TNF-α Polymorphisms in Chronic Beryllium Disease and Beryllium Sensitization

Erin C. McCanlies, PhD
Christine R. Schuler, PhD
Kathleen Kreiss, MD
Bonnie L. Frye, MS
James S. Ensey, BS
Ainsley Weston, PhD

Objective: *Tumor necrosis factor-alpha (TNF-α) is a potent cytokine involved in normal immune functions. The aim of this study was to investigate if there is an association between chronic beryllium disease or beryllium sensitization and two variants of the TNF-α gene located at -308 and -238 called TNF-α-308*02 and TNF-α-238*02.* ***Methods:*** *TNF-α-308 and TNF-α-238 genotyping was conducted in a large, population-based cohort consisting of 886 beryllium workers (92 individuals with chronic beryllium disease, 64 who were beryllium sensitized, and 730 individuals without sensitization or disease).* ***Results:*** *The odds of chronic beryllium disease in the presence of at least one TNF-α-308*02 or TNF-α-238*02 allele was not significant (OR = 1.0; 95% CI = 0.7, 1.7 and OR = 0.8; 95% CI = 0.4, 1.6). This was true regardless of whether a worker was homozygous or heterozygous for TNF-α-308*02 or TNF-α-238*02. Similarly, neither allele was associated with sensitization (P > 0.05).* ***Conclusions:*** *Unlike an earlier report, there was no association between these specific TNF-α alleles and either chronic beryllium disease or sensitization to beryllium.*
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Individuals who are exposed to beryllium dust or fumes may become sensitized to beryllium (BeS) and are at increased risk of developing chronic beryllium disease (CBD).[1–6] Epidemiologic and laboratory-based research has implicated an immunogenetic component in both BeS and CBD.[7–15] A number of genes have been evaluated in relationship to BeS and CBD. These include variants in *HLA-DP*, *HLA-DR*, *HLA-DQ*, and *TNF*-α. The association among BeS, CBD, and *HLA-DPB1*E69* has been well established.[7–15] Approximately 84% of beryllium workers with CBD have at least one copy of *HLA-DPB1*E69* compared with 36% of workers without BeS or CBD.[13,15] Although *HLA-DPB1*E69*, given beryllium exposure, accounts for a significant portion of workers with CBD and BeS, research evaluating the role of other genes in CBD and BeS continues.[9,13,15,16]

Human *TNF*-α is a 233-amino acid, non-glycosylated transmembrane polypeptide.[17] It is expressed by a variety of cell types, including macrophages, CD4+ and CD8+ T lymphocytes, and a range of epithelial cells.[18–20] *TNF*-α has several effects. First documented as a cytotoxin, it is now known to be a powerful cytokine responsible for induction of inflammation, tissue repair, hematopoiesis, immune response, and transcription.[21] *TNF*-α is located on 6p21 in the middle of the HLA gene cluster. There are at least nine single nucleotide polymorphisms (SNPs) in the *TNF*-α promotor region. All are adenine-guanine transversions, and they are thought to be functionally

From the Health Effects Laboratory Division (Dr McCanlies, Ms Frye, Mr Ensey); and the Division of Respiratory Disease Studies (Dr Schuler, Dr Kreiss, Dr Weston), National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, West Virginia.

Address correspondence and reprint requests to: Erin McCanlies, PhD, NIOSH-CDC, MS-L4050, 1095 Willowdale Road, Morgantown, WV 26505; E-mail: EIM4@CDC.GOV.

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significant.[22] The frequencies of the guanine variants range between 0.01 and 0.22. However, most of the research evaluating the role of *TNF*-α in disease has focused on polymorphisms at -308 and -238, also adenine-guanine transversions.[23–25] Significant associations have been reported with susceptibility to autoimmune diseases such as insulin-dependent diabetes mellitus and rheumatoid arthritis, as well as lung diseases such as asthma and sarcoidosis.[23,25–32] Thus, it was considered a candidate for genes that might be associated with CBD.

