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TITLE PAGE

Original Article

C-C chemokine receptor 5 (*CCR 5*) polymorphisms in beryllium disease

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Abstract

C-C chemokine receptor 5 (CCR5) is expressed on Th1 cells, which are involved in the pathogenesis of the granulomatous lung disease chronic beryllium disease (CBD). *CCR5* polymorphisms are associated with sarcoidosis severity. This study explores associations between *CCR5* polymorphisms and CBD and its disease progression.

We genotyped 8 *CCR5* polymorphisms in CBD (n=88), beryllium sensitized (BeS, n=86) and beryllium exposed non-diseased controls (controls, n=173) by SSP- PCR. Pulmonary function and bronchoalveolar lavage (BAL) data were examined for associations with genotypes.

There were no significant differences in genotype and allele frequency between CBD, BeS and controls. Associations were found in CBD with decline in FEV_1 & FVC and *CCR5 -3458TT* ($p<0.0001$), and an increase in A-a gradient at rest ($p=0.003$) & at maximum exercise ($p=0.01$) and the *-5663A allele*. Increased BAL lymphocyte numbers were associated with the *CCR5 -2459G /-2135T* ($p=0.01$) only in the combination of CBD & BeS.

This is the first study showing that *CCR5* polymorphisms are associated with worsening pulmonary function over time in CBD, suggesting that the *CCR5* gene is important in the progression of pulmonary function in CBD. Further studies would be useful to clarify the mechanism whereby CCR5 polymorphisms affect progression in CBD.

Key words: BAL, CCR5, chronic beryllium disease, lung function tests, polymorphism

Abbreviations:

A-a gradient: alveolar-arterial oxygen gradient

(A-a)PO_2m: alveolar-arterial oxygen gradient at maximum exercise

(A-a)PO_2r : alveolar-arterial oxygen gradient at rest

BAL: bronchoalveolar lavage

Be: Beryllium

Be-exp: beryllium exposed non-diseased controls

BeS: beryllium sensitized

BeLPT: beryllium lymphocyte proliferation test

BHL: bilateral hilar lymphadenopathy

CCR2: C-C chemokine receptor 2

CCR5: C-C chemokine receptor 5

CBD: chronic beryllium disease

DL_{COU}: diffusing capacity uncorrected for altitude

FEV_1: forced expiratory volume

FVC: forced vital capacity

LD: linkage disequilibrium

MHC: major histocompatibility complex

PaO_2m: partial pressure of oxygen at maximum exercise

PaO_2r: partial pressure of oxygen at rest

PCR: polymerase chain reaction

SSP: sequence-specific primers

TLC: total lung capacity

WLM: work load at maximum exercise

Introduction

Beryllium (Be) exposure in the workplace triggers a beryllium specific immune response in a minority of those exposed. Specifically, 2-10% of workers exposed develop beryllium sensitization (BeS) with lymphocytes that proliferate in response to beryllium in the beryllium lymphocyte proliferation test (BeLPT)[1;2]. Previous studies have shown that Be-antigen stimulated T cell proliferation is MHC Class II restricted[3]. Subsequently, trafficking of immune and inflammatory cells is initiated along with cytokine production, resulting in granuloma formation, the hallmark of chronic beryllium disease (CBD).

The chemokines involved in granuloma formation in CBD are unknown. The *C-C chemokine receptor 5* (*CCR5*), located on chromosome *3p21* and adjacent to the *C-C chemokine receptor 2* (*CCR2)* gene, is implicated in the chemotaxis and activation of leukocyte subsets, including $CD4^+$ Th1 lymphocytes[4]. The *CCR5* gene is polymorphic and some of the variants appear to be functional, although this is not well understood[5;6] .

Of relevance to CBD, we previously reported an association between *CCR5* polymorphisms and sarcoidosis. Although we did not find an association between *CCR5* polymorphisms and presence of disease in sarcoidosis compared to controls, we did find an association between a specific *CCR5 haplotype (HHC)* and chest radiographic stage of disease[7] . Others have reported an increased frequency of *CCR5* Δ*32* in sarcoidosis[8]. This may suggest that *CCR5* polymorphisms are a risk for disease or disease severity in granulomatous lung diseases.

An increase of CCR5 expression has been reported on bronchoalveolar lavage (BAL) cells in sarcoidosis[9-11]. There are no reports of an association between *CCR5* polymorphisms and CBD, which is histopathologically identical to sarcoidosis, sharing many clinical and

radiographic features of sarcoidosis. With this background, we hypothesized that *CCR5* polymorphisms and/or haplotypes would be associated with CBD and more severe forms of disease, but not with BeS in which pulmonary granulomatous disease is absent. The aims of this study were, to: 1) assess the contribution of *CCR5* polymorphisms and/or haplotypes as risk factors for CBD compared to BeS and Be-exposed non-diseased controls; 2) to evaluate the association between these polymorphisms and severity markers in CBD; and 3) to investigate the association between BAL cellularity and *CCR5* polymorphisms and/or haplotypes, to determine if there is a functional association between CCR5 polymorphisms and CBD.

Methods

Study design

We conducted a case control study comparing Caucasian subjects with CBD (n =88), BeS (n = 86) and subjects with Be exposure without evidence of sensitization or disease (controls, n = 173) to evaluate *CCR5* polymorphisms and their association with CBD. We only utilized Caucasian cases and controls to limit population stratification, as the number of non-Caucasian (e.g. American Black, Asian etc.) were too small to provide meaningful analysis. A case comparison study was used to evaluate *CCR5* polymorphisms and disease severity in CBD. Cases were enrolled from the clinic at National Jewish Health. Cases of CBD and BeS had two abnormal blood BeLPTs. If the cases had evidence of either non-caseating granulomas or a mononuclear cell infiltrate on lung biopsy, or BAL cell lymphocytosis ≥ 15% and an abnormal BAL LPT on bronchoscopy, they were diagnosed as CBD. If they had no evidence of these abnormalities, they were diagnosed as BeS. Controls were undergoing medical surveillance in the same Be-industries as cases[12-14] and had ≥ one normal BeLPT. Study subjects provided informed consent according to a protocol approved by the Institutional Review Board at National Jewish Health (NJH).

