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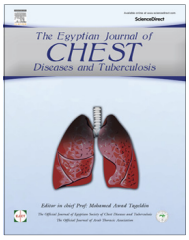

ORIGINAL ARTICLE

Role of glutathione S-transferase P-1 (GSTP-1) gene polymorphism in COPD patients

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Abstract *Background:* COPD is a multifactorial disease. It is a widely distributed disease with high morbidity and mortality, associated with different pathologies. The link between glutathione S-transferase P-1 (GSTP1) polymorphism and COPD needs to be clarified.

Objectives: To show the relationship between GSTP1 gene polymorphism and pathogenesis of COPD, and to clarify the role of smoking in GSTP1 gene polymorphism.

Patients and methods: This study carried out on 30 COPD patients met the clinical criteria of COPD set by GOLD 2013 and 20 healthy controls. Blood was sampled for studying GSTP1 gene polymorphism by PCR-RFLP.

Results: There was a significant difference between group A (COPD smokers) and group B (COPD non smokers) regarding the presence of non mutant and heterozygous mutation. GSTP1 mutation was significantly higher in group C (smoking controls) than group D (non smoking controls). There was a highly significant difference between smoking subjects than non smoking compared with mutation. There was no significant difference in mutation between smokers and ex-smokers. There was no significant difference between studied subjects having heterozygous mutation and subjects without as regards their spirometry results.

Conclusion: There is a significant association between GSTP1 gene polymorphism and the development of COPD, and smoking have a role in GSTP1 gene polymorphism. The polymorphism has no relation to disease severity.

Introduction

COPD is defined as a preventable and treatable disease, characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic

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inflammatory response in the airways and the lung to noxious particles or gases [1].

One of pathogenesis of COPD is oxidant/antioxidant imbalance. Indeed, it is well known that chronic tobacco smoking is a major risk factor for the development of COPD, and a defect in the detoxification of reactive species produced by cigarette smoke may predispose smokers to airflow obstruction and emphysema [2].

Potential explanations for why some cigarette smokers acquire chronic airways disease but not others include genetic differences in inflammatory and repair processes and/or modulation of these responses by infections or other environmental insults suggesting that individual susceptibility or genetic factors may play a role [3].

Several candidate genes such as α-1-antitrypsin (AAT), tumor necrosis factor α (TNF-α), heme oxygenase-1 (HMOX), cytochrome P450, and microsomal epoxide hydrolase (mEPHX) that could be involved in the development of COPD were investigated. Among the genes that contribute to xenobiotic metabolism and susceptibility to COPD, the glutathione S-transferase (GST) gene family is one of the most studied in diverse human populations [2].

GST is a family of enzymes comprising 16 genes in six subfamilies [alpha (GSTA), mu (GSTM), omega (GSTO), pi (GSTP), theta (GSTT), and zeta (GSTZ)], which is a group of phase II enzymes that catalyze the glutathione conjugation of many endogenous and exogenous electrophilic compounds, such as environmental toxins, and oxidative stress products [2,4]. The GSTM1, T1 and P1 enzymes are expressed in lung tissues [5], where the GSTP1 was found to be mainly present in the respiratory tract [6].

GSTP1 is found in humans with an A (Adenine) to G (Guanine) substitution at exon 5 causing Isoleucine (Ile) to Valine (Val) replacement at codon number 105 within the active site of the protein [7].

Aim of the work

The aim of the current study was to evaluate the role of GSTP1 gene in the pathogenesis of COPD patients and to clarify the role of smoking in GSTP1 gene polymorphism in exon 5.

Patients and methods

This work was carried out on 30 patients with COPD diagnosed and classified according to GOLD 2013 [1], referred to the Chest Department, Faculty Of Medicine, Menoufia University and Chest hospital of Shebin El Kom. The study also included 20 healthy controls.

Subjects were divided into:-

Group A: included 15 smoker patients with COPD and were subdivided into: 10 patients with moderate COPD and 5 patients with severe COPD.
Group B: included 15 non smoker patients with COPD and were subdivided into: 11 patients with moderate COPD and 4 patients with severe COPD.
Group C: included 10 healthy smoking volunteers who had no symptoms or signs of any chest disease and had normal ventilatory function tests as a control group.
Group D: included 10 healthy non smoking volunteers who had no symptoms or signs of any chest disease and had normal ventilatory function tests as a control group.

After having a written consent; each patient underwent:

1. Full history taking including smoking history & smoking index.
2. Full Clinical examination.
3. Chest X-ray (PA & Lat view) and high resolution computed tomography (HRCT) chest if needed.
4. Electrocardiography (ECG) & Echo if needed.
5. Arterial Blood Gases (ABG).
6. Pulmonary function tests.

