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# Immunogenetic factors in beryllium sensitization and chronic beryllium disease

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**Abstract**

Exposure to beryllium in the workplace can cause beryllium sensitization and chronic beryllium disease. Sensitization to beryllium can be detected in the laboratory using the beryllium lymphocyte proliferation test. It was shown that anti-HLA antibodies could block the beryllium-specific response in the beryllium lymphocyte proliferation test, thereby implicating HLA genes in chronic beryllium disease. A supratypic genetic marker, *HLA-DPB1*E69*, was found to be strongly associated with immunologic sensitization to beryllium and chronic beryllium disease in beryllium workers. However, there are 40 *HLA-DPB1* gene variants that have E69 but that also have other DNA sequence variations. The purpose of the study was to evaluate the evidence for potential differential susceptibility that may be associated with the physical characteristics of HLA protein molecules for which different *HLA-DPB1*E69* variants code; that is, do some *HLA-DPB1*E69* variants convey higher risk of beryllium sensitization and chronic beryllium disease than others. To do this, two approaches were pursued: first, detailed analysis of the findings from the published literature was performed, and second, computational chemistry was used to seek clues concerning the physical properties of the HLA protein molecules for which these alleles code. Among the 40 *HLA-DPB1* gene variants that code for E69, molecular epidemiological studies have suggested a risk hierarchy, where some variants appear to convey low to moderate risk of chronic beryllium disease (e.g., *HLA-DPB1*0201*, ~3-fold increased risk), some convey an intermediate

*Abbreviations:* ASDS, allele-specific DNA sequencing; BeLPT, beryllium lymphocyte proliferation test; CBD, chronic beryllium disease; D, aspartic acid; E, glutamic acid; HLA, human leukocyte antigen; K, lysine; NIOSH, National Institute for Occupational Safety and Health; OH, oligonucleotide hybridization; OR, odds ratio; PCR, polymerase chain reaction; R, arginine; SSP, sequence specific primer PCR; RFLP, restriction fragment length polymorphism

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risk (e.g., *HLA-DPB1*1901*, ∼5-fold) and others convey high risk (e.g., *HLA-DPB1*1701*, >10-fold). Molecular modeling has been used to further investigate a potential mechanistic basis for these observations. We found a strong correlation between the hierarchical order of risk of chronic beryllium disease associated with specific alleles and the predicted surface electrostatic potential and charge of the corresponding isotypes. Therefore, when alleles were grouped by the relative negative charge on the molecules for which they code, the data suggest that those alleles associated with the most negatively charged proteins carry the greatest risk of beryllium sensitization and disease.
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*Keywords:* Beryllium disease; Biomarkers; Genetic polymorphisms; Molecular epidemiology; Occupational disease

## 1. Introduction

Beryllium is a metal with many interesting properties making it suitable for an extensive variety of technological applications (Table 1). However, adverse health effects due to exposure to beryllium fumes, dust and very small particles were recognized more than 50 years ago [1–3]. Recently, it has been estimated that as many as 134,000 current workers in the United States may be exposed to beryllium and therefore at risk of chronic beryllium disease (CBD) [4].

CBD occurs in those who have developed a hypersensitivity to beryllium; the latter is generally identified through a blood test called the beryllium lymphocyte proliferation test (BeLPT) [5]. CBD primarily affects the lungs and is characterized by non-caseating granulomas and interstitial infiltrates, leading to fibrosis (Fig. 1). Symptoms of CBD are non-specific and include cough, shortness of breath, fatigue, and night sweats [6]. These symptoms are also typical of sarcoidosis, and it has been suggested that as many as 6% of those with sarcoidosis may actually have CBD [7,8].

Prior to widespread use of the BeLPT in the early 1990s, identification of CBD was dependent on recognition of symptoms, or significant radiographic and/or pulmonary function changes. Subsequent to the utilization of the BeLPT, many individuals are now diagnosed with subclinical disease. Prevalence of sensitization in cross-sectional surveys of exposed workers has ranged from 2 to 12%; further clinical evaluation has identified CBD in 49–100% of workers with an abnormal BeLPT [5,9–13]. It is likely that cross-sectional surveys underestimate the true risk to a population, and it is unknown

