PM$_{2.5}$ exposure and DLEC1 promoter methylation in Taiwan Biobank participants

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**Abstract**

**Background:** Particulate matter (PM) $< 2.5 \mu$m (PM$_{2.5}$) or fine PM is a serious public health concern. It affects DNA methylation and heightens carcinogenesis. Deleted in lung and esophageal cancer 1 (DLEC1) is a tumor suppressor gene. However, aberrant methylation of the gene is associated with several cancers. We evaluated the association between PM$_{2.5}$ and DLEC1 promoter methylation in Taiwanese adults based on regular outdoor exercise.

**Methods:** We obtained DNA methylation and exercise data of 496 participants (aged between 30 and 70 years) from the Taiwan Biobank (TWB) database. We also extracted PM$_{2.5}$ data from the Air Quality Monitoring Database (AQMD) and estimated participants' exposure using residential addresses.

**Results:** DLEC1 methylation and PM$_{2.5}$ were positively associated: beta coefficient ($\beta$) = $0.114 \times 10^{-3}$; p value = 0.046. The test for interaction between exercise and PM$_{2.5}$ on DLEC1 methylation was significant (p value = 0.036). After stratification by exercise habits, PM$_{2.5}$ and DLEC1 methylation remained significantly associated only among those who exercised regularly ($\beta$ = $0.237 \times 10^{-3}$; p value = 0.007). PM$_{2.5}$ quartile-stratified analyses revealed an inverse association between regular exercise and DLEC1 methylation at PM$_{2.5} < 27.37 \mu g/m^3$ ($\beta$ = $-5.280 \times 10^{-3}$; p value = 0.009). After combining exercise habits and PM$_{2.5}$ quartiles, one stratum (i.e., regular exercise and PM$_{2.5} < 27.37 \mu g/m^3$) was inversely associated with DLEC1 methylation ($\beta$ = $-5.160 \times 10^{-3}$; p value = 0.007).

**Conclusions:** We found significant positive associations between PM$_{2.5}$ and DLEC1 promoter methylation. Regular exercise at PM$_{2.5} < 27.37 \mu g/m^3$ seemingly regulated DLEC1 promoter methylation.

**Keywords:** PM$_{2.5}$, DLEC1, Methylation, Exercise, Taiwan Biobank

**Introduction**

PM$_{2.5}$ induces the generation of reactive oxygen species (ROS) which have detrimental effects [1], like immune response stress, inflammatory injury, DNA damage, and oxidative stress that enhance cancer formation [1–3]. PM$_{2.5}$ is a critical public health issue that accounts for most air pollution-related global deaths [1, 4]. It accounted for approximately 3.5 and 4.2 million global deaths in 1990 and 2015, respectively. Moreover, it was the fifth top cause of global mortality in 2015 [5]. While PM$_{2.5}$ could enhance inflammation and oncogenesis [3], exercise, on the other hand, could curb inflammation and related complications [6–9]. All mechanisms by which PM$_{2.5}$ and exercise affect tumorigenesis are not yet delineated.

DNA methylation is an epigenetic change that integrates the interactions between genes and the environment [10]. Abnormal patterns of this epigenetic marker are promising diagnostic and prognostic tumor markers.
because they are frequently detected even at the earliest stages of tumor formation [11, 12]. DNA methylation is one of the epigenetic mechanisms underlying the incidence of air pollution-induced allergic diseases [10] and human tumors [13, 14]. PM$_{2.5}$-induced DNA methylation alterations aggravate the risk of cancer by repressing tumor suppressor genes and activating oncogenes [15]. DNA methylation is believed to be an epigenetic indicator of exercise intervention [16, 17]. For instance, in a study where exercise-induced immunologic benefits attenuated the detrimental effects of air pollution on the lungs, DNA methylation was a marker of such benefits [18].

Deleted in lung and esophageal cancer 1 (DLEC1) is an important element in head and neck tumorigenesis [13, 19–22]. It is a bona fide tumor suppressor gene located on chromosome 3p22.3 [13, 19–24]. Its tumor-suppressing potentials were initially identified in lung, renal, and esophageal carcinomas [21]. Abnormalities in DLEC1 methylation are potential diagnostic and prognostic epigenetic tumor markers [25]. For instance, DLEC1 promoter methylation is a prognostic biomarker for gastric, lung, advanced ovarian, and endometrial cancer [11, 26–31]. It is also a diagnostic biomarker for prostate, breast, ovarian, colorectal, gastric, nasopharyngeal, and lung cancer [11, 25, 30, 32–39].

