Original research

HLA-DPB1 E69 genotype and exposure in beryllium sensitisation and disease

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ABSTRACT

Objectives Human leukocyte antigen-DP beta 1 (HLA-DPB1) with a glutamic acid at the 69th position of the ß chain (E69) genotype and inhalational beryllium exposure individually contribute to risk of chronic beryllium disease (CBD) and beryllium sensitisation (BeS) in exposed individuals. This retrospective nested case–control study assessed the contribution of genetics and exposure in the development of BeS and CBD.

Methods Workers with BeS (n=444), CBD (n=449) and beryllium-exposed controls (n=890) were enrolled from studies conducted at nuclear weapons and primary beryllium manufacturing facilities. Lifetime-average beryllium exposure estimates were based on workers' job questionnaires and historical and industrial hygienist exposure estimates, blinded to genotype and case status. Genotyping was performed using sequence-specific primer-PCR. Logistic regression models were developed allowing for over-dispersion, adjusting for workforce, race, sex and ethnicity.

Results Having no E69 alleles was associated with lower odds of both CBD and BeS; every additional E69 allele increased odds for CBD and BeS. Increasing exposure was associated with lower odds of BeS. CBD was not associated with exposure as compared to controls, yet the per cent of individuals with CBD versus BeS increased with increasing exposure. No evidence of a gene-by-exposure interaction was found for CBD or BeS.

Conclusions Risk of CBD increases with E69 allele frequency and increasing exposure, although no gene by environment interaction was found. A decreased risk of BeS with increasing exposure and lack of exposure response in CBD cases may be due to the limitations of reconstructed exposure estimates. Although reducing exposure may not prevent BeS, it may reduce CBD and the associated health effects, especially in those carrying E69 alleles.

Key messages

What is already known about this subject?

► HLA-DPB1 E69 genotype and beryllium exposure individually contribute to the development of chronic beryllium disease (CBD) and beryllium sensitisation (BeS).

What are the new findings?

► Having no E69 alleles was associated with lower odds of both CBD and BeS; every additional E69 allele increased odds for CBD and BeS. Increasing exposure was associated with lower odds of BeS but CBD was not associated with exposure compared with controls in this study; these findings were likely affected by differential exposure misclassification. The per cent of individuals with CBD versus BeS increased with increasing exposure, suggesting an impact of exposure with CBD.

How might this impact on policy or clinical practice in the foreseeable future?

► The new Occupational Safety and Health Administration standard of 0.2 µg/m^3 is a long-awaited step in helping to prevent BeS and CBD in the workplace. This study suggests that although reducing exposure may not prevent BeS, it may reduce the development CBD and the associated health effects, especially in those most susceptible.

INTRODUCTION

Beryllium is a lightweight, versatile metal that is used in many industrial applications from computer components to aerospace. Individuals exposed to beryllium can develop a cell-mediated immune response known as beryllium sensitisation (BeS) which can progress to chronic beryllium disease (CBD), a granulomatous lung disease with no known cure. Not all persons exposed to beryllium develop BeS or CBD, although <1% to up to 15% may develop these health effects.[1–10] Previous studies have shown that a genetic marker, a human leukocyte antigen-DP beta 1 (HLA-DPB1) on chromosome 6 with a glutamic acid substitution at the 69th position of the β chain (E69), is associated with susceptibility to BeS and CBD.[11–16] While a clear exposure response has been lacking, studies have shown an increasing risk for BeS and/or CBD with increasing exposure. Exposure response alone was examined in a cohort study of workers with six years or less tenure in the primary beryllium industry.[17] BeS prevalence increased with increasing exposure quartile for highest and average job exposures and CBD risk was associated with increasing cumulative exposure. A well-constructed cohort

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mortality study of workers at seven beryllium processing plants demonstrated increased risk of CBD mortality related to duration of employment but not to estimated cumulative or maximum exposure.[18] Two case–control studies of exposure and genetics in nuclear weapons workers[19 20] demonstrated that workers with higher mass-based inhalation exposures were more likely to be diagnosed with CBD and that the E69 genotype and beryllium exposure contributed individually to developing CBD. These two studies did not find a gene–environment interaction. A recent small case–control study from two beryllium processing facilities examined the association between genetics and beryllium exposure[21] and found no exposure response relationship for BeS or CBD with or without adjustment for E69 and other human leukocyte antigen-DR beta 1 (HLA-DRB1) polymorphisms. These previous studies in the nuclear weapons industry, the primary beryllium industry and in beryllium processing facilities[17 19–21] were limited by sample size. The present study combined populations from these previous reported studies to examine the contributions of the E69 genotype and exposure to risk of BeS and CBD in the largest population of beryllium-exposed workers to date from several different beryllium-using industries.

