Short-term personal $PM_{2.5}$ exposure and change in DNA methylation of imprinted genes: Panel study of healthy young adults in Guangzhou city, China

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Abstract

DNA methylation (DNAm) plays a significant role in deleterious health effects inflicted by fine particulate matter ($PM_{2.5}$) on the human body. Recent, studies have reported that DNAm of imprinted control regions (ICRs) in imprinted genes may be a sensitive biomarker of environmental exposure. Less is known about specific biomarkers of imprinted genes after $PM_{2.5}$ exposure. The relationship between $PM_{2.5}$ and its chemical constituents and DNAm of ICRs in imprinted genes after short-term exposure was investigated to determine specific human biomarkers of its adverse health effects. A panel study was carried out in healthy young people in Guangzhou, China. Mixed-effects models were used to evaluate the influence of $PM_{2.5}$ and its constituent exposure on DNAm while controlling for potential confounders. There was no significant correlation between DNAm and personal $PM_{2.5}$ exposure mass. DNAm changes in eight ICRs (*L3MBTL1*, *NNAT*, *PEG10*, *GNAS Ex1A*, *MCTS2*, *SNURF*/*SNRPN*, *IGF2R*, and *RB1*) and a non-imprinted gene (*CYP1B1*) were significantly associated with $PM_{2.5}$ constituents. Compared to non-imprinted genes, imprinted gene methylation was more susceptible to interference with $PM_{2.5}$ constituent exposure. Among those genes, *L3MBTL1* was the most sensitive to personal $PM_{2.5}$ constituent exposure. Moreover, transition metals derived from traffic sources (Cd, Fe, Mn, and Ni) significantly influenced DNAm of the imprinted genes, suggesting the importance of more targeted measures to reduce toxic constituents. Bioinformatics analysis

indicated that imprinted genes (*RB1*) may be correlated with pathways and diseases (non-small cell lung cancer, glioma, and bladder cancer). The present study suggests that screening the imprinted gene for DNAm can be used as a sensitive biomarker of $PM_{2.5}$ exposure. The results will provide data for prevention of $PM_{2.5}$ exposure and a novel perspective on potential mechanisms on an epigenetic level.

Capsule: Screening imprinted gene for DNAm can be used as a sensitive biomarker of $PM_{2.5}$ exposure.

Keywords: Particulate matter; Chemical constituents; Imprinted genes; Epigenetic modification; Sensitive biomarker.

1. Introduction

Epidemiological studies have demonstrated that particulate matter 2.5 ($PM_{2.5}$) exposure is closely related to lung function, respiratory system, cardiovasculature, and cancer (Achilleos et al., 2017; Hamra et al., 2014; Kingsley et al., 2017; Nelin et al., 2012; Ristovski et al., 2012; Sun et al., 2018). $PM_{2.5}$ exposure has led to approximately 6.67 million of premature deaths worldwide in 2019 (Health Effects Institute, 2020). $PM_{2.5}$ contains complex constituents that induce different toxicity, so that regulating the $PM_{2.5}$ mass alone may not be sufficient to protect human health (Krall et al., 2013; Tarantini

et al., 2009). Screening for specific biomarkers for both $PM_{2.5}$ and its constituents will be helpful for elucidating potential mechanisms of its adverse health effects on human health.

Telomere length, DNA methylation (DNAm), microRNAs, inflammatory effects, and oxidative stress are considered potential biomarkers of air exposure (Yang et al., 2017). However, sensitive and specific human biomarkers of $PM_{2.5}$ exposure is still needed. DNAm may be involved in regulating the process of oxidative damage and inflammation, which leads to disease (Chen et al., 2015; Dai et al., 2017; de et al., 2018; Lodovici and Bigagli, 2011; Ma et al., 2019; Shi et al., 2019). The reversibility of DNAm makes it more important to find specific biomarkers of DNAm and contributes to feasibility of disease prevention and treatment (Yoo and Jones, 2006). Although studies on $PM_{2.5}$ exposure and changes in gene methylation have been increasing, their results differed due to regional $PM_{2.5}$ differences and heterogeneity of the study population (Breton et al., 2012; Tarantini et al., 2013). Therefore, more data are still needed to screen and identify specific and sensitive biomarkers. Recently, studies have found that imprinted genes may be more vulnerable to environmental exposure (Cowley et al., 2018).

The haploid morphology of imprinted genes makes their methylation changes more biological (Smeester et al., 2014). Loss of imprinting, which is an epigenetic alteration in imprinted genes, is one of the most common changes in cancer. It is frequently associated with many health aspects, such as cell

111 function, body growth and development, and neural behavior (Jelinic and Shaw,
112 2007; Leick et al., 2012; Smeester et al., 2014). Imprinted genes are associated
113 with lung growth, respiratory diseases, and lung cancer. For example, imprinted
114 genes (*IGF2R*) are essential regulators of lung growth (Zhang et al., 2015),
115 imprinted genes (*PEG10*, *PEG3*, *MEST*, and *GNAS*) are affected in lung cancer
116 (Deng et al., 2014; Kim et al., 2015; Matouk et al., 2015), and *DLK1-DIO3*
117 imprinting is associated with respiratory diseases (Enfield et al., 2016).
118 Therefore, studying the relationship between imprinted gene methylation and
119 $PM_{2.5}$ exposure can provide innovative data and clues for exploring the potential
120 mechanism of methylation alteration triggered by $PM_{2.5}$ exposure. Studies on
121 imprinted genes have focused on the effects of exposure to a few toxic metals
122 (Cd, Pb, and As) on methylation of imprinted genes. Arsenic has been reported
123 to cause *ANO1*- and *FOXF1*-promoter hypermethylation in leukocytes, as well
124 as *INS*-promoter hypomethylation (Bailey et al., 2013; Smeester et al., 2011).
125 Imprinted control regions (ICRs) of imprinted genes have been reported to be
126 more sensitive to environmental changes (Cowley et al., 2018), which aides in
127 screening out specific biomarkers and understanding the mechanism of $PM_{2.5}$
128 action in human health (Monk et al., 2019).

