의학석사 학위논문

Influence of Genetic Polymorphisms on the Association between Phthalate Exposure and Pulmonary Function in Elderly Koreans

국내 노령인구에서 프탈레이트 노출과 폐기능 간 관련성에 대한 유전자 다형성의 영향

2012 년 8 월

서울대학교 대학원

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이 논문을 의학석사 학위논문으로 제출함
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2012 년 8 월

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논문 제목: Influence of Genetic Polymorphisms on the Association between Phthalate Exposure and Pulmonary Function in Elderly Koreans

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ABSTRACT

Introduction: Phthalates are prevalent environmental exposure chemical that have raised concern regarding their various health effects, including pulmonary function. Meanwhile, elderly people are more susceptible to environmental exposure, and their decreasing lung function is an important health issue. The purpose of current study was to investigate the association between urinary phthalate metabolite levels and indices for pulmonary function and evaluate effect modification by genetic polymorphisms of oxidative-stress related genes, catalase (CAT), superoxide dismutase (SOD2), and myeloperoxidase (MPO) in elderly Koreans.

Methods: We conducted a panel study on 418 individuals over 60 years old in Seoul, Korea, and repeatedly measured urinary phthalate metabolite levels and ran pulmonary function tests. Genetic polymorphisms of CAT (rs769218 and rs769217), SOD2 (rs4880, rs2758331, and rs5746136) and MPO (rs2071409, rs7208693) were determined. Mixed effect model was used to investigate association of urinary phthalate metabolite levels with indices for pulmonary function and to examine the effect of CAT, SOD2 and MPO polymorphisms on the association.

Results: Inverse association was demonstrated between sum of mono-(2-ethyl-5-hydroxyhexyl) phthalate and mono-(2-ethyl-5-oxohexyl) phthalate (∑DEHP) levels and FEV1/FVC (P=0.0275) or FEF25-75 (P=0.002) after adjusting for age, number

of months past after each previous measurements, sex, body mass index, cotinine, mean temperature and dew point. The effect of ∑DEHP on lung function was significant only in subjects with GG genotype of CAT rs769218 and CC genotype of CAT rs769217, TT genotype of SOD2 rs4880 and CC genotype of SOD2 rs2758331, AA genotype of MPO rs2071409 and GG genotype of MPO rs7208693. Having all significant genotypes in three genes compared to other combinations showed significant difference in the phthalate-pulmonary function association (p-value for interaction: 0.0176 for ∑DEHP and FEV1/FVC, 0.0663 and FEF25-75).

Conclusions: In conclusion, we found urinary phthalate metabolites levels associated with decreasing pulmonary function in elderly Koreans, and suggested the effect modification of certain CAT, SOD2 and MPO polymorphisms on the phthalates-lung function association.

Keywords: phthalates, pulmonary function, genetic polymorphisms, elderly

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LIST OF ABBREVIATIONS

MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate

MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate

DEHP: di(2-ethylhexyl) phthalate

MnBP: mono-n-butyl-phthalate

DnBP: di-n-butyl phthalate

∑DEHP: sum of MEHHP and MEOHP

FEV_1: forced expiratory volume in 1 second

FVC: forced vital capacity

FEV_1/FVC: ratio of FEV1 to FVC

$FEF_{25–75}$: forced expiratory flow between 25 and 75% of FVC

CAT: catalase

SOD: superoxide dismutase

MPO: myeloperoxidase

INTRODUCTION

Phthalate use and exposure are ubiquitous in industrialized countries, as they are most commonly used plasticizers, primarily in polyvinyl chloride (PVC). They are found in a wide variety of products including vinyl upholstery, food containers and wrappers, toys, floor tiles, lubricants, sealers and adhesives. Beyond use in PVC resins, they are also found in cosmetics such as perfume, eye shadow, moisturizer, nail polish, hair spray, and liquid soap, and as an inert ingredient in pesticides [1]. Phthalates contribute 10-60% of plastic products by weight because of their ability to increase flexibility and transparency [2].

Although most manifestation on the effect of phthalates on human health are focused on reproductive system such as decreased sperm production, infertility, and harming sexual development in male infants [3], increasing but limited evidence for effect of phthalate exposure on respiratory health has demanded attention for further investigation on the possible association [4-9].

Elderly are identified as susceptible group to environmental exposure [10], and investigating association between commonly exposed chemicals, namely phthalates, on elderly population's health is important especially in countries like South Korea where elderly population is rapidly increasing. It has not been demonstrated whether environmental exposure to phthalates contribute to reduction of pulmonary function in elderly, but the few epidemiologic studies on phthalate exposure and lung function in other subpopulation provide grounds for possible

association.

Oxidative stress is defined as an impaired balance between free radical production and antioxidant capacity resulting in excess oxidative products. Studies have demonstrated oxidative stress attributable to exposure of common environmental hazards including phthalates [11-14] and also investigated on induction of oxidative stress leading to airway inflammation [15, 16]. The role of key antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD2), and myeloperoxidase (MPO) are important in the process of oxidative stress. Activities of such enzymes in acute lung injury or lung carcinogenesis have been suggested in previous reports [17, 18]. In addition, studies on genetic polymorphisms of oxidative stress markers with possible effect on pulmonary function are much recommended [19-22].

With given background information, further investigation for effect of environmental phthalate exposure on pulmonary function in elderly population is necessary, and identifying possible biological explanation is just as important. Therefore, we conducted repeated pulmonary function tests and analyzed urinary samples in Korean elderly to evaluate on the association between phthalates exposure and pulmonary function, and attempted to explore the effect of genetic polymorphisms of oxidative stress markers, *CAT*, *SOD2* and *MPO*, on the association.

MATERIALS AND METHODS

1. Study subjects and sampling

Five hundred and sixty participants over 60 years old and residing in Seongbuk-gu, Seoul, Republic of Korea were enrolled in the Korean Elderly Environmental Panel (KEEP) study. In this research, we focused on 418 subjects with available measurements on their phthalate metabolite and pulmonary function test from September 2008 until November 2009. Among them, 410 subjects with available data on *CAT*, *SOD2* and *MPO* polymorphisms were selected for genotype and haplotype analysis.

We conducted pulmonary function test and obtained urine samples during three medical examinations. All urine samples were placed at -20℃ immediately following collection and stored until analysis for the three biomarkers: mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-n-butyl-phthalate (MnBP). Also, we used a structured questionnaire at initial examination, and obtained detailed information on demographics, lifestyle habits, medical history and environmental exposure.

The study protocol was approved by the institutional review board of Seoul National University Hospital (IRB No. H-0804-045-241). Written consent was received from all participants and was confirmed by the IRB.

2. Measurement of Urinary Biomarkers

Phthalates

In this study, we measured urinary levels of MEHHP and MEOHP as metabolites of di(2-ethylhexyl) phthalate (DEHP), and MnBP as di-n-butyl phthalate (DnBP) metabolite. We used high performance liquid chromatography tandem mass spectrometry (Agilent 6410 triple Quad LCMS, Agilent, Santa Clara, CA, USA) according to previously reported procedures [11]. To determine MEHHP and MEOHP levels, 500 μl of urine was buffered with 30 μl of 2.0 M sodium acetate (pH 5.0), and then spiked with a mixture of isotope phthalate monoester standards (100 ng/ml) and 10 μl of β-glucuronidase. The sample was incubated at 37 ℃ for 3 h to deconjugate the glucuronidated phthalate metabolites. After the incubation, 100 μl of 2 N HCl was added to collect phthalate monoester. The extract was dried with nitrogen gas and reconstituted with 1 ml of HPLC-grade H_2O in a 2 ml glass vial. The supernatant was purified by solid phase extraction with disposable Agilent SB-C18 1.8 μm (2.1 mm × 50 mm). The mobile phase was 0.1% acetic acid water: 0.01% acetic acid acetonitrile (90:10, v/v) at a flow rate of 0.25 ml/min and the eluate was monitored at target masses of 221, 293 and 291, and internal standard masses of 225, 297 and 295.

Cotinine

Urinary cotinine levels were determined for monitoring tobacco exposure. After

urine samples were centrifuged to remove particulate matter, cotinine level was analyzed by an enzyme-linked immunosorbent assay method based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen (Bio-Quant cotinine direct Elisa, San Diego, CA, USA). Brifly, a 10 μl of urine and 100 μl of Horseradish peroxidase-labeled Cotinine derivative are incubated in micro-plate wells coated with high affinity cotinine antibody for 60 min at room temperature (RT). The wells are washed thoroughly and 100 μl of chromogenic substrate added. After incubation for 30 min at RT, the color produced is stopped using 100 μl of stop solution and the wells are read at 450 nm and 650 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample.

