*JNCI J Natl Cancer Inst* (2016) 108(3): djv341

doi:10.1093/jnci/djv341
First published online November 11, 2015
Article

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# Fine Particle Pollution, Alanine Transaminase, and Liver Cancer: A Taiwanese Prospective Cohort Study (REVEAL-HBV)

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## Abstract

**Background:** Exposure to fine particulate matter ($PM_{2.5}$) may promote hepatic tumorgenesis through low-grade inflammation. Therefore, we assessed the association of long-term exposure levels of $PM_{2.5}$ and subsequent risk of hepatocellular carcinoma (HCC) and investigated the mediation effect of inflammation as represented by alanine aminotransferase (ALT) on this association.

**Methods:** Between 1991 and 1992, we recruited 23 820 participants in Taiwan with no history of HCC. Case patients of HCC were ascertained through computerized data linkage with the National Cancer Registry and death certification systems. Participants' exposures to $PM_{2.5}$ were based on a four-year average retrieved from stationary monitoring sites. Cox proportional hazards models were used to assess the association between $PM_{2.5}$ exposure and HCC incidence. Mediation effects of ALT on $PM_{2.5}$–associated HCC incidence were estimated.

**Results:** A total of 464 HCC cases were newly diagnosed with a median follow-up of 16.9 years. Statistically significantly increasing trends between $PM_{2.5}$ exposures and ALT were observed on the Main Island and Penghu Islets. The adjusted hazard ratio (HR) for HCC on the Penghu Islets was 1.22 (95% confidence interval [CI] = 1.02 to 1.47) per $PM_{2.5}$ interquartile range (IQR) increment (0.73 μg/m$^3$) exposure. We also found a positive association between $PM_{2.5}$ exposure (per IQR increment, 13.1 μg/m$^3$) and HCC incidence on the Main Island. Furthermore, ALT had a statistically significant mediation effect on $PM_{2.5}$-associated HCC incidence (HR = 1.17, 95% CI = 1.02 to1.52 on the Main Island; HR = 1.04, 95% CI = 1.03 to 1.07 on the Penghu Islets) per $PM_{2.5}$ IQR increment.

**Conclusions:** Long-term $PM_{2.5}$ exposure increased the risk for liver cancer, and chronic inflammation of the liver may underlie the pathogenesis.

Long-term exposure to ambient fine particulate matter ($PM_{2.5}$) has been associated with short- and long-term mortality, incidence of cardiovascular diseases (CVD) (1–3), and lung cancer (4–6). The World Health Organization (WHO) attributed 3.2 million worldwide deaths in 2010 to ambient particulate matter, (7) and the International Agency for Research on Cancer (IARC)

**Received:** April 6, 2015; **Revised:** July 11, 2015; **Accepted:** October 15, 2015

classifies ambient particulate pollution as a class 1 carcinogen associated with lung cancer (8).

Several human observational studies found that $PM_{2.5}$ exposure was positively associated with systematic inflammation markers (eg, C-reactive protein [CRP] and interleukin-6 [IL-6]) (9–15), which contributed to CVD risk. Experimental mice chronically exposed to concentrated $PM_{2.5}$ collected from ambient air had elevated levels of serum cytokines such as IL-6 and tumor necrosis factor alpha (TNF-α) (16). Evidence showed that exposure to ambient fine particulate matter increased serum levels of hepatic enzymes such as γ-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (17). Elevated serum ALT is a marker of liver damage, usually caused by liver inflammation resulting from the infection of hepatitis viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV). In addition, this marker has been documented as an independent predictor of hepatocellular carcinoma (HCC), the most common type of liver cancer and one of the leading cancer types in Taiwan. Other known risk factors for HCC include increasing age, male sex, and alcohol consumption, as well as chronic HBV and HCV infections. However, the association between long-term $PM_{2.5}$ exposure and HCC has never been investigated.

Therefore, we hypothesize that long-term $PM_{2.5}$ exposure may induce HCC through chronic liver inflammation. In this study we assess the association between chronic $PM_{2.5}$ exposure and HCC incidence in a prospective cohort study in Taiwan and also evaluate the role of ALT on $PM_{2.5}$-associated HCC incidence using mediation analyses.

