Microsomal Epoxide Hydrolase, Endotoxin, and Lung Function Decline in Cotton Textile Workers

Jingqing Hang, Wei Zhou, Xiaorong Wang, Hongxi Zhang, Bixiong Sun, Helian Dai, Li Su, and David C. Christiani

Shanghai Putuo District People's Hospital, Shanghai, China; Occupational Health Program, Department of Environmental Health, Harvard School of Public Health; and Pulmonary and Critical Care Unit, Massachusetts General Hospital/Harvard Medical School, Boston, Massachusetts

Occupational exposure to endotoxin in organic dust may induce lung function decline. Microsomal epoxide hydrolase (mEH) detoxifies reactive oxygen species generated by endotoxin exposure, and polymorphisms of the *mEH* gene are associated with altered enzyme activity. We investigated the associations between *mEH* polymorphisms, endotoxin exposure, and lung function decline in a 20-year prospective study of 265 workers exposed to endotoxin and 234 control subjects. *mEH Tyr113His* and *His139Arg* polymorphisms were genotyped by the 5′ nuclease assay, and data were analyzed using multivariate linear regression models, adjusting for important covariates. Overall, the annual decline rate of FEV_1 was 29.47 ml during the 20-year follow-up. Endotoxin exposure was associated with faster lung function decline among genotypes associated with slower enzyme activity: estimates (SE) of annual FEV_1 decline rates for endotoxin exposure were −2.33 (2.07), −2.81 (1.66), and −6.73 (2.83) ml for *Tyr/Tyr*, *Tyr/His*, and *His/His* genotype groups, respectively, for the *Tyr113His* polymorphism; and −1.82 (2.58) and −4.27 (1.33) ml for *Arg/Arg* + *His/Arg* and *His/His* genotypes, respectively, for the *His139Arg* polymorphism. We conclude that *mEH* polymorphisms modify the association between occupational endotoxin exposure and longitudinal lung function decline.

Keywords: chronic obstructive pulmonary disease; microsomal epoxide hydrolase; occupational lung disease; polymorphism

Epidemiologic studies have shown that chronic occupational exposure to organic dust may induce chronic airway disease (1–3). Among many agents present in organic dusts, there is substantial evidence to suggest that bacterial endotoxin is a major causative agent that contributes to airway inflammation and airflow obstruction (4, 5). The concentration of inhaled endotoxin in bioaserosol is associated strongly with the development of airway disease in cotton and agricultural workers (4, 6, 7). Exposure to endotoxin may result in endothelial cell damage (8), influx of blood leukocytes into the airway (9), production of different cytokines (10, 11), as well as increased production of oxygen metabolites, such as reactive oxygen species (ROS) that may induce oxidative lung injury (12, 13).

The epoxide hydrolases are a family of enzymes that hydrate simple epoxide to vicinal diols and arene oxides to trans-dihydrodiols (14). Among this family, microsomal epoxide hydrolase (mEH) plays an important role in the detoxification of ROS and reactive epoxide intermediates (15). In the coding region of the *mEH* gene, two relatively common genetic polymorphisms are characterized within exon 3 and exon 4. In exon 3, the *Tyr113His* (*C113T*) polymorphism results in a 40 to 50% decrease in enzyme activity, and, thus, the *His* allele has been called the "slow allele". In exon 4, the *His139Arg* (*G139A*) polymorphism is associated with a 25% increase of enzyme activity, with the *Arg* allele termed the "fast allele" (16). Slow activity *mEH* genotypes have been associated with chronic obstructive pulmonary disease (COPD) in some studies (17–21), but not in others (22–26).

Our previous studies have shown that long-term occupational exposure to cotton dust may induce a faster decline of lung function, and this decline probably is more closely related to high concentrations of endotoxin in cotton dust (6). mEHs metabolize the ROS generated by endotoxin exposure, therefore, polymorphisms of *mEH* with different enzyme activities may modify the associations between endotoxin exposure and lung function decline. Specifically, we hypothesized that the associations between endotoxin exposure and lung function decline will be stronger in genotypes with "slow" enzyme activities than in genotypes with "fast" enzyme activities. We tested these prior hypotheses in our 20-year longitudinal cohort of cotton textile workers.

METHODS

Additional detail is provided in the online supplement.