Creatinine

For the adjustment of urinary volume, we measured urinary creatinine levels using a HITACHI 7600 instrument (HITACHI, Tokyo, Japan). Briefly, 10 μl of urine and 300 μl of picric acid solution (Wako, Osaka, Japan) were mixed at room temperature for 3 min, and absorbance of the solution was measured at 505 nm. Seventy-five μl of alkaline solution (Wako, Osaka, Japan) was added for 4 min, and absorbance of the solution was measured at 570 nm. The mean of the two values measured at 505 nm and 570 nm was used as the creatinine level.

3. Measurement of Pulmonary Function

Spirometric Measurements

Spirometric testing was conducted every medical examination according to 2005 European Respiratory Society/American Thoracic Society recommendations. All tests were performed using a Microlab® (Sensormedic, Yorba Linda, CA, USA) spirometer by one trained technician, and one best result from three consecutive pulmonary function tests was taken for recording. From the test, we obtained values of forced expiratory volume in 1 second (FEV_1, L), forced vital capacity (FVC, L), a ratio of FEV_1 to FVC (FEV_1/FVC, %) and forced expiratory flow between 25 and 75% of the FVC ($FEF_{25–75}$, L/sec).

4. Measurement of *CAT*, *SOD2* and *MPO* polymorphisms

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and genetic polymorphisms of *CAT* (rs769218 and rs769217), *SOD2* (rs4880, rs2758331, and rs5746136) and *MPO* (rs2071409, rs7208693) were determined using the TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA, USA). SNPs from each gene were chosen with standards of having minor allele frequency > 0.05% and having reported to have been researched by the NCBI.

The final volume of polymerase chain reaction (PCR) was 5ul, containing 10ng

of genomic DNA and 2.5ul TaqMan Universal PCR Master Mix, with 0.13ul of 40X Assay Mix (Assay ID for rs769218, rs769217, rs4880, rs2758331, and rs5746136, C_3102900_10, C_3102907_10 C_8709053_10, C_16288770_10, and C_29322854_10, respectively). Thermal cycle conditions were as follows: 50℃ for 2 min to activate the uracil N-glycosylase and to prevent carry-over contamination, 95℃ for 10 min to activate the DNA polymerase, followed by 45 cycles of 95℃ for 15 s and 60℃ for 1 min. All PCR were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (ABI, Foster City, CA, USA) and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA). Negative controls were included to ensure accuracy of genotyping. For confirmation, five percent of the samples were randomly chosen and genotyped again, producing identical results.

When we tested for Hardy-Weinberg equilibrium (HWE) of *CAT*, *SOD2* and *MPO* gene polymorphisms, the study subjects were in HWE for all seven SNPs (all SNPs, $p>0.05$ by χ^2-test). Incomplete data missing at least one of two SNPs were excluded, and then individual haplotypes were estimated from genotype data using the PHASE program (ver. 2.0.2). Pairwise linkage disequilibrium (LD) between both polymorphic sites was estimated as relative disequilibrium (D′).

5. Measurement of seasonal factors

Daily average outdoor temperature and dew point data measured at the Songwol-dong monitoring center in Seongbuk-gu, which is the nearest monitoring center to the subjects' residence, were obtained from the Korea Meteorological Administration (http://www.kma.go.kr). Data were matched to the date each subject took measurements for urinary phthalate metabolite levels and pulmonary function test.

6. Statistical Analysis

Baseline characteristics determined on subjects' first visits were compared by sex. Urinary concentrations and distributions of MEHHP, MEOHP and MnBP were calculated and the concentrations under limit of detection (LOD) were assigned as a default value of LOD by 2 in the biomarker analysis. For the analysis of DEHP and DBP, the metabolites of DEHP (MEHHP and MEOHP) were evaluated individually and as the sum of the two (represented as "∑DEHP"). For each subject, the levels of MEHHP, MEOHP, ∑DEHP and MnBP that were averaged across repeatedly measured samples used to represent individual exposure to phthalates.

For association analyses, phthalate levels were log-transformed to improve normality. The effects of exposure to the phthalate metabolites, ∑DEHP and MnBP,

on pulmonary function were estimated using mixed model for repeated analysis with adjustment for age, sex, months past after each previous visit, body mass index, creatinine-adjusted cotinine level, mean temperature and mean dewpoint. We also estimated the association in separate sexes, and investigated on the interaction between sex and phthalate metabolites. Adjustment with months past after each previous visit was done to account for decline in lung function with aging [23].

To account for the non-random loss of follow-up due to different number of repeated measures [24], we adjusted for weight values yielded by inverse probability of attaining a follow-up response. With data from subjects with more than one visit, we performed logistic regression to predict follow-up probability (follow-up=1, missing=0), with covariates as previously measured age, sex, BMI, blood pressure, years of attending school, season of the year, and mean daily temperature [25, 26].

We also estimated changes of FEV_1/FVC and FEF_{25-75} by the increase of ∑DEHP and MnBP levels in different *CAT*, *SOD2* and *MPO* genotypes, and grouped their haplotypes based on effect estimates and p-values.

Because we used direct measurements of pulmonary function test instead of using answers to questionnaires on history of having lung disease, we performed sensitivity analysis to see differences upon including or excluding subjects with lung/bronchiolar diseases history. We also ran t-tests to check difference in levels of phthalate metabolites between subjects with and without record of pulmonary function tests.

To identify linkage disequilibrium, we calculated |D′| values between each *CAT, SOD2* and *MPO* haplotypes from our data, and drew LD plots for each *CAT, SOD2* and *MPO* genotypes using Japanese+Chinese SNP genotype data from HapMap (available at http://hapmap.ncbi.nlm.nih.gov) to confirm our analysis.

In all statistical analyses, we used SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Haploview version 4.2 (available at http://www.broadinstitute.org/haploview) was used for drawing LD plots.

RESULTS

1. Baseline characteristics by sex

Average number of repeated visits made by each subject was about 2.39 times. Age, BMI, pulmonary function, history of smoking and history of bronchial or lung disease assessed on first visit day were compared by sex (Table 1). Of all 418 subjects, 109 (26.08%) were male and 309 (73.92%) were female. Mean age of study participants were 70.85 ± 4.18 for males and 69.84 ± 5.23 for females (p=0.0437), and their BMI was 24.43 ± 2.71kg/m^3 and 25.06 ± 2.73kg/m^3 respectively (p=0.0371). Mean cotinine levels also differed by sex, and males showed higher levels of pulmonary function than females. Smoking status also varied by sex, but no gender difference was found in bronchial or lung disease status. Genotype distributions of *CAT, SOD2* and *MPO* SNPs showed no significant difference by gender.

2. Distribution of biomarker levels

From 418 subjects, we obtained 999 urinary samples for analysis, with 2.39 average samples per subject. Urinary mean concentrations and distributions by percentiles of MEHHP, MEOHP, ∑DEHP and MnBP levels were calculated (Table 2). Mean concentration values for all phthalate metabolites were higher than their

median values, indicative of the high level in the upper quantiles. LOD values for MEHHP, MEOHP, and MnBP were 0.160 μg/L, 0.206 μg/L, and 0.256 μg/L, respectively.

3. Effects of urinary phthalate metabolites on lung function

On association analysis by repeated measure analysis (Table 3a & 3b), negative relationship was found between ∑DEHP levels and two parameters of pulmonary function test, FEV_1/FVC and FEF_{25-75} (FEV_1/FVC: β=-0.632, P=0.0275; FEF_{25-75}: β=-0.077, P=0.002). As there were significant differences in the mean values of adjusted variables between males and females, we divided the subject by gender and performed same analysis by sex, and found that significance remained with female group only (P-values, for males: 0.3196 for FEV_1/FVC and 0.1365 FEF_{25-75}; for females: 0.0312 for FEV_1/FVC and 0.0023 FEF_{25-75}); however, on interaction analysis between sex and phthalate levels, significant interaction was not observed (p-values for interaction: 0.5179 for FEV_1/FVC, 0.5179 for FEF_{25-75}). No significant association was found between MnBP and any of the pulmonary function measurements, in all subjects (FEV_1/FVC: β=-0.212, P=0.4913; FEF_{25-75}: β=-0.025, P=0.3457) and in gender-divided groups.

