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# A report of cytokine polymorphisms and COPD risk in Xuan Wei, China

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## Abstract

Indoor air pollution has been documented as an important risk factor for chronic obstructive pulmonary disease (COPD), and inflammation is central to the development and progression of COPD. Single nucleotide polymorphisms (SNP) in some cytokine genes have been reported to be associated with COPD. We examined the association between 18 SNPs in 10 cytokine genes and COPD risk in a case-control study conducted in a population with high exposure to indoor smoky coal emissions. The study included 53 COPD cases and 122 healthy community controls. Carriers of the *CSF2* 117Ile allele had a 2.4-fold higher risk of COPD than the wild type (Thr/Thr) carriers (OR: 2.44; 95% CI: 1.10 – 5.41), and the AA genotype at *IL8 -351* was associated with an increased risk of COPD (OR: 2.71; 95% CI: 1.04 – 7.04). When the combined effects of *CSF2* 117Ile and *IL8 -351A* were examined, individuals carrying at least one variant in both genes had a five-fold increased risk of COPD (OR: 5.14, 95% CI: 1.32 – 29.86). This study suggests that polymorphisms in both *CSF2* and *IL8* may play a role in the pathogenesis of COPD, at least in highly exposed populations. However, in view of our relatively small sample size, this study should be replicated in other populations with substantial exposure to indoor air pollutants such as polycyclic aromatic hydrocarbons (PAH) and particulate matter.

### Keywords

COPD; Cytokine; CSF2; IL8; Single nucleotide polymorphism; Indoor air pollution

## Introduction

Chronic obstructive pulmonary disease (COPD) is a common disease characterized by airflow obstruction. It is the fourth leading cause of death in the United States and the first in rural areas of China. A number of factors have been documented as causal factors for COPD including tobacco smoking and indoor air pollution (IAP). In Xuan Wei, China, smoky coal is widely used for cooking and heating, often with poor ventilation (Mumford et al., 1987a), and

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rates of lung cancer were among the highest in China (Mumford et al., 1987c). Rates of COPD were also over twice the national average (Chapman et al., 2005d). A previous cross-sectional study found that smoky coal use was a strong risk factor for COPD in Xuan Wei, but tobacco smoking was found not to be associated significantly with COPD (He and Yang, 1994). In a cohort study in Xuan Wei, we found that reducing indoor coal smoke exposure by chimney installation was followed by a marked decrease in incidence of COPD, stronger than the effect of tobacco smoking (Chapman et al., 2005c). Further, indoor air monitoring has shown that levels of particulates and PAHs drop markedly with improved stove ventilation ($PM_{10}$: from 2.08 to 0.71 mg/$m^3$; Benzo(a)pyrene: from 1.66 to 0.25 ug/$m^3$) (Lan et al., 2002). Most men smoke tobacco in this area of China, and traditionally they have used a water pipe, which is likely to reduce exposure to COPD-causing agents. On balance, it is very likely that COPD in Xuan Wei has been caused mainly by IAP. Indoor smoky coal burning generates a high concentration of particulate matter and polycyclic aromatic hydrocarbons (PAH) (Mumford et al., 1987b), both of which are thought to provoke inflammatory and/or allergic disorders (van Eeden and Hogg, 2002;Tauchi et al., 2005). Genetic factors are likely to influence individual susceptibility. Coal and biomass fuel are widely used for cooking and heating in developing countries; the total population at risk is estimated at about 3 billion. Therefore, it is very important to identify the genetic susceptibility factors of IAP-induced COPD.

Inflammation is central to the development and progression of COPD. A variety of cytokines are secreted in the inflammatory process and play an important role in COPD (Reid and Sallenave, 2003). Colony stimulating factor 2 (CSF2), a pro-inflammatory cytokine, also named granulocyte macrophage-colony stimulating factor (GM-CSF), is capable of generating both granulocyte and macrophage colonies from precursor cells. A growing body of evidence indicates that CSF2 has important functions in host responses to external stimuli and in inflammatory/autoimmune conditions. A polymorphism of *CSF2* in codon 117 (Thr → Ile) has been associated with atopic asthma (Rohrbach et al., 1999a). Interleukin 8 (IL8), a chemokine member, mediates the activation and migration of neutrophils from peripheral blood into tissue. It plays a critical role in initiating and amplifying inflammatory processes. An A→T transversion in the promoter of *IL8* (-351) has been reported to be positively associated with bronchiolitis (Hull et al., 2000b) but inversely associated with bronchial asthma (Heinzmann et al., 2004b). In addition, single nucleotide polymorphisms (SNP) in some other cytokine genes have been associated with COPD including *TNF*, *LTA*, *IL1B*, *IL4*, *IL10*, and *IL13*. We report here the results of polymorphisms in these cytokine genes (*TNF*, *LTA*, *IL1B*, *IL4*, *IL10*, *IL13*, *CSF2* and *IL8*) and two IL8 receptor genes (*IL8RA* and *IL8RB*) in a population-based case-control study in Xuan Wei, China.