Cases of CBD and BeS underwent venipuncture, a BeLPT, pulmonary function testing, pre and post bronchodilator, maximum exercise testing with an indwelling arterial line, during initial (baseline) and subsequent clinical evaluations[15]. These data were obtained in the Pulmonary Physiology Unit at NJH using the same clinical protocols over time on the same equipment. Post-bronchodilator pulmonary function variables were used for analysis. Bronchoscopy with BAL, transbronchial biopsies, and BAL LPT were obtained for clinical purposes[15]. Control subjects underwent venipuncture and BeLPT. All subjects completed a modified version of the American Thoracic Society questionnaire[15].

CCR5 Genotypes

Genomic DNA was extracted from whole blood using the PAXgene kit (Qiagen Inc.Valencia, CA). Polymorphisms were determined using sequence-specific primers (SSP) and polymerase chain reaction (PCR) [16;17] to identify 8 *CCR5* single nucleotide polymorphisms: *–5663(A/G, rs2040388), –3900(C/A, rs2856757), –3458(T/G, rs2734225), –2459(G/A, rs1799987), –2135(T/C, rs1799988), -2086(A/G, rs1800023), -1835(C/T, rs1800024) and Δ32*, which were investigated in our previous study on sarcoidosis[7].

Data analysis

All genotype frequencies were tested for Hardy-Weinberg equilibrium using Chi-Square goodness-of-fit-tests. Normalizing transformations were performed on continuous outcome variables when necessary to better approximate model assumptions; these data are expressed as geometric means and standard deviations. Measures of linkage disequilibrium, D' and r^2, were calculated using Haploview (Whitehead Institute for Biomedical Research). Haplotype blocks were determined with Haploview using the confidence interval method[18]. Haplotype frequencies were estimated using Haplo.Score[19]. To adjust for uncertainty in haplotype assignments, weighted logistic regression models were used to assess associations with disease and disease severity. Comparisons were made when sufficient numbers of observed haplotypes were available. Linear regression was used to determine the influence of genotype/haplotype on cross-sectional continuous outcomes. Mixed effects models were used to assess longitudinal outcomes and was used to estimate effects based on different numbers of follow-up visits and different timings of the visits. Age at test, gender, smoking history, height, and time from first Be-exposure were included in the model along with the genotype/haplotype, and forced expiratory volume (FEV_1), forced vital capacity (FVC), total

lung capacity (TLC), diffusing capacity uncorrected for hemoglobin (DL_{COU}), and partial pressure of oxygen at rest and maximum exercise (PaO_2r, PaO_2m), alveolar-arterial oxygen gradient at rest and maximum exercise [$(A\text{-}a)PO_2r$, $(A\text{-}a)PO_2m$] at maximum exercise and at rest, and work load at maximum exercise (WLM) . The variable time from first exposure to visit date was restricted to visits between 10 and 40 years from first exposure, since there were few visits outside this range from which to make estimates. To avoid effects of corticosteroids on the severity of CBD, all subjects' data were included until commencement of corticosteroid use. We did not adjust for multiple comparisons as this adjustment is too conservative with the high correlation of outcomes. Statistical analysis was performed using Knowledge STUDIO (Angoss Software Corporation, Toronto, Canada), or SAS software (SAS Institute, Inc., Cary, NC version 9.1). All tests were two sided and $p< 0.05$ was used to determine statistical significance.

Results

Demographic Information

There were no significant gender differences between the three study groups. The control subjects (60.5±11.0 yrs.) were older than both the CBD (54.1±10.8 yrs.) or BeS subjects (53.5± 12.0 yrs.) ($p<0.0001$). Ever smoking was not significantly different across the groups. In the CBD group, 18 (20.5%) of patients had received corticosteroids. The BeS and controls were not treated with corticosteroids.

CCR5 polymorphisms

We compared *CCR5* promoter polymorphisms across groups, as summarized in **Table 1.** All populations were in Hardy-Weinberg equilibrium for all genotypes. *CCR5-2459A/G* and *-2135C/T* were in 100 % linkage disequilibrium (LD, D'=1). *CCR5-3900A* (p=0.03, OR=0.35, 95%CI=0.14-0.84), *-3458G* (p=0.02, OR=0.29, 95%CI=0.11-0.78), and *-2459A/-2135C* (p=0.03, OR=0.39, 95%CI=0.18-0.86) were decreased in CBD compared to BeS. However, no significant differences were found between CBD and controls, nor between BeS and controls. Consistent with the allele frequencies, similar differences were found between CBD and BeS when comparing the genotypes at the same three positions **(Table 1).** No other associations were found between any of the other CCR5 polymorphisms and disease.

The haplotype comprised of the *-5663/-3900/-3458,-2459/-2135/-2086* SNPs was identified using the confidence interval method in Haploview **(Figure 1). Table 2** shows the frequency of common (>1%) *CCR5* haplotypes. There were no significant differences in *CCR5* haplotypes between the three groups.

Longitudinal analysis of disease severity in CBD

Mixed effects models were used to fit lung function measurements from clinical visits, including FEV_1, FVC, TLC, DL_{COU}, PaO_2r, PaO_2m, (A-a)PO_2r , (A-a)PO_2m, and WLM, and covariates as noted above along with *CCR5* variants. The average number of visits was 3.96 with a maximum of 15 visits. First visit was on average 18.1 years (standard deviation=8.2) from first exposure and last visit was on average 27.2 years (standard deviation=8.8) from first exposure. **Tables 3a and 3b** show the average rates of change in these variables over time in relation to the *CCR5* genotypes of interest, adjusting for age at test, gender, smoking history, height, and time from first Be-exposure.