Spirometry was done for all subjects. All patients had FEV_1/FVC of less than 70% and post bronchodilator spirometry was performed after giving the patient a bronchodilator, such as an inhaled beta-agonist e.g. salbutamol 400 μg) [1].

The following parameters were measured:

(a) Forced expiratory volume in the first second (FEV_1) pre and post bronchodilator.
(b) Forced vital capacity (FVC).
(c) FEV_1/FVC ratio.
(d) Peak expiratory flow rate (PEFR).
(e) Forced expiratory flow from 25% to 75% (FEF25–75) of vital capacity.

The classification of Severity of Airflow Limitation in COPD patients with FEV1/FVC < 0.70 (Based on Post-Bronchodilator FEV1) according to GOLD staging system (GOLD 2013) [1] into:

GOLD 1: Mild FEV1 ⩾ 80% predicted.
GOLD 2: Moderate 50% ⩽ FEV1 < 80% predicted.
GOLD 3: Severe 30% ⩽ FEV1 < 50% predicted.
GOLD 4: Very Severe FEV1 < 30% predicted.

7 blood samples were drawn for studying glutathione S-transferase P1 gene polymorphism by PCR-RFLP. Blood collected on EDTA tubes was used for DNA extraction using gene JET whole blood DNA Mini Kits (Thermo Scientific, Sigma) stored at −20 for PCR amplification with an initial denaturation at 95 for 5 min and repeated 40 cycles of denaturation at 95 for 1 min, annealing at 56 for 1 min and extension at 72 for 1 min followed by a final extension at 72 for 5 min using forward primer (5-GTAGTTTGCCCAAGGTCAAG-3) and reverse primer (5-AGCCACCTGAGGGGTAAG-3). PCR product was digested by fast digest AIw26I restriction enzyme for 2 h at 37.

Results

Table 1 shows the distribution of patients and controls regarding their demographic criteria: age, sex, smoking status, smoking index. The age of the studied patient ranged from 43 to 75 years with mean age and standard deviation of 62.40 ± 9.32 in group A, 59.66 ± 6.33 in group B, 38.30 ± 5.86 in group C and 40.40 ± 7.73 in group D. Moreover, the number of male subjects was higher than females, 36

Table 1 Distribution of the studied groups regarding their demographic criteria.

	Groups							
	A (n = 15)		B (n = 15)		C (n = 10)		D (n = 10)	
	No	%	No	%	No	%	No	%
Age								
Mean ± SD	62.40 ± 9.32		59.66 ± 6.33		38.30 ± 5.86		40.40 ± 7.73	
Gender								
Male	15	100.0	6	40.0	10	100.0	5	50.0
Female	0	0.0	9	60.0	0	0.0	5	50.0
Smoking status								
Smoker	4		0		10		0	
Ex-smoker	11		0		0		0	
Non	0		15		0		10	

Table 2 Distribution of the studied groups regarding their Spirometry results.

Spirometry results	Groups								Fisher's exact	P value
	A (n = 15)		B (n = 15)		C (n = 10)		D (n = 10)			
	No	%	No	%	No	%	No	%		
Normal (n = 18)					**8**	**80.0**	10	100.0	2.22	**0.474**
Small airway obstruction (n = 2)					**2**	**20.0**	0	0.0		
Gold stage										
Moderate (n = 21)	**10**	**66.7**	11	73.3					0.15	**1.0**
Severe (n = 9)	**5**	**33.3**	4	26.7						

Bold values represent number of mutations.

and 14, respectively. Regarding smoking status the number of current smokers was 14 and ex-smoker was 11, while non smokers were 25.

Table 2 shows the comparison between the studied groups regarding their spirometric results, it shows the distribution of the studied groups regarding GOLD stages of patients in groups A and B (classified according to GOLD 2013). There were: 10 (66.7%) smoker patients in group (A) and 11 (73.7%) non-smoker patients in group (B) had moderate COPD. 5 (33.3%) smoker patients in group (A) had severe COPD. While from non-smoker patients in group (B) was 4 (26.7%) with severe COPD. There was a non significant difference between the studied groups (A) and (B) as regards the GOLD stage (P-value > 0.05). Regarding the control groups C and D there were two subjects (20%) of group C (control smokers) having small airway obstruction and 8 (80%) normal, while those from non-smoker controls in group (D) were 10 (100%) with normal pulmonary function tests. There was a non significant difference between the studied groups (C) and (D) as regards to their pulmonary function tests (P-value > 0.05).

Table 3 shows the distribution of all studied groups regarding mutation of GSTP1 gene polymorphism in each group. From 15 COPD smoker patients in group (A), 12 (80%) had heterozygous mutation, from 15 COPD non-smoker patients in group (B), 5 (33.3%) had heterozygous mutation, from 10 control subjects in group (C), 7 (70%) had heterozygous mutation, while 1 (10%) from 10 control subjects in group (D), a significant difference between groups A and B regarding type of mutation (P-value > 0.05), highly significant difference between groups A and D (P-value < 0.01) and a significant difference between groups C and D (P-value > 0.05).