4. Influence of *CAT, SOD2* and *MPO* polymorphisms

On estimating the effect of *CAT, SOD2* and *MPO* genotypes on the phthalate-pulmonary function (Table 4a & 4b), association between ∑DEHP levels and pulmonary function was significant only in subjects with *CAT* GG genotype of rs769218 (FEV_1/FVC: β=-1.331, P=0.0082; FEF_{25-75}: β=-0.094, P=0.0284) and CC genotype of rs769217 (FEV_1/FVC: β=-1.332, P=0.008; FEF_{25-75}: β=-0.094, P=0.0283), *SOD2* TT genotype of rs4880 (FEV_1/FVC: β=-0.803, P=0.329; FEF_{25-75}: β=-0.091, P=0.0014) and CC genotype of rs2758331 (FEV_1/FVC: β=-0.843, P=0.0105; FEF_{25-75}: β=-0.089, P=0.0017), *MPO* AA genotype in rs2071409 (FEV_1/FVC: β=-0.705, P=0.0225; FEF_{25-75}: β=-0.089, P=0.0007) and GG genotype in rs7208693 (FEV_1/FVC: β=-0.607, P=0.0644; FEF_{25-75}: β=-0.066, P=0.0232).

Effect estimates and the number of subjects were almost equal among the significant genotypes in each of the three genes, suggesting possible linkage. When we calculated |D′| values among the two SNPs in *CAT* and *MPO*, and among three SNPs in *SOD2*, both SNPs in *CAT*, *MPO* and two SNPs in *SOD2* (rs4880 and rs2758331) were found to be in LD (|D′|=1, p-value <0.0001 in both genes). The third SNP in SOD2, rs5746136, which did not show different nor significant effect estimates among different genotypes, had |D′| values of 0.77 and 0.86 with rs4880 and 2758331, respectively, and was excluded from the analysis in combining genotypes.

We compared our results with the Hapmap data, and found that our data was not different from Japanese+Chinese data obtained from Hapmap based on frequency of each genotype and LDs of *CAT* SNPs rs769218-rs769217 and *SOD2* SNPs rs4880-rs2758331 (|D′|=1, p-value <0.0001 in both genes for Hapmap) (Fig.1). Both r-square values for the two selected SNPs in *CAT* and *SOD2* in HapMap were calculated to be 1.0, confirming complete linkage. For *MPO*, we were not able to extract Hapmap genotype data for rs7208693, but LD plotting with nearest available SNP (rs8082134) showed |D′|=1 and r^2 =0.030. Because number of subjects and estimate size was similar among genotypes for rs2071409 and rs7208693, we assumed that the two SNPs in our data may also be closely linked.

Therefore, we interpreted that significant effect estimate in one genotype of a SNP from each gene in our data could represent the significant genotype in the other linked SNP. Using the linked structure, we paired the haplotypes producing risky diplotypes, i.e. GC-GC in *CAT*, TC-TC in *SOD2* and AG-AG in *MPO* (Table 5). We investigated if there is a dose-response relationship between number of risky diplotypes and effect estimates, and found that having all three significant diplotypes against other combinations (i.e. having none, one or two of the three significant diplotypes) showed marked difference in size of effect estimate and significance (Table 6a, 6b & 6c). This association also showed significant and borderline-significant p-values for interaction between the diplotype combination and phthalates (p=0.0176 for ∑DEHP and FEV_1/FVC, 0.0663 for ∑DEHP and FEF_{25-75}).

5. Comparison of association by smoking status and bronchial / lung disease history

We found that FEV_1/FVC measurements differed between current smokers, ex-smokers and never-smokers ($p < 0.0001$). Never-smokers had highest mean percentage of FEV_1/FVC (85.61%), followed by current smokers (82.42%) and ex-smokers (77.9%). No significant difference was observed between the groups for FEF_{25-75} measurements (Table 8a). In sensitivity analysis upon subject groups divided by smoking status, inverse relationship was found between ∑DEHP and pulmonary function parameters in never-smokers, and no significant association was found in current smokers and ex-smokers for both ∑DEHP and MnBP with FEV_1/FVC nor FEF_{25-75} (Table 8b).

We also investigated on any PTF measurement differences between those with no or already-cured history of bronchial or lung disease, with those currently diagnosed or in treatment. In all measurements summed, FEF_{25-75} measures were different among the two groups (none/cured: 2.12 L/s, diagnosed/in treatment: 1.77 L/s; p-value=0.0186) (Table 9a). Upon including or excluding subjects currently diagnosed or in treatment with lung/bronchiolar disease history, we found no difference in size, direction or significance of effect estimate (Table 9b).

DISCUSSION

1. Mean levels of phthalate metabolites

Mean levels of phthalate metabolites, MEHHP, MEOHP and MnBP in our study is 25.59μg/L, 21.51μg/L, and 59.27μg/L, respectively, and is in range with the previous reports in Europe, USA, Japan and Taiwan [27].

2. Association between phthalate exposure and pulmonary function, and influence by genetic polymorphisms of oxidative stress marker genes

Results from our finding shows that phthalate metabolite levels, namely ∑DEHP (sum of MEHHP and MEOHP), is in association with pulmonary function parameters, FEV_1/FVC and FEF_{25-75} in the repeated measure analysis in Korean elderly. This association was found more clearly with certain *CAT, SOD2* and *MPO* genotype groups, where two of the SNPs in each groups are in complete or near-complete linkage disequilibrium.

To our knowledge, there has been only a single reported epidemiologic study that investigated on gene-environment interaction, where authors attempted to find association between phthalate exposure and glutathione S-transferase M1 polymorphism in estrogen-dependent diseases, and found some significant interaction [28]. We also investigated on possible effect modification of genetic

polymorphism on phthalate-pulmonary function association, focusing on relevant polymorphisms for antioxidant markers. We have also found significant effect of genetic polymorphism, and p-values for gene-phthalate interactions were significant.

3. Possible mechanism for phthalate-pulmonary function association

DEHP is a high molecular weight phthalate that is first metabolized to hydrolytic monoesters in phase I biotransformation, followed by enzymatic oxidation of the alkyl chain, then transformed to more hydrophilic and oxidative metabolites. These products mostly undergo phase II biotransformation and produce glucuronide conjugates with increased water solubility and therefore increased urinary excretion [3]. Therefore, we assume that the notable effect of oxidative stress-related genetic polymorphisms on the significant association between ∑DEHP and pulmonary function is acceptable, based on the metabolic background.

Our result does not support for significant relationship between MnBP and pulmonary function measures. We assumed that the difference in the results may be due to the different metabolizing processes the short- and long-chain branched phthalates undertake; it is a well-documented fact that MEHHP and MEOHP, long-chain branched phthalates, continue further hydroxylation and oxidation, while short-chain branched MnBP does not [29, 30]. This assumption may be further supported by our extended gene-environment analysis, which showed influence of oxidative stress marker genes only in the ∑DEHP-pulmonary function association.

Additional epidemiological researches in various populations and settings may be needed to confirm the different association in short- and long-chain branched phthalates with lung function.

4. Possible mechanisms for influence of oxidative stress marker genes in the phthalate-pulmonary function association

Of the seven SNPs in our study, *SOD2* rs4880 and *MPO* rs7208693 were polymorphisms with missense mutation. By NCBI search, *SOD2* rs4880 (Ala16Val) was the only SNP with available information on clinical significance, categorized as "probable-pathogenic" based on 45 human researches (Table 11).

Researchers reported on influence of rs4880 in relation to susceptibility to cardiomyopathy and microvascular complications of diabetes mellitus, and suggested that the Val16 allele disrupts the alpha-helix structure of SOD2 and causes the protein to be retained at the level of the mitochondrial inner membrane, and that the mutant protein has lower activity and increases susceptibility to oxidative stress. Although inconsistent, influence of rs4880 was also reported in relation to response to chemotherapy in breast, colorectal and prostate cancer patients, aging and longevity [31]. In regard to pulmonary function, a single study reported association between increasing number of genotypes coding for high antioxidative enzyme activity, including rs4880 Val/Val, and FEV_1 % predicted in young adult smokers [32].

To summarize, existing reports sufficiently support our findings on the influence of rs4880 in the association between phthalate exposure and pulmonary function parameters. Only a single report was found for *MPO* rs7208693 that suggested its effect on susceptibility to benzene poisoning, but did not have adequate information [33]. Further investigation of clinical significance on this non-synonymous mutation would be encouraging.

No report was available for the other SNPs used in our analysis regarding associations with toxicological or epidemiological findings

5. Comparison of exposure effect by smoking versus phthalates on pulmonary function

Although our sensitivity analysis does not show significant difference in the phthalate-pulmonary function association between ex- and never-smokers, this result cannot be confirmative as sample sizes for current and ex-smokers are small in our study. Also, we were not able to take into account the duration of smoking in our analysis and are not exempt from possible measurement bias.