## Methods

### Study Population

As described previously (18–21), we recruited 23 820 participants from seven townships in Taiwan between 1991 and 1992. Three study townships were located on the Penghu Islets, while the other four were located on the Main Taiwan Island. A total of 1410 participants were excluded because of missing residential addresses. We further excluded participants who had missing information on smoking status (n = 39), alcohol consumption (n = 53), antibodies against HCV (anti-HCV) serostatus (n = 34), ratio of AST to ALT (n = 95), or who had residential addresses outside of Taipei, Hsinchu, Chiayi, Pingtung, or Penghu county (n = 165). Moreover, residents who were diagnosed with HCC based on health examination, data linkage with the Taiwan Cancer Registry, or had a history of HCC from questionnaire interviews at study entry were not eligible for this study. There were 22 062 participants remaining for the main analysis. Participants were followed through December 31, 2009. Public health nurses interviewed participants using structured questionnaires to collect demographic information and lifestyle factors for each resident. Each participant provided a 10 mL peripheral blood sample, which was fractionated and stored at -70 Celsius. Baseline serum samples were tested for hepatitis B surface antigen (HBsAg) to identify participants with chronic HBV infection using commercially available radioimmunoassay kits (Abbott Laboratories, North Chicago, IL); chronic HCV infection was identified based on anti-HCV serostatus tested using second-generation commercial enzyme immunoassays (Abbott Laboratories); and ALT levels were determined by a serum chemistry autoanalyzer (model 736, Hitachi, Tokyo, Japan). Each participant provided written informed consent for the questionnaire interview, health examinations, collection of bio-specimens, and computerized data linkage with the Taiwan Cancer Registry and national death certification system. All study protocols were approved by the Institutional Review Board of the College of Public Health, National Taiwan University.

### Exposure Assessment of Fine Particulate Matter

Hourly ambient $PM_{2.5}$ concentrations were measured by the Taiwan Environmental Protection Administration from January 1, 2006 to December 31, 2009. We used automatic monitoring data, which was available from 75 stationary sites covering the Penghu Islets and the Main Island. A modified ordinary kriging adopted from Liao et al. (22) was applied to approximate the long-term residential exposure to $PM_{2.5}$ for each participant. ArcView GIS (version 9.3) and its Geostatistical Analysts Extension (ESRI Inc., Redland, CA) were utilized to construct the semivariogram for spatial estimation of $PM_{2.5}$ levels. We used spherical spatial models with weighted least-squared methods to estimate patients' residential $PM_{2.5}$ levels. The median value of cross-validated R-squared for $PM_{2.5}$ exposure was 0.73 with an interquartile range (IQR) of 0.24. The mean levels of participants' $PM_{2.5}$ exposures were 32.23 μg/m³ (SD = 7.56 μg/m³, range = 15.9 to 45.8 μg/m³) and 24.22 μg/m³ (SD = 0.50 μg/m³, range = 23.5 to 28.9 μg/m³) for the Main Island and Penghu Islets, respectively. The median distance from participants' residential addresses to the nearest $PM_{2.5}$ monitoring station was 3.41 kilometers (IQR = 7.13 kilometers).

### Ascertainment of Hepatocellular Carcinoma

The ascertainment of newly developed HCC was described previously (23,24). At study entry, abdominal ultrasonography and confirmatory diagnoses were conducted in participants with family history of HCC or cirrhosis among first-degree relatives; had elevated serum levels of ALT, AST, or α-fetoprotein; and who were seropositive for HBsAg or anti-HCV. We excluded participants who were affected by HCC based on the health examination, history of HCC extracted from the questionnaire, or the data linkage with the Taiwan Cancer Registry. Newly diagnosed HCC during follow-up was ascertained by the regular health examinations, which included ultrasonography and serum α-fetoprotein level, and computerized data linkage with the Taiwan Cancer Registry and national death certification system from January 1, 1991 to December 31, 2009 in Taiwan. Medical confirmation of newly developed HCC was ascertained by pathologic examination of hepatic specimens, positive lesions confirmed by at least two different imaging techniques (angiogam, computer tomography, or abdominal ultrasonography), or positive lesions by one imaging technique accompanied by an α-fetoprotein level of 400 ng/mL or higher.