Beryllium stimulation of bronchoalveolar lavage (BAL) cells in patients with CBD was found to induce higher levels of *TNF*-α in samples that were positive for the *TNF*-α-*308*02* variant.[14] One molecular epidemiologic study comparing the frequency of *TNF*-α-*308*02* in 22 workers with CBD indicated that 23 workers with BeS and 93 workers exposed to beryllium without sensitization or CBD also reported that *TNF*-α-*308*02* occurred more often in both the BeS workers with and without CBD compared with those workers without sensitization or disease (OR = 7.8, 95% CI = 3.2–19.1).[9] It also reported a positive interaction between *TNF*-α-*308*02* and *HLA-DPB1*E69* in the individuals with CBD.

In a large, population-based study, we sought to further explore the possible association between CBD, BeS, and *TNF*-α polymorphisms at the promoter regions -238 and -308. We tested the hypothesis that the minor variants of *TNF*-α-*308* and *TNF*-α-*238* polymorphisms are associated with susceptibility to beryllium sensitization and CBD in 886 beryllium workers: 92 with a diagnosis of CBD, 64 who had BeS without disease, and 730 individuals without sensitization or CBD. We also evaluated whether there was an interaction between *TNF*-α-*308* or *TNF*-α-*238* and *HLA-DPB1*E69*.

Materials and Methods

Human Subjects

As previously described, three groups of workers from a large beryllium manufacturing company were eligible to participate in this study.[13] Briefly, two of these groups consisted of current and former workers who had participated in epidemiologic surveys conducted from 1992 to 1994 and 1998 to 2000 at the Ohio and Arizona plants, or a survey conducted in 2000 at a plant in Pennsylvania.[33–36] The third group consisted of former workers from the Ohio, Arizona, and Pennsylvania facilities who were known to have BeS or CBD and were not part of the described surveys of then-current workers. This last group will be referred to as KSD (known sensitized or diseased).

For this study, all participants completed a questionnaire on medical and work history and gave a blood sample for genetic analyses. Study participants not known to have beryllium sensitization or disease were also screened with the blood beryllium lymphocyte proliferation test (BeLPT). Those known to have BeS or CBD were not screened with the BeLPT. Workers identified as BeS were offered clinical evaluation to diagnose CBD. The National Institute for Occupational Safety and Health (NIOSH) Human Studies Review Board approved this research. Written informed consent was obtained from each participant.

Definitions of Health Outcomes

Beryllium Sensitization. The BeLPT measures the degree to which lymphocytes proliferate in vitro in response to stimulation from beryllium salts. The test was used to identify participants who were sensitized to beryllium. Blood samples were collected in heparinized tubes, and split samples were sent to two laboratories. A BeLPT was determined to be abnormal if at least two of six stimulation indices (based on beryllium concentration and exposure duration) were greater than three.[37] If only one of the individual stimulation indices were greater than three, or if other results were ambiguous (eg, technical problems), additional blood was drawn and the test was repeated. An individual was considered to be sensitized if any two BeLPTs were found to be abnormal.

Chronic Beryllium Disease. Participants who were found to be sensitized were referred for clinical follow-up to determine if they had CBD, which included BAL, transbronchial lung biopsy, and chest radiographs. Lavage cells were tested for evidence of lung sensitization with the BAL-BeLPT, and biopsy samples were evaluated microscopically. A person was diagnosed with CBD when granulomas or other abnormalities consistent with CBD (eg, lymphocytic alveolitis) were identified in biopsy samples, or radiological abnormalities were identified.

Blood Processing and DNA Extraction for Genetic Analysis

Blood (7 mL per sample) was drawn either into lavender-top Vacutainer tubes (Beckton Dickinson [BD], Franklin Lakes, NJ) containing an anticoagulant (K_3EDTA, 371 mM, 81 μL), or into cell preparation tubes (CPT) (BD) containing sodium citrate (100 mM, 450 μL) and a Ficoll-Hypaque gel (Pharmacia, Uppsala, Sweden). The citrate-containing tubes were centrifuged (1500 g, 20 minutes) in a swinging-bucket rotor. All blood-tubes were inverted to mix the ethylenediaminetetraacetic acid (EDTA) (for the lavender-top tubes) or the upper layer containing leukocytes and plasma (for the CPT tubes), and were transported by overnight express mail to the Molecular Carcinogenesis Laboratory at NIOSH in Morgantown, West Virginia. Leukocytes were isolated by centrifugation (800 g, 20 minutes to the pellet cells from the CPT tubes and 400 g mixed with Ficoll for 15 minutes for the lavender