On average, CBD subjects homozygous for the *-5663A* showed a significantly greater decrease in FEV_1 over time (p=0.01) compared to non-homozygous subjects **(Figure 2a)**. The carriers of the *A* allele also showed a greater decline in FEV_1 compared to non-carriers (p=0.001) (**Table 3a**). A similar trend was seen with these same alleles in relation to FVC. The carriers of the *-5663A* allele had a greater decline in Pao_2 at rest (p=0.01), as well as an increase in log (A-a)PO_2r (p=0.003) and (A-a)PO_2m (p=0.01), than the non-carriers **(Table 3b)**. Subjects homozygous for the *-3900C* allele had greater FEV_1 (p=0.0002) and FVC (p<0.0001) decline and greater increase in log (A-a)PO_2r at rest (p=0.04) than non-carriers **(Tables 3a and b)**. The subjects homozygous for the *-3458T* had greater FEV_1 (p<0.0001) and FVC (p<0.0001) declines than non-homozygous subjects. Those homozygous for the *-2459G/-2135T* (the two loci in complete linkage disequilibrium) had lower FEV_1 (p=0.04) and FVC (p=0.02) than non-homozygous subjects. Subjects with at least one Δ*32* allele had lower FEV_1 (p=0.002) and FVC (p=0.008) than non-carriers.

We evaluated the impact of *CCR5* haplotypes on change in lung function over time and similar results were noted (Table 3a and b). A carrier of *haplotype 1 (-5663G/-3900A/-3458G/-2459A/-2135C/-2086A)* had lesser decline in FEV_1 ($p<0.0001$), FVC ($p=0.0004$,Figure 2b) and TLC ($p=0.01$) and PaO_2 ($p=0.01$, Figure 2c) compared to non-carriers. A carrier of *haplotype 2 (-5663A/-3900C/-3458T/-2459G/-2135T/-2086G)* demonstrated increasing log (A-a)PO_2r ($p=0.03$) and (A-a)PO_2m ($p=0.01$) and greater decline in FEV_1 ($p=0.005$) over time than non-carriers.

CCR5 polymorphisms and bronchoalveolar lavage lymphocytes

Comparisons were made between BAL cell differential counts at the first visit to clinic and last visit, for 88 CBD and 78 BeS subjects. There were significant differences between geometric mean (standard deviation) lymphocyte% in BAL from CBD [n=88, 24.3%(2.35)] compared with BeS [n=78, 9.3%(2.24)], $p<0.0001$ at the first visit to clinic, and for CBD [n=51, 28%(1.87)] compared with BeS [n=47, 7%(0.22)], $p<0.0001$ at last visit.

The relationship between BAL lymphocyte% and *CCR5* polymorphisms was evaluated, comparing the *CCR5* polymorphisms, haplotypes and the BAL lymphocytes% in CBD and BeS combined at the first visit to clinic **(Table 4)**. The BAL lymphocytes% was significantly increased in individuals carrying the *CCR5 -2459G/-2135T* [n=104, 17.99%(2.53)] compared to the non-carriers [n=62, 12.30%(2.64)]; ($p=0.01$). Other *CCR5* polymorphism associations were found with both increased and decreased numbers of lymphocytes for CBD and BeS subjects combined. When subjects with CBD and BeS were analyzed separately, far fewer associations were demonstrated although similar trends were apparent (Online **Table 1).**

There was a significant difference in mean BAL lymphocyte% between carriers and non-carriers of *haplotype1*, in the combined CBD and BeS group (p=0.009, **Table 4**), with non-carriers of this haplotype having higher lymphocyte percentages. This haplotype includes all alleles which were associated with lower BAL lymphocyte percentage *(-5663G/-3900A/-3458G/-2459A/-2135C*). Carriers of *haplotype 2*, which includes *-2459A/-2135C,* had on average higher lymphocyte% than non-carriers of this haplotype in the combined CBD and BeS group. There were similar although non-significant trends seen in the BeS and CBD groups when analyzed separately (Online **Table 1)**.

Discussion

In this study, we describe the association between *CCR5* polymorphisms in Caucasians with CBD and markers of disease severity. There were few significant differences in genotype, allele frequency and haplotype frequencies between either CBD or BeS and controls. Interestingly, we found a greater decline in lung function and worsening gas exchange in CBD cases carrying specific *CCR5* polymorphisms and haplotypes. We also showed associations between BAL lymphocyte% and *CCR5* polymorphisms and haplotypes. Strikingly, these data support that the same *CCR5* polymorphisms associated with greater decline in CBD lung function are also associated with greater lymphocyte% in the lung. This suggests that *CCR5* variants are functional, impacting lymphocyte recruitment into the CBD lung and in turn greater decline in lung function and worsening gas exchange.

This is the first study of *CCR5* genotypes and haplotypes in CBD. Although the *CCR5-3900A,-3458G,-2459A/-2135C* alleles were slightly decreased in CBD compared to BeS, no *CCR5* polymorphisms or haplotype was significantly different between CBD and controls or BeS and controls. These findings suggest that *CCR5* polymorphisms are not a risk factor for CBD. This is consistent with studies of sarcoidosis, a similar granulomatous lung disease of unknown etiology. Although one study of Czech sarcoidosis cases found an increased rate of the *delta 32* variant in cases compared to controls[8], two studies in UK/Dutch and German populations found no association with any *CCR5* genotype or haplotypes, including the *delta 32*, and disease risk [7;20]. Of note, the frequencies of case and control genotypes reported in our study are similar to those noted in these prior studies[7;20].