Subjects

The study population was derived from a population-based, 20-year longitudinal cohort for respiratory disease among cotton textile workers in Shanghai, China, which was established in 1981. Details of this cohort have been described previously (4). The first survey included 919 subjects (447 cotton and 472 silk workers), and 4 follow-up surveys were performed every 5 years in the following 20 years. The study was approved by the Institutional Review Boards of the Harvard School of Public Health, the Putuo District People's Hospital, and the Human Resources Administration of China. Written informed consent was obtained from each participant.

Exposure Assessment and Pulmonary Function Tests

Airborne cotton dust was measured with a Vertical Elutriator (General Metalworks Corp., Mequon, WI), and gram-negative bacteria endotoxins were analyzed using the chromogenic Limulus amoebocyte lysate assay, as previously described (27, 28). Forced expiratory maneuver was performed at each survey (4). Annual decline in FEV_1 was calculated on the basis of the differences in preshift FEV_1 measurements between the last and first survey and was divided by 20 (years).

Genotyping

DNA was extracted from whole blood using a standard method. The *mEH Tyr113His* and *His139Arg* polymorphisms were genotyped by

(*Received in original form July 9, 2004; accepted in final form October 31, 2004*)

Supported by grant R01OH02421 from the National Institute for Occupational Safety and Health, grant TW00828 from the Fogarty International Institute of the National Institutes of Health (J.H.), and a Flight Attendants Medical Research Young Clinical Scientist Award (W.Z.).

Correspondence and requests for reprints should be addressed to David C. Christiani, M.D., M.P.H., Harvard School of Public Health, 665 Huntington Avenue, Building I-1407, Boston, MA 02115. E-mail: dchristi@hsph.harvard.edu

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 171. pp 165–170, 2005
Originally Published in Press as DOI: 10.1164/rccm.200407-888OC on November 5, 2004
Internet address: www.atsjournals.org

the 5′ nuclease assay (TaqMan) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The primers, probes, and reaction conditions are available on request.

Statistical Analysis

Population characteristics were tabulated, and significant differences in the distribution of the principal covariates were tested using the Chi-square, Fisher exact, and Student's *t* tests, where appropriate. Multivariate linear regression models were used to explore the association between *mEH* polymorphisms, alone or in combination with endotoxin exposure, and the annual decline rate of FEV_1 (as continuous variable), before and after adjusting for covariates, including age, sex, height, baseline FEV_1, smoking status, and packyears of smoking (29). For the *His139Arg* polymorphism, the *His/Arg* and *Arg/Arg* genotypes were combined because of low frequency for the *Arg/Arg* genotype. In all of the analyses, the *Tyr/Tyr* genotype of the *Tyr113His* polymorphism and *His/Arg* + *Arg/Arg* genotype of the *His139Arg* polymorphism were used as reference category, respectively, on the basis of the convention of faster enzyme activity for these genotypes. Three types of models were used to fit the data. Model I was fit to determine the overall association between *mEH* polymorphisms and lung function decline (main effect). At the same time, interactions among *mEH* polymorphisms, endotoxin exposure, smoking, as well as sex and age were determined, and they were retained in the model if reaching significance. Model II was to examine the modification effect of *mEH* polymorphisms on the association between endotoxin exposure and lung function decline, *i.e.,* stratified analysis of endotoxin exposure and lung function decline in each genotype of *mEH* polymorphisms. Model III was used to investigate the joint effects of *mEH* polymorphisms and endotoxin exposure on the decline of lung function, using the combined *mEH* "fast" genotype (the two polymorphisms were analyzed in different models) and non–endotoxin-exposed as the common reference group. Statistical analyses were undertaken using the SAS personal computer software (version 8.2, 1999) (SAS Institute, Cary, NC).

RESULTS

Endotoxin Exposure Data

A total of 802 fullshift air samples were collected over the first four surveys in the yarn preparation areas of the two cotton mills and the silk mill. For the two cotton mills, geometric mean dust exposure of the work areas ranged from 0.2 to 1.6 mg/m^3 and geometric mean endotoxin of the work areas ranged from 27 to 12,038 EU/m^3. Because cotton dust and endotoxin levels in the silk mill were identified as nearly zero over the period of the follow-up study (4, 30), we stratified this population into two groups (non–endotoxin-exposed and endotoxin exposed) on the basis of occupation (silk or cotton workers).

Population Characteristics

Five hundred one of 919 subjects participated in the last survey and donated blood samples, including 234 (49.58%) in the non–endotoxin-exposed group and 267 (59.73%) in the endotoxin-exposed group. There were no significant differences in age, sex, height, smoking status, and baseline FEV_1 between subjects with and without blood samples, in both nonexposed and exposed groups ($p > 0.05$).

