Journal of Hazardous Materials 463 (2024) 132780

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Research Paper

Epigenome-wide association study on short-, intermediate- and long-term ozone exposure in Han Chinese, the NSPT study

Xiyang Cai [a,1,2], Kaixuan Li [a,1,3], Xia Meng [b,1,4], Qinglin Song [a,1,5], Su Shi [b], Wenran Li [a,6], Yue Niu [b,7], Li Jin [c,d,e], Haidong Kan [b,f,*,8], Sijia Wang [a,e,g,**,9]

[a] CAS Key Laboratory of Computational Biology, Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China

[b] School of Public Health, Shanghai Institute of Infectious Disease and Biosecurity, Key Lab of Public Health Safety of the Ministry of Education and NHC Key Lab of Health Technology Assessment, Fudan University, Shanghai 200032, China

[c] State Key Laboratory of Genetic Engineering, Collaborative Innovation Center for Genetics and Development, and Human Phenome Institute, Fudan University, Shanghai, China

[d] Ministry of Education Key Laboratory of Contemporary Anthropology, Department of Anthropology and Human Genetics, School of Life Sciences, Fudan University, Shanghai, China

[e] Taizhou Institute of Health Sciences, Fudan University, Taizhou, Jiangsu, China

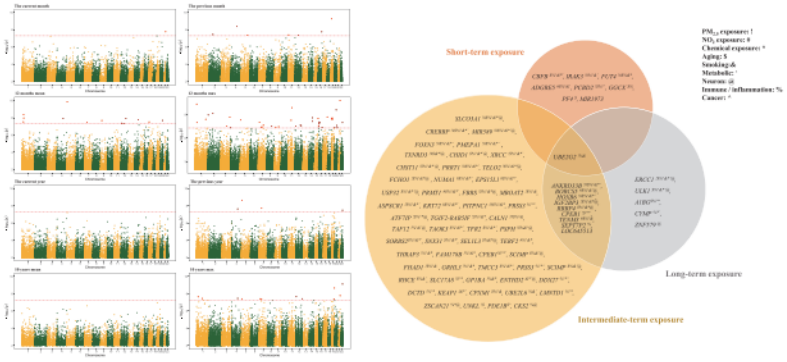
[f] Children's Hospital of Fudan University, National Center for Children's Health, Shanghai 201102, China

[g] Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming, China

HIGHLIGHTS

- EWAS in Chinese individuals identified DNAm changes associated with short-, intermediate- or long-term ozone exposure.
- Mid- and long-term exposure associated DNAm changes were apt to enhanced in response to longer exposure duration.
- Related genes were enriched in pathways involving telomere maintenance process and megakaryocyte differentiation.

GRAPHICAL ABSTRACT



* Correspondence to: Department of Environmental Health, School of Public Health, Fudan University, P.O. Box 249, 130 Dong-An Road, Shanghai 200032, China.
** Correspondence to: CAS Key Laboratory of Computational Biology, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai, China.
E-mail addresses: kanh@fudan.edu.cn (H. Kan), wangsijia@picb.ac.cn (S. Wang).
[1] These authors contributed equally to this work.
[2] **ORCID:** 0000–0002-3868–5426
[3] **ORCID:** 0000–0003-2869–0050
[4] **ORCID:** 0000–0002-0751–1722
[5] **ORCID:** 0009–0002-9613–2245
[6] **ORCID:** 0000–0002-1712–6895
[7] **ORCID:** 0000–0001-9819–8614
[8] **ORCID:** 0000–0002-1871–8999
[9] **ORCID:** 0000–0001-6961–7867

https://doi.org/10.1016/j.jhazmat.2023.132780
Received 9 June 2023; Received in revised form 9 October 2023; Accepted 12 October 2023
Available online 14 October 2023

ARTICLE INFO

Editor: Yuming Guo

Keywords:
Ozone
DNA methylation
EWAS
Functional analysis
Pathway enrichment

ABSTRACT

Epidemiological and epigenetic studies have acknowledged ambient ozone exposure associated with inflammatory and cardiovascular disease. However, the molecular mechanisms still remained unclear, and epigenome-wide analysis in cohort were lacking, especially in Chinese. We included blood-derived DNA methylation for 3365 Chinese participants from the NSPT cohort and estimated individual ozone exposure level of short-, intermediate- and long-term, based on a validated prediction model. We performed epigenome-wide association studies which identified 59 CpGs and 30 DMRs at a strict genome-wide significance ($P < 5 \times 10^{-8}$). We also conducted comparison on the DNA methylation alteration corresponding to different time windows, and observed an enhanced differentiated methylation trend for intermediate- and long-term exposure, while the short-term exposure associated methylation changes did not retain. The targeted genes of methylation alteration were involved in mechanism related to aging, inflammation disease, metabolic syndrome, neurodevelopmental disorders, and oncogenesis. Underlying pathways were enriched in biological activities including telomere maintenance process, DNA damage response and megakaryocyte differentiation. In conclusion, our study is the first EWAS on ozone exposure conducted in large-scale Han Chinese cohort and identified associated DNA methylation change on CpGs and regions, as well as related gene functions and pathways.

1. Introduction

Ambient air pollution gave estimated contribution to approximately 9 million deaths per year, corresponding to one sixth deaths worldwide, and ranked fifth in the leading risk factors for global mortality and disease [5]. Ozone, a potent photochemical oxidant in the ambient air pollution, is a major concern to human disease and death [26]. Recent study by Global Burden of Disease acknowledged that exposure to ozone pollution has caused 365,222 premature deaths per year globally [48]. It has been reported that oxidate stress pathway and pulmonary inflammation attribute the impact of ozone on chronic respiratory disease such as cardiovascular and respiratory system exerts. Other associated health risk included reproductive outcomes such as premature birth, and low birthweight [36].

Although many prior studies focused on the effect of ozone exposure on the risk of mortality and morbidity, much unknown remained on the underlying mechanisms for these association. DNA methylation (DNAm) is acknowledged as a regulator of transposable element and genes, characterizing the status of gene expression without altering DNA sequence [13]. DNAm has been identified as epigenetic factors to be altered by ozone exposure [8], as well as mediate the effects of air pollution exposure [35]. Investigation on the epigenetic effect might reveal the relationship between ozone exposure and health outcomes.

One limitation of former epigenetic studies on ozone exposure was that those studies mainly focused on the methylation change on a number of biomarkers of specific health outcome, such as coding region or regulatory elements. For instance, [43] measured Angiotensin-converting enzyme (*ACE*) and Endothelin 1 (*EDN1*) as core components in regulating blood pressure. Niu et al., [27] evaluated the methylation change on Nitric oxide synthase 2 (*NOS2A*) and Arginase 2 (*ARG2*) in the arginase–nitric oxide synthase pathway inducing respiratory inflammatory response. Bind et al., [2] investigated the five genes related to cardiovascular pathways, including tissue factor (*F3*), interferon gamma (*IFN-γ*), interleukin 6 (*IL-6*), toll-like receptor 2 (*TLR-2*), and intercellular adhesion molecule 1 (*ICAM-1*). Unlike the above hypothesis-driven schemes, an epigenome-wide association study made it possible for a comprehensive screen to capture the genome-wide DNAm alteration induced by ozone exposure. To the best of our knowledge, two recent studies have conducted genome-wide scans to detect differentially methylated loci associated with either prenatal exposure [20] or twelve-hours-before exposure in two hours duration [3], and a total of 5 CpGs and 4 differential methylation regions (DMRs) were identified. However, these studies focused on ozone exposure scenes far different from routine exposure of common population in daily lives, and were also limited by relatively small sample size. To this point, there is a lack of large-scale cohort-based epigenome wide methylation research on ozone exposure.

To elucidate mechanism underlying ozone exposure, we performed EWASs on ozone exposure and identified differential methylation probes on a large cohort of Han Chinese population in this study. The individual ozone exposure level was predicted by a validated model and we categorized the ozone exposure into three exposure time windows, including short- (1 month), intermediate- (1 year), and long-term (10 years). We identified differential DNAm variants associated with ozone exposure and proposed several potential underlying molecular mechanisms of ozone exposure, including oxidative stress, DNA damage repairment and megakaryocyte differentiation.

2. Material and methods

2.1. Study population

The Nation Survey of Physical Traits (NSPT) cohort was a population-based cohort study which enrolled participants of Chinese nationality in four sampling times from different suburban regions of China: Taizhou, Jiangsu in August 2015; Zhengzhou, Henan in July 2017; Nanning, Guangxi in March 2018 and Taizhou, Jiangsu in March 2019. Individuals were recruited as volunteers by random selection, and those with any critical illness were excluded from recruitment. Residential street address of each participant at the time point of recruitment was collected by questionnaire (Supplementary Table S1) and used for ozone prediction.

