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Genetic variants of microsomal epoxide hydrolase and glutamate-cysteine ligase in COPD

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ABSTRACT: The genetic factors that contribute to the development of chronic obstructive pulmonary disease (COPD) are poorly understood. Many candidate genes have been proposed, including enzymes that protect the lung against oxidative stress, such as microsomal epoxide hydrolase (EPHX1) and glutamate-cysteine ligase (GCL). To date, most reported findings have been for EPHX1, particularly in relation to functional variants associated with fast and slow metabolism of epoxide intermediates. The present study aimed to identify any association of variation in these genes with COPD susceptibility or severity.

In total, 1,017 white COPD patients and 912 nondiseased age and sex matched smoking controls were genotyped for six single nucleotide polymorphisms (SNPs) in EPHX1 (including the fast and slow variants and associated haplotypes), and eight SNPs in the two genes encoding GCL. GCL is a rate-limiting enzyme in the synthesis of glutathione, a major contributor to antioxidant protection in the lung.

No association of variation was found in EPHX1 or GCL with susceptibility to COPD or disease severity.

This is the largest reported study to date and is well powered to detect associations that have been previously suggested. The current data indicate that these genetic variants are unlikely to be related to susceptibility or disease severity in white chronic obstructive pulmonary disease patients.

KEYWORDS: Chronic obstructive pulmonary disease, genetic susceptibility, glutamate-cysteine ligase, microsomal epoxide hydrolase

Chronic obstructive pulmonary disease (COPD) is the sixth leading cause of morbidity and mortality in the western world and the prevalence of the disease is increasing as the population ages [1]. Cigarette smoking is the major environmental contributor to the disease in western societies yet only ~15% of cigarette smokers develop the disease, indicating that genetic factors play a part in determining susceptibility. With the notable exception of severe α_1-antitrypsin (α_1-AT) deficiency, these genetic factors are poorly understood.

Given that >95% of those who develop COPD are smokers and oxidative stress is thought to be important in the pathogenesis of the disease, genetic variation in enzymes that protect the lung

against smoke-induced oxidative stress has been a significant focus of study. Microsomal epoxide hydrolase (EPHX1), an enzyme involved in the first-pass metabolism of epoxide intermediates, has received particular attention as two functional variants of the gene, which confer slow and fast metabolic activity, have been identified [2]. The slow variant decreases activity by ~40–50%, whilst the fast variant increases activity by ~25%. Since the initial report of an association between increased susceptibility to COPD and His-His homozygosity at the "slow" variant locus (Tyr113His in exon 3) [3], subsequent studies have variously supported this association [4–6], or have reported an association with disease severity rather than susceptibility [7]; observed no association between this polymorphism and

disease susceptibility or severity [8–10]; or reported a protective effect of this polymorphism [11]. Similar disparities in study outcomes are reported for the ''fast'' variant (His139Arg in exon 4) with possession of the minor variant reported as being associated with protection [12, 13] or having no effect [3, 11].

The lack of replication and inconsistency in these studies most likely reflect a range of issues that can produce false-positive or false-negative outcomes in case–control studies of complex diseases where relatively weak associations between single genes and disease are expected [14]. These include lack of power due to insufficient numbers of cases and controls, poor matching of cases and controls, particularly for smoking [15], and genetic heterogeneity in and between different study populations. A recent study has also shown that, when using restriction fragment length polymorphism (RFLP) techniques, genotyping error arising from the presence of a single nucleotide polymorphism (SNP) at Lys119 near the exon 3 slow polymorphism can lead to significant overestimation of His113 homozygotes [10] with resulting lack of Hardy–Weinberg (HW) equilibrium in study populations. Indeed, in a meta-analysis of previous studies in which populations that were not in HW equilibrium were omitted, His113 homozygosity was reported as being protective against the development of COPD [11].

To address many of these issues, in a previous study the present authors collected samples from a cohort of 1,017 white COPD patients and 912 nondiseased control smokers, matched for ethnicity, age and sex [16]. This represented the largest collection reported to date in a candidate gene approach. By matching cases and controls from each centre in Europe the risk of population stratification was minimised. Recent data from genome-wide association studies have shown that, at least in the British population, there is very little evidence for stratification in nearly 3,000 controls analysed to date. A relatively small number of genes showed significant differences in allele frequencies, suggesting that the population is relatively homogeneous and not as variable as previously thought [17]. In the current study this resource was used to evaluate the relationship between EPHX1 variants with respect to both the occurrence and severity of COPD. The association between genetic variation in glutamate-cysteine ligase (GCL; also known as γ-glutamyl-cysteine synthetase) and risk of developing COPD was assessed. This is the rate-limiting enzyme in the synthesis of glutathione, a central contributor to the lung's anti-oxidant defence system. Recently, variation in the catalytic subunit of the gene has been shown to be associated with lung disease severity in cystic fibrosis [18]. To date, genetic variation in this gene has only been reported in one small study of COPD in which no association was found [19].

