non-asthmatic children at the IFN γ CpG⁻¹⁸⁶ locus. Further, among non-overweight asthmatic children, V levels were associated with lower methylation of IFN γ CpG⁻⁵⁴ (Additional file 1: Table S5). Methylation of ARG2 and IL4 was not associated with V levels among any of the phenotypes. Furthermore, when overweight was replaced by obesity, the observed associations of V with DNA methylation of NOS2A CpG⁺⁵⁰⁹⁹, IFN γ CpG⁻⁵⁴, and IFN γ CpG⁻¹⁸⁶ shown in Additional file 1: Table S5, remained strong (RR [95% CI]: 0.40 [0.26-0.63]; $p = 0.03$, 0.71 [0.54-0.92]; $p = 0.001$, 1.42 [1.02-1.97]; $p = 0.039$ for NOS2A CpG⁺⁵⁰⁹⁹, IFN γ CpG⁻⁵⁴, and IFN γ CpG⁻¹⁸⁶ respectively). Interestingly, a non-significant association, previously observed between V and lower methylation of IL4 CpG⁻³²⁶ among overweight asthmatic children, became significant when overweight was replaced by obesity (RR [95% CI]: 0.40 [0.24-0.68]; $p = 0.001$), high-BF (RR [95% CI]: 0.47 [0.27- 0.80]; $p = 0.006$), or high-WC in asthmatic children (RR [95% CI]: 0.21 [0.08- 0.53]; $p = 0.001$).

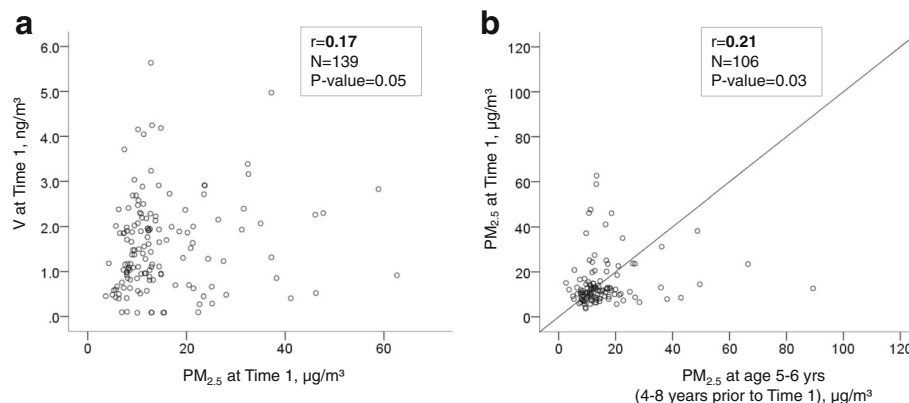


Fig. 3 Correlations between (a) $\text{PM}_{2.5}$ and V measured at Time 1, and (b) repeated residential indoor measures of $\text{PM}_{2.5}$, 4–8 years apart Of 139 children who had valid air pollution data at Time 1, 106 had previous residential indoor measurements that were collected 4–8 years ago using the same sampling methodology

Table 2 Associations between residential measures of PM_{2.5}, V and Day 6 DNA methylation in adjusted models (N:n = 146:229)

Gene	CpG sites	RR _{adj} ^a [95% CI]	
		PM _{2.5}	V
IL4	-326	1.16 [0.84–1.61]	0.80 [0.65–0.98]*
	-48	1.04 [0.76–1.43]	1.05 [0.83–1.34]
IFN γ	-186	0.87 [0.64–1.19]	1.23 [0.96–1.58]
	-54	1.03 [0.79–1.35]	0.81 [0.67–0.98]*
NOS2A	+5099	1.07 [0.79–1.45]	0.97 [0.80–1.19]
	+5106	1.07 [0.74–1.54]	0.98 [0.78–1.23]
ARG2	-32, -30, and -26 ^b	1.07 [0.97–1.17]	0.96 [0.89–1.02]