Findings on the relationship between air pollution and DNA methylation warrant critical replication and validation [40]. Exposure to smoky coals was associated with higher DLEC1 methylation in plasma and tissue samples from Chinese lung cancer patients [32], implying that air pollution-induced DLEC1 methylation could influence cancer etiology. Even though both DLEC1 methylation and PM$_{2.5}$ play prominent roles in carcinogenesis, their relationship has received limited research attention. Moreover, studies on DLEC1 methylation among Taiwanese are lacking. Furthermore, the combined effects of exercise and PM$_{2.5}$ exposure on health, alongside the underlying mechanisms require more investigations [8, 16, 41]. We evaluated the association between PM$_{2.5}$ and DLEC1 promoter methylation in relation to exercise among TWB participants.

**Methods**

**Data sources and study population**

We obtained data from two sources, namely the TWB database and AQMD. Recruitment of participants into the TWB project is restricted to Taiwanese aged between 30 and 70 years who have never been diagnosed with cancer. The TWB has 29 recruitment centers throughout Taiwan [42]. The TWB database (2008 to 2015) has data on methylation, exercise, sex, age, cigarette smoking, betel nut chewing, alcohol intake, and secondhand smoke exposure, and alcohol/betel nut intake. Five DLEC1 promoter CpG sites (cg0483533, cg16150706, cg11542528, cg20684180, and cg23881725) were available in the TWB database. It is worth stating that this dataset does not have air pollution data. So, we obtained annual averages (2006–2011) of daily concentrations of PM$_{2.5}$ (µg/m$^3$), SO$_2$ (ppb), CO (ppm), O$_3$ (ppb), and NOx (ppb) from the AQMD. The Environmental Protection Administration (EPA) in Taiwan monitors air quality through the Air Quality Monitoring Network (AQMN) [43, 44]. To date, the EPA has set up about 77 fully automated air quality monitoring stations nationwide for daily monitoring of air pollution [44]. We included 496 individuals with complete data in the final analyses.

**Main exposure and outcome**

The main exposure was PM$_{2.5}$ while the outcome was DLEC1 promoter methylation. We used the residential addresses of participants and estimated their PM$_{2.5}$ exposure. Health GeneTech Corp. performed all the DNA methylation experiments. In brief, a trained and qualified researcher with a medical background collected about 9 ml of venous blood from each participant into sodium citrate tubes. The blood samples were kept at 4 °C and transported to the laboratory for further experiments. DNA isolation and purification was done using an automated chemical extraction instrument called Chemagic® Prime™. Purified DNA samples were treated with sodium bisulfite using EZ DNA Methylation Kit (Zymo Research, CA, USA). DNA methylation was assessed with the Infinium® MethylationEPIC BeadChipEPIC array (Illumina Inc.) and presented as beta values, which range from 0 to 1 [45, 46]. Lower and higher beta values indicate lower and higher methylation levels, respectively. The Infinium MethylationEPIC BeadChipEPIC array targets more than 850,000 CpG sites across the genome. Quality control measures for methylation data were undertaken as previously stated [47, 48]. Ethical approval for this study was given by the Institutional Review Board of Chung Shan Medical University Hospital (CS2-20007).

**Covariates**

We used participants’ residential addresses and estimated the annual average exposure levels of SO$_2$ (ppb), CO (ppm), O$_3$ (ppb), and NOx (ppb). Self-filled TWB questionnaires contained data on exercise, sex, age, cigarette smoking, betel nut chewing, alcohol intake, and secondhand smoke exposure. Detailed descriptions of these variables and BMI are found somewhere else [47, 48]. Summarily, we defined outdoor exercise as engaging in any outdoor activities (e.g., Chinese martial arts, “Wai-Tan-Kung,” “Neidan-Kung,” “Falun Dafa,” “Taijiquan,” “Xiang Kong,” “Yuan Chin Dance,” “Qigong,” strolling, jogging, hiking, rope jumping, arm swing, soccer, golf, croquet, tennis, basketball, other ball games,
biking, mountain climbing, and hula hoop) lasting over 30 min, at least three times per week.

