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Association of IL8, CXCR2 and TNF-α polymorphisms and airway disease

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Abstract Chronic obstructive pulmonary disease (COPD) is a disease characterised by inflammation of the peripheral airways involving many inflammatory cells and mediators. IL8 is an important inflammatory mediator that is responsible for the migration and activation of neutrophils. Cellular activity of IL8 is mediated by the receptor CXCR2, and transcription of *IL8* is controlled by the cytokine tumour necrosis factor (TNFα). The aim of our study was to investigate the influence of single nucleotide polymorphisms in *IL8, CXCR2* and *TNF-α* on lung function and respiratory symptoms in subjects from Melbourne, Australia. A total of 1,232 participants completed a detailed respiratory questionnaire, spirometry and measurement of gas transfer. Genotyping for the *IL8* -251 T → A, *CXCR2* +785C → T and *TNF-α* -308G → A polymorphisms was performed using the tetra-primer ARMS-PCR method. The *TNF-α* A allele was associated with a reduced $FEF_{25–75}$ ($P = 0.03$). Inheritance of the *CXCR2* T allele was associated with significantly higher diffusing capacity ($P = 0.03$) and $FEF_{25–75}$ ($P = 0.02$). No association with the *IL8* -251 polymorphism was found. Our results suggest that *TNF-α* is associated with COPD-related phenotypes and the *CXCR2* +785 SNP may be important in protecting against pulmonary inflammation. These genes may be important candidates in the modulation of the inflammatory response in the airways.

Keywords TNF-α · CXCR2 · IL8 · Lung function · Polymorphism

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Introduction

Chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD) and asthma are characterised by airflow obstruction and a chronic persistent inflammatory process. The inflammatory process is a complex interaction between many cellular mechanisms, inflammatory mediators and effects (Barnes 2000).

IL8 is an important pro-inflammatory cytokine that primarily mediates the activation and migration of neutrophils into tissue from peripheral blood (Strieter 2002). High concentrations of IL8 have been found in the bronchoalveolar lavage fluid (BAL) of both asthma (Norzila et al. 2000) and COPD (Pesci et al. 1998; Rutgers et al. 2000) patients. Cellular activity of IL8 is mediated by two receptors, IL8 receptor 1 (CXCR1) and IL8 receptor 2 (CXCR2) (Renzoni et al. 2000). Mouse knockout studies have suggested CXCR2 is a major mediator of neutrophil migration (Cacalano et al. 1994) and, in humans with COPD exacerbations, the intensity of *CXCR2* mRNA expression was found to be significantly positively correlated with increased CXCR2 (Qiu et al. 2003). Another important inflammatory cytokine is tumour necrosis factor α (TNF-α), which is released from stimulated alveolar macrophages (Hajeer and Hutchinson 2000). The level of TNF-α is also elevated in BAL and sputum of patients with asthma (Thomas 2001) and COPD (Sun et al. 1998). Importantly TNF-α also

activates nuclear factor-κB (NF-κB), which switches on transcription of *IL8* (Rahman et al. 2002).

Several polymorphisms in *IL8* have been identified and one at position −251 (rs4073) of the *IL8* promoter has been shown to be associated with greater IL8 expression (Hull et al. 2000). Three novel polymorphisms have been identified in *CXCR2* including the *CXCR2* +785 C → T (rs2230054) polymorphism located in exon 11 that results in a silent codon change (Renzoni et al. 2000). For *TNF-α,* a polymorphism at position −308 (rs1800629) of the *TNF-α* promoter has been extensively studied because the *TNF-α* −308A allele has been shown to be associated with higher transcriptional activity (Hajeer and Hutchinson 2000).