tops), washed once by resuspension in isotonic phosphate buffered solution (PBS), and reprecipitated (800g, 20 minutes). Cell pellets were split into three approximately equal fractions. Two were stored at −70°C for future beryllium-related research, and the third was resuspended in cell lysis buffer (0.6% SDS/10 mM EDTA/10 mM Tris HCl, pH 7.5) containing RNase A (50 μg/mL). After incubation for 1 hour at 37°C, DNA was extracted after digestion of proteinase K (200 μg/mL, 37°C, 16–24 hours) as previously described.[13,38] The overall quality of DNA was assessed by determining $OD^{260/280}$ (for purity, with an expected ratio between 1.6 and 1.9), agarose gel-electrophoresis characteristics (for high molecular weight, with a narrow band expected >30 kb), and facile amplification of a >300-bp fragment by polymerase chain reaction (PCR). Samples that did not meet these criteria were repurified; DNA yields of 100 to 200 μg per 2.5 mL whole blood were typical. High-molecular-weight DNA was redissolved in Tris-EDTA (10:1 mM) and stored at -70°C; working solutions for PCR amplification (10 μg/mL, containing Tris Cl 10 mM and EDTA 1 mM) were prepared and stored at 0 to 4°C.

Polymorphism Analysis in the *TNF*-α Promotor Region

The two SNPs under study are both A/G substitutions at -308 (np3787) and -238 (np3857), respectively. Analysis was performed by real-time PCR using Applied Biosystems, Inc. (ABI) Custom SNP Genotyping Assays (ABD) (ABI, Foster City, CA). These are now available under ABD reference numbers TNFA2511-306R and TNFA2581-376R. For *TNF*-α-*308*, analysis was performed with primers and probes of the following sequences: F-CCAAAAGAAATGGAGGCAATAGGTT (TNFA2511-306RF) and R-GGACCCTGGAGGCTGAAC (TNFA2511-306RR). Probes were VIC-CCCGTCCCCATGCC (TNFA2511-306RV2) for *TNF*-α-*308*01* and FAM-CCCGTCCTCATGCC (TNFA2511-306RM2) for *TNF*-α-*308*02*.

For *TNF*-α-*238*, analysis was performed with primers and probes of the following sequences: F-CAGTCAGTGGCCCAGAAGAC (TNFA2581-376RF) and R-AGCATCAAGGATACCCCTCACA (TNFA2581-376RR). Probes were VIC-TCCCTGCTCCGATTC (TNFA2581-376RV2) for *TNF*-α-*238*01* and FAM-TCCCTGCTCTGATTC (TNFA2581-376RM2) for *TNF*-α-*238*02*.

Genomic DNA (50 ng) was added to the reaction mixture (25 μL total), which had been prepared according to the ABD instructions supplied by ABI, together with the ABD Custom assays. Reactions, performed in 96-well plates, were analyzed using an I-Cycler IQ (BioRad, Hercules, CA).

DNA Sequence Analysis

Two DNA sequence analysis methods were used in this study. The first was cycle sequencing for 281 nucleotides of the *TNF*-α promoter region including nucleotide positions -308 and -238. DNA samples (100 ng of *TNF*-α PCR product) were subjected to cycle sequencing in a total reaction volume of 20 μL containing the ABI terminator ready reaction mixture (8 μL) and primers (1 μL:25 pmol/reaction, F-TTCCTGCATCCTGTCTGGAAG and R-AAGCGGTAGTGGGCCCTG). Reactions were heated to 95°C for 2 minutes followed by 25 cycles of annealing (60°C for 4 minutes), extension (50°C for 5 seconds), and melting (96°C for 10 seconds). The reaction mixture was analyzed using an acrylamide gel (5%) in an ABI377 automated sequencer.

