Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 359 (2007) 136–142
BBRC
www.elsevier.com/locate/ybbrc

Genetic polymorphisms of *GSTP1* and *mEPHX* correlate with oxidative stress markers and lung function in COPD

Arpana Vibhuti [a,b], Ehtesham Arif [a,c], Desh Deepak [d], Bhawani Singh [d], M.A. Qadar Pasha [a,b,c,*]

[a] *Functional Genomics Unit, Institute of Genomics and Integrative Biology, New Delhi, India*
[b] *Department of Biotechnology, University of Pune, Pune, India*
[c] *Department of Medical Elementology & Toxicology, New Delhi, India*
[d] *Respiratory Unit, Dr. Ram Manohar Lohia Hospital, New Delhi, India*

Received 18 April 2007
Available online 22 May 2007

Abstract

The genetic susceptibility to COPD might depend on variations in detoxification enzymes that activate and detoxify cigarette smoke products, which otherwise generate oxidative stress causing pathogenesis. In a case-control study of 202 COPD patients and 136 normals, we examined the association of polymorphisms I105V, A114V of *GSTP1* and Y113H, H139R of *mEPHX* individually or in combination with disease and their contribution to oxidative stress markers such as MDA, GSH, GPx and airflow obstruction. Patients were over-represented by the alleles 105V, 114V of *GSTP1* and 113H, 139H of *mEPHX* ($\chi^2 = 10.63$, $p = 0.001$, $\chi^2 = 13.92$, $p < 0.001$, $\chi^2 = 13.02$, $p < 0.001$ and $\chi^2 = 4.48$, $p = 0.034$, respectively) and the haplotypes of same alleles i.e. 105V–114V and 113H–139H ($\chi^2 = 14.58$, $p < 0.001$ and $\chi^2 = 23.14$, $p < 0.001$). Moreover, there was marked over-representation of combination of genotypes, I105I+A114A of *GSTP1* (53% vs. 36%) in controls; whereas, the combinations with 105V/114V alleles (64% vs. 47%) of *GSTP1* (OR = 1.99; 95% CI = 1.28–3.09; $p = 0.002$) and the homozygotes H113H+H139H (27% vs.10%) of *mEPHX* (OR = 3.26; 95% CI = 1.73–6.15; $p = 0.0001$) in patients. Patients had significantly elevated MDA level ($p < 0.001$) and decreased GSH level ($p < 0.001$) and GPx activity ($p = 0.035$), respectively. Of note, the genotypes, I105V/V105V, A114V/V114V of *GSTP1* and Y113H/H113H of *mEPHX* associated with increased MDA level ($p = 0.04$, $p = 0.03$ and $p = 0.003$), decreased GSH level ($p = 0.019$, $p = 0.007$ and $p = 0.0006$) and lower FEV1 ($p = 0.23$, $p = 0.037$ and $p = 0.029$), respectively, in patients; so was the correlation of these biomarkers and lung function with the combinations of the genotypes. In conclusion, 105V/114V alleles of *GSTP1* and 113H/139H alleles of *mEPHX* and the combination of genotypes with same alleles associated with imbalanced oxidative stress and lung function in patients, signifying the importance in the disease.

Keywords: Microsomal epoxide hydrolase; Glutathione-*S*-transferase P1; Polymorphism; Lipid peroxidation; Reduced glutathione; Glutathione peroxidase; Lung function

Chronic obstructive pulmonary disease (COPD) is characterized by irreversible airflow limitation, abnormal permanent distal air-space enlargement and emphysema in the lungs [1]. Oxidative stress plays a major role in the pathogenesis of COPD [2]. Increased oxidative burden in COPD is because of both directly as a result of smoking and indirectly by the release of increasing amount of reactive oxygen species (ROS) from airways leukocytes [3]. It has been shown that ROS induce damage in all cellular macromolecules such as lipid, proteins and DNA [4]. In healthy individuals, a variety of antioxidant defense mechanisms serve multiple protective functions, among them glutathione redox system is the most abundant nonprotein thiol source in the cells [5]. Cigarette smoking, although, is

* Corresponding author. Address: Functional Genomics Unit, Institute of Genomics and Integrative Biology, New Delhi, India. Fax +91 11 27667471.
E-mail address: qpasha@igib.res.in (M.A. Qadar Pasha).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.05.076

the most important risk factor, only 10% of the chronic heavy smokers develop symptomatic COPD suggesting that there must be some genetic predisposing risk factors contributing to the susceptibility [6,7].