Smoking is nonetheless a major exogenous influential factor to pulmonary function decline [34, 35], and it is expected to produce more severe results in the elderly individual, and even more pronounced in those with chronic respiratory disease [36]. It has been reported that rate of decline in forced vital capacity by age is about 21 to 34 mL/year in men and 19 to 29 mL/year in women [37], and another

study also reported that decline in pulmonary function measures by smoking would be as much as up to 40% of the effect of aging [38]. Thus we can surmise that effect of smoking on pulmonary function in elderly to about 29 to 48 mL/year in men and 27 to 41 mL/year in women.

Our study shows no significant association results between phthalate exposure and FEV_1 and FVC, which are parameters for comparability with the smoking effect, and the effect estimates are in much larger scale (e.g. change in FEV_1 per IQR increase in ∑DEHP is -0.51 L (95% confidence limits: -1.3, 0.28)). Significant association results, however, shows some degree of phthalate effect on the pulmonary function; change in FEV_1/FVC per IQR increase in ∑DEHP is as much as -21.67% (-40.5, -2.84), and change in FEF_{25-75} per IQR increase in ∑DEHP is about -2.64 L/sec (-4.29, -0.99) (Table 3b).

6. Strength and limitation of the study

Using direct measurement of representative phthalate metabolites and pulmonary function over questionnaires on exposure or outcome history add to strength of our study, as our hypothesis was based on association between acute exposure and short-term outcome. Our sensitivity analysis result, having no difference in association with inclusion or exclusion of subjects with lung/bronchiolar diseases history, i.e. long-term outcomes, may add support to this assumption.

It has been reported that time of day urine samples are collected significantly influenced measured concentration of phthalate metabolites [39]. In the KEEP Study, provision and collection of urine sample containers was done only between 1000-1200 hours, thus is in exception to this information error.

Major limitations to our study are small sample size and cross-sectional nature of the analysis. Availability of greater number of subjects would have improved external validity on the effect of genetic polymorphisms on the exposure-outcome association. Also, although repeated measure analysis increased power to our finding, we cannot deduce causal relationship.

Study subjects were mostly female non-smokers without any lung disease, and although our research aimed at finding effect of environmental phthalate exposure on susceptible group such as the elderly, we cannot imply the finding on other pulmonary-function related susceptible groups such as smokers and lung disease patients.

Of the pulmonary function parameters, our results showed associations with FEV_1/FVC and FEF_{25-75}, but not with FEV_1 and FVC. FEV_1 and FVC are indices for obstructive and restrictive lung diseases, and FEV_1/FVC represents severity of either obstructive or restrictive obstruction. The less-clinically used index, FEF_{25-75}, is defined by flow (or speed) of air coming out of the lung during the middle portion of a forced expiration. It is known to be most sensitive to early diagnosis of small airway disease, but shows much variability even in normal persons, with its range depending largely on sex and age [40]. Although not directly

implicational, reports on correlation of FEF_{25-75} with bronchial hyperactivity in children with asthma or allergic rhinitis, even in normal FEV_1, could be related to our significant association finding with FEF_{25-75} [41, 42].

CONCLUSION

In conclusion, we found urinary phthalate metabolites levels associated with decreasing pulmonary function in elderly Koreans, and suggested the effect modification of certain CAT, SOD2 and MPO polymorphisms on the phthalates-lung function association.

Table 1. Baseline characteristics of study subjects by gender

		All (n=418)	Male (n=109)	Female (n=309)	P-value
		mean±SD / n (%)	mean±SD / n (%)	mean±SD / n (%)	
Age		70.11 ± 4.99	70.85 ± 4.18	69.84 ± 5.23	0.0437
BMI (kg/m^2)		24.89 ± 2.71	24.43 ± 2.60	25.06 ± 2.73	0.0371
Cotinine (mg /L)		316.32±1366.79	838.5±2114.4	95.48±787.1	0.0022
FEV_1 (L)		1.92 ± 0.44	2.34 ± 0.39	1.77 ± 0.36	<.0001
FVC (L)		2.27 ± 0.57	2.89 ± 0.47	2.05 ± 0.42	<.0001
FEV_1/FVC (%)		85.21 ± 7.00	81.49 ± 7.41	86.53 ± 6.36	<.0001
FEF_{25-75} (L/sec)		2.17 ± 0.72	2.37 ± 0.78	2.10 ± 0.69	0.0014
Smoking	Current smoker	26 (6.65)	25 (22.94)	1 (0.32)	
	Ex-smoker	26 (6.65)	23 (21.1)	3 (0.97)	<.0001
	Never-smoker	366 (93.35)	61 (55.96)	305 (98.71)	
Bronchial or lung disease	None / cured	409 (97.8)	108 (99.1)	301 (97.4)	0.4565
	Diagnosed / in treatment	9 (2.2)	1 (0.9)	8 (2.6)	
rs769218	GG	120 (28.78)	31 (28.44)	89 (28.9)	
	GA	225 (53.96)	57 (52.29)	168 (54.55)	0.6716
	AA	72 (17.27)	21 (19.27)	51 (16.56)	
rs769217	TT	69 (16.79)	21 (19.44)	48 (15.84)	
	CT	221 (53.77)	56 (51.85)	165 (54.46)	0.5392
	CC	121 (29.44)	31 (28.7)	90 (29.7)	
rs4880	TT	324 (77.88)	87 (79.82)	237 (77.2)	
	CT	86 (20.67)	21 (19.27)	65 (21.17)	0.5141
	CC	6 (1.44)	1 (0.92)	5 (1.63)	
rs2758331	CC	327 (78.61)	87 (79.82)	240 (78.18)	
	CA	84 (20.19)	21 (19.27)	63 (20.52)	0.6841
	AA	5 (1.2)	1 (0.92)	4 (1.3)	
rs5746136	GG	142 (34.22)	39 (35.78)	103 (33.66)	
	GA	207 (49.88)	54 (49.54)	153 (50)	0.6206
	AA	66 (15.9)	16 (14.68)	50 (16.34)	
rs2071409	AA	361 (86.99)	91 (84.26)	270 (87.95)	
	AC	52 (12.53)	17 (15.74)	35 (11.4)	0.4456
	CC	2 (0.48)	0 (0)	2 (0.65)	
rs7208693	GG	330 (79.52)	88 (80.73)	242 (79.08)	
	GT	77 (18.55)	20 (18.35)	57 (18.63)	0.5575
	TT	8 (1.93)	1 (0.92)	7 (2.29)	

FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, FEF_{25-75}: forced expiratory flow between 25% and 75% of forced vital capacity.

Table 2. Distribution of urinary phthalate metabolite levels

Biomarker	No. of observations	Mean (SD)	LOD	n < LOD (%)	Selected percentiles					
					10th	25th	50th	75th	90th	95th
MEHHP (μg/L)	999	25.59 (41.8)	0.16	1(0.10)	5.4	9.15	16.15	28.85	50.3	75.05
MEOHP (μg/L)	999	21.51 (39.8)	0.206	17(1.70)	5.32	7.81	13.1	23.74	42.03	61.64
∑DEHP (μg/L)	999	49.1 (75.8)	-	-	11.22	17.37	29.38	51.66	96.65	131
MnBP (μg/L)	999	59.27 (79.57)	0.256	1(0.10)	14.52	22.66	38.9	65.38	114.81	162.7

∑DEHP: sum of two DEHP metabolites, MEHHP and MEOHP

Table 3a. Association of urinary phthalate metabolite levels and pulmonary function measurements, by repeated measure analysis [1,2]

		All (n=418)		Female (n=309)		Male (n=109)	
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value
∑DEHP	FEV_1	-0.015 (0.012)	0.187	-0.011 (0.012)	0.3695	-0.034 (0.031)	0.2801
	FVC	-0.005 (0.015)	0.7163	0.002 (0.016)	0.8929	-0.021 (0.038)	0.573
	FEV_1/FVC	-0.632 (0.286)	0.0275	-0.646 (0.299)	0.0312	-0.759 (0.76)	0.3196
	FEF_{25-75}	-0.077 (0.025)	0.002	-0.081 (0.026)	0.0023	-0.093 (0.062)	0.1365
MnBP	FEV_1	0.001 (0.013)	0.9249	0.005 (0.013)	0.708	-0.006 (0.031)	0.8476
	FVC	0.007 (0.016)	0.6498	0.019 (0.018)	0.2824	-0.016 (0.037)	0.6748
	FEV_1/FVC	-0.212 (0.308)	0.4913	-0.478 (0.328)	0.1459	0.446 (0.758)	0.5575
	FEF_{25-75}	-0.025 (0.027)	0.3457	-0.033 (0.029)	0.2522	-0.015 (0.062)	0.8098

[1] adjusted for age, sex (in analysis for both sex), months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dewpoint

[2] all p-interaction values for ∑DEHP and sex are more than 0.1.