A total 499 of 501 subjects were genotyped successfully for both *Tyr113His* and *His139Arg* polymorphisms, including 234 in the non–endotoxin-exposed group and 265 in the endotoxin-exposed group. Details of this population were presented in Table 1. There were no significant differences between non–endotoxin-exposed and endotoxin-exposed groups in age, sex, height, smoking status, and baseline FEV_1 ($p > 0.05$). The smoking amount among smokers was higher in the endotoxin-exposed group, with mean (SD) packyears of 29.04 (20.47) in endotoxin-exposed and 24.88 (15.87) in nonexposed groups, respectively ($p < 0.05$).

Genotype Distributions

Genotype frequencies of both *Tyr113His* and *His139Arg* polymorphisms were in Hardy-Weinberg equilibrium in both non–endotoxin-exposed and exposed groups ($p > 0.05$ by Chi-square test). There were no significant differences in the frequencies of *mEH* genotypes between non–endotoxin-exposed and exposed groups. For the *Tyr113His* polymorphism, the frequencies of *Tyr/Yyr, Tyr/His*, and *His/His* genotypes were 33.76%, 50.00%, and 16.24% in the non–endotoxin-exposed group, and 30.19%, 49.43%, and 20.38% in the exposed group, respectively ($p = 0.44$). For the *His139Arg* polymorphism, genotype frequencies of *Arg/Arg*, *His/Arg*, and *His/His* were 0.85%, 23.08%, and 76.07% in the non–endotoxin-exposed group and 1.51%, 21.13%, and 77.36% in the exposed group, respectively ($p = 0.71$, Table 1).

mEH Polymorphisms and Lung Function Decline

Age, sex, height, smoking status, packyears of smoking, and baseline FEV_1 were all included in the multivariate linear regression model initially (Table 2). Sex and age were excluded in the final models because they were highly correlated with baseline FEV_1 ($r = 0.59$ between sex and baseline FEV_1 and $r = 0.74$ between height and baseline FEV_1, $p < 0.001$) and they turned out to be insignificant after baseline FEV_1 was included. In all of the analyses, age and packyears of smoking baseline FEV_1 were fitted as continuous variables.

The overall annual FEV_1 decline rate was −29.47 ml in the study population ("−" represents the direction of decline). Age, packyears of smoking, baseline FEV_1, and endotoxin exposure were all associated with an annual decline rate of FEV_1 ($p < 0.01$; Table 2). Among these, exposure to endotoxin caused a −3.23 ml (SE = 1.14) annual decline of FEV_1. There were no statistically significant associations between *mEH* polymorphisms and lung function decline (Model I). For the *Tyr113His* polymorphism, when compared with the *Tyr/Tyr* genotype, estimates of the annual decline rate of FEV_1 in *Tyr/His* and *His/His* genotypes were 0.03 ml (SE = 1.31) and −0.61 ml (SE = 1.69), respectively. For the *His139Arg* polymorphism, the estimate of the annual decline rate of FEV_1 for the *His/His* genotype was −1.87 ml (SE = 1.36) when compared with the combined *Arg/Arg* + *His/Arg* genotypes.

Second, we investigated the modification effect by *mEH* polymorphisms on the association between endotoxin exposure and the rate of lung function decline (Model II). Although endotoxin exposure was associated with a faster lung function decline in each *mEH* genotype group, the differences between exposed and nonexposed groups increased with the mEH enzyme activity decreased for both polymorphisms. For the *Tyr113His* polymorphism, the differences in estimates of the annual decline rate of FEV_1 between endotoxin-exposed and nonexposed groups were −2.33 ml (SE = 2.07, p = 0.26), −2.81 ml (SE = 1.66, p = 0.09), and −6.73 ml (SE = 2.83, $p < 0.05$) for the *Tyr/Tyr*, *Tyr/His*, and *His/His* genotype groups, respectively. For the *His139Arg* polymorphism, the difference between endotoxin-exposed and nonexposed groups was −1.82 ml (SE = 2.58, p = 0.48) and −4.27 ml (SE = 1.33, $p < 0.01$) for the *Arg/Arg* + *His/Arg* and *His/His* genotype groups, respectively (Table 3). When stratifying smoking status, we found that although cigarette smoking was related to additional lung function decline in all of the genotype groups, the modification effect of *mEH* polymorphisms on endotoxin exposure existed in both never and ever smokers (Table 3), and the three-way interactions between endotoxin exposure, smoking, and *mEH* polymorphisms were not statistically significant ($p > 0.05$).