METHODS

Study population

Four workforce populations were included in this retrospective nested case–control study (online supplemental figure 1). The first population, from National Jewish Health (NJH), was comprised of workers who participated in medical surveillance programmes for current and former beryllium workers from Department of Energy (DOE) and DOE vendor sites.[22 23] Beryllium-exposed control subjects were matched to identified BeS and CBD cases based on sex, race, site and decade of first beryllium exposure. The second population came from the Beryllium Biobank (BBB), a repository setup by the DOE to enrol cases of BeS, CBD and beryllium-exposed controls.[24] As with NJH, controls were matched to BeS and CBD cases based on sex, race, site and decade of first beryllium exposure. The third population consisted of BeS and CBD cases and beryllium-exposed controls who participated in a one-time study of workers from two primary beryllium processing plants in eastern Pennsylvania (PENN) that had operated in the past.[10 21 25] The last population was a group of workers who participated in the National Institute for Occupational Safety and Health (NIOSH) beryllium worker research programme,[5 6] from the primary beryllium industry. Only participants with white race were included in the NIOSH group in order to protect confidentiality.

All populations were screened with the blood beryllium lymphocyte proliferation test (BeLPT),[26] with medical follow-up to diagnose CBD, as part of medical surveillance or research programmes. Individuals with abnormal BeLPT results underwent clinical evaluation including bronchoalveolar lavage (BAL) and transbronchial biopsy to determine disease status. BeS cases had two or more abnormal blood BeLPT results and/or an abnormal BAL BeLPT with no evidence of CBD on clinical evaluation.[27] CBD subjects had evidence of BeS along with either (1) granulomas on biopsy; (2) both an abnormal BAL BeLPT and greater than 15% lymphocytes in BAL cells or (3) upper lobe fibrosis on chest radiograph in the presence of confirmed BeS.[27 28] Subjects with confirmed abnormal BeLPTs who did not complete a full clinical evaluation to rule out CBD were classified as BeS/CBD and excluded from gene-exposure analyses. Control subjects had normal BeLPT blood test results as part of medical surveillance or research studies. Control subjects were frequency matched by each site (except for the BBB which enrolled matched controls) approximately one to one to cases, based on sex, race, decade of hire and facility.

Genetic assessment

Genomic DNA was extracted from peripheral blood cells, with genotyping performed by sequence-specific primer-PCR (SSP-PCR) for HLA-DPB1 as described by Gilchrist *et al*[29] or based on the SSP-PCR method (Pel-Freez Clinical Systems, Brown Deer, Wisconsin, USA). For ambiguities or inconsistent patterns of primer amplifications, sequence-based typing was performed and confirmed by bi-directional sequencing-based typing of exon 2. The technicians performing genotyping were blinded to case and exposure status. Genes were typed to the allele level for the numerous HLA-DPB1 alleles. Participants were coded or grouped as follows: (1) E69 positive, indicating carriage of at least one E96 allele (ie, *0201, *0202, *0601, *0801, *0901, *1001, *1301, *1601, *1701, *1901 and so on); (2) E69 homozygous, specifying carriage of two copies of any E69-positive alleles; (3) E69 negative, non-carriage of any E69-positive alleles.

Identifying an exposure metric

Because study participants were enrolled over various time frames, work histories were collected using three different methods: (1) self-administered questionnaire, (2) interviewer-administered questionnaire or (3) company work history records. Common information available using these three methods included facility name, hire and termination dates, job titles with dates held, longest job held and highest exposure job. The longest held job title was categorised into one of 14 job categories as follows: machinist; industrial hygienist (IH)/safety; administration; custodial/maintenance; construction worker; in plant trades person; engineer/research and development; inspector/quality control; industrial production (non-machinist); non-production in plant; decontamination and decommissioning; laboratory; security and management.