Table 4 shows the distribution of all studied subjects regarding their smoking status compared with mutation. Twenty-five subjects have heterozygous mutation, eleven of them were smoking, eight were ex-smokers while six were non smokers. Twenty-five subjects have normal genotyping (non mutant), three of them were smoking, three were ex-smokers while nineteen were non smokers. There was a significant difference between subjects having heterozygous mutation and subjects without mutation as regards smoking status (P-value > 0.05), highly significant difference between them as regards non smoking status (P-value > 0.01) and non significant difference as regards ex-smoking status.

Table 5 shows the distribution of all studied groups regarding their spirometry results compared with mutation. There was no significant difference between subjects having heterozygous mutation and subjects without mutation as regards spirometry results (P-value < 0.05) (see Fig. 1).

Discussion

COPD is a multifactorial disease with possible genetic predisposition and involvement of various environmental factors. Among the genes that contribute to xenobiotic metabolism and susceptibility to COPD, GSTP1 gene is one of the most studied in diverse human populations [2]. GSTP1 was found to be mainly present in the respiratory tract and was proposed to be involved in the development of COPD [6].

Table 3 Distribution of the studied groups regarding their mutation.

Mutation	Groups								Fisher's exact	*P* value
	A (n = 15)		B (n = 15)		C (n = 10)		D (n = 10)			
	No	%	No	%	No	%	No	%		
Non mutant	3	20.0	10	66.7	3	30.0	9	90.0	F_1 = 6.65 F_2 = 0.33 F_3 = 11.78	P_1 = 0.025(S) P_2 = 0.653 P_3 < 0.001(HS)
Heterozygous	12	80.0	5	33.3	7	70.0	1	10.0	F_4 = 3.23 F_5 = 1.79 F_6 = 7.50	P_4 = 110 P_5 = 0.344 P_6 = 0.019(S)

F_1 = A, B F_2 = A, C F_3 = A, D F_4 = B, C F_5 = B, D F_6 = C, D.
S = significant HS = highly significant. Non mutant = mutation is not detected.
Heterozygous = two different alleles of the same gene.

Table 4 Distribution of the studied subjects regarding their smoking status compared with mutation.

Smoking	Mutation				*Z* test	*P* value
	Non mutant (n = 25)		Heterozygous (n = 25)			
	No	%	No	%		
Smoker	3	12.0	11	41.7	2.05	0.040(S)
Ex-smoker	3	12.0	8	33.3	1.46	0.143
Non	19	76.0	6	25.0	3.32	<0.001(HS)

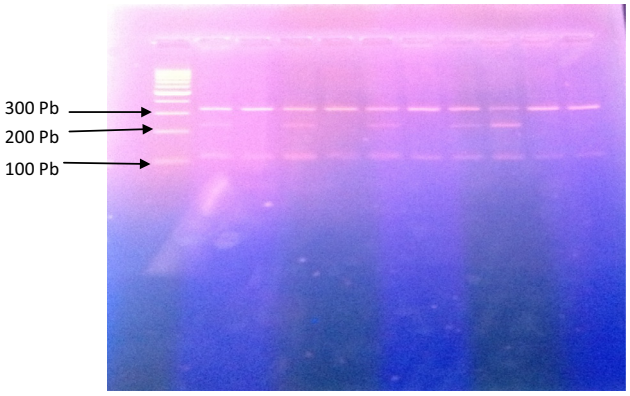

Figure 1 Shows gel electrophoresis of polymorphism of the GSTP1 gene. Lane 1 is the molecular marker of 100 bps. Lanes 2, 4, 6, 8 and 9 of 200 pb represents heterozygous mutations of GSTP1 by substitution of isoleucine (Ile) by valine (Val) at codon number 105. Lanes 3, 5, 7, 10 and 11 represent a non mutant type of GSTP1.

Smoking alters several genes that can be associated with health problems for smokers, such as increased risk of COPD [8].

Only a relatively small proportion of smokers actually develop airway obstruction. Genetic factors are related to this susceptibility and include genes regulating the protease–antiprotease and oxidant–antioxidant interactions [9].

Oxidant stress and reactive oxygen species, resulting from an oxidant/antioxidant imbalance, are believed to play an important role in the pathogenesis of COPD. Indeed, it is well known that chronic tobacco smoking is a major risk factor for the development of COPD, and a defect in the detoxification of reactive species produced by cigarette smoke may predispose smokers to airflow obstruction and emphysema [2].

A combination of oxidative attack and changes in antiprotease activity could amplify the lung tissue damage in COPD. This underlines that the interactions between genes as well as environmental factors may play an important role in the development of this pathogenesis [2] .