Statistical analyses
We stratified the basic characteristics of the participants into regular and no regular exercise. DLEC1 promoter methylation (mean ± standard error) was the average of the beta values of the 5 DLEC1 promoter CpG sites. We evaluated the differences in continuous variables (DLEC1 methylation levels, PM$_{2.5}$, SO$_2$, CO, O$_3$, NOx, and age) between the two exercise groups with the T test and the differences in the categorical variables (sex, cigarette smoking, alcohol/betel nut intake, BMI, and exposure to secondhand smoke) between the two groups with the chi-square test. Moreover, we determined the association between PM$_{2.5}$ and DLEC1 promoter methylation by employing multivariate linear regression analysis and adjusted for covariates (consisting of exercise, SO$_2$, CO, O$_3$, NOx, sex, age, cigarette smoking, BMI, secondhand smoke exposure, and alcohol/betel nut intake). We also used multivariate linear regression and determined the interaction between PM$_{2.5}$ and exercise on DLEC1 promoter methylation. We adjusted for cell-type composition in whole blood using the Reference-Free Adjustment for Cell-Type composition (ReFACTor) method [49]. For all analyses, a $p$ value $< 0.05$ was considered statistically significant.

| Variables                        | No regular exercise | Regular exercise | $p$ value |
|----------------------------------|---------------------|------------------|-----------|
| Mean DLEC1 promoter methylation (beta value) | 0.198 ± 0.001 | 0.193 ± 0.001 | < 0.001 |
| PM$_{2.5}$ ($\mu$g/m$^3$)       | 31.551 ± 0.455     | 32.756 ± 0.550  | 0.090     |
| SO$_2$ (ppb)                    | 4.137 ± 0.092      | 4.092 ± 0.099   | 0.741     |
| CO (ppm)                        | 0.560 ± 0.013      | 0.546 ± 0.013   | 0.450     |
| O$_3$ (ppb)                     | 27.860 ± 0.192     | 27.989 ± 0.221  | 0.660     |
| NO$_x$ (ppb)                    | 26.411 ± 0.746     | 25.581 ± 0.794  | 0.453     |
| Sex                              |                     |                  | 0.075     |
| Women                            | 150 (52.63)        | 94 (44.55)      |           |
| Men                              | 135 (47.37)        | 117 (55.45)     |           |
| Age (years)                      | 45.832 ± 0.635     | 55.185 ± 0.660  | < 0.001   |
| Cigarette smoking                |                     |                  | 0.011     |
| Never                            | 222 (77.89)        | 152 (72.04)     |           |
| Former                           | 27 (9.47)          | 39 (18.48)      |           |
| Current                          | 36 (12.63)         | 20 (9.48)       |           |
| Alcohol drinking                 |                     |                  | 0.633     |
| Never                            | 261 (91.58)        | 188 (89.10)     |           |
| Former                           | 8 (2.81)           | 7 (3.32)        |           |
| Current                          | 16 (5.61)          | 16 (6.50)       |           |
| Betel nut chewing                |                     |                  | 0.516     |
| No                               | 266 (93.33)        | 202 (95.73)     |           |
| Former                           | 11 (3.86)          | 5 (2.37)        |           |
| Current                          | 8 (2.81)           | 4 (1.90)        |           |
| BMI (kg/m$^2$)                   |                     |                  | 0.040     |
| Normal (< 18.5)                  | 130 (45.61)        | 94 (44.55)      |           |
| Underweight (18.5 ≤ BMI < 24)    | 17 (5.96)          | 3 (1.42)        |           |
| Overweight (24 ≤ BMI < 27)       | 76 (26.67)         | 71 (33.65)      |           |
| Obesity (BMI ≥ 27)               | 62 (21.75)         | 43 (20.38)      |           |
| Secondhand smoke exposure        |                     |                  | 0.009     |
| No                               | 247 (86.67)        | 198 (93.84)     |           |
| Yes                              | 38 (13.33)         | 13 (6.16)       |           |

Standard error (SE) represents continuous variables and percentage (%) represents categorical variables.
Air pollutant concentrations are annual averages (2006–2011) of daily concentrations. DLEC1 Deleted in lung and esophageal cancer 1, PM Particulate matter with diameter less than 2.5 microns, SO$_2$ Sulfur dioxide, CO Carbon monoxide, O$_3$ Ozone, NOx Nitrogen oxides, BMI Body mass index.
significant. All statistical analyses were executed with the SAS software; version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

We included a total of 496 participants (i.e., 211 with regular and 285 with no regular exercise habits). DLEC1 promoter methylation, age, cigarette smoking, BMI, and secondhand smoke exposure were significantly different (p value < 0.050) between the exercise groups (Table 1).

