Title

Exposure to $PM_{2.5}$ and Lung Function Growth in Pre- and Early Adolescent Schoolchildren

A Longitudinal Study Involving Repeated Lung Function Measurements in Japan

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Abstract

Rationale: Epidemiological evidence indicates ambient exposure to $PM_{2.5}$ have adverse effects on lung function growth in children, but it is not actually clear whether exposure to low level $PM_{2.5}$ results in long-term decrements in lung function growth in pre- to early adolescent schoolchildren.

Objectives: To examine long-term effects of $PM_{2.5}$ within the 4-yr average concentration range of 10 to 19 μg/m^3 on lung function growth with repeated measurements of lung function tests.

Methods: Longitudinal analysis of 6,233 lung function measurements in 1,466 participants aged 8 to 12 from 16 school communities in 10 cities around Japan, covering a board area of the country to represent concentration range of $PM_{2.5}$, was done with multilevel linear regression model. Forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), and maximal expiratory flow at 50% of FVC (V50) were used as lung function indicators to examine effects of 10-μg/m^3 increases in $PM_{2.5}$ concentration on relative growth per 10-cm increase in height.

Results: Overall annual mean $PM_{2.5}$ concentration was 13.5 μg/m^3 (range: 10.4 to 19.0 μg /m^3). We found no association between any of the lung function growth indicators and increases in $PM_{2.5}$ levels in children of either sex, even after controlling for potential confounders. Analysis with two-pollutant models with O_3 or NO_2 did not change the null results.

Conclusions: This nationwide longitudinal study suggests that concurrent, long-term exposure to $PM_{2.5}$ at concentrations ranging from 10.4 to 19.0 μg/m^3 has little effect on lung function growth in pre-adolescent boys and pre- to early adolescent girls.

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A growing body of epidemiological evidence has been accumulated regarding the effects of $PM_{2.5}$ exposure on lung health in children (1-19), and studies employing a longitudinal design with repeated measurements of lung function have been particularly useful in improving our understanding of the effects of $PM_{2.5}$ exposure on lung function growth (5-11, 17-19). Evaluating the impact of air pollution exposure on lung function development in school-age and adolescent children is essential, because lung function growth is closely linked with the adolescent growth spurt that occurs at about 10 years of age in girls and 12 years of age in boys (20). Recent longitudinal studies demonstrated that lung health during pre-adolescent and young adulthood is important to predict middle-age lung function or occurrence of respiratory disorders in later life. Trajectories of FEV1 from 7 to 53 years of age in the Tasmanian Longitudinal study identified 6 distinct trajectories and showed that not only 'persistently low' but also 'below average' trajectory had increased risk of developing chronic obstructive pulmonary disease (COPD) by middle age (21). In the same cohort, respiratory risk profiles at age 7 such as parental smoking or frequent asthma, bronchitis, allergy were associated with future decline in FEV1 and FVC at age 53 and with increased risk of COPD at age 53 (22). In the study from UK cohorts, 44 cytosine-phosphate-guanine dinucleotide (CPG) sites of DNA methylation measured in peripheral blood at age 10 was associated with lung function trajectories from age 10 to age 26, also indicating the importance of environmental insults during pre-adolescent period for lung health (23).

A key evidence with wider range of $PM_{2.5}$ levels has been reported from the Southern California Children's Health Study (CHS), and the latest results of the CHS showed that improvements in air quality resulted in improvements in 4-year growth of FEV1 and FVC in children between the ages of 11 and 15 across the 3 cohorts. In fact, the 3-year mean $PM_{2.5}$ levels improved significantly from cohort to cohort: in the 1994 cohort the levels ranged from 21.3 to 31.5 $\mu g/m^3$, in the 1997 cohort they ranged from 19.9 to 27.6 $\mu g/m^3$, and in the 2007 cohort the range was 11.9 to 17.8 $\mu g/m^3$ (10).

The preceding epidemiological evidence suggests that reductions in lung function growth could be observed in adolescents aged 10 and over when $PM_{2.5}$ levels exceed 20 $\mu g/m^3$. However, information is quite limited regarding the long-term effects of exposure to $PM_{2.5}$ within the range of 10 to 19 $\mu g/m^3$ on lung function growth as measured with repeated lung function tests in pre-adolescents and

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adolescents. Therefore, we carried out repeated lung functions tests in a longitudinal study of pre- and early adolescent schoolchildren living in Japanese communities where annual $PM_{2.5}$ levels ranged from 10 to 19 $\mu g/m^3$, and we report herein on the association between exposure to $PM_{2.5}$ and lung function growth in these children.

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Methods

Study design and participants

We initiated a prospective study of 3rd grade elementary school children aged 8-9 recruited from 9 public elementary schools of 6 cities in 2011 and 7 public elementary schools of 4 cities in 2012, in total 16 school communities in 10 cities in 2011-2012, covering a broad area of the country to represent wide concentration range of $PM_{2.5}$ in Japan to maximize geographical variability of $PM_{2.5}$ concentration over Japan (Fig. 1). Prior to the recruitment process, we evaluated the feasibility of conducting a longitudinal study that would be to maximize statistical power to increase the probability of detecting the effects on annual average of FEV1 growth comparable to those in the CHS 1993 cohort (4) under the $PM_{2.5}$ concentration distributions from April 2001 to March 2005 in Japan (24), indicating expected number of enrolled communities would be 10 with 100 to 150 participants per community. At one school, the study was postponed from 2011 to 2012 after obtaining informed consent from 3rd grade children in 2011 due to 2011 Great East Japan Earthquake, and we decided to enroll both 3rd- and 4th-grade children in 2012. Follow-up was planned annually until the children were in the 5th grade (aged 10-11), and twice when they were in the 6th grade (aged 12).

