Lack of association of human leukocyte antigen-B7 with COPD and rate of decline in lung function

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Summary *Background*: Although variation in the human leukocyte antigen (*HLA*) locus is associated with various diseases, there have been a limited number of studies that have examined the possible role of *HLA* in chronic obstructive pulmonary disease (COPD). Only *HLA-B7* has been shown to be correlated with low forced expiratory volume in 1 s (FEV_1) in Caucasians; however, this finding has not been replicated. The aim of this study was to investigate the contribution of the *HLA-B7* allele to COPD and to rate of decline of lung function.

Methods: We determined the prevalence of *HLA-B7* in a group of COPD patients and a non-obstructed control group of smokers by using a polymerase chain reaction-based genotyping assay. We also determined the prevalence of *HLA-B7* in smokers selected from the National Heart Lung and Blood Institute, Lung Health Study for having the fastest and slowest decline of lung function.

Results: No significant difference was found in the frequency of *HLA-B7* between the COPD and non-obstructed groups. There was also no significant association of *HLA-B7* with rate of decline of lung function.

Conclusion: These data indicate that *HLA-B7* does not contribute to COPD or rate of decline of FEV_1 in smokers.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality in developed countries.[1,2] This disease is characterized by irreversible airflow limitation, increased pulmonary

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resistance, and hyperinflation of the lung. Established risk factors for the pathogenesis of COPD are cigarette smoking and severe α_1-antitrypsin deficiency. However, clustering of COPD within families without deficiency of α_1-antitrypsin[3,4] and inter-individual variation in susceptibility to cigarette smoking[5] suggest that additional risk factors, possibly genetic factors, contribute to the development of COPD.

Despite the contribution of human leukocyte antigen (HLA) allelic variation to various diseases, the role of HLA class I alleles in the pathogenesis of COPD has not been extensively investigated. There have been a limited number of studies which describe association between HLA class I genes and COPD.[6,7] In a previous report, only the HLA-B7 allele was shown to be a risk factor for the development of COPD.[6] Although the finding was noteworthy because HLA-B7 is one of the predominant HLA types in Caucasians,[8] there has been no replication of this result. In this study, we have investigated the putative association of HLA-B7 with COPD by using a case–control approach in smokers. In addition, we investigated the contribution of HLA-B7 to rate of decline of lung function in smokers by using the Lung Health Study (LHS). The LHS was designed to investigate the impact of early intervention on the course of cigarette smoke-induced COPD.[9] Lung function, as measured by forced expiratory volume in 1s (FEV_1), normally increases to a maximal value at adulthood, remains stable for 10–15 years, and then declines,[10] and COPD may develop because of an accelerated rate of decline.

Therefore, the aim of this study was to clarify the role of HLA-B7 in genetic susceptibility to COPD and rate of decline of FEV_1 by employing two different study designs.

Methods

Subjects for the case and control study

Subjects for this study were recruited from patients admitted to St Paul's Hospital to undergo lobar or lung resection surgery for a localized lung cancer. Prior to surgery, all patients gave informed consent and completed an interviewer-administered questionnaire regarded smoking history, occupational exposure to dust or fumes, and respiratory symptoms. FEV_1, forced vital capacity (FVC) and FEV_1/FVC ratio were calculated. Patients in whom the lung lesion was obstructing a segmental or larger bronchus were excluded from this study as this may

influence lung function. All patients selected for this study were Caucasians. Any patients who had functional or pathologic evidence of a process other than those associated with COPD, and non-smokers were also excluded from this study. Of these who remained, individuals with $FEV_1 < 80\%$ predicted and $FEV_1/FVC < 70\%$ were classified as having COPD and placed into the obstructed category; those with $FEV_1 > 85\%$ predicted and $FEV_1/FVC > 75\%$ were classified as non-obstructed. In total, 113 patients were classified as obstructed and 61 patients were classified as non-obstructed. DNA for genotyping was extracted from frozen lung tissue or formalin-fixed lung tissue using a standard phenol/chloroform protocol or from blood using the QIAamp DNA BloodMaxi kit (QIAGEN Inc., Mississauga, Ontario). None of the individuals were homozygous for the Z allele of the α_1-antitrypsin gene.