Past and current IH data and information, historical industry data, published data and data collected for regulatory purposes were used to calculate industry-specific exposure estimates for each of the 14 job categories. For the NJH and BBB groups, an arithmetic mean of the available exposure measurements was calculated for each category within time periods based on years of data collection. Cumulative exposure estimates for each participant were calculated by multiplying the work-time in each job category by corresponding time period exposure estimate. Lifetime-average exposure estimates were calculated by dividing the cumulative exposure by the total number of years worked. Highest process exposure was estimated using the highest exposure job measurement associated with the longest held job. Highest process exposure was not able to be calculated for the BBB group. For the PENN population, historical air sampling data and work process descriptions along with work history were used to develop a job-exposure matrix[10] to calculate a daily-weighted average (DWA) for every year/combination and each subject was assigned cumulative exposure, lifetime-average exposure and highest process exposure. For the NIOSH group, DWAs were calculated as time weighted averages of breathing zone task samples and general area samples associated with each job group and time period. These estimates were assigned to participants' work histories and summarised to calculate the lifetime average and highest process exposures. Cumulative exposure measurements were not provided to protect data

confidentiality as tenure could be calculated resulting in an identifiable variable. All worker populations had estimates for lifetime-average exposure so that variable was chosen for the analysis.

Statistical analysis

To test for differences in the frequency of covariate values by E69 allele number and diagnosis, we used χ^2 tests on the contingency tables. The p values for sex, Hispanic ethnicity, lifetime-average exposure quartiles and worker cohort were calculated using the asymptotic χ^2 distribution. The p values for race were determined by Monte Carlo sampling with 10^7 samples. To test the effects of E69 genotype, lifetime-average exposure and their interaction on diagnosis, we used logistic regression models while controlling for demographic covariates and population. With these models we regressed dichotomous diagnosis (CBD vs control, BeS vs control or CBD vs BeS) on the number of E69 alleles and lifetime-average exposure, as well as their multiplicative interaction. We represented lifetime-average exposure in two ways: first on the $\log_{10}$ scale, and second by dividing absolute exposures into quartiles with Q1 <0.06 μg/m^3; Q2=0.06 <0.3 μg/m^3; Q3 >0.3–1.0 μg/m^3; Q4 >1 μg/m^3. These two representations of the data allowed for ease of illustrating results and to reduce the potential influence of small shifts in absolute exposure values due to differences in reconstructed exposures among populations. Our final reported models included categorical variables for race, sex and ethnicity and a separate fixed intercept for each worker group. These models were fitted using the glm function in R language (V.3.3.1) with the logit link function and the quasibinomial family to allow for overdispersion of the response. The positive convergence tolerance was set to be 1×10^{-13}, more stringent than the default of 1×10^{-8}. The 95% CIs were determined using the confint function, while the glht function in the multcomp package (V.1.4–6) was used to calculate the genotype-specific log odds within each exposure quartile.

For the CBD versus BeS case comparison analysis, we used χ^2 and Cochran Armitage test for trend. Our cut-off for statistical significance was set at 0.05 for all tests.

RESULTS

Population demographics

There were a total of 1846 individuals in the study population with genotyping results; 444 BeS, 63 BeS/CBD, 449 CBD and 890 beryllium-exposed controls. Exposure estimates were calculated on 90% of the study population as some subjects did not have IH measurements to associate with their work history and were excluded from the gene by exposure analyses. CBD cases had 16.7% missing exposures compared with 11.4% of BeS and 7.4% of controls, while Penn had 18.6% missing exposures compared with 9.6% from NJH, 8.0% from the BBB and none from NIOSH. The participants were predominantly men (86%) and Non-Hispanic white (94%), reflecting the confidentiality restrictions of the NIOSH group, geographical demographics, workforce characteristics and hiring practices of the beryllium industries studied. We found statistically significant differences between diagnosis and worker group, sex and quartile of exposure but not race or ethnicity (table 1).