To enroll as many children as possible, details of the study were given to parents at parent meetings and through information materials distributed at every school. Written informed consent was obtained from the parents of all participants. One thousand three hundred seven children out of 1381 were participated from the start year, and then 51 were enrolled in at later years while 131 were moved out to different school districts during study period. Additional 108 children were entered the study schools and participated thereafter (sFigure 1). About 80% (N=86) of the 108 children, were participated in the first half of the study, and attended the lung function testing 3 times or more.

The study was approved by the Ethical Committee of the Ministry of the Environment (approval number:11021001) centrally, and also approved at each regional study center.

Exposure measurement to selected air pollutants

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Ambient $PM_{2.5}$, ozone (O_3) and nitrogen dioxide (NO_2) were measured continuously over the study period at or near each school. The place to set the measuring equipment was decided so that the measurement data avoid the exhaust gases of heavy traffic and so on directly. Note that the measurement regarding O and P school was carried out near the schools (within 2 km) due to difficulty in setting the equipment at the schools. $PM_{2.5}$ was measured by beta ray attenuation method using PM-712 (Kimoto electric co. Ltd., Osaka, Japan). O_3 and NO_2 concentrations were measured by ultraviolet absorption and chemiluminescence methods using OA-781 and NA-721 (Kimoto electric co. Ltd.), respectively. The automated measurement of these air pollutants including quality assurance of measured data was in accordance with the manual for continuous monitoring of air pollution prepared by Ministry of the Environment, Japan (25).

Annual concentrations of suspended particulate matter (SPM), O_3, and NO_2 before the study period (i.e. when the study pupils were under 8 years old) and during it were calculated as 5-year means and range of yearly means on the basis of data obtained from the National Institute of Environmental Studies' environmental values database. SPM in Japan is classified as particulates whose aerodynamic diameter is less than 10 μm with a 100% cut-off level. Concentrations in the database were measured in accordance with the methods given in the Manual for Continuous Monitoring of Air Pollution, and the monitoring stations whose data were used were those closest to each elementary school.

Lung function tests and questionnaire on asthma and allergies

Lung function testing of the children in the 3rd to 6th grades was scheduled in the morning hours during the same season every year to minimize seasonal effects such as temperature and pollution levels; the same spirometers with Lilly type pneumotach sensors (Chest HI801, CHEST M.I., Inc., Tokyo) were used for testing at all locations. Except in 2 schools, pupils in the 6th grade were given an additional lung function test in February or March (i.e. a month before they moved on to junior high school): after having their height and weight measured, they were asked to perform a maximum forced expiratory maneuver. The tests were conducted by trained technicians following the testing protocol of the American Thoracic Society standards (26) modified for school-age children, in which

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no more than 6 blows are attempted, and FEV1, FVC, and maximal expiratory flow rate at 50% of FVC (V50) are determined from 3 satisfactory blows delivered by 2 pediatric pulmonologists.

A baseline questionnaire on asthmatic and allergic symptoms as classified in the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (27) was distributed to the participants' parents and returned on the day of the lung function testing. The questionnaire also contained questions on medical history, food allergies, exercise habits, exposure to environmental tobacco smoke (ETS), type of heating appliance used, and household pets in each household. A follow-up questionnaire was administered every time lung function testing was done.

Statistical analysis

The proxy measure of long-term exposure to $PM_{2.5}$ was defined as the arithmetic mean of the annual average of daily $PM_{2.5}$ concentrations over the study period at each school site. Most of the pupils lived within a 10-km radius of their school. Because O_3 and NO_2 are short-lived pollutants, their proxy measures were defined as the arithmetic mean of the annual average of hourly concentrations over the study period.

Of the participants, 834 underwent lung function testing 5 times, 358 were tested 4 times, 134 were tested 3 times, 89 were tested 2 times, and 51 were tested 1 time; all available data were utilized to construct a statistical model. It is known that this method is valid under the missing at random assumption (28). Lung function indicators were log-transformed in the following models because they were more linearly associated with height in both boys and girls in our preliminary analysis.

To assess the association between $PM_{2.5}$ concentrations and FVC, FEV1, and V50, the following multilevel linear regression model (29) was used for each sex:

$$\log Y_{cih} = a_{ci} + b_{ci}h + \gamma^T z_{ci} + e_{cih} \quad \text{(A)}$$

$$b_{ci} = B_c + e'_{ci} \quad \text{(B)}$$

$$B_c = \beta_0 + \beta_1 x_c + e''_c \quad \text{(C)}$$

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where c denotes school community, i denotes individual, h denotes height, Y_{cih} denotes the value of each lung function test, z_{ci} denotes confounding factors, x_c denotes the $PM_{2.5}$ concentration, and e_{cih}, e'_{ci}, e''_c denotes the independent random error terms following normal distributions. We note that unlike the model used in CHS (29), we used height instead of age in the first-stage model (A).

The first-stage model (A) was a linear regression of each lung function test (values were log-transformed) on height adjusting for confounders to assess community-specific associations between height and lung functions in both sexes. We assumed the association was linear after using fractional polynomials with 1 degree of freedom to check model fitting (30). We used the following confounders determined a priori in the main analysis: wheezing (yes/no) defined as the presence of wheezing or whistling in the chest more than once in the previous 12 months, sneezing (yes/no), itchy rash (yes/no) defined as having had at least 1 rash in the previous 6 months, exposure to ETS at home (yes/no) defined as either parent smoking in the same room as the child, household pet ownership (yes/no) defined as having a dog or a cat over the previous 12 months, habitual exercise (none/once or twice a week/more than twice a week) defined as doing vigorous exercise, and the Rohrer index (weight in kg divided by cubed height [m^3] multiplied by 10) used as an anthropometric index.