MATERIALS AND METHODS
Subjects
COPD cases and control subjects were recruited at six European centres. The number of subjects recruited from each centre was as follows. Barcelona (Spain): 70 controls and 138 cases; Bristol (UK): 152 controls and 129 cases; Dublin (Ireland): 195 controls and 196 cases; Edinburgh (UK): 81 controls and 168 cases; Leiden (the Netherlands): 216 controls and 188 cases; and Pisa (Italy): 198 controls and 198 cases.

Approval for the study was obtained from the appropriate committees at each recruitment centre. Informed consent was obtained from all subjects. Criteria for patient recruitment were: a firm clinical diagnosis of stable COPD; airflow limitation as indicated by forced expiratory volume in one second (FEV_1) $\leqslant$70% normal predicted values; FEV_1/forced vital capacity (FVC) <70% pred; no significant reversibility on bronchodilation; and a smoking history of $\geqslant$20 pack-yrs. Patients were excluded from the study if they had an established diagnosis of asthma, lung cancer, a history of atopy, known α_1-AT deficiency or a serum α_1-AT level <1.0 g·L^{-1}. Subjects were also excluded if they had had an acute exacerbation in the 4 weeks preceding assessment for the study.

Control subjects were recruited at each centre to match COPD patients for ethnicity, age, sex and smoking history. Exclusion criteria were as described for cases and also included a family history of COPD. Only individuals with no evidence of airflow obstruction (FEV_1 and FVC $\geqslant$80% and FEV_1/FVC >70%) were included in the control group. Only white subjects were recruited for cases and controls. Complete matching between cases and controls was not achieved, but this was taken into account during the analysis.

No previous evidence has been found for population stratification in this sample set, in keeping with the data obtained from a large sample of the British population [16, 17].

SNP mapping and haplotypes
DNA sequencing to identify SNPs was carried out using a previously described approach [16]. Primers were designed for PCR so that the exons and flanking regions of the genes could be screened. These SNPs were then genotyped using Taqman assays (Applied Biosystems, Foster City, CA, USA) in 291 independent samples from white subjects from a previous study of COPD to obtain more robust data on the frequencies of SNPs. All subjects had given informed consent and the study was approved by the ethics committee of each recruiting centre. Where SNPs were in full linkage disequilibrium in the sequenced samples, a single representative SNP was chosen for genotyping. Where the minor allele frequency of SNP fell below 5% it was removed from further analyses.

Genotyping of study samples
Genotyping of the study population for six SNPs in the EPHX1 gene and eight SNPs in the GCL genes (five in the GCL catalytic (GCLC) and three in the GCL modifier (GCLM) subunits) was carried out at Geneservices Ltd (Cambridge, UK) using Taqman assays. Primer and probe sequences are available on request. As a quality control measure, the 44 samples of known genotype from sequencing were included. These genotypes were unknown to Geneservices Ltd but were used as an internal quality control measure. If discrepancies were noted the analysis was repeated; this resulted in 100% concordance for all the assays.

Statistical analysis
Analysis of genetic variation in population groups
Each of the SNPs in the three genes were analysed for HW equilibrium. To examine linkage disequilibrium, the correlation coefficient between SNP pairs within each gene in cases and

controls was calculated. As matching was not completely achieved on recruitment, allele and genotype frequencies were adjusted for any residual confounding due to age, sex, smoking and centre using logistic regression. This also gave an increase in power by accounting for the matched design. Interactions of age, sex and smoking with centre were included as previously described [16]. Due to the study size it was possible to detect small differences between cases and controls. For instance, over the SNPs investigated, the (rare-type) allele frequency in controls ranged from a low of 7.4% to a high of 26.6% (both SNPs being in the GCL gene; tables 1 and 2). Using these extremes as examples and taking a 1% two-sided level of significance, the study had a power of 95% to detect an allele frequency of $\geqslant$11.4% in the cases compared with 7.4% in controls, and a frequency of $\geqslant$32.8% compared with 26.6%. The corresponding detectable percentages for a lower frequency in cases than controls are 4.2 and 20.8%, respectively.