### Statistical Analysis

To present effect modification and to minimize the confounding effect of region (Main Island or Penghu Islets) on $PM_{2.5}$-ALT and $PM_{2.5}$-HCC associations, all statistical analyses were primarily performed separately by region. Additionally, a unified analysis based on all participants (Main Island and Penghu Islets) with further adjustment for region was also performed. A continuous variable of the four-year average $PM_{2.5}$ level (2006–2009) was applied throughout the analyses. Time at risk for HCC was calculated from the enrollment date to the date of HCC diagnosis, the date of death, or the last date of linked data from the Taiwan

Cancer Registry, whichever came first. Follow-up period (years) was selected as the time scale in the main analyses. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for the association between long-term $PM_{2.5}$ exposure and HCC incidence, adjusting for age (30–39, 40–49, 50–59, or 60–65 years), sex (male or female), alcohol consumption habit (yes or no), smoking status (never or ever), ALT levels (<15.0, 15.0 to 44.9, or ≥45.0 U/L), as well as serostatus of HBsAg and anti-HCV (positive or negative). Verification of proportionality assumption was carried out by using Schoenfeld residuals followed by chi-squared test statistics before applying the analyses. We utilized a linear regression model to assess the association between $PM_{2.5}$ exposure and serum ALT levels. ALT values were added 0.5 followed by natural logarithm transformation. The linear regression models were adjusted for age, sex, alcohol consumption habit, smoking status, HBsAg serostatus, anti-HCV serostatus, and county (Taipei, Hsinchu, Chiayi, or Pingtung on the Main Island).

To evaluate the role of ALT levels on $PM_{2.5}$-associated HCC incidence, we applied mediation analysis using survival data as proposed by VanderWeele and Vansteelandt (25,26). This statistical approach has been successfully applied in epidemiological studies to demonstrate the role of mediators in well-known associations (27–29). Details of model fitting and assumptions can be found in the Supplementary Materials (available online). Natural indirect effects (NIEs), which assess the effect of $PM_{2.5}$ exposure on HCC incidence through elevated ALT, were derived from the model coefficients and residual variance from regression models. The 95% confidence intervals and *P* values of NIEs and natural direct effects (NDEs) were based on 5000 Monte-Carlo resamplings (30). In the sensitivity analysis, we further verified the linearity assumption for $PM_{2.5}$-ALT and $PM_{2.5}$-HCC associations using spline functions and did not find significant deviations. *P* values of less than .05 were considered statistically significant. All statistical tests were two-sided. All analyses were performed by the R statistical package (version 3.0.2).

## Results

A total of 464 newly developed HCC cases were identified during a median follow-up time of 16.9 years. The median $PM_{2.5}$ exposure level was statistically significantly higher on the Main Island (36.0 μg/m³) compared with the Penghu Islets (24.1 μg/m³, $P < .001$). Using the median levels of $PM_{2.5}$ in specific regions as cutoff values, participants who were exposed to higher $PM_{2.5}$ levels were consistently older and had a higher prevalence of HBsAg seropositivity as well as alcohol consumption in both the Penghu Islets and Main Island (Table 1). The cumulative incidence of HCC was positively associated with $PM_{2.5}$ exposure levels (above median vs below median) on the Main Island (1.8% vs 1.3 %, $P = .03$) and the Penghu Islets (3.9% vs 2.0%, $P < .001$). As shown in Figure 1, participants who resided on the Penghu Islets generally had higher cumulative incidences of HCC compared with those resided on the Main Island ($P < .001$). Residents who were exposed to higher $PM_{2.5}$ levels (above median vs below median) had an elevated cumulative incidence of HCC ($P = .02$ on the Main Island; $P < .001$ on the Penghu Islets).

