Lack of an Exposure Response and Interaction With HLA-DPβ1 and DRβ1 Polymorphisms in the Development of Beryllium Toxicity in a High Beryllium Exposure Cohort

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Objective: To evaluate interaction of HLA-DPβ1 and DRβ1 polymorphisms with metrics of beryllium exposure, in the development of beryllium sensitization (BeS) and chronic beryllium disease (CBD). **Methods:** A matched case-control study of 61 CBD, 41 BeS, and 259 controls from two beryllium-processing facilities. **Results:** BES and CBD were significantly associated with presence of DPβE69. Dose response of exposure was not observed for the development of BES and CBD with/without adjustment for DPβE69 ($P > 0.05$). The DRβE71 polymorphism was more common in BeS than CBD after adjusting for exposure and maybe a protective factor (aOR 0.4, 95% CI 0.2 to 0.9) against the progression of BeS to CBD. **Conclusion:** No exposure–response association was found, which may reflect that the workers in this high exposure cohort were above a threshold level where an exposure–response could be observed.

Keywords: beryllium, genetic–exposure interaction, human leukocyte antigen polymorphisms

On May 20, 2017, the new final occupational safety and health administration regulation on beryllium, which decreased the permissible exposure limit (PEL) to 0.2 $\mu g/m^3$ 8-hour time-weighted average, went in to effect.[1] However, there is still concern that genetically susceptible workers will not be adequately protected from developing beryllium toxicity.[2] Previous studies have shown inconsistency in the exposure–response effect of beryllium exposure on beryllium toxicity.[3–6] Higher exposure to beryllium does not always cause disease and even low exposure to or opportunistic contact with beryllium can cause sensitization and disease. Several studies reported increased risk with higher exposure to beryllium,[7–9] whereas other studies reported disease with low exposure to beryllium.[10–12] These studies have been based on job histories and assignment of certain job titles (ie, machinist) to a higher exposure category rather than to actual exposure measurements.[13] The exposure–response is complicated by the strong genetic susceptibility and the limited number of studies that have incorporated both genetics and exposure into their exposure analyses.[3–5,14] In addition, the physical form and solubility of beryllium and skin exposure may influence the development of chronic beryllium disease (CBD) and confound the ability to determine an exposure–response to beryllium air levels.[15,16]

Genetic susceptibility, particularly polymorphism DPβE69 in the HLA system, is an important risk for the development of both beryllium sensitization (BeS) and CBD.[17–19] The association of CBD and BeS with the DPβE69 polymorphism in the HLA system was first reported in 1993[20] and has been replicated in subsequent investigation across multiple different populations.[19] Further studies have shown that the non-0201 alleles of HLA-DPB1 Glu69 were important risk factors for BeS and CBD,[14,21–23] that certain genotypes such as HLA-DPB1∗02:01:02DPB1∗17:02 were at extremely high risk,[2] that HLA-DRArg74 was associated with BeS,[24] while DQ-B1-G86, DRB1-S11,[25] DRB1-S13, DQB1–06,[22] were associated with CBD, and DRB-Glu71[23] and TNF-α-308[24] were associated with both BeS and CBD. The pathophysiological effect of the genetic polymorphism has been explained as an enhancement of the presentation of the positively charged BE^{+2} to the cell and a subsequent increase in the risk of sensitization.[26]

The objective of this paper is to evaluate both the overall exposure–gene interaction and the interaction between different forms of beryllium and different beryllium metrics (ie, peak) and HLA-DPB1 Glu69 and other genetic polymorphisms in the development of BeS and CBD.

METHODS

Cohort Development

The study population consisted of workers in two beryllium-processing facilities in eastern Pennsylvania, USA. Facility A was open from 1958 to 1978, and facility B was open from 1935 to 2000. A total of 5490 individuals were identified from personnel records to have worked 2 or more days at these two facilities. The 2869 individuals not known to be dead in 1988 received a letter in 1996 to participate. A total of 1553 individuals participated in the medical screening, 560 only completed a questionnaire, 325 could not be located, 195 worked for the company but not at the beryllium processing facility and 256 declined all participation. The sample selection, HLA genotyping of DNA from white cells obtained from peripheral blood and medical examination have been described in previous publications in more detail.[23] Individuals provided their work history via a standardized questionnaire either through the mail or by phone prior to participating in the medical testing. The medical testing was provided at local hospitals near the two facilities or arrangements were made for the individual to have their blood collected for genetic analysis and their chest radiograph and

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Clinical Significance: Chronic beryllium disease (CBD) and sensitization (BeS) do not demonstrate the typical exposure response seen with other dust-related lung diseases such as asbestosis or silicosis. This paper shows that the risk of developing CBD and BeS is more dependent on the individual's genetic polymorphisms than their amount of beryllium exposure.

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spirometry performed at a medical facility located near where they currently lived. Individuals classified as definite CBD had granuloma on a lung biopsy and two positive beryllium lymphocyte proliferation tests (BeLPTs) or a positive bronchial lavage (BAL) LPT. Individuals classified as probable CBD had upper lobe fibrosis on a chest radiograph and two positive BeLPTs or a positive BAL LPT. Individuals classified as BeS had no granulomas on lung biopsy, no upper lobe fibrosis on a chest radiograph and either two positive BeLPTs or a positive BAL LPT.[23] Individuals who had HLA typing of their blood and had a normal chest radiograph, pulmonary function tests, and negative BeLPTs were eligible to be controls. The control group was matched by facility, sex, and year of birth within 5 years. Two, and if available three controls were chosen for each case. Because of the small number of women, who had worked at these facilities, no controls could be selected for two of the women who had BeS. We did not match on duration because we wished to use duration as one measure of exposure. Similarly we did not match on decade of hire since improvements in ventilation over the years this would have affected the exposure metrics between the CBD, BeS, and controls. The exposure metrics, see section below, took into account decade of hire and duration, both of which are important factors in estimating an individual's level of exposure. Complete data on medical testing, genetics, and exposure estimates were available for 61 CBD, 41 BeS, and 259 controls that were included in the final analysis. Figure 1 shows how the number of participants was derived from the overall workforce.

