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Altered Methylation in Tandem Repeat Element and Elemental Component Levels in Inhalable Air Particles

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Abstract

Exposure to particulate matter (PM) has been associated with lung cancer risk in epidemiology investigations. Elemental components of PM have been suggested to have critical roles in PM

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LH, CD, SW, JMC, AD, JS, and AAB developed and supervised the in-field operations.
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toxicity, but the molecular mechanisms underlying their association with cancer risks remain poorly understood. DNA methylation has emerged as a promising biomarker for environmental-related diseases, including lung cancer. In this study, we evaluated the effects of PM elemental components on methylation of three tandem repeats in a highly-exposed population in Beijing, China. The Beijing Truck Driver Air Pollution Study was conducted shortly before the 2008 Beijing Olympic Games (June 15-July 27, 2008) and included 60 truck drivers and 60 office workers. On two days separated by 1-2 weeks, we measured blood DNA methylation of *SATα*, *NBL2*, *D4Z4*, and personal exposure to eight elemental components in $PM_{2.5}$, including aluminum (Al), silicon (Si), sulfur (S), potassium (K), calcium (Ca) titanium (Ti), iron (Fe), and zinc (Zn). We estimated the associations of individual elemental component with each tandem repeat methylation in generalized estimating equations (GEE) models adjusted for $PM_{2.5}$ mass and other covariates. Out of the eight examined elements, *NBL2* methylation was positively associated with concentrations of Si (0.121, 95%CI: 0.030; 0.212, FDR=0.047) and Ca (0.065, 95%CI: 0.014; 0.115, FDR=0.047) in truck drivers. In office workers, *SATα* methylation was positively associated with concentrations of S (0.115, 95%CI: 0.034; 0.196, FDR=0.042). PM-associated differences in blood tandem-repeat methylation may help detect biological effects of the exposure and identify individuals who may eventually experience higher lung cancer risk.

Keywords

Tandem repeats; DNA methylation; lung cancer

Introduction

Particulate matter (PM) is a complex mixture of small particles composed of organic chemicals, soots, acids, metals, and soil or dust particles (EPA 2013). Ambient and occupational exposure to PM has been consistently associated with increased lung cancer risks (Dockery et al. 1993; Vineis and Husgafvel-Pursiainen 2005; Lepeule et al. 2012). Previous studies have observed association of increased lung cancer risk with occupational exposure to metals and other toxic components, such as aluminum (Al), silicon (Si), sulfur (S), and calcium (Ca) (Lee et al. 2002; Koh et al. 2011; Rachiotis et al. 2012; Tseng et al. 2012; Raaschou-Nielsen et al. 2013), indicating that such elemental components may have critical roles in determining PM toxicity. Although the carcinogenic potential of several toxic metals in PM has been well-recognized, the molecular mechanisms underlying their association with cancer risk remain poorly understood.

Experimental and epidemiologic studies suggest that PM mass and metal components may induce oxidative stress, immune deficiency, chronic inflammation, and other carcinogenesis-related biological processes that may alter gene expression via DNA methylation mechanism (Baccarelli and Bollati 2009). Therefore, DNA methylation, the addition of methyl groups to cytosine to form 5-methyl-cytosine (5mC), has emerged as one of the primary epigenetic mechanisms in the development of human cancers (Jones and Baylin 2002; Zhu et al. 2011). There are many different types of DNA repetitive elements, including interspersed repeats, such as long interspersed nucleotide (*LINEs*) and Alu repetitive elements (*Alu*), as well as tandem repeats that are present as long and uninterrupted clustered sequences (i.e. *SATα*,

NBL2, *D4Z4*) (Choi et al. 2009). In previous studies, blood DNA hypomethylation in LINE1 and Alu has been reported in individuals exposed to low-dose benzene (Bollati et al. 2007), while blood DNA hypermethylation in Alu and LINE1 has been found in coke-oven workers exposed to polycyclic aromatic hydrocarbon (PAH) (Pavanello et al. 2009). In elderly men exposed to low levels of arsenic, *LINE1* methylation tended to decrease, while *Alu* methylation tended to increase with increasing arsenic exposure (Lambrou et al. 2012). Based on previous findings showing blood LINE1 and *Alu* methylation can either decrease or increase in relation to different potential carcinogens, methylation assays have been widely proposed as biomarkers of environmental exposure (Choi et al. 2009).