Table 3b. Association of urinary phthalate metabolite levels (in interquartile range) and pulmonary function measurements, by repeated measure analysis [1,2]

		All (n=418)		Female (n=309)		Male (n=109)	
		change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value
∑DEHP (IQR)	FEV_1	-0.51 (-1.3, 0.28)	0.187	-0.38 (-1.17, 0.41)	0.3695	-1.17 (-3.21, 0.88)	0.2801
	FVC	-0.17 (-1.16, 0.82)	0.7163	0.07 (-0.98, 1.12)	0.8929	-0.72 (-3.22, 1.78)	0.573
	FEV_1/FVC	-21.67 (-40.5, -2.84)	0.0275	-22.15 (-41.84, -2.47)	0.0312	-26.03 (-76.06, 24.01)	0.3196
	FEF_{25-75}	-2.64 (-4.29, -0.99)	0.002	-2.78 (-4.49, -1.07)	0.0023	-3.19 (-7.27, 0.89)	0.1365
MnBP (IQR)	FEV_1	0.04 (-1.05, 1.13)	0.9249	0.21 (-0.87, 1.3)	0.708	-0.26 (-2.85, 2.34)	0.8476
	FVC	0.3 (-1.04, 1.64)	0.6498	0.81 (-0.7, 2.32)	0.2824	-0.68 (-3.78, 2.41)	0.6748
	FEV_1/FVC	-9.06 (-34.85, 16.73)	0.4913	-20.42 (-47.88, 7.04)	0.1459	19.05 (-44.42, 82.52)	0.5575
	FEF_{25-75}	-1.07 (-3.33, 1.19)	0.3457	-1.41 (-3.84, 1.02)	0.2522	-0.64 (-5.83, 4.55)	0.8098

[1] adjusted for age, sex (in analysis for both sex), months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dewpoint

[2] all p-interaction values for ∑DEHP and sex are more than 0.1.

(Interquartile range (IQR): ∑DEHP 34.29 μg/L, MnBP 42.72μg/L. Pulmonary function (PF) parameters: FEV_1 and FVC in liters (L), FEV_1/FVC in percentage (%),FEF_{25-75} in liters per second (L/s).

Table 4a. Effect of *CAT, SOD2* genotypes on phthalate-pulmonary function association [1]

***CAT* (rs769218)**

		GG (n=120)		**GA (n=225)**		**AA (n=72)**		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	-1.331 (0.496)	0.0082	-0.51 (0.436)	0.2426	0.484 (0.561)	0.3906	0.0718
	FEF_{25-75}	-0.094 (0.042)	0.0284	-0.06 (0.037)	0.1093	-0.089 (0.053)	0.0979	0.8371
MnBP	FEV_1/FVC	-0.519 (0.598)	0.3871	-0.212 (0.435)	0.6272	0.271 (0.603)	0.6537	0.6835
	FEF_{25-75}	-0.031 (0.051)	0.5511	-0.005 (0.037)	0.894	-0.059 (0.056)	0.2942	0.6698

***CAT* (rs769217)**

		TT (n=69)		**CT (n=221)**		**CC (n=121)**		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	0.515 (0.591)	0.3857	-0.389 (0.436)	0.3721	-1.332 (0.496)	0.008	0.0656
	FEF_{25-75}	-0.069 (0.054)	0.2073	-0.049 (0.038)	0.1974	-0.094 (0.042)	0.0283	0.6485
MnBP	FEV_1/FVC	0.293 (0.621)	0.6383	-0.175 (0.432)	0.6865	-0.499 (0.597)	0.4048	0.6998
	FEF_{25-75}	-0.07 (0.057)	0.2236	-0.002 (0.038)	0.9601	-0.03 (0.051)	0.5555	0.5561

***SOD2* (rs4880)**

		TT (n=324)		**CT (n=86)**		**CC (n=6)**		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	-0.803 (0.329)	0.0152	-0.234 (0.609)	0.7025	n/a		0.3831
	FEF_{25-75}	-0.091 (0.028)	0.0014	-0.057 (0.054)	0.2944	0.014 (0.169)	0.9394	0.1787
MnBP	FEV_1/FVC	-0.341 (0.353)	0.334	-0.151 (0.648)	0.8158	n/a		0.1661
	FEF_{25-75}	-0.039 (0.03)	0.1997	-0.023 (0.057)	0.6907	0.44 (0.159)	0.05	0.0585

***SOD2* (rs2758331)**

		CC (n=327)		**CA (n=84)**		**AA (n=5)**		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	-0.843 (0.328)	0.0105	-0.18 (0.601)	0.7652	n/a		0.3831
	FEF_{25-75}	-0.089 (0.028)	0.0017	-0.06 (0.054)	0.2655	n/a		0.1787
MnBP	FEV_1/FVC	-0.382 (0.352)	0.278	-0.072 (0.642)	0.911	4.861 (3.667)	0.3162	0.1661
	FEF_{25-75}	-0.038 (0.03)	0.2039	-0.027 (0.058)	0.6471	0.363 (0.189)	0.1956	0.0585

***SOD2* (rs5746136)**

		GG (n=142)		GA (n=207)		AA (n=66)		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	-0.653 (0.473)	0.1694	-0.582 (0.382)	0.1287	-0.767 (0.906)	0.4001	0.9149
	FEF_{25-75}	-0.089 (0.042)	0.0327	-0.062 (0.036)	0.0845	-0.077 (0.062)	0.2179	0.9265
MnBP	FEV_1/FVC	-0.358 (0.579)	0.5377	-0.298 (0.373)	0.4252	0.236 (1.07)	0.8261	0.7838
	FEF_{25-75}	-0.064 (0.051)	0.2099	-0.014 (0.035)	0.6839	0.001 (0.072)	0.9898	0.6861

MPO (rs2071409)

		AA (n=361)		AC (n=52)		CC (n=2)		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	-0.705 (0.308)	0.0225	-0.146 (0.763)	0.8493	n/a		0.3161
	FEF_{25-75}	-0.089 (0.026)	0.0007	-0.013 (0.078)	0.8689	n/a		0.3504
MnBP	FEV_1/FVC	-0.135 (0.334)	0.6858	-1.075 (0.821)	0.1952	n/a		0.9538
	FEF_{25-75}	-0.027 (0.028)	0.34	-0.068 (0.085)	0.4308	n/a		0.7155

MPO (rs7208693)

		GG (n=330)		GT (n=77)		TT (n=8)		
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	**P-int**
∑DEHP	FEV_1/FVC	-0.607 (0.327)	0.0644	-0.777 (0.631)	0.2217	-1.86 (1.513)	0.2862	0.4994
	FEF_{25-75}	-0.066 (0.029)	0.0232	-0.097 (0.049)	0.0523	-0.538 (0.235)	0.0837	0.7733
MnBP	FEV_1/FVC	-0.043 (0.343)	0.9003	-0.654 (0.738)	0.3779	-1.088 (1.58)	0.529	0.8163
	FEF_{25-75}	-0.003 (0.031)	0.9203	-0.085 (0.057)	0.1436	-0.342 (0.245)	0.2356	0.3243