Exposure Metrics

A detailed description of the development of the exposure measurements has been previously described.[23,27] We used historical air sampling data and work process descriptions to develop a task exposure matrix and then a job exposure matrix to calculate a daily-weighted average for every job/year combination and finally each person was assigned a cumulative, mean, and peak exposure. Exposure metrics were also calculated by solubility and physical form.

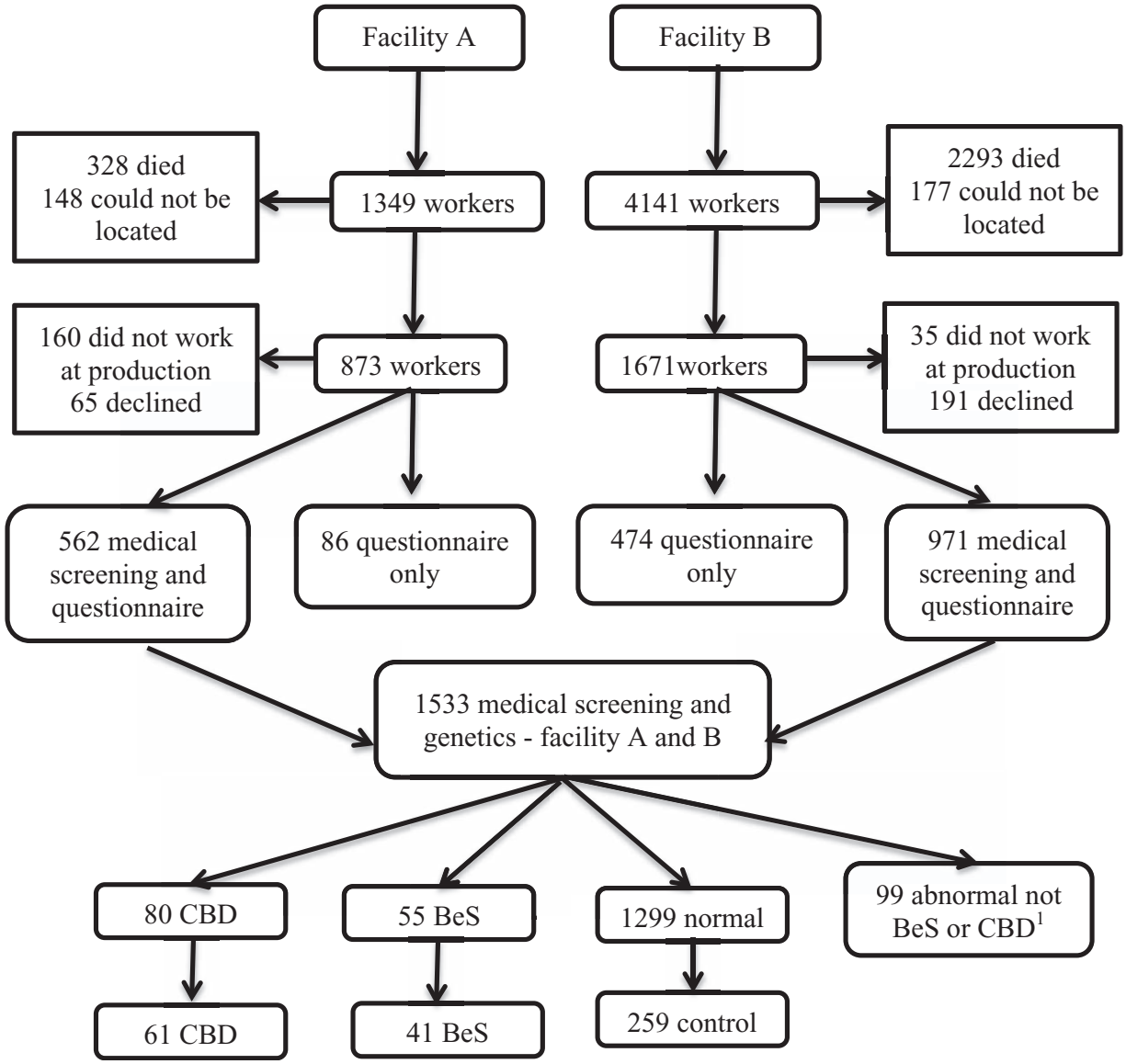

FIGURE 1. Study population and sample selection. [1]Ninety nine individuals excluded because of scarring on chest radiograph or borderline or single positive BeLPT. BeLPT, beryllium lymphocyte proliferation tests.

Genetic Testing

Genomic DNA was prepared 4 to 10 years after collection using Qiagen columns (QiaAmp 96 DNA Blood kit; Qiagen, Valencia, CA) from a venous whole blood sample that had been frozen the day after collection.[23] The DPB1 gene (exon 2 and 3) was characterized with high-resolution typing using the PCRSSP method (Pel-Freez Clinical Systems, Brown Deer, WI). For ambiguities or inconsistent patterns of primer amplifications, sequence-based typing was performed and confirmed by bi-directional sequencing-based typing of exon 2 (AlleleSEQR HLA-DPB1 SBT kit; Atria Genetics, South San Francisco, CA). Genetic analyses were performed without knowledge of the beryllium disease status of the participants.

Statistical Analysis

The non-parametric Wilcoxon rank-sum test (for two group differences) and Kruskal-Wallis tests (for multiple groups differences) were used to compare duration and type of exposure by beryllium and/or genetic status while Chi-square tests were used to compare the different genetic distributions by beryllium status. To estimate the effect of exposure and genetic polymorphisms on the risk of CBD and BeS, conditional logistic regression was performed. Analysis was also performed with the exposure levels categorized into quartiles (25th, 50th, and 75th percentiles), with the first quartile used as the reference group. This later analysis was performed to replicate the approach used by Van Dyke et al.[4] The conditional logistic regressions were done for CBD subjects and their matched controls (total of 216 subjects consisting of 61 CBD cases and 155 controls) and BeS subjects and their matched controls (total of 145 subjects consisting of 41 BeS and 104 controls).[23,27] The covariants in the three regression models were: (1) log cumulative exposure quartiles; (2) log cumulative exposure quartiles, DPβE69 and DPβE71 genetic polymorphisms, (3) log cumulative exposure quartiles, DPβE69 and DPβE71 genetic polymorphisms and interaction of DPβE71 with log cumulative exposure quartiles. The interaction between continuous values of log cumulative exposure and DPβE69, and DPβE71 genetic polymorphisms was also examined. Further analysis was also conducted for the 61 CBD and 41 BeS cases using unconditional logistic regression to assess factors influencing the development of BeS versus CBD.[23] All analyses were conducted with SAS 9.4. Software (SAS Institute Inc., Cary, NC).