E69 genotype

There was a statistically significant difference in the number of E69 alleles between CBD, BeS and controls with 87.5% of CBD cases, 79.1% of BeS cases and 38.3% of controls being E69

Table 1 Summary of E69 genotype, work site and demographic variables by diagnosis

Diagnosis, n (%)							
		Control	BeS	BeS/CBD	CBD	Total	χ^2 p value
E69 alleles	0	549 (77.3)	93 (13.1)	12 (1.7)	56 (7.9)	710	5.59 E−87
	1	302 (32.3)	291 (31.1)	37 (4.0)	305 (32.6)	935	
	2	39 (19.4)	60 (29.9)	14 (7.0)	88 (43.7)	201	
Site	BBB	100 (43.1)	86 (37.1)	19 (8.2)	27 (11.6)	232	4.45 E−28
	PENN	287 (67.2)	73 (17.1)	0	67 (15.7)	427	
	NIOSH	61 (38.9)	39 (24.8)	0	57 (36.3)	157	
	NJH	442 (42.9)	246 (23.9)	44 (4.3)	298 (28.9)	1030	
Sex	Male	784 (49.4)	364 (23.0)	52 (3.3)	385 (24.3)	1585	0.0211
	Female	106 (40.6)	80 (30.7)	11 (4.2)	64 (24.5)	261	
Race	White	885 (48.3)	437 (23.9)	61 (3.3)	448 (24.5)	1831	0.1437
	Black	4 (44.4)	4 (44.4)	0	1 (11.1)	9	
	Other	1 (25.0)	2 (50.0)	1 (25.0)	0	4	
	Missing	0	1 (50.0)	1 (50.0)	0	2	
Ethnicity	Hispanic	40 (40.8)	21 (21.4)	7 (7.1)	30 (30.6)	98	0.0567
	Non-Hispanic	850 (48.7)	421 (24.1)	55 (3.2)	419 (24.0)	1745	
	Missing	0	2 (66.6)	1 (33.3)	0	3	
Lifetime-average exposure quartile	Q1	125 (30.0)	177 (42.5)	30 (7.2)	84 (20.2)	416	9.04 E−34
	Q2	197 (47.9)	98 (23.8)	18 (4.4)	98 (23.8)	411	
	Q3	255 (56.4)	76 (16.8)	8 (1.8)	113 (25.0)	452	
	Q4	247 (65.9)	44 (11.7)	4 (1.1)	80 (21.3)	375	
Total		890	444	63	449	1846	

χ^2 p value for race was calculated after merging black and other categories and dropping the missing category. χ^2 p value for ethnicity was calculated after dropping the missing category. Q1 <0.06 μg/m^3; Q2=0.06 <0.3 μg/m^3; Q3 >0.3–1.0 μg/m^3; Q4 >1 μg/m^3.
BBB, beryllium biobank; BeS, beryllium sensitisation; CBD, chronic beryllium disease; NIOSH, National Institute for Occupational Safety and Health; NJH, National Jewish Health; PENN, Pennsylvania.

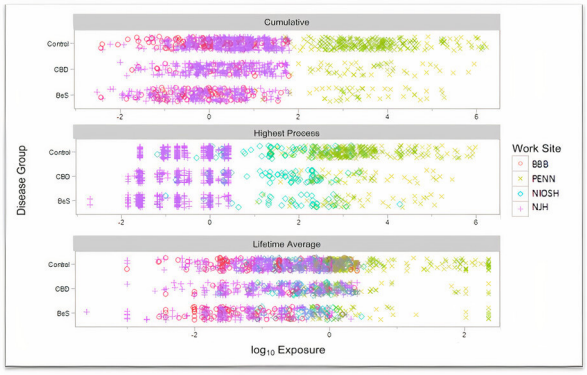

Figure 1 Diagnosis versus $\log_{10}$ exposure for each exposure metric. BBB, beryllium biobank; NIOSH, National Institute for Occupational Safety and Health; NJH, National Jewish Health; PENN, Pennsylvania.

positive (one or two alleles) ($p<0.0001$), similar to the frequencies in previous published studies.[11–16] There were also significant differences in allele number between worker groups, race, ethnicity and lifetime-average exposure quartile, but not with sex (online supplemental table 1).

Exposure estimates

Tenure at the beryllium facilities ranged from less than 1 year to 47 years. Figure 1 shows the distribution of $\log_{10}$ exposure for cumulative, highest process and lifetime-average exposures by disease group while figure 2 shows the $\log_{10}$ exposures by genotype. Median cumulative exposure was 3.66 µg/m^3-years (0–1 724 850); median highest process exposure was 1.0 µg/m^3 (0–962 808 µg/m^3); while median lifetime-average exposure was 0.3 µg/m^3 (0–229.24 µg/m^3). The PENN group had significantly higher exposures across all categories compared with the other populations (figure 1), with the NJH and BBB populations having lower exposures. We chose to use lifetime-average exposure for further analysis since it was common to all groups and was associated with outcomes in previous studies.[17 18] Controls had significantly higher median lifetime-average exposures (0.61 µg/m^3) compared with CBD (0.33 µg/m^3) and BeS (0.07 µg/m^3) ($p=0.0001$) and median lifetime-average exposures were significantly higher for CBD than BeS ($p=0.001$).