Lastly, we investigated a potential joint effect of *mEH* polymorphisms and endotoxin exposure on the rate of lung function

TABLE 1. BASIC CHARACTERISTICS OF SUBJECTS IN STUDY GROUPS

	Non–Endotoxin-Exposed[§] (*n = 234*)	Endotoxin-Exposed[§] (*n = 265*)	p Value[¶]
Sex, n (%)			
Male	100 (42.74)	111 (41.89)	0.85
Female	134 (57.26)	154 (58.11)	
Age, yr, mean (SD)*	56.00 (9.72)	56.67 (10.09)	0.56
Height, cm, mean (SD)*	163.42 (7.50)	163.65 (7.26)	0.53
Smoking status, n (%)			
Ever smoker	73 (31.20)	82 (30.94)	0.95
Never smoker	161 (68.80)	183 (69.06)	
Packyears, mean (SD)†	24.88 (15.87)	29.04 (20.47)	0.03
Baseline FEV_1, ml, mean (SD)	2,891.51 (0.68)	2,894.54 (0.71)	0.40
Annual decline rate of FEV_1, ml/yr, mean (SD)‡	−27.31 (14.94)	−31.37 (16.76)	< 0.01
Genotypes, n (%)			
Tyr113His polymorphism			
Tyr/Tyr	79 (33.76)	80 (30.19)	0.44
Tyr/His	117 (50.00)	131 (49.43)	
His/His	38 (16.24)	54 (20.38)	
His139Arg polymorphism			
Arg/Arg	2 (0.85)	4 (1.51)	0.71
His/Arg	54 (23.08)	56 (21.13)	
His/His	178 (76.07)	205 (77.36)	

* Age and height at the 2001 survey.
† Only for smokers.
‡ Annual decline rate of FEV_1 was expressed as: (FEV1 in 2001 − FEV_1 in 1981)/20.
§ On the basis of endotoxin exposure status.
¶ *t* Test for age, height, packyears of smoking, baseline FEV_1, and annual decline rate of FEV_1; Chi-square test for sex, smoking status, and genotypes.

decline (Model III). In this model, five (*Tyr113His* polymorphism) or three (*His139Arg* polymorphism) dummy variables were created for the combination of *mEH* genotypes and endotoxin exposure. The results from this model were similar to the results from the gene-endotoxin exposure interaction model. For the *Tyr113His* polymorphism, the combined *His/His* genotype and endotoxin-exposed group was associated with the fastest annual FEV_1 decline (−4.91 ml, SE = 2.26, p = 0.03), when compared with the reference group of the combined *Tyr/Tyr* genotype and non–endotoxin-exposed group. A similar association was found for the *His139Arg* polymorphism: the combined *His/His* genotype and endotoxin-exposed group was associated with the fastest annual FEV_1 decline (−4.37 ml, SE = 1.91, p = 0.02), when compared with the reference group of combined *Arg/Arg* + *His/Arg* genotype and nonexposed group (Table 4).

DISCUSSION

Occupational exposure to cotton dust has been shown to cause airway disease characterized by acute changes in airflow and by chronic obstructive lung disease (30). Endotoxin is the principal component of cotton responsible for the development of inflammation of the airway and airflow obstruction (5). LPS intraperitoneal injection results in a significant increase of ROS production by peritoneal leukocytes, generating an oxidative stress situation (12). *In vitro*, after the human monocyte THP-1 cell line was treated with different doses of LPS, LPS elicited a dose-dependent oxidative burst (13). The oxidant/antioxidant theory postulates that an excess of oxidants in the lung promotes cellular and tissue damage, and is the major initiator of the disease process (31, 32). Our previous results have suggested that chronic occupational endotoxin exposure is associated with faster decline of lung function (4).

Although endotoxin exposure is associated with oxidative stress and airway inflammation, not all workers who are exposed to endotoxin develop airway disease. Healthy volunteers who were challenged with LPS had different responsiveness to inhaled LPS (33), suggesting that host factors may play an important role in the response to endotoxin exposure. In this study,