The second-stage model (B) was a linear regression of community and individual specific estimates of lung function growth per 1-unit increase of height on communities. The third-stage model (C) was a linear regression of community-specific average growth on the $PM_{2.5}$ concentrations.

The main parameter of interest in the above model was the effect of 10-μg/m^3 increase in $PM_{2.5}$ on the relative growth of lung function per 10-cm increase in height, $\exp(\beta_1 \times 10^2)$. We calculated estimates and 95% confidence intervals, and the p-values of $\exp(\beta_1 \times 10^2)$.

As sensitivity/additional analyses, we also fitted the following models: (1) a 2-pollutant model with O_3 or NO_2 in addition to $PM_{2.5}$ in the third-stage model; (2) a model with additional covariates as potential confounders, namely ETS, household pet ownership, and indoor heating modality; (3) a subgroup (stratified) analysis to assess the effect modification by asthma and ETS; and (4) models excluding obese children with a Rohrer index greater than 160 kg/m^3 as extreme obesity could affect lung function testing. Furthermore, to ensure that inclusion of the later stage participants did not distort

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the study results, additional analysis was done by excluding those who participated in the latter half of the study (N=20).

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Results

Table 1 shows the baseline characteristics of the participants in the first-year survey (N=1,307). The mean age and height of the 3rd- and 4th-grade children were 9.05 years and 130.83 cm, and 9.96 years and 137.4, cm respectively.

Exposure estimates for the major pollutants are shown in Table 2, Figure 2 and sTable 1. The overall annual mean concentration of $PM_{2.5}$ over the study period was 13.5 μg/m^3 (range: 10.4 μg/m^3–19.0 μg/m^3). Annual mean $PM_{2.5}$ concentrations at 8 schools with 568 participants were below 12 μg/m^3, while those at 7 schools with 546 participants were above 15 μg/m^3.

In the boys, the relative effect of 10-μg/m^3 increase in $PM_{2.5}$ on the relative growth of lung function per 10-cm increase in height for FEV1, FVC and V50 was 1.00054 (95% CI: 0.99969–0.139%), 1.00038 (95% CI: 0.99958–1.00118), and 1.00071 (95% CI: 0.99900–1.00242); in the girls, it was 1.00012 (95% CI: 0.99916–1.00109), 1.00004 (95% CI: 0.99899–1.00108) and 0.99989 (95% CI: 0.99813–1.00165). These results indicate no associations in either sex between $PM_{2.5}$ exposure and relative growth in lung function, even after controlling for potential confounders (Table 3 and Fig. 3). These null results were not affected by analysis of our 2-pollutant models with O_3 or NO_2 (Table 3). Nor did sensitivity analyses change the results, including fully adjusted modeling for ETS, household pet ownership, use of indoor heating appliances, and sneezing; nor did stratified analysis for asthmatic and non-asthmatic participants, stratified analysis with or without ETS, or exclusion of extremely obese participants or later stage participants (data not shown).

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Discussion

This longitudinal analysis of the effects of $PM_{2.5}$ on lung function growth in pre-adolescent boys and pre- to early-adolescent girls aged 8-12 years involved 6,233 measurements in 1,466 participants, but did not support clear evidence of an association between $PM_{2.5}$ and relative growth of lung function measured by FEV1, FVC and V50 per 10cm growth of height both in male participants with pre-adolescent age and in female participants with pre- to early-adolescent age. Mean $PM_{2.5}$ concentrations over the study period ranged from 10 $\mu g/m^3$ to 19 $\mu g/m^3$ in the school communities studied. Our results were consistent even after control for the major co-pollutants NO_2 or O_3, and for additional potential confounders at the individual level. Nor were any effect modifications observed in association with the participants' asthmatic status or ETS at home.

Previous reports from the CHS have indicated significant associations between $PM_{2.5}$ and lung function growth, but the results were based on $PM_{2.5}$ concentrations as high as around 30 $\mu g/m^3$. In the 4th-grade participants of the 1993 cohort (10 years of age), no significant reductions in lung function were observed in association with $PM_{2.5}$ levels during a 4-year follow-up (4), but an extended follow-up until the participants were 18 years old indicated a significant difference in average growth as measured by FEV1 over the 8-year period between those from the most and least polluted communities (which had average annual $PM_{2.5}$ levels of 30 and 5 $\mu g/m^3$, respectively) (6). Further CHS data show improving 4-year growth as measured by FEV1 and FVC (aged 11-15) in line with improving $PM_{2.5}$ levels across the 3 cohorts: the 3-year mean $PM_{2.5}$ in the 5 participating communities ranged from 21.3 to 31.5 $\mu g/m^3$ in the 1994-1998 cohort, from 19.9 to 27.6 $\mu g/m^3$ in the 1997-2001 cohort, and from 11.9 to 17.8 $\mu g/m^3$ in the 2007-2011 cohort (10). This strongly suggests that exposure to $PM_{2.5}$ at annual average concentrations exceeding 20 $\mu g/m^3$ reduces lung function growth in children between the ages of 10 and 18 years. However, it remains unclear from the CHS whether long-term exposure to $PM_{2.5}$ at levels below 20 $\mu g/m^3$ causes reduced lung function growth in pre-adolescent children.