PM$_{2.5}$ was significantly associated with hypermethylation or higher levels ($\beta = 0.114 \times 10^{-3}$; p value = 0.046) while age was significantly associated with hypomethylation or lower levels of DLEC1 promoter methylation: $\beta = -0.206 \times 10^{-3}$; p value = < 0.001 (Table 2).

Exercise and PM$_{2.5}$ had a significant interaction on DLEC1 methylation (p value = 0.036). When we stratified participants by regular exercise habits, PM$_{2.5}$ and DLEC1 methylation remained positively associated. However, this association was significant only in the regular exercise group ($\beta = 0.237 \times 10^{-3}$, p value = 0.007). The inverse association between age and DLEC1 methylation remained significant in both exercise groups. The $\beta$ coefficient was $-0.212 \times 10^{-3}$ (p value < 0.001) for regular exercise and $-0.144 \times 10^{-3}$ (p value = 0.042) for no regular exercise (Table 3). There was a significant association between obesity and DLEC1 methylation in those who exercised regularly: $\beta = 3.870 \times 10^{-3}$, p value = 0.029.

When we stratified PM$_{2.5}$ concentrations into quartiles (Table 4), DLEC1 methylation in participants who exercised regularly was significant only at PM$_{2.5} < 27.37 \mu g/m^3$ ($\beta = -5.280 \times 10^{-3}$; p value = 0.009). The methylation was also significant at PM$_{2.5}$ levels 31.82 $\leq$ PM$_{2.5}$ $< 38.73 \mu g/m^3$ ($\beta = 3.980 \times 10^{-3}$; p value = 0.009) for SO$_2$, 27.37 $\leq$ PM$_{2.5}$ $< 31.82$, and PM$_{2.5}$ $\geq$ 38.73 $\mu g/m^3$ ($\beta = 3.850 \times 10^{-3}$; p value = 0.003, respectively) for NO$_x$, and 31.82 $\leq$ PM$_{2.5}$ $< 38.73$ and PM$_{2.5}$ $\geq$ 38.73 $\mu g/m^3$ ($\beta = -0.310 \times 10^{-3}$; p value < 0.001 and $\beta = -0.320 \times 10^{-3}$; p value = 0.003, respectively) for age.

Further stratification by exercise habits and PM$_{2.5}$ quartiles revealed significant lower DLEC1 promoter methylation levels in one stratum (regular exercise at PM$_{2.5}$ levels $< 27.37 \mu g/m^3$): $\beta = -5.160 \times 10^{-3}$; p value = 0.007 (Table 5).

Discussion

Based on the available literature, there are gaps in research focusing on the relationship between DLEC1 methylation and PM$_{2.5}$ exposure. To our utmost knowledge, the current study is the first to investigate such a relationship. PM$_{2.5}$ exposure was significantly associated with DLEC1 hypermethylation. Exercise seemingly modulated this relationship. That is, in relation to exercise, DLEC1 methylation was significantly associated with PM$_{2.5}$ at levels $< 27.37 \mu g/m^3$. In other words, the relationship between DLEC1 methylation and regular exercise disappeared as PM$_{2.5}$ levels increased, implying that exercise might significantly influence DLEC1 methylation only at lower levels of PM$_{2.5}$. This suggests that taking exercise when PM$_{2.5}$ levels are high might expose people to more PM$_{2.5}$ pollution, thereby abating the benefits of regular exercise. That is, the benefits of regular exercise might disappear when PM$_{2.5}$ concentrations increase. Thus, people should not be encouraged to exercise when PM$_{2.5}$ levels are high.

PM$_{2.5}$ induces the generation of ROS [1], which when unbalanced, could have oncogenic consequences [1], like immune response stress, inflammatory injury, DNA damage, and oxidative stress [1–3]. ROS imbalance results from improperly regulated ROS production [2]. It