129 The present study carried out personal monitoring in a panel of healthy
130 college students in Guangzhou, China, with the aim of reducing the impact of
131 individual differences in the study. Personal $PM_{2.5}$ samples, environmental data
132 and blood samples were collected four times after participants were exposed

to different environmental regions. $PM_{2.5}$ constituents were examined using ion chromatography, TOR, ICP-MS, and ICP-OES. MethylTarget was used to determine and analyze DNAm of imprinted genes in blood samples. Mixed-effects models were used to analyze the relationship between $PM_{2.5}$ and changes in DNAm. The experimental results provide fresh clues for exploring sensitive and specific biomarkers.

2. Materials and methods

2.1. Study participant recruitment

In this study, the participants were 19–23 years old students of Sun Yat-sen University. Participant health status criteria required that no symptoms were present in the week prior to testing (e.g., acute or chronic injury, wheezing, shortness of breath, and chest tightness). Individuals with these respiratory symptoms or a history of drinking and smoking and second hand smoking were excluded. Finally, 36 participants who met the study qualification criteria were enrolled as exposed subjects. The study protocol was approved by the Sun Yat-sen University Institutional Human Ethics Committee (Ethics Approval Number: L2016016).

2.2. Collection of $PM_{2.5}$ samples

Thirty-six participants were randomly divided into three exposure groups, each group consisting of 12 individuals. In the course of exposure measurements, each participant was asked to remain within 1.5 km of three

diverse stations in Guangzhou. Each group carried four samplers including two filter membranes within 8 h. Two samples were collected in Teflon filter membranes for measuring $PM_{2.5}$ concentration, while another two $PM_{2.5}$ samples were collected with two quartz filter membranes, which were used for chemical constituents. The subjects were required to avoid abnormal and strenuous exercise for four consecutive weekends between November and December 2016. At the same time, the following activities were allowed during the 8 h sampling period: playing cards, cell phone use, reading books, and walking.

The sampler used to collect personal $PM_{2.5}$ samples was BUCK-Libra Plus (A.P. BUCK, USA), which could be fitted with a Φ37 mm Teflon or quartz filter membrane for collecting $PM_{2.5}$ samples. The sampler's pump placed in the participant's backpack or handbag and filter film was fixed near the collar of the participant's shirt to collect $PM_{2.5}$ near the respiratory tract. Automatic weighing system (AWS-1, COMDE DERENDA, Germany, European standards, sensitivity: 0.001 mg) was used to weigh samples at the end of each sampling day. The equilibrium temperature (20 ± 1 ºC) and humidity (50 ± 5%) required balance before the membranes were weighed (Hu et al., 2018).

2.3. Air pollution measurements

Personal $PM_{2.5}$ samples were weighed in the laboratory to determine the total concentration and concentration of various $PM_{2.5}$ constituents using chemical analysis. Studies have shown that OC, EC, water soluble fractions

and trace metals accounted for the main portion of $PM_{2.5}$ (Tan et al., 2017). Inorganic constituents played a significant role in DNAm caused by environmental exposure. On one hand, previous studies have shown that imprinted gene methylation was significantly associated with inorganic toxicity exposure (Pb and Cd) in the environment (Cowley et al., 2018; Li et al., 2016; Wan et al., 2020). On the other hand, Lei et al. (2019) have found that *TNF-α* hypomethylation might mediate the relationship between *TNF-α* expression, metals (As, Ca, Cu, K, P, Pb, Si, Sr, Ti, and Zn), and EC in personal $PM_{2.5}$. In addition, OC included all organic carbon in organic compounds in $PM_{2.5}$. Like EC, OC may influence the relationship between DNAm and $PM_{2.5}$ exposure. Therefore, the present study sought to further investigate the relationship between ICR DNAm in imprinted genes and OC, EC, anions, and trace metals.

Carbonaceous fractions (CF) were detected by TOR (Atmoslytic, USA), including organic carbon (OC), elemental carbon (EC) and total carbon (TC). Ion Chromatograph (DIONEX, USA) was used to detect ions, including nitrate (NO_3^- and NO_2^-), sulfate (SO_4^{2-}), chloride (Cl^-), fluoride (F^-), and oxalic acid ($C_2O_4^-$). Chemical elements (crystal metals, transition metals, and metalloid elements) were detected by ICP-MS (Agilent, USA) and ICP-OES (PerkinElmer, USA). Crystal metals included aluminum (Al), strontium (Sr), magnesium (Mg), calcium (Ca), barium (Ba), sodium (Na), and potassium (K). Transition metals included zirconium (Zr), titanium (Ti), iron (Fe), scandium (Sc), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn),

molybdenum (Mo), cadmium (Cd), yttrium (Y), and wolfram (W). Metalloid elements included silicon (Si), lithium (Li), beryllium (Be), phosphorus (P), arsenic (As), rubidium (Rb), tin (Sn), antimony (Sb), cesium (Cs), cerium (Ce), thallium (Tl), lead (Pb), bismuth (Bi), lanthanum (La), samarium (Sm), thorium (Th), and uranium (U). In addition to these measurements, ambient temperature and relative humidity were also obtained from Guangzhou Meteorological Service.

2.4. DNA methylation

Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany), while MethylTarget (see Supplementary Materials for details) was used to determine methylation rates in target regions. MethylTarget can sequence multiple gene fragments simultaneously. In addition, inhibitor 5-azaC (Sigma, USA) was used to treat Beas-2B cells as negative experiments and complete methylation standard (Sigma, USA) was utilized as a positive control.

A literature review revealed 22 murine germline ICRs and three human-specific germline ICRs that were considered to be imprinted genes (Cowley et al., 2018). Five murine ICRs that were not confirmed to be imprinted in a human were excluded. In addition, two genes cannot detect DNAm due to low CpG islands. Thus, at last 18 germline ICRs that have been identified in humans were selected (Table 1), including *PEG13*, *DIRAS3*, *KvDMR*, *IGF2R*, *GNASXL*, *NNAT*, *GNAS Ex1A*, *MCTS2*, *PLAGL1*, *PEG10*, *INPP5F_V2*, *MEST*,

ZIM2/*PEG3*, *SNURF*/*SNRPN*, *L3MBTL1*, *H19*/*IGF2*, *RB1,* and *GRB10* (Cowley et al., 2018; Genevieve et al., 2005; Smeester et al., 2014). Previous studies have shown that the changes in methylation of these ICRs methylation were closely related to environmental pollutants. For example, ICRs are more susceptible to interference from Cd exposure (Cowley et al., 2018), while Pb exposure may change ICRs methylation (Li et al., 2016). Our previous study has shown that Pb exposure is significantly associated with imprinted gene methylation (Wan et al., 2020). In addition, DNAm of five non-imprinted genes was also determined and analyzed. Two predicted imprinted genes (*CYP1B1* and *APBA1*) were classified as non-imprinted genes during statistical analysis and three non-imprinted genes (*RASSF2*, *MGMT,* and *APC*) were potential $PM_{2.5}$ biomarkers in accordance with previous studies (Breton and Marutani, 2014; Ding et al., 2016; Ma et al., 2019). Therefore, it is necessary to further study whether the above 18 germline ICRs are more sensitive to $PM_{2.5}$ exposure when they are compared to non-imprinted genes.