RESULTS

Of the 361 subjects, 94.2% were men and 98.1% were white. There were more non-whites (4.9% vs 0.0% vs 1.9% [$P = 0.012$]) and women (9.8% vs 6.6% vs 5.0% [$P = 0.027$]) in the BeS versus the CBD or versus the control group. There were no significant differences in facility ($P = 0.585$) or history of smoking ($P = 0.873$) between the BeS, CBD, and control groups (Table 1).

Exposure

Table 2 shows the different metrics by outcome. For combined exposures, the control group had significantly longer duration and mixed cumulative exposure compared with BeS group ($P = 0.001$ and $P = 0.013$, respectively). There were also significant differences in exposures among control, BeS, and CBD groups with the highest levels in the control group, for mixed cumulative chemical exposure ($P = 0.023$) and cumulative, mean and peak mixed physical exposure ($P = 0.001$, 0.025, and 0.005, respectively) (Table 2). CBD generally had the second highest and BeS the lowest exposure metrics. However, individuals with BeS had higher mean and peak values for mixed physical exposure and dust exposure than those with CBD, though not statistically significant ($P > 0.05$).

Genetics

The DPβE69 genetic polymorphism frequency differed significantly between BeS, CBD, and Controls ($P < 0.001$) (Table 3). Individuals with CBD had the highest proportion of DPβE69 (91.8%), followed by those with BeS (78.1%). Thirty-nine percent of control individuals who tested positive for DPβE69 were not sensitized and remained disease free (Table 3). On further pair-wise comparisons, there were significant differences in the proportion of DPβE69 when comparing BeS versus CBD, BeS versus control, and CBD versus control. In Hardy-Weinberg Equilibrium (HWE) analyses, the frequencies of DPβE69 homozygotes and heterozygotes within the control subjects were in equilibrium.

The distributions of DPβE69 non-0201 alleles were significantly different between the three outcome groups ($P = 0.017$) as was the presence of homozygosity of DPβE69 ($P < 0.001$).

TABLE 1. Comparison of Demographic Characteristics Among Subjects With Chronic Beryllium Disease, Beryllium Sensitization, and Controls

Characteristics *N* (%)	CBD *N* = 61	BeS *N* = 41	Control *N* = 259	Total *N* = 361	*P* Value
Gender					0.0267*
Male	57 (93.4)	37 (90.2)	246 (95.0)	340 (94.2)	
Female	4 (6.6)	4 (9.8)	13 (5.0)	21 (5.8)	
Race					0.0122*
White	61 (100)	39 (95.1)	254 (98.1)	354 (98.1)	
Other	0 (0)	2 (4.9)	5 (1.9)	7 (1.9)	
Plant					0.5847
Plant 1	28 (45.9)	23 (56.1)	133 (51.3)	184 (51.0)	
Plant 2	33 (54.1)	18 (43.9)	129 (48.7)	177 (49.0)	
Smoking					0.8732
Never	20 (32.8)	16 (39.0)	78 (30.1)	114 (31.6)	
Ex-smoker	28 (45.9)	19 (46.3)	126 (48.6)	173 (47.9)	
Current smoker	10 (16.4)	5 (12.2)	38 (14.7)	53 (14.7)	
Unknown	3 (4.9)	1 (2.4)	17 (6.6)	21 (5.8)	
Total	61 (17.0)	41 (11.4)	262 (72.6)	361 (100)	

Comparison conducted by chi-square.
*Comparison conducted by Fisher method.

TABLE 2. Comparison of Magnitude and Type of Exposures Between CBD, BeS, and Control Groups