Previous genetic studies have revealed some information on the involvement of these genes in respiratory phenotypes. A study of the *IL8* -251 T → A polymorphism with risk of COPD (Arinir et al. 2005) failed to detect an association. A genome-wide scan has linked the region on chromosome 2q near the location of the *CXCR2* gene to spirometric phenotypes related to COPD (DeMeo et al. 2004; Silverman et al. 2002). No association studies of CXCR2 have been reported; however, a recent study reported an association between two novel polymorphisms in *CXCR1* and COPD (Stemmler et al. 2005). Additionally, due to its important role in the inflammatory process and its high level in the sputum of COPD patients, TNF-α has been well studied as an obvious candidate gene for COPD. To date there have been seven case-control studies of COPD and the *TNF-α* −308 polymorphism but the results have been inconsistent (Ferrarotti et al. 2003; Higham et al. 2000; Huang et al. 1997; Ishii et al. 2000; Keatings et al. 2000; Patuzzo et al. 2000; Sakao et al. 2001; Sandford et al. 2001).

Due to the overlapping and interactive roles of IL8, CXCR2 and TNF-α in the inflammatory process it is possible that polymorphisms in these genes may influence risk of airway inflammation and obstruction. To examine this we performed an association study using participants in an epidemiological study of risk factors for COPD. This representative sample of middle-aged and older adults from the population of Victoria, Australia, who because of their advanced age are likely to have developed airflow limitation and respiratory symptoms were genotyped for three previously described biallelic single nucleotide polymorphisms (SNPs): *IL8* -251 T → A, *CXCR2* +785C → T and *TNF-α* −308G → A. This paper presents an analysis of the effect of these polymorphisms, individually and in combination, on lung function and respiratory symptoms. The interaction with cigarette smoking has also been examined.

Materials and methods

Participants

Recruitment and pulmonary function testing are described in detail elsewhere (Matheson et al. 2005). Briefly, 1,232 subjects randomly selected from the general population were recruited to be part of the study. Ninety four percent ($n = 1{,}167$) of the participants were of Caucasian descent and, of these, 1,032 (88.4%) were successfully genotyped for *IL8*, *CXCR2* and *TNF-α*. In order to assess the association with COPD-related phenotypes, we removed those subjects with current asthma from the analysis (wheeze in the last 12 months and bronchial hyper-reactivity). The results for these remaining $n = 828$ subjects are presented. The study was approved by the Ethics Committee at The Alfred Hospital and Monash University in Melbourne Australia. All participants gave written informed consent.

Outcomes

Spirometry and diffusing capacity (TLco) was performed according to American Thoracic Society (ATS) criteria (American Thoracic Society 1994, 1995). Predicted values for FEV_1, FVC and $FEF_{25–75}$ were calculated from age, height and gender using the equations of Gore et al. (1995), and for TLco using the equations of Quanjer et al. (1983).

Genotyping

Genotyping for the *IL8* -251 T → A, *CXCR2* +785 C → T and *TNF-α* −308 G → A polymorphisms was performed using the PCR tetra-primer ARMS method (Ye et al. 2001). Primers were designed using the computer program made available by the authors of the method. The four primers required to genotype each of the polymorphisms are listed in Table 1. All genotyping was performed blind with respect to participant characteristics. Approximately 50 ng DNA was amplified in a 20 μl PCR mix. This mix contained 1× reaction buffer, 2.5 mM $MgCl_2$, 250 μM dNTP (GeneAmp, Applied Biosystems, Foster City, CA), 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and 0.5 μM of each primer. The outer primers were diluted to 1:10 of the concentration of the inner primers to enhance specificity. The PCR cycling conditions were 95°C for 10 min, followed by 35 cycles of 94°C for 1 min, 1 min at the annealing temperature specified in Table 1 and then 72°C for 1 min, followed by a final extension for 10 min at 72°C. The PCR products were resolved by electrophoresis on a 2% agarose gel stained with ethidium bromide. To validate the genotyping protocol, 10% of the total sample was re-genotyped using the single nucleotide primer extension method (SNuPe) (Amersham Biosciences, Little Chalfont, UK). The SNuPe method was performed as described in the manufacturer's instructions on an Amersham Biosciences MegaBACE system.