## Materials and methods

A population-based case-control study of lung cancer was conducted in Xuan Wei from 1995 through 1996 to investigate the role of genetic susceptibility factors on lung cancer risk in a highly exposed population. A total of 122 newly diagnosed lung cancer patients were enrolled. One control was selected for each lung cancer case, matching on sex, age (± 2 years), village and type of fuel used currently for cooking and heating at home. At the same time, all newly diagnosed chronic bronchitis and/or emphysema patients who had lived in Xuan Wei over their lifetime were collected from the Xuan Wei County Hospital. Chronic bronchitis and emphysema were combined into a single category – COPD, as in the cohort study (Chapman et al., 2005b). The participation rate was 100% and a total of 53 COPD patients were enrolled. The controls in the lung cancer case-control study were used as controls in the COPD study. Because lung cancer in Xuan Wei was predominantly driven by IAP rather than tobacco smoking and controls were matched to lung cancer cases on village and type of fuel, the controls had a high exposure to IAP just as the lung cancer cases had. DNA was extracted from sputum samples. A total of 18 SNPs in 10 genes were genotyped at the National Cancer Institute Core

Genotyping Facility (http://cgf.nci.nih.gov) and are listed in Table 1. The concordance rates between quality control samples were 99% – 100% for all assays. Unconditional logistic regression was used to estimate odds ratios (OR) and two-sided 95% confidence intervals (CI) of SNPs for COPD risk. Regression models were adjusted for age and sex.

## Results

Cases were comparable with the controls in terms of sex but were older than the controls (Table 1). The effect of smoking was weak and non-significant, consistent with previous reports on tobacco use and COPD in this region (He and Yang, 1994). We observed comparable exposures to smoky coal in cases and in controls (OR: 1.02; 95% CI: 0.89 – 1.18), as expected given that the controls were enrolled through matching to lung cancer cases on village and type of fuel used in a parallel study.

The genotype frequencies among the controls were consistent with Hardy-Weinberg proportions for all SNPs, except for *LTA* Ex1 +49 C>A (p = 0.02). The *CSF2* 117Ile allele carriers were found to have a 2.4-fold increased risk of COPD compared to the wild type carriers (Thr/Thr) (OR: 2.44; 95% CI: 1.10 – 5.41; p = 0.029) (Table 1). The *IL8* -351A allele was more common in cases than in controls (48% vs. 35%), and the AA genotype was associated with an increased risk of COPD (OR: 2.71; 95% CI: 1.04 – 7.04; p = 0.041). There was a significant linear trend for both SNPs, and adjustment for additional factors (i. e., tobacco smoking, smoky coal use, and other SNPs in metabolic and DNA repair genes) did not substantially alter the results. The other SNP in *IL8* (IVS1 -204 C>T) was in strong linkage disequilibrium with the *IL8* -351 T>A polymorphism ($D' = 1$, $r^2 = 0.94$), but was not significantly associated with COPD even though they acted in the same direction. Since the *IL8* polymorphisms were so strongly correlated, haplotype analysis did not provide any additional information. A SNP in the IL8 receptor alpha (*IL8RA* Ex2 +860 G>C) was borderline associated with COPD based on only 3 variant-carrying cases. Additionally, carrying at least one variant at *CSF2* Thr117Ile and *IL8* -351 T>A was associated with a five-fold risk of COPD (OR: 5.14; 95% CI: 1.32 - 29.86) (Table 1). None of the SNPs in the other genes were associated with COPD risk in this population (data not shown).

## Discussion

The similar distribution of smoky coal exposure between COPD cases and controls indicates that there was a high exposure pattern in the study population. It means that COPD cases were exposed to a high level of IAP, and that IAP should be a strong risk factor if it was to be compared to the exposure pattern in the general population. The similar distributions of tobacco smoking in two comparison groups implies that smoking is not the driving risk factor for COPD as documented in the cross-sectional study (He and Yang, 1994) and the cohort study (Chapman et al., 2005a) in Xuan Wei, which is analogous to the case of lung cancer (He and Yang, 1994), even though the sample size is small.

