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Personal Fine Particulate Matter Constituents, Increased Systemic Inflammation, and the Role of DNA Hypomethylation

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Abstract

Limited evidence is available on the effects of various fine particulate matter (PM_{2.5}) components on inflammatory cytokines and DNA methylation. We examined whether 16 PM_{2.5} components are associated with changes in four blood biomarkers, that is, tumor necrosis factor- α (TNF- α), soluble cluster of differentiation 40 ligand (sCD40L), soluble intercellular adhesion molecule-1 (sICAM-1), and fibrinogen, as well as their corresponding DNA methylation levels in a panel of 36 healthy college students in Shanghai, China. We used linear mixed-effect models to evaluate the associations, with controls of potential confounders. We further conducted mediation analysis to evaluate the potential mediation effects of components on inflammatory markers through change in DNA methylation. We observed that several components were consistently associated with TNF- α and fibrinogen as well as their DNA hypomethylation. For example, an interquartile range increase in personal exposure to PM_{2.5}-lead (Pb) was associated with 65.20% (95% CI: 37.07, 99.10) increase in TNF- α and 2.66 (95% CI: 37.07, 99.10) decrease in TNF- α methylation, 30.51% (95% CI: 0.72, 69.11) increase in fibrinogen and 1.25 (95% CI: 0.67, 1.83) decrease in *F3* methylation. PM_{2.5} components were significantly associated with *sICAM-1* methylation but not with sICAM-1 protein. DNA methylation mediated 19.89%–41.75% of the elevation in TNF- α expression by various PM_{2.5} constituents. Our findings provide clues that personal PM_{2.5} constituents exposure may contribute to increased systemic inflammation through DNA hypomethylation

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Supporting Information

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Primers used for DNA methylation analysis; Materials and Methods; Pearson correlation coefficients; supporting figures and tables (PDF)

The authors declare no competing financial interest.

Graphical Abstract

INTRODUCTION

Extensive epidemiological studies have linked PM_{2.5} exposure with the hospitalization and mortality caused by various cardiovascular diseases (CVDs).^{1,2} Systemic inflammation has been considered as one common underlying pathological pathway by which PM_{2.5} affects cardiovascular health.^{3,4} Previous studies found that short-term exposure to total PM_{2.5} mass may lead to the upregulation of inflammatory cytokines.⁵ However, the involved biological mechanism remains a matter of speculation. Epigenetic alterations can influence the expression of genes without modifying the genetic code,⁶ and typically DNA hypomethylation may be involved in the development of a wide range of CVDs.⁷ Our previous panel study hinted that DNA methylation plays an important role in mediating the effect of total PM_{2.5} mass exposure on inflammatory pathways.⁸

The cardiovascular effects of integrated PM_{2.5} have been largely evaluated,^{9,10} but which constituents are most responsible for these effects remain unidentified. This is partially due to the lack of methodology for accurate exposure assessment. Traditionally, PM_{2.5} exposure assessment relied on fixed monitoring network sites or stationary indoor monitors which usually generate temporally aggregated and averaged data for a certain spatial unit.¹¹ However, these methods ignore spatial heterogeneity and the difference of activity-patterns among individuals, which could introduce bias to the results of the health study. The bias thus could be magnified for health effect evaluation of specific PM_{2.5} constituents, given that PM_{2.5} composition normally varies with individual and regions.¹²

Therefore, we conducted personal monitoring in a panel of healthy college students in Shanghai, China. We aim to identify the key PM_{2.5} chemical components responsible for the effects of total PM_{2.5} exposure on circulating cytokines relevant to systemic inflammation. Moreover, we further explored the mediation effects of specific-gene methylation in the association between inflammatory protein expression and personal PM_{2.5} constituent exposure.

MATERIALS AND METHODS

Study Population and Design.