The second DNA-sequencing reaction was for exon 2 of HLA-DPB1. In this case, human constitutive genomic DNA was subjected to PCR. The reaction mixture (50 μL) contained primers (FDPI1-5′GAGAGTGGCGCCTCC3′ and RDPI2-5′CCCAAAGCCCTCACTC3′, 2 pM), genomic DNA (100 ng), MgCl2 (1.25 mM), dNTPs (0.8 mM), and Taq polymerase (1U, Perkin-Elmer Applied Biosystems, Foster City, CA). Amplification was allowed to proceed with an initial melt (95°C, 5 minutes), then 35 cycles of melting (95°C, 30 seconds), annealing (60°C, 35 seconds) and extension (72°C, 60 seconds), and a final extension at 72°C (10 minutes). The resulting PCR product (322bp) was checked for size and integrity by gel electrophoresis (2% agarose). A portion was then used as a template for DNA sequence determination using allele-specific primers. For sequencing in the reverse direction, allele-specific primers similar to, but not the same as, those developed by Wang et al in 1999 were used (5′GGTCACGGCCTCGTC3′, 5′GTCATGGGCCCGCC3′, 5′GGTCATGGGCCCGAC3′). For sequencing in the forward direction, four new primers were developed that recognize sequences between positions 7646 and 7665: 5′AATTACGTGTACCAGTTACG3′, 5′ATTACCTTTTCCAGGGACG3′, 5′AATTACGTGTACCAGGGAC3′, and 5′ATTACGTGCACCAGTTACG3′.

The sequencing reaction (20 μL) contained the DNA template (11 μL), primer (1 μL), and the ABI terminator ready reaction mixture (8 μL). Incubation for cycle sequencing proceeded for 25 cycles in a thermocycler (95°C for 2 minutes; 95°C for 10 seconds; 50°C for 5 seconds; 60°C for 4 minutes). The reaction mixture was analyzed using an ABI377 automated sequencer.

Data Analysis and Statistics

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the association between *TNF*-α and CBD or beryllium sensitization. χ^2 statistics were used to determine if the genotype frequencies reported in our population followed Hardy-Weinberg equilibrium (HWE) population laws.[39] To account for potential differences introduced by the different populations across the plant sites, overall odds ratios were calculated using Coch-

TABLE 1
Frequency of *TNF-α-308* and *TNF-α-238* Alleles in the Arizona, Ohio, and Pennsylvania Beryllium Workers

	*TNF-α-308*02* Alleles				*TNF-α-238*02* Alleles			
Plant	**N**	**0**	**1**	**2**	**N**	**0**	**1**	**2**
AZ								
Survey‡	138	97 (70.3)	38 (27.5)	3 (2.2)	138	122 (88.4)	16 (11.6)	0 (0.0)
KSD§	6	4 (66.7)	2 (33.3)	0 (0.0)	6	6 (100.0)	0 (0.0)	0 (0.0)
OH								
Survey‡	616	441 (71.6)	157 (25.5)	18 (2.9)	616	537 (88.0)	68 (11.2)	5 (0.8)
KSD§	13	9 (69.2)	3 (23.1)	1 (7.7)	13	13 (100.0)	0 (0.0)	0 (0.0)
PA	103	70 (68.0)	30 (29.1)	3 (2.9)	102	87 (85.3)	15 (14.7)	0 (0.0)

Values are numbers of participants with percentages in parentheses.

0 indicates the absence of the *TNF-α-308*02* or *TNF-α-238*02* allele; 1, inheritance of at least one *TNF-α-308*02* or *TNF-α-238*02* allele; 2, inheritance of two *TNF-α-308*02* or *TNF-α-238*02* alleles; Survey, participants recruited during cross-sectional and longitudinal population-based surveys; KSD, participants recruited because they were known to be sensitized or have CBD, but who were not members of a defined plant population (known sensitized or diseased); *TNF-α*, tumor necrosis factor-alpha; AZ, Arizona; OH, Ohio; PA, Pennsylvania.

ran-Mantel-Haenszel statistics, adjusting for plant site. If any of the four cells had expected values less than 5, exact test statistics were used. Logistic regression models were also fit to the data to test for an interaction effect between *HLA-DPB1*E69* and *TNF-α*. *HLA-DPB1*E69* and *TNF-α* were used as the predictor variables and sensitization and CBD as the outcome variables. Alternatively, we evaluated whether there was an interaction by calculating ORs and 95% CIs between *TNF-α* and CBD or beryllium sensitization stratified by the presence or absence of *HLA-DPB1*E69*. Exact *P* values were also calculated.