[1] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dewpoint

Table 4b. Effect of *CAT*, *SOD2* genotypes on relationship between interquartile increase of phthalate metabolites and pulmonary function parameters[1]

		change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value	p-int
CAT (rs769218)		**GG (n=120)**		**GA (n=225)**		**AA (n=72)**		
∑DEHP	FEV_1/FVC	-45.64 (-78.98, -12.3)	0.0082	-17.49 (-46.79, 11.81)	0.2426	16.6 (-21.11, 54.3)	0.3906	0.0718
	FEF_{25-75}	-3.22 (-6.05, -0.4)	0.0284	-2.06 (-4.54, 0.43)	0.1093	-3.05 (-6.61, 0.51)	0.0979	0.8371
MnBP	FEV_1/FVC	-22.21 (-72.24, 27.9)	0.3871	-9.07 (-45.48, 27.37)	0.6272	11.6 (-38.91, 62.07)	0.6537	0.6835
	FEF_{25-75}	-1.33 (-5.59, 2.95)	0.5511	-0.21 (-3.31, 2.88)	0.894	-2.52 (-7.21, 2.17)	0.2942	0.6698
CAT (rs769217)		**TT (n=69)**		**CT (n=221)**		**CC (n=121)**		
∑DEHP	FEV_1/FVC	17.66 (-22.06, 57.38)	0.3857	-13.34 (-42.64, 15.96)	0.3721	-45.67 (-79.01, -12.34)	0.008	0.0656
	FEF_{25-75}	-2.37 (-6, 1.26)	0.2073	-1.68 (-4.23, 0.87)	0.1974	-3.22 (-6.05, -0.4)	0.0283	0.6485
MnBP	FEV_1/FVC	12.54 (-39.48, 64.51)	0.6383	-7.49 (-43.65, 28.7)	0.6865	-21.35 (-71.3, 28.67)	0.4048	0.6998
	FEF_{25-75}	-3 (-7.76, 1.78)	0.2236	-0.09 (-3.27, 3.1)	0.9601	-1.28 (-5.55, 2.99)	0.5555	0.5561
SOD2 (rs4880)		**TT (n=324)**		**CT (n=86)**		**CC (n=6)**		
∑DEHP	FEV_1/FVC	-27.53 (-49.65, -5.42)	0.0152	-8.02 (-48.95, 32.91)	0.7025	n/a		0.3831
	FEF_{25-75}	-3.12 (-5, -1.24)	0.0014	-1.95 (-5.58, 1.67)	0.2944	0.48 (-10.88, 11.84)	0.9394	0.1787
MnBP	FEV_1/FVC	-14.59 (-44.12, 14.99)	0.334	-6.46 (-60.71, 47.81)	0.8158	n/a		0.1661
	FEF_{25-75}	-1.67 (-4.18, 0.85)	0.1997	-0.98 (-5.76, 3.79)	0.6907	18.83 (5.48, 32.11)	0.05	0.0585
SOD2 (rs2758331)		**CC (n=327)**		**CA (n=84)**		**AA (n=5)**		
∑DEHP	FEV_1/FVC	-28.91 (-50.95, -6.86)	0.0105	-6.17 (-46.56, 34.22)	0.7652	n/a		0.3831
	FEF_{25-75}	-3.05 (-4.93, -1.17)	0.0017	-2.06 (-5.69, 1.57)	0.2655	n/a		0.1787
MnBP	FEV_1/FVC	-16.35 (-45.79, 13.15)	0.278	-3.08 (-56.83, 50.68)	0.911	208 (-99.38, 514.7)	0.3162	0.1661

	FEF_{25-75}	-1.63 (-4.14, 0.89)	0.2039	-1.16 (-6.01, 3.7)	0.6471	15.53 (-0.32, 31.33)	0.1956	0.0585
SOD2 (rs5746136)		**GG (n=142)**		**GA (n=207)**		**AA (n=66)**		
∑DEHP	FEV_1/FVC	-22.39 (-54.18, 9.4)	0.1694	-19.96 (-45.63, 5.72)	0.1287	-26.3 (-87.19, 34.59)	0.4001	0.9149
	FEF_{25-75}	-3.05 (-5.87, -0.23)	0.0327	-2.13 (-4.55, 0.29)	0.0845	-2.64 (-6.81, 1.53)	0.2179	0.9265
MnBP	FEV_1/FVC	-15.32 (-63.77, 33.19)	0.5377	-12.75 (-43.96, 18.5)	0.4252	10.1 (-79.51, 99.67)	0.8261	0.7838
	FEF_{25-75}	-2.74 (-7, 1.54)	0.2099	-0.6 (-3.53, 2.33)	0.6839	0.04 (-5.99, 6.07)	0.9898	0.6861
MPO (rs2071409)		**AA (n=361)**		**AC (n=52)**		**CC (n=2)**		
∑DEHP	FEV_1/FVC	-24.17 (-44.87, -3.47)	0.0225	-5.01 (-56.29, 46.27)	0.8493	n/a		0.3161
	FEF_{25-75}	-3.05 (-4.8, -1.3)	0.0007	-0.45 (-5.69, 4.8)	0.8689	n/a		0.3504
MnBP	FEV_1/FVC	-5.78 (-33.73, 22.2)	0.6858	-46 (-114.67, 22.82)	0.1952	n/a		0.9538
	FEF_{25-75}	-1.16 (-3.5, 1.19)	0.34	-2.91 (-10.02, 4.21)	0.4308	n/a		0.7155
MPO (rs7208693)		**GG (n=330)**		**GT (n=77)**		**TT (n=8)**		
∑DEHP	FEV_1/FVC	-20.81 (-42.79, 1.16)	0.0644	-26.64 (-69.05, 15.77)	0.2217	-63.78 (-165.47, 37.91)	0.2862	0.4994
	FEF_{25-75}	-2.26 (-4.21, -0.31)	0.0232	-3.33 (-6.62, -0.03)	0.0523	-18.45 (-34.24, -2.65)	0.0837	0.7733
MnBP	FEV_1/FVC	-1.84 (-30.56, 26.88)	0.9003	-27.98 (-89.73, 33.85)	0.3779	-46.56 (-178.77, 85.82)	0.529	0.816
	FEF_{25-75}	-0.13 (-2.72, 2.47)	0.9203	-3.64 (-8.4, 1.14)	0.1436	-14.63 (-35.12, 5.9)	0.2356	0.3243

[1] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dewpoint

(Interquartile range (IQR): ∑DEHP 34.29 μg/L, MnBP 42.72 μg/L. Pulmonary function (PF) parameters: FEV_1 and FVC in liters (L), FEV_1/FVC in percentage (%), FEF_{25-75} in liters per second (L/s).

Ta**ble 5. Effect of *CAT, SOD2, MPO* haplotypes on phthalate-pulmonary function association** [1]

***CAT* (rs769218-rs769217)**						
		GC-GC (n=120)		**GC-AT, AT-AT,AT-AC (n=290)**		P-int
		β (SE)	*p*-value	β (SE)	*p*-value	
∑DEHP	FEV_1/FVC	-1.331 (0.496)	0.0082	-0.094 (0.355)	0.7913	0.1053
	FEF_{25-75}	-0.094 (0.042)	0.0284	-0.052 (0.031)	0.0931	0.3581
MnBP	FEV_1/FVC	-0.519 (0.598)	0.3871	-0.031 (0.358)	0.9301	0.9107
	FEF_{25-75}	-0.031 (0.051)	0.5511	-0.022 (0.031)	0.4759	0.9794
***SOD2* (rs4880- rs2758331)**						
		TC-TC (n=323)		**TC-CA, CC-CC, CA-CA (n=91)**		P-int
		β (SE)	*p*-value	β (SE)	*p*-value	
∑DEHP	FEV_1/FVC	-0.809 (0.33)	0.0146	-0.314 (0.571)	0.583	0.3537
	FEF_{25-75}	-0.091 (0.028)	0.0014	-0.044 (0.051)	0.3869	0.0967
MnBP	FEV_1/FVC	-0.345 (0.353)	0.3294	-0.045 (0.622)	0.9419	0.2837
	FEF_{25-75}	-0.039 (0.03)	0.1983	-0.012 (0.056)	0.8337	0.0585
***MPO* (rs2071409-rs7208693)**						
		GA-GA (n=278)		**GA-GC, GA-TA, GC-TA, TA-TA (n=134)**		P-int
		β (SE)	*p*-value	β (SE)	*p*-value	
∑DEHP	FEV_1/FVC	-0.723 (0.357)	0.0438	-0.478 (0.476)	0.3168	0.3521
	FEF_{25-75}	-0.077 (0.031)	0.0143	-0.081 (0.041)	0.0507	0.8819
MnBP	FEV_1/FVC	-0.033 (0.375)	0.929	-0.463 (0.543)	0.3958	0.9278
	FEF_{25-75}	-0.01 (0.033)	0.7653	-0.053 (0.047)	0.2578	0.687

[1] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dewpoint

Table 6a. Effect of grouped *CAT, SOD2, MPO* haplotypes on phthalate-pulmonary function association, by comparing having all 3 significant diplotypes versus having none, one or two of the 3 diplotypes [1]