2.5. Statistical analysis

Before statistical analysis, methylation data were processed using a natural logarithmic transformation to better approximate a normal distribution.

A mixed model was used to evaluate the association between personal $PM_{2.5}$ exposure and gene methylation, including random intercept for each participant. Three mixed-effects models were used to evaluate the consistency of personal $PM_{2.5}$ effects. In the single-constituent model, $PM_{2.5}$ and its

constituents were evaluated as independent fixed-effect variables. The constituent-$PM_{2.5}$ adjustment model considered $PM_{2.5}$ and its constituents simultaneously as an adjusted effect. The constituent-residual model was used to establish a constituent residual model to eliminate confusion caused by $PM_{2.5}$ and collinearity between $PM_{2.5}$ and its constituents (constituent-residual model) (Chen et al., 2015; Mostofsky et al., 2012; Wu et al., 2012). Three different fixed-effects models contained the same fixed-terms, including age, sex, body mass index (BMI), area, temperature, relative humidity, and time trend (time and a squared term of time). In addition, a random intercept was assigned to each subject, and it was assumed that the irregular covariance structure (UN) was equivalent to a random intercept to illustrate the correlation between repeated measurements for the same subject. For the constituent-residual model, constituent residuals were obtained by establishing a linear regression model with constituents as dependent variables and $PM_{2.5}$ as independent variables between $PM_{2.5}$ and its constituents. Then, the residuals were introduced into the single-constituent model to replace the constituents. The constituent residuals were considered as a rough measure of the independent contribution of each constituent to $PM_{2.5}$ (Wu et al., 2012).

All mixed-effects models were implemented using the mixed model in SAS 9.4 (SAS, Cary, NC, USA). A *p*-value of < 0.05 was considered statistically significant (two-tailed). In this work, final results were presented as the percent changes with 95% confidence intervals (CIs) in DNAm (%5mC), which were

associated with an interquartile range (IQR) increase in $PM_{2.5}$ and constituent concentrations.

2.6. Bioinformatics analysis

KOBAS 3.0 is an online platform for gene/protein functional annotation (annotation module) and functional gene set enrichment (enrichment module) (Xie et al., 2011). After entering the gene list or gene expression data into the enrichment module, enriched gene set, corresponding name, *p*-value or enrichment probability, and enrichment fraction were generated according to the results obtained from various methods. Genes significant for personal $PM_{2.5}$, were determined using the mixed-effects model and final results were obtained using a statistical test method (hypergeometric test/Fisher's exact test) and false discovery rate correction method (Benjamini and Hochberg; $p < 0.05$) with KOBAS (KEGG pathway and KEGG disease databases).

3. Results and discussion

3.1. Descriptive statistics

The present study collected and analyzed $PM_{2.5}$ and blood samples in 32 participants four times (four out of 36 participants did not complete the final test). Demographic characteristics for 32 participants are shown in Table 2. The study cohort included 17 males and 15 females with an average age of 21.19 ± 1.05 years (range 19–23 years) and BMI of 21.01 ± 2.82 Kg/m^2.

Table 2 shows descriptive statistics for average gene DNAm, which

revealed that DNAm of imprinted genes was generally higher than that of non-imprinted genes. DNAm was > 80.00% for *GRB10* (84.47 ± 2.63%), *INPP5FV_2* (88.36 ± 1.98%), and *PLAGL1* (81.53 ± 4.00%), while *GNAS Ex1A* (33.09 ± 3.27%) was the lowest. DNAm of imprinted genes was similar to previous studies that reported ranges between 33.00% and 88.00% (Gwen et al., 2018). However, the average methylation rate of non-imprinted genes *APC* (0.65 ± 0.11%), *MGMT* (2.01 ± 0.68%), and *RASSF2* (0.94 ± 0.11%) was generally low. *APBA1* reached 67.91% in the present study, while *APBA1* and *CYP1B1* have been predicted to be parent imprinted genes according to the imprinted genes selected from open databases (Smeester et al., 2014). Therefore, being an imprinted gene may be the reason for higher DNAm level for *APBA1* and *CYP1B1*. Table 3 presents statistical data for personal $PM_{2.5}$, chemical constituents, environmental temperature, and relative humidity. Personal $PM_{2.5}$ exposure (78.38 ± 30.45 μg/m^3) was higher than the national 24-h average threshold (China: 75.00 μg/m^3), Shanghai, North America, and Western Europe (Chen et al., 2015). In addition, ambient $PM_{2.5}$ concentration was obtained from Air Quality Monitoring System in Guangzhou during the period of personal exposure sampling. Personal $PM_{2.5}$ (78.38 ± 30.45 μg/m^3) was higher than ambient $PM_{2.5}$ (40.46 ± 14.32 μg/m^3; Table 3). This result was similar to that in previous studies (Hu et al., 2018; Lei et al., 2016).

3.2. Regression results

Figure 1 shows the changes in DNAm of imprinted genes associated with

an IQR increase in $PM_{2.5}$ constituents using three mixed-effects models. A significant correlation between gene and personal $PM_{2.5}$ exposure was not present, although chemical constituents were significantly associated with DNAm of eight ICRs and a non-imprinted gene in the three models ($p < 0.05$), including *L3MBTL1*, *NNAT*, *PEG10*, *GNAS Ex1A*, *MCTS2*, *SNURF*/*SNRPN*, *IGF2R*, *RB1*, and *CYP1B1* (non-imprinted gene). For instance, imprinted genes *L3MBTL1* and *PEG10* both had a significantly negative association with Fe and $C_2O_4^-$. An IQR increase in personal exposure to Fe (1.79 μg/m^3) was associated with a decrease of 4.93% (95%CI: -9.10, -0.76) and 1.72% (95%CI: -3.26, -0.18) in DNAm of *L3MBTL1* and *PEG10*, respectively. An IQR increase in personal exposure to $C_2O_4^-$ (0.14 μg/m^3) was associated with a decline of 1.96% (95%CI: -3.73, -0.19) and 0.73% (95%CI: -1.44, -0.01) in DNAm of *L3MBTL1* and *PEG10*, respectively. Additional results are shown in Figure S1 (Supplementary materials).