Tandem repeats located in (peri) centromeric regions maintain centromere function and stability of all human chromosomes (Armour 2006; Schmidt and Anderson 2006; Rando and Verstrepen 2007). Relative to most regions of the genome, tandem repeats display a greater propensity to mutate, and variable methylation may influence their mutagenicity rates. Tandem repeats located in coding regions have been shown influence on expression of genes, including cancer-related genes (Fondon and Garner 2004; Verstrepen et al. 2005; Zhou et al. 2011; Xiang et al. 2012). Several human studies investigated tandem repeat methylation in cancer tissues, and some reported hypomethylation in bladder and lung cancers (Choi et al. 2009; Carvalho et al. 2012), while others reported hypermethylation in Wilm's tumor and ovarian cancer (Nishiyama et al. 2005; Tsumagari et al. 2008; Choi et al. 2009). Global hypomethylation of DNA, such as *LINE1*, may lead to increase of its transcription, as well as increased transposition of other cancer-related genomic loci, which may ultimately result in mutational events (Choi et al. 2009). As both tandem and interspersed DNA repeats exhibit cancer-associated methylation changes, they may contribute together to cancer or environmental-related diseases through chromosomal rearrangements, gene expression changes, and other cancer-related changes (Choi et al. 2009; Ehrlich 2009). However, evidence of environmental exposure and methylation in tandem repeat element remains limited.

According to the World Bank Indicators, Beijing ranks among the 15 cities with the highest levels of air pollution worldwide (World Bank 2011). Traffic-derived PM and its elemental components are particularly important in Beijing due to very high population density and burgeoning vehicular traffic (Yu et al. 2011). Transported particles from industrial sources and windblown dust are also major sources of pollution (Yu et al. 2011). Thus, our study in a highly exposed population may help to identify changes in DNA methylation that might not be consistently demonstrated in populations with lower exposures.

In the present study, we investigated 60 truck drivers and 60 indoor workers from the Beijing Truck Driver Air Pollution Study (BTDAS) to evaluate whether the concentration in total $PM_{2.5}$ mass of eight elemental components, including aluminum (Al), silicon (Si), sulfur (S), potassium (K), calcium (Ca), titanium (Ti), iron (Fe), and zinc (Zn), is associated with methylation levels assessed in three tandem repeats that have been linked with cancer (Nishiyama et al. 2005; Tsumagari et al. 2008; Choi et al. 2009), i.e., satellite repeat alpha *SATα*, macrosatellite repeat *D4Z4* and non-satellite repeat *NBL2* (Kondo et al. 2000; Tremblay et al. 2010). Several of these components represent particles from different sources and with different composition (EPA 2006). Al, Ca, and to some extent Fe are from

crustal sources. They can represent road dust, which in addition to crustal elements contains organic and other material from traffic emissions, tire wear, and brake wear. Dust from the Gobi desert can also be high in these elements, and also in biological components. Fe can also be emitted from fuel combustion, and K is usually a tracer for biomass burning. S is predominantly from coal combustion. The two groups in BTDAS had both high exposure levels and were selected to sample on different types of exposures: truck drivers are directly exposed to traffic emissions, particularly, from diesel exhausts and road dusts; office workers were included as a sample representative of the highly-exposed urban residential population of Beijing. To enhance power to identify PM effects on tandem-repeat methylation, we studied each participant on two different examination days 1-2 weeks apart, and assessed their exposure on the days of the exam using personal measures of the eight elemental components.

Methods

Study Population and Design

The Beijing Truck Driver Air Pollution Study (BTDAS), conducted between June 15 and July 27, 2008, included 60 truck drivers and 60 indoor office workers. All study participants worked and lived in the Beijing metropolitan area and had been on their current jobs for at least two years. Truck drivers and indoor office workers were similar for their distributions by age, sex, smoking, and education. In-person interviews using a detailed questionnaire were conducted to collect information on demographics, lifestyle, and other exposures. Information on time-varying factors, including tea, alcohol, and smoking, was obtained for past usual exposure, as well as for each examination day. Because PM levels are highly variable on a day-to-day basis, we examined all participants on two work days separated by 1-2 week periods. Individual written informed consent was obtained from all participants prior to enrollment in the study. Institutional Review Board approval at all participating institutions was obtained prior to study participant recruitment.

