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Schoolchildren's antioxidation genotypes are susceptible factors for reduced lung function and airway inflammation caused by air pollution

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ABSTRACT

Background: We recently reported the relationship between exposure to ambient air pollutants and changes in lung function and nasal inflammation among schoolchildren. A study was conducted to investigate whether antioxidation genotypes influence these associations.
Methods: A follow-up study of 97 schoolchildren was conducted in New Taipei City, Taiwan. A structured respiratory health questionnaire was administered in September 2007, followed by monthly spirometry and measurement of nasal inflammation from October 2007 to November 2009. During the study period, complete daily monitoring data for air pollutants were obtained from the Environmental Protection Administration monitoring station and Aerosol Supersite. The genotypes of glutathione S-transferase (GST) subunits M1, T1, P1 and superoxide dismutases subunit 2 (SOD2) were characterized. Mixed-effects models were used, adjusting for known confounders.
Result: GSTM1 null children had significant $PM_{2.5}$-related increment in leukocyte (8.52%; 95% confidence interval (CI): 3.13–13.92%) and neutrophil (9.68%; 95% CI: 4.51–14.85%) in nasal lavage. Ozone levels were significantly and inversely associated with forced expiratory flow at 25% of forced vital capacity ($FEF_{25\%}$) (−0.43 L/s; 95% CI: −0.58, −0.28 L/s) in SOD2 Ala16 variant children.
Conclusion: In this longitudinal study of schoolchildren. Our data provide evidence that antioxidation genotype modifies the airway inflammation caused by $PM_{2.5}$. Antioxidation genotype also acts as an effect modifier, but not strong, in ozone-related small airway function response.

1. Introduction

Exposure to ambient air pollutants was associated with adverse airway cellular effects (Behndig et al., 2006), increase in respiratory symptoms (McConnell et al., 2003; Vichit-Vadakan et al., 2001), and decrease in lung function (Barraza-Villarreal et al., 2008; Delfino et al., 2008; Gotschi et al., 2008; Liu et al., 2009). We recently reported that schoolchildren had significant particulate matter with an aerodynamic diameter of 2.5 μm or less ($PM_{2.5}$)-related increments in the nasal inflammation (Chen et al., 2012), $PM_{2.5}$-related decrement in forced vital capacity (Chen et al., 2011), and ozone-related decrements in small airway functions (Chen et al., 2011).

Genetic factors probably contributed to the substantial variability between individuals with adverse respiratory outcomes following exposure to air pollutants (Romieu et al., 2002; Yang et al., 2008). However, the genes involved in using reactive oxygen/nitrogen species delivered with ambient air pollutants with strong oxidative potential and their associations are not well characterized in epidemiologic studies. The glutathione S-transferases (GSTs) are a superfamily of phase II enzymes that conjugate reactive intermediates with glutathione to produce less reactive water-soluble compound that are readily excreted. The most commonly studied genes involved in the antioxidant pathway are GSTM1, GSTT1, and GSTP1 (Hayes and Strange, 2000; Koyama and

Abbreviations: $PM_{2.5}$, particulate matter of aerodynamic smaller than 2.5 μm; GSTM1, glutathione S-transferase M1; GSTT1, glutathione S-transferase T1; GSTP1, glutathione S-transferase P1; SOD2, superoxide dismutases 2; EPA, Environmental Protection Administration; FVC, forced expiratory vital capacity; FEV_1, forced expiratory volume in 1 s; $FEF_{25\%}$, forced expiratory flow at 25% of FVC; $FEF_{50\%}$, forced expiratory flow at 50% of FVC; $FEF_{75\%}$, forced expiratory flow at 75% of FVC; $FEF_{25-75\%}$, average expiratory flow over the middle half of FVC

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Geddes, 1998; Mates et al., 1999; Onaran et al., 2001; Schunemann et al., 1997). In addition, the family of superoxide dismutases (SOD) is the only enzymatic system able to degrade superoxide anion into hydrogen peroxide (Fridovich, 1997). Despite their important roles, genes of these enzymes have not yet been widely studied.

We conducted a longitudinal study to investigate whether antioxidation genotypes might influence the air pollutants-related changes in nasal inflammation and lung function among schoolchildren.

2. Material and methods

2.1. Study design

A follow-up study of elementary and middle school students was conducted between October 2007 and November 2009. The children were enrolled in September 2007 after completing an initial questionnaire. The measurements of lung function and nasal inflammation were performed monthly during the study period. The study was approved by the Institutional Review Board of the National Taiwan University Medical Center (NTUH-REC No.200703055R).