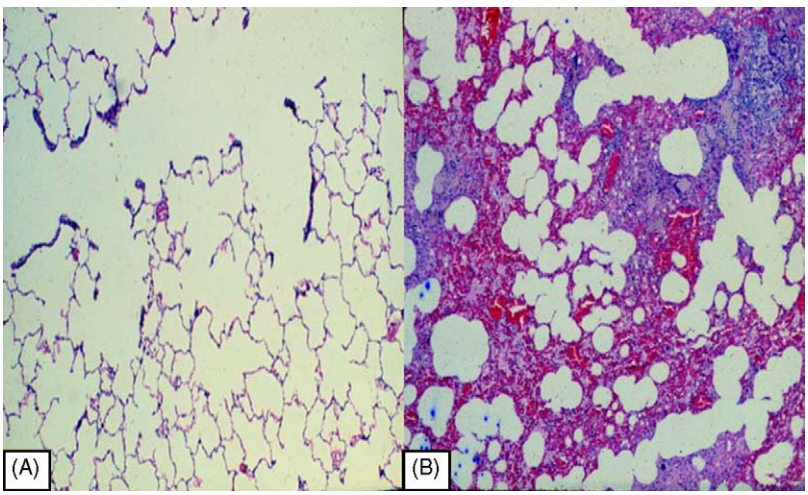


Fig. 1. Section of (A) normal lung and (B) a non-caseating granuloma stained with hematoxylin-eosin. Thickening of tissues in the granuloma leads to impaired gaseous exchange in the alveolae.

Table 1
Uses and properties of beryllium

| Technology | Application | Physical properties suiting application |
|---|---|---|
| Aerospace | Engines and rockets | Lw, Hc, D, Ag, St |
| | Brakes and landing gear | Lw, Hc, Sf, D, Ag, St, Wr |
| | Satellites and gyroscopes | Lw, Sf, St, Ds, Ag, Co |
| | Precision tools | Lw, Sf |
| | Altimeters | Fo, Sp |
| | Mirrors | Ds, Lw, Sf, St |
| Telecommunications | Undersea repeater housings | Cr, St |
| | Cell phones | Co, Fo, Sp, Er, Hc |
| | Personal computers | Co, Fo, Sp, Er, Hc, D |
| | Transistor mountings | Er, Hc |
| | Electrical connectors | Co, Fo, Sp, St |
| | Switches and springs | Co, Fo, Sp, St, D |
| | Electromagnetic shielding | Fo, Sp, St |
| Biomedical | X-ray tube windows | Lw, Xt |
| | Scanning electron microscopes | Xt |
| | Dental prostheses | Ca, Lw |
| | Medical lasers | Hc, Er |
| Defense | Tank mirrors | Lw, Sf, St |
| | Springs on submarine hatches | Co, Sp, St |
| | Mast mounted sights | Lw, Sf, St |
| | Missile guidance | Ds, Lw, Sf, St |
| | Nuclear triggers | Nm |
| Fire prevention | Non-sparking tools | Ca, Ns, St |
| | Sprinkler systems | Cr, Fo, Sp, St |
| Automotive | Air-bag triggers | Co, Fo, Sp |
| | Anti-lock braking systems | Co, Fo, Sp, St |
| | Steering wheel connectors | Ag, Co, Cr, Fo, Sp, St |
| Miscellaneous | Plastic molds | Hr |
| | Bellows | Fo, Sp |
| | Jewellery | Lw, Fo, No |
| | Golf clubs | Ca, St, Wr |
| | Bicycle frames | Lw, Sf, St, D |
| | Camera shutters | Lw, Sf |
| | Fishing rods | Fo, Sp, St |
| | Pen clips | Fo, Sp |

Ag: antigalling, Ca: castability, Co: electrical conductivity, Cr: corrosion resistant, D: use discontinued, Ds: dimensional stability, Er: electrical resistivity, Fo: formability, Hc: heat conductivity, Lw: light weight, Nm: neutron moderator, No: naturally occurring (as emerald and aquamarine), Ns: non-sparking, Sf: stiffness, Sp: springiness, St: strength, Wr: wear resistant, Xt: X-ray transparent.

whether all of those found to be sensitized but who do not have CBD will eventually develop disease.

Human leukocyte antigen (*HLA*) genes were implicated in CBD when it was demonstrated that the beryllium-specific proliferative response in peripheral blood lymphocytes could be blocked by antibodies directed against HLA molecules [14]. This observation led to two important questions: (1) is there an association between specific *HLA* genes and CBD? and (2) is there an association between specific *HLA* genes and beryllium sensitization? Several laboratories have answered the first question in the affirmative, finding an association between the supratypic marker *HLA-DPB1*E69* and CBD [15–22] (Fig. 2), with only one study failing to find an association with beryllium sensitization [19]. This marker codes for glutamic acid (E) in codon 69 of the *HLA-DPB1* gene, which is shared by 40 of 116 known variants