N: number of repeat subjects included for the analysis and n: number of observations from both Time 1 and Time 2

^aAdjusted for race/ethnicity, sex, age, heating season, asthma diagnosis, overweight, and DNA methylation at Day 0 (Two-pollutant models)

^bAverage of ARG2 CpG sites of -32, -30, and -26

*p-value < 0.05

PM_{2.5} and V exposures and lung function

In multivariable linear regression models, residential PM_{2.5} levels, measured over 6 days, appeared associated with decreased z-scores for FEV₁, FEV₁/FVC and FEF_{25–75}, including following adjustment for V levels (Table 3). In comparison, significant associations between V and lung function outcomes were not observed. Therefore, analysis for mediation by methylation of V on lung function was deferred.

Sensitivity analyses

First, after controlling for time spent home, the main findings in Table 2 remained similar although the association between V and methylation of IL4 CpG⁻³²⁶ became borderline significant (RR [95% CI]: 0.81 [0.66–1.00]; p = 0.052 for IL4 CpG⁻³²⁶ and 0.81 [0.67–0.97]; p = 0.026 for IFN γ CpG⁻⁵⁴). Second, with an adjustment of residential chronic exposure, assessed by previous PM_{2.5} levels measured 4–8 years prior to

Table 3 Associations between residential levels of PM_{2.5} and V and Day 6 lung function z-scores

Lung function	Beta coefficient ^a (95% CI)	
	PM _{2.5}	V
FVC	-0.07 (-0.19, 0.05)	0.07 (-0.02, 0.15)
FEV ₁	-0.15 (-0.29, -0.01)*	0.09 (-0.02, 0.19)
FEV ₁ /FVC	-0.17 (-0.31, -0.03)*	0.05 (-0.09, 0.19)
FEF _{25–75}	-0.18 (-0.32, -0.04)*	0.03 (-0.12, 0.18)

N: number of repeat subjects included for the analysis and n: number of observations from both Time 1 and Time 2

FVC Forced vital capacity, FEV₁ Forced expiratory volume in one second, FEF_{25–75} Forced expiratory flow at 25–75% of Forced Vital Capacity

^aTwo-pollutant models adjusted for heating season, asthma diagnosis, overweight, each lung function z-score measured on Day 1

^bBased on reference equation from the Global Lung Initiative 2012 [47]

*p < 0.05

Time 1, significant association between V and lower methylation of IFN γ CpG⁻⁵⁴ in Table 2 remained with a smaller RR (RR [95% CI]: 0.76 [0.63–0.92]; p = 0.005) while the association between V and methylation of IL4 CpG⁻³²⁶ lost statistical significance (p = 0.10), possibly due to a smaller sample (N_[subjects] = 115 and n_[observations] = 172). Third, when we controlled for food intake, the main findings in Table 2, persisted (Data not shown). Lastly, when the two heating vs nonheating season was replaced with four seasons, the significant associations of PM_{2.5} and lung function outcomes were replicated (data not shown).

Discussion

In this nested cohort of African American and Dominican children living in NYC, we found that that 6 day-integrated residential V, but not PM_{2.5}, was associated with lower buccal cell promoter DNA methylation of asthma T helper (Th) gene (i.e. IL4, IFN γ) loci, even after controlling for methylation levels 6 days previously. We also found that residential PM_{2.5} levels, but not V, were associated with lower lung function (i.e., z-scores for FEV₁, FEV₁/FVC and FEF_{25–75}). To our knowledge, this is the first study to report altered asthma gene DNA methylation related to residential V exposure, and lung function decrements associated with short-term residential exposure to PM_{2.5} among urban children.