Birth cohort studies have provided useful data on lung function growth, as they included participants of pre-adolescent age living in places with relatively low annual $PM_{2.5}$ levels. The PIAMA birth cohort

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study, for example, provided a longitudinal analysis of lung function tests carried out on 555 participants at the ages of 8 and 12 years, which indicated no significant association between growth in FEV1 and FVC and $PM_{2.5}$ at birth or during follow-up (31). This supported our results, but further analysis of 915 participants who attended at least 2 lung function tests at ages 8, 12 and 16 indicated that exposure to $PM_{2.5}$ at age 0-4 years resulted in reduced FEV1 growth from ages 8 to 16 with an adjusted difference of -0.26% per 1.2-$\mu g/m^3$ increase in $PM_{2.5}$. However, the only exposure variable was $PM_{2.5}$ concentrations near the time of the participants' birth, and no analysis was reported using exposure levels at the age of 4 years or later, or concurrent exposure from ages 8 to 16 (18). $PM_{2.5}$ concentrations in the PIAMA study were estimated from a land-use regression model extrapolated back from monitoring data collected between 2008 and 2010; the median (range of) $PM_{2.5}$ used in the further analysis were 16.4 (15.2-19.4) $\mu g/m^3$ for participants in the 0-4 age range, 16.4 (14.9-19.4) $\mu g/m^3$ for those aged 5-12, and 16.4 (14.9-18.6) $\mu g/m^3$ for those aged 13-16. In the BAMSE birth cohort study, no association was observed in 2,278 participants between longitudinal changes in FEV1 and FVC from the age of 8 to 16 and exposure to PM_{10} and NOx at the age of 0-1, 1-8 or 8-16 (17). Thus, the results are mixed when it comes to the effects of long-term exposure to $PM_{2.5}$ in the 10 to 19 $\mu g/m^3$ range on lung function growth in pre-adolescence to early adolescence.

Mechanistic understanding of what level of $PM_{2.5}$ during the childhood may or may not influence lung growth trajectories and other lung health outcomes is also of value to be elucidated through pathophysiological or molecular approaches. Although it remains speculative, airway dysanapysis, a physiological incongruence between the growth of the lung parenchyma and the caliber of the airway (32) (33) (34) , or epigenetic modification assessed by DNA methylation (23) (35, 36) may mediate the effects of $PM_{2.5}$ on lung growth during pre-adolescent and adolescent period.

One important question is whether a phase of lung function development during adolescent growth spurt is a critical time period vulnerable to air pollution exposure. Longitudinal analysis in a population-based British birth cohort study indicated that pubertal age defined by median age (IQR) at peak higher velocity of height growth was 13.5 (13.0-13.9) years for males and 11.7 (11.2-12.1) years for females, respectively (37). The study also showed that effect of pubertal growth was

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manifested by an increase in both FEV1 and FVC, indicating the importance of lung function growth in the pre-adolescent to adolescent age range. Extended follow-up of our cohort until adolescent age is expected to add to the epidemiological evidence of non-linear growth period of height and lung at relatively low levels of annual $PM_{2.5}$ concentration. In the ESCAPE project, random-effects meta-analysis combining lung function data on 5 European birth cohorts at the ages of 6 and 8 showed a small but statistically significant decrease in FEV1 per 5-μg/m^3 increase in estimated $PM_{2.5}$ levels in a participant's local area ($PM_{2.5}$ range: 7.4 to 17.3 μg/m^3), but no association was observed when the $PM_{2.5}$ at birth was applied (14). In a US birth cohort study, a 2-μg/m^3 increase in $PM_{2.5}$ 1 year prior to lung function testing at age 7 was also shown to result in a significant decrease in FEV1 ($PM_{2.5}$ range: 4.08 to 16.23 μg/m^3). However, neither $PM_{2.5}$ levels when participants were aged 0-1 nor average levels throughout their lifetime were associated with reductions in FEV1 (16). In contrast, the PIAMA birth cohort study indicated that exposure to $PM_{2.5}$ in early life (from birth to the age of 4) resulted in reduced FEV1 growth from ages 8 to 16 (median $PM_{2.5}$: 16.4 μg/m^3) (18). Also, in the Oslo birth cohort study, peak expiratory flow, forced expiratory flow at 25% and 50% of FVC measured in participants aged 9-10 years were significantly associated with exposure to $PM_{2.5}$, PM_{10} and NO_2 in the 1st year of life or throughout their lifetime (mean $PM_{2.5}$: 16.4 at age 0-1, and 14.5 μg/m^3 for lifetime), although no associations were observed for FEV1 or FVC (13). Relatively high levels of $PM_{2.5}$ in early life might relate to such effect. In our cohort setting, air pollutant levels before the study period were not directly measured, but historical data on SPM, O_3, and NO_2 levels were collected from the monitoring station closest to each study school. These showed steadily decreasing levels of SPM and NO_2, and increasing levels of O_3 over the years, although the degree of change varied from area to area (sTable2). For $PM_{2.5}$, annual $PM_{2.5}$ concentrations in Japan had downward trend since 2009 when the national air quality standard was introduced, and it had been reported as 20-25 μg/m^3 in a large metropolitan area such as Tokyo and Fukuoka in 2005 from the survey data by the Ministry of the Environment (24).

On the multilevel regression model used in this study, we used height rather than age as a factor to describe children's overall growth. We believe this is reasonable because lung function is expected

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to grow with the child's height. In fact, in our preliminary data analysis, height was more strongly associated with the results of each lung function test than age (data not shown). Furthermore, we also conducted an additional unplanned analysis using age instead of height in the model (A). The results were essentially the same as in our main analysis (sTable 3).