This longitudinal panel study was carried out during the period from December 17, 2014 to July 11, 2015. We excluded (1) participants with any diagnosed cardiopulmonary diseases (including asthma, rhinitis et al. $n = 2$); (2) past or current smoker or alcohol addict ($n = 0$); (3) those who are taking any medication/supplements ($n = 1$), and participants that experienced infection within 1 month of baseline physical exam ($n = 1$). Eventually, 36 healthy students from two universities located at downtown area of Shanghai were recruited in this study. To control for the aforementioned potential confounders, all participants were asked to record the information on any use of medications, health conditions, alcohol drinking, and physical activity during the follow ups. During the study period, we scheduled four rounds of repeated measurements for personal exposure and health end points, with at least 2-week interval. The study protocol was approved (IRB No. 2014-TYSQ-09-1) by the Institutional Review Board of the School of Public Health of Fudan University, and written informed consent was obtained from each subject. Detailed information about the study design was described in our previous publication.¹³

Exposure Measurements.

For each visit, all subjects were instructed to wear a designed sampling vest equipped with HOBO data loggers (Onset Computer Corporation, Pocasset, Massachusetts) and MicroPEM (RTI International, Research Triangle Park, NC, USA). HOBO data loggers were applied to record personal temperature and relative humidity every halfhour. MicroPEM with a 0.50 (± 0.05) L/min flow rate was used to log real-time $PM_{2.5}$ concentrations every 10 s based on an on-board micronephelometer and collect a Teflon-filtered (FPTMP325, Zefon, USA) sample through a one-stage $PM_{2.5}$ impactor. Under such a low flow rate, we collected a 72 h integrated filter sample for each personal measurement to ensure sufficient amount of loading mass for chemical constitutes analysis.

Fourteen elements were measured via polarized-energy dispersive X-ray fluorescence as described by Ngo,¹⁴ including arsenic (As), barium (Ba), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), potassium (K), manganese (Mn), phosphorus (P), lead (Pb), silicon (Si), strontium (Sr), titanium (Ti), and zinc (Zn). Elemental carbon (EC) concentrations were measured at Columbia University using a multiwavelength optical measurements.¹⁵ In the absence of measurements of personal organic carbon (OC) due to the limitations of our instruments and technology, we obtained hourly concentrations of OC measured at a fixed-site of the Environmental Science Research Institute (ESRI) of Shanghai which was 0.6 km away from the residence of our participants. The ambient OC was then used as a surrogate of OC exposure. Ambient OC concentrations were measured by a semicontinuous OC/EC analyzer (model 4G, Sunset Laboratory, OR, USA). More detailed quality assurance/quality control procedures of component measurements were routinely conducted as described in the Supporting Information SI Materials and Methods and our previous publication.¹⁶

DNA Methylation and Biomarker Measurements.

For each visit, we collected venous peripheral blood samples after 72 h personal sampling for DNA methylation and biomarkers measurements. According to our previous studies,^{8,17} we selected four inflammatory proteins, including tumor necrosis factor- α (TNF- α), intercellular adhesion molecule-1 (ICAM-1), soluble cluster of differentiation 40 ligand (sCD40L), and fibrinogen. The serum levels of TNF- α , ICAM-1, and fibrinogen protein were detected by Millipore MILLIPLEX MAP human cytokine/chemokine kit (Millipore Corporation, Billerica, MA, USA). sCD40L expression was determined using enzyme-linked immune sorbent assays as in our previous paper.¹

We measured DNA methylation levels of *TNF- α* , *ICAM-1*, and *sCD40L* at the two to five CpG positions. For fibrinogen protein, we measured DNA methylation levels of *F3* instead, as done in previous studies,^{17,18} because fibrinogen does not have corresponding methylation loci to examine. In addition, Bind et al.¹⁸ documented that *F3* hypomethylation may participate in pathways leading to increased fibrinogen in response to air pollution. The locations of the amplified region and each candidate CpG loci are listed in SI Table S1. Information about the primer sequences are provided in SI Table S2. Two milliliters plasma samples were used for DNA extraction using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany), and the concentration of purified DNA was determined using a ND1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). We modified aliquots of 300 ng of DNA with sodium bisulfite using the EpiTect Fast DNA Bisulfite Kit (Qiagen, Hilden, Germany) in a final elution volume of 10 μ L M-Elution buffer. The DNA methylation level was evaluated using highly quantitative bisulfite-PCR pyrosequencing by the PyroMark system (Qiagen, Hilden, Germany). The methylation degree of each CpG dinucleotide was presented as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines at position 5 (%5mC). More details of DNA methylation and cytokine measurements have been reported elsewhere^{8,17} and in the SI Materials and Methods.