Genetic polymorphisms in the detoxifying and antioxidant enzymes can be important markers as they might affect the function of proteins and significantly influence the detoxification and oxidative stress thereby playing a crucial role in COPD. Glutathione-*S*-transferases (*GSTs*) and microsomal epoxide hydrolase (*mEPHX*) are two such detoxifying candidates. *GSTs* consist of a supergene family of metabolic enzymes that catalyze the conjugation of reduced glutathione with various hydrophobic electrophilic compounds [8]. Among the various isoforms, *GSTP1* is expressed more abundantly in alveoli, alveolar macrophages, and respiratory bronchioles [9]. Activity of the enzyme is affected by substitution of isoleucine with valine (I105V) in exon 5 and alanine with valine (A114V) in exon 6 of this gene [10,11]. The *mEPHX* catalyses the hydrolysis of a wide range of exogenous arene and aliphatic epoxides to form water soluble dihydroidals [12]. In human *mEPHX*, substitution of tyrosine to histidine (Y113H) in exon 3 and histidine to arginine (H139R) in exon 4 influences enzyme activity [13]. The 113Y/139R variants are associated with fastest enzyme activity and 113H/139H variants with slowest, and the latter is closely linked to the pathogenesis of COPD [14,15].

Although oxidative stress and genetic variants are thought to be involved in COPD, the relationship between the two has not been explored yet. This study is, therefore, aimed at: to envisage the association of I105V, A114V polymorphisms of *GSTP1* and Y113H, H139R polymorphisms of *mEPHX*, individually and in combination with the disease; to examine whether the *GSTP1* and *mEPHX* polymorphisms individually and in combination influence oxidative stress markers such as malondialdehyde (MDA), reduced glutathione (GSH) and glutathione peroxidase (GPx) and lung function or severity of disease [forced respiratory volume in one second (FEV_1)].

Methods

Study subjects. The study was comprised of 202 COPD patients and 136 healthy controls with an age of $\geqslant$40 years. All the studied subjects were smokers and had a history of more than 10 pack years. Diagnosis of the COPD was based on presence of relentlessly progressive symptoms like cough, productive sputum and breathlessness over many years. Both groups were subjected to physical examination and chest X-ray. Spirometry was performed on masterscope spirometer 4.2 (Erich Jaeger Laboratories, Wurzburg, Germany) as per the American Thoracic Society criteria [16]. Patients had a reduction of both FEV_1 of <80% and the FEV_1/FVC of <70%. Patients with evidence of reversibility in airway obstruction were excluded. Reversibility was defined as >200 ml and >12% increase in FEV_1 after 30 min of nebulization of 2.5 mg of salbutamol (Asthalin, Cipla, India) [17,18]. Suspected subjects of bronchiectasis or tuberculosis were excluded based on the diagnosis by Chest X-ray followed by high resolution CT scan. All patients were clinically stable and none had a history of respiratory infection for at least four weeks period preceding the study. Informed written consent was obtained from all the subjects for participation in the study, which was approved by the Ethics Committee of our Institute and as well as of the Dr. Ram Manohar Lohia hospital.

Blood sample collection. Ten-milliliter fasting blood was obtained from each subject in anticoagulant coated tube. Plasma was separated for the estimation of MDA and GSH levels and GPx activity. The cells were used in DNA isolation. Samples were stored at −80 °C till further use.

Genotype analysis. DNA was isolated from peripheral blood leukocytes by a standard protocol [19]. The primers for the I105V (A → G, rs1695) and A114V (C → T, rs1138272) polymorphisms of *GSTP1* were designed using the primer select software of DNASTAR and were as follows: I105V, forward: 5′-TCCTTCCACGCACATCCTCT-3′; reverse: 5′-AGC CCC TTTCTTTGTTCAGC-3′; A114V, forward: 5′-GCTGGGAGGGA TGAGAGTAGG-3′; reverse: 5′-GCGCCCCACATATGCTGAGAG-3′. Fragments containing Y113H (T → C, rs1051740) and H139R (A → G, rs2234922) polymorphisms of *mEPHX* were amplified by a standard protocol with modifications [20]. The reaction mixture, 20 μl, contained 50 ng of DNA, 0.5 pmol of each primer, 1× buffer, 0.9 U of *Taq* DNA polymerase (Bangalore Genie, Bangalore, India), and 0.2 mM of dNTPs (Amersham Pharmacia, Uppsala, Sweden). For I105V, A114V and H139R polymorphisms, the cycling conditions were: 94 °C initial denaturation for 4 min, followed by 30 cycles of 94 °C denaturation for 30 s, 65 °C annealing for 30 s and 72 °C extension for 30 s followed by final extension of 7 min. In case of Y113H polymorphism only the annealing temperature differed, which was maintained at 60 °C. The reaction produced a DNA fragment of 290, 435, 190 and 210 bp for I105V, A114V, Y113H and H139R polymorphisms, respectively, which was screened by restriction fragment length polymorphism using two units either each of BsmAI, AciI, EcoRV and RsaI (New England Bio Labs, Cambridge, UK), respectively, per 25.0 μl of reaction mixture.

Biochemical parameters. Plasma MDA, GSH levels and GPx activity were measured by known methods [21–23] on a high-throughput Spectramax-plus384 Spectrophotometer (Molecular Devices, USA). The coefficients of variations of MDA, GSH and GPx were <5% for intra- and inter-batch assessment.

Correlation analysis. Various correlations between genotype, biochemical parameters and clinical characteristics have been performed. The individual genotype of each polymorphism and combination of genotypes between the two polymorphisms of each gene were investigated for correlations with MDA, GSH, GPx and FEV_1. These analyses provided comparisons between the individual genotype and the combinations with biochemical parameters.