Elemental Component Measurements

Personal $PM_{2.5}$ exposure was measured on both examination days using low weight gravimetric samplers worn by the study participants during the eight hours of work, as previously reported (Baccarelli et al. 2011). The sampler was carried in a belt pack with the inlet clipped near the breathing zone. Each sampler setup included an Apex pump (Casella Inc., Bedford, UK), a Triplex Sharp-Cut Cyclone (BGI Inc., Waltham, Massachusetts), and a 37-mm Teflon filter placed on top of a drain disc and inside a metal filter holder. The filters collecting $PM_{2.5}$ were taken from the gravimetric samplers, and kept under atmosphere-controlled conditions before and after sampling. PM elemental components were measured on the $PM_{2.5}$ collected on the filters using a XRF PANanalytical Epsilon 5 Analyzer (Almelo, Netherlands), as described previously (Chow and Watson 1998; Watson et al. 1999). For the present analysis, we selected eight elements, i.e., Al, Si, S, K, Ca, Ti, Fe, and Zn, that showed the highest reproducibility $r>0.75$) in our duplicate QC samples from a subset of 24 participants who wore two monitors at the same time (Supplementary Figure 1).

Sample Preparations and DNA Extraction

Peripheral blood was collected form each participant on both examination days. Buffy coat was separated within 2 hours and stored locally at -80°C. DNA was extracted from buffy coat using the Wizard Genomic DNA purification kit (Promega, Madison, WI) following manufacturer's instructions. Purified DNA was resuspended on the kit hydration solution, quantified and stored at -20°C until analysis.

DNA Methylation Analysis

500ng DNA was bisulfite treated using the EZ-96 DNA Methylation-Gold Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. Final elution was performed with 30 μl M-Elution Buffer. DNA methylation was quantified using bisulfite polymerase chain reaction (PCR) and pyrosequencing. The detailed primers and conditions were described previously (Choi et al. 2009). In brief, a 30 μl-PCR was carried out in 15 μl GoTaq Hot Start Green Master Mix (Promega, Madison, WI), 10 pmol forward primer, 10 pmol reverse primer, 1 μl bisulfite-treated genomic DNA and water. Pyrosequencing was performed using the PyroMark Q96 MD Pyrosequencing System (QIAGEN, Germantown, MD). The percentage of methylated and unmethylated cytosines was quantified for three CpG sites from *SATα*, four CpG sites from *NBL2*, and four CpG sites from *D4Z4*. The degree of methylation was expressed as percentage of methylated cytosines divided by the sum of methylated and unmethylated cytosines (%5mC) measured in each individual sample.

Statistical Analysis

The characteristics of truck drivers and office workers were summarized using standard descriptive statistics. An average of all CpG sites methylation levels at any of the tandem repeats investigated was used as outcome. Generalized estimating equations (GEE) (Zeger et al. 1988) were used to account for repeated measures and estimate group-specific tandem repeat methylation means and 95% Confidence Intervals (CIs). GEE take into account dependence between outcomes by treating repeated measures for each participant as a cluster. We fitted unadjusted models as well as models adjusted for sex (male/female) and age (continuous), two known factors that influence DNA methylation patterns (El-Maarri et al. 2007; Boks et al. 2009; Liu et al. 2010), BMI (continuous), cigarettes smoked during examination time (continuous), and usage of central heating (yes/no). We also used the GEE method to model the associations of inhaled toxic metals with each of the tandem repeat methylation scores, adjusted for measure day, sex, age, BMI, cigarettes smoked during examination time, and usage of central heating. In addition, $PM_{2.5}$ was adjusted in the models as a potential confounder (Mostofsky et al. 2012). Considering the high correlations between $PM_{2.5}$ and inhaled toxic metals (Supplementary Table 1), each component exposure was evaluated individually to minimize the effect of multi-collinearity. The Benjamini and Hochberg (BH) procedure was applied to account for multiple tests of significance. FDR of less than 0.05 was considered noteworthy. All analyses were performed in SAS 9.2 (SAS Institute Inc., Cary, NC).

