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ORIGINAL ARTICLE

Polymorphism of microsomal epoxide hydrolase is associated with chronic obstructive pulmonary disease and bronchodilator response

Chiung-Zuei Chen [a], Ru-Hsueh Wang [b], Cheng-Hung Lee [a], Chien-Chung Lin [a], Han-Yu Chang [a], Tzuen-Ren Hsiue [a,*]

[a] *Division of Chest Medicine, Department of Internal Medicine, National Cheng Kung University Medical College and Hospital, Tainan, Taiwan*
[b] *Department of Family Medicine, National Cheng Kung University Medical College and Hospital, Tainan, Taiwan*

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Background/Purpose: Microsomal epoxide hydrolase (EPHX) is an important enzyme that metabolizes harmful reactive epoxides from smoking. Genetic variations of this enzyme are thought to increase the risk of developing chronic obstructive pulmonary disease (COPD). The aim of this study was to confirm and advance our knowledge of the role of this genetic variation in COPD. In addition, the association between this gene and important COPD-related phenotype bronchodilator responses (BDRs) was studied.
Methods: This was a hospital-based case–control study. The EPHX1 genetic mutations of 105 smokers with COPD and 103 control smokers without COPD were evaluated by polymerase chain reaction, followed by restriction fragment length polymorphism analysis. The association of genetic mutations and COPD phenotypes was also studied.
Results: Subjects with EPHX1 113 (His^{113}/His^{113}) homozygote mutation had a strong correlation with COPD (odds ratio: 2.7, 95% confidence interval: 1.5–5.2). In addition, compared with other genotypes, the His^{113} homozygote mutation patients had significantly lower BDRs, as shown by the absolute and percentage changes from baseline, in COPD patients (91.7 ± 12.5 mL *vs.* 141.6 ± 15.1 mL, $p = 0.01$ and $8.3 \pm 1.2\%$ *vs.* $13.4 \pm 1.4\%$, $p = 0.006$).
Conclusion: A strong correlation between the EPHX1 113 mutant homozygote and smoking-related COPD was noted. This genetic polymorphism was also associated with lower BDRs in COPD patients.

* Corresponding author. Department of Internal Medicine, National Cheng Kung University Hospital, No. 138, Sheng-Li Road, Tainan, 704, Taiwan.
E-mail address: hsiue@mail.ncku.edu.tw (T.-R. Hsiue).

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Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world, and further increases in its prevalence and mortality can be predicted in the coming decades. The World Health Organization has predicted that it will be the third leading cause of death in the world by the year 2020.[1] However, there are still no effective drug therapies for COPD that alter disease progression. Genetic studies of COPD have provided a new approach to monitor disease with disease onset and disease progression, define disease subtypes, and predict responses to therapy. Genetic information may be used to develop more target drugs and finally may lead to new treatments.[2] Genetic association studies have implicated a variety of genes in COPD pathogenesis, such as tumor necrosis factor-α, glutathione S-transferase, and epoxide hydrolase (EPHX) 1 for COPD patients in Taiwan.[3,4]

Microsomal epoxide hydrolase (mEPHX) is an important enzyme that metabolizes harmful reactive epoxides from smoking. Genetic variations of this enzyme, including the His^{113}/His^{113} mEPHX exon-3 mutation (EPHX1 113 mutant homozygote), are thought to increase the risk of developing COPD.[5–7] The current problem in studies of genetic polymorphism of EPHX1 in COPD is the different results from various studies.[5–10] These may occur because of the phenotype definitions used or ethnic differences. A systematic review and meta-analysis of the published data may afford us a powerful tool to elucidate this association. However, two recent meta-analyses showed opposite results for the EPHX1 113 mutant homozygote in COPD. One reported it had a protective effect with an odds ratio (OR) of 0.5,[11] and the other reported it was a risk factor with an OR = 1.59.[12] These inconsistent results mean that more studies of the association between this genetic variation and COPD are necessary to clarify its role in COPD patients. In the present study, the relationship between the EPHX1 113 mutant homozygote and susceptibility to COPD was investigated.