3.2.1. Relationship between DNA methylation and personal $PM_{2.5}$ constituent exposure

A correlation between personal $PM_{2.5}$ exposure mass and gene methylation was not present, although constituents were significantly associated with eight ICRs and a non-imprinted gene. This suggested that constituents may be more closely related than personal $PM_{2.5}$ exposure mass and DNAm. The adverse health effects of $PM_{2.5}$ exposure may depend on toxic constituents. For example, Tarantini et al. (2009) found that *EDN1* DNAm is not

331 associated with PM_{10} and PM_1 but is associated with Zn. Wu et al. (2012) found
332 that inflammatory biomarkers have no significant correlation with $PM_{2.5}$ mass,
333 but are correlated with constituents (Mg, Fe, Ti, Co, Cd, Mn, and Se).

334 Although transition metals are only a small part of $PM_{2.5}$ (5%), they were
335 significantly associated with changes in methylation of imprinted genes, such
336 as *L3MBTL1* (Fe, V, Mn, and Cd) and *NNAT* (Mn, Cd, Cu, and Zn) (Fig. 1).
337 Research shown that transition metals may cause oxidative damage,
338 inflammatory effects, interfere with gene expression, and lead to lung disease
339 and cancer (Fortoul et al., 2015; Valavanidis et al., 2008). Lei et al. (2019) found
340 a significant negative correlation between transition metals in personal $PM_{2.5}$
341 and changes in *TNF-α* methylation. At the same time, there was a significant
342 positive correlation with the expression of inflammatory factors TNF-α proteins.
343 Other studies have shown that transition metals (Ni, Fe, and Zn) in $PM_{2.5}$
344 significantly affect heart rate variability in healthy populations (Wu et al., 2011).
345 Transition metals Fe and Zn in particular are, both important cofactors for many
346 enzymes. These enzymes can modify DNA and histones in epigenetic ways
347 (Vidal et al., 2015). Fe is also significantly associated with DNAm of *L3MBTL1*
348 and *PEG10*. The dynamic balance of Fe plays an important role in growth and
349 development. The overloaded Fe increases cancer risk and promotes tumor
350 growth (Beguin et al., 2014). There may also be $PM_{2.5}$ interactions between
351 different transition metals. For example, Fe can be replaced by Cd to bind to
352 membrane proteins, resulting in increased Fe and oxidative stress (Fortoul et

353 al., 2015). Fe deficiency anemia may increase the risk of Cd accumulation and
354 increase toxicity (Min et al., 2008). Moreover, Fe and Zn can reduce the adverse
355 health effects of Cd exposure. However, the current understanding of Fe toxicity
356 mechanisms remains unclear (Vidal et al., 2015). The present results indicate
357 that the changes in DNAm in response to Fe exposure may also be a potential
358 mechanism for iron to cause various adverse health effects.

359 Except for *L3MBTL1* that was positively correlated with $PM_{2.5}$ constituents,
360 methylation of most genes decreased after constituent exposure. The reactive
361 oxygen species produced by $PM_{2.5}$ exposure can cause oxidative DNA damage,
362 which interferes with the interaction between methyltransferase and DNA and
363 then reduces DNAm (Baccarelli et al., 2009; Bellavia et al., 2013; Valinluck et
364 al., 2004). *GNAS Ex1A* (belongs to the *GNAS* family) methylation was
365 negatively correlated with $C_2O_4^-$. *PEG10* methylation had a significant negative
366 correlation with $C_2O_4^-$ and Fe; *CYP1B1* methylation had a significant negative
367 correlation with Y and U. These results predicted that the expression of these
368 genes will be upregulated as a result of $PM_{2.5}$ exposure. Kingsley et al. (2017)
369 found $PM_{2.5}$ and black carbon at pregnant mothers' residence are related to the
370 expression of placental imprinted genes, while *PEG10* and *GNAS* expression
371 is positively correlated with $PM_{2.5}$. *CYP1B1* methylation decreases as these
372 constituents (Y and U) rise, which was also validated *in vitro* experiments. Our
373 previous *in vitro* results showed that $PM_{2.5}$ exposure induces a decrease in
374 *CYP1B1* methylation rate and mRNA increase in human lung cells (Ma et al.,

2019). In addition, *PEG10* is an oncogene at chromosome 7q21 and a reverse transcript derived imprinted gene. It is overexpressed in a variety of cancers and plays an important role in human lung cancer diffusion, progression, prognosis and metastasis (Deng et al., 2014; Ferguson-Smith et al., 2007; Matouk et al., 2015; Tsuji et al., 2010). Okabe et al. (2003) found that *PEG10* overexpression reduces SIAH1- mediated cell death and combines with *SIAH1* to participate in human hepatocyte carcinogenesis. Liu et al. (2011) identified *PEG10* as a biomarker of gallbladder adenocarcinoma. In addition, Lei et al. (2019) found that personal exposure to $PM_{2.5}$ constituents may promote systemic inflammation via DNA hypomethylation.

The relationship between constituents and *L3MBTL1* (Cd, Fe, Mn, and V) and *NNAT* (Cd, Mn, Cu, and Pb) indicate that traffic source is closely associated with DNAm, which is similar to previous research (Baccarelli et al., 2009; Lepeule et al., 2014; Nitschke et al., 2016; Nordling et al., 2008; Rice et al., 2015; Shi et al., 2019; Urman et al., 2014; Wu et al., 2013; Zhang et al., 2020). $PM_{2.5}$ constituents derived from traffic sources can affect DNAm changes and may be involved in adverse health effects (Chen et al., 2015). This implies the importance of more targeted measures to reduce toxic constituents (e.g., Cd, Fe, and Pb).

Figure 2 shows that *L3MBTL1* methylation had the greatest degree of change compared to *NNAT*, *RB1,* and *PEG10*. Moreover, it is significantly associated with more constituents, which indicates that *L3MBTL1* is the most

sensitive after exposure to personal $PM_{2.5}$.