The individual DNAm level was obtained using the Illumina Infinium Methylation EPIC BeadChips from blood samples, with 811,876 CpG probes retained after quality control. Outputs were the beta values that represent the percentage of methylation for every CpG probe (detailed in Supplementary). All covariates incorporated in our study were also collected by the personal questionnaire which includes sex, age, smoking status, smoke pack year, passive smoking, alcohol consumption, education and household income. BMI was derived from on-site measured height and weight. Genetic principal components (PCs) were calculated by principal component analysis on genotypes, to reveal and adjust for potential population structure. Blood leukocytes fractions (B cells, CD4 + and CD8 + T cells, NK cells, monocytes and neutrophils) were estimated based on DNAm measurement using EpiDISH [47]. A total of 3413 individuals with complete street address, matched DNAm data and other individual information were included for follow-up analysis (Fig. S1). The study was approved by the Ethics Committees of Fudan University (14117) and the Shanghai Institutes for Biological Sciences (ER-SIBS-261410), and all participants provided written informed consents.

2.2. Ozone Exposure assessment

2.2.1. Ozone predictions

The maximum daily 8-hour average (MDA8) ozone concentrations were predicted by the random forest method at a daily level and 1 km × 1 km resolution during 2005–2019 in mainland China with full spatio-temporal coverage. The details of model development and evaluation process were described and published in previous study [25] and summarized here. Random forest models were trained with ground-level MDA8 ozone measurements served as a dependent variable, and ozone simulations from the Community Multiscale Air Quality (CMAQ) multiscale chemical transport model, meteorological parameters, population density, elevation, and road network data served as independent variables. The overall cross validation R^2 and root-mean-square error (RMSE) values between modeled and observed ozone concentrations at daily level of random forest models were 0.80 and 20.93 $\mu g/m^3$, respectively. The monthly and annual mean ozone concentrations were calculated for each grid cell at 1-km spatial resolution.

2.2.2. Derived short-, intermediate- and long-term Ozone exposure

Monthly and annual mean ozone concentrations of grid cells were provided for each participant according to the individual residential information. Monthly ozone exposure levels were defined as estimated concentrations of the same month as the blood sampling time, and the prior 1–12 months to the sampling times. Annual ozone exposure levels were defined as the estimated concentrations of each year from 2005 to 2019. Based on the monthly and annual ozone exposure prediction, we divided the ozone exposure into three levels by the duration of exposure. 2 short-term ozone exposure levels were defined as ozone exposure of one-month, which includes the exposure level i) in the month (described as "the current month" in following) and ii) the previous month of the sampling points. 4 intermediate-term ozone exposure levels were defined as ozone exposure iii) in the year and iv) previous year (described as "the current year" and "the previous year"), as well as the v) mean and vi) maximum values of the 12 months prior to the sampling points (described as "12 months mean" and "12 months max"). 2 long-term exposure levels were defined as the vii) mean and viii) maximum value of the previous 10 years before the sampling points (described as "10 years mean" and "10 years max"). EWASs were conducted separately for 8 exposures level of short-, intermediate- and long-term.

For higher resolution in time windows, we calculated the mean value of each ascending time windows, from the current month, to the previous 11 months by a one-month step, and the average from current to previous X months was described as "X months prior" in the following.

2.3. Statistical analysis

2.3.1. Epigenome-wide association analysis (EWAS)

EWAS was performed to capture the association between ozone exposure and DNAm level at single CpG with generalized linear model using *limma* R package [37], with the percentage of methylation (beta value) as the response and the ozone exposure level as the predictor variable (R scripts available in Supplementary). To adjust for confounding, age, sex, smoking status (not, former or current smoker), smoke pack year, BMI, sampling point index (Taizhou2015, Zhengzhou2017, Nanning 2018 and Taizhou2019), blood leukocytes fractions and the first ten genetic PCs were incorporated as covariates in the EWAS model. The genome-wide significance threshold (P. Value $< 5 \times 10^{-8}$) was used to identify the CpGs significantly associated with ozone. The same EWAS model was performed in every subgroup to explore the heterogeneity (detailed in Supplementary). We also conducted sensitivity analysis to investigate the confounding effect of the covariate choice in EWAS model, by comparing the estimated effect sizes from regression models which excluded each covariate from the primary model, as well as additionally included potential factors to the primary model (detailed in Supplementary).

2.3.2. Comparison between different time windows

We extended the EWAS on a higher time-window solution to reveal any DNAm alteration pattern as exposed to ozone in a longer period. Specifically, with the estimated monthly ozone exposure in the prior 12 months, we calculated the mean value of each ascending time windows, from the current month to the previous 12 months by a one-month step. We then conducted EWAS on ozone exposure of each time window, with the same covariates set as the main EWAS model. Effect sizes and p-values were compared between EWAS on different time windows.

2.3.3. Differential methylation region analyses

In addition to the analyses of effect on individual CpG, we performed DMR analyses to investigate the effect of ozone exposure on locally dependent CpGs. To reduce false positive rates, we applied two algorithms to identify DMRs, *DMRcate* [33] and *comb-p* [31]. Both methods used the result of summary statistic data of EWAS on single probe level as input. *DMRcate* identified DMRs by applying tunable kernel smoothing process, whereas *comb-p* clustered local regions from the low p-value and examined the correlation to discover DMRs. We followed the same criteria as [21] to discover DMRs in both *DMRcate* and *comb-p*. First, more than three probes should be included in a DMR. Second, the length of a differential methylation region should not be over 500 base pairs (bp). Third, the multiple corrected p-value of differential methylation region should under 0.01 for both method: Benjamini-Hochberg FDR in *DMRcate* and Sidak -corrected p-value in *comb-p*.

2.3.4. Global and functional region analyses

To test whether ozone exposure altered the overall profile of DNAm, we examinate the change of global DNAm distribution using *GAMP* package in R [46]. We approximated the density and cumulative distribution of the DNAm using B-spline basis functions and obtained the B-spline coefficients as representatives of overall DNAm distribution. A joint test was carried out for significant association with ozone exposure. We also calculated the arithmetic mean value of methylation level of all probes and tested for association between global DNAm level and ozone exposure.

On a regional aspect, we classified the CpGs into several functional regions, by assigning each CpG to functional annotation using *annotatr* [4]. Arithmetic mean methylation level of CpGs at each functional region were calculated separately, and linear regression was performed to test the association between regional average methylation and ozone exposure. Association tests above were adjusted for sex, BMI, blood cell proportion and smoking.

2.4. Functional analysis on identified markers

Ozone associated CpGs were assigned to genes using *annotatr* R package. To facilitate additional epigenome-phenotypes associations, CpGs were queried against association studies databases, EWAS atlas (https://ngdc.cncb.ac.cn/ewas/atlas) and EWAS catalog (https://ewascatalog.org/), to expanding the associated phenotypic spectra. CpGs- and DMRs- mapped genes were annotated with airborne pollution related traits in a reverse phenotyping approach [42], by querying the mapped genes against EWAS databases as well as GWAS catalog (https://www.ebi.ac.uk/gwas/). We furtherly performed enrichment analysis to identify functional characteristics for the mapped genes by Metascape (https://metascape.org/).

3. Results

The NSPT cohort consisted of a total of 3413 Han Chinese individuals in 4 subgroups, 529 recruited in Taizhou in 2015, 961 in Zhengzhou in 2017, 1432 in Nanning in 2018 and 491 in Taizhou 2019. Matched Ozone exposure level, blood sample and other personal information were available for all participants. 1297 (38.00%) participants were males and 2116 (62.00%) were females, and the population aged from

18 to 83 years old (mean ± SD = 50.37 ± 12.63). 484 (37.32%) of the male participants and 2088 (98.68%) female participants were non-smoker. Characteristics summary of all personal information were shown in Table 1.

3.1. Distribution of ozone exposure across all time window

The distribution of derived short-, intermediate- and long-term ozone exposure level were shown in Fig. 1. The variations were relatively high for short-term exposure, as well as the maximum metrics of 12 months max and 10 years max. When stratified by sample subgroups, the intra-group distribution revealed relatively minor variations in each time window for all subgroups, compared to the high inter-group variants. Such difference of ozone exposure level and low inter-group overlap demonstrated an inter-group heterogeneity and spatially differed seasonal patterns, which could be explained by the seasonal, years and geographical difference. While the exposure levels peaked during April and September and decreased sharply in December to January in all region, the distribution showed unsynchronized peaks according to the recruitment time, as well as different levels according to geographical regions and years (Fig. 1A and B, and Fig. S2). Compared to the international guideline, the ozone concentrations in peak seasons in Taizhou 2015, Zhengzhou 2017, and Taizhou 2019 were all higher than the WHO recommended air quality guideline levels of peak season [30] Interim target 1 (100 $\mu g/m^3$), while lower than the Interim target 1 and higher than the Interim target 2 (70 $\mu g/m^3$) in Nanning 2018 (Supplementary and Fig. S2). Also, we observed a relatively higher correlation across different time windows within 4 months prior as well as above 8 months, but not between the two sides (Fig. 1C).