The strengths of this study include the 1) direct measurement of each child’s short-term (6 day) home exposure to air pollution, that may reduce misclassification of personal exposure and allow us to discern effects of individual key air pollutants (e.g., V and PM_{2.5}), 2) use of repeat prospective measures of environmental air pollutants, DNA methylation, and lung function, which allow us to detect changes in each within the same children and consider seasonal effects (6 months apart), 3) targeted focus on the methylation of specific loci that previously were implicated in air pollution-related asthma. We did so by using pyrosequencing technology to capture small differences in their DNA methylation in order to validate the importance of these small differences to pediatric urban asthma, and 4) utilization of a well-phenotyped prospective birth cohort study with detailed data on children’s clinical status and past residential PM_{2.5} levels; the latter of which correlated over a 4–8 year time period (Fig. 3) and allowed us to use as a surrogate for residential chronic exposure to PM_{2.5} in sensitivity analyses.

We chose to focus on altered methylation of mechanistically relevant gene loci that were implicated previously in air pollution-related asthma, given the emerging environmental epigenetic literature (Additional file 1: Table S1). In cohort studies of electric furnace steel plant workers, boilermaker welders, chronic obstructive pulmonary disease (COPD) patients in China, and

children in Southern California, exposure to $PM_{2.5}$ was associated with altered NOS2A promoter region DNA methylation [20, 27, 51, 52], suggesting environmental epigenetic mechanisms. We found that residential V, but not $PM_{2.5}$, was associated with lower methylation of several Th gene promoter loci, including IL4 CpG⁻³²⁶ and IFN γ CpG⁻⁵⁴. The findings may be supported by observations in mouse CD4⁺ splenic T cells following concomitant exposure of *Aspergillus fumigatus* and diesel exhaust particles (DEP) [24], mouse CD4⁺ T cells from lung-draining lymph nodes following chronic ovalbumin challenge [53], and numerous human cohort experimental studies [54] (Additional file 1: Table S1). Because DNA methylation in promoter regions is usually critical to gene silencing in human cells [55], its decrease is consistent with upregulated expression of the proallergic immune response. In comparison, lower methylation of IFN γ CpG⁻⁵⁴, while considered important to suppressing allergic immune responses in some mouse models [56], also has been observed paradoxically to enhance allergy or airway hyperresponsiveness [53, 57]. We did not identify associations between residential $PM_{2.5}$ and altered DNA methylation. One explanation may be the lack of short-term variability in $PM_{2.5}$ compared to V, given the moderate correlation over time (Additional file 1: Figure S3), thus making it hard to capture a methylation signal due to smaller variations in $PM_{2.5}$ levels. Alternatively, our results suggest that instead of $PM_{2.5}$, a complex particle mixture of various chemical constituents from multiple sources, a specific individual metal component, V, may be one of the important drivers of epigenetic changes despite V only contributes an average of less than 1% to total $PM_{2.5}$ mass.

Further, in exploratory analyses, we observed the associations between V and select DNA methylation differed by overweight asthma stratum. In particular, V was associated with lower methylation (and presumed greater gene activation) of NOS2A CpG⁺⁵⁰⁹⁹ only among overweight or obese asthmatic children. Also, the association between V and IL4 CpG⁻³²⁶ among overweight asthmatic children gained statistical significance when restricted to obese, high BF or high WC in asthmatics. These findings appear consistent with one small cross-sectional study that showed dysregulated DNA methylation in peripheral blood mononuclear cells (PBMCs) that varied by the asthma and obesity phenotype [46]. This group found that obese asthmatics, when compared to obese non-asthmatics, exhibited altered methylation in many pathways related to IL4, IFN γ and NOS2A gene functions (as shown in Additional file 1: Table S5), including IgE signaling, and interferon and chemokine activity [58, 59]. Interestingly, in the absence of asthma, the overweight or obese children were instead susceptible to hypermethylation of IFN γ CpG⁻¹⁸⁶ following V

exposure. This may be consistent with enhanced inflammation and differential regulation of T helper responses previously observed in obesity [60]. Despite the smaller sample size in stratified analysis, our results were consistent across overweight, obese, high BF and high WC classification, suggesting that obesity may enhance susceptibility to the effect of V on epigenetic changes.