Results

Of the 886 genetic study participants, *TNF-α-308* and *TNF-α-238* results were obtained on 876 and 869 individuals, respectively. Two ABDs that interrogated *TNF-α-308* and *TNF-α-238* were validated by DNA sequence determination of 120 samples (>13% of samples). DNA-sequence analysis and ABD analysis were 100% concordant. DNA sequence determination indicated that the *TNF-α-308* and the *TNF-α-308* ABDs used here were not subject to genotype misclassification error.

Table 1 shows the frequency of *TNF-α-308*02* and *TNF-α-238*02* across all three plants. Overall, the majority of participants across all groups and plants did not carry either the *TNF-α-308*02* or the *TNF-α-238*02* minor alleles. Between 67% and 72% of the workers were *TNF-α-308*01*; a greater number were *TNF-α-238*01* (85% to 100%).

Because the KSD group was recruited based on known sensitization or disease, and not because they were members of a defined survey population, there was some concern that the frequency of *TNF-α-308*02* and *TNF-α-238*02* between the survey (population-based) group and the KSD group would be different. Using the Fisher exact test, we compared the frequency of the alleles for each group for the Arizona and Ohio plants and found that they were not significantly different. The KSD workers in Ohio had the highest frequency of homozygous *TNF-α-308*02* (7.7%), although this was not significantly different than the Ohio survey participants (2.9%). For those in Arizona, only 2.2% of the survey participants were homozygous *TNF-α-308*02* compared with 0% of the KSD group. This difference was also not significant. When the frequency of the *TNF-α-238*02* allele was considered in a similar set of comparisons, not significant results were also observed. Thus, the KSD groups were analyzed with the population-based groups.

Tables 2 and 3 report the odds of CBD and BeS in the presence of *TNF-α-308*02* and *TNF-α-238*02*, adjusting for plant site. There was no association between the *TNF-α* al-

TABLE 2
Distribution of the *TNF-α-308* Alleles in Participants With CBD, BeS, and who are Non-Sensitized (Adjusting for Plant)

		TNF-α-308 Alleles				
	N	**0**	**1**	**2**	**OR (95% CI)***	**HWE *P* Value**
CBD	91	65 (71.4)	24 (26.4)	2 (2.2)	1.0 (0.7, 1.7)	0.9
BeS	63	39 (61.9)	20 (31.8)	4 (6.4)	1.6 (0.9, 2.7)	0.5
Non-sensitized	722	517 (71.6)	186 (25.8)	19 (2.6)	1.0	0.6

Values are numbers of participants with percentages in parentheses.

*Referent was non-sensitized group. Odds ratios calculated based on the presence of at least one *TNF-α-308*02* allele (carrier).

0 indicates the absence of the *TNF-α-308*02* allele; 1, inheritance of one *TNF-α-308*02* allele; 2, inheritance of two *TNF-α-308*02* alleles; *TNF-α*, tumor necrosis factor-alpha; CBD, chronic beryllium disease; BeS, beryllium sensitization; HWE, Hardy-Weinberg equilibrium.

TABLE 3
Distribution of the *TNF*-α-*238* Alleles in Participants With CBD, BeS, and who are Non-Sensitized (Adjusting for Plant)

		TNF-α-*238* Alleles				
	N	0	1	2	OR (95% CI)*	HWE *P* Value
CBD	90	82 (91.1)	7 (7.8)	1 (1.1)	0.8 (0.4, 1.6)	0.1
BeS	62	54 (87.1)	8 (12.9)	0 (0.0)	1.2 (0.6, 2.7)	0.6
Non-sensitized	717	629 (87.7)	84 (11.7)	4 (0.6)	1.0	0.5

Values are numbers of participants with percentages in parentheses.

*Referent was non-sensitized group. Odds ratios calculated based on the presence of at least one *TNF*-α-*238*02* allele (carrier).

0, the absence of the *TNF*-α-*238*02* allele; 1, the inheritance of one *TNF*-α-*238*02* allele; 2, the inheritance of two *TNF*-α-*238*02* alleles; *TNF*-α, tumor necrosis factor-alpha; CBD, chronic beryllium disease; BeS, beryllium sensitization; HWE, Hardy-Weinberg equilibrium.

leles and either CBD or BeS. This was true regardless of whether a worker was homozygous or heterozygous for *TNF*-α-*308*02* or *TNF*-α-*238*02*. Our observation that the frequency of the alleles does not deviate from what would be expected, as demonstrated by the not significant HWE *P* values, also implies that they are not associated with BeS or CBD.