Table 2: Multiple linear regression showing the association of PM$_{2.5}$ and outdoor exercise with DLEC1 promoter methylation in participants

| Variables                        | $\beta (\times 10^{-3})$ | p value |
|----------------------------------|--------------------------|---------|
| PM$_{2.5}$                       | 0.114                    | 0.046   |
| Regular outdoor exercise (reference: No) |                         |         |
| Yes                              | -1.080                   | 0.246   |
| SO$_2$                           | 0.321                    | 0.323   |
| CO                               | -9.120                   | 0.199   |
| O$_3$                            | 0.030                    | 0.889   |
| NO$_x$                           | 0.119                    | 0.330   |
| Sex (reference: Women)           |                          |         |
| Men                              | 0.513                    | 0.589   |
| Age                              | -0.206                   | < 0.001 |
| Cigarette smoking (reference: Never) |                         |         |
| Former                           | 1.560                    | 0.250   |
| Current                          | 2.590                    | 0.088   |
| Alcohol drinking (reference: Never) |                        |         |
| Former                           | -3.400                   | 0.176   |
| Current                          | 1.790                    | 0.309   |
| Betel nut chewing (reference: Never) |                        |         |
|Former                           | -1.560                   | 0.524   |
|Current                          | -3.990                   | 0.175   |
|BMI (reference: Normal)           |                          |         |
|Underweight                      | -0.725                   | 0.739   |
|Overweight                       | -0.092                   | 0.926   |
|Obesity                          | 1.760                    | 0.113   |
|Secondhand smoke exposure (reference: No) |                    |         |
|Yes                              | -0.258                   | 0.855   |

DLEC1 Deleted in lung and esophageal cancer 1, PM Particulate matter with diameter less than 2.5 microns, SO$_2$ Sulfur dioxide, CO Carbon monoxide, O$_3$ Ozone, NO$_x$ Nitrogen oxides, BMI Body mass index
also occurs when free radicals do not properly neutralize or detoxify oxidative effects [2]. Oxidative stress resulting from ROS is believed to be the main driving force for most air pollution-related adverse health effects [40]. PM2.5 is a critical issue that has increased oral, lung, breast, ovarian, and hepatic cancer morbidity and mortality in Taiwan [4, 50–52].

DLEC1 exhibits its cancer-inhibiting potentials by decreasing the invasiveness and metastasis of tumor cells [21, 22, 53] and also by enhancing apoptosis and arresting the G1 phase of the cell cycle [54, 55]. However, an abnormal methylation profile (like DLEC1 hypermethylation) is significantly linked to head and neck, ovarian, lung, renal, nasopharyngeal, oral, adrenocortical, hepatocellular, esophageal, gastric, and squamous cell carcinoma [11, 28, 30–32, 38, 39, 53, 54, 56–64]. In light of this, we believe that the PM2.5-related DLCE1 hypermethylation observed in our study could also heighten the risk of cancer.

Transcriptional suppression of DLEC1 by promoter hypermethylation is also believed to be an early event in carcinogenesis [62]. This is evident in adrenocortical, lung, nasopharyngeal, and esophageal squamous cell carcinoma [26, 30, 31, 57, 61]. Upregulation of DLEC1 is associated with reduced growth and invasiveness while downregulation is associated with cell proliferation, invasiveness, and poor disease prognosis [22, 31, 53, 58, 62]. DLEC1 expression could be restored with demethylating agents when downregulated, as demonstrated in lung, prostate, oral, nasopharyngeal, renal, ovarian, adrenocortical, and uterine tumors [11, 22, 25, 31, 53, 60–62, 65]. This reversible nature of DLEC1 suggests that it could be a potential treatment target for these cancers.

The detrimental effects of air pollution on health could be attenuated by exercise [18]. This could be achieved, in part, through protective immunologic responses and DNA methylation [18]. In our study, DLEC1 methylation was inversely associated with