Biostatistical analysis. Statistical Package for the Social Sciences for windows (SPSS 10) and EPIINFO 6 software were used to carry out the statistical analysis. Genotype distribution, combinations of genotypes between the two polymorphisms of each gene and haplotypes were analyzed between the two groups. Genotype and allele frequencies of *GSTP1* and *mEPHX* polymorphisms were compared between the two groups using Pearson's χ^2 test and Fisher's exact test, respectively. Deviation of the observed genotype frequencies from Hardy-Weinberg equilibrium was checked using χ^2 goodness of fit test. Haplotype frequencies of the polymorphisms were estimated and compared between cases and controls by χ^2 test and permutation analysis was performed by SNP Alyze software (version 3.1; Dynacom, Mobara-shi, Japan). To determine the extent of association of the I105V, A114V of *GSTP1* and Y113H, H139R of *mEPHX* polymorphisms the Lewontin's coefficient (D') and squared Correlation coefficient (r^2) for pairwaise linkage disequilibrium (LD) were calculated by using SNP Alyze software. Genotype conversions were looked for association with clinical and biochemical parameters. The combinations of genotypes were also analyzed for association with biochemical parameters, if any. Difference between the two groups was analyzed by unpaired *t*-test with two tailed values. The biochemical parameters were expressed as mean ± SD. Analysis of covariance for adjustment of age, gender, smoking habits, BMI and FEV_1 (% predicted) was carried out by the general linear model procedure to examine the independent effects of polymorphisms on dependent variables. For calculation of odds ratio 95% confidence interval (CI) the genotype data was adjusted for potential confounding factors by a multivariate logistic regression analysis. Where appropriate, *p* values for pairwise differences

were corrected for multiple comparisons by using Bonferroni correction. A p value of <0.05 was considered statistically significant.

Results

Clinical characteristics and biochemical parameters

Clinical characteristics and biochemical parameters are presented in Supplement Table. COPD patients had significantly lower body mass index (BMI) ($p < 0.001$) and higher obstruction in pulmonary function ($p < 0.001$) than controls. The MDA level was significantly higher ($p < 0.001$), whereas, GSH level and GPx activity were significantly lower ($p < 0.001$ and $p = 0.035$, respectively) in patients than controls. A percent difference of 13.6, 45.1, 21.1, 27.7 and 7.1 was obtained for BMI, FEV_1 (% predicted), MDA, GSH level and GPx activity, respectively.

Genotypes distribution

The results are presented in Table 1. The two groups were in Hardy-Weinberg equilibrium for the polymorphisms I105V, A114V, Y113H, and H139R. Genotype distribution for the same polymorphisms differed significantly ($\chi^2 = 10.43$, $p = 0.005$; $\chi^2 = 11.41$, $p = 0.003$; $\chi^2 = 10.38$, $p = 0.005$ and $\chi^2 = 5.98$, $p = 0.045$, respectively) between the two groups; the homozygotes V105V, V114V, H113H and H139H being higher in patients than controls. Moreover, the I105V+V105V, A114V+V114V and Y113H+H113H genotypes were also greater, whereas the H139R+R139R genotypes were lesser in patients ($\chi^2 = 6.71$, $p = 0.009$, $\chi^2 = 10.81$, $p = 0.001$, $\chi^2 = 7.36$, $p = 0.008$ and $\chi^2 = 5.67$, $p = 0.017$, respectively). As a consequence, the alleles 105V, 114V, 113H and 139H were over-represented in patients ($\chi^2 = 10.63$, $p = 0.001$, $\chi^2 = 13.92$, $p < 0.001$, $\chi^2 = 13.02$, $p < 0.001$ and $\chi^2 = 4.48$, $p = 0.034$, respectively).

Haplotype and pairwise LD analysis

Haplotypes for the I105V, A114V polymorphisms of *GSTP1* and Y113H, H139R polymorphisms of *mEPHX* are presented in Table 2A. Maximum likelihood procedure suggested four haplotypes between the two polymorphisms