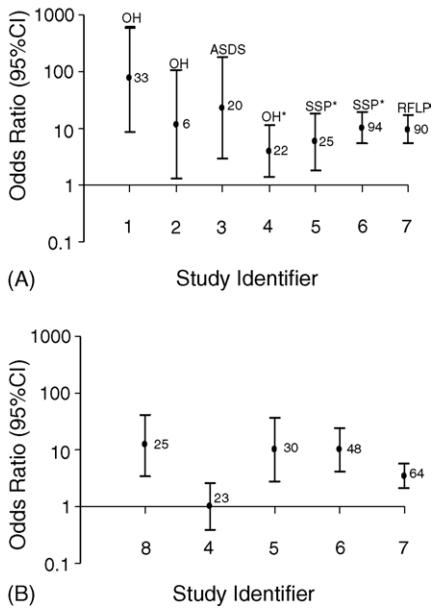


Fig. 2. Results of molecular epidemiologic studies that have sought associations between *HLA-DPB1* alleles that code for E69 and (A) CBD and (B) beryllium sensitization. Study identifiers: 1, Richeldi et al. [15]; 2, Richeldi et al. [16]; 3, Wang et al. [17]; 4, Saltini et al. [19]; 5, Rossman et al. [20]; 6, Maier et al. [21]; 7, McCanlies et al. [22]; 8, Wang et al. [18]. Numbers proximal to point estimates are numbers of cases.

or *HLA-DPB1* haplotypes [23]. These 40 E69-coding alleles fall into two broad categories as follows: the *HLA-DPB1*0201* family of alleles that have an overall population frequency of approximately 0.15; and a group of less common E69 allelomorphs (*HLA-DPB1*0601/*0901/*1001/*1301/*1701/*1901*; $F = 0.002–0.04$).

Studies implicating the *HLA-DPB1*E69* supratypic marker have led to two conflicting assertions: the first assertion is that the *HLA-DPB1*0201* family of alleles carries the highest risk of CBD [15,19], and the second assertion is that the rare *HLA-DPB1*E69* variants carry the highest risk of CBD [17,18]. This discrepancy may be the result of the variety of methods used for allelic discrimination. These include oligonucleotide hybridization (OH) [15,16,19], allele-specific DNA sequencing (ASDS) [17,18], sequence specific primer (SSP) polymerase chain reaction (PCR) [20,21] and PCR restriction fragment length polymorphism (RFLP) [22]. In addition, early studies were performed when as few as 20 of the *HLA-DPB1* variants were known and allelic discrimination was relatively poor [15–18].

We sought to use the tools of molecular modeling to investigate the physical characteristics of HLA protein molecules for which the different *HLA-DPB1* alleles code. The rationale for using a molecular modeling approach was to determine if there were some characteristics common to the protein products of genes under study that would correlate with the molecular epidemiologic risk data. Using this approach it might be possible in the future to study the potential interaction of positively charged beryllium ions ($Be^{2+}$) and negatively charged amino acid residues (e.g., E69) that are found in the antigen-binding groove of the HLA molecules. Here we present a systematic analysis of the findings of published molecular epidemiologic reports on CBD, and introduce a computational chemistry approach in an attempt to illuminate the question of whether some *HLA-DPB1*E69* alleles convey a higher risk of CBD and beryllium sensitization. This information may be of use for both risk assessment and for the development of intervention strategies or treatment modalities.

## 2. Materials and methods

### 2.1. Literature search and statistics

The OVID MEDLINE database was searched for reports published between 1 January 1989 and 19 July 2004, using the search terms: beryllium, genetics, berylliosis and HLA. The terms HLA and beryllium and genetics and beryllium were merged. This strategy yielded 223 citations, which were further reduced to seven molecular epidemiologic case-control type studies with data for allelic frequencies [15–21]. Only three of these studies provided a complete set of allele-specific data. Thus, the main analysis was predicated on data from these studies [19–21].

Crude odds ratios for allelic frequency and outcome were determined using Mantel–Haenszel analysis [24]. To investigated the relationship between charge and disease status (e.g. CBD, BeS, versus control), charge was determined by summarizing the frequency of the alleles within each charge group for each study. The Mantel–Haenszel $\chi^2$ was used to calculate odds ratios and the Breslow–Day test for homogeneity of the odds

ratio was used to determine if the tables could be combined for much of the analysis.

The correlation and regression analyses of ORs were carried out using the log-transformed data. This corresponds to the logit formulation of the logistic regression model, in which the outcome (disease or sensitization) is related to the risk factor (charge). The regression and ANOVA analyses were carried out on ungrouped data using the Grace software [25]. In addition, since the data were naturally stratified by charge, grouping by charge was carried out to test if the fitted model was the correct one. This was accomplished by analyzing lack-of-fit and pure error terms following the strategy of Pollard [26].