| Variables                        | No regular outdoor exercise | Regular outdoor exercise |
|----------------------------------|-----------------------------|--------------------------|
|                                  | β (× 10^-3) | p value    | β (× 10^-3) | p value    |
| PM2.5                            | 0.012      | 0.880      | 0.237      | 0.007      |
| SO2                              | 0.251      | 0.535      | 0.505      | 0.357      |
| CO                               | 1.250      | 0.890      | −25.170    | 0.033      |
| O3                               | 0.345      | 0.224      | −0.607     | 0.078      |
| NOx                              | 0.045      | 0.771      | 0.203      | 0.314      |
| Sex (reference: Women)           |             |            |            |            |
| Men                              | 1.220      | 0.322      | −0.897     | 0.567      |
| Age                              | −0.212     | < 0.001    | −0.144     | 0.042      |
| Cigarette smoking (reference: Never) |         |            |            |            |
| Former                           | 0.613      | 0.761      | 2.210      | 0.256      |
| Current                          | 3.050      | 0.104      | 0.412      | 0.880      |
| Alcohol drinking (reference: Never) |         |            |            |            |
| Former                           | −4.540     | 0.186      | 0.435      | 0.910      |
| Current                          | 1.580      | 0.527      | 2.670      | 0.295      |
| Betel nut chewing (reference: Never) |         |            |            |            |
| Former                           | −1.650     | 0.586      | −0.137     | 0.975      |
| Current                          | −1.430     | 0.695      | −0.009     | 0.084      |
| BMI (reference: Normal)          |             |            |            |            |
| Underweight                      | −0.352     | 0.884      | −8.510     | 0.119      |
| Overweight                       | −1.310     | 0.340      | 1.130      | 0.446      |
| Obesity                          | 1.160      | 0.428      | 3.870      | 0.029      |
| Secondhand smoke exposure (reference: No) |       |            |            |            |
| Yes                              | −1.990     | 0.241      | 3.790      | 0.152      |
| Outdoor exercise*PM2.5           |             | p value = 0.036 |            |            |

DLEC1 Deleted in lung and esophageal cancer 1, PM Particulate matter with diameter less than 2.5 microns, SO2 Sulfur dioxide, CO Carbon monoxide, O3 Ozone, NOx Nitrogen oxides, BMI Body mass index, PM2.5*Exercise interaction between PM2.5 and exercise
exercise, suggesting that exercise could attenuate PM2.5-related DLEC1 hypermethylation and the subsequent adverse effects. The possible mechanism through which exercise could modulate PM2.5-induced DLEC1 methylation is unclear. As previously stated, PM2.5 exacerbates inflammation and oxidative stress that could induce epigenetic alterations, especially DNA methylation [3, 66, 67]. Conversely, exercise regulates inflammation [6–9] and protects against air pollution-related health outcomes through altered DNA methylation profiles [18]. Inflammation is suggested as a possible contributor to epigenetic changes resulting from exercise interventions [66]. For instance, available literature corroborates the idea that exercise-associated inflammatory effects could be among the mechanisms that regulate DNA methylation [68]. Therefore, exercise might affect DLEC1 methylation by suppressing PM2.5-induced inflammation.

Previously, smoking and DLCE1 methylation were not significantly related [11, 28, 34]. It is important to note that in our study, smoking, SO2, CO, O3 showed significant associations with DLEC1 only after PM2.5 was stratified into quartiles. The attainment of a significant association between DLEC1 methylation and smoking after stratification into quartiles implies that PM2.5 might aggravate smoking-related DLEC1 methylation effects. Therefore, smoking which is already a harmful lifestyle habit could be more detrimental in air polluted areas. So far, the relation of age with DLEC1 methylation has not been concordant. For example, in esophageal cancer, age was significantly associated with DLCE1 methylation and expression [57]. On the other hand, in lung and gastric cancer, both factors had no significant association [11, 29]. In the current study, we found significant inverse associations between DLEC1 methylation and age, suggesting that DLEC1 methylation might decrease with