Results

Characteristics of Study Participants

The characteristics of the 60 office workers and 60 truck drivers are shown in Table 1. Truck drivers were moderately older than office workers. Truck drivers had higher BMI, higher $PM_{2.5}$ mass exposure, smoked more cigarettes during the examination time, and a higher proportion of central heating use.

Personal Inhaled Element Component Levels

Table 2 shows the levels and distribution of personal time-weighted average exposure to inhaled elemental components estimated during eight work hours. All the eight components were higher in truck drivers than that in office workers.

Tandem Repeat Methylation in Truck Drivers and Office Workers

Truck drivers and office workers showed no significant differences in sites-combined average methylation levels of *D4Z4*, *NBL2*, and *SATα* tandem repeats in unadjusted analysis, as well as in analysis adjusted by measure day, age, sex, BMI, number of cigarettes smoked during examination time and usage of central heating (Table 3). Further analysis on tandem repeat methylation levels at individual CpG sites within each of the tandem repeat did not show any statistically significant difference between truck drivers and office workers (Supplementary Table 2).

Association of Tandem Repeat Methylation with Personal Elemental Component Level

We evaluated the association of elemental component exposure measures with tandem repeat methylation by fitting models in office workers or truck drivers separately. All results are expressed as changes in methylation of tandem repeat associated with 10% increase in the elemental components exposure and are covariate-adjusted and considered noteworthy at FDR<0.05. *NBL2* methylation was positively associated with concentrations of Si (0.121, 95%CI: 0.030; 0.212, FDR=0.047) and Ca (0.065, 95%CI: 0.014; 0.115, FDR=0.047) in truck drivers (Table 4). In office workers, *SATα* was positively associated with concentrations of S (0.115, 95%CI: 0.034; 0.196, FDR=0.042) (Table 5). No noteworthy association was found for *D4Z4* (data not shown).

We further examined the interaction effects of group (truck drivers or office workers) or sex with inhaled elemental components on tandem repeat methylation by introducing either an interaction term with group or an interaction term with sex into the model, respectively. We did not observe noteworthy interactions between metal exposures and group (Supplementary Table 3) as well as between metal exposures and sex (Supplementary Table 4).

Discussion

In the present investigation on two groups of highly-exposed individuals in Beijing, China, we found that *NBL2* was positively associated with concentrations of Si and Ca in truck drivers, which may represent exposure to either asphalt- or cement-paved road dusts related with traffic emissions during driving (EPA 2011). We also found that *SATα* was positively

associated with concentrations of S in office workers, which is likely to represent the exposure to coal combustions, a typical urban air pollution source in China. In fact, the levels of S by XRF analysis indicates predominately sulfate (SO_4^{2-}) in particles derived mainly from coal combustion processes. Gaseous sulfur dioxide (SO_2) is the precursor of sulfate in particles, which is also emitted also from coal combustion processes such as coal-fired power plants. In 2011, China's coal consumption accounted for 47% of global consumption, which is almost as much as that produced in the rest of the world combined (EIA 2013). The growth of coal demand in China is the result of a more than 200% increase in Chinese electric generation since 2000, fueled primarily by coal (EIA 2013).

Evidence in human subjects is rapidly mounting to establish associations of DNA methylation changes with environmental exposures (Tarantini et al. 2009; Hou et al. 2011). Such changes can persist over time even in the absence of the conditions that established them and even accumulate in response to continuous exposure (Anway et al. 2005; Richards 2006; Dolinoy et al. 2007), which might be causally involved in carcinogenesis (Ehrlich 2009; Wolff et al. 2010). For example, in 63 healthy foundry workers with high levels of metal-rich PM exposure, we observed that blood methylation in interspersed repeat elements (*Alu*, *LINE1*) was negatively associated with PM exposure (Tarantini et al. 2009). In the same population, we further examined DNA methylation in four tumor suppressor genes, and found association of PM exposure with hypermethylation of *p16* and *APC*, and hypomethylation of *RASSF1A* and *p53* (Hou et al. 2011). Other studies have reported associations of air pollution with changes in gene-specific methylation in the same cohort, such as decreased level of inducible nitric oxide synthase (*iNOS*) and several asthma-relate genes (Madrigano et al. 2012; Sofer et al. 2013). We also found that Alu and LINE1 blood methylation levels were higher in coke-oven workers at high risk of lung cancer due to PAH exposure (Pavanello et al. 2009). Taken together, this growing body of evidence suggests that repetitive elements show specific responses in blood DNA across different types of exposures.