Statistical Analysis.

We used a linear mixed-effect (LME) model to estimate association between constituent-specific PM_{2.5} concentrations and inflammatory markers as well as DNA methylation. All cytokine concentrations were natural log-transformed before statistical analysis to better approximate a normal distribution. In the basic single-constituent model, PM_{2.5} constituents entered the model as fixed-effect independent variables one at a time, and several covariates including age, gender, body mass index (BMI), 24 h temperature, 24 h relative humidity, and season were also incorporated as fixed-effect predictors. In addition, a random participant-specific intercept was introduced to explain the within-subject correlations of repeated measurements. Moreover, a “constituent-PM_{2.5} joint model” with the adjustment of total PM_{2.5} mass (72 h average) was also built to estimate the effects of constituents independent of PM_{2.5}. The Benjamini–Hochberg false discovery rate (FDR) was used for the correction of simultaneous multiple comparisons in dealing with multiple constituent-specific associations estimations.

All LME models were conducted using the package “lme4” in R (version 3.5.0, R development Core Team). The effect estimation for blood biomarkers and methylation were expressed as the mean percent changes and the absolute changes with their 95% confidence intervals (CIs) associated with an interquartile-range (IQR) increase in PM_{2.5} constituent concentrations. Statistical tests were two-sided, and *P*-value 0.05 and 0.10 were considered “significant” and “marginal significant”, respectively.

We also conducted mediation analyses to evaluate the potential mediation effects of DNA methylation on the association between a component and an inflammatory biomarker. For this analysis, we used the existing “mediation” package in R. Detailed information about the methods and materials are described in SI Materials and Methods.

RESULTS

Descriptive Statistics.

Details of the descriptive characteristics of 36 subjects were shown in SI Table S3. Females accounted for nearly 61% (22/36). The average ($\pm$ standard deviation, SD) age of our participants was 24 ± 2 years and the average ($\pm$ SD) BMI was 21 ± 3 kg/m², respectively (SI Table S3). Results from self-reported questionnaires showed that none of them participated in strenuous physical activity, smoked, drank alcohol, or took medication or supplements during the study period. Because one participant missed the last visit, a total of 143 valid measurements on both exposure and health were eventually collected.

Table 1 provides the summary statistics of personal PM_{2.5} constituents, temperature, and relative humidity. During the study period, the 72 h mean ($\pm$ SD) mass concentration of total PM_{2.5} was $45.73 (\pm 31.63) \mu\text{g}/\text{m}^3$. Concentrations of PM_{2.5} constituents vary considerably. Carbonaceous components (OC and EC) were the most abundant composition with a mean ($\pm$ SD) of $8.00 (\pm 4.92) \mu\text{g}/\text{m}^3$ and $6.14 (\pm 2.84) \mu\text{g}/\text{m}^3$, respectively. Among elements of PM_{2.5}, Fe was the most major component with a mean ($\pm$ SD) of $0.85 (\pm 0.45) \mu\text{g}/\text{m}^3$, followed by Si with $0.67 (\pm 0.35) \mu\text{g}/\text{m}^3$. Our findings were comparable to previous studies reported in Hong Kong¹² and Beijing.¹⁹ In addition, we observed correlations among constituents varied with constituents but they were all statistically significant at 1% levels (SI Table S4).

Table 2 provides descriptive statistics for four circulating inflammatory cytokines and average DNA methylation. Mean ($\pm$ SD) concentrations of TNF- α , sCD40L, fibrinogen, and ICAM-1 were $4.22 (\pm 5.27) \text{ pg}/\text{mL}$, $121.50 (\pm 86.61) \text{ pg}/\text{mL}$, $3.03 (\pm 3.58) \mu\text{g}/\text{mL}$, and $172.80 (\pm 98.31) \text{ ng}/\text{mL}$, respectively.⁸ The methylation levels and correlation coefficients of CpG loci pairs for *TNF- α* , *ICAM-1*, *CD40L* genes have been reported in our previous work,⁸ and the corresponding detailed information for *F3* refers to SI Table S5 and SI Figure S1. The mean ($\pm$ SD) level of *TNF- α* , *CD40L*, *F3*, and *ICAM-1* genes at different CpG loci were $17.28 (\pm 6.60) \%5\text{mC}$, $48.25 (\pm 12.46) \%5\text{mC}$, $3.67 (\pm 2.62) \%5\text{mC}$, and $3.49 (\pm 2.29) \%5\text{mC}$, respectively. Although the methylated proportions differed across candidate loci, they were highly correlated with each other (all correlation coefficients ranged from 0.58 to 0.92, *P* < 0.05). They may share most of the same functional complexes and traits; we hence introduced the average methylation levels across sites into the model. The correlation