Another objective was to evaluate adverse effects of a specific metal component of V on lung function in relation to $PM_{2.5}$. Our findings that $PM_{2.5}$ seemed to drive decrements in lung function are novel because 1) to date, no studies have looked at lung function in relation to short-term exposure in children. We did this by controlling for previous lung function z-scores measured on Day 1 to assess changes in lung function over a short period, and 2) we used direct measurement of $PM_{2.5}$ through residential indoor monitoring, rather than estimates from land-use regression models employed in most studies [3, 61]. In comparison, counter to our prediction based on recent reports of V effects on lung function in children [13, 61], we were not able to detect any associations between residential V levels and lung function z-scores. But our study differed in terms of the shorter timeline of exposure to V and by the differences detected in the obstructive airway physiology characteristic of asthma (i.e., reduced FEV₁), and not FVC.

The focus on quantifying differences in pre-selected CpG specific targets also allows us to compare their potential differential impact across loci, across a short timeline of exposure, and in limited case, across studies. For example, we observed relatively weak correlations between neighboring CpG sites within the same gene at Time 1 (Additional file 1: Figure S5). This observation may challenge previous reports that DNA methylation at adjacent CpG sites tends to display similar amounts of methylation [62]. However, they are consistent with emerging evidence in humans that suggest individual CpG sites may methylate to a different extent [49]. Another group also reported, in a controlled exposure study of allergen and diesel exhaust, substantial differences in single CpG site methylation in human bronchial epithelial cells [32]. This suggests that neighboring CpG sites may respond to exposures independently.

We acknowledge several limitations. First, methylation was measured in buccal cells, previously shown to inform on airway molecular changes [20, 25], and did not compare to the target lung tissue. However, lung tissue cannot be accessed repeatedly in children, and previous studies have documented high correlations in gene expression between tissues obtained from the buccal mucosa and lung [63, 64]. The cells themselves represent a relatively homogenous population of epithelial cells, as we demonstrated. Second, we monitored residential indoor air for exposure assessment, but not outdoor or

school environments that may have important confounding effects. However, urban children in this study spent the majority (68% on daily average) of their time home, and studies have shown that outdoor PM_{2.5} and V readily penetrate indoors [65, 66]. Further, sensitivity analysis with an adjustment of time-spent home showed that our findings persisted. Third, we recognize that other CpG loci upstream or downstream of targets, and certainly other genes, may contribute to the air pollution epigenetic effects, and that the presence of single nucleotide polymorphisms (SNPs) could impact methylation levels. However, the effect sizes of variants measured in genome-wide association studies also seem small, driving our approach to capture additional small epigenetic responses. Last, other metal components of PM_{2.5} such as nickel and iron, that may important along with V in respiratory health [9, 12, 13], were not available. Nonetheless, with targeted epigenetic loci previously implicated in asthma and the use of pyrosequencing technology, we demonstrated for the first time that short-term residential V exposure is associated with changes in the degree of methylation of important asthma genes in children. This furthers our understanding of epigenetic regulation and its susceptibility to exposure to a specific air pollutant. Further, we explored associations between V and methylation by asthma-obesity and observed differential DNA methylation patterns by asthma phenotype. While intriguing, the interpretation of these results should be considered with caution due to a relatively small sample size in stratified analyses.

Additional discussions of the ambient air pollution levels, DNA methylation values, and the further study limitations are presented in Additional file 1.

Conclusions

We found associations between short-term V exposure and DNA methylation of asthma gene loci. Short-term residential indoor measures of PM_{2.5}, but not V, may have been associated with lower lung function among urban children. While previous studies have suggested possible links between metal exposure and particulate matter and differences in DNA methylation pattern relevant to asthma, none to our knowledge have specified the key component of these pollutants. Further, to the best of our knowledge, this is the first study to investigate the effect of short-term V exposure on altered DNA methylation of asthma genes, and suggests that V may be important to urban asthma via epigenetic regulation. Although requiring further investigation in a large cohort, our results suggest asthma phenotypes may need to be considered in environmental and epigenetic studies of asthma. In turn these findings ultimately may help guide policy-related or medical interventions against specific pollutants, and against obesity, as well as provide methods for early identification of at-risk children.

