# Association Between IL-1A Single Nucleotide Polymorphisms and Chronic Beryllium Disease and Beryllium Sensitization

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**Objective:** To determine if single nucleotide polymorphisms (SNPs) in interleukin (IL) IL-1A, IL-1B, IL-1RN, IL-2, IL-9, and IL-9R were associated with chronic beryllium disease (CBD) and beryllium sensitization (BeS). **Methods:** Forty SNPs in six IL genes were evaluated in 85 individuals with CBD, 61 individuals with BeS, and 730 individuals without BeS or CBD (nonsensitized) using a 5′ nuclease polymerase chain reaction assay. Logistic regression was used to evaluate the association between IL SNPs, CBD, and BeS, adjusting for plant-site and *HLA-DPB1*$^{Glu69}$ in additive, dominant, and recessive inheritance models. **Results:** IL-1A-1142, IL-1A-3769, and IL-1A-4697 were significantly associated with CBD in both the additive and dominant models compared to individuals with BeS or the nonsensitized. **Conclusions:** These results indicate that genetic variations in the IL-1A gene may play a role in the development of CBD but not BeS.

## Learning Objectives

- Review the features of beryllium sensitization (BeS) and chronic beryllium disease (CBD), including the previous research findings suggesting a possible role of interleukin (IL) gene variants.
- Discuss the new findings on IL gene single nucleotide polymorphisms (SNPs) and their associations with BeS and/or CBD.
- Summarize the contribution of the new study to understanding of the genetics of BeS and CBD.

Exposure to beryllium can trigger a cell-mediated, type IV delayed hypersensitivity immune reaction.[1,2] Some individuals sensitized to beryllium (BeS) go on to develop chronic beryllium disease (CBD). The immune reaction to beryllium has led researchers to study the role of immune response genes and their variations in the development of CBD and BeS. Previous population-based studies mainly focused on the association of specific human leukocyte antigen (HLA) alleles with CBD.[3–11] The results of these studies have shown that *HLA-DPB1* gene variants, particularly those coding for a glutamic acid at position 69 (*HLA-DPB1*$^{Glu69}$), are associated with CBD and BeS.[5,9–13] However, CBD is a complex disease and likely influenced by the effects and interactions of many genes. In addition to exposure to beryllium, immune/inflammation-related genes and modifier genes may play a role in sensitization and disease progression. We previously investigated polymorphisms (−308 and −238) in the proinflammatory cytokine TNF-$\alpha$. Neither was found to be associated with BeS or CBD. Besides TNF-$\alpha$, cytokine genes are also of interest because of their role in granulomatous inflammation and fibrosis—a hallmark of CBD.

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The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Erin McCanlies and coauthors Berran Yucesoy, Anna Mnatsakanova, James E. Slaven, Michael E. Andrew, Bonnie L. Frye, Christine R. Schuler, Kathleen Kreiss, and Ainsley Weston have no financial interest related to this research.

The JOEM Editorial Board and planners have no financial interest related to this research.

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DOI: 10.1097/JOM.0b013e3181e48ec8

This study was designed to extend our previous efforts by investigating the role of genetic variations in a selected group of interleukin (IL) genes (IL-1A, IL-1B, IL-1RN, IL-2, IL-9, IL-9R) that play a role in a number of cellular mechanisms. The IL-1 family is involved in various immune/inflammatory processes and hematopoiesis, whereas IL-2 is important for the proliferation of T and B lymphocytes and is required for other activities crucial to the regulation of the immune response. IL-9 is a pro-fibrotic cytokine that modulates inflammatory and fibrogenic processes. IL-9R modulates the biological effect of IL-9 and is involved in lung inflammation and hyperresponsiveness.[14,15] Variations in these genes have been linked to a number of inflammatory diseases such as rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease.[16] The role these cytokines play in immune, inflammatory, and fibrotic processes makes them biologically plausible candidates for genetic association studies in BeS and CBD. Based on this, we investigated the possible impact of 40 single nucleotide polymorphisms (SNPs) in the IL-1 (IL-1A, B, and RN), IL-2, IL-9, and IL-9R genes on the occurrence of BeS and CBD in a large cohort of beryllium workers.