Exposure		Mean (SD)				*P* Value			
		CBD $N=61$	BeS $N=41$	Control $N=259$	Total $N=361$	Overall**	CBD vs BeS	CBD vs Control	BeS vs Control
Duration in years		8.98 (10.37)	5.49 (8.17)	12.19 (12.33)	10.87 (11.81)	**0.001**	0.102	0.056	**0.001**
Exposure metric									
All forms	Cumulative (μg-yr/m^3)	291.40 (708.91)	145.54 (302.10)	743.99 (2137.37)	599.55 (1849.99)	**0.024**	0.293	0.126	**0.013**
	Log cumulative (μg-yr/m^3)	4.13 (1.87)	3.60 (1.98)	4.53 (2.22)	4.36 (2.15)	**0.024**	0.293	0.126	**0.013**
	Mean (μg/m^3)	7.66 (20.59)	8.17 (17.89)	7.32 (16.41)	7.47 (17.30)	0.493	0.482	0.234	0.905
	Peak (μg/m^3)	14.26 (31.05)	11.39 (22.09)	28.40 (132.70)	24.08 (113.51)	0.148	0.918	0.086	0.230
Type of exposure: chemical									
Soluble (beryllium fluoride and hydroxide)	Cumulative (μg-yr/m^3)	6.11 (39.81)	4.09 (10.23)	53.03 (298.64)	39.54 (254.27)	0.146	0.290	0.057	0.290
	Mean (μg/m^3)	0.11 (0.38)	0.19 (0.54)	0.50 (4.73)	0.40 (4.02)	0.321	0.590	0.127	0.742
	Peak (μg/m^3)	1.77 (11.47)	0.66 (1.23)	3.58 (20.83)	2.94 (18.28)	0.237	0.492	0.092	0.636
Non-soluble (beryllium metal and oxide)	Cumulative (μg-yr/m^3)	85.49 (228.73)	71.91 (169.96)	133.80 (383.62)	118.61 (343.51)	0.154	0.608	0.079	0.517
	Mean (μg/m^3)	1.24 (4.68)	1.25 (2.69)	1.19 (3.02)	1.21 (3.31)	0.483	0.313	0.271	0.879
	Peak (μg/m^3)	3.51 (14.16)	2.86 (4.03)	4.61 (10.92)	4.23 (11.00)	0.059	0.175	0.018	0.907
Chemical mixed (soluble/non-soluble)	Cumulative (μg-yr/m^3)	199.76 (650.69)	69.54 (167.73)	547.65 (1904.53)	434.57 (1645.28)	**0.023**	0.314	0.131	**0.013**
	Mean (μg/m^3)	6.62 (20.88)	6.81 (18.22)	5.72 (15.65)	6.00 (16.89)	0.215	0.377	0.258	0.136
	Peak (μg/m^3)	11.54 (28.81)	9.54 (22.60)	26.20 (132.44)	21.83 (113.20)	0.060	0.222	0.131	**0.042**
Type of exposure: physical									
Mixed (dust/fume)	Cumulative (μg-yr/m^3)	49.81 (125.21)	31.29 (108.82)	198.92 (778.43)	154.68 (665.73)	**0.001**	0.368	**0.011**	**0.001**
	Mean (μg/m^3)	0.97 (3.11)	2.79 (12.78)	1.84 (8.74)	1.80 (8.64)	**0.025**	0.745	**0.044**	**0.037**
	Peak (μg/m^3)	4.77 (15.19)	5.65 (19.33)	8.06 (21.09)	7.23 (20.01)	**0.005**	0.646	**0.019**	**0.011**
Dust (beryllium metal, hydroxide, or oxide)	Cumulative (μg-yr/m^3)	105.79 (355.72)	54.08 (175.84)	178.16 (653.42)	151.84 (576.59)	0.569	0.589	0.650	0.310
	Mean (μg/m^3)	4.03 (13.46)	4.74 (13.52)	3.97 (13.55)	4.06 (13.50)	0.901	0.984	0.706	0.755
	Peak (μg/m^3)	7.66 (19.86)	9.00 (18.65)	19.33 (122.88)	16.18 (104.65)	0.063	0.190	**0.019**	0.677
Fume (beryllium fluoride)	Cumulative (μg-yr/m^3)	95.18 (385.64)	10.67 (30.60)	277.07 (1446.64)	216.08 (1238.81)	0.146	0.839	0.091	0.223
	Mean (μg/m^3)	2.52 (16.82)	0.20 (0.53)	1.08 (5.54)	1.22 (8.34)	0.379	0.577	0.170	0.650
	Peak (μg/m^3)	7.14 (25.52)	1.24 (3.36)	12.11 (54.73)	10.04 (47.64)	0.140	0.618	0.076	0.263

BeS, beryllium sensitization; CBD, chronic beryllium disease. *P* values in bold are <.05

*Comparison was conducted by the Wilcoxon rank-sum test or Kruskal Wallis non-parametric test.

**Three groups comparison of CBD, BeS, and controls.

TABLE 3. Comparison of Gene Distribution Between CBD, BeS, and Control

Gene	Frequency (%)				*P* Value*			
	CBD $N=61$	BeS $N=41$	Control $N=259$	Total $N=361$	Overall†	CBD vs BeS	CBD vs Control	BeS vs Control
DPβ E69								
Positive	56 (91.8)	32 (78.1)	101 (39.0)	189 (52.4)	**<0.0001**	**0.048**	**<0.001**	**<0.001**
Negative	5 (8.2)	9 (21.9)	158 (61.0)	172 (47.6)				
DPβ E69 homozygosity								
Homozygous	10 (16.4)	8 (19.5)	17 (6.6)	35 (9.7)	**<0.0001**	0.104	**<0.001**	**<0.001**
Heterozygous	46 (75.4)	24 (58.5)	84 (32.4)	154 (42.7)				
Negative	5 (8.2)	9 (22.0)	158 (61.0)	172 (47.6)				
DPβ E69–0201 alleles								
0201 alleles	28 (45.9)	17 (41.5)	72 (27.8)	117 (32.4)	**0.017**	0.136	**<0.001**	**<0.001**
Non-0201 alleles	28 (45.9)	15 (36.6)	29 (11.2)	72 (20.0)				
DPβE69 negative	5 (8.2)	9 (21.9)	158 (61.0)	172 (47.6)				
DRβ E71								
Positive	16 (26.2)	20 (48.8)	60 (23.2)	96 (26.6)	**0.003**	**0.020**	0.613	**0.001**
Negative	45 (73.8)	21 (51.2)	199 (76.8)	265 (73.4)				
DRβ Serine 11								
Positive	44 (72.1)	32 (78.1)	174 (67.2)	250 (69.3)	0.325	0.501	0.456	0.163
Negative	17 (27.9)	9 (21.9)	85 (32.8)	111 (30.7)				
DRβ Serine 13								
Positive	38 (62.3)	30 (73.2)	162 (62.6)	230 (63.7)	0.408	0.253	0.971	0.188
Negative	23 (37.70)	11 (26.8)	97 (37.4)	131 (36.3)				
DRβ Arginine 74								
Positive	10 (16.4)	5 (12.2)	47 (18.1)	62 (17.2)	0.634	0.557	0.748	0.350
Negative	51 (83.6)	36 (87.8)	212 (81.9)	299 (62.8)				
DRβ Asparagine 37								
Positive	18 (29.5)	20 (48.8)	94 (36.3)	132 (36.6)	0.138	0.048	0.318	0.126
Negative	45 (70.5)	21 (51.2)	165 (63.7)	229 (63.4)				
DRβ Histidine 32								
Positive	27 (44.3)	21 (51.2)	114 (44.0)	162 (44.9)	0.686	0.490	0.972	0.389
Negative	34 (55.7)	20 (48.8)	145 (56.0)	199 (55.1)				
DRβ Phenyl alanine 47								
Positive	46 (75.4)	30 (73.2)	196 (75.7)	272 (75.3)	0.942	0.799	0.965	0.730
Negative	15 (24.6)	11 (26.8)	63 (24.3)	89 (24.7)				
DRβ Tyrosine 26								
Positive	10 (16.4)	5 (12.2)	50 (19.3)	65 (18.0)	0.511	0.557	0.600	0.274
Negative	51 (83.6)	36 (87.8)	209 (80.7)	296 (82.0)				

BeS, beryllium sensitization; CBD, chronic beryllium disease *P* values in bold are <.05
*Comparisons conducted by Chi Squares tests.
†Three groups comparison of CBD, BeS, and controls.