We also investigated the association between long-term $PM_{2.5}$ exposure and serum ALT levels. As shown in Table 2, $PM_{2.5}$ exposure was positively associated with ALT levels. ALT levels in logarithmic scales increased by 28.36% (95% CI = 6.82 to 54.25) on the Main Island and by 6.62 % (95% CI = 4.36 to 8.93) on the Penghu Islets per IQR increment of $PM_{2.5}$. Similar results were found in a restricted population of nonsmokers and nondrinkers who were seronegative for HBsAg and anti-HCV on both the Main Island and Penghu Islets. We also found that $PM_{2.5}$ exposure was consistently negatively associated with the ratio of AST to ALT (AAR), an index of hepatic fibrosis (Supplementary Table 1, available online).

In Table 3, we incorporated an interaction term for $PM_{2.5}$ and region (Penghu Islets vs Main Island) to account for the potential effect modification by region. We found a statistically significant interaction between $PM_{2.5}$ and region on HCC risks ($P_{interaction} = .03$). HCC incidence was positively associated with

**Table 1.** Baseline characteristics and number of newly developed HCC cases in the REVEAL-HBV cohort according to region and $PM_{2.5}$ exposure levels

| | Main Island | | | Penghu Islets | | |
|---|---|---|---|---|---|---|
| | Low exposure (<36.0 μg/m³) (n = 6620) | High exposure (≥36.0 μg/m³) (n = 6554) | | Low exposure (<24.1 μg/m³) (n = 4520) | High exposure (≥24.1 μg/m³) (n = 4368) | |
| Variables | No. (%) | No. (%) | P* | No. (%) | No. (%) | P* |
| Age, y | | | | | | |
| 30–39 | 2273 (34.3) | 1362 (20.8) | <.001 | 1488 (32.9) | 1139 (26.1) | <.001 |
| 40–49 | 1763 (26.6) | 1715 (26.2) | -- | 1295 (28.7) | 1014 (23.2) | -- |
| 50–59 | 1985 (30.0) | 2369 (36.1) | -- | 1094 (24.2) | 1327 (30.4) | -- |
| 60–65 | 599 (9.0) | 1108 (16.9) | -- | 643 (14.2) | 888 (20.3) | -- |
| Male sex | 3107 (46.9) | 3606 (55.0) | <.001 | 2343 (51.8) | 2057 (47.1) | <.001 |
| BMI, kg/m² (mean ± SD) | 24.06 ± 7.22 | 23.94 ± 6.18 | .24 | 24.08 ± 3.58 | 24.34 ± 7.33 | .003 |
| Ever smoker | 1922 (29.0) | 2026 (30.9) | .02 | 1271 (28.1) | 1290 (27.2) | 0.51 |
| Alcohol consumption habit | 469 (7.1) | 777 (11.9) | <.001 | 556 (12.3) | 572 (13.1) | .27 |
| Serum alanine aminotransferase, U/L (mean ± SD, logarithm scale) | 2.31 ± 0.72 | 2.22 ± 0.69 | <.001 | 2.49 ± 0.78 | 2.60 ± 0.78 | <.001 |
| HBsAg sero-positivity | 1106 (16.7) | 1121 (17.1) | .46 | 799 (17.7) | 834 (19.1) | .09 |
| Anti-HCV sero-positivity | 430 (6.5) | 290 (4.4) | <.001 | 141 (3.1) | 345 (7.9) | <.001 |
| Incident cases of hepatocellular carcinoma | 86 (1.3) | 117 (1.8) | .03 | 92 (2.0) | 169 (3.9) | <.001 |

* *P* values were based on the Wilcoxon rank-sum test for body mass index and alanine transaminase, Pearson's chi-squared test for age, or Fisher's exact test for sex, smoke, alcohol consumption, hepatitis B virus serostatus, anti–hepatitis C virus serostatus, and hepatocellular carcinoma status. All statistical tests were two-sided. HBsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; IU = international unit.