In our current study, we found strong associations between changes in static lung function and measures of gas exchange, and *CCR5* polymorphisms in CBD. Specifically, the *-5663*

AA, -3900 CC, -3458 TT, -2459GG/-2135TT homozygous genotypes and carriage of the *delta 32* allele were associated with greater decline in FEV_1, FVC, while the *-5663 A* and *-3900 C* alleles were associated with worsening measures of gas exchange at rest and during exercise. No associations were noted between any genotypes and DL_{CO}, which may be due to the variability in the measurement of this parameter. Interestingly, some of the genotypes associated with worse lung function over time were the same as those noted to be slightly increased in CBD compared to BeS (*-3900 CC*, *-3458 TT, -2459GG/-2135TT*); this overrepresentation of genotypes in CBD may be due to the association with a more severe disease phenotype in our CBD population than disease susceptibility. Most of the genotypes associated with greater decline in lung function were included in *haplotype 2(-5663A/-3900C/-3458T/-2459G/-2135T/-2086G)*. Carriers of this haplotype had greater decline in FEV_1 and increase in (A-a)PO_2r and (A-a)PO_2m. The polymorphisms composing *haplotype 2* in our current study are also represented in the *HHC* haplotype that we found overrepresented in higher sarcoidosis chest radiograph staging (stage II, III and IV) compared to lower staging (stage 0 and I)[7] . Similarly, the sarcoidosis *HHC* haplotype carriers also had significantly lower FEV_1 and FVC at presentation compared with non-*HHC* carriers[7]. Conversely, our CBD cases carrying the *-5663G/-3900A/-3458G/-2459A/-2135C/-2086A* (*haplotype 1*) had better lung function with higher FEV_1, FVC, TLC and $Pa0_2$r than non-carriers. These results suggest that *CCR5* variants may be important in granulomatous lung disease progression, as indicated by chest radiography in sarcoidosis, and lung function changes in both sarcoidosis and CBD. While a second CCR5 sarcoidosis study by Fischer et al. showed the *CCR5 -5663A* and *-3900C* allele were increased in female patients with Lofgren's syndrome[20], they did not assess lung function or radiographic stage. As a result, it is difficult to compare our studies with theirs.

In addition to associations between lung function and *CCR5* genotypes/haplotypes, we noted associations between BAL %lymphocytes and these same *CCR5* genotypes/haplotypes. Specifically, the BAL %lymphocytes was significantly increased in the carriers of *CCR5 -2459G /-2135T* and *haplotype 2* compared to the non-carriers of these alleles. Conversely, the carriers of *CCR5 -5663G, -3900A, -3458G* and *-2459A/-2135C* alleles and *haplotype 1*, containing all these alleles, showed lower BAL %lymphocytes compared to non-carriers. It is possible that these data reflect functional alterations in CCR5 expression important in cell trafficking and cell to cell interactions. In this regard, CCR5 is expressed at high levels on CD4+ Th1 cells and may play a role in enhanced recruitment and activation of lymphocytes and monocytes to the lung and these cells' subsequent immune response[21]. It is possible that enhanced recruitment and activation of BAL lymphocytes may explain the association of the same *CCR5* genotypes and haplotypes with more rapid disease progression.

Previously, Katcher et al have reported a positive correlation between BAL %lymphocytes and the number of CCR5 expressing CD4+ BAL T cells in sarcoidosis[10]. Cappeli et al reported that increased CCR5 expression was observed in both lymphocytes and macrophages of all sarcoidosis patients, along with a trend to decreased positivity from stage I to III of disease[22]. They also showed that CCR5 expression was significantly reduced in lymphocytes from IPF compared to controls[9]. However, there have been no studies to date evaluating the association between CCR5 expression on BAL cells and *CCR5* genotypes or haplotypes in sarcoidosis or other lung disease, including CBD.

The functional correlates of most of the *CCR5* polymorphisms are unknown, except for the Δ*32*deletion. Homozygosity for this 32 base pair deletion results in a truncation of the protein lacking the transmembrane domain and thus cannot be expressed on the cell

surface[23]. The *CCR5* promoter polymorphism *-2459G/A* have been investigated primarily in HIV infection[6;24-26]. Interestingly, the *CCR5 -2459 AA* genotype has been associated with over-expression of CCR5 and HIV progression[6;25]. This is discordant with our findings. To clarify the discrepancy of associations with this *CCR5 -2459 G/A* polymorphism between CBD and HIV infection, functional assay of CCR5 expression in CBD patients will be needed but these experiments are beyond the scope of our current study.

Although we considered using Bonferroni adjustment of the p-values for multiple comparisons made in the longitudinal analyses, we chose not to do so as it would have been too conservative. That is with the consistency of the correlations in the outcome variables, and the correlation between these variables, the Bonferroni adjustment would have overadjusted our p-values. If only one or two significant associations had been found, these associations could have been discounted as possibly being due to chance alone because of the multiple comparisons undertaken in this analysis. However, there were many associations found between disease severity indicators and the *CCR5* polymorphisms that all showed consistent direction, which supports our not using Bonferroni corrections.

In summary, this is the first study to report associations between CCR5 polymorphisms and CBD. Although we found no overall association between *CCR5* polymorphisms and either CBD or BeS, we found associations between worsening change of lung function and *CCR5* polymorphisms and *CCR5 haplotype 2* in CBD. Furthermore, we have shown the same variants associated with increased BAL lymphocyte%, supporting the concept of a functional consequence of this genotype in CBD. Further studies will be needed to show how these alterations in BAL effected worsening functional changes.

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Figure legends

Figure 1. *CCR5* linkage disequilibrium and haplotype block structure in cases and controls derived using the confidence interval methods. Two measures of pairwise linkage disequilibrium used in the Haploview program are D' and LOD scores. Pairwise linkage disequilibrium plots demonstrate the D' for the eight polymorphisms in Caucasian cases and controls. – Numbers in squares indicate D' for adjacent pairs, while blank squares indicate a D' of 1. Red squares indicate the greatest linkage disequilibrium, with an upper confidence bound (UCI) for D' equal to 1.0 and a LOD score >2. The light blue squares indicate less information about linkage disequilibrium with an UCI for D = 1.0, but a LOD score <2, while the white squares have an UCI of <1.0 for D' and a LOD score <2. A haplotype block is identified between the *-5663, -3900, -3458, -2459, -2135* and *-2086* polymorphisms using the confidence interval method.