Table 1 Details of the primers, annealing temperature and fragment sizes for the single nucleotide polymorphisms (SNPs) genotyped using the tetra-primer-PCR method

SNP	Primer sequence	T_m	Fragment size
TNF-α −308G → A (rs1800629)	Forward inner primer (T allele): 5′-TGGAGGCAATAGGTTTTGAGGGGCAGGA	68°C	154 bp (A allele)
	Reverse inner primer (A allele): 5′-TAGGACCCTGGAGGCTGAACCCCGTACC		224 bp (G allele)
	Forward outer primer (5′–3′): 5′-ACCCAAACACACGCCTCAGGACTCAACA		323 bp (two outer primers)
	Reverse outer primer (5′–3′): 5′-AGTTGGGGACACGCAAGCATGAAGGATA		
IL8 -251T → A (rs4073)	Forward inner primer (T allele): 5′-GTTATCTAGAAATAAAAAAGCATACAA	58°C	169 bp (T allele)
	Reverse inner primer (A allele): 5′-CTCATCTTTTCATTATGTCAGAG		228 bp (A allele)
	Forward outer primer (5′–3′): 5′-CATGATAGCATCTGTAATTAACTG		349 bp (two outer primers)
	Reverse outer primer (5′–3′): 5′-CACAATTTGGTGAATTATCAAA		
CXCR2 +785C → T (rs2230054)	Forward inner primer © allele): 5′-TCTTTGCTGTCGTCCTCATCTTCCTGATC	63°C	226 bp © allele)
	Reverse inner primer (T allele): 5′-AGGACCAGGTTGTAGGGCAGCCAGAAA		281 bp (T allele)
	Forward outer primer (5′–3′): 5′-CTGCCTGTCTTACTTTTCCGAAGGACCG		451 bp (two outer primers)
	Reverse outer primer (5′–3′): 5′-TCTTGAGGAGTCCATGGCGAAACTTCTG		

Statistical analysis

Statistical analysis was performed using the statistical package STATA (version 6 STATA Corporation, TX). Hardy Weinberg Equilibrium (HWE) was tested by means of a χ^2 goodness of fit test. Multiple regression or logistic regression analysis was used to examine the association between genotype and continuous or binary outcomes. Outcomes investigated included: lung function (including FEV_1 and TLco) and respiratory symptoms in the last 12 months (including wheeze, morning and chronic cough, morning and chronic sputum, dyspnoea grade 3 and nocturnal chest tightness). Covariates included in the models were age, gender, smoking status and pack years of smoking. Pack-years were calculated as number of cigarettes smoked per day divided by 20 multiplied by the number of years of smoking.

Results

General characteristics

The mean (SD) age of the subjects was 57.8 (±7.4) years. There was no significant excess of either gender in the sample, with 52.5% males ($n=435$). The prevalence of current smokers was 11% ($n=91$), past smokers 38.4% ($n=318$) and never smokers 50.6% ($n=419$). The lung function parameters of the study group are presented in Table 2 and the prevalence of respiratory symptoms for the study group are presented in Table 3.

Genotyping and allele frequencies

Figures 1, 2 and 3 depict representative gel electrophoresis results for the three genes. Table 4 shows the genotype and allele frequencies for the *TNF-α*, *CXCR2* and *IL8* polymorphisms. Both the *CXCR2* and *TNF-α* polymorphisms were in HWE (CXCR2 +785 $P=0.53$; TNF− $P=0.62$); however, *IL8* was not found to be in HWE ($P=0.02$). To confirm the genotyping results of IL8, 10% of the sample were repeated using an alterative

Table 2 Lung function in the study group ($n=828$)

Parameter	Mean ±SD
FEV_1	3.26 ± 0.81
FVC	4.27 ± 0.99
FEV_1/FVC	76.1 ± 7.4
$FEF_{25–75}$	2.87 ± 1.14
Tlco	23.5 ± 5.56
Kco	4.03 ± 0.64
Log dose response slope	1.69 ± 1.02

Table 3 Respiratory symptoms in the study group ($n=828$)