TABLE 2. ESTIMATES (SE) OF ANNUAL DECLINE RATE OF FEV_1 OVER THE 20-YEAR FOLLOW-UP*

	Annual Decline Rate in FEV_1	p Value
Intercept	38.29 (20.31)	0.06
Age, yr†	−0.66 (0.08)	< 0.001
Sex (male vs. female)	2.68 (2.32)	0.25
Height, cm†	0.07 (0.13)	0.56
Smoking status (ever smokers vs. never smokers)	−1.00 (2.35)	0.67
Packyears of smoking†	−0.25 (0.06)	< 0.001
Baseline FEV_1, ml	−0.01 (0.001)	< 0.001
Endotoxin-exposed vs. nonexposed	−3.23 (1.14)	< 0.01
Tyr*113His* polymorphism - *Tyr/His* vs. *Tyr/Tyr*	0.03 (1.31)	0.98
Tyr113His polymorphism - *His/His* vs. *Tyr/Tyr*	−0.61 (1.69)	0.72
His139Arg polymorphism - *His/His* vs. *Arg/Arg* + *His/Arg*	−1.87 (1.36)	0.17

* Using multivariate liner regression models; "−" represents the direction of decline.
† Continuous variable.

TABLE 3. ESTIMATES (SE) OF ANNUAL DECLINE RATE OF FEV_1 OVER THE 20-YEAR FOLLOW-UP IN RELATION TO ENDOTOXIN EXPOSURE BY SMOKING STATUS AND *mEH* POLYMORPHISMS*

Genotype	Total (*n = 499*)	Ever Smoker (*n = 155*)	Never Smoker (*n = 344*)
Tyr113His polymorphism			
Tyr/Tyr	−2.33 (2.07)	−6.35 (4.24)	0.51 (2.07)
Tyr/His	−2.81 (1.66)	−5.40 (3.50)	−1.49 (1.87)
His/His	−6.73 (2.83)†	−9.02 (5.57)	−6.61 (2.84)†
His139Arg polymorphism			
Arg/Arg + His/Arg	−1.82 (2.58)	−3.28 (5.67)	0.27 (2.65)
His/His	−4.27 (1.33)‡	−6.47 (2.69)†	−2.79 (1.44)§

* Using multivariate linear regression models, adjusting for age, packyears of smoking, and baseline FEV_1; the "−" represents the direction of decline.

† $p < 0.05$ (endotoxin-exposed vs. non–endotoxin-exposed).

‡ $p < 0.01$ (endotoxin-exposed vs. non–endotoxin-exposed).

§ $p = 0.05$ (endotoxin-exposed vs. non–endotoxin-exposed).

we investigated the relationship between *mEH* polymorphisms and the annual decline rate of lung function in a population chronically exposed to endotoxin. Our results suggest that the *mEH His/His* genotypes of both *Tyr113His* and *His139Arg* polymorphisms associated with lower enzyme activities may increase lung function decline induced by endotoxin exposure. The modification effects of *mEH* polymorphisms on the association between endotoxin exposure and faster decline of lung function existed in both never and ever smokers.

The mEH enzyme is an important Phase II biotransformation enzyme, and it is highly expressed in several human tissues including bronchial epithelial cells (34), where it catalyzes the hydrolysis of various epoxides and reactive epoxide intermediates into less reactive and more water-soluble dihydrodiols, which then are excreted from the body (14, 35, 36). Hence, mEH is a protective enzyme involved in general oxidative defense against a number of environmental and endogenous stimulants including endotoxin exposure. Genotypes that are associated with "slow" enzyme activity will lead to inefficient metabolizing of ROS generated by endotoxin exposure, and may eventually induce faster lung function decline. Previous *in vitro* studies have suggested that the *His/His* genotypes of both *Tyr113His* and *His139Arg* polymorphisms are associated with decreased enzyme activity (16). Therefore, we observed the fastest annual decline rate of lung function associated with endotoxin exposure in the *His/His* genotypes of both *mEH* polymorphisms (Table 3).

Inconsistent associations between *mEH* polymorphisms and the development of COPD were suggested in different studies. The "slow" *mEH* genotypes were associated with higher susceptibility or severity of COPD in five studies (17–21), but not in others (22–26). In one study with 591 white COPD patients who smoked, the "slow" *mEH* genotypes were associated with rapid decline of lung function (19). The inconsistent results may be due to the differences in the study population or environmental exposure levels. Unlike our 20-year prospective population, the majority of the previous studies were hospital-based case-control studies with the outcome of the development of COPD. Our results support the hypothesis that *mEH* genotype alone may only have a slight effect on lung function decline; however, *mEH* polymorphisms may modify the effects of endotoxin exposure on lung function decline. In the *mEH* polymorphisms-endotoxin joint effects analyses, the joint effects of endotoxin exposure and "slow" *mEH* genotypes are significantly related to faster decline in lung function, with a stronger effect than genotype or endotoxin exposure alone (Table 4), further suggesting the importance of investigating both genetic and environmental factors (endotoxin exposure and smoking) in lung function decline and the development of COPD.