CSF2 is a mediator involved in the recruitment and activation of leucocytes including eosinophils and neutrophils, the latter of which is critical for COPD. Increased levels of CSF2 have been found in the epithelium and bronchoalveolar lavage fluid from subjects with chronic bronchitis and asthma (Balbi et al., 1997). Inhibition of the cytokine results in a reduced influx of neutrophils and increased susceptibility to respiratory infections (Shibata et al., 2001). The polymorphism at codon 117 of *CSF2* encodes a nonconservative amino acid change, a hydrophilic (Thr) to a hydrophobic (Ile) amino acid, in a conserved and functionally important region. In contrast to Asian populations, the Thr allele is uncommon in Caucasians and was found to be a risk factor for atopic asthma in a Swiss population (Rohrbach et al., 1999b). However, in our population the *CSF2* 117Ile allele was significantly associated with an

increased risk of COPD, suggesting that it plays a different role in COPD and in atopic asthma. It is also possible that if the observed association is due to linkage disequilibrium with an unknown variant, the associations with risk may differ between ethnic populations due to different linkage disequilibrium patterns.

IL8 is a potent neutrophil chemoattractant that is involved in both inflammatory and non-inflammatory processes. The -351 substitution is located in the *IL8* promoter region, which contains several binding sites for transcription factors and harbors an interferon-stimulated response element (Yamaoka et al., 2004). In an in vitro assay, the *IL8* –351A variant showed greater expression than the T allele with the highest values being observed for the AA genotype (Hull et al., 2000a). The -351A variant was reported to be associated with an increased risk of respiratory syncytial virus (RSV) bronchiolitis (Hull et al., 2000c). Although one study reported that the -351A variant was associated with a reduced risk of asthma (Heinzmann et al., 2004a), this finding was not replicated in a larger Korean study (Park et al., 2004). We observed an increased risk of COPD with the AA genotype, which is consistent with the functional data for the polymorphism and the association observed for RSV bronchiolitis. The association with *IL8* –351A may be due to its linkage to another functional variant based on a haplotype analysis (Hull et al., 2001). The tight linkage pattern through the 5′ region in our population supports the hypothesis and extensive haplotype analysis of *IL8* is warranted to extend the finding.

It was reported that IL8 and CSF2 have synergistic effects in attracting neutrophils in the female reproductive tract (Shen et al., 2004). Thus, we speculated that the *CSF2* 117Ile and *IL8* -351A variants may have a synergistic effect on COPD risk through the assembling of more pro-inflammatory cells and the worsening of the ill-regulated inflammation process. Although our p-value for interaction was not significant, we found that individuals carrying at least one at-risk allele in both genes had a significantly higher risk of COPD than individuals carrying no at-risk alleles.

Our study is limited by relatively small sample size, and the possibility of false positive findings cannot be excluded. Nonetheless, our study, like others, suggests that genetic variation in *CSF2* and *IL8* is important in the pathogenesis of COPD. With the specific exposure pattern in our population, the polymorphisms in *CSF2* and *IL8* may be important for the development of COPD among populations with high exposure to IAP. These results should be replicated in larger studies, both in subjects with high air pollution exposure and in the general population.

## References

Balbi B, Bason C, Balleari E, Fiasella F, Pesci A, Ghio R, Fabiano F. Increased bronchoalveolar granulocytes and granulocyte/macrophage colony-stimulating factor during exacerbations of chronic bronchitis. Eur Respir J 1997;10:846–850. [PubMed: 9150323]

Chapman RS, He X, Blair AE, Lan Q. Improvement in household stoves and risk of chronic obstructive pulmonary disease in Xuanwei, China: retrospective cohort study. BMJ 2005c;331:1050. [PubMed: 16234255]

Chapman RS, He X, Blair AE, Lan Q. Improvement in household stoves and risk of chronic obstructive pulmonary disease in Xuanwei, China: retrospective cohort study. BMJ 2005b;331:1050. [PubMed: 16234255]

Chapman RS, He X, Blair AE, Lan Q. Improvement in household stoves and risk of chronic obstructive pulmonary disease in Xuanwei, China: retrospective cohort study. BMJ 2005a;331:1050. [PubMed: 16234255]

Chapman RS, He X, Blair AE, Lan Q. Improvement in household stoves and risk of chronic obstructive pulmonary disease in Xuanwei, China: retrospective cohort study. BMJ 2005d;331:1050. [PubMed: 16234255]

He, X.; Yang, R. Lung cancer and indoor air pollution from coal burning. Yunnan Science and Technology Press; 1994.