coefficients for the four proteins versus the DNA methylation levels at corresponding upstream candidate CpG sites are shown in SI Table S6. Significant negative correlations were found between *TNF- α* methylation and TNF- α protein, between *F3* methylation and fibrinogen protein.

Regression Results.

The effect on four circulating inflammatory biomarkers varied with constituents and with biomarkers. The percent changes in four inflammatory proteins per an IQR increase in specific 16 PM_{2.5} constituents' concentrations are illustrated in Figure 1. We observed significant positive associations of all PM_{2.5} chemical components with at least one circulating biomarker. sICAM-1 protein was not associated with any constituent; in contrast, TNF- α protein was significantly correlated with all PM_{2.5} constituents. Especially, an IQR increase in personal exposure to Pb was associated with increases of 42.43% (95% confidence interval (CI): 4.27, 94.57) ($P = 0.03$) and 65.20% (95% CI: 37.07, 99.10) ($P < 0.01$) in sCD40L and TNF- α , respectively. An IQR elevation in personal exposure to Si was associated with increases of 44.46% (95% CI: 16.12, 79.71) in fibrinogen.

Figure 2 presents the changes in DNA methylation associated with an increase in the 72 h average concentration of PM_{2.5} constituents. We observed a significant elevation in all 16 components associated with the immediate decrease in at least one circulating inflammatory methylation, except for Cr which was negatively linked with the *sICAM-1* methylation with marginal significance ($P = 0.10$). For example, an IQR increase in personal exposure to K and Pb was associated with decreases of 1.28 (95% CI: 0.45, 2.11) ($P < 0.01$) and 1.25 (95% CI: 0.67, 1.84) ($P < 0.01$) in CD40L methylation and *F3* methylation (%5mC), respectively. Si was associated with both 1.21 (95% CI: 0.65, 1.76) ($P < 0.01$) and 3.25 (95% CI: 1.67, 4.82) ($P < 0.01$) decrease in *sICAM-1* and *TNF- α* methylation (%5mC), respectively.

Notably, after correcting for multiple comparisons (Table S7), we found robust associations between PM_{2.5} constituents and TNF- α cytokines; while, the important associations between PM_{2.5} constituents and sCD40L cytokines as well as *CD40L* methylation turned to be borderline significant or insignificant. The magnitude of the associations between PM_{2.5} constituents and fibrinogen, ICAM-1 cytokines, and DNA methylation was slightly attenuated, and the 95% CIs became wider. Furthermore, except for the sCD40L protein, the significant associations between PM_{2.5} constituents and other inflammation markers as well as the methylation of candidate genes did not substantially change in the constituent-PM_{2.5} joint model (SI Figures S2 and S3).

The average methylation level of TNF- α (Pearson $r = -0.46$, $P < 0.01$) and *F3* (Pearson $r = -0.21$, $P < 0.05$) were significantly inversed with their corresponding proteins. These relationships suggested that hypomethylation in *TNF- α* and *F3* genes has the potential to indirectly mediate the effects of the PM_{2.5} constituent on the protein. Table 3 and SI Table S8 show the results of mediation analysis. The mediated effects of *TNF- α* methylation in the associations between PM_{2.5} constituents and TNF α protein varied greatly among various constituents. Reduced methylation of *TNF- α* significantly mediated from 19.89% (Cu) ($P = 0.03$) to 41.75% (Ca) ($P = 0.05$) of the association between personal exposure to PM_{2.5}

components and the elevation in TNF- α protein. However, we did not observe a significant mediation effect of *F3* methylation ($P > 0.10$).