Symptom	*n* (%)
Wheeze	189 (22.8)
Nocturnal chest tightness	101 (12.2)
Nocturnal SOB	59 (7.1)
Morning cough	120 (14.5)
Chronic cough	70 (8.5)
Morning phlegm	54 (6.5)
Chronic phlegm	23 (2.8)
Dyspnoea	15 (1.8)

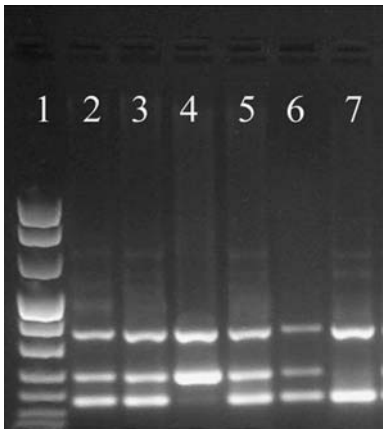

Fig. 1 Electrophoresis gel of the *IL8* −251 T → A PCR-tetra primer method. Bands: *Top* Control (349 bp), *middle* A allele (228 bp), *bottom* T allele (169 bp). Lanes: *1* DNA size marker; *2*, *3*, *5*, *6* A/T heterozygotes; *4* AA homozygote; *7* TT homozygote

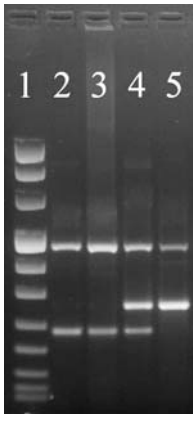

Fig. 2 Electrophoresis gel of the *CXCR2* +785 C → T PCR-tetra primer method. Bands: *Top* Control (451 bp), *middle* T allele (281 bp), *bottom* C allele (226 bp). Lanes: *1* DNA size marker; *4* T/C heterozygote; *2*, *3* CC homozygotes; *5* TT homozygote

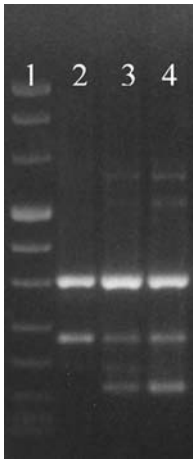

Fig. 3 Electrophoresis gel of the *TNF*-α −308 G → A PCR-tetra primer method. Bands: *Top* Control (323 bp), *middle* G allele (224 bp), *bottom* A allele (154 bp). Lanes: *1* DNA size marker; *3*, *4* G/A heterozygotes, *2* GG homozygote

Table 4 Genotype and allele frequencies

SNP	Genotype	Genotype frequency *n* (%)	Allele	Allele frequency (%)
IL8 −251 A > T	A/A	183 (22.1)	A	44.8
	A/T	375 (45.3)	T	55.3
	T/T	270 (32.6)		
CXCR2 +785 C > T	C/C	215 (26.0)	C	50.4
	T/C	405 (48.9)	T	49.6
	T/T	208 (25.1)		
TNF-α −308 A > G	A/A	24 (2.9)	A	17.8
	A/G	246 (29.7)	G	82.3
	G/G	558 (67.4)		

method called SNuPe. All results were found to be concordant.

Lung function parameters and *TNF*-α, *CXCR2* and *IL8*

The association between the lung function indices and the polymorphisms are presented in Table 5. There was no association between any of the lung function parameters and IL8. Individuals with the *CXCR2* +785-TT genotype had a significantly better TLco %predicted and the *CXCR2*-TC genotype was associated with significantly better $FEF_{25–75}$ %predicted. When the TT and TC genotypes were combined, the T allele was associated with a significantly better $FEF_{25–75}$ (0.16, 95%CI 0.008–0.31, $P = 0.04$). For *TNF*-α, individuals with the GA genotype had a significantly reduced $FEF_{25–75}$ %predicted. When the A/A and A/G genotypes were combined, inheritance of at least one A allele was associated with borderline statistically significantly reduced $FEF_{25–75}$ (−0.14, 95%CI −0.28–0.005, $P = 0.06$). However, there was no association with any of the other lung function parameters.