Haplotype analysis

For full analysis of the relationships between COPD and EPHX1 and GCL variants, a staged, haplotype-based approach was used, as described previously [16]. First, the SNP groupings on which to perform the haplotype analysis were identified. To allow for any combination, the present authors screened all the possible SNP groups in the EPHX1 gene and in the GCLM and GCLC genes for differences between cases and controls. This was carried out by performing an omnibus test [20] and employing a p-value of <0.01 as a cut-off value for statistical significance. Exact p-values based on 10,000 permutations were generated. Extremely rare haplotypes, occurring at a frequency of <1% within each of these groups, were excluded. Individual haplotypes were compared between COPD cases and controls using the pseudo Chi-squared test. A 1% level of significance was taken.

RESULTS

Characteristics of the study population

The characteristics of the control and COPD groups recruited to the study are summarised in table 3. Targeted recruitment of controls yielded groups with similar age and sex profiles, although complete matching of control and COPD groups was not achieved as a high proportion of smokers aged >65 yrs had evidence of some obstruction in pulmonary function and were therefore excluded from recruitment as controls. This incomplete matching was adjusted for in the analysis of the data. Within the COPD group, those recruited were almost equally distributed across the Global Obstructive Lung Disease severity categories of moderate, severe and very severe (see table 1S of online supplementary data).

SNP mapping

EPHX1

In total, 25 SNPs were identified in the EPHX1 gene on sequencing (see table 2S of online supplementary data). Two of these SNPs (rs45616640 and rs45505095) had a minor allele frequency of <5% so were excluded from further analysis. Two further SNPs (rs45467394 and rs4149227) were unsuitable for Taqman assay design. Complete linkage disequilibrium was observed between SNPs at sites -699/-613/-362 and -290/-200 in the 5' flanking region leaving 19 SNPs with poor linkage disequilibrium occurring at a frequency of >5%. As screening for 19 SNPs would result in a large number of haplotypes occurring at a frequency <1%, thus reducing the power of the study, a selection of six SNPs was made based on either previous associations or with potential functional variants. The SNPs chosen included three that fully covered variation in the promoter region (rs2854450, rs2854451 and rs3753658), the slow and fast functional SNPs (rs1051740 and rs2234922, respectively) that have been shown to affect activity of EPHX1 and one SNP (rs4653695) from the 3' untranslated end of the gene.

GCL

Six SNPs were identified in the GCLC subunit and six in the GCLM subunit. The location and nucleotide substitution of these SNPs are indicated in the supplementary data (table 3S). In the GCLC subunit, two SNPs (rs2100375 and rs1901773) were in complete linkage disequilibrium, so only the rs2100375 was assessed in further analyses. In 291 independent samples, the remaining five SNPs were found to contribute to nine haplotypes that occurred with a frequency of >1% and accounted for 99% of the observed variation in the gene, thus, providing almost complete coverage of the gene. These five SNPs were genotyped in the study population. In the GCLM subunit, three SNPs (rs41303970, rs743119 and rs2273406) were in complete linkage disequilibrium, so rs41303970 was chosen to infer information for all three sites in subsequent analyses. SNP rs35267053 had a minor allele frequency of <5%, so was not included in further investigations. The three remaining SNPs were found to contribute to four haplotypes that occurred with a frequency of >1% and accounted for 99% of the observed variation in the 291 samples screened. All three SNPs were genotyped in the final sample resource.

Analysis of variation in EPHX1 and GCL genes in COPD and controls

The allele and genotype frequencies of the six EPHX1 SNPs that were screened in the full study population are shown in

TABLE 1	Glutamate-cysteine ligase (GCL) allele frequencies in controls and chronic obstructive pulmonary disease (COPD) subjects					
SNP no.	SNP	dbSNP reference	Control	COPD	p-value	
					Crude	Corrected#
GCLC						
1c	C-129T	rs17883901	0.074	0.068	0.48	0.67
2c	G24434A	rs2100375	0.279	0.277	0.86	0.65
3c	G36138A	rs16883912	0.102	0.102	0.95	0.41
4c	A37764G	rs1555903	0.091	0.087	0.62	0.58
5c	G39514A	rs524553	0.222	0.231	0.50	0.15
GCLM						
1m	C-590T	rs41303970	0.159	0.152	0.62	0.30
2m	C7623T	rs7515191	0.354	0.336	0.26	0.21
3m	G14613T	rs769211	0.266	0.250	0.29	0.25

SNP: single nucleotide polymorphism; dbSNP: SNP database; GCLC: GCL catalytic subunit; GCLM: GCL modifier subunit. #: adjusted by logistic regression for age, sex, centre and smoking.