$PM_{2.5}$ on the Main Island and Penghu Islets after adjustment for potential risk factors. On the Main Island, the β coefficient for HCC associated with each IQR increment of $PM_{2.5}$ exposure was 0.19 (95% CI = -0.05 to 0.42, $P$ = .12). On the Penghu Islets, however, $PM_{2.5}$ exposure was statistically significantly associated with HCC incidence. The β coefficient for HCC development was 0.21 (95% CI = 0.02 to 0.39, $P$ = .03) per IQR increment of $PM_{2.5}$ exposure (0.73 μg/m³), which was equivalent to an adjusted hazard ratio (HR) of 1.22 (95% CI = 1.02 to 1.47) per IQR increment. Additionally, a full evaluation of effect modification by demographics on the $PM_{2.5}$-HCC association was performed, and we found that region, sex, and alcohol consumption habit statistically significantly modified this association (Supplementary Table 2, available online). We further assessed the association of ALT levels with HCC risk among all participants. The adjusted hazard ratios of developing HCC were 2.49 (95% CI = 2.01 to 3.09) for patients with ALT levels between 15.0 and 45.0 U/L and 5.17 (95% CI = 3.88 to 6.90) for ALT levels higher than 45 U/L, when compared with patients with normal ALT levels (<15 U/L).

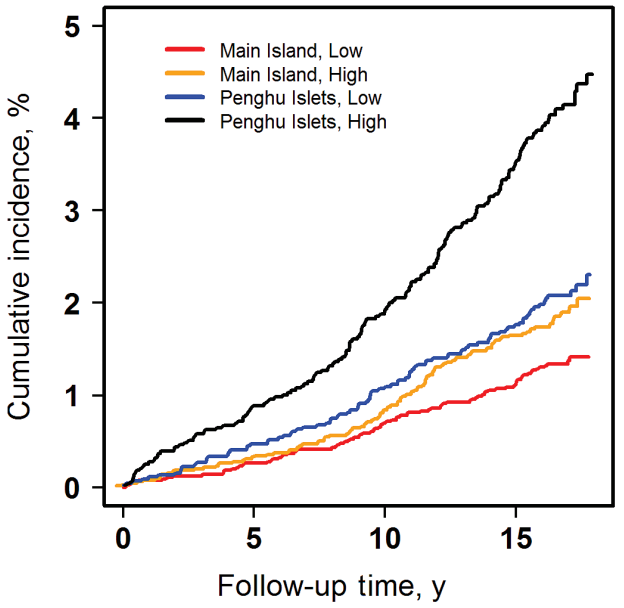


**Figure 1.** Cumulative incidence of hepatocellular carcinoma by levels of fine particulate matter and region. $PM_{2.5}$ levels were dichotomized by the median values on the Main Island (low, <36.0 μg/m³; high, ≥36.0 μg/m³) and Penghu Islets (low, <24.1 μg/m³; high, ≥24.1 μg/m³). $P$ value for the Nelson-Aalen curve was less than .001 using a two-sided log-rank test.

We also examined the mediation effect of serum ALT levels on $PM_{2.5}$-associated HCC incidence as shown in Table 4. A statistically significant NIE of $PM_{2.5}$ exposure on HCC incidence through elevated ALT levels was found on the Main Island (HR = 1.17, 95% CI = 1.02 to 1.52, $P$ = .05) and the Penghu Islets (HR = 1.04, 95% CI = 1.03 to 1.07, $P$ < .001) for each incremental IQR increase of $PM_{2.5}$. We also found that $PM_{2.5}$ had a positive NDE on HCC incidence, although it did not reach statistical significance.

## Discussion

In this study, we found that long-term $PM_{2.5}$ exposure was associated with baseline serum ALT levels in a dose-dependent manner and was also positively associated with subsequent HCC risk during long-term follow-up. Using mediation analysis, we also found a statistically significant mediation effect of serum ALT levels on $PM_{2.5}$-associated HCC risks. Serum ALT levels serve as a surrogate marker of chronic liver tissue inflammation and are frequently used in clinical settings. The statistically significant mediation effect indicates that $PM_{2.5}$ exposure may induce chronic hepatic inflammation that eventually leads to the development of HCC. To our knowledge, this was the first study to examine the association between long-term $PM_{2.5}$ exposure and incidence of HCC that also clarified the role of chronic inflammation in this association.