In COPD patients, the bronchodilator response (BDR) has been proposed to predict the survival and the annual rate of decline of lung function.[13–17] There is some evidence that oxidative stress may contribute to reversible airway narrowing.[18,19] EPHX1 is an antioxidant-related gene,[20] and a recent study has found an association of BDR and EPHX1 gene polymorphism at amino acid position 622.[21] To replicate this finding for the role of EPHX1 in BDR in additional populations, we studied the association between the EPHX1 113 mutant homozygote and the reversibility of airflow obstruction (the BDR) in this study.

Methods

Study population

This study was a hospital-based case—control study that consisted of 208 patients. The case group included 105 male adults (age $\geq$ 40 years) with smoking-related COPD; most of these patients visited National Cheng-Kung University Hospital for their chronic airway symptoms such as coughing and breathlessness. They were diagnosed as having COPD on the basis of medical history, chest radiography, and spirometry, according to the World Health Organization 2006 Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines.[1] Case group patients had a smoking history of $\geq$10 pack-years, with chronic airway obstruction, defined as a post-bronchodilator ratio of forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) $<$ 70%. Subjects were excluded if they had a history of asthma. The control group included 103 smokers or ex-smokers who were male adults (age $\geq$ 40 years with a smoking history of $\geq$10 pack-years) without airway obstruction demonstrated by spirometry as a ratio of FEV_1/FVC $\geq$ 70%. Most of the subjects visited our hospital for health checkups. Only male patients were enrolled in this study, because our hospital had few female smokers or COPD patients. The study was approved by the Ethics Committee of National Cheng-Kung University Medical Collage and Hospital, and signed informed consent was obtained from all patients.

DNA preparation

Genomic DNA was isolated from total blood cells by Puregene commercial DNA isolation kits (Gentra Systems, Minneapolis, MN, USA). Genomic DNA (20 ng) was amplified by polymerase chain reaction (PCR). The PCR was performed in a volume of 40 μL reaction mixture containing 1.5 mM $MgCl_2$, 100 ng primer, 500 μM dNTP and 0.6 IU *Taq* DNA polymerase (GeneMark, Taiwan). The amplification protocol was done using a DNA Thermal Cycler (GeneAmp PCR system 9700; Perkin Elmer Applied Biosystems, Norwalk, CT, USA).

PCR restriction fragment length polymorphism analysis of mEPHX gene

The polymorphism of mEPHX at exon 3 (EPHX1 113) was determined by PCR and restriction fragment length polymorphism. The PCR conditions consisted of an initial single cycle of 10 minutes at 94°C, followed by 40 cycles of 94°C for 45 seconds, 52°C for 20 seconds and 72°C for 5 seconds. For the PCR-based genotyping assays for mEPHX (exon 3), the primer pair was synthesized with sense: 5′-GATCGATA AGTTCCGTTTCACC-3′ and antisense: 5′-ATCCTTAGTCTT GAAGTGAGGAT-3′. The PCR product was digested to completion with *Escherichia coli* J62 pLG74 RV (New England Biolabs, Beverly, MA, USA), and separated by electrophoresis through a 3% agarose gel, stained with ethidium bromide and transilluminated with ultraviolet light. The exon-3 wild type allele was expected to yield 140 and 22 base pair fragments, and the mutation allele revealed a 162 base pair fragment.

Pulmonary function tests

The procedures for spirometry have been previously described in detail.[22] Spirometry was performed before and 30 minutes after bronchodilator therapy with salbutamol (400 μg). Patients were asked to omit short-acting bronchodilators for 8 hours and long-acting bronchodilators for 12 hours before testing. The BDR was expressed by two common indexes (absolute and relative BDRs) used in clinical practice. The absolute BDR was calculated as the

difference in the absolute number (mL) of the prebronchodilator and postbronchodilator values (postbronchodilator FEV_1 − prebronchodilator FEV_1), and the relative BDR was calculated as a percentage of the prebronchodilator value ([postbronchodilator FEV_1 − prebronchodilator FEV_1]/ prebronchodilator FEV_1) × 100.