However, there were no statistically significant differences between CBD and BeS in the presence of the alleles or zygosity.

DRβE71 genetic polymorphism frequency also differed significantly among the three groups ($P=0.003$) (Table 3). There were significant differences in the proportion of DRβE71 polymorphism between BeS and CBD (48.8% vs 26.2%, $P=0.020$), and BeS and controls (48.8% vs 26.6%, $P=0.001$).

Genetic–Exposure Interaction

Table 4 shows the descriptive levels of exposure among CBD, BeS, and control individuals by their DPβE69status. Although not significant, control subjects who were DPβE69 positive, had had a lower exposure than control subjects who were DPβE69 negative. In individuals with CBD and BeS, exposure metrics were in the opposite direction with exposure having been non-significantly greater in those who were DPβE69 positive versus DPβE69 negative. Table 5 shows that control individuals with the non-0201 allele had had a non-significant but higher exposure than those with the 0201 alleles. And CBD and BeS individuals with the non-0201 allele had had lower non-significant cumulative but not mean or peak exposure compared with those with 0201 alleles (Table 5).

When we restricted the analysis to the 189 subjects who were positive for DPβE69 (Table 6), controls continued to have longer duration of exposure followed by CBD and BeS ($P=0.022$). Individuals with CBD had higher non-significant exposures compared with individuals with BeS, particularly in the cumulative and peak measures of exposures.

We found no significant associations between CBD and level of exposure either categorized by quartiles (Table 7, Model 1). In the adjusted analysis, Model 2, adjustment for presence of DPβE69 did not change the relationship between level of exposure and CBD, however, DPβE69 was a significant predictor for CBD, (adjusted odds ratio [aOR] 28.6, 95% CI 6.7 to 121.5, $P<0.001$). There was no significant association between the DRβE71 polymorphism and CBD (aOR 1.7, 95% CI 0.8 to 3.7, $P=0.203$) adjusting for log cumulative exposure and DPβE69 (Table 7, Model 2).

Both the DPβE69 and DRβE71 polymorphisms were significantly associated with BeS (aOR 7.8, 95% CI 2.7 to 22.4, $P<0.001$; and aOR 2.6, 95% CI 1.1 to 6.2, $P=0.035$, respectively) (Table 7, Model 2). For the risk of developing BeS versus CBD, the presence of DRβE71 was a significant protective factor (aOR 0.4, 95% CI 0.2 to 0.9, *P* 0.024) adjusting for log cumulative exposure (Table 7, Model 2). Exposure was not associated with development

TABLE 4. Comparison of Cumulative, Log Cumulative, Mean, and Peak Exposure Between CBD, BeS, and Control Groups Based on HLA-DPβE69 Presence

Outcome	Exposure	*N*	HLA-DPβE69	Mean (SD)	*P* Value*
CBD	Cumulative	56	Positive	307.65 (737.52)	0.969
		5	Negative	109.36 (109.36)	
	Log Cumexp	56	Positive	4.15 (1.89)	0.969
		5	Negative	3.94 (1.77)	
	Mean	56	Positive	8.16 (21.42)	0.752
		5	Negative	2.10 (2.13)	
	Peak	56	Positive	15.28 (32.23)	0.703
		5	Negative	2.92 (2.43)	
BeS	Cumulative	32	Positive	166.95 (337.12)	0.306
		9	Negative	69.40 (87.12)	
	Log Cumexp	32	Positive	3.76 (1.98)	0.306
		9	Negative	3.03 (1.97)	
	Mean	32	Positive	9.43 (19.79)	0.181
		9	Negative	3.67 (7.36)	
	Peak	32	Positive	13.42 (24.44)	0.171
		9	Negative	4.20 (7.20)	
Control	Cumulative (μg-yr/m^3)	101	Positive	312.17 (759.35)	0.406
		158	Negative	1020.03 (2635.02)	
	Log Cumexp	101	Positive	4.27 (2.10)	0.406
		158	Negative	4.70 (2.28)	
	Mean	101	Positive	6.83 (16.25)	0.720
		158	Negative	7.62 (16.55)	
	Peak	101	Positive	11.84 (22.37)	0.450
		158	Negative	38.98 (168.32)	

Exposures metrics are expressed as μg-yr/m^3 unit for cumulative and Log Cumexp and μg/m^3 for mean and peak. BeS, beryllium sensitization; CBD, chronic beryllium disease.
*Comparison was conducted by Wilcoxon rank-sum test.

of CBD versus BeS nor was there a significant interaction between log cumulative exposure and DRβE71 ($P > 0.05$).

Table 7 (Model 3) showed the regression results with gene and log cumulative exposure quartiles interaction terms added. The BeS and CBD patients in the highest log cumulative exposure quartile (more than or equal to 5.76 μg/m^3) were all DPβE69 positive. Therefore, we could only examine DPβE71 and exposure interaction terms. In supplementary Table 1, http://links.lww.com/JOM/A668 we examined the DPβE69 and DPβE71 polymorphisms and exposure interaction using exposure as a continuous variable.

In Supplementary Tables 2–4, http://links.lww.com/JOM/A668, the results are shown for DPβE69 negative, DRβE71 positive, and DRβE71 negative polymorphisms, and the different measures of exposure, respectively. The results were non-significant except for those related to mixed dust/fume exposure (Supplemental Table 4, http://links.lww.com/JOM/A668).

DISCUSSION

Our results continue to show the importance of DPβE69 presence, alleles, and zygosity in the development of beryllium toxicity. In addition, we demonstrated the significance of DRβE71 in reducing the likelihood the development of BeS progression to CBD. We were unable to identify a significant exposure–response association of beryllium exposure for the development of CBD or BeS even after controlling for the DPβE69 and DRβE71 polymorphisms. The mean levels of exposure in our cohort were above the new occupational safety and health administration PEL of 0.2 μg/m^3 8-hour time-weighted average and also generally above the previous PEL of 2.0 μg/m^3 8-hour time-weighted average. It is possible that above a certain threshold level of exposure that an exposure–response cannot be identified. Additionally, no effort to ensure skin protection was used at the two facilities in the cohort and we cannot factor in the amount of skin exposure into the exposure analysis.