Previous findings suggest that air pollution may increase serum levels of hepatic enzymes. Markevych et al. found that $PM_{2.5}$ was positively associated with γ-glutamyl transferase (GGT) in a German population (17). They also observed that other common air pollutants, $PM_{10}$ and $NO_2$, were consistently associated with GGT, ALT, and AST. Additionally, the positive relationship between $PM_{2.5}$ and ALT found in our study was robust, even among participants who were seronegative for HBsAg and anti-HCV, as well as nonsmokers without an alcohol consumption habit. The restriction analysis minimized the potential confounding because of hepatitis infection, which was not controlled by Markevych et al.

Evidence from epidemiological studies found that exposure to particulate matter is positively associated with systematic inflammation markers. In an elderly panel cohort study (10), participants who were exposed to a higher concentration of $PM_{0.25}$, a subfraction of $PM_{2.5}$, had elevated serum IL-6 levels. By analyzing the composites of $PM_{0.25}$, this study found that polycyclic aromatic hydrocarbons, which represent one of the primary organic aerosol components and tracers, were associated with higher levels of serum IL-6. In an experimental mouse

**Table 2.** Association between long-term $PM_{2.5}$ exposure and serum alanine transaminase level

| Region | Whole population (n = 22 062) | | | Restricted population (n = 11 840)* | | |
|---|---|---|---|---|---|---|
| | % increase (95% CI) in logarithm ALT per IQR increase of $PM_{2.5}$†,‡ | P§ | $P_{interaction}$§ | % increase (95% CI) in logarithm ALT per IQR increase of $PM_{2.5}$‡,‖ | P§ | $P_{interaction}$§ |
| Main Island | 28.36 (6.82 to 54.25) | .007 | <.001 | 20.24 (-6.13 to 54.00) | .14 | <.001 |
| Penghu Islets | 6.62 (4.36 to 8.93) | <.001 | -- | 13.01 (9.94 to 16.0) | <.001 | -- |
| Combined Region | 44.17 (23.21 to 68.68) | <.001 | -- | 68.26 (36.27 to 107.7) | <.001 | -- |

* Analyses were restricted to participants who were nonsmokers, were seronegative for HBsAg and anti-HCV, and did not have habitual alcohol consumption at study entry. ALT = alanine transaminase; CI = confidence interval; HBsAg = hepatitis B virus surface antigen; HCV = hepatitis C virus; IQR = interquartile range.
† Models were adjusted for age, sex, alcohol consumption, smoking, HBsAg serostatus, anti-HCV serostatus, and county at study entry.
‡ The IQRs were 0.73 μg/m³ (Penghu Islets), 13.1 μg/m³ (Main Island), and 12.2 μg/m³ (combined region) based on the whole population (n = 22 062).
§ $P$ value (two-sided) for coefficient was based on least squares estimation. $P_{interaction}$ was calculated by using two-sided Wald test on the cross-product of $PM_{2.5}$ and region (Main Island or Penghu Islets).
‖ Models were adjusted for age, sex, alcohol consumption, smoking, and county at study entry.