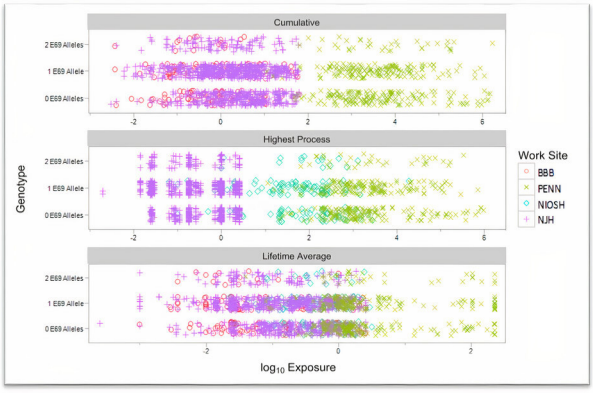

Figure 2 E69 genotype versus $\log_{10}$ exposure for each exposure metric. BBB, beryllium biobank; NIOSH, National Institute for Occupational Safety and Health; NJH, National Jewish Health; PENN, Pennsylvania.

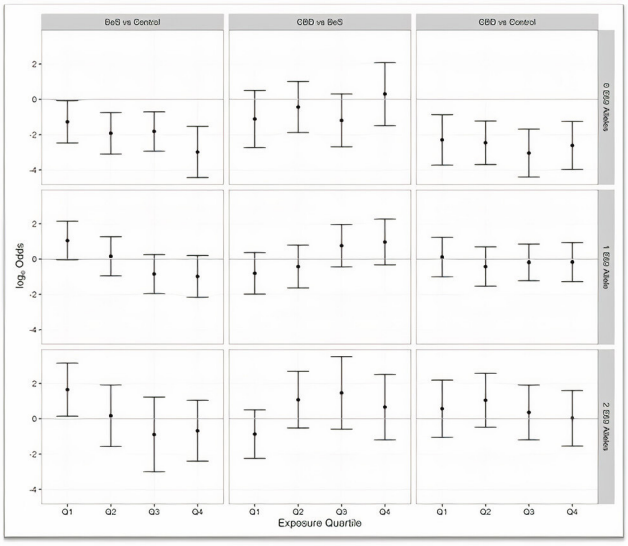

Figure 3 Log odds of BeS or CBD at each quartile of lifetime-average exposure for the given number of E69 alleles (Q1 <0.06 µg/m^3; Q2=0.06 <0.3 µg/m^3; Q3 >0.3–1.0 µg/m^3; Q4 >1 µg/m^3). BeS, beryllium sensitisation; CBD, chronic beryllium disease.

Genotype and log exposure

At the mean of $\log_{10}$ lifetime-average exposure, subjects with no E69 alleles had lower odds of both CBD (OR=0.05; 95% CI=(0.01 to 0.29)) and BeS (OR=0.14; 95% CI=(0.05 to 0.44)) compared with controls. However, each additional E69 allele increased the odds for CBD (OR=5.92; 95% CI=(4.31 to 8.15)) and BeS (OR=3.93; 95% CI=(2.88 to 5.38)). Averaging over all E69 genotypes, every 10-fold increase in lifetime-average exposure was associated with lower odds of BeS (OR=0.47; 95% CI=(0.36 to 0.63)) compared with controls, while CBD was not significantly associated with increasing exposure compared with controls (OR=0.85; 95% CI=(0.65 to 1.11)). None of the demographic covariates (race, sex and Hispanic ethnicity) were significantly associated with CBD or BeS diagnosis. However, we retained them in the model because they may influence disease progression and are correlated with genotype (online supplemental table 2).

At the mean of $\log_{10}$ lifetime-average exposure, subjects with zero E69 alleles were not significantly less or more likely to have CBD than BeS (OR=0.34; 95% CI=(0.07 to 1.68)). However, these odds increased significantly with increasing allele number (OR=1.70; 95% CI=(1.18 to 2.44)). Averaging over all E69 genotypes, every 10-fold increase in lifetime-average exposure was positively associated with CBD compared with BeS (OR=1.85; 95% CI=(1.38 to 2.48)), although there was no evidence of genotype by exposure interaction (OR=1.21; 95% CI= (0.86 to 1.74)).