There are several strengths and limitations with this study. First, we secured a representative sample of the Japanese population with a wide range of $PM_{2.5}$ by closely following up the participants and maintaining a high participation rate. More than 95% of the participants underwent lung function tests at least twice, 81.3% were tested 4 or 5 times. Only 3.5% was moved out after single testing. The relatively large sample size (6,233 measurements in 1,466 participants) reduced the risk of random errors. Use of pollutant concentrations monitored at each school site during the whole study period is another strength, although school-based community level concentration instead of individual level could lead to increased chance of getting null results due to random misclassification of exposure. Lack of at-home or indoor measurement also could contribute to random misclassification of exposure. Lung function testing is not easy to perform on children, especially young ones, but all testing was carried out by trained technicians following a standardized protocol. The tests were strictly scheduled throughout the study in the morning hours during the same season every year, and they were all performed with the same spirometers equipped with Lilly type pneumotach sensors to reduce the possibility of measurement errors. Unmeasured confounding factors may distort the study results naturally although known, major ones were already considered in the analysis. The result of this study could be implicative to examine the adequacy of air quality standard of $PM_{2.5}$ although generalizability issue must be taken into account for applying our study results to a different population due to differences in ethnicity or composition of $PM_{2.5}$. Range of exposure in this study is equivalent to annual air quality standard levels of various countries including US (12 $\mu g/m^3$), Japan (15 $\mu g/m^3$), and the EU (20 $\mu g/m^3$ 3-yr Average Exposure Indicator goals).

In conclusion, our nation-wide large longitudinal cohort study of pre-adolescent males and pre- to early adolescent females in Japan with repeated measurements of lung function suggests that concurrent, exposure to $PM_{2.5}$ in the range of 10.4 -19.0 $\mu g/m^3$ during pre-adolescent and early

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adolescent period has little effect on lung function growth. Further studies with extended follow-up of this population until pubertal age is needed to elucidate the effects of $PM_{2.5}$ exposure on lung function growth.

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Figure legends

Figure 1. Study sites

Figure 2. Annual average concentrations of PM_{2.5} (left), ozone (center) and NO₂ (right) by year during the study period (μg/m³)

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Figure 3. Relative growth of lung function indicators (FEV1, FVC and V50) per 10cm growth of height by sex

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Table 1. Participant characteristics at the time of the first survey

School	City	Prefecture	# of participants at baseline	Male (%)	Age in years (SD)	Height in cm (SD)	Rohrer index (SD)	ETS (%)	Pets (%)	Wheezing (%)	Sneezing (%)	Itchy rash (%)	Exercise (%)
A	Sapporo	Hokkaido	138	74 (53.6)	9.15 (0.61)	132.0 (6.2)	121.8 (14.9)	21 (15.2)	20 (14.5)	20 (14.5)	75 (54.3)	35 (25.4)	20 (14.5)
B	Sendai	Miyagi	109	50 (45.9)	8.95 (0.58)	131.2 (6.5)	122.6 (14.7)	12 (11.0)	8 (7.3)	14 (12.8)	59 (54.1)	28 (25.7)	17 (15.6)
B' [a)]			92	49 (53.3)	9.96 (0.60)	137.4 (7.4)	121.8 (14.2)	8 (8.7)	6 (6.5)	11 (12.0)	51 (55.4)	20 (21.7)	11 (12.0)
C	Sakata	Yamagata	42	21 (50.0)	9.12 (0.54)	131.8 (5.8)	128.6 (20.1)	9 (21.4)	5 (11.9)	4 (9.5)	17 (40.5)	7 (16.7)	10 (23.8)
D			37	23 (62.2)	9.01 (0.46)	130.2 (6.4)	127.8 (17.0)	6 (16.2)	0 (0)	3 (8.1)	14 (37.8)	3 (8.1)	12 (32.4)
E			58	33 (56.9)	9.10 (0.42)	132.1 (6.3)	125.6 (15.6)	16 (27.6)	9 (15.5)	3 (5.2)	22 (37.9)	6 (10.3)	21 (36.2)
F			14	8 (57.1)	9.07 (0.39)	134.3 (6.0)	121.8 (14.2)	3 (21.4)	3 (21.4)	0 (0)	8 (57.1)	5 (35.7)	6 (42.9)
G	Itabashi	Tokyo	91	52 (57.1)	9.01 (0.67)	129.1 (5.8)	123.4 (14.5)	27 (29.7)	10 (11.0)	6 (6.6)	46 (50.5)	23 (25.3)	17 (18.7)
H			35	17 (48.6)	8.92 (0.66)	129.8 (8.1)	126.0 (14.4)	8 (22.9)	7 (20.0)	6 (17.1)	19 (54.3)	11 (31.4)	5 (14.3)
I	Shibuya		37	17 (45.9)	9.05 (0.89)	131.7 (7.3)	122.4 (14.9)	3 (8.1)	6 (16.2)	3 (8.1)	22 (59.5)	6 (16.2)	4 (10.8)
J			56	26 (46.4)	9.14 (0.68)	132.1 (6.0)	119.4 (14.7)	12 (21.4)	7 (12.5)	8 (14.3)	30 (53.6)	13 (23.2)	8 (14.3)
K	Nagano	Nagano	81	45 (55.6)	9.22 (0.62)	131.5 (7.0)	123.7 (16.7)	9 (11.1)	10 (12.3)	10 (12.3)	42 (51.9)	20 (24.7)	14 (17.3)
L	Matsumoto		78	29 (37.2)	9.36 (0.69)	132.4 (6.8)	120.4 (13.3)	13 (16.7)	12 (15.4)	5 (6.4)	36 (46.2)	18 (23.1)	12 (15.4)
M	Okayama	Okayama	101	50 (49.5)	8.61 (0.36)	128.3 (5.4)	124.9 (12.7)	8 (7.9)	17 (16.8)	7 (6.9)	36 (35.6)	11 (10.9)	9 (8.9)
N	Fukuoka	Fukuoka	82	35 (42.7)	8.98 (0.66)	131.2 (6.9)	120.5 (14.5)	21 (25.6)	11 (13.4)	8 (9.8)	40 (48.8)	20 (24.4)	7 (8.5)
O			144	75 (52.1)	9.04 (0.76)	129.8 (7.3)	125.6 (16.3)	39 (27.1)	19 (13.2)	12 (8.3)	69 (47.9)	30 (20.8)	15 (10.4)
P	Amami	Kagoshima	112	63 (56.3)	9.17 (0.80)	130.5 (7.3)	129.0 (17.1)	37 (33.0)	13 (11.6)	23 (20.5)	52 (46.4)	19 (17.0)	12 (10.7)