Because of the importance of cigarette smoking in the inflammatory process, an interaction with smoking was examined by fitting a genotype × smoking interaction term in the models. However, there were no significant interactions found between any of the genes and cigarette smoking. Nor was there any evidence of an interaction between any of the genes when gene × gene interaction terms were fitted to the models (data not shown).

Respiratory symptoms and *TNF*-α, *CXCR2* and *IL8*

No significant associations were found between any of the respiratory symptoms and the *IL8*, TNF-α or *CXCR2* polymorphisms. Those individuals with the *TNF*-α-AA genotype had an increased risk of dyspnoea but this did not reach statistical significance (Table 6). Those with self-reported respiratory symptoms had significantly worse lung function than those without symptoms (data not shown).

Table 5 Lung function parameters by *CXCR2* and *TNF-α* genotype

Polymorphism	Genotype (mean ± SD)			Regression coefficient	(95% CI) *P* value[a]
TNF-α −308	A/A ($n=24$)	A/G ($n=246$)	G/G ($n=558$)	A/A vs G/G genotype	G/A vs G/G genotype
FEV_1	3.15 ± 0.87	3.26 ± 0.81	3.26 ± 0.81	0.05 (−0.15, 0.25)	−0.05 (−0.12, 0.03)
FEV_1/FVC	76.7 ± 5.08	75.5 ± 6.9	76.4 ± 7.65	0.53 (−2.34, 3.41)	−0.80 (−1.86, 0.26)
$FEF_{25–75}$	2.76 ± 0.98	2.79 ± 1.12	2.91 ± 1.16	0.004 (−0.39, 0.40)	−0.15 (−0.29,−0.0005)*
TLco	22.7 ± 5.74	23.6 ± 5.56	23.6 ± 5.56	0.53 (−0.84, 1.89)	−0.22 (−0.72, 0.28)
CXCR2 +785	C/C($n=215$)	T/C ($n=405$)	T/T ($n=208$)	T/T vs C/C genotype	T/C vs C/C genotype
FEV_1	3.22 ± 0.83	3.26 ± 0.80	3.29 ± 0.82	0.04 (−0.05, 0.13)	0.06 (−0.02, 0.14)
FEV_1/FVC	75.3 ± 7.64	76.7 ± 7.3	75.8 ± 7.2	0.22 (−1.12, 1.56)	1.37 (0.21, 2.53)*
$FEF_{25–75}$	2.75 ± 1.16	2.94 ± 1.10	2.87 ± 1.20	0.07 (−0.12, 0.26)	0.20 (0.04, 0.36)*
TLco	23.2 ± 5.48	23.3 ± 5.5	24.3 ± 5.75	0.95 (0.32, 1.59)*	0.26 (−0.29, 0.81)

*$P < 0.05$
[a]Adjusted for age, sex, height, smoking status and pack years

Discussion

Genes involved in the inflammatory process are important potential modifiers of individual susceptibility to reduced lung function and respiratory symptoms. IL8 and TNF-α are important cytokines involved in inflammation of the airways. IL8 is a potent neutrophil chemoattractant and its cellular activity is mediated by the receptor CXCR2. TNF-α is released from macrophages and can switch on transcription of *IL8*. The complex interaction between these three proteins makes them interesting potential candidate genes for modulating risk of inflammatory disease. This is one of the first studies to examine polymorphisms in the *IL8* and *CXCR2* genes and risk of reduced lung function and respiratory symptoms. The *TNF-α* gene has previously been investigated in relation to COPD. However, only one previous study has looked at this gene in an unselected community-based sample to determine if these genes have a role in some of the intermediate phenotypes associated with airway inflammation, such as reduced lung function.