## MATERIALS AND METHODS

### Participants

As previously described, three groups of workers from a large beryllium manufacturing company were eligible to participate in this study.[5] Briefly, two of these groups consisted of current and former workers who had previously participated in epidemiologic surveys conducted in 1992–1994 and 1998–1999 at plants in OH and AZ, and a survey conducted in 2000 at a plant in PA.[17–20] The third group consisted of former workers from the OH and AZ facilities who were known to have BeS or CBD but who were not part of any of the above epidemiologic survey cohorts. In total, there were 876 beryllium workers in this study: 85 diagnosed with CBD, 61 with BeS, and 730 workers without CBD or BeS, who will be referred to as nonsensitized.

For this study, all participants completed a medical and work history questionnaire and gave a blood sample for genetic analyses. Study participants not already known to have BeS or CBD were also screened with the beryllium lymphocyte proliferation test (BeLPT). Those known to have BeS or CBD were not retested. Workers identified as BeS were offered clinical evaluation to

diagnose CBD. The National Institute for Occupational Safety and Health Human Subjects Review Board approved this research. Written informed consent was obtained from each participant.

## Definition of Health Outcomes

### Beryllium Sensitization

As previously described,[5] the BeLPT is used to identify participants sensitized to beryllium. Split samples from the initial blood draw were sent to two laboratories. A BeLPT was determined to be abnormal if at least two of the six concentration (1, 10, or 100 $\mu$M of beryllium sulfate)/duration (5 or 7 days) ratios were $\geq$3.0, borderline negative if a single ratio was $\geq$3.0, and normal if no ratios were $\geq$3.0. Additional blood was drawn and the test repeated for confirmation of an abnormal test result (eg, if an individual's initial split sample blood draw returned one abnormal and one normal result), for clarification of a borderline result, or when the laboratory deemed the results uninterpretable. A person was considered to be "sensitized" to beryllium (BeS) if two or more BeLPTs were found to be abnormal, either from separate laboratories or from repeated testing at the same laboratory. Participants who did not meet this definition were classified as "nonsensitized."

### Chronic Beryllium Disease

Participants with abnormal BeLPTs were referred for voluntary clinical evaluation, including bronchoscopy for collection of transbronchial biopsies and bronchoalveolar lavage. Biopsy specimens were examined for granulomas, mononuclear cell interstitial infiltrate, and fibrosis; lavage cells were tested for evidence of lung sensitization with the bronchoalveolar lavage lymphocyte proliferation test. A sensitized individual was considered to have CBD if granulomas or other pathologic abnormalities consistent with that diagnosis were present. A few individuals were diagnosed through identification of radiographic abnormalities consistent with CBD, mostly before the onset of the use of diagnostic bronchoscopy in the 1980s.

## Laboratory Methods

Leukocyte DNA was isolated from whole blood as previously described.[5,21] Forty SNPs in six IL genes were evaluated. Genotyping was performed using a 5′ nuclease polymerase chain reaction assay. Primers and probes were designed using the Assay-by-design™ service (PE Applied Biosystems, Foster City, CA). Table 1 lists the candidate genes and SNPs selected for evaluation.

## Genetic Models

The genetic data were analyzed under additive, dominant, and recessive inheritance models. These models identify the mode of inheritance that best captures the effect and the intensity of the gene-disease association. To determine if the additive model most accurately describes the effect, two odds ratios (ORs) are estimated and compared, the OR for carriage of the heterozygous SNP versus carriage of the homozygous major SNP and the OR for carriage of the homozygous minor SNP versus heterozygous. If the two ORs calculated in the additive model are approximately equal then the results indicate that the additive model is valid and the ORs calculated indicates the intensity of the association. If the two ORs are different then either the dominant or recessive models may more accurately describe the data.

The dominant model estimates the odds of disease with carriage of at least one minor SNP versus carriage of the homozygous major SNP, whereas the recessive model estimates the odds of disease with carriage of the homozygous minor SNPs versus carriage of at least one major SNP. A low or nonsignificant OR generated from the recessive model indicates that the dominant model better describes the effect. Similarly, a low or nonsignificant OR for the dominant model indicates that the recessive model more accurately reflects the effect.