Table 1
Distribution of genotypes and alleles between controls and patients

Gene	Control ($n = 136$)	Patients ($n = 202$)	χ^2	OR (95% CI)^{corr}	p^*
GSTP1					
I105I	90 (66)	105 (52)		1	
I105V	42 (31)	75 (37)		1.05 (1.00–.07)	
V105V	4 (3)	22 (11)		2.15 (1.04–.22)	
			10.43		0.005
I105V+V105V	46 (34)	97 (48)	6.71	1.81 (1.15–2.83)	0.009
105I	222 (82)	285 (70)	10.63	1.85 (1.28–2.69)	0.001
105V	50 (18)	119 (30)			
GSTP1					
A114A	106 (78)	123 (61)		1	
A114V	24 (18)	57 (28)		1.56 (1.08–2.70)	
V114V	6 (4)	22 (11)		2.47 (1.15–6.12)	
			11.41		0.003
A114V+V114V	30 (22)	79 (39)	10.81	2.27 (1.38–3.72)	0.001
114A	236 (87)	303 (75)	13.92	2.19 (1.44–3.32)	0.000
114V	36 (13)	101 (25)			
mEPHX					
Y113Y	54 (40)	52 (26)		1	
Y113H	51 (37)	75 (37)		1.09 (0.959–1.91)	
H113H	31 (23)	75 (37)		1.81 (0.984–2.06)	
			10.38		0.005
Y113H+H113H	82 (60)	150 (74)	7.36	1.90 (1.19–3.03)	0.008
113Y	159 (59)	179 (45)	13.02	1.77 (1.30–2.41)	0.000
113H	113 (41)	225 (55)			
mEPHX					
H139H	72 (53)	133 (66)		1	
H139R	56 (41)	59 (29)		1.95 (1.16–4.53)	
R139R	8 (6)	10 (5)		1.48 (0.966–2.34)	
			5.98		0.045
H139R+R139R	64 (47)	69 (34)	5.67	1.71 (1.10–2.67)	0.017
139H	200 (73)	325 (81)	4.48	1.48 (1.03–2.13)	0.034
139R	72 (27)	79 (19)			

OR (95% CI)^{corr}; odds ratio (95% confidence interval) corrected for age, gender, BMI, FEV1 and smoking habits.
p^* by χ^2 test.

Table 2
Haplotype association and LD analysis in the two groups

(A) Comparison of haplotypes of I105V, A114V polymorphisms of *GSTP1* and Y113H, H139R polymorphisms of *mEPHX* between patients and controls

Haplotype		Overall	Patients	Control	χ^2Value	p	p[a]
GSTP1							
I105V	A114V						
105I	114A	0.6551	0.5708	0.7158	16.061	<0.0001	<0.001
105I	114V	0.1671	0.2425	0.1155	33.332	<0.0001	<0.0001
105V	114A	0.1585	0.1443	0.1686	0.7926	0.3733	0.397
105V	114V	0.0193	0.0424	0.0001	14.585	<0.0001	0.001
Overall					48.289	<0.0001	
mEPHX							
Y113H	H139R						
113Y	139H	0.3688	0.3273	0.4214	7.548	<0.0001	0.0141
113Y	139R	0.1424	0.1241	0.1597	2.103	0.1469	0.2031
113H	139H	0.4031	0.4781	0.3111	23.141	<0.0001	<0.0001
113H	139R	0.0857	0.0705	0.1078	3.465	0.0627	0.0791
Overall					23.578	<0.0001	

(B) Pairwaise LD analysis of the two polymorphisms between patients and controls

	Pair1	Pair2	D' Value	r^2 Value	χ^2Value	p
Patients						
	I105V	A114V	1	0.0671	22.672	<0.0001
	Y113H	H139R	0.3394	0.0338	15.437	<0.0001
Controls						
	I105V	A114V	0.1709	0.0190	7.839	0.001
	Y113H	H139R	0.0381	<0.0001	0.1229	0.7259

[a] p values are obtained by permutation test for each haplotype frequency compared between the two groups.

of *GSTP1* or *mEPHX*. The haplotypes 105V–114V of *GSTP1* and 113H–139H of *mEPHX* were significantly more frequent in patients ($\chi^2 = 14.58$, $p = 0.001$ and $\chi^2 = 23.14$, $p < 0.0001$, respectively). On the contrary, the haplotypes 105I–114A of *GSTP1* and 113Y–139H of *mEPHX* were significantly more frequent in controls ($\chi^2 = 16.06$, $p < 0.001$ and $\chi^2 = 7.548$, $p = 0.014$). The overall distribution of the haplotypes for I105V, A114V polymorphisms of *GSTP1* and Y113H, H139R polymorphisms of *mEPHX* also differed significantly between the two groups ($\chi^2 = 48.28$, $p < 0.0001$ and $\chi^2 = 23.57$, $p < 0.0001$, respectively, Table 2A). The mutant haplotypes dominated in patients.

The LD was significantly high for the I105V and A114V polymorphisms of *GSTP1* in patients ($D' = 1$, $r^2 = 0.067$, $\chi^2 = 22.67$, $p < 0.0001$), the LD was even significant in controls ($D' = 0.171$, $r^2 = 0.019$, $\chi^2 = 7.83$, $p = 0.001$; Table 2B). In case of *mEPHX*, the LD was significantly high for the Y113H and H139R polymorphisms in patients ($D' = 0.339$, $r^2 = 0.033$, $\chi^2 = 15.43$, $p < 0.0001$), only.

Combinations of genotypes

Distributions of the possible combinations of genotypes between the two polymorphisms of each gene are presented in Fig. 1. The results revealed a marked over-representation (53% vs. 36%) of combination of genotypes, I105I+A114A, of *GSTP1* in controls, whereas the remaining combinations were over-represented (64% vs. 47%) in patients (OR = 1.99; 95% CI = 1.28–3.09; $p = 0.002$; Fig. 1A). It is of consequence to add that each of the genotypes in these combinations have single or both slow alleles (105V/114V) in patients. The distribution of the combination of genotypes, H113H+H139H, of *mEPHX* was greater (27% vs.10%) in patients (OR = 3.26; 95% CI = 1.73–6.15; $p = 0.0001$; Fig. 1B).