a): B indicates 3rd-grade students and B' indicates 4th-grade students, as both 3rd- and 4th-grade children were enrolled in 2012.

ETS: exposure to environmental tobacco smoke at home; Pets: household pet ownership over the previous 12 months; wheezing, sneezing, and itchy rash are defined according to the Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire; Exercise: habitual exercise 3 times a week or more

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Table. 2 4-year average concentrations of $PM_{2.5}$ (left), ozone (center) and NO_2 (right) during the study period

City	**School**	**4-year average concentration**		
		$PM_{2.5}$ ($\mu g/m^3$)	**Ozone (ppb)**	**NO_2 (ppb)**
Sapporo	**A**	10.4	30	11
Sendai	**B**	10.8	32	11
	B'	10.8	32	11
Sakata	**C**	11	36	3
	D	10.5	38	5
	E	11.4	38	3
	F	10.6	36	3
Itabashi	**G**	16.4	27	21
Shibuya	**H**	16.5	26	20
	I	15.4	29	20
	J	16.2	26	23
Nagano	**K**	12.9	29	9
Matsumoto	**L**	11.4	30	9
Okayama	**M**	16.4	32	11
Fukuoka	**N**	19	31	15
	O	16.5	33	10
Amami	**P**	12.5	35	3

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Table 3. Effect of 10 μg/m^3 increase in $PM_{2.5}$ on relative growth of lung function indicators (FEV1, FVC and V50) per 10cm growth of height: stratified analysis by sex.

Sex	Outcome	Adjusted estimate of $\exp(\beta_1 \times 10^2)$*	95% CI		p-value
			Lower	Upper	
Single pollutant model					
Male	FVC	1.00038	0.99958	1.00118	0.351
	FEV1	1.00054	0.99969	1.00139	0.211
	V50	1.00071	0.99900	1.00242	0.417
Female	FVC	1.00004	0.99899	1.00108	0.944
	FEV1	1.00012	0.99916	1.00109	0.803
	V50	0.99989	0.99813	1.00165	0.905
2-pollutant model					
+ O_3					
Male	FVC	1.00005	0.99997	1.00014	0.230
	FEV1	1.00007	0.99997	1.00016	0.166
	V50	1.00006	0.99986	1.00025	0.570
Female	FVC	1.00005	0.99995	1.00015	0.364
	FEV1	1.00004	0.99994	1.00014	0.432
	V50	1.00000	0.99980	1.00020	0.997
+ NO_2					

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Male	FVC	1.00004	0.99994	1.00014	0.392
	FEV1	1.00006	0.99995	1.00017	0.279
	V50	1.00006	0.99984	1.00027	0.604
Female	FVC	1.00008	0.99997	1.00019	0.162
	FEV1	1.00007	0.99996	1.00018	0.207
	V50	1.00003	0.99981	1.00026	0.765

*adjusted for Rohrer index, allergic symptoms (wheezing, itchy rash), habitual exercise, exposure to environmental tobacco smoke at home

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Figure 1. Study sites

245x275mm (118 x 118 DPI)

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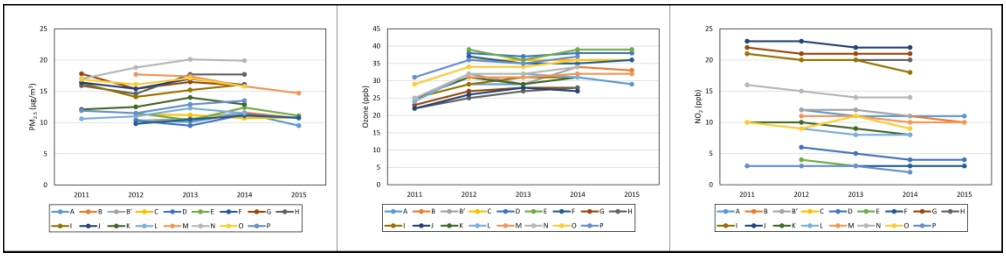

Figure 2. Annual average concentrations of $PM_{2.5}$ (left), ozone (center) and NO_2 (right) by year during the study period ($\mu g/m^3$)

3242x808mm (47 x 47 DPI)