Genotype and exposure quartile

The interactive effect of genotype and exposure quartile on the odds of CBD or BeS is presented in figure 3. Consistent with the results above, the log-odds of CBD and BeS compared with control are both negative among the population with no E69 alleles across all exposure quartiles. Examining results by individual quartile, the log odds of BeS compared with the controls significantly differed from zero only for the lowest quartile (Q1), and only for two E69 alleles, where the log odds are negative. Log odds of CBD versus BeS increased from Q1 to Q3/Q4 for subjects with either 1 or 2 E69 alleles.

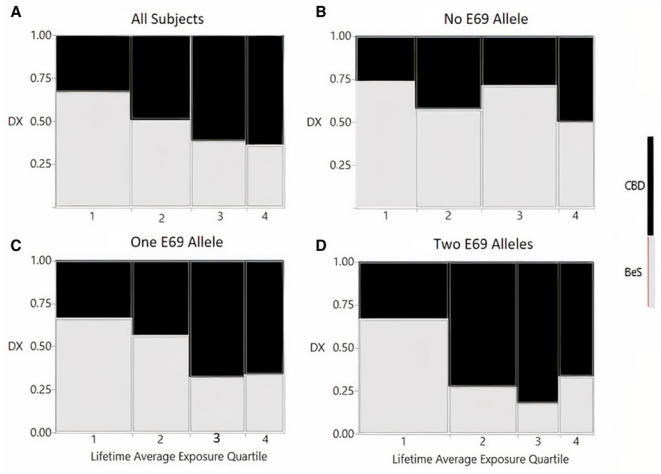

Figure 4 Subjects with CBD versus BeS by lifetime-average exposure quartile (Q1 <0.06 µg/m^3; Q2=0.06 <0.3 µg/m^3; Q3 >0.3–1.0 µg/m^3; Q4 >1 µg/m^3). Results are shown for: (A) all subjects; (B) subjects with no E69 alleles; (C) subjects with one E69 allele and (D) subjects with two E69 alleles. BeS, beryllium sensitisation; CBD, chronic beryllium disease.

Based on the results of the CBD versus BeS comparison by allele number, figure 4 shows that with increasing quartile of exposure; (a) the per cent of CBD cases compared with BeS cases increased significantly for all subjects regardless of E69 allele number ($p<0.001$); (b) for the subset of those with no E69 alleles, there were no differences in per cent CBD cases across exposure quartiles ($p=0.2553$); (c) for those with one E69 allele, the per cent of CBD increased across exposure quartiles ($p<0.0001$) and (d) for those with two E69 alleles, the per cent of CBD was significantly lower for subjects in exposure Q1 compared with the higher quartiles ($p<0.0001$).

DISCUSSION

This study examined the relationship between beryllium exposure estimates and HLA-DPB1 E69 genotypes in a combined population of four different beryllium-exposed workforces. In this largest genetic by exposure study to date, we showed increasing odds of BeS and CBD with increasing number of E69 alleles, which is consistent with prior literature.[16 19 20 30 31] Controls had significantly higher median lifetime-average exposures compared with CBD and BeS and exposures were significantly higher for CBD compared with BeS. Across all genotypes, higher exposure was associated with lower odds of BeS compared with controls, while CBD was not significantly associated with exposure compared with controls. However, the odds of CBD versus BeS increased significantly with increasing allele number and exposure but there was no evidence of genotype by exposure interaction. When examining quartiles of lifetime-average exposure, the per cent of CBD compared with BeS also increased significantly with increasing exposure quartile for the group as a whole and for subjects carrying an E69 allele.

Lack of statistically significant increased risk of CBD with increasing exposure was in contrast to other studies looking at genetic and exposure disease relationships.[19 20] A smaller study of nuclear industry workers using reconstructed exposure estimates[20] found that increasing lifetime weighted average exposure (cumulative exposure/total years worked) increased the odds of CBD compared with controls, but not BeS, regardless of E69 genotype. A similar study examining genetics and exposure[19] found that higher lifetime weighted exposure and E69 carriage were independently associated with increased odds of BeS and CBD.