### 2.2. Molecular modeling

Since experimentally determined coordinates for HLA-DP molecules were not available, HLA-DR coordinates of conserved regions were used, specifically, Protein Data Bank entry 1FV1 (HLA-DRA*0101/B5*0101) [27]. SYBYL 6.6 Software (Tripos Inc., St. Louis, MO) was used to convert the HLA-DR template to HLA-DP, following a protocol described elsewhere [28]. In brief, the modeled atoms (substituted side-chains and full residues in the gap region) were subjected to 50 iterations of steepest descent minimization in the all-atom AMBER force field, followed by exhaustive Powell minimization until full convergence by the energy gradient was reached [29]. These minimization procedures were performed to alleviate possible inaccuracies of the model. In other words, to obtain a representative approximation to the actual molecule. The electrostatic potential and isopotential surface maps were calculated using the Insight II program (Accelrys, San Diego, CA). Debye–Huckel boundary conditions and four-stage electrostatic grid focusing were employed. The PARSE parameters were set with a 1.4 Å rolling probe radius, and a 2 Å Stern layer was used [28]. The dielectric constants of protein and water were assigned 4 and 80, respectively.

## 3. Results

Studies by Wang et al. [17,18] identified specific *HLA-DPB1*E69* alleles but not specific *HLA-DPB1*K69* or *HLA-DPB1*R69* alleles. Therefore to calculate crude odds ratios for CBD and beryllium sensitization for individual *HLA-DPB1*E69* alleles from these data, alleles coding for either a lysine residue or an arginine residue at codon 69 were collapsed into one reference group for beryllium sensitization and one for CBD. Although numbers were small, the data were suggestive of an allelic hierarchy, where the odds ratio for risk of CBD associated with *HLA-DPB1*0201* was smallest (3.0; 1.2–7.7), the odds ratio associated with *HLA-DPB1*1701* was greatest (46.0; 5.2–408.7), and with *HLA-DPB1*1901* < *HLA-DPB1*1301* < *HLA-DPB1*0901* = *HLA-DPB1*1001* < *HLA-DPB1*0601* were ranked in between.

Next, homologous computational chemical models of the different HLA proteins that would be formed from the specific *HLA-DPB1* allelomorphs were developed to determine the physical characteristics that might account for the allelic hierarchy observed in molecular epidemiologic studies. Two of these models, the non-E69 allele derived from *HLA-DPB1*0401* (generically *HLA-DPB1*K69*) and the high risk E69 allele *HLA-DPB1*1701*, are shown to illustrate the differences in surface charge potential (Fig. 3). The antigen-binding groove portion of these molecules is indicated by the presence of a myelin molecule, which is shown as a stick structure. Dark areas of the surface indicate increasingly negative surface potential and light areas are positive, with white being neutral. It can be seen that the allele associated with the lower risk, *HLA-DPB1*0401*, gives rise to a protein with a neutral to slightly positive binding groove (Fig. 3A), whereas the high risk *HLA-DPB1*1701* allele gives rise to a protein with a very negatively charged binding groove (Fig. 3B).

We then sought to test the hypothesis that overall electrostatic charge in the antigen-binding region was related to risk among different *HLA-DPB1* alleles. To increase the numbers of molecular epidemiology observations, data derived from the laboratories of Saltini et al. [19], Rossman et al. [20] and Wang et al. [17,18] were combined in a Mantel–Haenszel analysis based on total numbers of alleles. Overall results were similar to those originally obtained [28,30]. Then a correlation was sought between the log of the odds ratios and formal charge. These data, shown in Fig. 4, indicate that alleles with the highest reported odds of beryllium sensitization and CBD also have the most negative surface potential [28,29].