2.2. Study subjects

The participants of this study have been reported in detail previously (Chen et al., 2012). In brief, a modified and validated Chinese version of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire, including information on children respiratory health history, was answered by the parents of schoolchildren. A total of 133, 1059, and 2745 children were grouped as having current asthma, having current allergic rhinitis but not asthma, and healthy controls, respectively. Children were randomly selected from each group to participate in a longitudinal follow up to achieve approximately one-third in each atopy stratum. Among the selected children, 33 current asthmatics, 30 current allergic rhinitis, and 37 healthy children voluntarily participated. Informed consent was obtained from each participant's parents.

2.3. Exposure assessment

This study was conducted in children' schools located within a 2.5-km radius of the Taiwan Environmental Protection Administration (EPA) monitoring station and the Aerosol Supersite in Sinjhuang District, New Taipei City, Taiwan. The latter was established to continuously monitor environmental particulate matter. The monitoring data of PM_{10} and $PM_{2.5}$ were obtained from the Aerosol Supersite. Levels of $PM_{2.5–10}$ were derived by subtracting the $PM_{2.5}$ level from the PM_{10} level. Data for ozone, carbon monoxide, nitrogen dioxide, and sulfur dioxide were obtained from the EPA monitoring station. The concentrations of air pollutants were measured continuously and reported hourly. The 8-h moving average was calculated for ozone. For all other air pollutants, the 24-h mean was calculated. Personal air pollutant exposure was assumed to follow the daily data as described above.

2.4. Genotyping

Cotton swabs containing oral mucosa were collected at the beginning of the follow-up study in October 2007. Genomic DNA was extracted using standard genomic DNA extraction methods. The genotypes of GSTM1 and GSTT1 were characterized using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). GSTP1 (Ile105Val, rs1695) and SOD2 (Val16Ala, rs4880) were assayed by TaqMan real-time PCR technology (Applied Biosystems, Foster City, California) according to manufacturer's instructions.

2.5. Outcome measurements

Trained interviewers visited the children's schools and conducted spirometry and nasal lavage. The measurements were evaluated on the last Wednesday, Thursday, and Friday of every month during the follow-up period.

2.5.1. Spirometry

Each child was tested with a spirometer while standing (Chestgraph HI-101; CHEST MI, Tokyo, Japan), according to the standardization of spirometry of the American Thoracic Society (Miller et al., 2005). Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), forced expiratory flow at 25%, 50%, and 75% of FVC ($FEF_{25\%}$, $FEF_{50\%}$, and $FEF_{75\%}$), and average expiratory flow over the middle half of FVC ($FEF_{25–75\%}$) were recorded. Quality control consisted of a 3-L syringe calibration and a leak test before each test day.

2.5.2. Nasal lavage

10 mL of warm (37 °C) phosphate-buffered saline was applied to the nostrils while the child was seated (Koren et al., 1990). Lavage fluid was centrifuged at $1{,}000 \times g$ for 10 min at 4 °C. The supernatants were aliquoted and stored at −20 °C. The cell pellet was resuspended in 200 lL of phosphate-buffered saline, and 2 cytospin slides were prepared from each sample using a cytocentrifuge (Cytospin4; Thermo Shandon Ltd., Cheshire, United Kingdom).

2.5.3. Nasal cytology

Cytospin slides were stained with Liu's stain and were analyzed with a light microscope. The differential cell count of epithelial cells, neutrophils, eosinophils, and monocytes was performed on 100 cells on each of the 2 separate cytospins, and the average number of cells was calculated.

2.5.4. Cytokine and chemokine assessment

Levels of interleukin-4, interleukin-5, interleukin-8, interleukin-13, chemokine ligand 11, and interferon-gamma in nasal lavage were measured using an enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, Minnesota) according to the manufacturer's instructions. Results were calculated as the average of duplicates. When the values were under detection limits, one-third of the detection limits were used for data analysis.

2.6. Statistical method

A mixed-effect model was used to analyze the association between exposure to ambient air pollutant and changes in lung function and nasal inflammation. The exposure measurements were obtained on the day of and 1, 2, and 3 days before the outcome measurements (i.e., using 0-, 1-, 2-, 3-day lag assumptions). Confounding was evaluated as previously described (Chen et al., 2012, 2011). For power consideration, genes were grouped their carriers of the minor allele in analysis with dominant coding. Interaction terms of air pollutants and genotypes were added to the model to examine whether interaction existed. The genotype-stratified analysis would be used if the interaction existed. In addition, coefficients of each subgroup of genotypes were compared using t test (Romieu et al., 2004; Rosner, 2010). Analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).