Three mixed-effects models were used to analyze the relationships between $PM_{2.5}$ constituents and each *L3MBTL1* gene site (Supplementary materials: Figure S2). Three gene sites (Chr20: 43514646, Chr20: 43514599, and Chr20: 43514724) were found to have a significant correlation with more constituents, of which 15 constituents are also significantly related to the average of *L3MBTL1* methylation. Moreover, the relationship between changes in DNAm at each site is also consistent with *L3MBTL1*. For example, *L3MBTL1* and the site are negatively correlated with $C_2O_4^-$, and the sites have the same change trend as *L3MBTL1*. Therefore, these sites may be precise targets for the prevention and treatment of diseases resulting from $PM_{2.5}$ exposure.

L3MBTL1 is a tumor suppressor gene located at 20q12. It is a paternal imprinted gene. The L3MBTL1 protein that it encodes is a transcriptional repressor, which can act as a chromatin reader to maintain chromatin structure (Adams-Cioaba and Min, 2009; Trojer et al., 2007; Zeng et al., 2012), inhibit transcription of many genes, and be an indispensable mitosis protein. Combined with the present results, it can be speculated that *L3MBTL1* may be a sensitive environmental sensor, which warrants further study of its environmental sensitivity and its role in environmental factors, such as atmospheric pollutants.

3.2.2. Comparison between imprinted and non-imprinted genes

In this study, 18 imprinted genes were detected and analyzed, of which

eight imprinted genes were significantly related to $PM_{2.5}$ constituents. In addition, a non-imprinted gene (*CYP1B1*) was significantly associated with $PM_{2.5}$ constituents. However, *CYP1B1* is currently a predicted imprinted gene. This result shows that compared with non-imprinted genes, the relationship between imprinted gene methylation and $PM_{2.5}$ constituent is closer. This implies that imprinted genes are more susceptible to interference from constituents after $PM_{2.5}$ exposure.

Among these genes, imprinted genes are associated with more constituents than non-imprinted genes. Both *L3MBTL1* and *NNAT* are related to more than ten constituents, and DNAm changes greatly with constituents (Fig. 1). Among the non-imprinted genes, only *CYP1B1* has a significant correlation with Y and U, while the change in *L3MBTL1* is greater than that in *CYP1B1* when constituent Y increases an IQR (0.14 ng/m^3). Therefore, imprinted genes are more susceptible to environmental interference. According to the imprinting rules (Smeester et al., 2014), imprinted genes express genetic information from paternal or maternal line through single alleles. One of the alleles is methylated and silently expressed, while only one DNA strand is methylated, which is easily disturbed by environmental factors. This may be the reason why DNAm of imprinted genes is more susceptible to interference than that of non-imprinted genes after $PM_{2.5}$ exposure. Studies have found that DNAm of imprinted genes (*H19*/*IGF2*) changes after exposure to toxic metals (e.g., cadmium and lead) (Cowley et al., 2018; Li et al., 2016; Nye et al., 2016).

441 Cowley et al. (2018) found that in neonatal cord and maternal blood,
442 differentially methylated regions are more common in 15 imprinted control
443 regions (ICRs) of maternal origin than in non-imprinted genes at similar sites,
444 indicating that ICRs are more sensitive after Cd exposure. This also shows the
445 particularity of imprinted gene methylation and underscores its sensitivity to
446 environmental interference.

447 *3.3. Bioinformatics analysis*

448 KEGG pathway and disease enrichment analysis for genes was conducted
449 using the KOBAS platform. Fourteen pathways were found to be associated
450 with these genes, such as bladder, non-small cell lung, pancreatic and prostate
451 cancer (Table 4). In addition, 18 diseases were also associated with these
452 genes, such as retinoblastoma, small cell lung cancer, and cancers of the lung
453 and pleura.

454 Dai et al. (2017) found similar pathways enriching CpGs that are
455 associated with $PM_{2.5}$ constituents. Moreover, pathways correlated with Fe and
456 Ni were also discovered. Similarly, both constituents (Fe and Ni) were
457 significantly associated with *L3MBTL1* and *PEG10*, which may indicate their
458 important effect on diseases. Based on the current reports, the changes in
459 $PM_{2.5}$-induced methylation may affect multiple genes through multiple pathways
460 (Li et al., 2017; Smeester et al., 2014). The mechanism is also more complex
461 because $PM_{2.5}$ constituents are carcinogens. In disease outcomes, lung and
462 pleural cancers were associated with *RB1*. *RB1* is a tumor suppressor gene,

and pRb protein, which it encodes, plays a key role in cell cycle, especially during the S-G2 transition period. Furthermore, pRb is involved in regulation of life activities, such as cell differentiation, apoptosis, and DNA injury response through downstream pathways (Anwar et al., 2014). Smeester et al. (2014) found that Cd induces disease through two signaling pathways of TP53 and AhR and significantly decreases *RB1* expression. In addition, a number of studies have shown that occupational exposure to Cd is associated with cancers, such as lung and bladder cancers (Arita and Costa, 2009; Waalkes, 2003). There was a significant correlation between Cd and *RB1* in the present results (single-constituent model), which may suggest that Cd affects changes in *RB1* methylation to regulate *RB1* expression and thus increase the risk of diseases, such as lung cancer. $PM_{2.5}$ constituents may affect multiple genes to mediate disease through multiple pathways. The altered methylation imprinted gene is a reversible biomarker linking trajectories between environmental exposure and disease development. Therefore, these results deserve further study.

4. Conclusions

In summary, this research utilized a panel study of mixed models and mediation analysis to evaluate the relationship between DNAm of imprinted genes and personal $PM_{2.5}$ and chemical constituent exposure. The results provide novel clues and data on a healthy population that might be used to

elucidate potential mechanisms of adverse health effects of $PM_{2.5}$ exposure. There was no significant correlation between mass of personal $PM_{2.5}$ exposure and DNAm of the candidate genes, but constituents were significantly associated with eight ICRs and a non-imprinted gene. $PM_{2.5}$ exposure resulted in a decrease of most candidate gene methylation. Methylation of imprinted genes was more sensitive to constituents than that of non-imprinted genes, and the change in *L3MBTL1* methylation was the most sensitive. DNAm of imprinted genes may mediate the occurrence of many diseases induced by $PM_{2.5}$ constituents. The results suggest that DNAm of imprinted genes changes significantly with environmental interference in early embryonic development and in adults. Since the number of blood samples was limited in this study, it cannot reflect the lag effect between DNAm and personal $PM_{2.5}$ and constituent exposure or evaluate seasonal change characteristics. Moreover, chemical analysis did not involve more organic analysis. Therefore, future studies need to further verify the experimental conclusions and explore the potential mechanism and specific biomarkers of $PM_{2.5}$ exposure by increasing the sample size, including more chemical constituents, considering the lag effect, and using *in vitro* experiments. In addition, we have not found a cause-effect relationship between ICRs and personal constituents, which should be of interest for future studies. Whether there is a cause-effect relationship between personal $PM_{2.5}$ constituents and DNAm is also worthy of further research. Therefore, additional studies should pay more attention to the cause-effect

relationship between personal $PM_{2.5}$ constituents and ICR methylation.