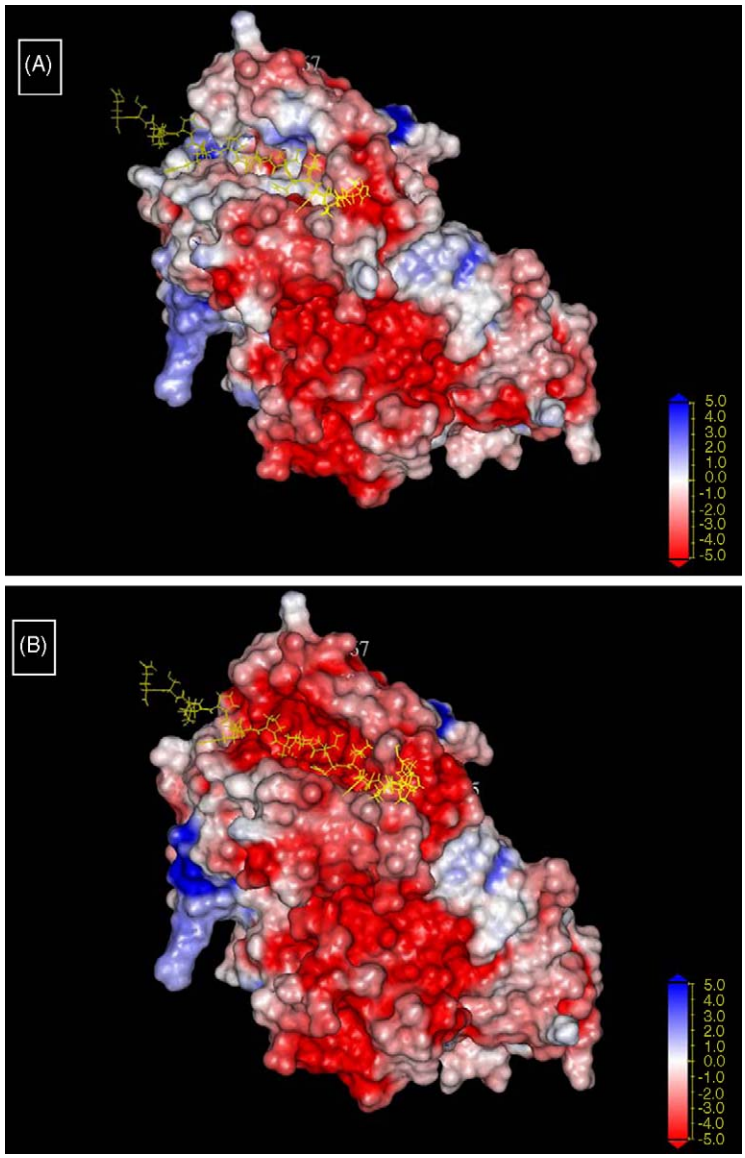


Fig. 3. HLA molecules modeled by homology. Surface electrostatic potential is indicated by color where blue intensity indicates increasing positive charge, red intensity indicates increasing negative potential, and white is neutral. The stick model represents an antigen in the antigen-binding groove (myelin). An HLA molecule derived from a low risk allele (*HLA-DPB1*0401*, surface charge −3) appears in panel (A); an HLA molecule derived from a high risk allele (*HLA-DPB1*1701*, surface charge −9) appears in panel (B).

Based on these results a more detailed analysis was made of allele-specific data presented in studies reported by Saltini et al. [19], Rossman et al. [20], and Maier et al. [21]. These studies were selected because they are the only studies in the literature that report a complete set of allele-specific data. First, analyses that considered associations between alleles within the −7 charge group that code for E69 (e.g., *HLA-DPB1*0201*, *HLA-DPB1*1301* and *HLA-DPB1*4801*) and those not coding for E69 (e.g., *HLA-DPB1*0301*, *HLA-DPB1*1401* and *HLA-DPB1*2001*) showed clear and distinct differences with respect to disease risk

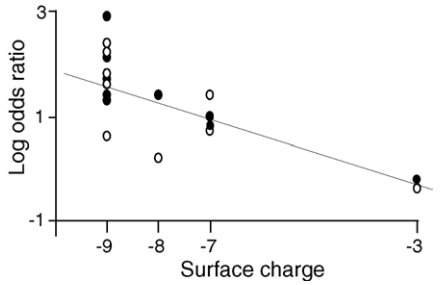


Fig. 4. Correlation between odds ratios calculated from the pooled data of Wang et al. [17,18], Saltini et al. [19] and Rossman et al. [20] and electrostatic negative charge on HLA. Association between OR of CBD and surface charge (●) and association between OR of beryllium-sensitization and surface charge (◯), see also Snyder et al. [28].

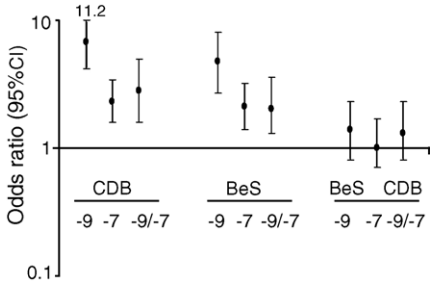


Fig. 5. Odds ratios and 95% confidence intervals plotted for the data presented in Table 3. In the figure the data are presented by disease status.