Statistical analysis

Associations between specific genotypes and phenotypes were analyzed for significance by the two-tailed $\div^2$ test and unpaired *t* test for univariate analysis. A logistic regression model was used to calculate ORs adjusted for age and cumulative cigarette consumption in pack-years between smokers with and without COPD. Multivariate analysis was used to assess the contributions of genetic factors, age, smoking (status and cumulative amount by pack-years) and baseline covariates (prebronchodilator FEV_1 for BDRs) to BDRs in COPD patients. We used the fit model of standard least squares on the JMP software program (SAS Institute Inc. Cary, NC, USA) doing multivariate analysis. In each analysis, a difference with $p < 0.05$ was accepted as significant.

Results

The characteristics of the control and COPD subjects are summarized in Table 1. No significant differences were observed in smoking history between the control and COPD groups. The mean age was lower in the control group than the COPD group; we adjusted for this incomplete matching in the analysis of the data. The Hardy–Weinberg equilibrium was used to test the genotypes in the control group and no obvious deviation was found.

In the present study, subjects with the EPHX1 113 mutant homozygote (His^{113}/His^{113}) had a strong correlation with COPD (OR: 2.7, 95% confidence interval: 1.5–5.2), the frequency of this genotype was significantly higher in the COPD group than the control group [61/105 (58.1%) *vs.* 36/103 (35.0%), p = 0.001) (Table 1).

We reported that the EPHX1 113 mutant homozygote was associated with lower BDRs in COPD patients. COPD patients with the His^{113} homozygote mutation ($n = 61$) had significantly lower BDRs (absolute and relative), as shown by the absolute change and percentage change from baseline, than COPD patients with other genotypes ($n = 44$) (91.7 ± 12.5 mL *vs.* 141.6 ± 15.1 mL, $p = 0.01$ and 8.3 ± 1.2% *vs.* 13.4 ± 1.4%, $p = 0.006$) (Table 2).

Table 1 Patient characteristics of COPD and control smokers.

	COPD ($n = 105$)	Controls ($n = 103$)	*P* value
Age (yr)	69.4 ± 1.1	60.0 ± 1.1	<0.001
Smoking/ pack-years	43.0 ± 2.2	37.5 ± 2.2	0.07
FEV_1 % pred	56.7 ± 1.8	93.1 ± 1.8	<0.001
FVC % pred	75.0 ± 1.8	92.5 ± 1.8	<0.001
FEV_1/FVC	50.5 ± 0.9	79.6 ± 0.9	<0.001
Genotypes			
His^{113}/His^{113}	61/105 (58.1%)	36/103 (35.0%)	0.001
His^{113}/Tyr^{113}	28/105 (26.7%)	43/103 (41.7%)	0.01
Tyr^{113}/Tyr^{113}	16/105 (15.2%)	24/103 (23.3%)	0.29

Values are expressed in mean ± standard error. COPD = chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in one second; pred = predicted; FVC = forced vital capacity.

Discussion

A genetic variation of EPHX1 is thought to increase the risk of developing COPD and is significantly associated with rapid decline in lung function and disease severity in COPD patients, because of its effect in decreasing enzyme activity.[5–7,23–25] However, various studies of genetic polymorphism of EPHX1 in COPD have shown different results.[5–10] Different phenotype definitions used in these studies may be one of the important reasons for different results for the same genetic variation.[2,12]