DISCUSSION

Previously, we have reported positive associations between integrated PM_{2.5} exposure and the levels of inflammatory cytokines in this group of participants.⁸ Our current results identified that specific constituents of PM_{2.5} exposure substantially contributed to these associations, which has implications for developing air pollution abatement strategies to maximize public health benefits. Moreover, this study linked PM_{2.5} constituents to inflammation methylation on genes related to inflammation pathways, and the mediation analysis provided further insight into the potential importance of the association between PM_{2.5} exposure and gene-specific methylation.

Systemic inflammation has been suggested as a common underlying pathway of cardiovascular diseases caused by PM_{2.5} exposure.²⁰ In this study, we selected an informative set of circulatory cytokines (TNF- α , sICAM-1, sCD40L, and fibrinogen) which are well-established indicators of systemic inflammation and could predict cardiovascular disease risk.^{21,22} These biomarkers have been linked with PM_{2.5} concentrations;²³ however, the results are not consistent.²⁴ The inconsistency of the observations may be partially due to the outdoor fixed-site measurements that were used to assess exposure in these studies which may cause exposure misclassification.²⁵ In our study, personal sampling was conducted to minimize the bias. Our observation that TNF- α and sCD40L were associated with a set of PM_{2.5} components further confirmed our previous findings of acute inflammation following personal exposure to total PM_{2.5}.⁸ In contrast, none of components were identified to be significantly associated with sICAM-1. This suggests that other components (such as ion fraction) that were not examined in this study may be responsible for sICAM-1 expression; that is, Bind et al.²⁶ observed that exposure to sulfate could cause sICAM-1 protein overexpression.

We found consistent positive associations between the following PM_{2.5} components and selected inflammatory biomarkers in both the basic single-constituent model and the constituent-PM_{2.5} joint model. EC was found to be associated with sCD40L, fibrinogen, and TNF- α increase. The effect on TNF- α and fibrinogen remained robust after accounting for multiple comparisons and adjustment for PM_{2.5}, supporting the finding from our prior study in a panel of 28 urban residents in Shanghai.²³ Similar results were reported elsewhere.^{27–29} In the metropolis cities, EC is a strong marker of vehicle exhaust. Lall et al. found that 2.8 $\mu\text{g}/\text{m}^3$ increased in traffic-related PM_{2.5} exposures were positively associated with same day total cardiovascular admissions (RR = 1.041; 95% CI, 1.005–1.077) across disease-specific subcategories: stroke and heart failure.³⁰ Thus, our results suggest that the traffic emission might be partly responsible for the influence of PM_{2.5} exposure on cardiovascular system disease through inflammatory response.

Although crustal elements only account for a small portion of PM_{2.5} mass, five of them were observed to have stronger influences on inflammatory biomarkers, including K, Ca, P, Si, and Sr. Similar effects were also found in transition metals (Cu and Ti) and heavy metals (As

and Pb). Among these elements, K and P could be traced to biomass burning, Pb and As are widely recognized as markers of industry sources and mobile combustion,³¹ and Ca, Sr, and Si mostly are attributed to emissions from mixed sources of construction dust, soil, and industry processes.^{32,33} Our analyses are in line with the previous findings. For example, Wu et al. found that a significant increase in fibrinogen is associated with dust and soil-source high loading of Ca, Ba, Sr and Ti in Beijing among a panel of 40 healthy university students.³⁴ Toxicological and pathological studies documented significant associations of Cu, Ca, K, Si, and Ti with inflammatory responses, and these components may possess the ability to induce toxic effects in a cardiopulmonary system.^{35,36} Rich et al. reported that increased acute cardiovascular hospitalization rates were associated with increased concentrations of road dust, which showed high explained variations and concentrations for Si, Ca, Ti, and Cu.^{37,38} Thus, our findings provide evidence that exposure to the aforementioned trace elements could increase cardiovascular risks. These results also highlight that specific sources of pollution (i.e., dust, motor vehicles, biomass combustion, and industry) should be of particular concern.