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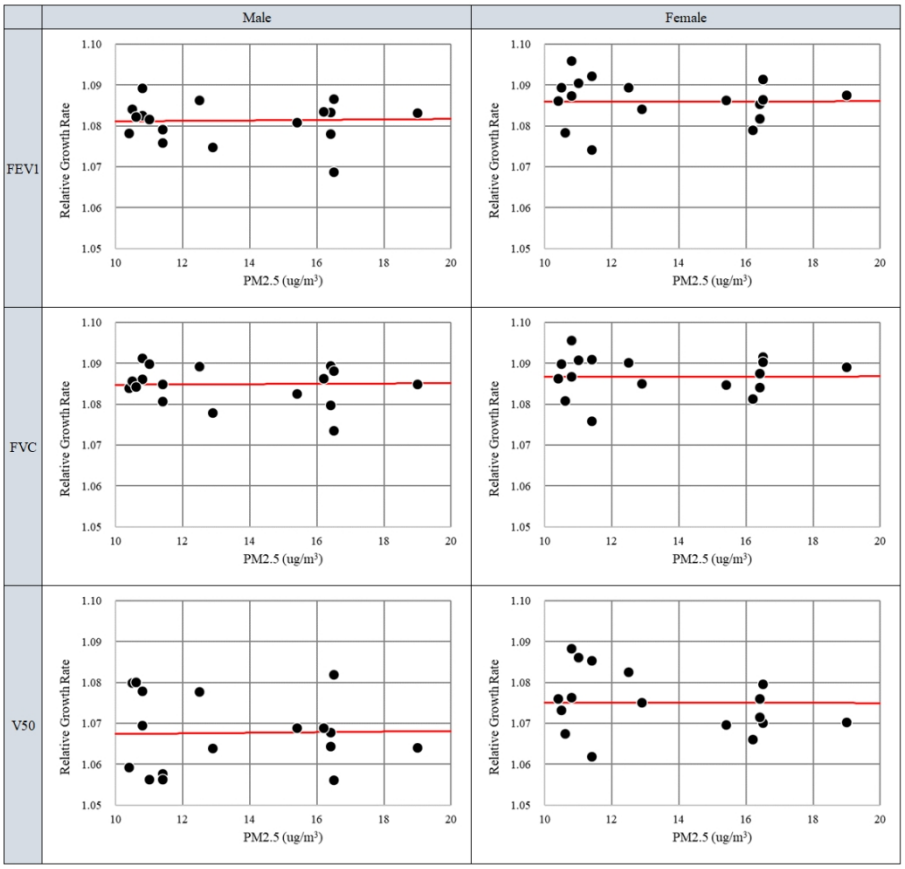

Figure 3. Relative growth of lung function indicators (FEV1, FVC and V50) per 10cm growth of height by sex

682x618mm (47 x 47 DPI)

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Title

Exposure to PM2.5 and Lung Function Growth in Pre- and Early Adolescent Schoolchildren

A Longitudinal Study Involving Repeated Lung Function Measurements in Japan

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sTable 1. Annual average concentrations of $PM_{2.5}$ (figures in the upper line for each location), ozone (middle line) and NO_2 (lower line) by year during the study period (μg/m³ for $PM_{2.5}$; ppb for ozone and NO_2)

School	City	**2011**	**2012**	**2013**	**2014**	**2015**	**Average**
			10.4	10.1	11.5	9.5	10.4
A	Sapporo	-	29	29	31	29	30
			12	11	11	11	11
			10.3	10.6	11.6	10.7	10.8
B		-	32	29	34	33	32
	Sendai		12	12	11	10	11
			10.3	10.6	11.6		10.8
B’		-	32	29	34	-	32
			12	12	11		11
			11.3	11.2	10.7	10.8	11.0
C		-	36	36	36	36	36
			4	3	3	3	3
			10.3	9.5	11.3	10.7	10.5
D		-	38	37	38	38	38
			6	5	4	4	5
	Sakata		11.5	10.4	12.4	11.1	11.4
E		-	39	36	39	39	38
			4	3	3	3	3
			9.8	10.5	11.1	10.8	10.6
F		-	37	35	35	36	36
			3	3	3	3	3
		17.8	15.4	16.5	15.9		16.4
G	Itabashi	23	27	28	28	-	27
		22	21	21	21		21
		15.9	14.6	17.7	17.7		16.5
H		22	25	27	28	-	26
		21	20	20	20		20
		16.3	14.1	15.2	16.1		15.4
I	Shibuya	25	29	31	31	-	29
		21	20	20	18		20
		16.4	15.4	17.0	16.0		16.2
J		22	26	28	27	-	26
		23	23	22	22		23
		12.1	12.5	14.0	12.9		12.9
K	Nagano	25	31	29	31	-	29
		10	10	9	8		9
		10.6	11.0	12.3	11.5		11.4
L	Matsumoto	24	32	32	31	-	30
		10	9	8	8		9
			17.7	17.4	15.8	14.7	16.4
M	Okayama	-	31	31	32	32	32
			11	11	10	10	11
		17.0	18.8	20.1	19.9		19.0
N		25	32	32	34	-	31
	Fukuoka	16	15	14	14		15
		16.9	16.1	17.1	15.8		16.5
O		29	34	34	36	-	33
		10	9	11	9		10
P	Amami	11.9	11.5	12.9	13.5	-	12.5

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	31 3	36 3	35 3	37 2	35 3

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sTable 2. Concentration distributions (5-year means and range of yearly means) of suspended particulate matter (SPM, μg/m^3), ozone (O_3, ppb) and nitrogen oxide (NO_2, ppb) before the study period, as measured at the monitoring station closest to each study site