(ORs = 2.6; 1.4–4.7 [$p = 0.001$] for CBD and 2.9; 1.4–5.6 [$p = 0.002$] for beryllium sensitization), implying that the charge distribution, with the 69th glutamic acid residue residing with the antigen-binding groove, is an important factor in and of itself. Therefore all subsequent comparisons of −7 alleles with −9 alleles were made between alleles coding for E69 only.

The two largest groups of alleles that code for glutamic acid at codon 69 are those with a surface charge of −9 or −7. The alleles with a surface charge of −7 include and are principally composed of the *HLA-DPB1*0201* family of alleles (Table 2). Thus, in terms of risk assessment these data pose three important new questions. First, do alleles with a surface charge of −9 impart a significantly greater risk of CBD than alleles with a surface charge of −7? Second, do alleles with a surface charge of −9 impart a significantly greater risk of beryllium sensitization than alleles with a surface charge of −7? And third, if either of the preceding questions are true, are workers who are beryllium-sensitized and have alleles that code for HLA molecules that have a surface charge of −9 more likely to have CBD than those beryllium workers who are sensitized with alleles that code for HLA molecules that have a surface charge of −7? In the absence of longitudinal study design, this third question is a surrogate for the question of whether alleles coding for nine charged HLA molecules are more important for progression from sensitization to CBD than alleles coding for seven charged HLA molecules. To address these questions, allele-specific data only were compiled from the reports of Saltini et al. [19], Rossman et al. [20] and Maier et al. [21] (Table 2, it should be noted that inference of allele data from Maier et al., may not be completely accurate since allele frequencies were estimated from phenotypic frequencies). In addition, from these data, odds ratios were calculated to compare alleles in the charge categories of −9 and −7 for their associations with CBD and beryllium sensitization as well as the relative association between CBD and beryllium sensitization. The analyses, comparing −9 E69 alleles to −7 E69 alleles, were performed in several ways: using all non-E69 alleles as the reference category, limiting the reference category to −3 alleles, or −3 plus −5 alleles. For examination of the associations with CBD, −9 alleles were consistently associated with higher odds of disease than −7 alleles (Fig. 5: OR = 6.8; 4.2–11.2 versus OR = 2.3; 1.6–3.4), and the difference was statistically significant (OR = 2.8; 1.6–5.0; $p < 0.0001$). A similar trend was observed for beryllium sensitization (Fig. 5: OR = 4.7; 2.7–8.1 versus OR = 2.1; 1.4–3.2), and there was again a statistically significant difference between −7 alleles and −9 alleles with greater risk associated with −9 alleles (OR = 2.0; 1.1–3.6). Thus, alleles with a surface charge of −9 had higher odds ratios for their associations with both CBD and beryllium sensitization than do alleles with a surface charge of −7.

The next analysis considered the proportions of alleles in the CBD group with respect to those in the beryllium-sensitized group directly. There was no clear distinction between −7 alleles and −9 alleles between beryllium sensitization and CBD; that is −9 alleles were not more likely to be found in CBD compared to beryllium-sensitized workers (Fig. 5: OR = 1.4; 0.8–2.3).

Table 2
Numbers of specific alleles reported in groups of beryllium workers[a]

| Allele designation | Control ($n = 290$) | Disease group BeS ($n = 101$) | CBD ($n = 141$) |
|---|---|---|---|
| −3[b] | | | |
| *HLA-DPB1*0401* | 221 | 60 | 57 |
| *HLA-DPB1*1501* | 5 | 2 | 0 |
| *HLA-DPB1*2301* | 8 | 1 | 0 |
| *HLA-DPB1*3901* | 0 | 0 | 1 |
| *HLA-DPB1*4001* | 1 | 0 | 0 |
| Subtotal | 235 (41%) | 63 (31%) | 58 (21%) |
| −5[b] | | | |
| *HLA-DPB1*0101* | 32 | 11 | 8 |
| *HLA-DPB1*0402* | 75 | 9 | 40 |
| *HLA-DPB1*1101* | 10 | 5 | 4 |
| *HLA-DPB1*1801* | 2 | 0 | 1 |
| *HLA-DPB1*2601* | 1 | 0 | 0 |
| *HLA-DPB1*3301*[c] | 2 | 0 | 0 |
| *HLA-DPB1*7701* | 1 | 0 | 0 |
| Subtotal | 123 (21%) | 25 (12%) | 53 (19%) |
| −6[b] | | | |
| *HLA-DPB1*0501* | 13 (2%) | 9 (4%) | 5 (2%) |
| *HLA-DPB1*0202*[c] | 6 | 1 | 2 |
| −7[b] | | | |
| *HLA-DPB1*0201*[c] | 91 | 42 | 60 |
| *HLA-DPB1*0301* | 54 | 10 | 16 |
| *HLA-DPB1*1301*[c] | 9 | 11 | 10 |
| *HLA-DPB1*1401* | 10 | 1 | 3 |
| *HLA-DPB1*2001* | 4 | 2 | 0 |
| *HLA-DPB1*3501* | 1 | 0 | 0 |
| *HLA-DPB1*4801*[c] | 0 | 0 | 1 |
| *HLA-DPB1*7801* | 1 | 0 | 0 |
| Subtotal | 170 (29%) | 66 (33%) | 90 (32%) |
| −8[b] | | | |
| *HLA-DPB1*1901*[c] | 2 | 1 | 2 |
| Subtotal | 8 (1%) | 2 (1%) | 4 (1%) |
| −9[b] | | | |
| *HLA-DPB1*0601*[c] | 4 | 15 | 16 |
| *HLA-DPB1*0801*[c] | 1 | 1 | 0 |
| *HLA-DPB1*0901*[c] | 1 | 4 | 11 |
| *HLA-DPB1*1001*[c] | 14 | 9 | 22 |
| *HLA-DPB1*1601*[c] | 4 | 2 | 6 |
| *HLA-DPB1*1701*[c] | 7 | 5 | 16 |
| Subtotal | 31 (5%) | 36 (18%) | 71 (25%) |
| Total | 580 (100%) | 201[d] (100%) | 282 (100%) |