| Table 4: Multiple linear regression showing the association between outdoor exercise and DLEC1 promoter methylation stratified by PM2.5 quartiles |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables       | PM2.5 < 27.37   | 27.37 ≤ PM2.5 < 31.82 | 31.82 ≤ PM2.5 < 38.73 | PM2.5 ≥ 38.73   |
| β (× 10^-3)     | p value         | β (× 10^-3)     | p value         | β (× 10^-3)     | p value         |
| Regular outdoor exercise (reference: No) |                           |                           |                           |                           |
| Yes             | −5.280          | 0.009            | 0.323           | 0.872          | −1.280           | 0.433           | 1.680           | 0.428           |
| SO2             | 5.900           | 0.275            | −0.940          | 0.121          | 3.980           | 0.009           | 0.434           | 0.622           |
| CO              | 109.490         | 0.123            | 5.060           | 0.784          | −19.800         | 0.291           | −21.790         | 0.244           |
| O3              | 0.180           | 0.724            | 3.850           | 0.003          | 0.725           | 0.059           | −1.700          | 0.035           |
| NOx             | −1.640          | 0.105            | 0.744           | 0.201          | 0.252           | 0.323           | −0.557          | 0.376           |
| Sex (reference: Women) |                           |                           |                           |                           |
| Men             | 1.580           | 0.427            | −1.150          | 0.563          | 3.960           | 0.037           | 0.711           | 0.743           |
| Age             | −0.095          | 0.286            | −0.150          | 0.098          | −0.310          | < 0.001         | −0.320          | 0.003           |
| Cigarette smoking (reference: Never) |                           |                           |                           |                           |
| Former          | 0.510           | 0.861            | 3.440           | 0.213          | −0.542          | 0.818           | 5.950           | 0.063           |
| Current         | 3.960           | 0.172            | −1.010          | 0.745          | 1.810           | 0.557           | 1.670           | 0.672           |
| Alcohol drinking (reference: Never) |                           |                           |                           |                           |
| Former          | −4.110          | 0.358            | −2.550          | 0.606          | −6.610          | 0.164           | −5.120          | 0.618           |
| Current         | 4.380           | 0.183            | 0.484           | 0.900          | 6.970           | 0.056           | −6.840          | 0.093           |
| Betel nut chewing (reference: Never) |                           |                           |                           |                           |
| Former          | −10.910         | 0.135            | 1.990           | 0.672          | −2.710          | 0.485           | −7.910          | 0.276           |
| Current         | 2.360           | 0.677            | −9.510          | 0.103          | −2.410          | 0.679           | −9.910          | 0.210           |
| BMI (reference: Normal) |                           |                           |                           |                           |
| Underweight     | −3.110          | 0.434            | −1.760          | 0.656          | −2.110          | 0.642           | 11.390          | 0.055           |
| Overweight      | −2.750          | 0.206            | −1.650          | 0.457          | −0.709          | 0.699           | 2.010           | 0.345           |
| Obesity         | 0.747           | 0.755            | 0.964           | 0.685          | 1.010           | 0.642           | 4.360           | 0.071           |
| Secondhand smoke exposure (reference: No) |                           |                           |                           |                           |
| Yes             | 1.590           | 0.548            | −1.520          | 0.573          | −3.030          | 0.379           | 1.010           | 0.761           |

DLEC1 Deleted in lung and esophageal cancer 1, PM Particulate matter with diameter less than 2.5 microns, SO2 Sulfur dioxide, CO Carbon monoxide, O3 Ozone, NOx Nitrogen oxides, BMI Body mass index
increasing age. Age was also inversely associated with DNA methylation in a genome-wide DNA methylation study [69]. In the context of the current study, the underpinning mechanism cannot be clearly stated.

DNA methylation is a reliable molecular predictor of cancer because it is the most common epigenetic variation that could be detected even at premalignant or early malignant stages [13, 70]. Moreover, in noncancerous tissues, it could reveal previous exposure to carcinogens and so, is a possible indicator of disease risk [71]. Hence, the observed DLEC1 hypermethylation due to PM$_{2.5}$ and smoking might serve as an early predictor of adverse health conditions. Moreover, hypomethylation of DLEC1 due to regular exercise at low PM$_{2.5}$ levels shows that exercise intervention could help reverse the methylation status of DLEC1 and possibly upregulate the gene. As a limitation, we could not adjust for occupational exposure because we did not have related information. Moreover, we did not evaluate DLEC1 expression due to the unavailability of data in the TWB dataset. Nevertheless, many studies found significant associations between DLEC1 hypermethylation and down-regulation [26, 30, 31, 57, 61].

**Conclusions**

This study shows that PM$_{2.5}$ might affect DLEC1 methylation in individuals with no personal history of cancer. Exercise might regulate the effect of PM$_{2.5}$ on DLEC1

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**Table 5** Multiple linear regression showing DLEC1 promoter methylation in relation to regular exercise habits and PM$_{2.5}$ quartiles