In addition to endotoxin exposure, cigarette smoking is another important environmental factor for lung function decline. The mEH enzyme is also involved in the metabolism of smoking-induced highly reactive epoxide intermediates. However, unlike

TABLE 4. JOINT EFFECTS OF *mEH* POLYMORPHISMS AND ENDOTOXIN EXPOSURE ON ESTIMATES (SE) OF ANNUAL DECLINE RATE OF FEV_1 OVER THE 20-YEAR FOLLOW-UP*

Genotype	Non–Endotoxin-Exposed	Endotoxin-Exposed	Marginal
Tyr113His polymorphism			
Tyr/Tyr	Ref†	−2.13 (2.02)	Ref
Tyr/His	0.24 (1.86)	−2.66 (1.82)	−0.14 (1.30)
His/His	1.63 (2.51)	−4.91 (2.26)‡	−0.86 (1.67)
Marginal	Ref	−3.34 (1.14)§	
His139Arg polymorphism			
Arg/Arg + His/Arg	Ref†	−1.04 (2.35)	Ref
His/His	−0.40 (1.94)	−4.37 (1.91)‡	−1.91 (1.34)
Marginal	Ref	−3.34 (1.14)§	

Definition of abbreviations: marginal = no interaction term in the model; ref = reference group.

* Using multivariate linear regression models, adjusting for age, smoker status (ever smokers and never smokers), packyears of smoking, and baseline FEV_1; "−" represents the direction of decline.

† *Tyr/Tyr* genotype of *Tyr113His* polymorphism and *Arg/Arg + His/Arg* genotype of *His139Arg* polymorphism in non–endotoxin-exposed groups serve as reference.

‡ $p < 0.05$ (compared with ref).

§ $p < 0.01$ (compared with ref).

the metabolism of mEH on endotoxin-generated ROS, mEH has both Phase I and Phase II activity for tobacco chemical (37), which may be one reason that we did not find statistically significant interactions between cigarette smoking and *mEH* genotypes (data not shown). In this study, we found that although cigarette smoking induced additional lung function decline in different genotype groups, the modification effect of *mEH* polymorphisms on endotoxin exposure existed in both never smokers and ever smokers. The associations between *mEH* genotypes and endotoxin exposure were consistent in different models, including stratified analyses by *mEH* genotypes (Table 3) as well as gene-endotoxin exposure joint analyses (Table 4). We cannot exclude the possibility of gene-smoking interactions existing in nonoccupational populations, because our results were generated from the occupational cohort.

There are several limitations to our study. First, this is a moderate sample size study, especially in the stratified analyses by genotype groups. In this study, the study population was derived from a 20-year longitudinal cohort. In the initial enrollment, subjects were excluded if they had a history of respiratory diseases, and only healthy subjects were included in this cohort. We have complete demographic, smoking, and genotype data, and adjusted possible confounders in the multivariate liner regression models. Although a higher proportion of people in the endotoxin-exposed group donated blood samples (59.73%) than in the nonexposed group (49.58%), blood donations were random and not on the basis of genotypes, and the demographic distributions (age, sex, height, smoking, and baseline FEV_1) were similar between those with and without blood samples. Second, the use of area rather than personal endotoxin exposure data may have introduced some exposure misclassification. However, we collected complete environmental monitoring data, which allowed us to stratify the population into endotoxin-exposed and nonexposed groups, and to investigate the association between endotoxin exposure and lung function decline. Lastly, the enzyme activity associated with *mEH* genotypes was derived from *in vitro* studies, which might induce some misclassification for the function of *mEH* genotypes. We have observed consistent results for both of the *mEH* polymorphisms and in different multivariate regression models.

In conclusion, *mEH* polymorphisms play an important role in the association between endotoxin exposure and lung function decline. Our results suggest that *mEH* "slow" genotypes increase the annual decline rate of lung function for subjects who are occupationally exposed to airborne endotoxin.

Conflict of Interest Statement: J.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; W.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; X.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; H.Z. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; B.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; H.D. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; L.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this article; D.C.C. does not have a financial relationship with a commercial entity that has an interest in the subject of this article.