There is considerable evidence to suggest that IL8 is an important cytokine released in response to environmental insults. For example, levels of IL8 are elevated in the BAL of smokers (Kuschner et al. 1996), and IL8 release is increased from the macrophages of cigarette smokers and COPD patients (Lim et al. 2000). Several SNPs have been described in *IL8* (Renzoni et al. 2000; Rovin et al. 2002). The *IL8* -251A allele has been associated with increased production of IL8 (Hull et al. 2000). To our knowledge only one study has investigated IL8 and risk of COPD, which did not find an association with the *IL8* -251 polymorphism (Arinir et al. 2005).

Table 6 Respiratory symptoms by *TNF-α* genotype

TNF-α -308	Cases *n* (%)	Controls *n* (%)	OR (95%CI)[a]
Wheeze	$n=189$	$n=639$	
A/A ($n=24$)	7 (3.7)	17 (2.7)	1.27 (0.51, 3.18)
A/G ($n=246$)	50 (26.5)	196 (30.7)	0.81 (0.56, 1.19)
G/G ($n=558$)	132 (69.8)	426 (66.7)	1.0
Dyspnoea (grade 3)	$n=15$	$n=813$	
A/A ($n=24$)	2 (13.3)	22 (2.7)	4.25 (0.74, 24.4)
A/G ($n=246$)	3 (20.0)	243 (29.9)	0.58 (0.15, 2.29)
G/G ($n=558$)	10 (66.7)	548 (67.4)	1.0
Chronic cough	$n=70$	$n=758$	
A/A ($n=24$)	2 (2.9)	22 (2.9)	0.85 (0.19, 3.79)
A/G ($n=246$)	20 (28.6)	226 (29.8)	0.90 (0.52, 1.57)
G/G ($n=558$)	48 (68.6)	510 (67.3)	1.0
Chronic phlegm	$n=23$	$n=805$	
A/A ($n=36$)	1 (4.4)	23 (2.9)	1.41 (0.18, 11.3)
A/G ($n=311$)	7 (30.4)	239 (29.7)	1.04 (0.42, 2.60)
G/G ($n=685$)	15 (65.2)	543 (67.5)	1.0

*$P < 0.05$
[a]Logistic regression adjusted for age, sex, current smoking and pack years

Similarly, we did not find any association between the *IL8* -251 polymorphism and respiratory symptoms or lung function.

Homozygotes for the *CXCR2* +785 T allele were found to have significantly higher TLco %predicted, and heterozygotes for the *CXCR2* +785 T allele had significantly better $FEF_{25–75}$ %predicted. COPD is characterised both by decreased expiratory flow rates and abnormal gas exchange and these results suggest that the *CXCR2* +785 T allele may be associated with protection against these abnormalities. The only published genome-wide scan for loci linked to COPD-related phenotypes found evidence of linkage of the *CXCR2* region (chromosome 2q, LOD score 4.12 at 222 cM) to a number of quantitative spirometric abnormalities (DeMeo et al. 2004; Silverman et al. 2002). No association of FEV_1/FVC with the *CXCR2* gene was found in the present analysis, but the associations with $FEF_{25–75}$ %predicted and TLco %predicted further support a role for this gene in the inflammatory component of airflow limitation.

The *CXCR2* +785 polymorphism does not result in an amino acid substitution, so it is most likely that this polymorphism is nearby an as yet undiscovered polymorphism that has functional consequences for gene transcription, protein expression or stability. A previous study of this polymorphism found an association between systemic sclerosis and individuals homozygous for the *CXCR2* +785C allele (Renzoni et al. 2000). Two polymorphisms in the CXCR1 gene were found to be over represented in a study of COPD subjects (Stemmler et al. 2005). Overall, our results provide evidence to suggest that CXCR2 is an important and interesting new candidate gene potentially involved in regulating the inflammatory process in the airways.

Individuals with at least one *TNF*-α −308A allele were found to have a significantly reduced $FEF_{25–75}$ %predicted. Furthermore, the *TNF*-α −308 AA genotype was found to be associated with an increased risk of dyspnoea. These symptoms are generally associated with small airways disease, which is caused by cigarette smoking and has been shown to progress to COPD (Hogg 2004). Despite this relationship we did not find any evidence of an interaction between the *TNF*-α −308 genotype and smoking status.