		GC-GC & TC-TC & GA-GA (n=64)		**others (n=340)**		
		β (SE)	*p*-value	β (SE)	*p*-value	P-int
∑DEHP	FEV_1/FVC	-2.169 (0.711)	0.0032	-0.221 (0.314)	0.4827	0.0176
	FEF_{25-75}	-0.155 (0.059)	0.0103	-0.049 (0.028)	0.0749	0.0663
MnBP	FEV_1/FVC	-0.382 (1.037)	0.7138	-0.24 (0.321)	0.4554	0.8347
	FEF_{25-75}	-0.012 (0.086)	0.8915	-0.034 (0.028)	0.2233	0.9048

[1] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dew point

Table 6b. Effect of grouped *CAT, SOD2, MPO* haplotypes on phthalate-pulmonary function association, by comparing each of the possible combinations of having significant diplotypes [1]

		Having all 3 diplotypes (GC-GC & TC-TC & GA-GA) (n=64)		**Having 2 of the 3 diplotypes (n=195)**		**Having 1 of the 3 diplotypes (n=124)**		**Having none of the 3 diplotypes (n=21)**		P-int
		β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	β (SE)	*p*-value	
∑DEHP	FEV_1/ FVC	-2.169 (0.711)	0.0032	-0.34 (0.425)	0.4238	-0.088 (0.51)	0.864	-0.9 (1.344)	0.5105	0.0378
	FEF_{25-75}	-0.155 (0.059)	0.0103	-0.04 (0.038)	0.297	-0.072 (0.042)	0.094	-0.015 (0.153)	0.9227	0.0948
MnBP	FEV_1/ FVC	-0.382 (1.037)	0.7138	-0.499 (0.41)	0.2248	0.336 (0.569)	0.5564	-2.097 (1.588)	0.2016	0.4978
	FEF_{25-75}	-0.012 (0.086)	0.8915	-0.055 (0.036)	0.1327	0.007 (0.048)	0.886	-0.101 (0.184)	0.5893	0.3367

[1] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dew point

Table 6c. Effect of grouped *CAT, SOD2, MPO* haplotypes in diplotype combinations, on relationship between interquartile increase of phthalate metabolites and pulmonary function parameters[1]

		Having all 3 diplotypes (GC-GC & TC-TC & GA-GA) (n=64)		**Having 2 of the 3 diplotypes (n=195)**		**Having 1 of the 3 diplotypes (n=124)**		**Having none of the 3 diplotypes (n=21)**		P-int
		change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value	change in PF (95% CI)	*p*-value	
∑DEHP	FEV_1/FVC	-74.38 (-122.16, -26.59)	0.0032	-11.66 (-40.22, 16.9)	0.4238	-3.02 (-37.29, 31.26)	0.864	-30.86 (-121.19, 59.47)	0.5105	0.0378
	FEF_{25-75}	-5.31 (-9.28, -1.35)	0.0103	-1.37 (-3.93, 1.18)	0.297	-2.47 (-5.29, 0.35)	0.094	-0.51 (-10.8, 9.77)	0.9227	0.0948
MnBP	FEV_1/FVC	-16.32 (-103.15, 86.83)	0.7138	44.37 (86.08, 2.52)	0.2248	17.54 (-10.62, 45.65)	0.5564	24.35 (199.89, -151.28)	0.2016	0.4978
	FEF_{25-75}	-0.51 (-7.71, 7.2)	0.8915	3.68 (8.28, -0.93)	0.1327	1.54 (0.95, 2.12)	0.886	2.05 (10.51, -6.41)	0.5893	0.3367

[1] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dew point (Interquartile range (IQR): ∑DEHP 34.29 μg/L, MnBP 42.72 μg/L. Pulmonary function (PF) parameters: FEV_1 and FVC in liters (L), FEV_1/FVC in percentage (%),FEF_{25-75} in liters per second (L/s).

Table 7. Mean levels of pulmonary function tests by genotypes.

			FEV_1/FVC		FEF_{25-75}	
		N	mean (SD)	p-value	mean (SD)	p-value
CAT	GG	298	84.73 (8)	0.8628	2.09 (0.75)	0.5892
(rs769218)	GA	528	84.91 (7.64)		2.11 (0.7)	
	AA	172	85.12 (6.45)		2.16 (0.76)	
CAT	TT	166	85 (6.48)	0.8799	2.14 (0.76)	0.6686
(rs769217)	CT	518	85.02 (7.65)		2.13 (0.7)	
	CC	299	84.75 (8)		2.09 (0.75)	
SOD2	TT	778	84.73 (7.67)	0.195	2.1 (0.73)	0.3894
(rs4880)	CT	199	85.8 (7.24)		2.18 (0.7)	
	CC	17	84.35 (4.74)		2.1 (0.53)	
SOD2	CC	787	84.78 (7.65)	0.2808	2.1 (0.73)	0.3075
(rs2758331)	CA	193	85.67 (7.27)		2.19 (0.71)	
	AA	14	83.71 (4.87)		2.06 (0.57)	
SOD2	GG	344	84.56 (7.5)	0.0616	2.08 (0.72)	0.0044
(rs5746136)	GA	495	85.43 (7.21)		2.18 (0.75)	
	AA	157	83.96 (8.64)		1.98 (0.65)	
MPO	AA	361	84.84 (7.57)	0.9533	2.09 (0.72)	0.3095
(rs2071409)	AC	52	85.06 (7.53)		2.2 (0.74)	
	CC	2	85 (4)		2.1 (0.73)	
MPO	GG	330	84.82 (7.41)	0.5243	2.11 (0.72)	0.9751
(rs7208693)	GT	77	85.26 (8.22)		2.12 (0.76)	
	TT	8	86.5 (7.12)		2.15 (0.63)	

Table 8a: Mean levels of pulmonary function indices by smoking status

		All visits			First visit			Second visit			Third visit		
		N	**Mean (SD)**	**P-value**	**N**	**Mean (SD)**	**P-value**	**N**	**Mean (SD)**	**P-value**	**N**	**Mean (SD)**	**P-value**
FEV_1/ FVC	Current smoker	26	82.42 (8.39)	<0.0001	26	82.23 (8.41)	<0.0001	19	79.26 (8.54)	<0.0001	15	86.73 (6.56)	
	Ex-smoker	26	77.9 (9.79)		26	78.38 (7.16)		20	75.2 (12.33)		21	79.86 (9.79)	0.0004
	Never-smoker	366	85.61 (6.98)		366	85.91 (6.58)		268	83.86 (6.32)		238	87.12 (7.84)	
FEF_{25-75}	Current smoker	26	2.34 (0.83)	0.0312	26	2.36 (0.79)	0.2629	19	2.16 (0.83)	0.5098	15	2.56 (0.89)	
	Ex-smoker	26	2.04 (0.81)		26	2.03 (0.68)		20	1.95 (0.88)		21	2.14 (0.92)	0.1259
	Never-smoker	366	2.1 (0.71)		366	2.17 (0.72)		268	1.97 (0.66)		238	2.15 (0.74)	

Table 8b: Association of urinary phthalate metabolite levels and pulmonary function measurements, by smoking status[a]

		Current smoker (n=26)		Ex-smoker (n=26)		Never-smoker (n=366)		p- int
		β (SE)	p- value	β (SE)	p- value	β (SE)	p- value	
∑DEHP	FEV_1/FVC	-0.208 (1.757)	0.9066	0.066 (1.641)	0.9683	-0.615 (0.288)	0.0335	0.4176
	FEF_{25-75}	0.011 (0.159)	0.9433	-0.015 (0.114)	0.8956	-0.085 (0.026)	0.0009	0.5052
MnBP	FEV_1/FVC	3.462 (1.839)	0.0714	1.839 (2.204)	0.4103	-0.426 (0.307)	0.1653	0.1197
	FEF_{25-75}	0.228 (0.168)	0.1875	0.149 (0.151)	0.332	-0.04 (0.027)	0.1382	0.3399

[a] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dew point

Table 9a: Mean levels of pulmonary function indices by history of bronchial or lung disease

		All visits			First visit			Second visit			Third visit		
		N	**Mean (SD)**	**P-value**	**N**	**Mean (SD)**	**P-value**	**N**	**Mean (SD)**	**P-value**	**N**	**Mean (SD)**	**P-value**
FEV_1 /FVC	None / cured	409	84.92 (7.54)	0.5467	409	85.23 (6.98)	0.7038	299	83.15 (7.34)	0.0452	266	86.44 (8.2)	0.2584
	Diagnosed / in treatment	9	84 (8.22)		9	84.33 (8.54)		8	77.88 (6.56)		8	89.75 (4.95)	
FEF_{25-75}	None / cured	409	2.12 (0.73)	0.0186	409	2.17 (0.72)	0.3182	299	2 (0.68)	0.0087	266	2.18 (0.76)	0.5623
	Diagnosed / in treatment	9	1.77 (0.7)		9	1.93 (0.73)		8	1.35 (0.55)		8	2.02 (0.69)	