SNP no.	SNP ID	Genotype	Control	COPD	p-value	
					Crude	Corrected[#]
GCLC						
1c	C129T	CC/CT/TT	0.86/0.14/0.004	0.87/0.12/0.01	0.41	0.22
2c	G24434A	GG/GA/AA	0.54/0.37/0.09	0.51/0.42/0.07	0.02	0.12
3c	G36138A	GG/GA/AA	0.80/0.19/0.01	0.81/0.18/0.01	0.57	0.19
4c	A37764G	AA/AG/GG	0.82/0.17/0.004	0.84/0.16/0.01	0.29	0.14
5c	G39514A	GG/GA/AA	0.61/0.34/0.05	0.58/0.39/0.04	0.07	0.05
GCLM						
1m	C-590T	CC/CT/TT	0.73/0.23/0.04	0.73/0.24/0.03	0.42	0.31
2m	C7623T	CC/CT/TT	0.43/0.44/0.13	0.44/0.45/0.11	0.34	0.42
3m	G14613T	GG/GT/TT	0.54/0.39/0.07	0.56/0.39/0.06	0.38	0.42

TABLE 2 Glutamate-cysteine ligase (GCL) genotype frequencies in controls and chronic obstructive pulmonary disease (COPD) subjects

SNP: single nucleotide polymorphism; GCLC: GCL catalytic subunit; GCLM: GCL modifier subunit. [#]: adjusted by logistic regression for age, sex, centre and smoking.

tables 4–6. For ease of presentation, screened SNPs are numbered sequentially from 1 to 6 with the gene location relative to the transcription start site, base substitution and the National Centre for Biotechnology Information SNP database (dbSNP) reference number for each SNP indicated. All of the SNPs were within the accepted criteria for HW equilibrium and the frequencies of the SNPs were similar in the populations from all six recruitment centres.

No difference in allele frequency was observed between patient and control groups for any individual EPHX1 SNP nor was there any difference (at the 1% level set for acceptance of significance) in SNP genotype frequencies between patients and controls (table 4–6). Similarly, when classified into functional phenotypes on the basis of genotype at the slow and fast loci, as described by SMITH and HARRISON [3], no difference was observed between controls and COPD patients. Neither were functional phenotypes found to be associated with disease severity within the cases (see table 4S of online supplementary data). The common six SNP haplotypes (occurring with a frequency >1%) associated with the slow and fast functional variants were also evaluated, but no difference was found in haplotypes between control and COPD groups (table 7).

As with the EPHX1 gene, the frequencies of the six SNPs screened in the GCLC and GCLM genes were similar in the populations from all six recruitment centres and, with the exception of SNP 1m (C-590T GLCM subunit), all were within the accepted criteria for HW equilibrium. For SNP 1m, the frequency of the minor allele homozygotes was slightly higher than expected (4.3 versus 2.5%) in the control population (p=0.002). This was unlikely to be due to genotyping error as the blinded controls for this assay were accurately assigned. The frequency of the minor allele is <5% and small deviations in observed numbers may cause significant deviation from HW equilibrium. The possibility of a copy number variant, which often contributes to deviations from HW equilibrium, cannot be fully excluded although no evidence for this was found in preliminary experiments using real-time PCR (data not shown).

No difference in allele frequency was observed between case and control groups for any individual SNP in either the GCLC or GCLM genes, nor was there any difference in SNP genotype frequencies (tables 1 and 2).

Linkage disequilibrium analysis (see tables 5S and 6S in the online supplementary data) demonstrated strong linkage between SNPs 2c and 5c (rs2100375 and rs524553) in the GCLC subunit and between all three SNPs in the GCLM subunit in both case and control groups. No strong linkage was observed between SNPs evaluated in the EPHX1 gene in either cases or controls.

Haplotype analysis of SNP combinations in each of the three genes did not identify any combinations that were related to COPD. Similarly, when evaluated on the basis of disease severity within the COPD group, no association between haplotypes and disease severity was observed for any of the three genes evaluated.