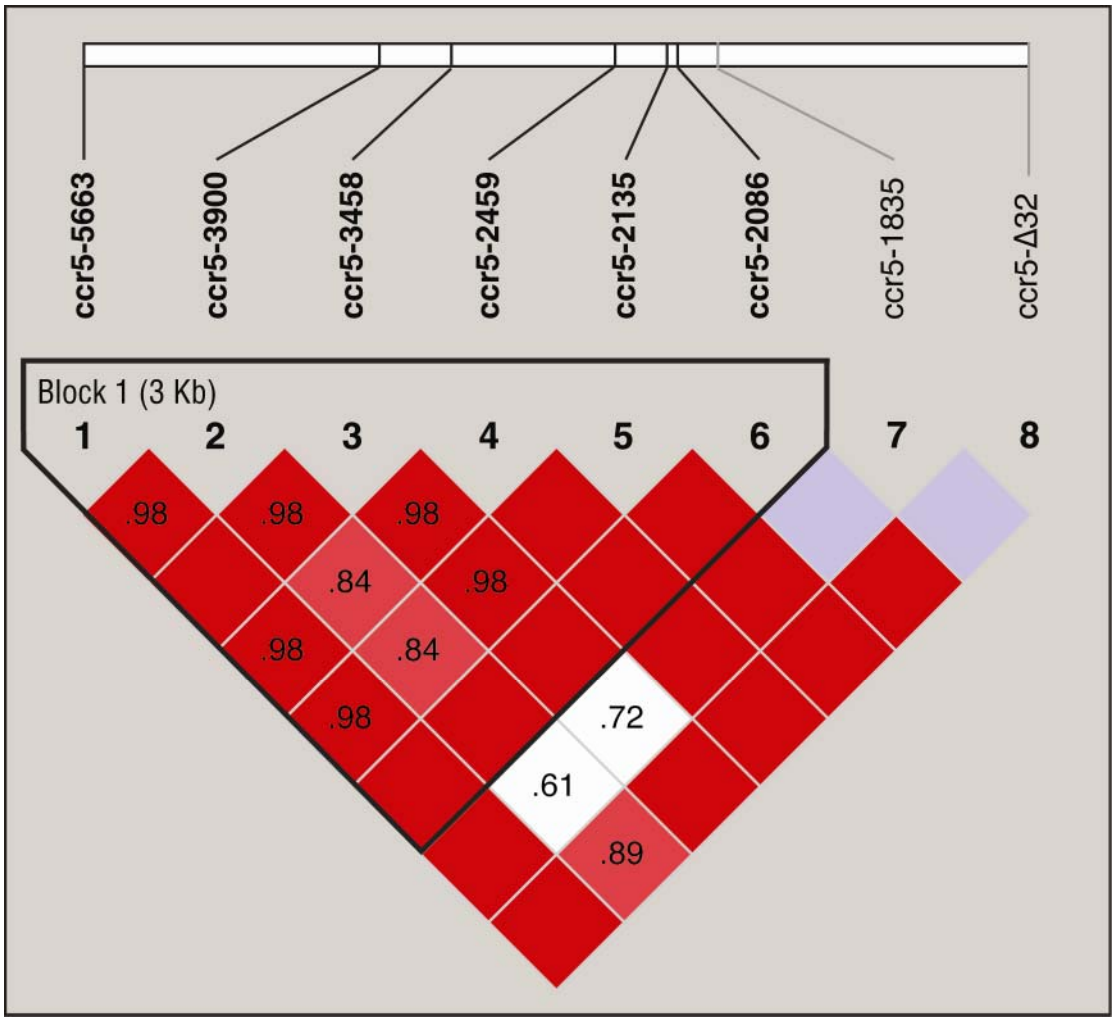
ccr5-5663
ccr5-3900
ccr5-3458
ccr5-2459
ccr5-2135
ccr5-2086
ccr5-1835
ccr5-Δ32
Block 1 (3 Kb)
1
2
3
4
5
6
7
8
.98
.98
.98
.84
.98
.98
.84
.98
.72
.61
.89

Figure 2a Average change in FEV_1 (L/year) in those homozygous for the -*5663 AA* (——) vs those with -*5663AG/GG* (------), over time modeled between 10 and 40 years since first beryllium exposure adjusted for gender, height, smoking history (Current, Former, Never), age at test. This figure demonstrates that those individuals with the -*5663AA* genotype have a greater decline in FEV_1 compared to the -*5663AG and GG* (p=0.01). Estimates are based on mixed effects models. Vertical bars extend +/- 2 standard errors from the average.