3.2. Epigenome-wide analyses

In total, EWAS identified 59 differentially methylated CpGs where methylation levels were associated with ozone exposure at a genome-wide significance (P. Value $< 5 \times 10^{-8}$), of which 8 CpGs associated with one-month short-term exposure, 48 CpGs associated with one-year intermediate-term exposure and 12 CpGs associated with ten-years long-term exposure (Fig. 2 and Table S2). Of the 8 short-term exposure associated CpGs, 1 and 7 were associated with exposure in the month and previous month of sampling point, respectively. Of the 48 CpGs associated with intermediate-term exposure, 41 and 13 CpGs associated with maximum and mean exposure level derived from previous 12 months measurement, and 1 CpG from the previous year average prediction. The 12 long-term exposure related CpGs were found associated with maximum exposure level of the previous 10 years.

26 out of the 59 identified CpGs were validated in multiple subgroups, with effects in consistent direction and at a nominal significance (P. Value < 0.05). Meanwhile, several CpGs showed diverse methylation alteration across the 4 subgroups. Specifically, a proportion of CpGs were significantly associated in samples from Nanning 2018 subgroup, while showed negligible or reversed effect in other subgroups (Fig. S3).

Sensitivity analysis demonstrated that the effect sizes on the identified CpGs were robust to intrinsic and extrinsic factors including age, sex, smoking (both active and passive smoking), PM2.5 exposure, alcohol consumption, education level and household income. On the other hand, the effect sizes were sensitive to blood leukocytes fractions, which implies that different blood cells may exhibit varying patterns of methylation changes on the identified CpGs, in response to ozone exposure, suggesting a blood-cell-type specific effect of ozone exposure (Fig. S4).

For independent replication, we aligned the 59 CpGs with the two previous EWAS studies on prenatal ozone exposure [20] and two hours ozone exposure in randomized controlled trial study [3], along with 3 gene-specific association studies [2,27,43] for cross-study validation, but the identified CpGs did not overlap with the reported variants from previous EWASs. We further tested for significance of the reported CpGs

Table 1
Characteristics summary of individual in NSPT cohort.

	Total (N = 3413)	Taizhou, 2015 (N = 529)	Zhengzhou, 2017 (N = 961)	Nanning, 2018 (N = 1432)	Taizhou, 2019 (N = 491)
Sex, N (%)					
Male	1297 (38.0)	212 (40.1)	393 (40.9)	541 (37.8)	151 (30.8)
Female	2116 (62.0)	317 (59.9)	568 (59.1)	891 (62.2)	340 (69.2)
Age, Mean (sd)					
Mean (SD)	50 (13)	48 (13)	44 (13)	55 (11)	53 (9.2)
BMI, Mean (sd)					
Mean (SD)	25 (3.5)	25 (3.7)	25 (3.7)	24 (3.3)	25 (3.4)
Smoke status, Mean (sd)					
Never Smoker	2572 (75.4)	385 (72.8)	723 (75.2)	1085 (75.8)	379 (77.2)
Former moker	160 (4.7)	33 (6.2)	41 (4.3)	70 (4.9)	16 (3.3)
Current Smoker	681 (20.0)	111 (21.0)	197 (20.5)	277 (19.3)	96 (19.6)
Smoke pack year Mean (sd)					
Mean (SD)	0.54 (1.2)	0.57 (1.2)	0.52 (1.1)	0.52 (1.3)	0.61 (1.3)
Passive smoking					
Yes	1798 (52.7)	285 (53.9)	442 (46.0)	794 (55.4)	277 (56.4)
No	1615 (47.3)	244 (46.1)	519 (54.0)	638 (44.6)	214 (43.6)
Alcohol consumption, N (%)					
Rarely	2669 (78.2)	383 (72.4)	788 (82.0)	1114 (77.8)	384 (78.2)
Once a week	193 (5.7)	26 (4.9)	82 (8.5)	73 (5.1)	12 (2.4)
2–3 times a week	81 (2.4)	0 (0.0)	32 (3.3)	37 (2.6)	12 (2.4)
> 3 times a week	305 (8.9)	95 (18.0)	25 (2.6)	115 (8.0)	70 (14.3)
NA	165 (4.8)	25 (4.7)	34 (3.5)	93 (6.5)	13 (2.6)
Education, N (%)					
Uneducated	285 (8.4)	86 (16.3)	22 (2.3)	92 (6.4)	85 (17.3)
Primary	830 (24.3)	127 (24.0)	108 (11.2)	443 (30.9)	152 (31.0)
Junior secondary	1229 (36.0)	182 (34.4)	298 (31.0)	567 (39.6)	182 (37.1)
Senior secondary	521 (15.3)	62 (11.7)	207 (21.5)	215 (15.0)	37 (7.5)
Tertiary and above	397 (11.6)	47 (8.9)	300 (31.2)	28 (2.0)	22 (4.5)
NA	151 (4.4)	25 (4.7)	26 (2.7)	87 (6.1)	13 (2.6)
Annual Household income in CNY, N (%)					
< 2500	45 (1.3)	7 (1.3)	3 (0.3)	34 (2.4)	1 (0.2)
2500–4999	63 (1.8)	7 (1.3)	1 (0.1)	53 (3.7)	2 (0.4)
5000–9,999	161 (4.7)	17 (3.2)	23 (2.4)	115 (8.0)	6 (1.2)
10,000–19999	269 (7.9)	21 (4.0)	51 (5.3)	177 (12.4)	20 (4.1)
20,000–34,999	461 (13.5)	60 (11.3)	123 (12.8)	226 (15.8)	52 (10.6)

(continued on next page)

Table 1 (*continued*)

	Total (N = 3413)	Taizhou, 2015 (N = 529)	Zhengzhou, 2017 (N = 961)	Nanning, 2018 (N = 1432)	Taizhou, 2019 (N = 491)
> 35,000	1903 (55.8)	388 (73.3)	723 (75.2)	462 (32.3)	330 (67.2)
NA	511 (15.0)	29 (5.5)	37 (3.9)	365 (25.5)	80. 16.3)

in our EWAS. 75 out of 278 CpGs reached nominal significance and the permutation analysis determined that the reported CpGs were merely replicated by EWAS on the current month and were not replicated by the remaining EWASs (Supplementary and Fig. S5). However, we reasoned that the cross-study inconsistency could be result from that the previous studies measured ozone exposure utterly different from the ambient ozone exposure scenario in this study.

3.3. Comparison on methylation effect between short-, intermediate- and long-term ozone exposure

52 of 59 associated CpGs were nominal significant (P. Value < 0.05) in all three exposure durations. And one CpG, cg16369592, showed consistent hypomethylation effect across all exposure terms at genome-wide significance. Those CpGs identified in the intermediate-term exposure EWAS were all associated with long-term exposure at a nominal significance, 7 out of which were simultaneously acknowledged as "long-term exposure associated" at a genome-wide significance. Among the intermediate-term exposure associated CpGs, 28 CpGs showed an increasing hypomethylation effect for exposure of longer period. As for the 4 CpGs exclusively associated with long-term ozone exposure, 3 hypomethylated and 1 hypermethylated, all showed an enhanced methylation effect from short- to long-term ozone exposure.

Meanwhile, of the remaining 7 CpGs exclusively identified in short-term exposure EWAS, effect sizes were not different from zero and even tend to reverse in direction in intermediate- and long-term exposure results. By a further comparison on the short-term exposure associated CpGs across moving time windows, we found that the significance of CpGs decrease sharply in results of longer exposure duration. As for exposure approaching intermediate-term, results were coherent with intermediate- and long-term exposure, with approximate effect sizes and significance.

In general, the effect sizes of CpGs in intermediate- and long-term exposure revealed a gradually enhanced differentiated methylation trend with the extension of time scale, while the short-term exposure associated methylation changes did not retain in response to longer exposure (Fig. 3).

3.4. Differentially methylated regions analysis

We applied the two cluster-based DMR analyses, and identified 104 significant (FDR-P. Value < 0.01) DMRs from *DMRcate*, and 164 significant (Sidak $p < 0.01$) DMRs from *comb-p*, associated with any ozone exposure. To reduce false positive, we considered the DMRs shared by the results from both methods as validated findings (Table S4C). Of the 30 overlapped DMRs, 2 were associated with short-term ozone exposure, 27 with intermediate-term exposure and 4 with long-term exposure. Notably, only 1 DMRs (chr15:83240550–83240792) overlapped with the significant CpG (cg03152456), while the other 29 DMRs were novel to the EWAS identified CpGs. While we attempted to validate the DMRs with independent studies, the DMRs did not overlap with the identified variants from two previous EWAS studies [3,20].