DISCUSSION
The EPHX1 gene has been a significant focus for studies on genetic susceptibility in COPD in the past 10 yrs. It is a promising candidate gene as it is strongly expressed in the

TABLE 3 Characteristics of control and chronic obstructive pulmonary disease (COPD) study subjects

	Control	COPD	p-value
Subjects n	912	1017	
Male %	63.6	69.8	0.0038
Age yrs	60.8±8.9	65.9±8.2	<0.0001
Smoking pack-yrs	38.7±17.4	48.7±22.8	<0.0001
FEV1 % pred	95.4±11.0	43.2±15.2	<0.0001
FEV1/FVC	77.8±4.9	47.6±12.1	<0.0001

Data are presented as mean±SD, unless otherwise stated. FEV1: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity.

TABLE 4		Microsomal epoxoide hydrolase minor allele frequencies in controls and chronic obstructive pulmonary disease (COPD) subjects				
SNP no.	SNP ID	dbSNP reference	Control	COPD	p-value	
					Crude	Corrected#
1	C-399T	rs2854450	0.217	0.206	0.40	0.30
2	G-362A	rs2854451	0.245	0.242	0.82	0.95
3	G-290T	rs3753658	0.124	0.132	0.43	0.63
4	T6651C	rs1051740	0.301	0.294	0.60	0.86
5	A13426G	rs2234922	0.204	0.208	0.76	0.90
6	A20094C	rs4653695	0.107	0.111	0.65	0.91

SNP: single nucleotide polymorphism; dbSNP: SNP database. #: adjusted by logistic regression for age, sex, centre and smoking.

bronchial epithelium and its biological role in detoxifying epoxides is extremely relevant in protecting the lung against smoke-induced damage. In addition, there is significant individual variation in enzymatic activity, and genetic polymorphisms that modify enzymatic activity have been identified [21].

The majority of studies on genetic variants of EPHX1 in COPD have evaluated polymorphisms in the coding region of the gene that are associated with slow (Tyr113His) and fast (His139Arg) enzymatic activity, with conflicting results. Many of these studies have limitations with respect to power (with numbers of subjects in the case and control groups ranging 60–200); matching for ethnicity, age, sex and smoking history also vary considerably. In addition, studies that analysed polymorphic variation using RFLP techniques are likely to have over-estimated the proportion of slow (113His) homozygotes [10].

The present study overcomes many of these limitations by evaluating EPHX1 polymorphisms in case and control groups with sufficient power to readily replicate previously reported associations. All subjects were white and any potential effects of population stratification were minimised by recruiting cases and controls from the same populations. In addition, targeted recruitment of controls facilitated the enrolment of controls with a similar profile to COPD patients with respect to sex, age and smoking.

Analysis of EPHX1 polymorphisms showed no relationship between susceptibility to COPD and variation at the Tyr113His or His139Arg loci. Given the significantly greater level of power and case–control matching in the present study it is unlikely that effects of the magnitude observed in previous, less powered studies have been missed. All EPHX1 SNPs evaluated were in HW equilibrium in control populations. This, along with the inclusion of previously sequenced samples as quality controls in the genotyping analysis, indicates that the current analysis is not affected by genotyping error.

Furthermore, detailed analysis of haplotypes corresponding to the fast (His139Arg) and slow (Tyr113His) variants failed to identify haplotypes that were associated with disease. The present authors also analysed variation in the 5′ promoter region of the EPHX1 gene, which has been shown to modulate enzyme expression by up to 30% [21], but no association with disease susceptibility was found.

The current results are at variance with two adequately powered recent studies which report the protective effects of the His139Arg [12] and Try113His [11] alleles, although neither study replicated the other's results. In a study of 304 emphysema patients and 441 controls, HERSH et al. [12] report a protective effect (odds ratio 0.73, p=0.03) with the fast variant of codon 139, while no association was observed with variation in the 113 codon. By contrast, BROGGER et al. [11] evaluated the same polymorphisms in 492 white smokers and ex-smokers (244 COPD patients and 248 controls) and reported a protective effect of His/His homozygosity at the 113 locus, with no effect of variation at the 139 locus. The present study did not replicate either of these observations, nor were they noted in the majority of the smaller studies [3–10].