It is not clear how systemic inflammation and adverse cardiovascular outcomes are triggered by PM_{2.5} exposure. A recent research has identified that DNA methylation can be a potential biological mechanism to explain adverse health effects from air pollution.³⁹ This hypothesis could be supported by our observation that a similar set of compositions was associated with hypomethylation of genes coding *TNF-α* and *F3* proteins. Our mediation analysis further confirmed that decreased *TNF-α* methylation potentially mediated the elevated level of the *TNF-α* protein of several PM_{2.5} constituent exposures, including EC, As, Ca, Cu, K, P, Pb, Si, Sr, Ti, and Zn. Especially, the mediation effects for these compositions were more than 30%. However, the mediated effect of *F3* methylation was not significant, which may be due to the much smaller variation of *F3* methylation (2.62 vs 6.60 for *TNF-α* methylation). We did not observe any mediation effects of sCD40L and sICAM-1, which may be impacted by other biological processes.

The current study has several strengths. First, this longitudinal panel study measured PM_{2.5} chemical components in individual levels, which offers an accurate exposure assessment. Second, this study simultaneously evaluated the variation of circulating gene-specific methylation and the expression of the associated inflammatory cytokine after PM_{2.5} constituent exposure, which provides a unique opportunity for a preliminary assessment of the potential mediation of methylation epigenetics in the cardiovascular effects of PM_{2.5}. Third, all the participants were healthy nonsmoking young college students in the present study, which minimizes the impacts of several confounders, such as unhealthy lifestyle, medication use, chronic disease, ect.

Several limitations still exist in our study. First, because the personal exposure measurement is expensive and burdensome, we only recruited 36 participants, which somewhat limited our statistical power and precision. Second, OC concentrations monitored at a fixed-site were used to substitute personal OC exposure levels, which may slightly attenuate the accuracy of assessment of the relationships between the OC fraction and the biomarkers. Third, we only obtained the 72 h concentrations of personal PM_{2.5} constituent exposure, other exposure time windows (e.g., 24 h) thus cannot be tested in this study. Finally, we did

not evaluate the effects of ions (e.g., SO_4^{2-} , NO_3^-) and gaseous air pollutants (SO_2 , NO_2 and O_3), which might also influence DNA methylation and the protein levels, due to the limitation of instruments.

In summary, this longitudinal panel study highlighted that *TNF- α* hypomethylation might play a modest mediate role in the effects of personal exposure to carbon and trace element components exposure (EC, As, Ca, Cu, K, P, Pb, Si, Sr, Ti, and Zn) on increased *TNF- α* expression. Our results provide evidence for the speculation that methylation epigenetic regulation may be one of the key physiological mechanisms linking $\text{PM}_{2.5}$ exposure and adverse cardiovascular outcome. More epidemiological and toxicological studies with comprehensive analysis of the exposure, biomarker variables, and organ and tissue responses to specific $\text{PM}_{2.5}$ constituents and mixtures are required to replicate our findings and to decipher the biological mechanism in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Percent changes in four inflammation proteins associated with an interquartile range increase in personal 72 h average concentrations of PM_{2.5} constituents in the single-constituent model. Abbreviations: TNF- α , tumor necrosis factor alpha; *sICAM-1*, soluble intercellular adhesion molecule-1; *sCD40L*, soluble cluster of differentiation 40 (CD40) ligand; CF, carbonaceous fractions; HM, heavy metals.

Figure 2. Changes in DNA methylation (5mC) of four inflammation genes associated with an interquartile range increase in personal 72 h average concentrations of PM_{2.5} constituents in the single-constituent model. Abbreviations: *TNF-α*, tumor necrosis factor alpha; *sICAM-1*, soluble intercellular adhesion molecule-1; *CD40L*, cluster of differentiation 40 (CD40) ligand; *F3*, tissue factor; CF, carbonaceous fractions; HM, heavy metals.

Table 1.

