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Association of glutathione-S-transferase omega haplotypes with susceptibility to chronic obstructive pulmonary disease

DILYARA G. YANBAEVA[1], EMIEL F. M. WOUTERS[1,2], MIEKE A. DENTENER[1], MARTIJN A. SPRUIT[2], & NIKI L. REYNAERT[1]

[1]*Department of Respiratory Medicine, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University Medical Centre, Maastricht, The Netherlands, and* [2]*Department of Research, Development & Education, Centre for Integrated Rehabilitation of Organ failure (CIRO), Horn, The Netherlands*

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Abstract

Cigarette smoking is the main risk factor for developing the inflammatory lung disease chronic obstructive pulmonary disease (COPD). Differences in susceptibility among smokers have been attributed to a genetic predisposition. A recent publication on the Framingham Heart Study found a strong association of the Asn142Asp SNP in *Glutatthione-S-transferase Omega* (*GSTO) 2* with forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC). FEV_1 is the main parameter reflecting the degree of airflow limitation in patients with COPD. Therefore the present study was undertaken to investigate whether the Asn142Asp polymorphism in *GSTO2* occurs more frequently in patients with COPD than healthy subjects and to replicate the finding that it strongly correlates with FEV_1. Furthermore, the Ala140Asp substitution in *GSTO1* was examined. Genotyping was carried out in 195 healthy controls and 355 patients with COPD. The results demonstrate that the Asn142Asp polymorphism in *GSTO2* and the *GSTO1*140Asp/*GSTO2*142Asp haplotype were associated with increased risk of COPD. However, single-marker and haplotype-based analyses failed to reveal an association between lung function parameters and investigated non-synonymous coding SNPs in the *GSTO* genes. In conclusion, GSTO2 is a candidate gene for COPD, but is not associated with FEV_1.

Keywords: *GST-O, COPD, polymorphism, FEV_1*

Introduction

Chronic obstructive pulmonary disease (COPD) is a leading global cause of morbidity and mortality [1]. It is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, cigarette smoke most notably [2]. The inflammatory response in the airways is known as chronic bronchitis, whereas in the parenchyma it is associated with destruction of lung tissue or emphysema. The main characteristic of COPD is airflow limitation that is not fully reversible and progressive [2]. Although cigarette smoking is the main risk factor for developing COPD, only a proportion of smokers become COPD patients, indicating that other factors may be involved, including a genetic predisposition [3].

An array of polymorphisms has been associated with COPD, including members of the Glutathione-S-transferase (GST) family, namely *GSTT1*, *GSTP1* and *GSTM1* [4]. GSTs are enzymes that conjugate electrophilic compounds to glutathione, allowing further metabolism and excretion. The omega class, on which there is only one study in COPD to date [5], contains two expressed genes, *GSTO1* and *GSTO2*, that lie 7.5 kb apart on chromosome 10q24.3 and share 64% amino acid sequence homology. GST-O is a member of the GST family based on homology, but does not share substrate specificity. They display monomethylarsonate reductase, dehydroascorbate reductase as well as glutathione-dependent thioltransferase activities [6]

Correspondence: Niki L. Reynaert, Maastricht University, Department of Respiratory Medicine, PO Box 616, 6200 AZ Maastricht, The Netherlands. Tel: +31-43-3882270. Fax: +31-43-3875051. Email: n.reynaert@pul.unimaas.nl

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and are therefore closely related to glutaredoxins, enzymes that catalyse disulphide and protein-glutathione mixed disulphide reductions.

In lung samples of COPD patients, both glutaredoxin 1 [7] and GST-O1 [5] protein levels have been found to be decreased compared to healthy controls. Glutaredoxins and GST-Os are associated with the homeostasis of the major antioxidant in the lungs, glutathione and could thus contribute to the oxidant–antioxidant imbalance that is believed to play an important role in the pathogenesis of COPD.

A recent publication on the Framingham Heart Study (FHS) that reported genome-wide association analysis (GWA) for measures of lung function found a strong association of the non-synonymous coding SNP (rs156697, Asn142Asp) in *GSTO2* with mean forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) measurements and was thus named credible candidate gene for pulmonary function [8].

FEV_1 is the major lung function parameter reflecting the degree of airflow limitation in patients with COPD and therefore the present study was undertaken to investigate whether the Asn142Asp polymorphism in *GSTO2* occurs more frequently in COPD patients than healthy subjects, as well as to replicate the finding that it strongly correlates with FEV_1. Furthermore, the Ala140Asp substitution in *GSTO1* was examined.