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844 **Table 1. Imprinting control regions in humans.**

Genes	Hg38	Status	Expressed Allele
PEG13	chr8:140097739-140100885	Imprinted	Paternal
DIRAS3	chr1:68050554-68050790	Imprinted	Paternal
KvDMR	chr11:2699181-2700857	Imprinted	Paternal
IGF2R	chr6:160005233-160006470	Imprinted	Paternal
GNASXL	chr20:58853970-58856184	Imprinted	Paternal
NNAT	chr20:37520202-37521734	Imprinted	Paternal
GNAS Ex1A	chr20:58888598-58892684	Imprinted	Paternal
MCTS2	chr20:31547274-31547489	Imprinted	Paternal
PLAGL1	chr6:144007780-144008710	Imprinted	Paternal
PEG10	chr7:94655547-94657215	Imprinted	Paternal
INPP5F_V2	chr10:119818018-119818873	Imprinted	Paternal
MEST	chr7:130490899-130493270	Imprinted	Paternal
ZIM2/*PEG3*	chr19:56839916-56840916	Imprinted	Paternal
SNURF/*SNRPN*	chr15:24954889-24955907	Imprinted	Paternal
L3MBTL1	chr20:43514571-43514951	Imprinted	Paternal
H19/*IGF2*	chr11:1999841-2000164	Imprinted	Maternal
RB1	chr13:48318500-48319721	Imprinted	Maternal
GRB10	chr7:50782056-50783174	Imprinted	Isoform Dependent
APBA1	chr9:69515972-69516184; chr9:69516268-69517258; chr9:69671889-69673165	Non-imprinted	
CYP1B1	chr2:38231000-38231681	Non-imprinted	
APC	chr5:112707383-112707974	Non-imprinted	
RASSF2	chr20:4823013-4823551	Non-imprinted	
MGMT	chr10:129466685-129467446	Non-imprinted	

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Table 2. Study participant demographics.

Demographics	N (%)	Mean	SD	Min	Median	Max	IQR
Subjects							
Male	17 (53%)						
Female	15 (47%)						
Age (year)		21.19	1.05	19.00	21.00	23.00	1.75
BMI (Kg/m^2)		21.01	2.82	16.69	20.73	28.91	2.95
DNA methylation (%)							
Imprinted genes							
INPP5F_V2		88.36	1.98	79.84	88.43	93.23	2.81
GRB10		84.47	2.63	79.07	84.43	89.71	3.99
PLAGL1		81.53	4.00	68.08	81.81	89.96	5.43
GNASXL		76.33	3.41	51.77	76.36	84.64	3.18
IGF2R		76.86	6.52	64.58	75.68	98.45	4.62
NNAT		70.03	3.95	52.57	70.51	78.93	4.62
PEG13		67.95	3.24	53.42	68.01	75.75	3.43
RB1		62.89	4.46	46.96	63.06	73.01	6.77
KvDMR		61.75	4.09	49.45	61.28	73.79	4.91
L3MBTL1		58.61	7.31	42.59	58.00	81.63	8.98
MEST		58.43	2.85	52.44	58.00	66.16	3.69
ZIM2/PEG3		56.11	4.73	41.58	56.40	68.90	6.11
DIRAS3		55.92	3.34	44.66	55.95	64.21	4.35
PEG10		52.90	2.82	43.96	53.00	61.03	3.67
MCTS2		52.12	4.30	43.22	52.06	79.72	4.46
SNURF/SNRPN		49.54	4.58	38.08	49.80	61.33	5.96
H19/IGF2		39.87	2.05	36.14	39.58	47.65	2.73
GNAS Ex1A		33.09	3.27	25.00	32.93	43.77	4.77
Non-imprinted genes							
APBA1		67.91	5.24	42.54	69.53	77.38	6.30
CYP1B1		6.67	0.86	5.00	6.41	9.29	1.07
APC		0.65	0.11	0.40	0.65	1.06	0.12
RASSF2		0.94	0.11	0.65	0.94	1.23	0.12
MGMT		2.01	0.68	0.27	1.97	6.12	0.73

Abbreviations: SD, standard deviation; IQR, interquartile range.

Table 3. Distribution of personal $PM_{2.5}$ exposure, chemical constituents, and weather variables during study period in Guangzhou, China (2016).

Variables	Mean	SD	Min	Median	Max	IQR
Particle						
Personal $PM_{2.5}$, $\mu g/m^3$	78.38	30.45	18.17	88.74	116.55	32.79
Carbonaceous fractions						
TC, $\mu g/m^3$	42.09	10.20	23.97	45.74	57.87	17.63
OC, $\mu g/m^3$	32.59	7.55	18.57	34.77	43.93	11.36
EC, $\mu g/m^3$	9.50	3.37	3.85	10.98	13.95	7.08
Ions						
NO_3^-, $\mu g/m^3$	10.80	7.24	2.44	9.51	26.92	10.49
SO_4^{2-}, $\mu g/m^3$	10.59	4.78	3.66	10.03	16.34	10.29
Cl^-, $\mu g/m^3$	0.83	0.87	0.17	0.42	2.94	0.77
F^-, $\mu g/m^3$	0.11	0.13	0.03	0.05	0.52	0.05
$C_2O_4^-$, $\mu g/m^3$	0.11	0.14	0.01	0.05	0.48	0.14
NO_2^-, $\mu g/m^3$	0.09	0.14	0.01	0.04	0.51	0.01
Crystal metals						
Al, $\mu g/m^3$	3.92	6.16	0.97	1.67	24.91	0.98
Mg, $\mu g/m^3$	1.37	0.79	0.71	1.16	3.74	0.70
Ca, $\mu g/m^3$	1.12	0.77	0.61	0.83	3.48	0.34
K, $\mu g/m^3$	0.70	0.33	0.21	0.58	1.20	0.66
Na, $\mu g/m^3$	0.58	0.21	0.27	0.54	0.87	0.42
Ba, ng/m^3	12.56	2.87	7.71	13.03	17.00	3.89
Sr, ng/m^3	2.35	0.85	1.06	2.06	4.30	0.89
Transition metals						