Moreover, we carried out an analysis based on genomic regions to investigate the association between ozone exposure and global or regional methylation levels. Our findings indicated no significant association (P. value > 0.05) between global methylation and any level of ozone exposure. However, we did observe a modification in the methylation level of both the gene body and promoter region (Table S4D). These functional regions displayed a hypomethylated pattern under intermediate- and long-term ozone exposure, suggesting a possible association with the upregulation of gene expression. Furthermore, although we found a significant association between regional methylation change and short-term exposure, the direction of the methylation effect was inconsistent between exposure in the previous and current month.

3.5. Gene annotation and enrichment analysis

We mapped the 59 CpGs and 30 DMRs to a total of 102 genes by *annotatr*, and these genes were deduced as the potential regulatory target. To investigate the potential function of the targeted genes, we leveraged databases of association studies, and overlapped the genes with traits reported by previous GWAS and EWAS (Fig. 4 and Table S5). Consistency in associations was found in the suggested genes approximate to the top CpG sites, with other pollutants exposures such as $PM_{2.5}$, NO_2, and smoking, as well as other contaminants. Specifically, 39 genes had been previously reported related to $PM_{2.5}$ or $PM_{2.5}$ element exposure, 21 genes related to NO_2 exposure, and 14 genes were simultaneously identified to be related to both air pollution exposure.

In addition, 54 genes were associated with exposure to a substantial number of environmental contaminants including inorganic contaminant (mercury and arsenic) and organic contaminant (e.g., polychlorinated biphenyls, perfluorooctanoate and bisphenol A), and 57 genes were identified associated with smoking. Moreover, 56 genes were

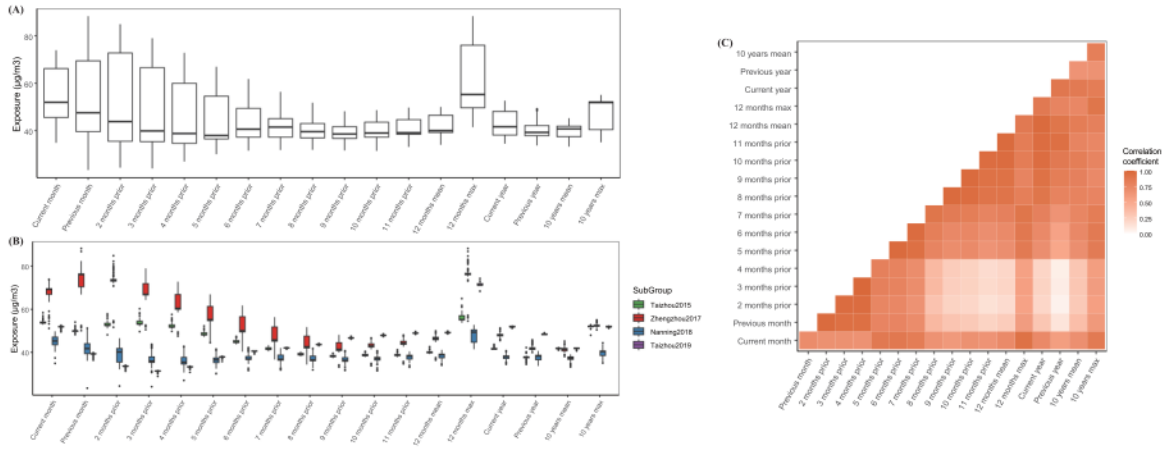


Fig. 1. Distribution of ozone exposure level across time windows. (A) Bar plot of the overall distribution of ozone exposure level. (B) Bar plot of the distribution of ozone exposure level in each subgroup. (C) Correlation of ozone exposure level across time windows.

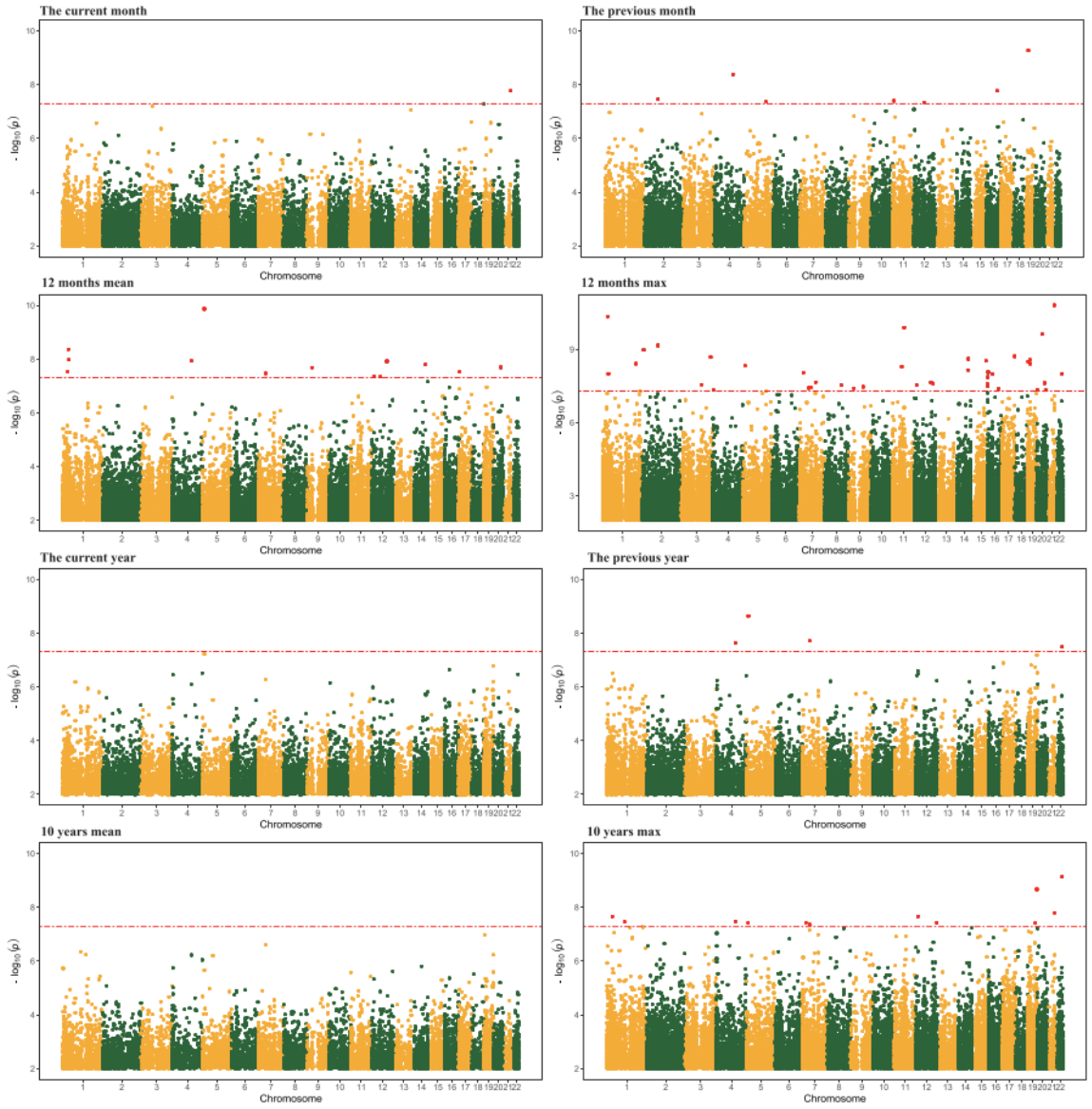


Fig. 2. Manhattan plot of EWAS on Ozone exposure. The red line corresponds to the genome-wide significant threshold ($P \leq 5 \times 10^{-8}$). Significant CpGs were plotted in red. Yellow and green represented chromosomes.

previously implicated in association with aging, 28 genes in association with diabetes and metabolic syndrome, and 24 genes in association with neurodevelopmental, congenital or mental disorders. Remarkably, there is a notable overlap of 67 genes with findings from studies on immune-related traits and inflammation disease, including asthma, allergic sensitization, atopic dermatitis, systemic lupus erythematosus and acute lymphoblastic leukemia. 58 genes were involved in oncogenesis process of certain cancers or identified as pan-cancer biomarkers.

Specifically, cg16369592 and its mapped gene *UBE2G2*, which were associated with any-term ozone exposure (Fig. 3), were reported in association studies on $PM_{2.5}$ [2], smoking [18] and SETD1B-related syndrome [19].