Suggestive interactions between genetics and exposure that we did observe were that despite having the highest exposure, control individuals without either the DPβE69 or DRβE71 polymorphisms remained free of CBD and BeS. Additionally, we observed non-significant differences in exposures in individuals with positive DPβE69 and DRβE71. Those with CBD generally had higher combined, chemical, and physical cumulative exposures compared with those with BeS. However, the gene–exposure interaction terms were generally non-significant except for DRβE71 and mixed dust exposure (Table 7 and Supplementary Tables 2–4, http://links.lww.com/JOM/A668). Although our analysis was conducted using one of the largest cohorts (361 subjects) to date for examining the effect of beryllium exposure and genetics, the lack of statistical significance for a dose–response relationship with or without adjustment for genetics, may be attributable to insufficient sample size. Our calculation showed that to detect a significant difference in the prevalence of CBD at different levels of exposure defined in our paper (less than 2.78, 2.78 to less than 4.44, 4.44 to less than5.76, more than or equal to 5.76) with 80% power, would require 679 individuals with CBD and 228 with BeS in each exposure level.

Consistent with a previous report on this cohort, the highest exposure metrics were found in control individuals, followed by CBD and BeS.[27] This mainly reflects a longer duration of work for the controls as the mean exposures, although greater in controls than individuals with CBD or BeS, did not differ as much as the cumulative values.

Several previous studies have reported that higher exposure to beryllium does not always cause disease and that even low exposure to or opportunistic contact with beryllium can cause sensitization and even disease.[10–12,28,29] The results in our paper agree with the lack of a dose–response as reported in the above studies.[30–32] We could not corroborate the findings reported by Van Dyke et al[4] of an exposure–response association in the development

TABLE 5. Comparison of Cumulative, Log Cumulative, Mean, and Peak Exposure Between CBD, BeS, and Control Groups Based on HLA-DPβE69 Allele Type

Outcome	Exposure	*N*	HLA-DPβE69	Mean (SD)	*P* Value*
CBD	Cumulative, μg-yr/m^3	28	Non-0201	176.31 (483.19)	0.185
		28	0201	438.99 (915.84)	
		5	Negative	109.36 (118.91)	
	Log Cumexp	28	Non-0201	3.69 (1.79)	0.185
		28	0201	4.61 (1.90)	
		5	Negative	3.94 (1.77)	
	Mean, μg/m^3	28	Non-0201	10.27 (28.03)	0.947
		28	0201	6.05 (11.83)	
		5	Negative	2.10 (2.13)	
	Peak, μg/m^3	28	Non-0201	18.43 (39.74)	0.582
		28	0201	12.12 (22.71)	
		5	Negative	2.92 (2.43)	
BeS	Cumulative	15	Non-0201	132.27 (141.99)	0.315
		17	0201	197.55 (447.69)	
		9	Negative	69.40 (87.12)	
	Log Cumexp	15	Non-0201	4.03 (1.90)	0.315
		17	0201	3.52 (2.07)	
		9	Negative	3.03 (1.97)	
	Mean	15	Non-0201	12.91 (24.7)	0.327
		17	0201	6.36 (14.26)	
		9	Negative	3.67 (7.46)	
	Peak	15	Non-0201	14.68 (25.50)	0.374
		17	0201	12.31 (24.20)	
		9	Negative	4.20 (7.20)	
Control	Cumulative	29	Non-0201	407.15 (1036.79)	0.411
		72	0201	273.91 (618.90)	
		158	Negative	1020.03 (2635.02)	
	Log Cumexp	29	Non- 0201	4.60 (2.07)	0.411
		72	0201	4.14 (2.11)	
		158	Negative	4.70 (2.28)	
	Mean	29	Non-0201	8.15 (20.56)	0.600
		72	0201	6.30 (14.29)	
		158	Negative	7.62 (16.55)	
	Peak	29	Non- 0201	13.27 (23.32)	0.334
		72	0201	11.26 (22.12)	
		158	Negative	38.98 (168.32)	

Exposures metrics are expressed as μg-yr/m^3 unit for cumulative and Log Cumexp and μg/m^3 for mean and peak. BeS, beryllium sensitization; CBD, chronic beryllium disease.
*Comparison was conducted by Kruskal Wallis non-parametric test.

of CBD and BeS. Possible explanations for the difference are: (1) the mean beryllium exposure in our cohort were higher (0.79, 1.59, and 3.81 vs 0.001, 0.03, and 0.17 μg/m^3 for 25th, 50th, and 75th percentiles, respectively) than in Van Dyke's cohort; and/or (2) our exposure metrics were based on job personnel records and collected well before medical examinations were conducted to determine disease status. In contrast, Van Dyke assigned exposures based on a job history obtained via an interview of the subject, which was conducted after the medical examination. This approach may have introduced recall bias and potential exposure misclassification[4,27]; and (3) we assigned controls through exact matching based on sex, plant, and year of birth, while Van Dyke et al[4,5] assigned controls through frequency matching based on sex, race, work status, and decade of hire.

As previously reported CBD group had the highest proportion of DPβE69 but the highest proportion of homozygosity occurred in individuals with BeS[14,21] and there was a higher proportion of DPβE69 non-0201 alleles in CBD and BeS cases compared with controls.[14,21,26] Control individuals with DPβE69 non-0201 alleles had higher exposures than those with DPβE69 0201 alleles the opposite of what we expected since DPβE69 non-0201 alleles, are more common in individuals with CBD and BeS (Table 3).

The importance of DRβE71 in beryllium toxicity, in accordance with a previous report on this cohort, was reconfirmed in this analysis.[23] There was a significantly higher proportion of DRβE71 in individuals with BeS cases (48.8%) compared with CBD and controls (proportions respectively 26.2% and 23.2%). In further analyses, we also found that all diseased individuals (CBD and BeS) had either or both DPBβE69 or DRβE71 polymorphisms, compared with only 32.4% of controls.