Fe, μg/m^3	2.64	1.09	1.06	2.37	4.90	1.79
Ti, μg/m^3	0.86	0.55	0.14	0.78	2.52	0.44
Zn, μg/m^3	0.21	0.09	0.07	0.22	0.38	0.16
V, ng/m^3	88.82	14.08	61.05	93.36	116.60	19.34
Zr, ng/m^3	29.22	7.17	15.44	31.47	38.68	13.82
Cr, ng/m^3	25.60	4.64	18.34	25.68	37.13	4.72
Cu, ng/m^3	25.19	10.19	8.62	24.85	51.82	11.49
Mn, ng/m^3	21.30	9.24	5.86	20.98	37.49	11.47
Ni, ng/m^3	14.08	31.74	2.91	4.45	128.70	4.97
Mo, ng/m^3	2.21	0.61	1.32	2.33	3.23	1.31
W, ng/m^3	1.26	0.19	0.99	1.25	1.55	0.41
Cd, ng/m^3	1.04	0.61	0.32	0.97	2.50	0.69
Y, ng/m^3	0.46	0.09	0.27	0.49	0.57	0.14
Co, ng/m^3	0.33	0.13	0.11	0.31	0.57	0.19
Sc, ng/m^3	0.17	0.13	0.08	0.14	0.57	0.05
Other metals/metalloid elements						
Si, μg/m^3	3.10	1.41	1.43	2.72	6.46	1.26
P, ng/m^3	54.18	23.08	25.36	45.53	103.97	28.96
Pb, ng/m^3	39.67	14.22	24.01	35.99	69.90	25.50
As, ng/m^3	29.14	3.08	21.58	29.74	35.55	2.53
Sn, ng/m^3	7.90	4.98	2.14	7.76	21.95	7.66
Sb, ng/m^3	8.23	3.19	3.74	8.69	13.58	6.11

Rb, ng/m^3	2.34	1.01	0.67	2.26	3.91	1.79
Bi, ng/m^3	1.42	0.75	0.24	1.43	2.72	1.20
Li, ng/m^3	1.26	0.43	0.74	1.18	1.98	0.77
Ce, ng/m^3	0.67	0.19	0.28	0.70	0.98	0.18
La, ng/m^3	0.42	0.13	0.17	0.43	0.61	0.16
Tl, ng/m^3	0.25	0.11	0.14	0.23	0.48	0.11
Cs, ng/m^3	0.21	0.11	0.09	0.16	0.46	0.16
Th, ng/m^3	0.15	0.05	0.07	0.15	0.24	0.10
U, ng/m^3	0.10	0.03	0.05	0.11	0.14	0.04
Sm, ng/m^3	0.05	0.02	0.01	0.04	0.08	0.03
Be, ng/m^3	0.04	0.02	0.02	0.03	0.08	0.03
Weather condition[a]						
relative humidity, %	58.54	9.18	46.75	60.29	73.84	15.69
Temperature, ºC	23.90	4.47	16.26	25.65	28.15	9.01
Ambient concentration of Air pollutants[b]						
Ambient $PM_{2.5,}$ μg/m^3	40.46	14.32	15.33	43.11	56.22	29.61

[a]Mean of temperature and relative humidity recorded at Guangzhou Meteorological Service (http://www.tqyb.com.cn/gz/weatherAlarm/) during the period of personal exposure sampling.

[b]Mean of ambient $PM_{2.5}$ recorded from Air Quality Monitoring System in Guangzhou (http://112.94.64.160:8023/gzaqi_new/RealTimeDate.html) during the period of personal exposure sampling.

858 **Table 4. KEGG pathway and disease enrichment analysis.** Results are
859 ranked by *p*-value.

Term	*p*-Value	Corrected *p*-Value
KEGG Pathways		
Tryptophan metabolism	0.01	0.03
Bladder cancer	0.01	0.03
Ovarian steroidogenesis	0.01	0.03
Non-small cell lung cancer	0.01	0.04
Steroid hormone biosynthesis	0.01	0.04
Glioma	0.01	0.04
Pancreatic cancer	0.01	0.04
Melanoma	0.01	0.04
Chronic myeloid leukemia	0.01	0.04
Metabolism of xenobiotics by cytochrome P450	0.01	0.04
Chemical carcinogenesis	0.01	0.04
Small cell lung cancer	0.01	0.05
Prostate cancer	0.01	0.05
Endocrine resistance	0.01	0.05
Lysosome	0.02	0.06
Cell cycle	0.02	0.06
Breast cancer	0.02	0.06
Gastric cancer	0.02	0.06
Cushing syndrome	0.02	0.06
Hepatitis C	0.02	0.06
Cellular senescence	0.02	0.06
Hepatitis B	0.02	0.06
Hepatocellular carcinoma	0.02	0.06
Kaposi sarcoma-associated herpesvirus infection	0.02	0.07
Viral carcinogenesis	0.02	0.07
Epstein-Barr virus infection	0.02	0.07
Human T-cell leukemia virus 1 infection	0.03	0.07
Human cytomegalovirus infection	0.03	0.07
Endocytosis	0.03	0.08
MicroRNAs in cancer	0.04	0.08
Human papillomavirus infection	0.04	0.09
KEGG Diseases		
Retinoblastoma	0.00	0.03
Chronic myeloid leukemia	0.00	0.03
Small cell lung cancer	0.00	0.03
Osteosarcoma	0.00	0.03
Anterior segment dysgenesis	0.00	0.03
Bladder cancer	0.00	0.03
Esophageal cancer	0.00	0.03