Tandem repeats are a distinct family of repetitive elements that, albeit widely represented in the human genome and involved in cancer etiology, have yet not been studied in human investigations of the effects of environmental carcinogens. Tandem repeats located in (peri) centromeric regions maintain centromere function and stability of all human chromosomes (Armour 2006; Schmidt and Anderson 2006; Rando and Verstrepen 2007). Relative to most regions of the genome, tandem repeats display a greater propensity to mutate, and variable methylation may influence their mutagenicity rates. Furthermore, tandem repeat elements located in coding regions have been shown to influence expression of genes, including cancer-related genes, such as *PCA3* and *PTTG1IP* (Fondon and Garner 2004; Verstrepen et al. 2005; Zhou et al. 2011; Xiang et al. 2012). Vinces et al. suggested that promoter-associated tandem repeats may facilitate evolutionary tuning of gene expression by mediating elevated responsiveness to changing environmental conditions (Vinces et al. 2009). Yauk et al. indicated that expanded simple tandem repeats (ETSR) could serve as a sensitive biomarker of environmental exposure, and observed mutation and hypermethylation of ETSR in spermatogonial stem cells from mice exposed to particulate air pollution (Yauk et al. 2002; Yauk 2004; Yauk et al. 2008). Our study investigated a

group of healthy individuals exposed to high levels of PM, well above the levels that are documented to determine lung carcinogenicity (Pope et al. 2004). We showed positive association of *NBL2* and *SATα* with several PM elemental components in separate analyses, but no association of *D4Z4* with any of the eight elemental components in office workers, truck driver and all subjects combined. In the same population, we have observed negative associations of *SATα* and *NBL2* with personal $PM_{2.5}$ or ambient PM_{10} levels, but we did not observe association of *D4Z4* with PM exposure (data not shown). Although most previous studies have reported decreased *NBL2* methylation, Nishiyama et al. detected both increased and decreased *NBL2* in different cancer tissues, suggesting that during carcinogenesis, such opposite epigenetic changes may share a common step to affect chromatin structure leading to hypo- or hypermethylation of cytosine residues (Nishiyama et al. 2005). Taken together, our findings indicate that *SATα* and *NBL2* methylation might be more sensitive than *D4Z4* methylation to the overall particle and elemental components levels.

Our results are in line with previous studies showing association of increased lung cancer risks with exposure to several components, such as Ca, Si, and S. Cement factory workers are exposed to a mixture of components, including Ca, Si, Al and Fe, and such occupational exposure may cause lung cancer (Dietz et al. 2004). Several human studies have reported decreased lung function (Al-Neaimi et al. 2001; Meo et al. 2002; Mwaiselage et al. 2004; Zeleke et al. 2010; Nordby et al. 2011) and increased lung cancer risk (Koh et al. 2011; Rachiotis et al. 2012) in cement factory workers. Silicon dioxide (SiO_2), a ubiquitous substance, can be inhaled and get embedded deep into the alveolar sacs to start an inflammation reaction releasing chemokine (Deb et al. 2012). The persistent chronic irritation caused by cement could induce repeated cycles of cell death, cell proliferation, and other inflammatory responses, which may ultimately result in cancer (Rachiotis et al. 2012). In addition, as noted previously, Si and Ca are markers for road dust exposure in truck drivers, and such road dust has a wide range of organic compounds from vehicle emissions attached, some of which are carcinogenic (EPA 2006). Although, to the best of our knowledge, there is no study on the association of lung cancer with exposure to S, increased lung cancer risk has been observed in the pulp and paper industry (Lee et al. 2002), and in females in Taiwan (Tseng et al. 2012) with exposure to SO_2. The reported chromosomal aberrations (Nordenson et al. 1980; Meng and Zhang 1990), mucociliary clearance, impairment of alveolar macrophage function and increased epithelial permeability (Beeson et al. 1998) in humans exposed to SO_2 suggest that it may exert a carcinogenic effect through both genotoxic and non-genotoxic mechanisms. In addition, Ghio et al. has reported that the amount of S on a particle filter is a good proxy for the amount of soluble transition metals on the particles, which in turn was highly correlated with the ability of the particle to generate damaging oxidant compounds (Ghio et al. 1999). This is because acidic sulfate particles participate in the conversion of metals from insoluble oxides (Ghio et al. 1999). Soluble transition metals can catalytically induce oxidative stress via Fenton chemistry, resulting in substantial lung inflammation, as reported in other studies. For example, Duvall et al. reported that cultured human airway epithelial cells, when exposed to particle collected in different cities, showed differential responses, and IL8 generation was strongly associated with the sulfate content of the particles (Duvall et al. 2008).