Descriptive Statistics on Personal PM_{2.5} and PM_{2.5} Chemical Constituents, And Weather Variables for the Study Participants (*n* = 36) in Shanghai, China, 2014–2015^a

variables	mean	SD	min	median	max	IQR
PM _{2.5} , µg/m ³	45.73	31.63	8.43	36.18	139.95	32.92
Carbonaceous Fractions						
OC, µg/m ³	8.00	4.92	1.07	6.91	25.22	5.75
EC, µg/m ³	6.14	2.84	1.06	5.73	15.33	3.11
Crustal Elements						
Ca, ng/m ³	393.13	196.83	78.12	352.53	1145.61	192.99
K, ng/m ³	550.21	377.78	51.57	442.86	1856.69	296.93
Si, ng/m ³	2.63	1.99	0.00	2.29	9.39	1.92
Ba, ng/m ³	31.97	19.02	7.41	26.84	108.79	15.35
Al, ng/m ³	666.87	350.94	74.09	622.59	1588.21	489.50
P, ng/m ³	17.56	10.61	2.57	15.60	87.86	11.62
Transition Metals						
Fe, ng/m ³	848.07	450.93	116.77	754.74	2492.30	518.56
Zn, ng/m ³	159.33	114.85	23.03	126.31	713.79	100.58
Cu, ng/m ³	18.74	11.19	3.80	16.56	90.81	10.80
Ti, ng/m ³	21.100	10.07	1.94	20.85	45.44	11.98
Cr, ng/m ³	7.88	5.28	0.71	7.06	30.84	5.10
Mn, ng/m ³	64.89	32.93	11.30	60.06	205.23	41.90
Heavy Metals						
As, ng/m ³	2.43	2.04	0.00	1.80	10.18	2.37
Pb, ng/m ³	38.79	34.11	0.37	28.35	145.08	31.20
temp, °C	22.44	4.64	10.24	24.60	28.54	6.81
relative humidity, %	55.13	11.13	31.04	54.20	75.96	17.58
temp, °C ^b	22.40	4.37	10.50	24.16	29.22	6.35
relative humidity, % ^b	55.31	9.80	26.31	56.48	75.66	6.44

^aDefinition of abbreviations: SD, standard deviation; IQR, interquartile range.

^bData are presented as the 24 h average of weather conditions before blood sample collection

Table 2.

Descriptive Statistics on Inflammation-Related DNA Methylations and Cytokines for the Study Participants ($n = 36$) in Shanghai, China, 2014–2015^a

variables	mean	SD	min	median	max
Cytokines					
TNF- α , pg/mL	4.22	5.27	0.66	2.44	38.88
ICAM-1, ng/mL	172.80	98.31	24.92	143.80	574.40
sCD40L, pg/mL	121.50	86.61	0.33	111.00	372.00
fibrinogen, μ g/mL	3.03	3.58	0.88	1.88	26.83
Gene-Specific Methylation					
<i>TNF-α</i> methylation, %5mC	17.28	6.60	6.61	15.14	35.43
<i>ICAM-1</i> methylation, %5mC	3.49	2.29	0.00	2.91	8.88
<i>CD40L</i> methylation, %5mC	48.25	12.46	25.73	55.71	63.64
<i>F3</i> methylation, %5mC	3.67	2.62	0.00	2.92	9.40

^aDefinition of abbreviations: SD, standard deviation; TNF- α , *TNF* encoding tumor necrosis factor- α ; ICAM-1, *ICAM1* encoding intercellular adhesion molecule-1; sCD40L, *CD40LG* encoding soluble cluster of differentiation 40 (CD40) ligand; *F3*, *F3* encoding tissue factor.

Table 3.

Mediated Effects of *TNF- α* Methylation in the Associations between Personal PM_{2.5} Compositions Exposure and *TNF- α* Protein

PM _{2.5} constituents → <i>TNF-α</i> methylation → <i>TNF-α</i> protein				
pathway	mean (%)	95% CI lower (%)	95% CI upper (%)	<i>P</i> -value
EC	29.34	−7.90	76.00	0.10
As	33.50	9.30	69.00	0.02
Ca	41.75	−0.53	139.00	0.05
Cu	19.89	3.89	39.00	0.03
K	27.00	13.10	48.00	0.00
P	26.53	6.84	48.00	0.02
Pb	25.21	9.88	48.00	0.00
Si	34.70	17.50	59.00	0.00
Sr	27.18	6.27	57.00	0.01
Ti	32.40	12.10	74.00	0.00
Zn	20.27	0.43	40.00	0.04