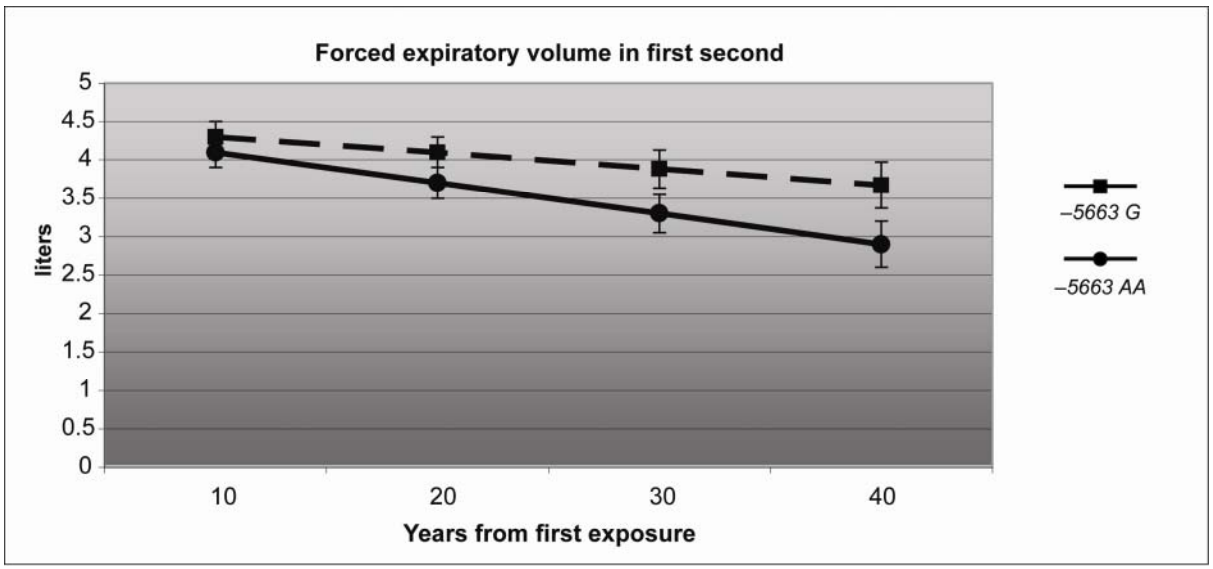

Figure 2b Average change in FVC(L/year) in those with at least one *Haplotype 1* -*5663/-3900/-3458/-2459/-2135/-2086 GAGACA* haplotype (----) vs no -*5663/-3900/-3458/-2459/-2135/-2086 GAGACA* haplotype (——) , over time modeled between 10 and 40 years since first beryllium exposure adjusted for gender, height, smoking history (Current, Former, Never), age at test. This figure demonstrates a lesser decline in FVC in the *Haplotype 1* heterozygotes and homozygotes compared to those with no *Haplotype 1* (p=0.01). Estimates are based on mixed effects models. Vertical bars extend +/- 2 standard errors from the average.

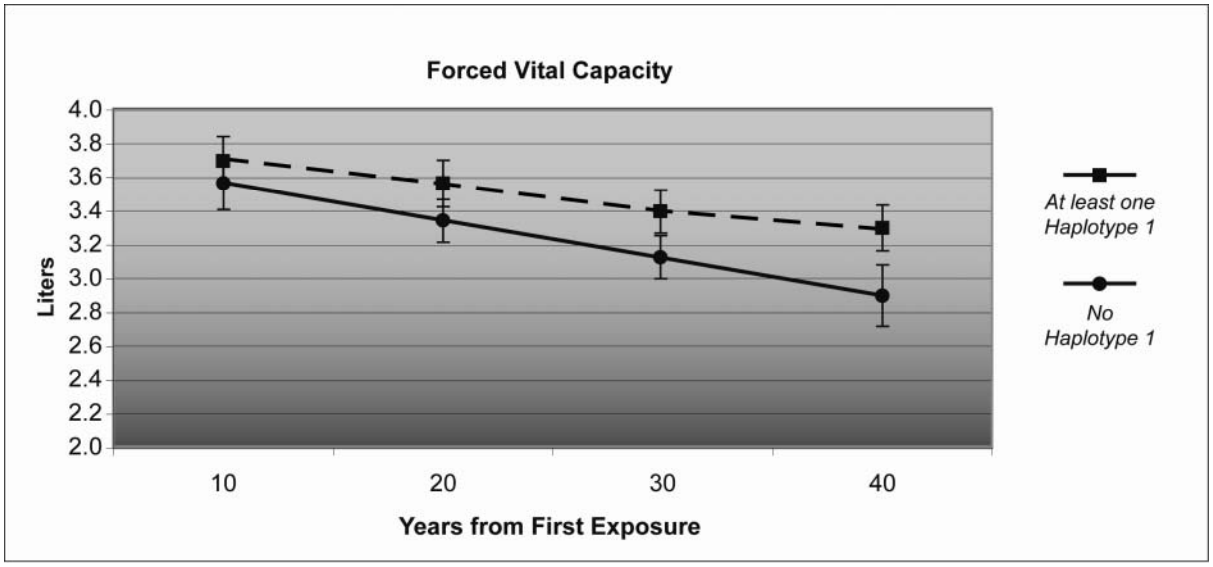

Figure 2c Average change in PaO_2 at rest (mm Hg/year) in those with at least one *Haplotype 1*

-5663/-3900/-3458/-2459/-2135/-2086 GAGACA haplotype (----) vs no *-5663/-3900/-3458/-2459/-2135/-2086 GAGACA* haplotype (——) , over time modeled between 10 and 40 years since first beryllium exposure adjusted for gender, , smoking history (Current, Former, Never), age at test. This figure demonstrates a lesser decline in PaO_2 in the *Haplotype 1* heterozygotes and homozygotes compared to those with no *Haplotype 1* (p=0.01). Estimates are based on mixed effects models. Vertical bars extend +/- 2 standard errors from the average.

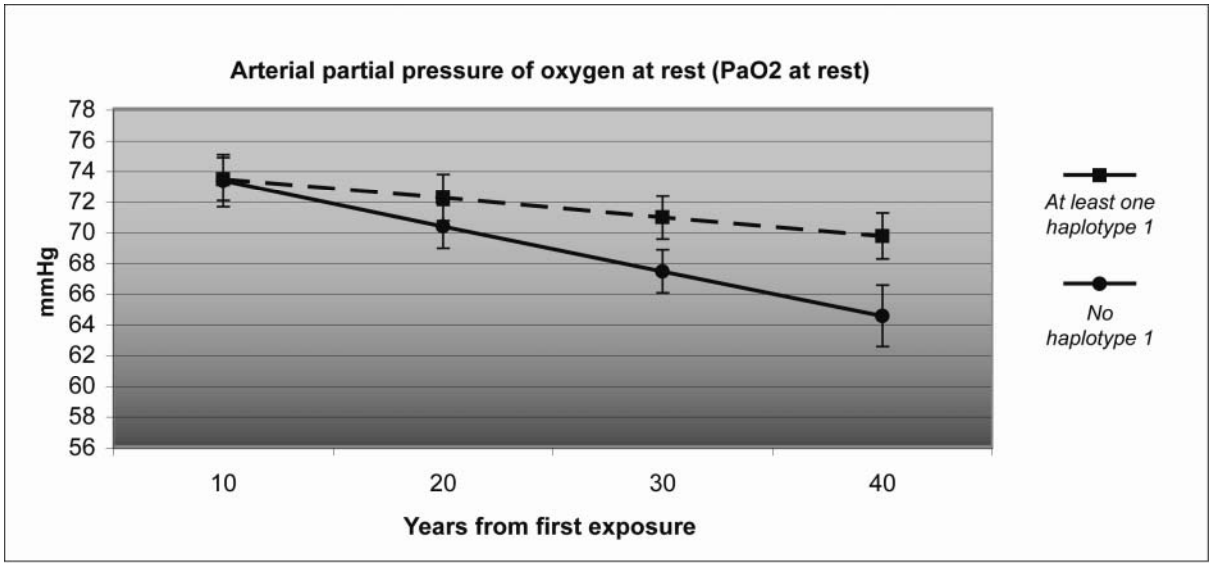
Arterial partial pressure of oxygen at rest (PaO2 at rest)
78
76
74
72
70
68
66
64
62
60
58
56
mmHg
10
20
30
40
Years from first exposure
At least one haplotype 1
No haplotype 1