While in the discussion above we presented the potential effects of Ca, Si and S, these components may be tracers of specific pollution sources responsible for the observed effects. For instance, Ca and Si are important soil components; however, in the case of truck drivers it is most likely that they represent exposures to traffic. Ca is an element associated with road dust or released by combustion of motor oil additives, detergent additives, or lubricant oil (Cadle et al. 1997; Bhagwan et al. 2000; Lough et al. 2005); thus high exposures to Ca in truck drivers indicate high exposures to road dust and exhaust emissions. Si is another soil element but it is also found in road dust particles, because it is released from brake wear (Lough et al. 2005). Road dust and traffic particles are toxic because they encompass many toxic constituents (Gent et al. 2009), and also other components – such as manganese (Mn), Chromium (Cr), Copper (Cu), Antimony (Sb), and Tin (Sn) – which were not considered in our statistical analysis, as well as latex, soot, PAHs and other organics (Cheung et al. 2010; Amato et al. 2011). In the case of office workers who spent most of their time indoors, S represents the impact of outdoor particle sources which include traffic, power plants and industries. Our previous studies indicate that indoor S is an excellent tracer of $PM_{2.5}$ particles penetrating from outdoors for the following reasons (Sarnat et al. 2002): first, S does not have significant indoor sources; second, sulfate particles have an aerodynamic size that is representative of $PM_{2.5}$ particles; third, sulfate particles are stable and have a high penetration efficiency (indoor/outdoor infiltration efficiency); and finally, the S indoor/outdoor ratio depends on the ventilation of the building. High indoor/outdoor ratios correspond to leaky buildings where a large fraction of pollution penetrates. Therefore, high indoor levels of S reflect high exposures to pollution originating outdoors.

In our study, we also found non-noteworthy (i.e., FDR>0.05) association of *NBL2* with concentrations of Al (another road dust element) in truck drivers, and non-noteworthy association of $SAT\alpha$ with concentrations of Ti and Zn (both are road dust elements) in office workers. Al is one of the most benign industrial metals, and Al workers were suggested to be prone to respiratory diseases (Abbate et al. 2003). In a mice study, Al has been suggested to induce systemic oxidative stress and inflammatory, which may potentiate cancer development (Mazzoli-Rocha et al. 2010). In Al-exposed workers, Elserougy et al. reported an elevated level of CRP (Elserougy et al. 2012), an inflammation marker that has been repeated associated with increased lung cancer risk in a human studies (Chaturvedi et al. 2010; Xu et al. 2013). Zn inhalation has been shown to induce inflammation and oxidative stress in animal studies (Kodavanti et al. 2002; Kodavanti et al. 2003). Kodavanti et al. have also demonstrated that leachable Zn from PM induced both pulmonary and systemic changes in multiple *in vivo* toxicology experiments (Kodavanti et al. 2008). Consistent with our discussion of pollution sources above, at least some of these associations could be traced back to the sources of particle exposures. In particular, Ti, Al, and Zn are all enriched in urban road dust and may represent tracers of this type of exposure in the study groups.

Our study has several strengths. We conducted technical validation of eight personal elemental components measures and observed high reproducibility of our measurements (r>0.75). All participants were evaluated using standardized protocols for blood collection and storage. Blood DNA samples were randomized across plates to limit potential bias from plate effects and laboratory personnel were blinded to exposure groups and exposure study.