**TABLE 1.** Genes and SNPs Analyzed in the Study

| Gene | Position | SNP ID |
|---|---|---|
| IL-1A | −1613 | 1800587 |
| | +1142 | 1609682 |
| | +1170 | 1894399 |
| | +1375 | 3856838 |
| | +3769 | 3783539 |
| | +4125 | 17561 |
| | +4697 | 3783543 |
| IL-1B | −1061 | 3087258 |
| | −581 | 1143627 |
| | +289 | 1143629 |
| | +2538 | 3136558 |
| | +3423 | 1143634 |
| | +5260 | 1143642 |
| | +5511 | 1143643 |
| | +6380 | 1071676 |
| IL-1RN | −379 | 4251961 |
| | −87 | 4251968 |
| | −31 | 2234677 |
| | +36 | 2234678 |
| | +158 | 16065 |
| | +619 | 4251969 |
| | +1129 | 315919 |
| | +1185 | 2637988 |
| | +11614 | 419598 |
| | +12986 | 432014 |
| | +13307 | 380092 |
| IL-2 | −385 | 2069762 |
| | +4464 | 2069772 |
| | +5621 | 2069776 |
| IL-9 | +992 | 1859430 |
| | +1155 | 2069870 |
| | +1252 | 31564 |
| | +1419 | 2069879 |
| | +1872 | 2069882 |
| | +3115 | 2069884 |
| | +3338 | 2069885 |
| IL-9R | +1206 | 2077051 |
| | +1578 | 731476 |
| | +5694 | 3093494 |
| | +6425 | 3093499 |

## Statistical Analysis

Deviations from Hardy-Weinberg equilibrium were tested using chi-square goodness of fit test.[22] Logistic regression was used to test for multicollinearity between significant IL-1A alleles and to evaluate if the IL SNPs were associated with either CBD or BeS. For those alleles found to be associated with CBD or BeS, logistic regression was used to calculate ORs and 95% confidence intervals, adjusted for plant-site and *HLA-DPB1*$^{Glu69}$ status for the three different genetic models, additive, dominant, and recessive. Plant-site is adjusted for because the plant populations have different racial/ethnic distributions, and the frequency of ILs is known to differ by race.[23,24] Analyses were conducted using SAS version 9.1 statistical software.[25]

## RESULTS

### Associations Between IL SNPs, CBD, and BeS

All the genotype frequencies except for IL-1A-1613, IL-1RN-379, and IL-1RN-12986 were in Hardy-Weinberg equilibrium in the nonsensitized participants.

The frequency of three alleles (IL-1A-1142, IL-1A-3769, and IL-1A-4697) was found to differ significantly when individuals with CBD, BeS, and the nonsensitized were compared. The frequency of the homozygous minor, heterozygous, and homozygous major alleles for each of these IL-1A SNPs in individuals with CBD, BeS, and the nonsensitized are shown in Table 2. The test for multicollinearity between these three alleles was also found to be highly significant. After adjusting for *HLA-DPB1*$^{Glu69}$ and plant-site, all three IL-1A SNPs showed significant associations with CBD in one additive model and the dominant model (Table 3). In both the additive and dominant model, IL-1142 occurred twice as often in individuals with CBD compared with the nonsensitized, and three times more often in individuals with CBD compared to those with BeS. Similar associations were found for the IL-1A-3769 and IL-1A-4697 SNPs. In the additive and dominant models, both the IL-1A-3769 SNP and IL-1A-4697 SNP occurred more than twice as often in individuals with CBD compared to those with BeS and slightly less than twice as often in those with CBD compared with the nonsensitized. There were no significant associations observed for the recessive model. Furthermore, none of the IL SNPs were found to be associated with BeS.

**TABLE 2.** Frequency of Significant IL-1A SNPs in Individuals With CBD, BeS, and Nonsensitized

| SNP | Genotype | CBD (*N*, %) | BeS (*N*, %) | Nonsensitized (*N*, %) |
|---|---|---|---|---|
| IL-1A-1142 | CC | 9 (11.8) | 4 (7.8) | 83 (12.8) |
| | AC | 44 (57.9) | 18 (35.3) | 273 (41.9) |
| | AA | 23 (30.3) | 29 (56.9) | 295 (45.3) |
| IL-1A-3769 | AA | 10 (11.9) | 4 (6.9) | 75 (10.4) |
| | AG | 47 (56.0) | 22 (37.9) | 326 (45.1) |
| | GG | 27 (32.1) | 32 (55.2) | 322 (44.5) |
| IL-1A-4697 | CC | 9 (10.7) | 4 (6.8) | 83 (11.3) |
| | CT | 48 (57.1) | 22 (37.3) | 329 (44.7) |
| | TT | 27 (32.1) | 33 (55.9) | 324 (44.0) |

C, cytosine; A, adenine; G, guanine; T, thymine.