Glioma	0.00	0.03
Cancers of the lung and pleura	0.00	0.03
Cancers of the urinary system	0.00	0.03
Hepatocellular carcinoma	0.00	0.03
Cancers of eye, brain, and central nervous system	0.00	0.03
Cancers of soft tissues and bone	0.00	0.03
Congenital malformations of eye	0.01	0.03
Cancers of the digestive system	0.01	0.04
Chromosomal abnormalities	0.01	0.04
Cancers of hematopoietic and lymphoid tissues	0.01	0.04
Other congenital disorders	0.01	0.05
Cancers	0.03	0.07

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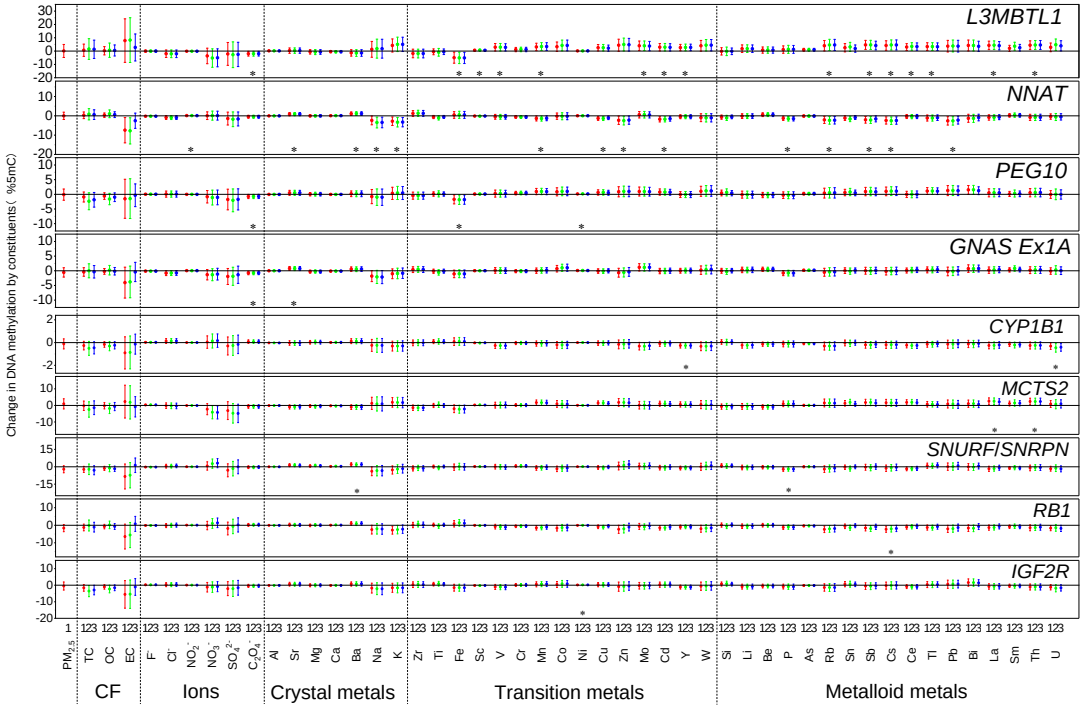
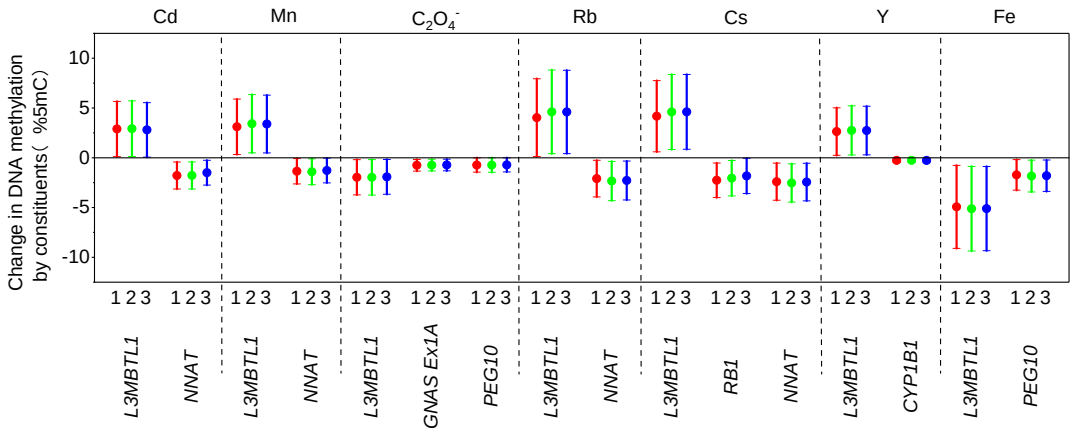


Fig. 1. Changes in DNA methylation (5%mC) of nine genes associated with IQR increase in $PM_{2.5}$ constituents in three mixed-effects models. Nine genes included *L3MBTL1*, *NNAT*, *PEG10, GNAS Ex1A*, *CYP1B1*, *MCTS2*, *SNURF*/*SNRPN*, *RB1,* and *IGF2R*. Notes: Numbers on the abscissa represent three mixed models (1: single-constituent model; 2: constituent-$PM_{2.5}$ adjustment model; and 3: constituent-residual model). CF represents carbonaceous fractions of $PM_{2.5}$. *means significant correlation in three mixed models simultaneously (*$p < 0.05$).



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871 Fig. 2. Comparison of DNA methylation change degree in different genes.

872 Compared to other genes, degree of change in *L3MBTL1* is greatest when

873 constituents are the same (e.g., Cd, Mn, $C_2O_4^-$, Rb, Cs, Y, and Fe). Numbers

874 on the abscissa represent three mixed models (1: single-constituent model; 2:

875 constituent-$PM_{2.5}$ adjustment model; and 3: constituent-residual model). There

876 is a significant correlation between genes and constituents ($p < 0.05$).

Declaration of competing interest

The authors declare they have no actual or potential competing financial interests.

Author statement

Yaohui Liang: Experiments, Data Curation, Writing - Original Draft. Liwen Hu: Conceptualization, Methodology, Investigation. Jun Li: Project administration. Fei Liu: Software. Kevin C. Jones: Conceptualization. Daochuan Li: Methodology. Jing Liu: Formal analysis. Duohong Chen: Resources, Data Curation. Jing You: Supervision. Zhiqiang Yu: Resources. Gan Zhang: Funding acquisition. Guanghui Dong: Resources, Funding acquisition. Huimin Ma: Methodology, Writing - Review & Editing, Funding acquisition.