The lower odds of BeS with increasing exposure may suggest that any exposure can lead to BeS but the development of CBD may be more dependent on higher lifetime exposure. We observed an increasing odds of CBD versus BeS with increasing exposure and E69 alleles, showing the effect of exposure among those who are most genetically susceptible. We found no significant exposure response among CBD and BeS subjects with no E69 alleles. However, for those who were E69 heterozygous, there was a significant increase in CBD compared with BeS with increasing exposure quartile. For homozygotes, the per cent of CBD to BeS was significantly lower in the first quartile compared with second through fourth quartiles, perhaps suggesting a threshold effect for the most susceptible.

We expected to find more evidence of an exposure response by genotype with our larger and diverse population compared with previous studies.[17 19–21] Our lack of association between increasing exposure and BeS or CBD compared with controls is most likely due to exposure misclassification which contributed to attenuation bias in the exposure response relationship. By defining a common exposure metric available for all the data sets, we may have lost more detailed exposure reconstructions for individual workforces. The use of work histories and self-reported jobs via questionnaires to calculate average and cumulative exposures and the use of industrial hygiene data from multiple time periods and facilities in the development of the specific job exposure estimates likely resulted in misclassification on a µg/m^3 scale, but less misclassification on a relative scale or between quartiles for comparing study participants. Although we made every attempt to calculate an analogous continuous measure for analysis, the differences within individual workforces in regard to beryllium work performed, time frames of exposure, available work histories and industrial hygiene sampling data may have affected the uniformity of the exposure estimates. There were different levels of accuracy and precision of data available for the different workforces as well as for different jobs and beryllium materials processed within the sites. While production jobs in all workforces had detailed industrial hygiene measurements to use in the exposure reconstructions, other job exposures such as management and administrative, had limited sampling. In addition, there were different magnitudes of exposure across workforces with the PENN group having significantly higher exposures than the other three groups. These results are not surprising as the PENN subjects came from two beryllium processing facilities,[25] that operated from the 1930s through the 2000s before Occupational Safety and Health Administration (OSHA) exposure standards. In a study of exposure and disease risk in this group,[10] the authors were unable to show an exposure response between air concentrations of beryllium and BeS or CBD. A recent study examining both exposure and genetics in this same population[21] also found no exposure response for BeS or CBD with or without adjusting for E69. The authors suggest that the exposures in the study group may have been too high to observe an exposure response relationship. These two worker groups,(PENN) previously reported,[10 21] are part of our larger population. The estimated mean lifetime weighted exposure was 26.8 µg/m^3 for the PENN group compared with less than 1.0 µg/m^3 for the other three workforces. There was the potential for disease misclassification as exposed workers can develop sensitisation or disease in less than 2 months of exposure or years after exposure ceases.[32] In addition, progression from BeS to CBD has been estimated at 6%–8% per year.[33] Subjects tested

after relatively short tenures or while still exposed in the NJH, BBB and NIOSH populations could have progressed to BeS and/or CBD after study activities were completed. However, the misclassification would be minimal as the prevalence of BeS and CBD in these study populations ranged from 2% to 15%. Finally, our lack of association between increasing exposure and CBD may be due to differential follow-up. In this study controls had significantly higher lifetime-average exposures than both BeS and CBD. Although exposures were reconstructed until the date of medical surveillance blood test for current workers in the NIOSH and NJH populations, the former workers in the PENN, NJH and BBB populations were tested after employment ended so their entire tenure was included in the exposure reconstruction. CBD cases may have left employment sooner due to undiagnosed disease while BeS and controls continued to be exposed. However, it is important to note that there were no differences in tenure between CBD and controls in any of our populations.

The new OSHA standard of 0.2 µg/m^3 as a full-shift time-weighted average[34] is a long-awaited step towards the prevention of BeS and CBD in the workplace. This study and others have shown that reducing exposure may not prevent BeS but may be able to reduce CBD and its associated health effects, especially in those most susceptible. It is noteworthy that there were 84 (22.4%) of our CBD cases with lifetime-average exposure estimates in the lowest exposure quartile, eight of whom carried no E69 alleles. In this quartile, the estimated exposures were less than 0.6 µg/m^3 suggesting that even with the lowered beryllium exposure standard not all CBD may be prevented. Future work should focus on other genetic risk factors contributing to the risk and progression of BeS and CBD such as HLA DRB1,[21 25] as well as genome wide association studies.

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