## DISCUSSION

Our results indicated that the three IL-1A SNPs, IL-1A-1142, IL-1A-3769, and IL-1A-4697, were associated with CBD when compared with both the BeS and the nonsensitized. No associations were found when the BeS were compared with the nonsensitized. The significant multicollinearity test result indicated that these three SNPs are highly linked. Therefore, any one of these SNPs may be the best candidate, or another SNP that is linked to

**TABLE 3.** Adjusted* OR and 95% CI for Significant IL-1A SNPs for Different Genetic Models

| SNP/Genetic Model | CBD vs Nonsensitized OR (95% CI) | BeS vs Nonsensitized OR (95% CI) | CBD vs BeS OR (95% CI) |
|---|---|---|---|
| IL-1A-1142 | | | |
| Additive | | | |
| AC vs AA | 2.23 (1.27–3.93)† | 0.68 (0.36–1.27) | 3.04 (1.34–6.91)† |
| CC vs AC | 0.76 (0.34–1.69) | 0.76 (0.24–2.41) | 1.04 (0.26–4.11) |
| Dominant | | | |
| CC, AC vs AA | 2.03 (1.18–3.48)† | 0.63 (0.35–1.15) | 3.02 (1.36–6.70)† |
| Recessive | | | |
| CC vs AC, AA | 1.05 (0.48–2.28) | 0.64 (0.22–1.85) | 1.49 (0.40–5.57) |
| IL-1A-3769 | | | |
| Additive | | | |
| AG vs GG | 1.75 (1.03–2.96)† | 0.70 (0.39–1.24) | 2.29 (1.08–4.85)† |
| AA vs AG | 0.95 (0.43–2.12) | 0.84 (0.26–2.66) | 1.15 (0.31–4.33) |
| Dominant | | | |
| AA, AG vs GG | 1.73 (1.45–2.88)† | 0.65 (0.37–1.13) | 2.51 (1.21–5.19)† |
| Recessive | | | |
| AA vs AG, GG | 1.36 (0.64–2.88) | 0.74 (0.26–2.17) | 1.86 (0.53–6.50) |
| IL-1A-4697 | | | |
| Additive | | | |
| CT vs TT | 1.91 (1.13–3.23)† | 0.69 (0.38–1.20) | 2.56 (1.21–5.41)† |
| CC vs CT | 0.82 (0.37–1.81) | 0.73 (0.23–2.29) | 1.18 (0.31–4.39) |
| Dominant | | | |
| CC, CT vs TT | 1.72 (1.04–2.85)† | 0.62 (0.36–1.08) | 2.56 (1.24–5.29)† |
| Recessive | | | |
| CC vs CT, TT | 1.04 (0.48–2.25) | 0.63 (0.22–1.82) | 1.51 (0.42–5.43) |

*All ORs adjusted for plant and *HLA-DPB1*$^{Glu69}$.
†Significant ORs.
CI, confidence intervals.

these. It is also possible that all three may be necessary to observe the associations we found. The fact that the ORs calculated for the additive model were not equal indicated that either the dominant or recessive model may better describe the mode of inheritance. The significant results observed for the dominant model indicates that the dominant model may best describe the disease-gene relationship: carriage of at least one of the three minor IL-1A SNPs was associated with CBD.