The non-significant ORs in Tables 4 and 5 illustrate no interaction between either *TNF*-α-*308*02* and *HLA-DPB1*E69*, or *TNF*-α-*238*02* and *HLA-DPB1*E69* in CBD and BeS. These results were confirmed by logistic regression (data not shown).

Discussion

This study is the second to evaluate *TNF*-α in relationship to CBD and BeS. In contrast to the original study that reported an association between *TNF*-α-*308*02* and having a positive BeLPT response (with and without CBD) (OR = 7.8; 95% CI = 3.2, 19.1),[9] the current study found no relationship. The original study also reported that *TNF*-α-*308*02* was independently associated with CBD (OR = 4.0, $P < 0.05$), and in the presence of *HLA-DPB1*E69*, the odds of disease were even higher (OR = 9.7, $P < 0.05$).[9] Although we saw an association between *HLA-DPB1*E69* and CBD and BeS, we did not observe a significant interaction between

TABLE 4
The Association Between CBD and *TNF*-α-*308*02*, *TNF*-α-*238*02* Stratified by Inheritance of *HLA-DPB1*E69*

	*HLA-DPB1*E69* = 0					*HLA-DPB1*E69* = 1				
	CBD	Non-Sensitized	OR	95% CI	*P* Value*	CBD	Non-Sensitized	OR	95% CI	*P* Value*
TNF-α-*308*02*‡										
+	3	137	0.55	0.16–1.95	0.42	23	67	1.13	0.64–1.99	0.66
−	14	353				52	171			
TNF-α-*238*02*‡										
+	1	51	0.54	0.07–4.14	1.0	7	37	0.56	0.24–1.31	0.25
−	16	439				68	201			

*Exact P values (Fisher exact test).

0 indicates the absence of the *HLA-DPB1*E69* allele; 1, the inheritance of at least one *HLA-DPB1*E69* allele; +, the inheritance of at least one *TNF*-α**02* allele; −, the absence of the *TNF*-α**02* allele; *TNF*-α, tumor necrosis factor-alpha; CBD, chronic beryllium disease.

TABLE 5
The Association Between BeS and *TNF*-α-*308*02*, *TNF*-α-*238*02* Stratified by Inheritance of *HLA-DPB1*E69*

	*HLA-DPB1*E69* = 0					*HLA-DPB1*E69* = 1				
	BeS	Non-Sensitized	OR	95% CI	*P* Value*	BeS	Non-Sensitized	OR	95% CI	*P* Value*
TNF-α-*308*02*										
+	9	137	1.93	0.80–4.69	0.14	15	67	1.37	0.69–2.72	0.37
−	12	353				28	171			
TNF-α-*238*02*										
+	1	51	0.43	0.06–3.27	0.71	7	36	1.06	0.44–2.55	0.82
−	20	439				37	201			

*Exact P values (Fisher exact test).

0 indicates the absence of the *HLA-DPB1*E69* allele; 1, inheritance of at least one *HLA-DPB1*E69* allele; +, the inheritance of at least one *TNF*-α**02* allele; −, the absence of the *TNF*-α**02* allele; *TNF*-α, tumor necrosis factor-alpha; BeS, beryllium sensitization.

*HLA-DPB1*E69* and either of the *TNF*-α polymorphisms.[13]

Differences between our results and those observed in the previous study may be, in part, methodological.[9] In the previous study, detection of *TNF*-α-*308*01* and *TNF*-α-*308*02* was accomplished by PCR amplification and oligonucleotide hybridization with nonradiomatic enzyme immunoassay. We used TaqMan (ABI), in which DNA-sequence probes for each alleloform carry fluorescent molecules with differing spectrometric characteristics. In this case, the wavelength of the fluorescence emission determines the genotype. This method is less susceptible to interpretation errors and, therefore, misclassification errors than hybridization and nonradiomatic enzyme immunoassay. However, to examine the possibility of sample misclassification in our population, we determined the DNA-sequence for exon 2 of *HLA-DPB1* on a subset ($n = 108$) of the same samples for which the *HLA-DPB1*E69* results had been determined previously.[13] In this case, no discrepant results were found indicating that our samples had not been misclassified. The fact that the genetic data were in compliance with HWE population laws provides additional evidence to support the validity of the assays and our results.