Correlation of genotypes with clinical and biochemical parameters

Table 3 represents the influence of genotypes on BMI, spirometric and biochemical values in the two groups. No significant influence of these genotypes on BMI is visible although a trend can be seen. In case of lung function only the A114V/V114V genotypes compared to A114A genotype of *GSTP1* and Y113H/H113H genotypes compared to Y113Y genotype of *mEPHX* associated with significantly lower FEV_1 (% predicted) in patients ($p = 0.037$ and $p = 0.029$, respectively). The genotypes, moreover, had equally considerable influence on biochemical parameters. Of note in patients, the genotypes, I105V/V105V, A114V/V114V of *GSTP1* and Y113H/H113H of *mEPHX* associated with increased MDA level ($p = 0.04$, $p = 0.03$ and $p = 0.003$) and decreased GSH level ($p = 0.019$, $p = 0.007$ and $p = 0.0006$), respectively; while in controls only Y113H/H113H genotypes as compared to Y113Y genotype of *mEPHX* associated significantly with

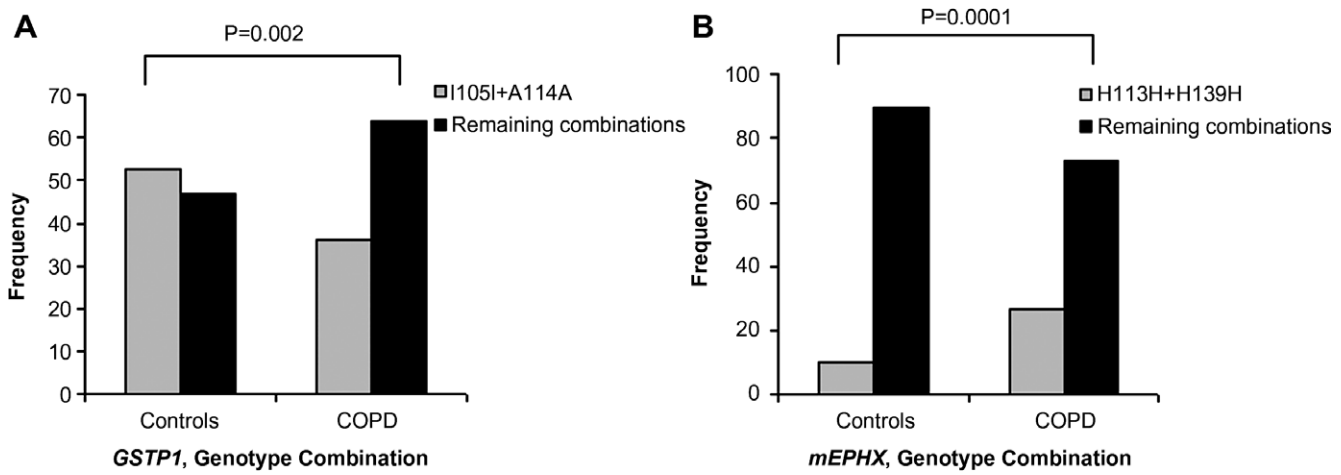

Fig. 1. Distribution of combinations of genotypes between the two polymorphisms of each gene, either *GSTP1* or *mEPHX* in two groups. (A) Frequency of I105I+A114A genotype combination of *GSTP1*. (B) Frequency of H113H+H139H genotype combination of *mEPHX*.

Table 3
Comparison of clinical characteristics with the *GSTP1* I105V, A114V and *mEPHX* Y113H, H139R genotypes in the controls and patients