Heinzmann A, Ahlert I, Kurz T, Berner R, Deichmann KA. Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis. J Allergy Clin Immunol 2004b;114:671–676. [PubMed: 15356575]

Heinzmann A, Ahlert I, Kurz T, Berner R, Deichmann KA. Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis. J Allergy Clin Immunol 2004a;114:671–676. [PubMed: 15356575]

Hull J, Ackerman H, Isles K, Usen S, Pinder M, Thomson A, Kwiatkowski D. Unusual haplotypic structure of IL8, a susceptibility locus for a common respiratory virus. Am J Hum Genet 2001;69:413–419. [PubMed: 11431705]

Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 2000a;55:1023–1027. [PubMed: 11083887]

Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 2000c;55:1023–1027. [PubMed: 11083887]

Hull J, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. Thorax 2000b;55:1023–1027. [PubMed: 11083887]

Lan Q, Chapman RS, Schreinemachers DM, Tian L, He X. Household stove improvement and risk of lung cancer in Xuanwei, China. J Natl Cancer Inst 2002;94:826–835. [PubMed: 12048270]

Mumford JL, He XZ, Chapman RS, Cao SR, Harris DB, Li XM, Xian YL, Jiang WZ, Xu CW, Chuang JC, Wilson WE, Cooke M. Lung cancer and indoor air pollution in Xuan Wei, China. Science 1987a; 235:217–220. [PubMed: 3798109]

Mumford JL, He XZ, Chapman RS, Cao SR, Harris DB, Li XM, Xian YL, Jiang WZ, Xu CW, Chuang JC, Wilson WE, Cooke M. Lung cancer and indoor air pollution in Xuan Wei, China. Science 1987c; 235:217–220. [PubMed: 3798109]

Mumford JL, He XZ, Chapman RS, Cao SR, Harris DB, Li XM, Xian YL, Jiang WZ, Xu CW, Chuang JC, Wilson WE, Cooke M. Lung cancer and indoor air pollution in Xuan Wei, China. Science 1987b; 235:217–220. [PubMed: 3798109]

Park BL, Kim LH, Choi YH, Lee JH, Rhim T, Lee YM, Uh ST, Park HS, Choi BW, Hong SJ, Park CS, Shin HD. Interleukin 3 (IL3) polymorphisms associated with decreased risk of asthma and atopy. J Hum Genet 2004;49:517–527. [PubMed: 15372320]

Reid PT, Sallenave JM. Cytokines in the pathogenesis of chronic obstructive pulmonary disease. Curr Pharm Des 2003;9:25–38. [PubMed: 12570672]

Rohrbach M, Frey U, Kraemer R, Liechti-Gallati S. A variant in the gene for GM-CSF, I117T, is associated with atopic asthma in a Swiss population of asthmatic children. J Allergy Clin Immunol 1999b;104:247–248. [PubMed: 10400873]

Rohrbach M, Frey U, Kraemer R, Liechti-Gallati S. A variant in the gene for GM-CSF, I117T, is associated with atopic asthma in a Swiss population of asthmatic children. J Allergy Clin Immunol 1999a;104:247–248. [PubMed: 10400873]

Shen L, Fahey JV, Hussey SB, Asin SN, Wira CR, Fanger MW. Synergy between IL-8 and GM-CSF in reproductive tract epithelial cell secretions promotes enhanced neutrophil chemotaxis. Cell Immunol 2004;230:23–32. [PubMed: 15541716]

Shibata Y, Berclaz PY, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU.1. Immunity 2001;15:557–567. [PubMed: 11672538]

Tauchi M, Hida A, Negishi T, Katsuoka F, Noda S, Mimura J, Hosoya T, Yanaka A, Aburatani H, Fujii-Kuriyama Y, Motohashi H, Yamamoto M. Constitutive expression of aryl hydrocarbon receptor in keratinocytes causes inflammatory skin lesions. Mol Cell Biol 2005;25:9360–9368. [PubMed: 16227587]

van Eeden SF, Hogg JC. Systemic inflammatory response induced by particulate matter air pollution: the importance of bone-marrow stimulation. J Toxicol Environ Health A 2002;65:1597–1613. [PubMed: 12396870]

Yamaoka Y, Kudo T, Lu H, Casola A, Brasier AR, Graham DY. Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in Helicobacter pylori infection. Gastroenterology 2004;126:1030–1043. [PubMed: 15057743]

**Table 1**

The distribution of demographic variables and the association between smoking, and cytokine polymorphisms and COPD risk [a].