| Variables                                                                 | $\beta \times 10^{-3}$ | $p$ value |
|--------------------------------------------------------------------------|-------------------------|-----------|
| Regular outdoor exercise, PM$_{2.5}$ (reference: No regular outdoor exercise, PM$_{2.5}$ $\geq$ 38.73) |                         |           |
| No regular outdoor exercise, 31.82 $\leq$ PM$_{2.5}$ $<$ 38.73            | 2.150                   | 0.198     |
| No regular outdoor exercise, 27.37 $\leq$ PM$_{2.5}$ $<$ 31.82            | 0.136                   | 0.934     |
| No regular outdoor exercise, PM$_{2.5}$ $<$ 27.37                         | $-0.808$                | 0.634     |
| Regular outdoor exercise, PM$_{2.5}$ $\geq$ 38.73                        | 1.310                   | 0.444     |
| Regular outdoor exercise, 31.82 $\leq$ PM$_{2.5}$ $<$ 38.73              | 0.570                   | 0.750     |
| Regular outdoor exercise, 27.37 $\leq$ PM$_{2.5}$ $<$ 31.82              | 0.410                   | 0.827     |
| Regular outdoor exercise, PM$_{2.5}$ $<$ 27.37                           | $-5.160$                | 0.007     |
| SO$_2$                                                                   | 0.209                   | 0.544     |
| CO                                                                       | $-6.270$                | 0.379     |
| O$_3$                                                                    | $-0.010$                | 0.964     |
| NOx                                                                      | 0.038                   | 0.759     |
| Sex (reference: Women)                                                   |                         |           |
| Men                                                                      | 0.652                   | 0.490     |
| Age                                                                      | $-0.207$                | $<0.001$  |
| Cigarette smoking (reference: Never)                                     |                         |           |
| Former                                                                   | 1.470                   | 0.274     |
| Current                                                                  | 2.340                   | 0.123     |
| Alcohol drinking (reference: Never)                                      |                         |           |
| Former                                                                   | $-3.390$                | 0.174     |
| Current                                                                  | 1.690                   | 0.336     |
| Betel nut chewing (reference: Never)                                     |                         |           |
| Former                                                                   | $-1.820$                | 0.459     |
| Current                                                                  | $-4.270$                | 0.145     |
| BMI (reference: Normal)                                                  |                         |           |
| Underweight                                                              | $-1.070$                | 0.620     |
| Overweight                                                               | $-0.264$                | 0.791     |
| Obesity                                                                  | 1.620                   | 0.143     |
| Secondhand smoke exposure (reference: No)                                |                         |           |
| Yes                                                                      | $-0.309$                | 0.826     |

DLEC1 Deleted in lung and esophageal cancer 1, PM Particulate matter with diameter less than 2.5 microns, SO$_2$ Sulfur dioxide, CO Carbon monoxide, O$_3$ Ozone, NOx Nitrogen oxides, BMI Body mass index
methylation, especially at low concentrations of PM$_{2.5}$. Our findings support prior reports that DNA methylation in noncancerous tissues could reveal previous exposure to carcinogens. Hence, DLEC1 promoter hypermethylation might elucidate the epigenetic mechanism through which PM$_{2.5}$ enhances disease onset and might be a possible biomarker of disease risk.

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Authors’ contributions
Conceptualization, Ying-Hsiang Chou, Disline Manli Tantoh, Ming-Chi Wu, Yeu-Sheng Tyan, Pei-Hsin Chen, Oswald Ndzi Nfor, Shu-Yi Hsu, Chao-Yu Shen, Chien-Ning Huang, and Yung-Po Liaw; methodology, Ying-Hsiang Chou, Disline Manli Tantoh, Ming-Chi Wu, Yeu-Sheng Tyan, Pei-Hsin Chen, Oswald Ndzi Nfor, Shu-Yi Hsu, Chao-Yu Shen, Chien-Ning Huang, and Yung-Po Liaw; formal analysis, Pei-Hsin Chen and Yung-Po Liaw; validation, Ying-Hsiang Chou, Disline Manli Tantoh, Ming-Chi Wu, Yeu-Sheng Tyan, Pei-Hsin Chen, Oswald Ndzi Nfor, Shu-Yi Hsu, Chao-Yu Shen, Chien-Ning Huang, and Yung-Po Liaw; supervision, Chao-Yu Shen, Chien-Ning Huang, and Yung-Po Liaw; writing—original draft, Ying-Hsiang Chou and Disline Manli Tantoh; writing—review and editing, Ying-Hsiang Chou, Disline Manli Tantoh, Ming-Chi Wu, Yeu-Sheng Tyan, Pei-Hsin Chen, Oswald Ndzi Nfor, Shu-Yi Hsu, Chao-Yu Shen, Chien-Ning Huang, and Yung-Po Liaw. The authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from Taiwan Biobank but restrictions apply to the availability of these data, which were used under license for the current study, and are not publicly available. Data are however available from the authors upon reasonable request and with permission of Taiwan Biobank.

Ethics approval and consent to participate
The Chung Shan Medical University Institutional Review Board (CS2-20007) granted ethical approval for this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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