We conduct enrichment analysis for the assigned genes, to investigate the coordination of gene functions at pathway level. The top enriched GO pathways involved in cellular events of telomere maintenance, chromosome regulation, embryonic development, TGF-β signaling and regulation of transcription by RNA polymerase II (Fig. 5). In detail, *XRCC3*, *ERCC1* and *TERF2* participated in regulating telomere maintenance process and DNA damage response, including telomere loop disassemble, telomere trimming, telomeric circle formation and protection against non-homologous end-joining. Genes including *HOXB3*, *HOXB6*, *PRMT1* and *TERF2* involved in embryonic development. The telomere maintenance and chromosome regulation pathways, as well as the embryonic development pathways were also confirmed in REACTOME. *CREBBP*, *RBBP4*, *CHST11* and *PMEPA1* participated in TGF-β signaling pathways. And a gene set of *CREBBP*, *CBFB*, *ATF7IP*, *ERCC1* and *TAF12* were enriched in DNA-templated transcription initiation (Table S6). Additionally, *PF4*, *GP1BA*, *PRMT1* and *CBFB* were enriched in megakaryocyte differentiation pathways in Reactome, playing a role in hematopoiesis by regulating megakaryocyte differentiation.

4. Discussion

We proposed a paradigm with a) prediction on individual level ozone exposure measurements based on cohort data of healthy participants, b) epigenome wide association study to identify related DNAm change at both CpG and genomic region levels, c) sensitivity analysis to investigate robustness and heterogeneity, d) follow-up functional analysis to reveal underlying biological mechanism. With a relatively large sample size,

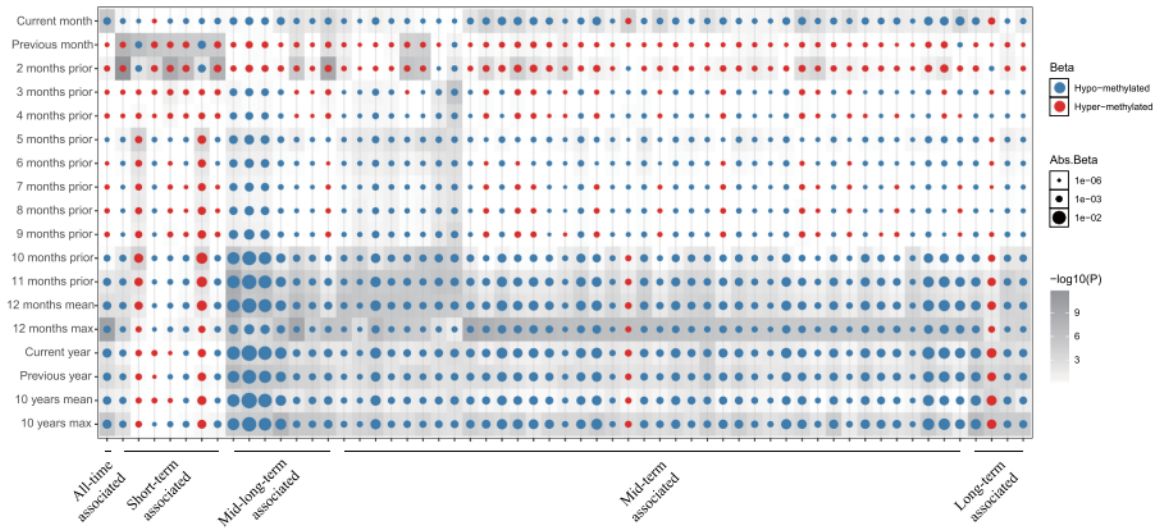


Fig. 3. Comparison of effect size estimate on identified CpG across time windows. Ticks on the X axes represented for a total of 59 identified CpGs. Red and blue dots represented positive and negative effect size, and the size corresponding to the absolute value of effect size. The background color represented the p-value derived from association tests.

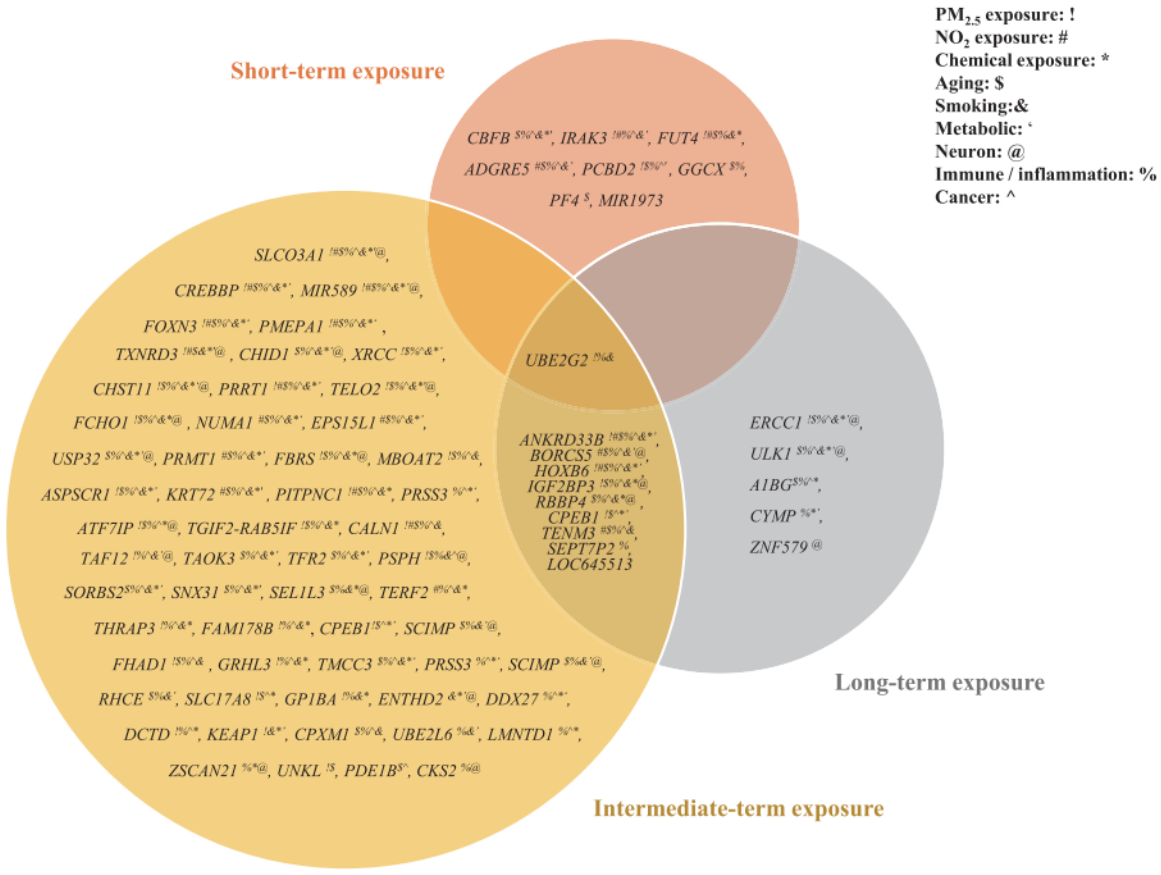


Fig. 4. Venn diagram of genes identified from EWAS on Ozone exposure and overlapping with previous association studies. Superscripts indicated that the gene was previously reported to be associated with certain traits.

we identified differentially methylated CpGs and genomic region associated with short-, intermediate- and long-term ambient ozone exposure, reaching epigenome-wide significance. To our knowledge, this study is the first cohort-based EWAS on ozone exposure, as well as the first EWAS on a wide range of exposure time windows. With a relatively large sample size, we identified epigenome-wide significant CpGs and DMRs, using the model-predicted ozone exposure level.