Materials and methods

Subjects

We carried out a case-control association study in 550 Caucasian subjects, including 355 patients with clinically stable moderate-to-severe COPD entering pulmonary rehabilitation (Center for Integrated Rehabilitation of Organ failure (CIRO), Horn, The Netherlands). Clinical history of COPD and disease severity were assessed according to the published Global Initiative for Chronic Obstructive disease (GOLD) guidelines [2]. All patients were current or former smokers. To provide a control population from the same geographical area, 195 apparently healthy (ex)-smoking volunteers were recruited through an advertisement in a local newspaper and applicants of the COSMO study [9]. The ethical review board of the University Hospital Maastricht approved the study and all subjects gave their written informed consent.

Clinical examination and measurements

FEV_1 and FVC were calculated from the flow-volume curve using standardized spirometry. FEV_1 was performed after inhalation of a β_2-agonist via a metered dose inhaler. BMI was calculated as body weight divided by squared height (kg/m^2). Genotyping for both SNPs was carried out by *Taqman*® allelic discrimination assays by means of an ABI 7900HT instrument according to the manufacturer's specifications (Applied Biosystems, Foster City, CA). Negative and positive controls were included. Average genotyping rate was 99%. IL-6 was determined in duplicate in EDTA plasma by Pelkine high-sensitivity sandwich enzyme-linked immunosorbent assay (ELISA) (Sanquin, Amsterdam, The Netherlands). C-reactive protein (CRP) was assessed in EDTA plasma in duplicate by high-sensitivity particle enhanced immunoassay (COBAS Mira Radiometer, Copenhagen). Fibrinogen was assessed using a coagulation analyser (Sysmex CA-7000; Dade-Behring, Liederbach, Germany) according to the Clauss method and calculated from EDTA to citrate plasma values.

Power calculation

Power was estimated using Quanto [10]. Assuming disease prevalence of 10%, case-control ratio in our population (1:0.55), minor allele frequencies 0.33 and 0.40 for rs4925 and rs156697 (from the Hap Map reference panel), additive inheritance mode and odds ratio of 1.5, the case-control sample afforded an estimated power of 86% and 88%, respectively, at two-sided significance level 0.05.

Statistical analysis

Variables are presented as proportions (percentage), mean $\pm$ SD or median (range) depending on their measurement scale and distribution. Differences between COPD patients and healthy controls were analysed using Student's *t*-test and Mann-Whitney test for continuous variables and χ^2 square test for categorical variables.

To determine the linkage disequilibrium (LD) we calculated the *D'* statistics between two SNPs. Hardy–Weinberg equilibrium was tested by the exact χ^2 statistic. Genetic effects have been tested under the additive genetic model. Differences in haplotype distributions were tested using the global statistic of Schaid et al. [11]. Genotype and haplotype-specific effects were estimated using linear and logistic regression for quantitative (all, but COPD) and binary outcome (COPD) [12]. Post-bronchodilator FEV_1, FVC and COPD were treated as primary outcomes. Post-bronchodilator FEV_1 percentage predicted, FVC percentage predicted and systemic inflammation levels were chosen as secondary outcomes. Regression models were adjusted for age and sex (excepting percentage predicted outcomes), BMI, pack-years smoked, smoking status and hypertension. Models with FEV_1 and FVC were additionally adjusted for height and affection status (for the total group).

Table I. Baseline characteristics of COPD patients and healthy controls.*

Characteristics	Controls ($n=195$)	COPD ($n=355$)	p-value
Years of age	54.3 (7.3)	64.2 (9.4)	0.000
Male, n (%)	94 (48)	219 (62)	0.002
Current smoker, n (%)	115 (59)	90 (25)	0.000
Tobacco consumption, pack years-smoked	29.6 (14.8)	39.9 (18.5)	0.000
FEV1, l	3.2 (0.7)	1.1 (0.5)	0.000
FEV1,% predicted	103.3 (15.1)	41.9 (16.0)	0.000
FEV1/FVC,%	78.3 (5.3)	42.0 (11.9)	0.000
FVC, l	4.1 (0.9)	3.0 (0.9)	0.000
FVC,% predicted	109.3 (16.8)	86.3 (21.1)	0.000
BMI, kg/m^2	26.4 (3.7)	25.0 (5.0)	0.000
Hypertension,[a] n (%)	33 (17)	88 (25)	0.01
CRP, mg/L	1.3 (0.2–3.19)	4.6 (1.3–11.09)	0.000
IL-6, pg/mL	0.7 (0.45–1.38)	2.1 (1.15–4.24)	0.000
Fibrinogen, g/L	3.3 (3.00–3.60)	3.6 (3.23–3.82)	0.000

*Data presented as mean (SD), median (inter quartile range) or n (%).
[a]Hypertension was defined as blood pressure >140/90 mmHg or need for anti-hypertensive treatment.