Table 9b: Association of urinary phthalate metabolite levels and pulmonary function measurements, on including or excluding subjects diagnosed or in current treatment of bronchial / lung disease[a]

		All subjects (n=418)		Excluding bronchial / lung disease history (n=409)	
		β (SE)	p- value	β (SE)	p- value
∑DEHP	FEV_1/FVC	-0.632 (0.286)	0.0275	-0.581 (0.29)	0.0456
	FEF_{25-75}	-0.077 (0.025)	0.002	-0.079 (0.025)	0.0017
MnBP	FEV_1/FVC	-0.212 (0.308)	0.4913	-0.166 (0.315)	0.5974
	FEF_{25-75}	-0.025 (0.027)	0.3457	-0.023 (0.027)	0.3886

[a] adjusted for age, sex, months past after each previous visit, BMI, creatinine-adjusted cotinine levels, mean temperature and mean dew point

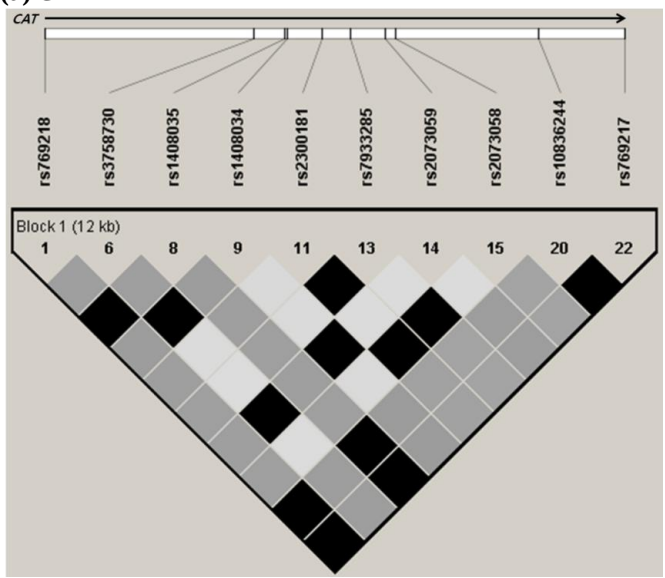
Table 10. Genetic information on selected *CAT, SOD2, MPO* SNPs

Gene (ID)	SNP no.	Chromosome no.	Allele	Position	Function	SNP to mRNA	Allele change, at mRNA level	Residue change, at protein level	Clinical Significance *
CAT (847)	**rs769218**	11	GA	intron					n/a
	rs769217	11	CT	codon389	cds-synon	forward	GA**C**→GA**T**	D[Aspt]→D[Asp]	n/a
SOD2 (6648)	**rs4880**	16	CT	codon16	missense	forward	G**T**T→G**C**T	V[Val]→A[Ala]	Probable-pathogenic
	rs2758331	16	AC	intron					n/a
	rs5746136	16	CT	downstream	UTR-3	forward	n/a	n/a	n/a
MPO (4353)	**rs7208693**	17	GT	codon53	missense	reverse	**G**TC→**T**TC	V[Val]→F[Phe]	n/a
	rs2071409	17	GT	intron					n/a

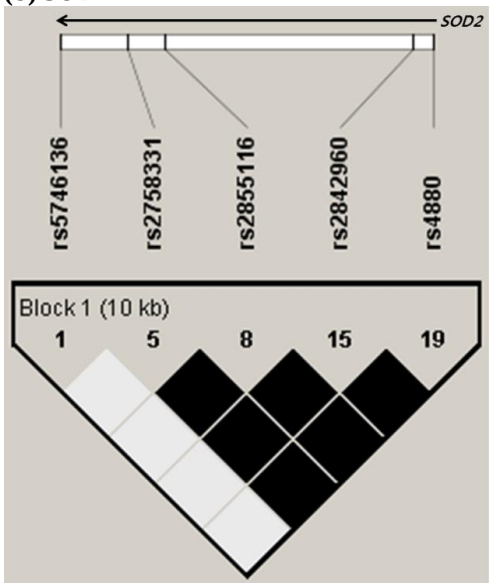
* supported values of clinical significance are categorized into 'unknown', 'untested', 'non-pathogenic', 'probable-non-pathogenic', 'probable-pathogenic', 'pathogenic', 'drug-response', 'histocompatibility', and 'other'.

Fig.1. Linkage disequilibrium plots for selected *CAT* (rs769218-rs769217) and *SOD2* (rs4880-rs27583331-rs5746136) SNPs using Japanese+Chinese SNP genotypes from HapMap data [1]

(a) *CAT* [2]



(b) *SOD2* [2]



[1] LD plots are displayed in r^2 color scheme (black: $r^2=1$, shades of gray: $0<r^2<1$, white: $r^2=0$), and SNPs with only $|D'|=1$ are presented. Arrows indicate direction of transcription.

[2] Ranges for r^2 in dark and light-grey colors are 0.356-0.397 and 0.135-0.149 respectively, and all LOD scores are greater than 3.

Four SNPs (rs2076556, rs2268063, rs710395, rs1055981), although with minor allele frequency (MAF) >0.001, were excluded from plotting as SNP pairing with these SNPs produced |D'| <1.

[3] Range for r^2 in light-grey colors is 0.081-0.085. LOD scores are greater than 3 in black colors, and 1.54-1.58 in light-grey colors

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국문 초록

서론: 프탈레이트는 흔한 환경 노출과 이에 따른 폐기능 감소 등의 건강 영향에 대해 우려를 일으키는 화학물질이며, 노령 인구는 이러한 영향에 대해 취약한 군이므로 이들 집단에서 프탈레이트 노출에 의한 폐기능 감소 또한 중요한 건강문제로 대두되고 있다. 국내 노령인구에서 프탈레이트 대사물질의 수치와 폐기능 지표 간 관련성을 조사하고, 이러한 관련성에 대한 산화손상 관련 유전자의 (*CAT, SOD2, MPO*) 가능한 효과 조정을 평가한다.

방법: 서울시에 거주하는 60 세 이상의 노인 418 명에 대상자에 대해 패널 연구를 수행하여, 요중 프탈레이트 대사물질 측정과 폐기능 검사를 반복하였고, *CAT* (rs769218, rs769217), *SOD2* (rs4880, rs2758331, rs5746136), *MPO* (rs2071409, rs7208693) 유전자의 유전자 다형성을 조사하였다. 혼합효과모델을 적용하여 프탈레이트 대사물질 수치와 폐기능 지표 간 관련성 및 이러한 관련성에 대한 *CAT, SOD2, MPO* 다형성의 영향을 분석하였다.

결과: Mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-

ethyl-5-oxohexyl) phthalate를 합한 수치와 (∑DEHP) FEV_1/FVC 및 FEF_{25-75}의 관련성은 연령, 이전 측정 후 지난 개월 수, 성별, 체질량지수, 코티닌 수치, 평균 기온 및 이슬점을 보정한 상태에서 유의한 역의 상관관계를 나타냈다 (P-values: FEV_1/FVC 0.0275, FEF_{25-75} 0.002). 프탈레이트 노출의 폐기능에 대한 영향은 *CAT* rs769218 중 GG 유전형, *CAT* rs769217의 CC 유전형, *SOD2* rs4880의 TT 유전형, *SOD2* rs2758331의 CC 유전형, *MPO* rs2071409의 AA 유전형, *MPO* rs7208693의 GG 유전형에서만 유의하게 나타났다. 세 가지 유전자에서 세 개의 유의한 이배체를 모두 가진 경우 그렇지 않은 경우에 비해 프탈레이트-폐기능 관련성에 있어서 차이를 나타내었다 (P-values for interaction: ∑DEHP-FEV_1/FVC 0.0176, ∑DEHP-FEF_{25-75} 0.0663)

결론: 국내 노령인구에서 요중 프탈레이트 대사물질 수치의 증가와 폐기능의 감소는 유의한 관련성을 나타내었으며, 이러한 관련성에 대한 몇몇 *CAT, SOD2, MPO* 유전자 다형성의 취약성 영향을 확인할 수 있었다.폐기능의 감소는 유의한 관련성을 나타내었으며, 이러한 관련성에 대한 몇몇 *CAT, SOD2, MPO* 유전자 다형성의 취약성 영향을 확인할 수 있었다.

주요어 : 프탈레이트, 폐기능, 유전자 다형성, 노령인구

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