This study has both strengths and limitations. One of its strengths is the large, population-based sample and power. Based on the results reported by Saltini et al in 2001, we estimated that we had more than 99% power to see an association should one have been present. The power still remained high (80%) if an OR of two were considered. One limitation may be the misclassification of health outcome status. This may be particularly true among some of the longest tenured participants who were known to have had sensitization years ago, but who have not been recently evaluated for CBD.

Maier et al, in 2001, reported an association between *TNF*-α-*308*02* and high levels of beryllium-antigen-stimulated *TNF*-α. The high *TNF*-α levels were positively correlated with the severity of the CBD. It is tempting to extrapolate these results and assume that *TNF*-α-*308*02* must be associated with CBD; however, this is not the case. On closer examination, it can be seen that the frequency of the *TNF*-α-*308*02* allele in their population was very similar to what we found in the individuals with CBD in our population (22.5% [Maier et al in 2001] vs 28.6% [current study]). Maier et al also reported that their gene frequencies were in HWE, as we have found. This would be expected if the allele were not associated with disease. Hence, the results of Maier et al support our conclusions, although they did not specifically test if there was an association between *TNF*-α-*308*02* and *TNF*-α-*238*02* and sensitization or CBD.

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References

1. Bauer RA, Sawyer RT, Daniloff E, Balkissoon R, Rose CS, Newman LS. Bronchoalveolar lavage macrophage-lymphocyte clusters in granulomatous disease are linked to lymphocytosis. *Sarcoidosis Vasc Diffuse Lung Dis*. 2000;17:174–180.
2. Cullen MR, Cherniack MG, Kominsky JR. Chronic beryllium disease in the United States. *Seminars in Respiratory Medicine*. 1986;7:203–209.
3. McCanlies EC, Kreiss K, Andrew M, Weston A. HLA-DPB1 and chronic beryllium disease: a HuGE review. *Am J Epidemiol*. 2003;157:388–398.
4. Meyer KC. Beryllium and lung disease. *Chest*. 1994;106:942–946.
5. Deubner DC, Goodman M, Iannuzzi J Variability, predictive value, and uses of the beryllium blood lymphocyte proliferation test (BLPT): preliminary analysis of the ongoing workforce survey. *Appl Occup Environ Hyg*. 2001;16:521–526.
6. Deubner DC. Beryllium. *J Occup Environ Hyg*. 2005;2:D74–D78.
7. Wang Z, White PS, Petrovic M, et al. Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. *J Immunol*. 1999;163:1647–1653.
8. Wang Z, Farris GM, Newman LS, et al. Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology*. 2001;165:27–38.
9. Saltini C, Richeldi L, Losi M, et al. Major histocompatibility locus genetic markers of beryllium sensitization and disease. *Eur Respir J*. 2001;18:677–684.
10. Rossman MD, Stubbs J, Lee CW, Argyris E, Magira E, Monos D. Human leukocyte antigen Class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *Am J Respir Crit Care Med*. 2002;165:788–794.
11. Richeldi L, Sorrentino R, Saltini C. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science*. 1993;262:242–244.
12. Richeldi L, Kreiss K, Mroz MM, Zhen B, Tartoni P, Saltini C. Interaction of genetic and exposure factors in the prevalence of berylliosis. *Am J Ind Med*. 1997;32:337–340.
13. McCanlies EC, Ensey JS, Schuler CR, Kreiss K, Weston A. The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. *Am J Ind Med*. 2004;46:95–103.
14. Maier LA, Sawyer RT, Bauer RA, et al. High beryllium-stimulated TNF-alpha is associated with the -308 TNF-alpha promoter polymorphism and with clinical severity in chronic beryllium disease. *Am J Respir Crit Care Med*. 2001;164:1192–1199.
15. Maier LA, McGrath DS, Sato H, et al. Influence of MHC class II in susceptibility to beryllium sensitization and chronic beryllium disease. *J Immunol*. 2003;171:6910–6918.
16. Maier LA. Genetic and exposure risks for chronic beryllium disease. *Clin Chest Med*. 2002;23:827–839.
17. Pennica D, Nedwin GE, Hayflick JS, et al. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature*. 1984;312:724–729.
18. Jung HC, Eckmann L, Yang SK, et al. A distinct array of proinflammatory cyto-