Controlling for genetic susceptibility, we observed that among those positive for DPβE69 there was a non-statistically significant trend suggesting an exposure–response in regard to peak exposure (Table 4). Previous reports have suggested the importance of the type of beryllium exposure in the development of CBD and BeS, as well as progression to CBD in sensitized individuals.[15,19] We found no significant difference by exposure, that is, peak exposure, peak chemical mixed, and peak soluble. However, we did find that CBD individuals generally had higher metrics of exposure compared with BeS subjects, which suggests an exposure–response for the development of CBD from BeS (Table 4).

Although we found no significant association of exposure with disease development and no significant genetic and exposure interactions in our multivariable model, individuals who were

TABLE 6. Comparison of Magnitude and Type of Exposures Between CBD, BeS, and Control Groups Based on Individuals With *DPβE69* Positive

Exposure		Mean (SD)				*P* Value*			
		CBD $N=56$	BeS $N=32$	Control $N=101$	Total $N=189$	Overall**	CBD vs BeS	CBD vs Control	BeS vs Control
Duration in years		9.18 (10.71)	5.08 (8.16)	11.51 (12.22)	9.73 (11.38)	**0.022**	0.088	0.274	**0.006**
Exposure metric									
All forms	Cumulative (μg-yr/m^3)	307.65 (737.52)	166.95 (337.12)	312.17 (759.35)	286.24 (698.21)	0.333	0.576	0.378	0.159
	Log cumulative (μg-yr/m^3)	4.15 (1.89)	3.76 (1.98)	4.27 (2.10)	4.15 (2.02)	0.333	0.576	0.378	0.159
	Mean (μg/m^3)	8.16 (21.42)	9.43 (19.79)	6.83 (16.25)	7.67 (18.45)	0.474	0.230	0.410	0.557
	Peak (μg/m^3)	15.28 (32.23)	13.42 (24.44)	11.84 (22.37)	13.12 (25.90)	0.616	0.603	0.322	0.860
Type of exposure: chemical									
Soluble (beryllium fluoride and hydroxide)	Cumulative (μg-yr/m^3)	1.10 (3.01)	4.28 (10.37)	18.53 (66.87)	10.95 (49.66)	0.337	0.316	0.948	0.866
	Mean (μg/m^3)	0.07 (0.22)	0.16 (0.42)	0.12 (0.35)	0.11 (0.33)	0.396	0.562	0.890	0.587
	Peak (μg/m^3)	1.87 (11.96)	0.65 (1.20)	0.58 (1.19)	0.98 (6.57)	0.390	0.456	0.846	0.760
Non-soluble (beryllium metal and oxide)	Cumulative (μg-yr/m^3)	91.20 (237.73)	84.56 (189.00)	114.29 (427.32)	102.41 (346.01)	0.533	0.354	0.310	0.977
	Mean (μg/m^3)	1.32 (4.87)	1.53 (2.98)	1.36 (2.75)	1.38 (3.53)	0.245	0.117	0.380	0.230
	Peak (μg/m^3)	3.69 (3.69)	3.49 (4.32)	3.44 (4.77)	3.52 (8.89)	0.066	**0.032**	0.061	0.471
Mixed (soluble/non-soluble)	Cumulative (μg-yr/m^3)	215.31 (677.34)	78.12 (184.61)	175.27 (612.94)	170.69 (584.59)	0.475	0.178	0.192	0.229
	Mean (μg/m^3)	7.10 (21.74)	7.84 (20.22)	5.37 (16.18)	6.30 (18.60)	0.741	0.238	0.259	0.438
	Peak (μg/m^3)	12.45 (29.91)	11.25 (25.12)	10.05 (22.90)	10.96 (25.41)	0.620	0.211	0.236	0.333
Type of exposure: physical									
Mixed (dust/fume)	Cumulative (μg-yr/m^3)	53.06 (130.02)	31.86 (115.96)	116.65 (399.76)	83.45 (305.76)	0.095	0.366	0.204	**0.042**
	Mean (μg/m^3)	0.96 (3.18)	2.73 (14.00)	2.42 (12.25)	2.04 (10.75)	0.174	0.629	0.195	0.100
	Peak (μg/m^3)	5.10 (15.81)	6.33 (21.57)	5.25 (17.09)	5.39 (17.47)	0.250	0.511	0.328	0.117
Dust (beryllium metal, hydroxide, or oxide)	Cumulative (μg-yr/m^3)	115.23 (370.04)	69.23 (197.02)	100.34 (468.13)	99.48 (404.05)	0.752	0.622	0.775	0.460
	Mean (μg/m^3)	4.39 (14.00)	6.06 (15.09)	3.34 (10.74)	4.11 (12.54)	0.929	0.806	0.709	0.970
	Peak (μg/m^3)	8.21 (20.65)	11.36 (20.55)	8.16 (16.75)	8.72 (18.58)	**0.050**	**0.020**	0.080	0.267
Fume (beryllium fluoride)	Cumulative (μg-yr/m^3)	97.05 (401.09)	9.96 (31.47)	34.71 (145.23)	48.99 (243.93)	0.893	0.764	0.641	0.957
	Mean (μg/m^3)	2.69 (17.55)	0.15 (0.41)	0.57 (3.67)	1.13 (9.92)	0.738	0.574	0.458	0.887
	Peak (μg/m^3)	7.70 (26.58)	1.33 (3.75)	3.22 (12.57)	4.23 (17.28)	0.876	0.752	0.612	0.949

BeS, beryllium sensitization; CBD, chronic beryllium disease. *P* values in bold are <.05

*Comparison was conducted by the Wilcoxon rank-sum test or Kruskal Wallis non-parametric test.