Individuals from the LHS

Individuals were selected from the participants in the National Heart, Lung and Blood Institute, Lung Health Study. The design of this multi-center, randomized clinical trial has been described in detail elsewhere.[11] Study participants were healthy current smokers, 35–60 years of age, who had spirometric signs of early COPD. During 5 years of follow up, 3216 subjects continued to smoke and from this cohort we selected the 303 with the fastest rate of decline of FEV_1 (decline in $FEV_1 > 3.0\%$ of predicted value per year) and the 324 who had no decline of FEV_1 (increase in $FEV_1 > 0.4\%$ of predicted value per year). In total, 613 of these samples were successfully genotyped for HLA-B7. Of this group, we studied the Caucasian individuals separately. Other ethnic groups were of insufficient sample size to provide statistically meaningful comparison, and were excluded from statistical analysis. Therefore, 581 Caucasian (277 fastest decline and 304 non-decline) subjects remained for statistical analysis. None of the individuals were homozygous for the Z allele of the α_1-antitrypsin gene.

Genotyping

The subjects were genotyped by polymerase chain reaction (PCR) assay by using the published HLA-B7 group-specific primer.[12] The sense oligonucleotide primer and anti-sense primer were designed from exon 3 of the HLA-B gene which was amplified as a 119bp PCR product. The sequence of sense primer was 5′-CAA GTG GGA GGC GGC CCG TGA-3′ and anti-sense primer was 5′-TGG TAC CAG CGC GCT

CCA GCT-3′. In order to verify that all samples contained amplifiable DNA, internal control primers that amplified a 394bp fragment of the Coxsackie-adenovirus receptor gene (exon 2)[13] were added in each reaction mixture. The sequence of the control sense primer was 5′-CTG GGC ATC TCT TGA GTT TGG A-3′, and the anti-sense primer was 5′-ACT GGC AAG GTG ATG GAC ACA T-3′.

Optimized PCR conditions for *HLA-B7* typing were as follows: the PCR reaction mixture in a final volume of 20 μl consisted of 100 ng genomic DNA, 1.5 mM MgCl$_2$ for frozen tissue and blood-derived DNA samples and 2.5 mM MgCl$_2$ for formalin-fixed samples, 200 μM each of dATP, dCTP, dGTP and dTTP, 0.4 μM of each *HLA-B7* primer and 0.5 μM of each control primer, and 0.5 U DNA *Taq* Polymerase (Hotstar Taq, Qiagen Inc., Mississauga, Ontario). PCR amplification was performed in a PCR EXPRESS Thermal Cycler (Thermo Hybrid, Ashford, Middlesex, UK). PCR conditions for cycling were optimized using the touch down method[14] as follows: initial denaturation step at 95 °C for 15 min, followed by 5 cycles of 94 °C for 30 s (denaturation), 68 °C for 30 s (annealing), and 72 °C for 45 s (extension), followed by 10 cycles with a decreased annealing temperature at 67 °C, 20 cycles at 66 °C and a final extension for 10 min at 72 °C. PCR products were loaded on 2% agarose gels stained with ethidium bromide and visualized in ultra-violet illumination.

Statistical analysis

The results are presented as the prevalence of *HLA-B7* positive individuals (containing both heterozygotes and homozygotes for *HLA-B7*). The differences in genotype frequencies between the groups were assessed by χ^2 tests. The associations were also analyzed by logistic regression to adjust for potential confounding factors such as age, sex and smoking history (pack years). Since differences in cigarette consumption over the course of the Lung Health Study could also influence the rate of decline of lung function we also included the average cigarettes smoked per day over the course of the study as a confounding variable. All tests were performed using the JMP Statistics software package (SAS Institute Inc.). *P*-values of <0.05 were considered to be statistically significant. Values for continuous variables are expressed as mean (±SE).

Results

The characteristics of the 174 subjects in the case–control study and the 581 LHS participants are shown in Tables 1 and 2, respectively. There were significant differences in several potentially confounding factors between each group. Therefore, the frequencies of genotypes between groups were analyzed by logistic regression to adjust for these factors. In the case–control study, the frequencies of *HLA-B7* positivity in the COPD and non-obstructed groups were 23.9% and 34.4%, respectively, (Fig. 1a) but the difference between the groups was not significant. The odds ratio adjusted for age, sex and smoking history was 0.53 (95% CI 0.25–1.10, $P = 0.09$). In the LHS cohort, the frequencies of *HLA-B7* positivity in the fastest and non-decline groups were 23.8% and 26.3%, respectively, (Fig. 1b) and there was also no significant difference between these two groups. The odds ratio for *HLA-B7* positivity adjusted for age, sex, smoking history, cigarettes smoked per day over the course of the study, bronchodilator response, methacholine response and baseline lung function was 0.83 (95% CI 0.53–1.29, $P = 0.41$). The allele frequencies of *HLA-B7* in these study groups, if Hardy–Weinberg equilibrium is assumed, were 0.12 and 0.13 for the case–control and LHS groups, respectively. These allele frequencies are similar to the reported allele frequency (0.134) in Caucasians.[8]