Characteristics	*GSTP1* I105V				*GSTP1* A114V			
	Controls		Patients		Controls		Patients	
	I105I ($n = 90$)	I105V/V105V ($n = 46$)	I105I ($n = 105$)	I105V/V105V ($n = 97$)	A114A ($n = 106$)	A114V/V114V ($n = 30$)	A114A ($n = 123$)	A114V/V114V ($n = 79$)
BMI (kg/m^2)	25.5 ± 4.9	24.7 ± 5.8	22.2 ± 4.9	21.3 ± 4.4	25.4 ± 5.4	24.8 ± 5.4	21.9 ± 4.7	21.5 ± 4.8
FEV1 (% predicted)	110.7 ± 22.4	109.1 ± 16.7	61.9 ± 29.6	57.7 ± 24.1	110.1 ± 21.2	109.4 ± 16.5	63.9 ± 28.5	56.2 ± 22.4*
MDA (μmoles/L)	0.56 ± 0.28	0.62 ± 0.34	0.71 ± 0.28	0.79 ± 0.29*	0.58 ± 0.23	0.61 ± 0.32	0.70 ± 0.29	0.79 ± 0.28*
GSH (μmoles/L)	4.2 ± 2.5	3.9 ± 2.4	3.0 ± 1.6	2.5 ± 1.4*	4.1 ± 2.6	4.0 ± 2.3	3.1 ± 1.5	2.5 ± 1.6†
GPx (U/ml)	19.2 ± 3.9	18.9 ± 3.5	17.9 ± 8.5	17.4 ± 6.0	19.3 ± 3.6	18.7 ± 3.9	18.1 ± 8.3	17.3 ± 6.3
	mEPHX Y113H				*mEPHX* H139R			
	Controls		Patients		Controls		Patients	
	Y113Y ($n = 54$)	Y113H/H113H ($n = 82$)	Y113Y ($n = 52$)	Y113H/H113H ($n = 150$)	H139H ($n = 72$)	H139R/R139R ($n = 64$)	H139H ($n = 133$)	H139R/R139R ($n = 69$)
BMI (kg/m^2)	25.6 ± 5.5	24.7 ± 5.4	22.0 ± 4.8	21.3 ± 4.7	25.2 ± 4.4	25.1 ± 6.2	21.7 ± 4.9	21.8 ± 4.5
FEV1 (% predicted)	112.1 ± 23.8	108.5 ± 18.1	64.7 ± 25.2	55.4 ± 26.8*	108.9 ± 20.9	111.5 ± 18.9	59.6 ± 25.4	60.1 ± 26.2
MDA (μmoles/L)	0.59 ± 0.31	0.62 ± 0.28	0.68 ± 0.29	0.81 ± 0.27†	0.61 ± 0.30	0.60 ± 0.29	0.74 ± 0.30	0.75 ± .24
GSH (μmoles/L)	4.7 ± 2.7	3.4 ± 2.2*	3.2 ± 1.5	2.4 ± 1.4¶	4.0 ± 2.2	4.1 ± 2.6	2.9 ± 1.6	2.7 ± 1.5
GPx (U/ml)	19.1 ± 3.6	19.0 ± 3.7	18.7 ± 8.2	16.4 ± 6.4*	18.6 ± 3.5	19.3 ± 3.9	17.5 ± 8.5	18.0 ± 6.1

n = number of subjects; L = liter, values are expressed in mean ± SD.
* $p < 0.05$.
† $p < 0.01$.
¶ $p < 0.001$.

decreased GSH level ($p = 0.04$). GPx activity decreased with Y113H/H113H as compared to Y113Y genotype of *mEPHX* in patients ($p = 0.039$).

Correlation of combinations of genotypes with clinical and biochemical parameters

The combinations of genotypes between the two polymorphisms of each gene were analyzed for their contribution to clinical and biochemical characteristics (Table 3). A comparison of the combination of genotypes I105I+A114A against remaining combinations of *GSTP1* revealed that the latter combinations associated with decreased FEV_1 (% predicted, $p = 0.044$), increased MDA level ($p = 0.001$) and decreased GSH level ($p = 0.005$) and GPx activity in patients. In case of *mEPHX*, the H113H+H139H genotypes combination compared to remaining combinations, associated with decreased FEV_1 (% predicted, $p = 0.011$), increased MDA level ($p = 0.006$) and decreased GSH level ($p = 0.0005$) and GPx activity ($p = 0.088$) in patients. Controls also showed similar trends, though, nonsignificant.

Discussion

COPD being a complex disease, role of multiple genes is expected [7,24]. This study investigated the contribution of genes encoding xenobiotic metabolizing enzymes, which metabolize the tobacco constituents. It is reported that emphysema is caused by cigarette smoke generated epoxides, which can be detoxified principally by *GSTs* or *mEPHX* [14]. However, variation in the respective genes can adversely influence its own activity and thereby oxidative stress, which is what we attempted to evaluate. Our evaluation of the polymorphisms revealed over-representation of 105V, 114V alleles of *GSTP1* and 113H, 139H alleles of *mEPHX* in patients (Table 1). The literature, however, is not without controversy. The 105V allele was associated with lung cancer [11]; whereas, predominance of I105I genotype in COPD in Japanese [25]. This discrepancy might arise from racial/varied selection of study subjects. Our results on *EPHX*, in spite, of ethnic variations are in agreement with previous reports which suggested correlation between slow metabolizing form of *mEPHX* containing 113H/139H alleles with emphysema [13,14].

The significance of our genotypic findings was further strengthened by the LD and haplotype analysis (Table 2). The haplotypes 105V–114V and 105I–114V of *GSTP1* were significantly more frequent in patients, whereas, the haplotypes 105I–114A and 105V–114A were over-represented in controls. Such distribution of haplotypes in patients is indicative of the lower enzymatic activity, as a consequence incomplete catabolization of the toxicants, whose presence may lead to oxidative stress. In fact, one of the haplotypes, 105V–114A as obtained in controls has been reported to have seven-fold higher catalytic activity for the diol epoxides of polycyclic aromatic hydrocarbons [10,26]. Similarly, in case of *mEPHX*, the slowest enzyme activity-associated haplotype, 113H–139H, was significantly more frequent in patients. Furthermore, our analyses of the combinations of genotypes of the two polymorphisms of each gene in the two groups were in agreement with the findings on individual genotypes or haplotypes. Of note, the alleles (105V, 114V, 113H and 139H) that associate with slow enzyme activity were more frequent in the combinations of the genotypes of patients (Fig. 1). The findings, therefore, point to a possible additive effect of the slowest enzyme activity-associated alleles of both the genes in the susceptibility to COPD.