[a] Compiled from reports by Saltini et al. [19], Rossman et al. [20], and Maier et al. [21]. *n*: number of workers.
[b] −3, −5, −6, −7, −8, −9 are the charge categories.
[c] E69 alleles.
[d] One allele was reported as *HLA-DPB1*1201*. According to the HLA database [23], this allele was first reported in 1989; however, the entry was later found to be in error. This allele was excluded from our analysis.

Table 3
Odds ratios[a] and % for homogeneity

| Comparison groups | OR[b] (95% CI[c]) | $\chi^2$ (homogeneity) | $p$-Value |
|---|---|---|---|
| −9/−3,−5 | | | |
| CBD[d] | 6.8 (4.2–11.2) | 4.2 | 0.12 |
| BeS[e] | 4.2 (2.7–8.1) | 2.5 | 0.28 |
| CBD → BeS | 1.4 (0.8–2.3) | 4.4 | 0.11 |
| −7/−3,−5 | | | |
| CBD | 2.3 (1.6–3.4) | 1.2 | 0.55 |
| BeS | 2.1 (1.4–3.2) | 5.3 | 0.07 |
| CBD → BeS | 1.0 (0.7–1.7) | 6.1 | 0.05 |
| −9/−7 | | | |
| CBD | 2.8 (1.6–5.0) | 1.4 | 0.49 |
| BeS | 2.0 (1.1–3.6) | 1.4 | 0.49 |
| CBD → BeS | 1.3 (0.8–2.3) | 0.1 | 0.97 |

[a] Mantel–Haenszel $\chi^2$ was used to calculate odds ratios and the Breslow–Day test was used to determine homogeneity of the odds ratio.
[b] OR: odds ratio.
[c] CI: confidence interval.
[d] CBD: chronic beryllium disease.
[e] BeS: beryllium sensitization.

The Breslow–Day test for homogeneity of the odds ratio was used to determine the validity of combining data from these three studies. It was expected that values involving data from one study [19] for beryllium sensitized would introduce heterogeneity because that study was the only study not to find an association between *HLA-DPB1*E69* and beryllium sensitization. In fact only one of the comparisons reached significance (Table 3). Overall the data suggest that charge might not affect progression from beryllium sensitization to CBD, although longitudinal follow-up will be needed to confirm or deny this.

## 4. Discussion

Review and analysis of published molecular epidemiologic data suggested a hierarchy of risk for CBD and beryllium sensitization among alleles coding for a glutamic acid residue in the 69th position of the mature protein [30,31]. We used computational chemistry to investigate the physical–chemical properties of HLA-DP molecules to provide an explanation for this putative risk hierarchy. It was observed that when alleles were grouped by total surface charge on the HLA molecules for which they code that they fit into relatively discrete risk groups. However, the supratypic marker *HLA-DPB1*E69* was found to provide an almost overwhelming local effect in the antigen-binding groove. Further analysis of the allelic frequencies based on coding for E69 and overall negative charge showed that odds of CBD and beryllium sensitization increased with greater negative HLA surface charge.