| Variable | Controls(%) | Cases(%) | OR b | 95% CI | p value |
|---|---|---|---|---|---|
| Age | | | | | |
| <55 | 51 (42) | 13 (24) | | | |
| >=55 | 71 (58) | 40 (76) | | | 0.029 c |
| Sex | | | | | |
| Female | 43 (35) | 19 (36) | | | |
| Male | 79 (65) | 34 (64) | | | 0.939 c |
| Pack-year | | | | | |
| Never | 53 (43) | 22 (42) | Ref. | | |
| <25 | 36 (30) | 11 (21) | 1.12 | 0.28-4.39 | 0.875 |
| >=25 | 33 (27) | 20 (38) | 1.82 | 0.48-6.90 | 0.376 |
| *CSF2* Ex4 +23 C>T (rs25882) Thr117Ile | | | | | |
| CC | 42 (38) | 11 (22) | Ref. | | |
| CT | 53 (48) | 28 (57) | 2.34 | 1.01-5.38 | 0.046 |
| TT | 15 (14) | 10 (20) | 2.75 | 0.95-7.95 | 0.061 |
| CT+TT | 68 | 38 | 2.44 | 1.10-5.41 | 0.029 |
| Trend | | | | | 0.039 |
| *IL8* -351 T>A (rs4073) | | | | | |
| TT | 48 (43) | 15 (30) | Ref. | | |
| TA | 49 (44) | 22 (44) | 1.41 | 0.65-3.08 | 0.380 |
| AA | 15 (13) | 13 (26) | 2.71 | 1.04-7.04 | 0.041 |
| TA+AA | 64 | 35 | 1.72 | 0.84-3.53 | 0.141 |
| Trend | | | | | 0.047 |
| IVS1 -204 C>T (rs2227306) | | | | | |
| CC | 47 (43) | 18 (37) | Ref. | | |
| CT | 48 (44) | 19 (39) | 0.99 | 0.46-2.15 | 0.988 |
| TT | 15 (14) | 12 (24) | 2.04 | 0.79-5.25 | 0.139 |
| CT+TT | 63 | 31 | 1.24 | 0.62-2.51 | 0.544 |

| Variable | Controls(%) | Cases(%) | OR b | 95% CI | p value |
|---|---|---|---|---|---|
| Trend | | | | | 0.204 |
| *IL8RA* Ex2 +860 G>C (rs2234671) Ser276Thr | | | | | |
| GG | 92 (82) | 44 (94) | Ref. | | |
| GC | 20 (18) | 3 (6) | 0.31 d | 0.06-1.14 | 0.088 |
| *CSF2* Ex4 +23 C>T/*IL8* -351 | | | | | |
| T>A [e] | | | | | |
| 1 | 22 | 3 | Ref. | | |
| 2 | 45 | 19 | 3.25 [d] | 0.81-19.18 | 0.115 |
| 3 | 42 | 27 | 5.14 [d] | 1.32-29.86 | 0.013 |
| Trend | | | | | 0.0096 |

[a] all other genotyped SNPs in the study and frequencies of minor allele are *TNF* (-1036 C>T (rs1799724) (q = 0.15); -487 G>A (rs1800629)) (q = 0.05); *LTA* (Ex1 +49 C>A (rs2239704) (q = 0.27); IVS1 +90 A>G (rs909253) (q = 0.46)); *IL1B* (-1060 C>T (rs16944) (q = 0.40)); *IL4* (-588 T>C (rs2243250) (q = 0.18); Ex1 -168 T>C (rs2070874) (q = 0.28)); *IL10* (-1116 A>G (rs1800896) (q = 0.08); -626 A>C (rs1800872) (q = 0.31)); *IL13* (-1069 C>T (rs1800925) (q = 0.12); Ex4 +98 G>A (rs20541) (q = 0.29)); and *IL8RB* (Ex3 +811 C>T (rs2230054) (q = 0.27); Ex3 +1235 T>C (rs1126579) (q = 0.35); Ex3 -1010 G>A (rs1126580) (q = 0.19)).

[b] based on logistic regression adjusting for age and sex.

[c] based on Pearson Chi square test.

[d] based on exact logistic regression (used due to small cell size), adjusting for age and sex.

[e] the combination of *CSF2* Ex4 +23 C>T and *IL8* -351 T>A: 1, *CSF2* CC/*IL8* TT; 2, *CSF2* CC/*IL8* (TA+AA) or *CSF2* (CT+T T)/*IL8* TT; 3, *CSF2* (CT+TT)/*IL8* (TA+AA).