Apart from the association of the genotypes with the disease, the various correlation analyses between genetic variants and biochemical and clinical parameters were highly categorical. In COPD, increased oxidative stress is because of increased burden of free radicals originating from activated neutrophils [27]. In the present study, significantly higher MDA level and lower antioxidants such as GSH level and GPx activity were found in patients (Supplement Table). These results are in agreement with previous studies, which reported elevated plasma lipid peroxidation products in patients with stable COPD [28] and rapid depletion of intracellular GSH and a reduction in the activity of GPx with exposure to cigarette smoke or its condensate in epithelial cells *in-vitro* and in rat lungs *in-vivo* [29,30]. Additionally, we investigated the correlation between the variants and the biomarkers, we observed increased MDA level and decreased GSH level, GPx activity with the presence of 105V and 114V alleles of *GSTP1* and the alleles 113H and 139H of *mEPHX* individually (Table 3). Although, separate reports on gene polymorphisms and the biomarkers are available [2,3,14,25] but none showed their correlation; ours is the first report on the association of these polymorphisms with MDA, GSH levels and GPx activity in COPD and our findings are of significance. In case of correlation of genotype with clinical characteristics, we found association of mutant alleles of *GSTP1* and slowest enzyme activity alleles of *mEPHX* polymorphisms with low BMI and high airflow obstruction. An earlier study reported association of low BMI with increased risk of COPD in men [31]. Yet another highlight of our study was the correlation between the combination of the genotypes and the oxidant-antioxidants or lung function. We observed increased MDA level and decreased GSH level, GPx activity in the presence of combinations of genotypes consisting of 105V/114V alleles of *GSTP1* and the H113H+H139H of *mEPHX*. These combinations also associated significantly with decreased lung function (Table 3) and the *mEPHX* finding is in agreement with Sandford et al. [15], who reported association of the hapolotype of same alleles with lung function. Incidentally, the correlation of combination of genotypes with lung function is reported for the first time by us. We have been able to prove the importance of the polymorphisms individually or in combination with lung function. In addition, our correlations of the genetic variants with biomarkers strengthens our findings, as it is well established that MDA and other oxidative stress markers add to the severity of emphysema and thus lung function [2,3,7].

In conclusion, slow enzyme activity-associated alleles 105V, 114V of *GSTP1* and 113H, 139H of *mEPHX* and their haplotypes were over-represented in patients. The same alleles associated with airflow obstruction, low BMI, elevated MDA level and lower GSH level, GPx activity. Moreover, the genotype combinations and their correlations with respective biomarkers further strengthened our findings. The study provides an inference that gene polymorphisms affecting the function of proteins might alter the level of oxidative stress. The reduction in the capacity of antioxidative enzymes and increase in toxic lipid peroxidation products may relate to the progression of the disease. The findings, as are of consequence, deserve to be tested in a larger cohort.

Acknowledgments

CSIR, India supported this work (Toxicogenomics Taskforce, CMM–0003). We thank the staff of Respiratory

Unit, Dr. Ram Manohar Lohia Hospital, New Delhi for their co-operation and Professor S.K. Brahmachari, Director, IGIB for providing an opportunity to work on this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.05.076.