In the present study, the new definition of COPD from the GOLD 2006 guidelines[1] was used to set up the inclusion criteria for our study group. According to the new guidelines, postbronchodilator FEV_1/FVC and FEV_1 measurements are recommended for the diagnosis and assessment of severity of COPD. The degree of reversibility of airflow limitation, such as a significantly increased FEV_1 after bronchodilator, is no longer recommended for the diagnosis or to differentiate COPD from asthma. This is because there is increasing evidence that up to 30% of COPD patients with non-fully reversible airway limitation have significant BDR following administration of a short-acting β_2 agonist (positive BDR).[14] There were 34 COPD patients (34/105, 32.4%) with significant BDR (relative BDR > 12% or absolute BDR >200 mL) in our study; none of these patients had a history or an established diagnosis of asthma. However, most previous studies have excluded patients with significant BDRs from their study group[3,8–10,20]; however, studies with this exclusion criterion miss many COPD patients with BDR phenotypes, which may cause varying results. In addition, phenotype definition is a crucial issue for genetic investigations of complex human diseases such as COPD. One genome-wide study has suggested that postbronchodilator spirometric measures are optimal phenotypes for COPD genetic studies because they appear to provide more powerful phenotypes (nearly doubled) for genetic studies of COPD than conventional prebronchodilator spiromtery.[26] However, postbronchodilator spirometry has been used in only a few COPD genetic studies. Other than the present, only one previous study[11] has used GOLD guidelines with postbronchodilator spirometry to analyze the association between the EPHX1 and COPD phenotypes.

Our result is consistent with a recent meta-analysis that has reported that the EPHX1 113 mutant homozygote is significantly associated with an increased risk of COPD (OR: 1.57, 95% confidence interval: 1.24–1.98) in Asian populations.[12]

In COPD patients, a lower reversibility of airflow limitation (lower BDR) is related to more severe emphysema[27]

Table 2 Comparison of EPHX1 genotypes with BDRs in COPD patients.

	His^{113}/His^{113} ($n = 61$)	His^{113}/Tyr^{113} and Tyr^{113}/Tyr^{113} ($n = 44$)	*P* value
Age	68.3 ± 1.4	71.0 ± 1.7	0.23
Smoking/pack-years	43.1 ± 3.1	43.0 ± 3.7	0.97
FVC (% pred)	75.3 ± 2.5	74.6 ± 3.0	0.80
FEV_1 (% pred)	55.0 ± 2.7	59.0 ± 3.2	0.30
FEV_1/FVC	49.7 ± 1.5	51.6 ± 1.7	0.41
Absolute BDR (mL)	91.7 ± 12.5	141.6 ± 15.1	0.01
Relative BDR (%)	8.3 ± 1.2	13.4 ± 1.4	0.006

Values are expressed in mean ± standard error. EPHX = epoxide hydrolase; BDR = bronchodilator response; COPD = chronic obstructive pulmonary disease; FVC = forced vital capacity; pred = predicted; FEV_1 = forced expiratory volume in one second.

and is proposed to predict a rapid decline in FEV_1 and poor survival.[13,14] In addition, reversibility of airflow limitation is also associated with airway eosinophils and predicts the clinical and functional responses to inhaled corticosteroids in COPD patients.[28,29] Therefore, BDR may be a useful indicator not only for predicting the clinical outcome and survival but also identifying patients who respond to pharmacological treatment. The precise mechanisms underlying the reversibility of airflow limitation in COPD are still debated; genetic study of COPD phenotypes may provide opportunities to understand disease mechanisms. However, there have been few genetic studies of BDR phenotypes in COPD patients.[2] In the present study, we reported that the EPHX1 113 mutant homozygote was significantly associated with a decreased BDR in COPD patients, as shown by the absolute and percentage changes from baseline (91.7 ± 12.5 mL *vs*. 141.6 ± 15.1 mL, $p = 0.01$; and 8.3 ± 1.2% *vs*. 13.4 ± 1.4%, $p = 0.006$). Our data suggest a possible involvement of oxidative stress mechanisms in the pathogenesis of BDR in COPD.

One limitation of the present study was that only male adults were enrolled. The major population of COPD and chronic smokers in our population is male, especially in Southern Taiwan (<10% of recorded COPD patients and chronic smokers in our hospital were female). Another limitation is that this was a case–control study with a relatively small number of recruited subjects.

In conclusion, in our population, a strong correlation between the EPHX1 113 mutant homozygote and smoking-related COPD was noted, and this genetic polymorphism was also associated with decreased BDR in COPD patients.

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