The question of whether greater negative charge could potentially predict risk of CBD in beryllium-sensitized workers remains to be addressed, but these data did not indicate that workers with CBD were more likely to have the highest negative charge. Before examining the results of published studies in this light, a difference should be noted between the overall frequencies of *HLA-DPB1*E69* in previous studies [15–21] and those recently published by the National Institute for Occupational Safety and Health (NIOSH) [22]. We compared the data available from the literature for the supratypic marker *HLA-DPB1*E69* in a non-allele-specific way as a surrogate risk factor for CBD in workers who were already sensitized, but there was no association (OR = 1.3; 0.9–1.9; $p = 0.148$) [15–21]. This result was not consistent with our recent results that suggest that it does (OR = 2.9; 1.3–6.1; $p = 0.006$) [22]. These analyses of the literature data provide a framework for our future allele-specific DNA sequencing analysis of beryllium worker DNA samples in the ongoing NIOSH study.

Other studies that have considered specific residues in HLA-DP, other than E69, have additionally identified positions 8, 9, 11, 36, 55, 56, 84, 85, 86, 87 as being important for risk of CBD [15,17,21]. These residues are either directly in the antigen-binding groove or close-by. However, on consideration of the data presented here, it should be noted that *HLA-DPB1*0301* (K69), which is relatively common ($F \sim 0.08$), has a −7 charge by virtue of having D55 and E56 but appears not to convey an elevated risk of CBD. Therefore, although these ancillary residues contribute significantly to the allelic risk hierarchy, E69 appears to be necessary to render HLA-DPB1 as a risk factor for beryllium sensitization and CBD.

It should be noted that there are several limitations in the analyses of published molecular epidemiologic data presented here. First, it was only possible to deduce allele frequencies from available published reports.

Consequently it was not possible to consider the effect of zygosity, but since it has been shown that *HLA-DPB1*E69* homozygotes are at increased risk compared to heterozygotes this factor needs to be taken into account [17,21,22]. Second, allele frequencies from at least two of the literature reports were deduced from phenotypic frequencies which may not be entirely accurate [17,21]. Third, the numbers of known allelotypes have steadily grown since the first study in 1993 when ∼20 were known, to 2004 when 110 were known, and it is likely that some level of allelic misclassification exists in the literature reports from which the data were drawn (see footnote of Table 2). Fourth, the studies considered here were all case-control studies based on identification of workers with beryllium sensitization and CBD, and it is possible that some workers have changed categories from not sensitized to beryllium-sensitized or from beryllium-sensitized to CBD. One of the populations reported here documents this, as some of the controls in the first study [17] were classified as beryllium-sensitized in the later study [18]. Fifth, there are gene–environment interaction issues that have not been addressed in the published study reports [17–21]. Sixth, one of the studies from which data were extracted to perform our analyses did not find an association between E69 and beryllium sensitization [19], whereas the other two did [20,21]. Although this was the smallest study, it could be a source of bias as indicated by the test for homogeneity.

To address many of these issues, the NIOSH study: (1) addresses zygosity, (2) derives two haplotypes for each sample, (3) uses a rigorous allele-specific DNA sequencing approach, (4) has a longitudinal study design, and (5) can integrate job-related information and extensive beryllium exposure data from the related epidemiologic studies among these workers. The latter two points are very important in that longitudinal follow-up will reduce outcome misclassification, and sensitization and CBD do not occur in the absence of exposure to beryllium. Approximately 15–25% of those with sensitization and CBD do not carry an E69 allele, and consideration of exposure-related factors will be important in increasing our understanding.

Methods in computational chemistry will be used to further elucidate the role of E69 alleles in beryllium sensitization and CBD. The binding affinities will be compared to affinities of other metal ions that may compete for these sites. Thus questions concerning specificity of beryllium binding on HLA-DP can be addressed. Specifically, putative ion binding sites on allotypes of HLA-DP proteins will be identified and beryllium-binding affinities will be determined using free-energy perturbation techniques [32].

The results of the molecular epidemiologic and computational chemistry analyses presented here have allowed us to generate three new hypotheses that may guide future studies designed to develop strategies for risk assessment [33]. The major research questions that require confirmation, to follow up the analyses presented here, are: does the overall surface charge of the HLA molecules for which the *HLA-DPB1*E69* alleles code have important implications for the degree of risk that they convey for (1) beryllium sensitization, (2) CBD, and (3) progression from sensitization to CBD. Furthermore, the results of computational chemistry specifically suggest that preferential cation binding to specific HLA amino acid sequences in a putatively metal free antigen-binding pocket might selectively reflect the innate specificity of antigen recognition. In addition, it may be possible to use a computational chemistry approach to identify candidate susceptibility genes for further investigation of other occupational diseases.

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