Acknowledgment: The authors thank the members of the Shanghai field team; Professors Gu Xue-qi, Lu Pei-lian, and Ye Ting-ting from Shanghai Medical University; the Shanghai Textile Bureau; and workers and members of the First and Second Textile Mills and the First Silk Mill of Shanghai, China. They also thank Janna Frelich for data management and Dr. Stephen Olenchock of the National Institute for Occupational Safety and Health for endotoxin analyses.

References

1. Chan-yeung M, Schulzer M, MacLean L, Dorken E, Tan F, Lam S, Enarson D, Grzybowski S. A follow-up study of the grain elevator workers in the Port of Vancouver. *Arch Environ Health* 1981;36:75–81.
2. Glindmeyer HW, Lefante JJ, Jones RN, Rando RJ, Weill H. Cotton dust and across-shift change in FEV_1 as predictors of annual change in FEV_1. *Am J Respir Crit Care Med* 1994;149:584–590.
3. Schwartz DA, Donham KJ, Olenchock SA, Popendorf W, van Fossen DS, Burmeister LF, Merchant JA. Determinants of longitudinal changer in spirometric functions among swine confinement operators and farmers. *Am J Respir Crit Care Med* 1995;151:47–53.
4. Christiani DC, Wang XR, Pan LD, Zhang HX, Sun BX, Dai H, Eisen EA, Wegman DH, Olenchock SA. Longitudinal changes in pulmonary function and respiratory symptoms in cotton textile workers: a 15-yr follow-up study. *Am J Respir Crit Care Med* 2001;163:847–853.
5. Rylander R, Bake B, Fischer JJ, Helander IM. Pulmonary function and symptoms after inhalation of endotoxin. *Am Rev Respir Dis* 1989;140: 981–986.
6. Wang XR, Eisen EA, Zhang HX, Sun BX, Dai HL, Pan LD, Wegman DH, Olenchock SA, Christiani DC. Respiratory symptoms and cotton dust exposure; results of a 15 year follow up observation. *Occup Environ Med* 2003;60:935–941.
7. Schwartz DA, Thorne PS, Yagla SJ, Burmeister LF, Olenchock SA, Watt JL, Quinn TJ. The role of endotoxin in grain dust-induced lung disease. *Am J Respir Crit Care Med* 1995;152:603–608.
8. Lantz RC, Birch K, Hinton DE, Burrell R. Morphometric changes of the lung induced by inhaled bacterial endotoxin. *Exp Mol Pathol* 1985; 43:305–320.
9. Toward TJ, Broadley KJ. Chronic lipopolysaccharide exposure on airway function, cell infiltration, and nitric oxide generation in conscious guinea pigs: effect of rolipram and dexamethasone. *J Pharmacol Exp Ther* 2001;298:298–306.
10. Nightingale JA, Rogers DF, Hart LA, Kharitonov SA, Chung KF, Barnes PJ. Effect of inhaled endotoxin on induced sputum in normal, atopic, and atopic asthmatic subjects. *Thorax* 1998;53:563–571.
11. Michel O, Nagy AM, Schroeven M, Duchateau J, Neve J, Fondu P, Sergysels R. Dose–response relationship to inhaled endotoxin in normal subjects. *Am J Respir Crit Care Med* 1997;156:1157–1164.
12. Victor VM, De la Fuente M. Several functions of immune cells in mice changed by oxidative stress caused by endotoxin. *Physiol Res* 2003;52: 789–796.
13. Ryan KA, Smith MF Jr, Sanders MK, Ernst PB. Reactive oxygen and nitrogen species differentially regulate Toll-like receptor 4-mediated activation of NF-kappa B and interleukin-8 expression. *Infect Immun* 2004;72:2123–2130.
14. Curtis JO, Hassett C, Hosagrahara V. Epoxide hydrolase – polymorphism and role in toxicology. *Toxicol Lett* 2000;112–113:365–370.
15. Fretland AJ, Omiecinski CJ. Epoxide hydrolases: biochemistry and molecular biology. *Chem Biol Interact* 2000;129:41–59.
16. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum Mol Genet* 1994;3:421–428.
17. Smith CA, Harrison DJ. Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema. *Lancet* 1997;350:630–633.
18. Xiao D, Wang C, Du MJ, Pang BS, Zhang HY, Xiao B, Liu JZ, Weng XZ, Su L, Christiani DC. Association between polymorphisms in the microsomal epoxide hydrolase (mEH) gene and chronic obstructive pulmonary disease. *Zhonghua Yi Xue Za Zhi* 2003;83:1782–1786.
19. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Pare PD. Susceptibility genes for rapid decline of lung function in the lung health study. *Am J Respir Crit Care Med* 2001;163:469–473.
20. Rodriguez F, Jardi R, Costa X, Juan D, Galimany R, Vidal R, Miravitlles M. Detection of polymorphisms at exons 3 (Tyr113→His) and 4 (His139→Arg) of the microsomal epoxide hydrolase gene using fluorescence PCR method combined with melting curves analysis. *Anal Biochem* 2002;308:120–126.
21. Yoshikawa M, Hiyama K, Ishioka S, Maeda H, Maeda A, Yamakido M. Microsomal epoxide hydrolase genotypes and chronic obstructive pulmonary disease in Japanese. *Int J Mol Med* 2000;5:49–53.
22. Takeyabu K, Yamaguchi E, Suzuki I, Nishimura M, Hizawa N, Kamakami Y. Gene polymorphism for microsomal epoxide hydrolase and susceptibility to emphysema in a Japanese population. *Eur Respir J* 2000;15: 891–894.
23. Yim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG. Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. *Thorax* 2000;55: 121–125.
24. Budhi A, Hiyama K, Isobe T, Oshima Y, Hara H, Maeda H, Kohno N. Genetic susceptibility for emphysematous changes of the lung in Japanese. *Int J Mol Med* 2003;11:321–329.