Cytokines play a leading role in the inflammatory events leading to the formation of pulmonary granulomas and the development of fibrosis.[26,27] IL-1 is an important cytokine in the generation of early-phase protective immunity and is known to trigger granuloma formation by stimulating T helper cells.[26] In challenged mice, IL-1A was associated with an immune-induced fibrotic response; the size of the granuloma correlated with IL-1A activity.[28,29] Furthermore, it has been shown that if the IL-1 receptor was blocked, tissue damage was significantly reduced.[29] The release of IL-1A from macrophages in humans with lung diseases such as sarcoidosis and CBD, as well as differences in the development and severity of the former in different racial and ethnic groups, also suggests that variation in IL-1 gene expression may be important in the etiology of these diseases.[27,30,31]

Rybicki et al[31] evaluated a number of gene variants associated with the T cell receptor, ILs, and interferon regulatory factor in blacks with sarcoidosis and an unrelated healthy control group. Of the IL variants analyzed, the IL-1A-137 SNP was most strongly associated with sarcoidosis.[31] In Whites, the IL-1A-899 1.1 genotype was found to occur significantly more often in patients with sarcoidosis compared with healthy controls.[30] However, to our knowledge, the IL-1A SNPs have not been studied in individuals with CBD or BeS.[31] Our results show that IL-1A gene polymorphisms may increase susceptibility to CBD and influence the development of disease after sensitization has occurred. Our data are consistent with these alleles playing a more important role in CBD, which is characterized by the presence of granulomas, than in BeS, which is not characterized by the presence of granulomas.

The role of IL-1B in granulomatous formation is less clear. Kline et al[32] found that higher levels of IL-1B were released by alveolar macrophages in individuals with interstitial lung disease, particularly sarcoidosis and idiopathic pulmonary fibrosis, compared with normal subjects. However, when alveolar macrophage-derived cytokine gene expression was studied in patients with CBD, sarcoidosis, and normal individuals, there was no difference in IL-1B gene expression.[33] Consistent with the latter findings, we also did not find an association between any of the eight IL-1B SNPs we evaluated and CBD or BeS. Inconsistent results across these studies may be linked to cytokine redundancy and the induction of regulatory mediators that limit IL-1 activity.

Previously, we found that carriage of *HLA-DPB1*$^{Glu69}$ was associated with both CBD and BeS, with the strongest relationships in those with CBD.[5] However, here we found that the IL-1A SNPs were more frequent in individuals with CBD compared with those with BeS. Although BeS is a precondition for CBD, the formation of granulomas in CBD likely requires an independent inflammatory response controlled by genes unrelated to beryllium recognition. This idea is supported by previous research that found that some gene variations modify not only the risk of disease but also the severity/progression of disease.[16] For example, *TGFβ1* genotypes have been found to be associated with more severe granulomatous disease in individuals with CBD.[34,35] Similarly, Yucesoy et al[36,37] evaluated a number of different cytokines in former miners with silicosis of varying severity and found that workers with the *TNF-238*02* allele were about four times more likely to have severe disease, but half as likely to have moderate disease. Production and secretion of cytokines are under complex biological control in the lung. Based on the nature of stimulus, the patterns of specific expression of cytokines can vary in different stages of diseases. IL-1A variations may participate to a lesser degree in the early stages of immune reactivity where a redundant cytokine network is less dominant.

IL-2, IL-9, and IL-9R are cytokines that are mainly produced by lung lymphocytes. Increased local production of IL-2 has been reported in the lungs of patients with sarcoidosis, tuberculosis, and CBD,[38] whereas animal models indicate that IL-9 is involved in inflammation and hyperresponsiveness. IL-9R is thought to play a role in the fibroproliferative response observed in some interstitial lung diseases.[26] Genetic variations in these cytokine genes have not been studied in relation to granulomatous lung diseases. We did not find an association between SNPs in IL-2, IL-9, or IL-9R and either CBD or BeS in our participants.

Besides magnitude of exposure and host factors, physicochemical properties of beryllium also appear to influence immune reactivity and susceptibility to sensitization and development of CBD.[39] It is possible that functional polymorphisms in cytokine genes may result in different binding properties and activation patterns of transcription factors central to the regulation of cytokine expression depending on cell type, the tissue, and exposure characteristics.[39]

In conclusion, our results suggest that in combination with other risk factors such as the presence of the *HLA-DPB1*$^{Glu69}$ allele, variations in the IL-1A gene may contribute to the etiology of CBD. The strength of our study is the source of the participants, primarily several surveys of beryllium production workers, and its fairly large sample size. However, additional studies are needed to confirm these associations in an independent population of beryllium-exposed workers. Currently, the molecular mechanism of BeS and progression from sensitization to CBD remains poorly understood. Mapping integrated regulatory functions of cytokines and the HLA region genes will be central to the understanding of the pathogenesis of these processes.

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