Gene annotation revealed that the methylation change on several genes were simultaneously affected by ozone exposure as well as exposure to other air pollution, including $PM_{2.5}$, NO_2, multiple chemical component and smoking. The overlap targeted genes suggested that air pollution exposure of various contaminants might share commonality in epigenomic effect and trigger similar biological response likewise, or even share certain risks on health outcomes. Moreover, combined with

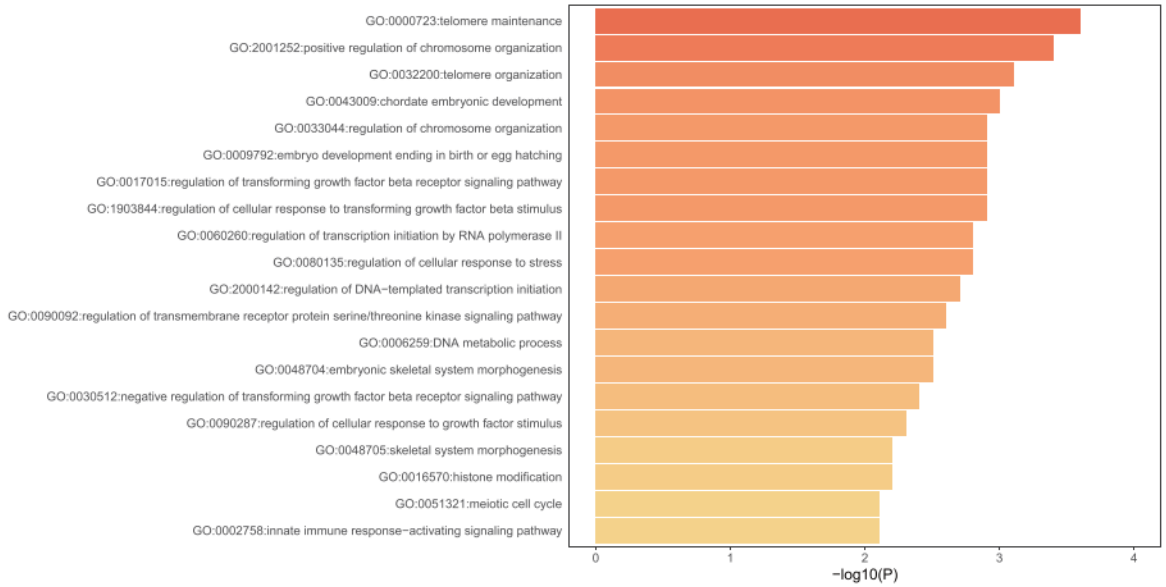


Fig. 5. Enriched GO pathways of identified ozone exposure related genes. Enrichment analysis was conducted on the mapped genes. The p-value was derived by comparing the observed frequency of genes involving in a pathway term with the frequency expected by chance.

previous association studies, the ozone exposure related genes were implicated to be involved in aging and a wide range of diseases, including diabetes and metabolic syndrome, neurodevelopmental, congenital or mental disorders, immune-related traits and inflammation disease, and oncogenesis. It has been acknowledged by various evidence that air pollution exposure accelerates aging and have impact on molecular phenotypes such as telomere length and mitochondrial DNA copy number [15,38,41]. Particularly, exposure to ambient ozone was associated with shortened telomere length and decreased mitochondrial copy number, which as aging biomarkers, mediated the increase risk of atherosclerotic cardiovascular diseases [22]. Consistently, our studies also linked ozone exposure to telomere maintenance and aging, and highlighted that ozone exposure associated DNAm alteration might participate in the accelerated aging process. Moreover, inflammatory response was found to be significant health outcomes induced by ozone exposure, even in short-term and low-concentrations [44]. Ozone-induced inflammation can lead to the production of reactive oxygen species (ROS) and oxidative stress, causing damage to cells and tissues. This oxidative stress can trigger the activation of signaling pathways involved in inflammation, such as nuclear factor-kappa B (NF-κB) and activator protein-1 (AP-1), leading to the production of various inflammatory mediators [11]. In this study, we observed that epigenomic effect of ozone exposure aligned with genes related to immune system and inflammation response, and the inflammatory response to ozone exposure could be responsible to the increased risk of inflammation symptoms including asthma and allergic sensitization, or even metabolic [40] and neurological symptoms [11]. More importantly, chronic exposure to ozone and the resulting persistent inflammation may contribute to the development or progression of respiratory diseases. Prolonged inflammation can lead to structural changes in the airways, remodeling, and fibrosis, which can impair lung function over time [12].

On top of the evidence from previous association studies, we also identified a gene sets (*XRCC3*, *ERCC1* and *TERF2*) enriched in coordinated pathways involved in telomere maintenance process and DNA damage response, including telomere loop disassemble, telomere trimming, telomeric circle formation and protection against non-homologous end-joining, which might help reveal the underly mechanism of biological response induced by ozone exposure. As mentioned above, ozone exposure has been acknowledged as inducing ROS and oxidative stress, furtherly leading to DNA damage and resulting in premature telomere shortening and cellular aging. It is worth mentioning that, firstly, such impact on telomere length is not exclusive for ozone exposure, but common for various air pollution exposure. Many studies have reported the association between telomere length and air pollutants including NO_x, NO_2, $PM_{2.5}$ and PM_{10} [14,39], black carbon [24], and polycyclic aromatic hydrocarbons [32]. Secondly, regulation on telomere maintenance is a complex process influenced by various factors, especially may differ across cell types and tissues. Oxidative stress is just one of the many factors that can contribute to telomere attrition, and it is much not likely caused by ozone exposure solely. Another interesting finding from enrichment analysis is we identified a gene set (*PF4*, *GP1BA*, *PRMT1* and *CBFB*) participating in hematopoiesis by regulating megakaryocyte differentiation. Megakaryocyte differentiation is responsible for the production of blood platelets and functions in both health (hemostasis) and disease (thrombosis) [29]. ROS affects megakaryocyte differentiation, maturation, polyploidy and proplatelet fragmentation [9] and the relation between acute ozone exposure and prothrombosis has been proposed based on proteomic alteration [28]. As the gene set identified by our study were significantly associated with short-term ozone exposure, we believed these finding provided further evidence on epigenomics and gene regulation involving megakaryocyte differentiation, linking short-term ozone exposure to thrombosis.

Sensitivity analysis revealed that some of the identified CpGs were sensitive to regional heterogeneity (Fig. S3, S4A). We reasoned that there were disparate distributions of ozone exposure levels across subgroups (Fig. 1B), and the subtle overlapping might result in regional heterogeneity and led to divergent effects on methylome, even though we have adjusted the group index as covariance. Similar heterogeneity were also brought up by a previous EWAS on $PM_{2.5}$ exposure in Italy and Nederland [34]. Also, the effect sizes of several CpGs were sensitive to blood leukocytes fractions (Fig. S4A), which implies that different blood cells may exhibit varying patterns of methylation changes on the identified CpGs, in response to ozone exposure, suggesting a blood-cell-type specific effect of ozone exposure. On the other hand, the findings were robust to intrinsic and extrinsic factors including age, sex, smoking (both active and passive smoking), alcohol consumption, education level and household income (Fig. S4). Lastly, despite that we spotted overlapped genes in response to both ozone and PM2.5 exposure, the ozone-related DNAm was not confounded by PM2.5 (Fig. S4. B), which suggested shared biological mechanism between ozone exposure and other air

pollutant.

On top of the identified genes and pathways, our study provided insight in the effect of ozone exposure on DNAm across different time windows. To date, most of the studies focused on ozone exposure of single period, and our study proposed a comprehensive investigation on the shared and differed pattern across ozone exposure in different time windows based on the broadest range of time windows from short- to long-term, including monthly, annual and 10-years exposure. We found that the methylation alteration on the identified CpGs showed diverse patterns between intermediate- to long-term zone exposure and short-term exposure, with one exception is cg16369592 (*UBE2G2*), which were concordantly hypomethylated in response to all-time ozone exposure. The consistency between effects of intermediate- to long-term ozone exposure, might to some extent result from highly correlated ozone exposure level in intermediate- to long-term (Fig. 1C). On the other hand, such concordance suggested an imprinting of long-lasting ozone exposure on epigenome through methylation. As for the short-term associated CpGs, the effects ceased with the expansion of the time windows (Fig. 3), which suggest that the methylome effect of short-term ozone exposure was mostly sensitive to the exposure level and did not retain over time. Previous studies have proved that two-hours ozone exposure could induce the differentiated expression of genes and proteins [8,28], and within 24-hours exposure could cause inflammatory effects [1,10,6]. These findings, together with our EWAS results, suggested that the short-term exposure and intermediate- to long-term exposure may induce divergent methylation alteration.

The results showed EWAS on maximum exposure levels (12 months max and 10 years max) identified the most signals than the mean exposure levels. Initially, we obtained both the mean and maximum exposure level to investigate whether the most severe exposure and/or the average exposure within the period effects the methylation change. As the previous studies suggested, metrics of peak exposure might better reflect the health damage caused by ozone exposure, and WHO concluded that there was higher certainty on the relationship between all-cause mortality and "warm-season average" exposure (peak of the year) than annual average exposure [16,23,45,7]. Hence, the controversy between EWAS on mean and max groups was generally consistent with the previous understanding. However, the controversy could be caused by both biological response and statistics. The maximum metrics showed larger variation than the average metrics (Fig. 1), which might bring in statistics bias issue in the significance.