**Three groups comparison of CBD, BeS, and controls.

TABLE 7. Multivariable Conditional Logistic Regression Using Log Cumulative Exposure Quartiles

	Model 1		Model 2		Model 3	
Variable	**OR (95% CI)**	***P*-Value**	**OR (95% CI)**	***P*-Value**	**OR (95% CI)**	***P*-Value**
Development of CBD*						
Exposure						
Log cumulative exposure <2.78	Ref		Ref			
2.78 ≤ Log cumulative exposure < 4.44	1.2 (0.5–2.9)		0.8 (0.3–2.5)		0.8 (0.2–2.6)	0.66
4.44 ≤ Log cumulative exposure < 5.76	1.1 (0.4–2.7)	0.490	1.1 (0.4–3.1)	0.847	1.2 (0.4–3.7)	0.80
Log cumulative exposure ≥ 5.76	0.6 (0.2–1.5)		0.7 (0.2–2.0)		1.0 (0.3–3.6)	0.94
Genetic						
DPβE69 positive (Ref = negative)			**28.6 (6.7–121.5)**	**<.001**	**34.7 (7.5–161.2)**	**<0.001**
DRβE71 positive (Ref = negative)			1.7 (0.8–3.7)	0.203	3.3 (0.2–43.5)	0.37
Genetic* exposure						
DRβE71 Positive and 2.78 ≤ Log cumulative exposure < 4.44					1.0 (0.1–19.77)	0.99
DRβE71 positive and 4.44 ≤ Log cumulative exposure < 5.76					0.4 (0–9.2)	0.60
DRβE71 positive and log cumulative exposure ≥ 5.76					0.2 (0–4.6)	0.30
Development of BeS*						
Exposure						
Log cumulative exposure <2.78	Ref		Ref			
2.78 ≤ Log cumulative exposure < 4.44	0.8 (0.2–2.6)		0.6 (0.1–2.7)		1.6 (0.3–8.5)	0.60
4.44 ≤ Log cumulative exposure < 5.76	0.8 (0.3–2.1)	0.233	0.8 (0.2–2.7)	0.403	1.2 (0.2–6.5)	0.82
Log cumulative exposure ≥ 5.76	0.3 (0.1–1.0)		0.3 (0.1–1.2)		0.4 ((0.1–2.4)	0.29
Genetic						
DPβE69 positive (Ref = negative)			**7.8 (2.7–22.4)**	**<0.001**	**9.1 (3.0–27.4)**	**<0.001**
DRβE71 positive (Ref = negative)			**2.6 (1.1–6.2)**	**0.035**	**7.2 (1.3–39.1)**	**0.02**
Genetic* exposure						
DRβE71 positive and 2.78 ≤ Log cumulative exposure < 4.44					0.1 (0–1)	0.05
DRβE71 positive and 4.44 ≤ Log cumulative exposure < 5.76					0.4 (0–4.6)	0.48
DRβE71 positive and log cumulative exposure ≥ 5.76					0.4 (0–6.8)	0.53
Development of BeS vs CBD†						
Exposure						
Log cumulative exposure <2.78	Ref		Ref		Ref	
2.78 ≤ Log cumulative exposure < 4.44	1.6 (0.5–4.5)		1.5 (0.5–4.5)		0.8 (0.2–3.0)	0.73
4.44 ≤ Log cumulative exposure < 5.76	1.2 (0.4–3.3)	0.715	1.2 (0.4–3.6)	0.857	1.1 (0.3–4.6)	0.89
Log cumulative exposure ≥ 5.76	1.9 (0.5–6.9)		1.6 (0.4–5.9)		1.2 (0.2–6.4)	0.80
Genetic						
DRβE71 positive			**0.4 (0.2–0.9)**	**0.024**	**0.2 (0–0.9)**	**0.04**
Genetic* exposure						
DRβE71 Positive and *2.78* ≤ Log cumulative exposure < 4.44					6.7 (0.6–70.1)	0.11
DRβE71 Positive and 4.44 ≤ Log cumulative exposure < 5.76					1.4 (0.1–13.7)	0.79
DRβE71 Positive and log cumulative exposure ≥ 5.76					3.3 (0.2–51.7)	0.40

Model 1 included log cumulative exposure quartiles. Model 2 included log cumulative exposure quartiles, *DPβE69* and *DRβE71*. Model 3 included log cumulative exposure quartiles, DPβE69, DRβE71, and DRβE71× log cumulative exposure quartiles. *P* values in bold are <.05

*Indicates conditional logistic regression and.

†Indicates unconditional logistic regression.

negative for DPBβE69 had non-statistically significant higher cumulative exposure than controls that carried the DPBβE69 polymorphism. This result is consistent with the absence of this susceptibility polymorphism being protective for beryllium toxicity and explains the significantly higher cumulative exposure in controls.

We found that the DRβE71 polymorphism had the possibility of being protective in the progression of BeS to CBD. This association should be examined in a larger cohort, and consideration be given to a possible mechanism. Both homozygosity of DPBβE69[22,33] and longer duration or higher exposures have been identified as possibly increasing the risk of progressing from BeS to CBD.[34,35]

Our results imply that there are other important factors in addition to the magnitude and type of exposure and DPBβE69 and DRβE71 polymorphisms. Other factors that might influence the interaction of exposure and genetics are the structural changes in peptides and protein related to beryllium exposure and an autoimmunity process[36,37] and/or the local environment of the epitopes.[25,38]

Limitations of our study include potential measurement error of exposure based on the use of limited historical exposure sampling data and no assessment of exposure from skin absorption. Another limitation of our study was the potential differences between the two facilities. Our analyses showed that both the type and levels of

exposures in facility B were substantially higher and that employees at facility B had significantly higher cumulative, mean, and peak exposure compared with facility A.[39] The longer duration, different production process, and earlier starting of operations at facility B likely contributed to the different exposure levels, as industrial hygiene controls were improved over time. To address these differences, we matched cases with controls within each facility to control for any effect specific to one facility. All workers, whether or not they still worked were invited to participate. Therefore workers who left work because of beryllium related changes were still invited to participate. However the healthy worker effect may have still been a factor since 47.7% of the workers had died before our medical screening was initiated so those workers who died from CBD prior to the initiation of our medical screening would not have been included. A final limitation is the sample size of our cohort.

CONCLUSION

Although our results show no clear exposure–response association between the magnitude or type of exposure and beryllium disease, we found that the control individuals with the highest exposure are those who did not have the HLA-DPβE69 polymorphism and may remain healthy because they are not genetically susceptible (Table 3B). In addition, we found that HLA-DRβE71 was increased in those with BeS and may decrease the risk of progressing from BeS to CBD. The significance of DRβE71 in the development of beryllium toxicity especially in the absence of DPβE69, as well as the role of this polymorphism in decreasing the risk of progressing from CBD to BeS, warrants further research.

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