**Table 3.** Association between long-term $PM_{2.5}$ exposure and incidence of hepatocellular carcinoma

| Variable | β coefficient (95% CI) | P* |
|---|---|---|
| $PM_{2.5}$, 1 μg/m³ increment | 0.01 (0.00 to 0.03) | .12 |
| Region, Penghu Islets vs Main Island | -6.15 (-12.23 to -0.08) | .05 |
| $PM_{2.5}$ x Region | 0.27 (0.02 to 0.52) | .03 |
| Age, y | | |
| 40–49 vs 30–39 | 1.11 (0.73 to 1.49) | <.001 |
| 50–59 vs 30–39 | 1.80 (1.44 to 2.15) | <.001 |
| >60 vs 30–39 | 2.01 (1.63 to 2.39) | <.001 |
| Sex, male vs female | 0.42 (0.17 to 0.66) | <.001 |
| Hepatitis B virus surface antigen, positive vs negative | 1.96 (1.77 to 2.15) | <.001 |
| Antibodies against hepatitis C virus, positive vs negative | 1.45 (1.22 to 1.67) | <.001 |
| Serum ALT levels, U/L | | |
| 15–44 vs <15 | 0.91 (0.70 to 1.13) | <.001 |
| >45 vs <15 | 1.64 (1.35 to 1.92) | <.001 |
| Alcohol drinking habit, yes vs no | 0.29 (0.04 to 0.54) | .02 |
| Cigarette smoking habit, yes vs no | 0.25 (0.03 to 0.48) | .03 |
| Main effect of $PM_{2.5}$ by region calculated from the above model | | |
| $PM_{2.5}$ (on Main Island), per IQR increment (13.1 μg/m³) | 0.19 (-0.05 to 0.42) | .12 |
| $PM_{2.5}$ (on Penghu Islets), per IQR increment (0.73 μg/m³) | 0.21 (0.02 to 0.39) | .03 |

* P value (two-sided) for each coefficient was based on the Wald test using the maximum likelihood estimation. ALT = alanine transaminase; CI = confidence interval; IQR = interquartile range.

**Table 4.** Mediation effect of serum alanine transaminase levels on $PM_{2.5}$-associated incidence of hepatocellular carcinoma

| | Natural indirect effect | | Natural direct effect | |
|---|---|---|---|---|
| Region | HR (95% CI) per IQR increment of $PM_{2.5}$* | P | HR (95% CI) per IQR increment of $PM_{2.5}$* | P |
| Main Island | 1.17 (1.02 to 1.52) | .05 | 1.18 (0.64 to 2.07)† | .54 |
| Penghu Islets | 1.04 (1.03 to 1.07) | <.001 | 1.27 (0.98 to 1.61)‡ | .09 |
| Combined | 1.21 (1.06 to 1.41) | .005 | 1.28 (0.88 to 1.92)† | .14 |

* The interquartile ranges were 0.73 μg/m³ (Penghu Islets), 13.1 μg/m³ (Main Island), and 12.2 μg/m³ (combined region), based on all participants (n = 22 062). P value (two-sided) was based on tests of 5000 Monte-Carlo simulations. CI = confidence interval; HR = hazard ratio; IQR = interquartile range.
† Direct effects of $PM_{2.5}$ exposure on the incidence of hepatocellular carcinoma were based on participants who were age 40 to 49 years, males, smokers, positive for HBsAg serostatus, positive for anti-HCV serostatus, had alcohol consumption habit, and resided in Pingtung county.
‡ Direct effects of $PM_{2.5}$ exposure on the incidence of hepatocellular carcinoma were based on participants who were age 40 to 49 years, males, positive for HBsAg serostatus, positive for anti-HCV serostatus, and had alcohol consumption habit.

study, compared with the control group, Wilson et al. (16) found statistically significantly higher levels of serum cytokines such as IL-6 and TNF-α among mice exposed to concentrated ambient particulate matter collected from a severely air-polluted area. Based on observational and experimental studies, because $PM_{2.5}$ can potentially induce systematic inflammation markers, it is plausible that $PM_{2.5}$ may induce hepatic inflammation as assessed by ALT levels.

We found a positive association between cigarette smoking (ever vs never) and HCC risk among males (adjusted HR = 3.00, 95% CI = 1.05 to 8.58), even after the adjustment for important risk factors including $PM_{2.5}$ and ALT. Cigarette smoking reduced ALT levels by 5.66% (95% CI = -17.47% to 7.84 %) in the logarithmic scale, but the association did not reach statistical significance (*P* = .39). In the literature, the association between cigarette smoking and ALT levels has remained inconsistent (31,32) and is considered to be modified either by NADH dehydrogenase (33) or hepatitis serostatus (34). The null association of cigarette smoking and ALT levels observed in our study does not conflict with previous findings.