Additional file

Additional file 1: Table S1. Promoter region CpG locations and rationale. **Table S2.** Primers for PCR and pyrosequencing. **Table S3.** Intraclass correlation coefficients (ICC) among repeated measures of buccal cell DNA methylation. **Table S4.** Associations between residential PM_{2.5} and Day 6 DNA methylation: by asthma and overweight. **Table S5.** Associations between residential V and Day 6 DNA methylation: by asthma and overweight. **Figure S1.** Targeted CpG sites in promoter region. **Figure S2.** Seasonal variations in (a) PM_{2.5} and (b) vanadium (V). **Figure S3.** Repeated residential indoor measures of (a) PM_{2.5} and (b) vanadium (V), 6 months later. **Figure S4.** Distribution of percent DNA methylation of IL4, IFN γ , NOS2A, and ARG2 at Day 6. **Figure S5.** Correlation matrix for Day 6-buccal cell DNA methylations of IL4, IFN γ , NOS2A, and averaged ARG2 at Time 1. (DOCX 286 kb)

Abbreviations

%BF: Body fat percentages; ARG: Arginase; BC: Black carbon; BMI: Body mass index; CCEH: Columbia Center for Children's Environmental Health; COPD: Chronic obstructive pulmonary disease; DEP: Diesel exhaust particle; EC: Elemental carbon; ETS: Environmental tobacco smoke; FEF₂₅₋₇₅: Forced Expiratory Flow at 25–75% of Forced Vital Capacity; FeNO: Fractional exhaled nitric oxide; FEV₁: Forced expiratory volume in one second; FOXP3: Forkhead box transcription factor 3; FVC: Forced vital capacity; GEE: Generalized estimating equation; GLI: Global lung initiative; ICC: Intraclass correlation coefficient; IFN γ : Interferon gamma; IL4: Interleukin 4; IgE: Immunoglobulin E; IQR: Interquartile range; LINE-1: Long interspersed nuclear element-1; NO₂: Nitrogen dioxide; NOS2A: Inducible nitric oxide synthase; NYC: New York City; PAH: Polycyclic aromatic hydrocarbon; PBMCs: Peripheral blood mononuclear cells; PEFR: Peak expiratory flow rate; PFT: Pulmonary function test; PM: Particulate matter; PTFE: Polytetrafluoroethylene; RR: Relative risk; SNPs: Single nucleotide polymorphisms; V: Vanadium; WC: Waist circumference

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Availability of data and materials

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

Authors' contributions

KHJ contributed to data analyses and interpretation of results, and co-wrote this manuscript with RLM. DT contributed to the collection of samples and analysis of DNA methylation. SLD contributed to the collection of samples, interpretation of results, and critical revision of the manuscript. MP contributed to the analytical approach and critical revision of the manuscript. JB contributed to sample collection. JRJ contributed to the analysis of DNA methylation. LH managed the database. JR contributed to the analysis of vanadium and particulate matter. FPP contributed to the study design and critical revision of the manuscript. SNC contributed to the study design, analysis of vanadium and particulate matter, and provided a critical revision of the manuscript. RLM conceived of the study, supervised all study elements, helped analyze the data, and co-wrote the manuscript. All authors reviewed and approved the final manuscript.

Competing interests

None of the authors have financial relationships with a commercial entity that has an interest in the subject of this manuscript. The authors declare that they have no competing interests.

Consent for publication

No data on an individual level is provided and thus this is not applicable.

Ethics approval and consent to participate

This study has been approved by Columbia University Human Subjects protocol AAAI0459. Written informed consent to participate has been obtained from study participants or their legal guardian.

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