Table 1. Genotype and allele carriage frequencies of *CCR5* polymorphisms

Position		Controls (N=173)	CBD (N=88)	BeS (N=86)	p-value (CBD vs BeS)
Genotype					
-5663	*AA*	33.5	36.4	22.1	NS
	AG	45.1	37.5	52.3	
	GG	21.4	26.1	25.6	
-3900	*AA*	36.4	44.3	44.2	***p=0.03**
	AC	48.0	33.0	46.5	
	CC	15.6	22.7	9.3	
-3458	*GG*	39.5	45.5	45.3	***p=0.02**
	GT	48.9	34.1	47.7	
	TT	11.6	20.5	7.0	
-2459/-2135	*AA / CC*	29.5	36.4	36	***p=0.03**
	AG / CT	52.0	36.4	51.2	
	GG / TT	18.5	27.2	12.8	
-2086	*AA*	39.9	45.5	45.3	NS
	AG	52.6	44.3	50	
	GG	7.5	10.2	4.7	
-1835	*CC*	83.2	84.1	81.4	NS
	CT	15.6	14.8	18.6	
	TT	1.2	1.1	0.0	
Δ32	*GG*	79.8	84.1	75.6	NS
	G/deletion	19.1	15.9	22.1	
	deletion/deletion	1.2	0.0	2.3	
Allele carriage					
-5663	*A*	78.6	73.9	74.4	NS
	G	66.5	63.6	77.9	NS
-3900	*A*	84.4	77.3	90.7	**p=0.03**
	C	63.6	55.7	55.8	NS
-3458	*G*	87.9	79.5	93.0	**p=0.02**
	T	60.5	54.6	54.7	NS
-2459/-2135	*A / C*	81.5	72.7	87.2	**p=0.03**
	G / T	70.5	63.6	64.0	NS
-2086	*A*	92.5	89.8	95.3	NS
	G	60.1	54.3	54.7	NS
-1835	*C*	98.8	98.9	100.0	NS
	T	16.8	15.9	18.6	NS
Δ32	*G*	98.8	100.0	97.7	NS
	deletion	20.2	15.9	24.4	NS

The values are given as percentages (%).

*p-values for genotypes were obtained by 2x3 tables, comparing the genotype numbers in CBD and BeS.

Table 2. Common *CCR5* haplotype frequencies in CBD, BeS and Controls

Haplotype	***-5663***	***-3900***	***-3458***	***-2459***	***-2135***	***-2086***	Controls (N=346)	CBD (N=164)	BeS (N=172)
1	G	A	G	A	C	A	43.2	44.3	50.6
2	A	C	T	G	T	G	34	32.4	29
3	A	A	G	A	C	A	8.5	8.6	9.4
4	A	A	G	G	T	A	7.8	7.9	7.4
5	A	C	G	A	C	A	3.1	1.1	1.1
6	A	C	T	G	T	A	1.7	4.5	1.2

Only the common haplotypes (>1%) are shown in this table.

The values are shown as percentages

Table **3a Estimates of change in lung function indices measured by average change in, FEV_1, FVC, TLC and $DLCO_U$ by *CCR5* polymorphisms and haplotypes in CBD between 10 to 40 years from first beryllium exposure**

Carriers	FEV_1 (L/y)	p-value	FVC (L/y)	p-value	TLC (L/y)	p-value	$DLCO_U$ (mL/min/mmHg/y)	p-value
-5663AA	-.02(.007)	**0.01**	-.02(.008)*	**0.01**	-.02(.01)	0.08	.10(.09)	0.3
-5663A	-.03(.009)	**0.001**	-.02(.01)**	0.12	-.006(.01)	0.61	.08(.11)	0..48
-3900CC	-.03(.008)	**0.0002**	-.04(.01)	**<.0001**	-.02(.01)	0.22	-.05(.11)	0.64
-3900C	-.02(.007)	**0.007**	-.02(.01)	0.06	-.004(.01)	0.7	-.01(.09)	0.93
-3458TT	-.03(.008)	**<.0001**	-.05(.01)	**<.0001**	-.02(.01)	0.2	.05(.11)	0.63
-3458T	-.01(.01)	0.12	-.009(.01)	0.28	-.009(.01)	0.4	.0001(.09)	0.99
-2459GG/-2135TT	-.02(.008)	**0.04**	-.03(.009)	**0.02**	-.01(.01)	0.36	-.09(.10)	0.36
-2459G/-2135T	-.01(.01)	0.06	-.006(.01)	0.56	-.02(.01)	0.07	.03(.10)	0.74
-2086AA	.01(.01)	0.12	.009(.01)	0.28	.008(.01)	0.4	.0001(.09)	0.99
-1835CC	.008(.02)	0.53	.007(.02)	0.68	.01(.02)	0.44	-.17(.16)	0.29
Δ32	-.03(.008)	**0.002**	-.03(.12)	**0.008**	-.02(.01)	0.12	-.06(.12)	0.58
Haplotype1	.03(.007)	**<.0001**	.03(.009)	**0.0004**	.01(.01)	**0.01**	-.06(.09)	0.51
Haplotype 2	-.02(.008)	**0.005**	-.009(.01)	0.35	-.004(.01)	0.71	.03(.10)	0.76