Several limitations of this study should be acknowledged. First of all, we only collected the residential address at the time point of recruitment, but not the whole address history for the past 10 years. Therefore, there was a potential misclassification risk for the residence-based ozone prediction, especially for long-term, as the prediction might be less accurate if an individual was based at multiple places during the period. Although the findings of long-term exposures were overall consistent with the findings of 1 year mid-term exposure, and thus we believed it was unlikely to be false positive results, we might miss additional methylome hits suffered from the misclassification risk. If possible, future studies are warranted to keep track of residential information and improve the precision of exposure level. Also, the discrepancy between ambient ozone measurement and individual ozone exposure level could be induced by the frequency of outdoor activity, as those who were more outdoor active would expose to more severe ambient ozone, which might bring error to exposure measurement. Secondly, gene expression and immune markers were not available in the current stage of NSPT cohort, and thus we were not capable to examine the role of identified CpGs in gene expression regulation or immune reaction with paired omics data. Thirdly, DNAm was profiled in blood leukocytes; the identified CpGs, genes or pathways may not be shared across all ozone exposure associated health outcomes, especially tissue-specific diseases. Last but not least, the inconsistency across subgroups and time windows, as well as the lack of cross-study replication implied a potential risk of false positive finding. More valid studies are expected to cross examinate the methylation effect of short-, intermediate- and long-term ozone exposure.

We would like to elucidate a few notable strengths of our study. For one, the proposed ambient ozone prediction model provided a cost-efficient way to elevate individual-level ozone exposure in cohort study. Using this method, it guaranteed a further feasibility in conducting epigenome-wide association study with a relatively large sample and statistical power. Besides, it provided higher spatial resolution than station monitoring measurement-based imputation methods on deducting individual exposure [17] and thus beneficial for better precision. Last but not least, this study not only is the first report on the association between ambient ozone exposure and DNAm in genome wide, but also made comparison on the exposure in a series of time windows, and investigated the dynamic changes of differentiated methylation across short-, intermediate- and long-term exposure.

5. Conclusions

To sum up, this study conducted the first EWASs on short-, intermediate- and long-term ozone exposure in a Han Chinese cohort, with relatively large sample size. In total 59 CpGs and 30 DMRs were identified to be associated with ozone exposure. The targeted genes of methylation alteration were involved in mechanism related to aging, immune-related traits and inflammation disease, metabolic syndrome, neurodevelopmental, congenital or mental disorders, and oncogenesis. Underlying pathways were enriched in biological activities including telomere maintenance process, DNA damage response and megakaryocyte differentiation.

CRediT authorship contribution statement

Xiyang Cai: Conceptualization, Methodology, Data curation on DNA methylation and Ozone exposure measurement, Formal analysis on EWAS and follow-up, Visualization, Writing – original draft, Writing – review & editing. **Kaixuan Li:** Formal analysis on EWAS and follow-up, Writing – original draft. **Xia Meng:** Methodology on Ozone exposure measurement, Data curation, Formal analysis, Writing - review & editing. **Qinglin Song:** Formal analysis on EWAS and follow-up, Visualization. **Shi Su:** Methodology on $PM_{2.5}$ exposure measurement, Formal analysis. **Wenran Li:** Formal analysis, Writing – review & editing. **Yue Niu:** Formal analysis, Writing – review & editing. **Li Jin:** Resources, Supervision. **Haidong Kan:** Conceptualization, Writing – review & editing, and Supervision. **Sijia Wang:** Conceptualization, Writing – review & editing, Supervision. All authors have read and approved the final draft of the paper.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The summary statistics were provided in Supplementary.

Acknowledgements

This work is supported by the Strategic Priority Research Program of Chinese Academy of Sciences (Grant No. XDB38020400), the National Natural Science Foundation of China (NSFC) (92249306, 82030103), the National Key Research and Development Project (2018YFC0910403), Science and Technology Commission of Shanghai Municipality Major Project (2017SHZDZX01), Shanghai Science and Technology Commission Excellent Academic Leaders Program (22XD1424700), CAS Young Team Program for Stable Support of Basic

Research (YSBR-077).

Environmental Implication

Ozone, is considered as a potent photochemical oxidant in the ambient air pollution and a major concern to human disease and death. This study explored the methylome effect of short-, intermediate- and long-term ambient ozone exposure in Chinese population. Gene function analysis suggested that ozone exposure is associated with various health condition involving aging, inflammation disease, metabolic syndrome, neurodevelopmental disorders, and oncogenesis. Pathway analysis also highlighted that ozone exposure effected DNA damage repairment and thrombosis. This study brought novel insight on the epigenetic effect of ozone exposure, and hopefully raised awareness on the health risk of ambient ozone exposure.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2023.132780.

References

[1] Arjomandi, M., Wong, H., Donde, A., Frelinger, J., Dalton, S., Ching, W., Power, K., Balmes, J.R..-H., and Physiology, C. (2015). Exposure to medium and high ambient levels of ozone causes adverse systemic inflammatory and cardiac autonomic effects. *308*, H1499-H1509.

[2] Bind, M.-A., Lepeule, J., Zanobetti, A., Gasparrini, A., Baccarelli, A.A., Coull, B.A., Tarantini, L., Vokonas, P.S., Koutrakis, P., and Schwartz, J.J.E. (2014). Air pollution and gene-specific methylation in the Normative Aging Study: association, effect modification, and mediation analysis. *9*, 448–458.

[3] Bind, M.-A., Rubin, D., Cardenas, A., Dhingra, R., Ward-Caviness, C., Liu, Z., Mirowsky, J., Schwartz, J., Diaz-Sanchez, D., and Devlin, R.J.Sr (2020). Heterogeneous ozone effects on the DNA methylome of bronchial cells observed in a crossover study. *10*, 1–15.

[4] Cavalcante, R.G., and Sartor, M.A.J.B. (2017). Annotatr: genomic regions in context. *33*, 2381–2383.

[5] Cohen, A.J., Brauer, M., Burnett, R., Anderson, H.R., Frostad, J., Estep, K., Balakrishnan, K., Brunekreef, B., Dandona, L., and Dandona, R.J.Tl (2017). Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *389*, 1907–1918.

[6] Devlin, R.B., Duncan, K.E., Jardim, M., Schmitt, M.T., Rappold, A.G., and Diaz-Sanchez, D.J.C. (2012). Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. *126*, 104–111.

[7] Di, Q., Wang, Y., Zanobetti, A., Wang, Y., Koutrakis, P., Choirat, C., Dominici, F., Schwartz, J.D., 2017. Air pollution and mortality in the Medicare population. N Engl J Med 376, 2513–2522.

[8] Du, X., Niu, Y., Wang, C., Wang, W., Liu, C., Meng, X., Chu, C., Chen, R., and Kan, H.J.E.I. (2022). Ozone exposure and blood transcriptome: A randomized, controlled, crossover trial among healthy adults. *163*, 107242.

[9] Eliades, A., Matsuura, S., and Ravid, K.J.Jocp (2012). Oxidases and reactive oxygen species during hematopoiesis: a focus on megakaryocytes. *227*, 3355–3362.

[10] Frampton, M.W., Pietropaoli, A., Dentler, M., Chalupa, D., Little, E.L., Stewart, J., Frasier, L., Oakes, D., Wiltshire, J., and Vora, R.J.It (2015). Cardiovascular effects of ozone in healthy subjects with and without deletion of glutathione-S-transferase M1. *27*, 113–119.

[11] González-Guevara, E., Martínez-Lazcano, J.C., Custodio, V., Hernández-Cerón, M., Rubio, C., and Paz, C.J.It (2014). Exposure to ozone induces a systemic inflammatory response: possible source of the neurological alterations induced by this gas. *26*, 485–491.

[12] Gowers, A.M., Cullinan, P., Ayres, J.G., Anderson, H.R., Strachan, D.P., Holgate, S. T., Mills, I.C., Maynard, R.L.J.R., 2012. Does outdoor air pollution induce new cases of asthma? Biological plausibility and evidence; a review. Respirology 17, 887–898.

[13] Greenberg, M.V., and Bourc'his, D.J.M (2019). The diverse roles of DNA methylation in mammalian development and disease. *20*, 590–607.

[14] Hautekiet, P., Nawrot, T.S., Janssen, B.G., Martens, D.S., De Clercq, E.M., Dadvand, P., Plusquin, M., Bijnens, E.M., and Saenen, N.D.I. (2021). Child buccal telomere length and mitochondrial DNA content as biomolecular markers of ageing in association with air pollution. *147*, 106332.

[15] Hu, C., Sheng, X., Li, Y., Xia, W., Zhang, B., Chen, X., Xing, Y., Li, X., Liu, H., and Sun, X.J.C. (2020). Effects of prenatal exposure to particulate air pollution on newborn mitochondrial DNA copy number. *253*, 126592.

[16] Huangfu, P., Atkinson, R., 2020. Long-term exposure to NO2 and O3 and all-cause and respiratory mortality: a systematic review and meta-analysis. Environ Int 144, 105998.