We also recognize that our study is subject to a number of limitations. Because of the relatively small sample size, we cannot exclude false negative finding, particularly for the lack of association between *D4Z4* and the exposures, as well as chance findings. Therefore, whether *D4Z4* blood methylation is sensitive to elemental components in air particles should be further evaluated in future investigations on larger samples of exposed individuals. The study was conducted in a short period of time in the summer of 2008. In our previous study, we observed differences in global DNA methylation (*LINE1* and *Alu*) by season (Baccarelli et al. 2009). Whether our findings can be extended to the winter season in Beijing remains to be determined. In bladder normal tissues, Choi et al. found correlation of *LINE1* and *Alu* with *SATα*, but not with *D4Z4* and *NBL2*, suggesting that changes in DNA methylation of tandem DNA repeats could be different from interspersed repeats (Choi et al. 2009). A recent epigenome-wide analysis of repeated elements showed wide differences in the inter-individual variability of DNA methylation of repeated elements (Flanagan et al. 2006). In particular, methylation in satellite sequences exhibited the largest differences between individuals, although variations at different degrees were found also in all the other classes of repeated elements in the human genome. Our results indicate that part of this inter-individual variability might be due to environmental factors. In our study, we did not measure copy number variation at the three loci, limiting our ability of determining its correlations with exposure levels in relation to DNA methylation alterations, which warrants future studies.

In summary, our results indicate that increased methylation in *SATα* and *NBL2* can be detected in blood leukocytes. If confirmed, this finding may help identify individuals in human populations that suffer biologically-relevant effects from exposure to metals and other toxic components. Future studies are warranted to determine whether PM-induced changes in DNA methylation of tandem DNA repeats associated with future risk of lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1
Characteristics of the study participants

	Office Workers (n=60)	Truck Drivers (n=60)
Sex, n(%)		
Male	40 (66.7	40 (66.7)
Female	20 (33.3)	20 (33.3)
Age [Years], mean ± SD	30.3 ± 8.0	33.5 ± 5.7
BMI [kg/m^2], mean ± SD	22.8 ± 3.4	24.3 ± 3.2
Cigarettes smoked during Examination time, mean ± SD	0.5 ± 1.7	2.3 ± 4.2
Usage of central heating, n(%)		
Yes	6 (10.0)	16 (26.7)
No	54 (90.0)	44 (73.3)
Personal $PM_{2.5}$, mean ± SD	94.6 ± 64.9	126.8 ± 68.8

Table 2

Level of inhaled elemental components during work hours on the examination days

	Office Workers								Truck Drivers							
	Obs	Mean	SD	10pct	25pct	Median	75pct	90pct	Obs	Mean	SD	10pct	25pct	Median	75pct	90pct
Inhaled elemental components[a] (μg/m^3) on the examination days, from personal monitors																
Al	118	0.54	0.25	0.23	0.37	0.50	0.70	0.86	120	1.36	0.93	0.44	0.59	1.29	1.86	2.32
Ca	118	0.32	0.18	0.15	0.19	0.28	0.41	0.54	120	2.09	2.20	0.29	0.40	1.58	3.08	4.52
Fe	118	0.38	0.21	0.17	0.24	0.34	0.44	0.69	120	1.01	0.64	0.38	0.50	0.82	1.34	1.75
K	118	0.76	0.77	0.18	0.27	0.56	0.82	2.07	120	1.31	1.07	0.34	0.44	0.89	2.06	2.81
S	118	6.19	5.05	0.61	1.66	5.30	8.64	13.77	120	8.43	4.92	2.30	4.87	6.98	12.47	16.14
Si	118	0.79	0.53	0.28	0.45	0.68	1.03	1.43	120	2.37	1.76	0.64	0.82	2.09	3.54	4.13
Ti	118	0.02	0.01	0.00	0.01	0.02	0.03	0.04	120	0.06	0.04	0.02	0.03	0.05	0.08	0.10
Zn	118	0.15	0.17	0.02	0.05	0.08	0.22	0.37	120	0.27	0.22	0.06	0.09	0.17	0.41	0.68

[a] Measured during the work hours of examination days using light-weight personal monitors.