References

[1] N.M. Siafakas, P. Vermeire, N.B. Pride, P. Paoletti, J. Gibson, P. Howard, J.C. Yernault, M. Decramer, T. Higenbottam, D.S. PostmaOn behalf of the task force. Optimal assessment and management of chronic obstructive pulmonary disease (COPD), The Eur. Resp. Soc. Task Force, Eur. Respir. J. 8 (1995) 1398–1420.
[2] I. Rahman, W. MacNee, Oxidant antioxidant imbalance in smokers and chronic obstructive pulmonary disease, Thorax 51 (1996) 348–350.
[3] J.R. Hoidal, R.B. Fox, P.A. LeMarbe, R. Perri, J.E. Repine, Altered oxidative metabolic responses *in vitro* of alveolar macrophages from asymptomatic cigarette smokers, Am. Rev. Res. Dis. 123 (1981) 85–89.
[4] L. Packer, Oxidants, antioxidant nutrients and the athlete, J. Sports Sci. 15 (1997) 353–363.
[5] A. Meister, M.E. Anderson, Glutathione, Annu. Rev. Biochem. 52 (1983) 711–760.
[6] J.M. Sethi, C.L. Rochester, Smoking and chronic obstructive pulmonary disease, Clin. Chest. Med. 21 (2000) 67–86.
[7] G.L. Snider, Chronic obstructive pulmonary disease: risk factors, pathophysiology and pathogenesis, Annu. Rev. Med. 40 (1989) 411–429.
[8] J.D. Hayes, R.C. Strange, Glutathione-*S*-transferase polymorphisms and their biological consequence, Pharmacology 61 (2000) 154–166.
[9] A.M. Cantlay, C.A. Smith, W.A. Wallace, P.L. Yap, D. Lamb, D.J. Harrison, Heterogeneous expression and polymorphic genotype of glutathione-*S*-transferases in human lung, Thorax 49 (1994) 1010–1014.
[10] A.S. Johansson, G. Stenberg, M. Widersten, B. Mannervik, Structure activity relationships and thermal stability of human glutathione transferase P1-1 governed by the H-site residue 105, J. Mol. Biol. 278 (1998) 687–698.
[11] D. Ryberg, V. Skaug, A. Hewer, D.H. Phillips, L.W. Harries, C.R. Wolf, D. Ogreid, A. Ulvik, P. Vu, A. Haugen, Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk, Carcinogenesis 18 (1997) 1285–1289.
[12] T. Watabe, M. Kanai, M. Isobe, N. Ozawa, The hepatic microsomal biotransformation of delta 5-steroids to 5 alpha, 6 beta-glycols via alpha- and beta-epoxides, J. Biol. Chem. 256 (1981) 2900–2907.
[13] C. Hassett, L. Aicher, J.S. Sidhu, C.J. Omiecinski, Human microsomal epoxide hydrolase: genetic polymorphism and functional expression *in vitro* of amino acid variants, Hum. Mol. Genet. 3 (1994) 421–428.
[14] C.A. Smith, D.J. Harrison, Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema, Lancet 350 (1997) 630–633.
[15] A.J. Sandford, T. Chagani, T.D. Weir, J.E. Connett, N.R. Anthonisen, P.D. Pare, Susceptibility genes for rapid decline of lung function in the lung health study, Am. J. Resp. Crit. Care. Med. 163 (2001) 469–473.
[16] American Thoracic Society. Standardization of spirometry, Am. Rev. Resp. Dis. 136 (1987) 1285–1298.
[17] R. W Light, S.A. Conrad, R.B. George, The one best test for evaluating the effects of bronchodilator therapy, Chest 72 (1977) 512–516.
[18] A.L. Reis, Response to Bronchodilators, in: Pulmonary Function Testing: Guidelines and Controversies, Academics Press, New York, 1982, pp. 15–221.
[19] S.A. Miller, D.D. Dykes, H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, Nucleic Acids Res. 16 (1988) 1215.
[20] J.J. Yim, G.Y. Park, C.T. Lee, Y.W. Kim, S.K. Han, Y.S. Shim, C.G. Yoo, 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 55 (2000) 121–125.
[21] J.R. Wright, H.D. Colby, P.R. Miles, Cytosolic factors which affect microsomal lipid peroxidation in lung and liver, Arch. Biochem. Biophys. 206 (1981) 296–304.
[22] D.J. Jollow, J.R. Mitchell, N. Zampaglione, J.R. Gillette, Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite, Pharmacology 11 (1974) 151–169.
[23] J. Mohandas, J.J. Marshall, G.G. Duggin, J.S. Horvath, D.J. Tiller, Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney, possible implications in analgesic nephropathy, Biochem. Pharmacol. 33 (1984) 1801–1807.
[24] A. Ahsan, R. Ram, M.A. Baig, M.A. Qadar Pasha, ACE I allele and eNOS G allele crosstalk may have a role in chronic obstructive pulmonary disease, Clin. Biochem. 37 (2004) 1037–1040.
[25] T. Ishii, T. Matsuse, S. Teramoto, H. Matsui, M. Miyao, T. Hosoi, H. Takahashi, Y. Fukuchi, Y. Ouchi, Glutathione *S*-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease, Thorax 54 (1999) 693–696.
[26] K. Sundberg, A.S. Johansson, G. Stenberg, M. Widersten, A. Seidel, B. Mannervik, B. Jernstrom, Differences in the catalytic efficiencies of allelic varients of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons, Carcinogenesis 19 (1998) 433–436.
[27] J.E. Repine, A. Bast, I. Lankhorst, Oxidative stress in chronic obstructive pulmonary disease, Am. J. Resp. Crit. Care. Med. 156 (1997) 341–357.
[28] P.N. Dekhuijzen, Antioxidant properties of *N*-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease, Eur. Resp. J. 23 (2004) 629–636.
[29] X.Y. Li, K. Donaldson, I. Rahman, W. MacNee, An investigation of the role of glutathione in the increased permeability induced by cigarette smoke *in vivo* and *in vitro*, Am. J. Resp. Crit. Care. Med. 149 (1994) 1518–1525.
[30] I. Rahman, X.Y. Li, K. Donaldson, D.J. Harrison, W. MacNee, Glutathione homeostasis in alveolar epithelial cells *in vitro* and lung *in vivo* under oxidative stress, Am. J. Physiol. 269 (1995) L285–L292.
[31] R.I. Harik-Khan, J.L. Fleg, R.A. Wise, Body mass index and the risk of COPD, Chest 121 (2002) 370–376.