Lakhdar et al. [2] made a study concerning the relationship between GSTP1 polymorphism and COPD in a Tunisian

Table 5 Distribution of the studied groups regarding their Spirometry results compared with mutation.

Spirometry	Mutation				Fisher's exact	*P* value
	Non mutant (n = 25)		Heterozygous (n = 25)			
	No	%	No	%		
Groups (C&D)						
Small airway obstruction	0	0.0	2	25.0	3.33	0.147
Normal	12	100.0	6	75.0		
GOLD stage groups (A&B)						
Moderate	11	84.6	10	58.8	2.33	0.127
Severe	2	15.4	7	41.2		

population and concluded that subjects with GSTP1 Val105 allele are at higher risk of COPD.

So, in the present study, we attempted to analyze the relationship between GSTP1 gene polymorphism and COPD as GSTP1 was found to be mainly present in the respiratory tract and to clarify the role of smoking in GSTP1 gene polymorphism.

In the current study, the age of the studied patients ranged from 43 to 75 years old with mean age and standard deviation of 62.40 ± 9.32 in group A, 59.66 ± 6.33 in group B, 38.30 ± 5.86 in group C and 40.40 ± 7.73 in group D. Moreover, the number of male subjects was higher than females (Table1). This result comes in agreement with Lakhdar et al. [2] who explained this result by the fact that the major population of COPD patients and chronic smokers are males in Egypt. Therefore, it is difficult to find a balanced set of female subjects for genotype studies. Secondly, there was a difference in mean age between cases and controls. In fact, younger controls were more likely to provide a blood sample than older controls, and were thereby over sampled.

Many studies have reported on the association between GST gene polymorphisms and pulmonary disorders but with controversial results [7].

In the present study, on comparing results of GSTP1 gene polymorphism among the studied COPD patient groups, it was found that heterozygote mutation was significantly higher in group A (COPD smokers) than in group B (COPD non smokers). This result comes in accordance with Bentley et al. [10] who declared that in GSTP1, the heterozygous mutation due to substitution of Ile amino acid at codon number 105 by Val which causes altered affinity for specific substrates, was associated with chronic smoking COPD patients (Table 3).

Similarly, Lakhdar et al. [2] found the Tunisians carrying a GSTP1 Val105 allele were at higher risk of COPD. In addition, the meta-analysis completed by Yan et al. [11] suggested that the GSTP1 105Val/Val genotype was an important genetic contributor to COPD susceptibility. In contrast, Yim et al. [12] showed no association between GSTP1 gene polymorphism at exon 5 and COPD in Koreans, while Ishii et al. [13] showed an increased prevalence of homozygous mutation (similar alleles) in GSTP1 polymorphism at exon 5 in Japanese COPD patients.

Regarding (Table 4) of the current study, the number of detected GSTP1 mutations was significantly higher in smoking patients than those not smoking or quit smoking. Out of twenty-five heterozygous mutations detected, eleven subjects were smoking (41.7%), six were not smoking (25%) and eight were ex-smokers (33.3%).

This result comes in accordance with Spira [14] who stated that some people who smoke respond differently at a genetic level to a cigarette smoke. These people may be at a heightened risk of developing COPD because genes that normally protect lung cells against environmental toxins are altered, including some genes linked to COPD. Moreover, Zuntar et al. [7] reported that there were no significant differences between healthy smokers and COPD-smokers.

Regarding (Table 5), the current study showed no significant difference between studied subjects having heterozygous mutation and subjects without as regards spirometry results.

This result comes in accordance with the International Journal of COPD., [15] a study search that identified 104 publications reporting a total of 130 genes and 48 intergenic regions studied in 20,288 individuals including GSTP1 exon 5 polymorphism. This great study declared that none of the studied genes was significantly associated with forced expiratory volume in one second (FEV1) or FEV/forced vital capacity (FVC) ratio after correction for multiple testing.

Moreover, Rodriguez et al. [16] declared that no significant association was observed in different genotypes of the GSTP1 with lung function in the group of patients with COPD. They reported that the GSTP1 genotypes studied did not seem to influence the severity of lung function impairment in patients with COPD.

However, in a large study designed by He et al. [17] involving 1098 individuals with different degrees of lung function impairment, the results demonstrated a significant association of mutation with rapid decline in lung function in smokers with mild to moderate airflow obstruction.

Conclusion

From the present study we concluded that there is a relationship between GSTP1 gene polymorphism and development of COPD. Smoking has a role in GSTP1 gene polymorphism. GSTP1 gene polymorphism in COPD patients has no relation to the GOLD stage. Further works using a larger population and studying more candidate genes taking in account the gene-environment interaction remains necessary in order to elucidate the genetic pathogenesis of COPD.

Conflict of interest

There is no conflict of interest.

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