Table 3

Mean tandem repeat methylation levels (all sites combined) in truck drivers and office workers

	Office workers (obs=120)		Truck drivers (obs=120)	
	Mean	**95% CI**	**Mean**	**95% CI**
Unadjusted				
D4Z4	66.81	(65.62 ; 68.01)	67.13	(65.88 ; 68.38)
NBL2	82.06	(81.16 ; 82.97)	82.90	(82.14 ; 83.66)
SATα	73.55	(72.17 ; 74.94)	73.23	(71.59 ; 74.87)
Adjusted [a]				
D4Z4	66.57	(65.13 ; 68.02)	66.84	(65.52 ; 68.16)
NBL2	81.37	(80.28 ; 82.45)	82.70	(81.83 ; 83.58)
SATα	72.92	(70.97 ; 74.88)	73.31	(71.56 ; 75.07)

[a] Adjusted for measure day, age, sex, BMI, number of cigarettes smoked during examination time and usage of central heating

Table 4

Change in methylation of *NBL2* associated with 10% increase in inhaled elemental components [a]

	Office Workers (obs=120)				Truck Drivers (obs=120)			
	β	95%CI	p-value	FDR	β	95%CI	p-value	FDR
Inhaled elemental components[b]								
Al	-0.077	(-0.219 ; 0.066)	0.291	0.810	0.096	(-0.007 ; 0.199)	**0.067**	0.180
Ca	0.023	(-0.092 ; 0.138)	0.692	0.810	0.065	(0.014 ; 0.115)	**0.012**	**0.047**
Fe	0.013	(-0.095 ; 0.121)	0.810	0.810	0.054	(-0.027 ; 0.135)	0.189	0.377
K	-0.056	(-0.181 ; 0.068)	0.375	0.810	0.035	(-0.046 ; 0.117)	0.396	0.528
S	0.020	(-0.046 ; 0.085)	0.554	0.810	-0.003	(-0.092 ; 0.087)	0.954	0.954
Si	0.010	(-0.063 ; 0.082)	0.795	0.810	0.121	(0.030 ; 0.212)	**0.009**	**0.047**
Ti	0.060	(-0.030 ; 0.151)	0.192	0.810	0.044	(-0.045 ; 0.133)	0.331	0.528
Zn	-0.024	(-0.092 ; 0.043)	0.479	0.810	-0.004	(-0.059 ; 0.051)	0.890	0.954

[a] Based on 240 total observations (120 study days for office workers and 120 study days for truck drivers). P-values were obtained from GEE models, which were adjusted for occupation, $PM_{2.5}$, measure day, age, sex, BMI, number of cigarettes smoked during examination time and usage of central heating.

[b] Measured during the work hours of examination days using light-weight personal monitors.

Table 5

Change in methylation of *SATα* associated with 10% increase in inhaled elemental components [a]

	Office Workers (obs=120)				Truck Drivers (obs=120)			
	β	95%CI	p-value	FDR	β	95%CI	p-value	FDR
Inhaled elemental components[b]								
Al	0.025	(-0.183 ; 0.233)	0.814	0.814	0.110	(-0.098 ; 0.319)	0.299	0.893
Ca	-0.058	(-0.246 ; 0.129)	0.541	0.814	0.027	(-0.095 ; 0.148)	0.669	0.893
Fe	0.055	(-0.153 ; 0.263)	0.606	0.814	0.053	(-0.102 ; 0.208)	0.503	0.893
K	0.033	(-0.164 ; 0.230)	0.742	0.814	-0.053	(-0.224 ; 0.118)	0.541	0.893
S	0.115	(0.034 ; 0.196)	**0.005**	**0.042**	-0.004	(-0.158 ; 0.150)	0.964	0.964
Si	-0.036	(-0.198 ; 0.126)	0.664	0.814	0.020	(-0.181 ; 0.220)	0.846	0.964
Ti	0.156	(-0.014 ; 0.325)	**0.072**	0.288	0.044	(-0.113 ; 0.202)	0.581	0.893
Zn	0.061	(-0.042 ; 0.164)	0.244	0.650	-0.110	(-0.216 ; -0.003)	**0.044**	0.355

[a] Based on 240 total observations (120 study days for office workers and 120 study days for truck drivers). P-values were obtained from GEE models, which were adjusted for occupation, $PM_{2.5}$, measure day, age, sex, BMI, number of cigarettes smoked during examination time and usage of central heating.

[b] Measured during the work hours of examination days using light-weight personal monitors.