Table 1 Characteristics of the study subjects in the COPD and non-obstructed groups. Continuous variables shown as mean±SEM.

	COPD ($n = 113$)	Non-obstructed ($n = 61$)	*P*-value
Male/Female	85/28	32/29	0.002
Age (years)	64.3±0.8	62.3±1.4	0.18
Smoking history (pack years)*	57.2±2.9	40.6±4.3	0.001
FEV$_1$ (% predicted)	59.3±1.3	98.8±1.3	—
FEV$_1$/FVC$_1$ (%)	57.5±0.9	79.6±0.5	—

*Number of packs of cigarettes smoked per day × number of years smoking.

Table 2 Characteristics of the fast and non-decliner study groups.

	Fast decliners ($n = 277$)	Non-decliners ($n = 304$)	P-value
Male/Female	161/116	202/102	0.04
Age (years)	49.5 ± 0.4	47.6 ± 0.4	0.0007
Smoking history (pack years)*	41.6 ± 1.0	37.7 ± 1.0	0.007
Cigarettes smoked per day[†]	25.6 ± 0.6	22.4 ± 0.6	0.0003
Baseline FEV_1(%) predicted[‡]	72.5 ± 0.5	75.7 ± 0.5	<0.0001
Rate of decline in lung function[§]	-4.14 ± 0.06	1.08 ± 0.04	<0.0001
Bronchodilator response (%)[¶]	3.5 ± 0.3	5.8 ± 0.3	<0.0001
Methacholine response (O'Connor slope)**	-23.5 ± 2.0	-7.8 ± 0.9	<0.0001

Values for continuous variables are means $\pm$ SE

*Number of packs of cigarettes smoked per day $\times$ number of years smoking.

[†]Average over the five years of the Lung Health Study.

[‡]Lung function at the start of the Lung Health Study.

[§]Change in lung function per year over a 5 year period measured as % predicted FEV_1.

[¶]Percent change in FEV_1 from baseline in response to a bronchodilator.

**Responsiveness of the airways to methacholine expressed as percent decline in FEV_1 per final cumulative dose of methacholine administered.
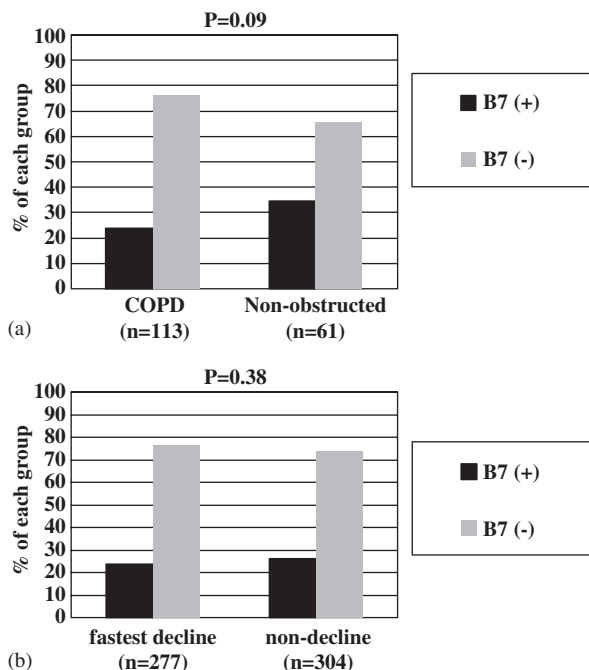

Fig. 1 Prevalence of *HLA-B7* positivity in the study groups. (a) Comparison between the COPD and non-obstructed groups. The *P*-value shown is from the logistic regression model adjusting for age, sex and smoking history. (b) Comparison between the fastest decliner and non-decliner groups. The *P*-value shown is from the logistic regression model adjusting for age, sex, smoking history, methacholine response, bronchodilator response and baseline lung function.