The *TNF*-α −308 polymorphism has been investigated in several previous studies of COPD. No association between the *TNF*-α −308A allele and COPD has been shown in any of the previous studies of Caucasian COPD subjects (Higham et al. 2000; Keatings et al. 2000; Patuzzo et al. 2000; Sandford et al. 2001). However, the results are less consistent for studies of Asian populations. Two studies, one in a Taiwanese population (Huang et al. 1997) and one in a Japanese population (Sakao et al. 2001), reported a significantly higher prevalence of the *TNF*-α −308A allele in the cases compared to controls. However, this result was not replicated in a subsequent Japanese population (Ishii et al. 2000). All of these studies, with the exception of the lung health study (LHS) (Sandford et al. 2001), were likely to be affected by a lack of statistical power due to the low frequency of the *TNF*-α −308 AA genotype. This is also a likely explanation for our finding of an association with *TNF*-α −308 AG heterozygotes, but not *TNF*-α −308 AA homozygotes with $FEF_{25–75}$.

The inflammatory process is extremely complex, with interactions between many cytokines including TNF-α and IL8. TNF-α stimulates the release of NF-κB, which in turn switches on transcription of IL8, which attracts neutrophils into the airways (Rahman et al. 2002). These protein interactions prompted an investigation of interaction between polymorphisms by fitting a gene × gene interaction term in the model. However, no evidence of an interaction between any of the polymorphisms was found for any of the traits examined.

In view of the lack of HWE shown by *IL8*, the association reported here should be interpreted with some reservations, but should not preclude *IL8* from further investigation in relation to COPD. Lack of HWE could be due to genotyping error or population stratification. To eliminate genotyping error as a possible explanation for the lack of HWE, we genotyped 10% of the sample using an alternative method, and did not find any discrepant results. We observed an excess of homozygotes, which can be caused by population dynamics such as inbreeding, or population stratification known as Wahlund's principle (Schaid and Jacobsen 1999). However, the likelihood of inbreeding in this randomly selected population from Melbourne is small. Population stratification due to sampling of different ethnic groups is another possible explanation for the deviation from HWE. All subjects in this analysis were of Caucasian descent; however, the multi-cultural structure of the Melbourne community means that population stratification cannot be entirely ruled out.

We found the overall prevalence of asthma symptoms such as wheeze and nocturnal dyspnoea to be quite high in our study group. However, the prevalence of asthma in Australia is among the highest in the world (Masoli et al. 2004). To examine this we compared the prevalence of self-reported respiratory symptoms between participants in the laboratory phase of the study and participants in only the initial screening questionnaire phase. We observed a significantly higher prevalence of wheeze in those that attended the laboratory but other respiratory symptoms and self-reported COPD were not associated with attendance. We did find significantly more current smokers among the non-participants and slightly more males (data not shown). However, this bias towards symptomatic non-smokers is unlikely to have affected the associations between genes and lung function. Due to the number of analyses conducted with many different outcomes, it might be argued that adjustment for multiple comparisons is necessary. However, for each gene there was an a priori hypothesis of an individual association with reduced lung function and respiratory symptoms. This was based on the role of these genes and their products in the inflammatory

process. The *P* values for the analyses are presented without adjustment and the results should be viewed as hypothesis-generating rather than proof that these polymorphisms are associated with lung function.

In conclusion, the association of three cytokine gene polymorphisms with lung function and respiratory symptoms has been analysed in a community-based study of older adults. Evidence of an association between the *TNF-α* −308A allele and small airways disease was found, as indicated by a reduced $FEF_{25–75}$ accompanied by respiratory symptoms. Furthermore, we have also found evidence that *CXCR2* may be associated with better lung function, suggesting the gene may be important in down-regulating the airway inflammatory response. This analysis suggests these genes are possibly important in modulating the inflammatory processes in the airways response to environmental insults and the lung ageing process.

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