kines is expressed in human colon epithelial cells in response to bacterial invasion. *J Clin Invest*. 1995;95:55–65.
19. Varela LM, Ip MM. Tumor necrosis factor-alpha: a multifunctional regulator of mammary gland development. *Endocrinology*. 1996;137:4915–4924.
20. Ware CF, Crowe PD, Grayson MH, Androlewicz MJ, Browning JL. Expression of surface lymphotoxin and tumor necrosis factor on activated T, B, and natural killer cells. *J Immunol*. 1992;149:3881–3888.
21. Zhang M TK. Tumor necrosis factor. In: Thomson AW, ed. *The Cytokine Handbook*. New York: Academic press; 1998. p 517–548.
22. Hajeer AH, Hutchinson IV. TNF-alpha gene polymorphism: clinical and biological implications. *Microsc Res Tech*. 2000;50:216–228.
23. Abraham LJ, Kroeger KM. Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *J Leukoc Biol*. 1999;66:562–566.
24. Allen RD. Polymorphism of the human TNF-alpha promoter—random variation or functional diversity? *Mol Immunol*. 1999;36:1017–1027.
25. Speer CP. Inflammatory mechanisms in neonatal chronic lung disease. *Eur J Pediatr*. 1999;158(Suppl 1):S18–S22.
26. Campbell DA, Li Kam Wa E, Britton J, Holgate ST, Markham AF, Morrison JF. Polymorphism at the tumour necrosis factor locus and asthma. *Monogr Allergy*. 1996;33:125–137.
27. Isomaki P, Punnonen J. Pro- and anti-inflammatory cytokines in rheumatoid arthritis. *Ann Med*. 1997;29:499–507.
28. James DG. Sarcoidosis 2001. *Postgrad Med J*. 2001;77:177–180.
29. Kornman KS, di Giovine FS. Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Ann Periodontol*. 1998;3:327–338.
30. Moffatt MF, Cookson WO. Linkage and candidate gene studies in asthma. *Am J Respir Crit Care Med*. 1997;156:S110–S112.
31. Wilson AG, di Giovine FS, Duff GW. Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. *J Inflamm*. 1995;45:1–12.
32. Wilson AG, Duff GW. Genetics of tumour necrosis factor in systemic lupus erythematosus. *Lupus*. 1996;5:87–88.
33. Henneberger PK, Cumro D, Deubner DD, Kent MS, McCawley M, Kreiss K. Beryllium sensitization and disease among long-term and short-term workers in a beryllium ceramics plant. *Int Arch Occup Environ Health*. 2001;74:167–176.
34. Kreiss K, Mroz MM, Newman LS, Martyny J, Zhen B. Machining risk of beryllium disease and sensitization with median exposures below 2 micrograms/m3. *Am J Ind Med*. 1996;30:16–25.
35. Kreiss K, Mroz MM, Zhen B, Wiedemann H, Barna B. Risks of beryllium disease related to work processes at a metal, alloy, and oxide production plant. *Occup Environ Med*. 1997;54:605–612.
36. Schuler CR, Kent MS, Deubner DC, et al. Process-related risk of beryllium sensitization and disease in a copper-beryllium alloy facility. *Am J Ind Med*. 2005;47:195–205.
37. Kreiss K, Newman LS, Mroz MM, Campbell PA. Screening blood test identifies subclinical beryllium disease. *J Occup Med*. 1989;31:603–608.
38. Weston A, Willey JC, Modali R, et al. Differential DNA sequence deletions from chromosomes 3, 11, 13, and 17 in squamous-cell carcinoma, large-cell carcinoma, and adenocarcinoma of the human lung. *Proc Natl Acad Sci U S A*. 1989;86:5099–5103.
39. Russell PJ. Population genetics. In: Davies G DK, ed. *Genetics*. New York: Harper-Collins; 2006. p 707–758.