Discussion

The association between HLA types and numerous diseases have been extensively studied because

HLA mediated responses are integral to the human immune system and are involved in chronic inflammatory reactions. However, the role of polymorphisms of *HLA* genes in COPD has been poorly studied. The association between COPD and an *HLA* gene was described in 1983 by Kauffmann and colleagues.[6] They found a significantly higher *HLA-B7* frequency in a group of non-smokers who had low FEV_1 compared with a group of heavy smokers who had normal FEV_1 (odds ratio (OR) = 3.8) in a French population. This finding suggests that *HLA-B7* is a susceptibility allele for COPD independent of exposure to cigarette smoke. In 1990, Maranetra and colleagues showed a significant increase in *HLA-Bw60* frequency in a group with low ventilatory drive compared with normal ventilatory drive (OR = 42) among COPD patients in the Thai population.[7] This result suggests that *HLA-Bw60* might be related to ventilatory response to CO_2 in COPD, although not correlated with the risk of COPD itself. This finding, however, may not be relevant to other populations since *HLA-Bw60* is commonly found in South East Asians (allele frequency 8–16%) but is rarer in other populations e.g. in Caucasians the frequency is 3–5%.[15] Recently, HLA typing was also performed in a Japanese patient group who had diffuse pan-bronchiolitis (DPB) which is a distinctive form of obstructive lung disease of unknown etiology.[16,17] The results demonstrated an increased prevalence of HLA-Bw54 in the DPB patients compared with the control group (relative risk = 13.3 and OR = 3.4). HLA-Bw54 is specifically found in East Asian populations and is seldom found in other ethnic groups. The fact that DPB is also limited to East

Asians may be a result of the strong association between HLA-Bw54 and DPB.

On the basis of findings mentioned above, we focused on the *HLA-B7* allele as a potential risk factor for COPD in Caucasian populations. However, our data did not show a significant association between *HLA-B7* and COPD. Although there was no significant difference between the case and control groups, the genotype frequency of *HLA-B7* was slightly higher in the non-obstructed group (34.4%) than in the COPD group (23.9%), which was a trend opposite to the results of Kauffmann et al.[6] In this study, we also investigated whether *HLA-B7* was associated with an accelerated rate of decline of lung function. This phenotype is likely to result in the development of COPD if the rate of decline remains constant. This is the first study to investigate the influence of an *HLA* gene on this phenotype; however, we also found no contribution of *HLA-B7* to rate of decline of lung function even with the use of a large sample size. With any negative study it is important to calculate the power of the experimental design to detect a difference. Given the numbers in this study we should have been able to detect a relative risk of $\geqslant 3.1$ for *HLA-B7* as a risk factor for COPD and $\geqslant 2.0$ for *HLA-B7* as a risk factor for rapid decline. Therefore, although we cannot rule out the possibility that the lack of association seen in this study was due to type 2 error we did have sufficient power to detect an effect of the magnitude (OR = 3.8) reported by Kauffmann et al.[6] However, another possible cause of a false negative result could be the differences in recruitment and phenotype definition between the present study and that of Kauffmann et al.[6]

In this study there were significant differences between the COPD patients and controls and between the fast and slow decliners in regard to important confounding factors such as exposure to cigarette smoke. It is likely that these differences contributed to the development of COPD and a rapid rate of decline of lung function. Therefore, we have attempted to correct for these differences in the logistic regression analyses. However, while cigarette smoking is clearly the most important environmental risk factor for COPD we believe that it does not completely explain the pathogenesis of this disease. For example, epidemiological studies have shown that only 10–20% of smokers develop symptomatic COPD[18] and differences in cigarette smoke exposure account for only ~15% of the variation in lung function.[19] In the subset of the LHS investigated in this study, rate of decline of lung function was poorly correlated with pack-years (correlation coefficient, $R^2 = 0.02$) and cigarette consumption over the course of the study ($R^2 = 0.02$). Therefore, we believe that it is important to look for additional factors involved in the pathogenesis of COPD and the decline in lung function.

In conclusion, we consider that the lack of association in this study indicates that *HLA-B7* does not contribute substantially to the risk of COPD and an accelerated rate of decline of lung function.

Acknowledgments

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