Sequencing of the EPHX1 gene revealed that variation in this gene is high, with 25 polymorphisms being identified. This may explain some of the conflicting results reported in studies of the slow and fast variants as these polymorphisms could contribute to disease susceptibility on a background of other specific SNP variants or haplotypes. However, this seems unlikely as the present authors have captured the most significant haplotypes corresponding to the fast and slow variants. It may be that variation in other genes plays a role as studies on the impact of the slow and fast EPHX1 genetic variants indicate that they may have only a modest impact on *in vivo* activity levels and that

TABLE 5		Microsomal epoxoide hydrolase genotype frequencies in controls and chronic obstructive pulmonary disease (COPD) subjects				
SNP no.	SNP ID	Genotype	Genotype frequency		p-value	
			Control	COPD	Crude	Corrected#
1	C-399T	CC/CT/TT	0.62/0.33/0.05	0.63/0.33/0.04	0.50	0.17
2	G-362A	GG/GA/AA	0.57/0.36/0.06	0.58/0.35/0.07	0.83	0.98
3	G-290T	GG/GT/TT	0.77/0.21/0.02	0.75/0.23/0.02	0.27	0.12
4	T6651C	TT/TC/CC	0.49/0.41/0.10	0.50/0.41/0.09	0.80	0.98
5	A13426G	AA/AG/GG	0.64/0.31/0.05	0.63/0.33/0.04	0.72	0.97
6	A20094C	AA/AC/CC	0.80/0.19/0.01	0.79/0.21/0.01	0.30	0.43

SNP: single nucleotide polymorphism. #: adjusted by logistic regression for age, sex, centre and smoking.

TABLE 6	Microsomal epoxide hydrolase functional phenotype frequencies in controls and chronic obstructive pulmonary disease (COPD) subjects			
Phenotype	**SNP4/SNP5 genotype**	**Control**	**COPD**	**p-value**
Normal	TT/AA or TC/AG	0.433	0.453	0.37
Slow	TC/AA or CC/AA	0.364	0.345	0.41
Fast	TT/AG or TT/GG	0.204	0.201	0.90

SNP: single nucleotide polymorphism; normal: no mutation in gene (exon 3:Tyr/Tyr and exon 4: His/His), or heterozygote for both exon 3 (Tyr113/His113) and exon 4 (His139/Arg139) mutations; slow: one slow allele (exon 3: Tyr113/His113) or two slow alleles; fast: at least one fast mutation (exon 4: His139/Arg139 or Arg139/Arg139) and no exon 3 mutation (Tyr113/Tyr113).

TABLE 7	Haplotypes associated with microsomal epoxide hydrolase slow 6651C (113His) and fast 13426G (139Arg) variants		
Haplotype	**Frequency**		**p-value**
	Controls	**COPD**	
6651C (113His)			
CAGCAA	0.0998	0.0955	0.613
CGGCAA	0.0818	0.0775	0.5974
TGGCAA	0.0262	0.0349	0.1197
CGTCAA	0.0195	0.0142	0.2036
13426G (139Arg)			
CGGTGA	0.0656	0.0725	0.3944
TGGTGA	0.0488	0.0357	0.0425
CAGTGA	0.0247	0.0285	0.3959

COPD: chronic obstructive pulmonary disease. Relevant nucleotide substitutions are underlined. None of the haplotypes were significantly different between COPD and controls. Haplotypes with frequencies <1% in both cases and controls are not shown.

post-transcriptional modification may be important [21–23]. Therefore, it seems unlikely that these particular variants contribute to COPD susceptibility, although the possibility of rare variants playing a role cannot be excluded and significantly larger studies are needed to test this reliably.

The rate limiting enzyme in glutathione synthesis, GCL, is also a potential candidate gene for susceptibility to COPD. Significantly decreased glutathione levels are observed in the lungs of GCLM knockout mice [24] and message expression for this subunit is decreased in alveolar macrophages from healthy smokers [25]. In addition, the presence of GCL protein in the bronchial airways appears to be diminished in smokers and COPD patients compared with nonsmoking controls [26, 27]. Both subunits of this enzyme were genotyped and investigated with the role of the genetic variation observed as a potential risk factor for disease. As with EPHX1, no association was found with disease and SNPs in genes encoding either subunit of the GCL enzyme. This confirms the only other reported study of GCL genetic variation in COPD, which investigated a single SNP in the GCLC gene (rs17883901; SNP 1c) in 322 subjects from the Han Chinese population, and failed to find evidence for an association with COPD [19]. The present study has extended these previous findings to include more SNPs throughout the GCLC gene, as well as considering variation in the gene for the GCLM subunit.

In conclusion, no evidence was found for a significant association of polymorphisms in microsomal epoxide hydrolase or glutamate-cysteine ligase with susceptibility to chronic obstructive pulmonary disease in a white population.

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