[17] Hüls, A., Vierkötter, A., Gao, W., Krämer, U., Yang, Y., Ding, A., Stolz, S., Matsui, M., Kan, H., and Wang, S.J.T.Joid (2016). Traffic-related air pollution contributes to development of facial lentigines: further epidemiological evidence from Caucasians and Asians. *136*, 1053–1056.

[18] Joehanes, R., Just, A.C., Marioni, R.E., Pilling, L.C., Reynolds, L.M., Mandaviya, P. R., Guan, W., Xu, T., Elks, C.E., and Aslibekyan, S.J.Ccg (2016). Epigenetic signatures of cigarette smoking. *9*, 436–447.

[19] Krzyzewska, I., Maas, S., Henneman, P., Lip, K..v.d., Venema, A., Baranano, K., Chassevent, A., Aref-Eshghi, E., Van Essen, A., and Fukuda, T.J.Ce (2019). A genome-wide DNA methylation signature for SETD1B-related syndrome. *11*, 1–15.

[20] Ladd-Acosta, C., Feinberg, J.I., Brown, S.C., Lurmann, F.W., Croen, L.A., Hertz-Picciotto, I., Newschaffer, C.J., Feinberg, A.P., Fallin, M.D., and Volk, H.E.. (2019). Epigenetic marks of prenatal air pollution exposure found in multiple tissues relevant for child health. *126*, 363–376.

[21] Li, Q.S., Sun, Y., and Wang, T.J.Ce (2020). Epigenome-wide association study of Alzheimer's disease replicates 22 differentially methylated positions and 30 differentially methylated regions. *12*, 1–14.

[22] Li, R., Chen, G., Liu, X., Pan, M., Kang, N., Hou, X., Liao, W., Dong, X., Yuchi, Y., and Mao, Z.J.M. (2022). Aging biomarkers: Potential mediators of association between long-term ozone exposure and risk of atherosclerosis. *292*, 512–522.

[23] Lim, C.C., Hayes, R.B., Ahn, J., Shao, Y., Silverman, D.T., Jones, R.R., Garcia, C., Bell, M.L., Thurston, G.D., 2019. Long-term exposure to ozone and cause-specific mortality risk in the United States. Am J Respir Crit care Med 200, 1022–1031.

[24] McCracken, J., Baccarelli, A., Hoxha, M., Dioni, L., Melly, S., Coull, B., Suh, H., Vokonas, P., and Schwartz, J.J.Ehp (2010). Annual ambient black carbon associated with shorter telomeres in elderly men: Veterans Affairs Normative Aging Study. *118*, 1564–1570.

[25] Meng, X., Wang, W., Shi, S., Zhu, S., Wang, P., Chen, R., Xiao, Q., Xue, T., Geng, G., and Zhang, Q.J.P. (2022). Evaluating the spatiotemporal ozone characteristics with high-resolution predictions in mainland China, 2013–2019. *299*, 118865.

[26] Murray, C.J., Aravkin, A.Y., Zheng, P., Abbafati, C., Abbas, K.M., Abbasi-Kangevari, M., Abd-Allah, F., Abdelalim, A., Abdollahi, M., and Abdollahpour, I.J.T (2020). Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *396*, 1223–1249.

[27] Niu, Y., Chen, R., Xia, Y., Cai, J., Lin, Z., Liu, C., Chen, C., Peng, L., Zhao, Z., Zhou, W., et al., 2018. Personal ozone exposure and respiratory inflammatory response: the role of DNA methylation in the arginase–nitric oxide synthase pathway. Environ Sci Technol 52, 8785–8791.

[28] Niu, Y., Li, H., Wang, W., Wang, C., Liu, C., Du, X., Zhang, Q., Li, J., Shi, S., and Meng, X.J.M. (2022). Ozone exposure and prothrombosis: Mechanistic insights from a randomized controlled exposure trial. *429*, 128322.

[29] Noetzli, L.J., French, S.L., Machlus, K.R., 2019. New insights into the differentiation of megakaryocytes from hematopoietic progenitors. Arterioscler Thromb Vasc Biol 39, 1288–1300.

[30] Organization, W.H., 2021. WHO Global Air Quality Guidelines: Particulate Matter (PM2. 5 and PM10), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. World Health Organization.

[31] Pedersen, B.S., Schwartz, D.A., Yang, I.V., and Kechris, K.J.B. (2012). Comb-p: software for combining, analyzing, grouping and correcting spatially correlated P-values. *28*, 2986–2988.

[32] Perera, F., Lin, C.-j, Qu, L., Tang, D., 2018. Shorter telomere length in cord blood associated with prenatal air pollution exposure: benefits of intervention. Environ Int 113, 335–340.

[33] Peters, T.J., Buckley, M.J., Chen, Y., Smyth, G.K., Goodnow, C.C., and Clark, S. (2021). Calling differentially methylated regions from whole genome bisulphite sequencing with DMRcate. *49*, e109-e109.

[34] Plusquin, M., Guida, F., Polidoro, S., Vermeulen, R., Raaschou-Nielsen, O., Campanella, G., Hoek, G., Kyrtopoulos, S.A., Georgiadis, P., Naccarati, A.J.Ei, 2017. DNA methylation and exposure to ambient air pollution in two prospective cohorts, 108, 127–136.

[35] Poursafa, P., Kamali, Z., Fraszczyk, E., Boezen, H.M., Vaez, A., and Snieder, H. (2022). DNA methylation: a potential mediator between air pollution and metabolic syndrome. *14*, 1–13.

[36] Rappazzo, K.M., Nichols, J.L., Rice, R.B., and Luben, T.. (2021). Ozone exposure during early pregnancy and preterm birth: A systematic review and meta-analysis. *198*, 111317.

[37] Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *43*, e47-e47.

[38] Scholten, R.H., Møller, P., Andersen, Z.J., Dehlendorff, C., Khan, J., Brandt, J., Ketzel, M., Knudsen, L.E., and Mathiesen, L. (2021). Telomere length in newborns is associated with exposure to low levels of air pollution during pregnancy. *146*, 106202.

[39] Walton, R.T., Mudway, I.S., Dundas, I., Marlin, N., Koh, L.C., Aitlhadj, L., Vulliamy, T., Jamaludin, J.B., Wood, H.E., Barratt, B.M., 2016. Air pollution, ethnicity and telomere length in east London schoolchildren: an observational study. Environ Int 96, 41–47.

[40] Wang, W., Zhang, W., Hu, D., Li, L., Cui, L., Liu, J., Liu, S., Xu, J., Wu, S., and Deng, F. (2022). Short-term ozone exposure and metabolic status in metabolically healthy obese and normal-weight young adults: A viewpoint of inflammatory pathways. *424*, 127462.

[41] Wang, X., Hart, J.E., Liu, Q., Wu, S., Nan, H., Laden, F.J.Ei, 2020. Association of particulate matter air pollution with leukocyte mitochondrial DNA copy number, 141, 105761.

[42] Wilczewski, C.M., Obasohan, J., Paschall, J.E., Zhang, S., Singh, S., Maxwell, G.L., Similuk, M., Wolfsberg, T.G., Turner, C., and Biesecker, L. (2023). Genotype first: Clinical genomics research through a reverse phenotyping approach. *110*, 3–12.

[43] Xia, Y., Niu, Y., Cai, J., Lin, Z., Liu, C., Li, H., Chen, C., Song, W., Zhao, Z., Chen, R., et al. (2018). Effects of personal short-term exposure to ambient ozone on blood pressure and vascular endothelial function: a mechanistic study based on DNA methylation and metabolomics. *52*, 12774–12782.

[44] Xia, Y., Niu, Y., Cai, J., Liu, C., Meng, X., Chen, R., and Kan, H.J. (2021). Acute effects of personal ozone exposure on biomarkers of inflammation, oxidative stress, and mitochondrial oxidative damage—Shanghai Municipality, China, May–October 2016. *3*, 954.

[45] Yang, C., Yang, H., Guo, S., Wang, Z., Xu, X., Duan, X., Kan, H., 2012. Alternative ozone metrics and daily mortality in Suzhou: the China air pollution and health effects study (CAPES). Sci Total Environ 426, 83–89.

[46] Zhao, N., Bell, D.A., Maity, A., Staicu, A.M., Joubert, B.R., London, S.J., and Wu, M. C.. (2015). Global analysis of methylation profiles from high resolution CpG data. *39*, 53–64.

[47] Zheng, S.C., Breeze, C.E., Beck, S., and Teschendorff, A.E.. (2018). Identification of differentially methylated cell types in epigenome-wide association studies. *15*, 1059–1066.

[48] Zhou, Y., Duan, W., Chen, Y., Yi, J., Wang, B., Di, Y., He, C.J.E., and Health (2022). Exposure risk of global surface o3 during the boreal spring season. *14*, 431–446.