25. Zhang R, Zhang A, He Q, Lu B. Microsomal epoxide hydrolase gene polymorphism and susceptibility to chronic obstructive pulmonary disease in Han nationality of North China. [Article in Chinese] *Zhonghua Nei Ke Za Zhi* 2002;41:11–14.
26. Cheng SL, Yu CJ, Chen CJ, Yang PC. Genetic polymorphism of epoxide hydrolase and glutathione S-transferase in COPD. *Eur Respir J* 2004; 23:818–824.
27. Christiani DC, Ye TT, Wegman DH, Eisen EA, Dai HL, Lu PL. Pulmonary function among cotton textile workers: a study of variability in symptom reporting, across-shift drop in FEV_1, and longitudinal change. *Chest* 1994;105:1713–1721.
28. Olenchock SA, Christiani DC, Mull JC, Ye-TT, Lu PL. Airborne endotoxin concentrations in various work areas within two cotton mills in the People's Republic of China. *Biomed Environ Sci* 1990;3:443–451.
29. Kleinbaum DG, Kupper LL, Muller KE, Nizam A. Applied regression analysis and other multivariable methods. Pacific Grove: Duxbury Press; 1998.
30. Christiani DC, Ye TT, Zhang S, Wegman DH, Eisen EA, Ryan LA, Olenchock SA, Pothier L, Dai HL. Cotton dust and endotoxin exposure and long-term decline in lung function: results of a longitudinal study. *Am J Ind Med* 1999;35:321–331.
31. Farber JL. Mechanisms of cell injury by activated oxygen species. *Environ Health Perspect* 1994;102(S10):17–24.
32. Tsuji C, Minhaz MU, Shioya S, Fukahori M, Tanigaki T, Nakazawa H. The importance of polymorphonuclear leukocytes in lipopolysaccharide-induced superoxide anion production and lung injury: *ex vivo* observation in rat lungs. *Lung* 1998;176:1–13.
33. Kline JN, Cowden JD, Hunninghake GW, Schutte BC, Watt JL, Wohlford-Lenane CL, Powers LS, Jones MP, Schwartz DA. Variable airway responsiveness to inhaled lipopolysaccharide. *Am J Respir Crit Care Med* 1999;160:297–303.
34. Willey JC, Coy E, Brolly C, Utell MJ, Frampton MW, Hammersley J, Thilly WG, Olson D, Cairns K. Xenobiotic metabolism enzyme gene expression in human bronchial epithelial and alveolar macrophage cells. *Am J Respir Cell Mol Biol* 1996;14:262–271.
35. Archelas A, Furstoss R. Epoxide hydrolases: new tools for the synthesis of fine organic chemicals. *Trends Biotechnol* 1998;16:108–116.
36. Omiecinski CJ, Aicher L, Holubkov R, Checkoway H. Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. *Pharmacogenetics* 1993;3:150–158.
37. Georgiadis P, Demopoulos NA, Topinka J, Stephanou G, Stoikidou M, Bekyrou M, Katsouyianni K, Sram R, Autrup H, Kyrtopoulos SA. Impact of phase I or phase II enzyme polymorphisms on lymphocyte DNA adducts in subjects exposed to urban air pollution and environmental tobacco smoke. *Toxicol Lett* 2004;149:269–280.