The positive relationship between systematic inflammation markers and HCC risk is supported by human evidence. In a case-control study nested in a large cohort in the European Prospective Investigation into Cancer and Nutrition (EPIC) (35), higher incidence rates of HCC were found among participants who had elevated levels of serum IL-6 and CRP measured at study entry. A similar trend was also found for the incidence of biliary tract cancer outside of the liver. Several mechanisms are proposed to link inflammation markers and HCC. Intestine-specific homeobox (*ISX*), a transcription factor highly expressed in intestines (36), has been suggested to be involved in inflammation-associated tumorgenesis (37). Inflammatory cytokines such as IL-6 and TNF-α statistically significantly induce ISX mRNA expression in a dose-dependent manner in hepatic cancer cells. *Isx* expression was higher among HCC patients compared with non-HCC controls. Participants with higher *Isx* expression at enrollment had decreased survival time during follow-up. Overexpressed *Isx* increased cell cycle progression, whereas knocking down *Isx* using RNA interference techniques attenuated tumorgenesis capability in Hep G2 cells. These findings suggest that *ISX*, a proinflammatory homeobox gene, is essential for hepatic tumor growth. Furthermore, several studies found that transcription factor *STAT3* explained substantially early tumor growth in colitis-associated cancer induced by IL-6 (38–40) and suggested that the NK-kB-IL-6-STAT3 cascade is one of the essential pathways for tumorgenesis in intestinal epithelial cells.

Several limitations should be considered when interpreting the findings of this study. First, as $PM_{2.5}$ information was unavailable prior to June 2005 in Taiwan, residential exposures to $PM_{2.5}$ from 2006 to 2009 were used as the long-term exposure to fine particulate pollution. Certain levels of exposure misclassification may have been introduced because exposure levels may vary between participants' residential and working places. Additionally, the average $PM_{2.5}$ levels from 2006 to 2009 may not be the perfect index for long-term exposure to $PM_{2.5}$. However, previous evidence has suggested that short periods of $PM_{2.5}$ exposure may serve as a good surrogate for long-term exposure because of the high correlation of annual exposure levels and uniform exposure ranking between different geographical locations over time (3,5,41). Second, we cannot exclude the possibility of competing risks from cardiovascular-related mortality because of $PM_{2.5}$ exposure, which may bias the findings in this study. Specifically, it is well known that short- (42) and long-term (5,43–45) $PM_{2.5}$ exposures are positively associated with cardiovascular mortality, and these findings were primarily based on survivors who were more resistant to $PM_{2.5}$-associated cardiovascular mortality. The findings based on this study may not be generalizable to the general population. Third, we did not adjust for socioeconomic status (SES) in the analysis, which may raise concerns of unmeasured confounding given limited studies that found SES to be inversely associated with HCC (46,47). However, the role of SES in HCC risk is potentially through its positive association with obesity, alcoholism, and hepatitis B or C infection, which are well-established risk factors for liver cancer. Because we have included BMI, alcohol consumption, and hepatitis virus infections as covariates in this analysis, the unmeasured confounding from SES should be minimized. On the other hand, our study has several strengths, including a large sample size, a long-term follow-up period, and a prospective design.

In conclusion, in this study, we found that $PM_{2.5}$ exposure was positively associated with HCC risks, and elevated ALT levels may serve as the mediator for the association between $PM_{2.5}$ and HCC. Additional observational studies should be conducted to validate this finding. Moreover, toxicological experiments will facilitate the understanding of the $PM_{2.5}$-HCC etiology.

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## Funding

This work was supported by grant NSC-101-2625-M-006-009, NSC-101-2621-M-006-001, NSC-101-2314-B-001-005-MY3 from the Taiwan National Science Council (NSC), Academia Sinica, and the Ministry of Health and Welfare.

## Notes

The contents of this report are solely the responsibility of the authors and do not necessarily represent the official views of the sponsoring institutions. The funders had no role in the design of the study; the collection, analysis, or interpretation of the data; the writing of the manuscript; nor the decision to submit the manuscript for publication.

The authors have no conflicts of interest to disclose.

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