Results

The demographic and clinical characteristics of participants included in this study are presented in Table I. COPD patients were older, more likely to be men and had more pack-years smoked compared to healthy smokers and, on average, had moderate-to-severe airflow obstruction.

Allele frequencies and genotype distributions are reported in Table II. There was no significant deviation from Hardy-Weinberg equilibrium in cases or controls. There were differences in allele and genotype frequencies between COPD patients and healthy smokers for the *GSTO2* SNP rs156697. The minor allele of this SNP was associated with 1.4 times higher risk of being a COPD patient compared to a healthy smoker (Table II, $p=0.047$). The association was stronger under the dominant model ($p=0.037$, OR = 1.60; 95% CI = 1.03–2.49). None of the SNPs was associated with FEV_1 or FVC in the total study group (Table II).

The two polymorphisms were in moderate LD with one another ($D'=0.4$) and generated four common haplotypes (Table III). Haplotype analysis revealed an even stronger association of *GSTO* genes with COPD (p overall = 0.016) but not with lung function phenotypes in the total group (Table III). Further analysis showed that the haplotype H2 (*GSTO1*140Asp/*GSTO2*142Asp) was associated with increased risk of COPD when tested against all other haplotypes ($p=0.003$) and vs the most common referent haplotype H1 ($p=0.002$, OR = 2.4; 95% CI = 1.43–4.02) (Table III).

To evaluate the possible association with percentage predicted FEV_1 and FVC, we performed additional analysis in the total group, but no further associations were found. Additionally, no evidence for association was detected both in genotype and haplotype-based analyses when actual and predicted FEV_1 and FVC were analysed in controls or COPD patients only, as well as when men and women were considered separately (data not shown). Neither of

Table II. Association of rs4925 and rs156697 and primary outcomes.*

			COPD		FEV_1		FVC	
Parameters	Controls, n	COPD, n	OR (95% CI)	p-value	Diff (95% CI)	p-value	Diff (95% CI)	p-value
GSTO1 Asp140Ala (rs4925)								
Asp/Asp	88	170						
Asp/Ala	85	139	0.93 (0.67–1.30)	0.68	−0.03 (−0.09–0.03)	0.36	−0.02 (−0.11–0.07)	0.73
Ala/Ala	19	41						
Ala, %	32%	32%		0.88				
GSTO2 Asn142Asp (rs156697)								
Asn/Asn	97	141						
Asn/Asp	77	160	1.39 (1.00–1.93)	0.047	0.02 (−0.04–0.07)	0.59	−0.03 (−0.12–0.05)	0.44
Asp/Asp	21	53						
Asp, %	31%	38%		0.019				

*Logistic regression analysis was adjusted for baseline age, sex, BMI, smoking status, pack-years smoked and hypertension.

Table III. Association between *GSTO* haplotypes and COPD.*

Haplotype	Allele GSTO1 Rs4925	Allele GSTO2 Rs156697	Estimated frequency Controls	Estimated frequency COPD	COPD Score statistic[a]	COPD *p*-value[a]	COPD OR[b] (95% CI)	COPD *p*-value[b]	FEV1** Score statistic[a]	FEV1** *p*-value[a]	FEV1** Diff±SE[b]	FEV1** *p*-value[b]	*FVC*** Score statistic[a]	*FVC*** *p*-value[a]	*FVC*** Diff±SE[b]	*FVC*** *p*-value[b]
H1 11	140Asp	142Asn	0.55	0.45	−1.49	0.14	1.00	—	0.01	0.99	1.00	—	0.13	0.89	1.00	—
H2 12	140Asp	142Asp	0.13	0.23	2.94	0.003	2.40 (1.43–4.02)	0.002	1.66	0.10	0.09±0.06	0.13	0.48	0.63	0.07±0.09	0.47
H3 21	140Ala	142Asn	0.15	0.17	−0.29	0.77	1.51 (0.87–2.60)	0.19	−0.73	0.47	0.01±0.05	0.77	0.21	0.84	0.06±0.08	0.42
H4 22	140Ala	142Asp	0.17	0.14	−0.32	0.74	0.94 (0.59–1.50)	0.94	−0.82	0.41	−0.03±0.06	0.53	−0.99	0.32	−0.1±0.09	0.27
Global[c]								0.016				0.38				0.65

*All tests were adjusted for baseline measures of age, sex, pack-years smoked, current smoking status and body mass index and hypertension.
**Regression models with FEV1 and FVC as outcome variables were additionally adjusted for height and affection status (COPD/control).
[a]Score test *p*-values refer to tmhe comparison between the given haplotype against all others (haplo.score function).
[b]Individual haplotype effects and *p*-values were estimated by logistic and linear regression relative to the most common haplotype H1 (haplo.glm function).
[c]Global test *p*-value from haplo.score for overall association between haplotypes and trait.

the SNPs was associated with plasma levels of CRP, IL-6 or fibrinogen in COPD (data not shown).