* Increased rate of decline of 20 ml/year if AA homozygous compared to non-AA homozygous.
** Increased rate of decline of 20 ml/year if both A-containing genotypes compared with GG.
The same conventions are applied to all other cells

Haplotype 1(-5663G/-3900A/-3458G/-2459A/-2135C/-2086A), Haplotype 2 (-5663A/-3900C/-3458T/-2459G/-2135T/-2086G)

Table 3b **Estimates of change in lung function indices measured by average change in Pao_2, A-a gradient, and Workload by *CCR5* polymorphisms and haplotypes in CBD between 10 to 40 years from first beryllium exposure**

Carriers	Pao_2max*	p-value	Pao_2rest	p-value	A-a gradient**** max*	p-value	A-a gradient**** rest	p-value	WLM	p-value
-5663AA	-.12(.24)	0.62	-.21(.21)**	0.33	1.01(1.02)	0.49	1.01(1.02)	0.49	.36(.71)	0.61
-5663A	-.13(.20)	0.5	-.40(.16)***	**0.01**	1.04(1.01)	**0.01**	1.06(1.02)	**0.003**	-.73(.56)	0.19
-3900CC	.07(.23)	0.77	-.26(.17)	0.12	1.01(1.02)	0.62	0.99(1.01)	0.73	-1.7(.64)	**0.01**
-3900C	.15(.20)	0.45	-.08(.17)	0.65	1.02(1.01)	0.07	1.04(1.02)	**0.04**	-.13(.57)	0.82
-3458TT	.11(.23)	0.62	-.23(.17)	0.18	0.99(1.02)	0.45	1(1.02)	0.98	-1.8(.65)	**0.01**
-3458T	-.07(.19)	0.71	-.06(.17)	0.71	1.22(1.17)	0.21	1.17(1.12)	0.14	-.31(.56)	0.58
-2459GG/-2135TT	-.005(.21)	0.98	.25(.16)	0.11	1(1.02)	0.98	1.01(1.02)	0.79	-1.2(.58)	**0.05**
-2459G/-2135T	-.28(.21)	0.17	-.19(.18)	0.3	1.02(1.01)	**0.05**	1.03(1.02)	0.06	.14(.60)	0.81
-2086AA	.07(.19)	0.71	.06(.17)	0.7	1.02(1.02)	0.15	1.03(1.02)	0.09	.31(.56)	0.58
-1835CC	.25(.31)	0.42	.22(.27)	0.4	0.99(1.02)	0.63	0.97(1.03)	0.34	-.43(.84)	0.61
Δ32	0.41	0.07	-.08(.20)	0.69	1(1.01)	0.96	1(1.02)	0.95	-1.7(.67)	**0.01**
Haplotype1	-.18(.18)	0.32	.42(.16)	**0.01**	1(1.01)	0.7	0.99(1.02)	0.7	.73(.53)	0.17
Haplotype 2	-.12(.19)	0.55	-.08(.18)	0.67	1.03(1.01)	**0.01**	1.04(1.02)	**0.03**	.18(.57)	0.75

*at maximum exercise;

** Increased rate of decline of 0.21 mm Hg/year if *AA* homozygous compared to non-*AA* homozygous.
*** Increased rate of decline of 0.40 mm Hg/year if both A-containing genotypes compared with *GG*.
The same conventions are applied to all other cells

**** Geometric mean geometric standard deviation
Haplotype 1(-5663G/-3900A/-3458G/-2459A/-2135C/-2086A), Haplotype 2 (-5663A/-3900C/-3458T/-2459G/-2135T/-2086G)

Table 4. The association between the carriage of *CCR5* polymorphisms and the percentage of BAL lymphocytes in CBD and BeS.

		Carrier			Non-carrier				
Position		**N**	**Geometric Mean**	**Geometric Standard Deviation**	**N**	**Geometric Mean**	**Geometric Standard Deviation**	**Mean Difference (95% CI)**	p-value*
-5663	*A*	121	16.78	2.56	45	12.94	2.69	1.29(0.56,1.08)	0.13
	G	120	14.15	2.64	46	20.29	2.39	0.7(0.51,0.97)	**0.03**
-3900	*A*	140	14.44	2.61	26	22.87	2.32	0.63(0.43,0.94)	**0.03**
	C	90	17.64	2.51	76	13.60	2.69	0.77(0.58,1.03)	0.08
-3458	*G*	143	14.73	2.61	23	22.65	2.44	0.65(0.43,0.99)	**0.04**
	T	88	17.81	2.51	78	13.46	2.66	1.32(0.99,1.77)	0.06
-2459/-2135	*A / C*	135	14.59	2.64	31	21.12	2.27	0.69(0.47,1)	**0.05**
	G / T	104	17.99	2.53	62	12.30	2.64	0.69(0.51,0.93)	**0.01**
-2086	*A*	154	15.33	2.64	12	20.29	2.10	0.75(0.43,1.32)	0.32
	G	88	17.81	2.51	78	13.46	2.66	0.76(0.56,1.01)	0.06
-1835	*C*	165	N/A	N/A	1	N/A	N/A	N/A	N/A
	T	29	14.15	2.64	137	15.96	2.61	1.13(0.77,1.66)	0.5
delta 32	*G*	164	N/A	N/A	2	N/A	N/A	N/A	N/A
	deletion	35	13.07	2.72	131	16.44	2.56	1.25(0.88,1.80)	0.21
Haplotype 1		114	13.46	2.66	52	20.09	1.77	0.69(0.53,.91)	**0.009**
Haplotype 2		76	18.17	2.51	90	13.46	2.51	1.32(1.03,1.70)	**0.03**

• using Student's t-tests

Haplotype 1(-5663G/-3900A/-3458G/-2459A/-2135C/-2086A), Haplotype 2 (-5663A/-3900C/-3458T/-2459G/-2135T/-208