School	City	**pollutant**	**2001-2005**	**2006-2010**	**2011-2015**
A	Sapporo	SPM	16 (14 – 18)	14 (13 - 16)	12 (10 - 14)
		O_3	25 (23 - 27)	29 (26 - 31)	28 (26 - 29)
		NO_2	20 (18 - 21)	15 (13 - 18)	13 (11 - 14)
B, B’	Sendai	SPM	20 (12 - 23)	19 (16 - 21)	13 (12 - 14)
		O_3	27 (24 - 30)	32 (31 - 33)	31 (29 - 35)
		NO_2	17 (15 - 18)	13 (11 - 15)	10 (9 - 11)
C – F	Sakata	SPM	17 (16 - 19)	15 (14 - 15)	13 (13 - 14)
		O_3	37 (33 - 39)	36 (32 - 39)	36 (33 - 42)
		NO_2	9 (8 - 9)	6 (5 - 7)	4 (3 - 6)
G	Itabashi	SPM	32 (30 - 34)	25 (22 - 30)	22 (20 - 23)
		O_3	29 (27 - 30)	30 (29 - 33)	31 (29 - 32)
		NO_2	29 (27 - 30)	24 (22 - 27)	19 (19 - 21)
H – J	Shibuya	SPM	41 (34 - 48)	31 (27 - 32)	23 (21 - 26)
		O_3	26 (24 - 28)	29 (27 - 31)	31 (28 - 35)
		NO_2	29 (27 - 31)	24 (22 - 27)	19 (18 - 20)
K	Nagano	SPM	21 (19 - 23)	16 (14 - 18)	15 (14 - 16)
		O_3	34 (31 - 37)	34 (32 - 36)	35 (33 - 37)
		NO_2	11 (9 - 12)	8 (7 - 10)	6 (6 - 7)
L	Matsumoto	SPM	21 (19 - 23)	17 (15 - 20)	16 (15 - 17)
		O_3	28 (25 - 31)	33 (30 - 35)	31 (27 - 32)
		NO_2	15 (14 - 17)	11 (8 - 13)	9 (9 - 10)
M	Okayama	SPM	31 (29 - 35)	26 (19 - 31)	18 (17 - 19)
		O_3	27 (6 - 28)	30 (7 - 33)	29 (27 - 31)
		NO_2	16 (16 - 17)	12 (11 - 14)	9 (8 - 11)
N	Fukuoka	SPM	29 (26 - 38)	34 (31 - 37)	26 (24 - 29)
		O_3	26 (24 - 27)	30 (27 - 32)	30 (26 - 32)
		NO_2	25 (23 - 27)	21 (20 - 23)	17 (15 - 19)
O		SPM	36 (34 - 38)	28 (16 - 37)	17 (16 - 18)
		O_3	30 (30 - 31)	34 (27 - 38)	32 (30 - 35)
		NO_2	19 (17 - 21)	14 (10 - 17)	12 (12 - 13)
P	Amami	SPM	34 (33 - 37)	43 (40 - 48)	21 (20 - 22)
		O_3	23 (20 - 27)	23 (20 - 31)	27 (24 - 30)
		NO_2	13 (12 - 15)	10 (4 - 13)	6 (3 - 8)

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sTable 3. Effect of 10 μg/m^3 increase in $PM_{2.5}$ on relative growth of lung function indicators (FEV1, FVC and V50) per 1year growth of age: stratified analysis by sex.

Sex	Outcome	Adjusted estimate of exp(β_1×10)*	95% CI		p-value
			Lower	Upper	
Single pollutant model					
Male	FVC	0.99954	0.99731	1.00177	0.6833
	FEV1	0.99994	0.99751	1.00238	0.9631
	V50	1.00017	0.99682	1.00352	0.9221
Female	FVC	0.99917	0.99763	1.00071	0.2926
	FEV1	0.99904	0.99751	1.00058	0.2211
	V50	0.99883	0.99667	1.00100	0.2901

*adjusted for Rohrer index, allergic symptoms (wheezing, itchy rash), habitual exercise, exposure to environmental tobacco smoke at home

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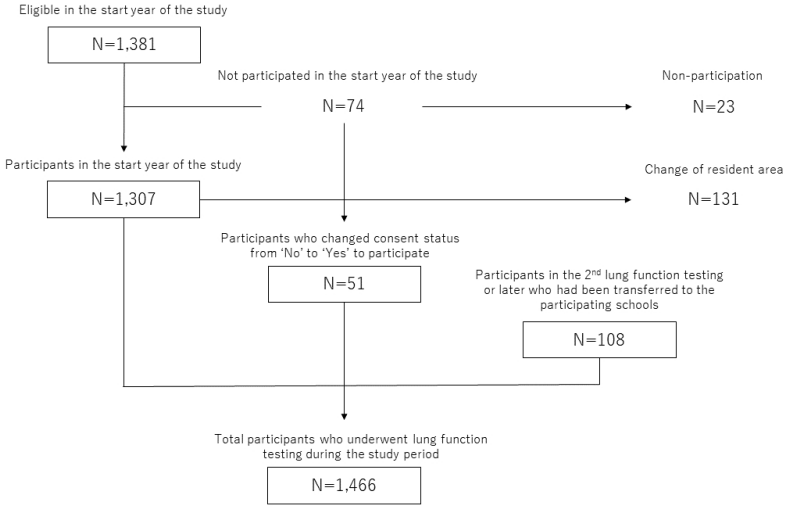

sFigure 1. A flow diagram of the study participants

855x481mm (38 x 38 DPI)

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