Discussion

GWA studies have emerged as a powerful approach to identify genes involved in complex diseases. Recently, the FHS reported a positive association of the Asn142Asp *GSTO2* polymorphism with main lung function parameters, FEV_1 and FVC [8]. We aimed to test *GSTO1* and *2* as new candidate genes for COPD and to replicate the results of this first GWA for pulmonary function measures. Although both single-marker and haplotype-based analyses failed to reveal an association between lung function parameters and investigated non-synonymous coding SNPs in the *GSTO* genes, the Asn142Asp polymorphism in the *GSTO2* and haplotype H2 (*GSTO1*140Asp/*GSTO2*142Asp) were associated with increased risk of COPD in this case-control study.

The main difference between the FHS and the study presented here is that while FHS is a prospective study of a general population, our study has a case-control design, resulting in significant differences in lung function distribution between studies. To overcome this, we have chosen to analyse FEV_1 and FVC in the total study group which reflects a full scale of outcome variables. All analyses in the total group were additionally adjusted for affection status (COPD/control). Another interesting difference between studies is that FHS selected mean pre-bronchodilator FEV_1 and FVC values over two examinations as outcomes, whereas we have tested single-point post-bronchodilator spirometry values as primary phenotypes. Therefore, subjects in the FHS with a declined FEV_1 could have either been asthmatics, where FEV1 reverses after administering the bronchodilator, or COPD patients where the bronchodilator would have a minimal effect. Since we did not observe a relation of the Asn142Asp polymorphism in *GSTO2* with FEV_1 in COPD patients, the association with FEV_1 could potentially be found in asthmatics. Many polymorphisms in *GST* genes have been identified in both lung diseases and *GSTO* in asthma has also not been investigated, warranting further studies. Lastly, the lack of association with lung function could be due to the smaller sample size of the present study.

Regardless of the lack of association with FEV_1 and FVC, this study does demonstrate a significant association between the Asn142Asp polymorphism in *GSTO2* as well as the haplotype H2 (*GSTO1*140Asp/*GSTO2*142Asp) and COPD. Both SNPs investigated are non-synonymous

coding SNPs. Gene expression studies for the Asn142Asp SNP in *GSTO2* in COS-1 cells have demonstrated protein expression levels to be 24% lower compared to wild type [13]. Studies on the Asp140Ala SNP in *GSTO1* are conflicting, with one study reporting decreased thioltransferase activity by 25% compared to wild type [14], whereas another study observed no activity differences [6]. Furthermore, decreased expression of *GSTO1* has recently been observed in lungs of patients with COPD [5]. The possible link between SNPs in *GSTOs* and altered levels of proteins and activities in disease remains to be tested.

Both GST-O enzymes play an important role in the biotransformation of arsenic, which is present in cigarette smoke. To date, few studies have explored an association between arsenic exposure and respiratory symptoms [15]. An increased risk for COPD among people with arsenical skin lesions has not been investigated but is certainly plausible since there is a strong link between arsenic exposure and lung cancer [16] and the high incidence of lung cancer in COPD patients [17].

Our results do not exclude other disease-related SNPs in the region of the investigated polymorphisms. According to Hapmap data, the Asn142Asp SNP is in high LD with several other SNPs in both *GSTO* genes. For example, the *GSTO1* intron SNP rs1147611 is a possible candidate with a high conservation score of 0.53, whereas Asn142Asp itself has a score of 0.002 [18]. Interestingly, around *GSTO1* and *2* there are also five protein-coding genes with yet unknown functions. We cannot exclude that a causal SNP is located in those uninvestigated genes.

We did not observe an association between the investigated SNPs and plasma levels of CRP, IL-6 or fibrinogen, which were elevated in COPD as a sign of systemic inflammation. Future studies on the proinflammatory mediator interleukin 1β would furthermore be of interest, since GSTO1-1 has been implicated in the post-translational processing leading to the release of this cytokine [19].

Taken together, this study demonstrates that *GSTO2* is a candidate gene for COPD, but that it is not associated with FEV_1, in the investigated COPD patients and healthy smokers from the Netherlands.

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