Final Performance Report
Genetic/Exposure Interaction in Beryllium Disease

5 R01 – OH 007495
8/1/2003 – 7/31/2007

Kenneth D. Rosenman, MD, Principal Investigator, Michigan State University
Vicki Stover Hertzberg, PhD, Co-Investigator, Emory University
Dimitrios Monos, PhD, Co-Investigation, Children's Hospital of Philadelphia
Carol Rice, PhD, Co-Investigator, University of Cincinnati
Milton Rossman, MD, Co-Investigator, University of Pennsylvania

Contact Information for Principal Investigation and Institution to which award was made.

Kenneth D. Rosenman
Michigan State University
Department of Medicine
117 West Fee
East Lansing, MI 48824-1315
517-353-1846
FAX: 517-432-3606
Rosenman@msu.edu

A. List of Terms/Abbreviations

BeLPT – beryllium lymphocyte proliferation testing

Be S – beryllium sensitization

CBD – chronic beryllium disease

CI – confidence interval

HLA – human leukocyte antigen

OR – odds ratio

B. Abstract

Rationale: CBD is a hypersensitivity granulomatous disease affecting the lungs of people exposed to the metal Beryllium (Be^{2+}).

Objective: To study the polymorphisms of the HLA class II molecules that influence whether an individual will develop CBD and/or BeS in a large cohort of beryllium-exposed individuals with a high prevalence of CBD and BeS.

Methods: DNA-based typing of HLA-DPß1 and HLA-DRß1 loci at the allele level was performed on 65 CBD, 44 BeS and 288 non-effected Be-exposed age, gender and facility-matched controls.

Measurements and Main Results: The DPβE69 residue, particularly if present on non-*0201 alleles, was associated with both CBD and BeS. The DPβDE55, 56 epitope was associated with CBD but not BeS among DPβE69 positive, DPB1*0201 negative subjects. The DRβE71 residue was significantly increased among DPβE69 negative subjects, in both CBD (100%), *pcor* = 0.045 and BeS cases (100%), *pcor* = 1.1×10^{-4} compared to controls (19.2%). DPβE69 and/or DRβ71 were significantly increased in CBD (100%) and in BeS subjects (100%) versus controls (50.3%), p = 7.1×10^{-14} and p =

$2.37x10^{-11}$, respectively. DRßN37 was significantly increased among DPßE69 negative subjects with BeS, but residues E71, N37 and H32 in pairs, were in strong LD in both BeS and controls and therefore it is unclear as to whether any of these three residues is stronger than the other in terms of predisposing to BeS among DPβE69 negative subjects. Conclusions: Specific residues DPβE69, DRβE71 and DRβN37 influence sensitization to beryllium and the development of CBD. Our results support an interactive relationship of residues and/or genes. Some of these interactions elicit increased susceptibility while others exhibit a protective role against the development of CBD and/or BeS.

C. Highlights/Significant Findings

Our study cohort is one of the largest involving the characterization of HLA class II molecules in CBD and BeS cases. Members of our cohort had longer latency periods from first and last exposure to beryllium than most previously reported cohorts. It is also one of the most complete, in terms of typing of DRB1 and DPB1 genes at the allele level. A summary of our findings are:

- Sixty-five of 65 (100%) CBD subjects, 44 of 44 (100%) BeS subjects were positive for DPβE69 and/or DRβ71.
- Among BeS subjects who were DPβE69 negative, the DRβE71 residue was significantly increased.
- There was no difference in the risk of developing CBD vs. BeS by zygosity.
- E69 alleles that code for -9 or -7 charges were significantly associated with CBD and BeS with the -9 charge being a significantly greater risk then the -7 charge.
- The distribution of DPB1*0201 alleles was decreased in the CBD subjects as compared to control group.
- No exposure-genetic interaction was identified.

D. Translation of Findings

The results of this study do not change the basic approach to reducing beryllium related disease, which is engineering controls to minimize exposure. Although genetic testing is a sensitive test for identifying individuals who are at risk of developing the condition, the testing is still too specific to allow its use in pre placement medical screening.

E. Outcome/Relevance/Impact

The observation that almost every beryllium-affected individual is positive for specific HLA polymorphisms, while only 50% of controls are positive for the same residues, is extremely important in the context of identifying additional genes involved in the disease process. It appears that these HLA residues are necessary but not sufficient for the disease phenotype. Stratification of beryllium exposed populations based on the specific HLA polymorphisms should reveal differences in genome-wide association studies, between control and all the other subjects that will go beyond HLA polymorphisms and will be important for predicting disease susceptibility and/or protection.

The results of this study continue to expand knowledge about the genetics of beryllium disease. HLA molecules definitively play a significant role in this immunologically mediated disease and identifying the specific HLA components has contributed to the identification of future directions that should be pursued for a more comprehensive understanding of its pathophysiology. Ideally these pursuits will contribute to interventions that will prevent the occurrence of beryllium disease as well as insight into other conditions with similar pathological mechanisms.

F. Scientific Report

Specific Aims:

1. Investigate the specific genetic markers that are associated with:
   a. An increased risk of developing chronic beryllium disease (CBD)
   b. An increased risk of developing a positive lymphocyte transformation test to beryllium (sensitization).
   c. An increased risk of developing more clinically severe (CBD)

2. Evaluate the interaction between levels of exposure and genetic markers associated with:
   a. An increased risk of developing (CBD)
   b. An increased risk of developing a positive lymphocyte transformation test to beryllium (sensitization)
   c. An increased risk of developing more clinically severe (CBD)

Background: Workers exposed to beryllium have developed both sensitization (BeS) and chronic beryllium disease (CBD) (1). As early as the 1950's it was hypothesized that the disease was immunologically mediated after it was observed that CBD occurred after both high and low levels of exposure (2). Genetic susceptibility to the development of CBD was reported in 1993 (3) and to BeS in 2002 (4). These results, which found an increased risk of CBD and BeS with the presence of glutamic acid at position 69 on the HLA-DPβ chain, have been confirmed by subsequent investigators (5-7). The associations between glutamic acid at position 69 and CBD and BeS are biologically plausible. Studies with blocking monoclonal antibodies have strongly suggested that the presence of glutamic acid at position 69 has functional significance (8-10). The hypothesized mechanism is that beryllium is a positive bivalent cation which may influence peptide binding directly or indirectly to the HLA molecules. This influence may occur by changing the local electrostatic environment and affecting peptide binding to the groove of the HLA class II molecules (11), which in sequence may also change the interaction of the T-cell receptor of CD4+ T-cells with the HLA-peptide complex.

Because only 80-90% of individuals with CBD or BeS are HLA-DPβE69 positive and 30-40% of beryllium exposed workers who do not develop CBD or BeS are HLA-

DPβE69 positive, it makes sense that other genetic and/or exposure metrics play a role in determining which individuals develop CBD and BeS. This has led to investigations of other genetic markers, including: HLA-DRB1 polymorphisms (12), butyrophilin-like 2 (BTNL2) polymorphisms (13), transforming growth factor beta (TGF-ß1) polymorphisms (14), and tumor necrosis factor (TNF) polymorphisms (15). Among the DPβE69 negative subjects, findings have been reported related to the HLA-DRB1 gene, such as the association of DRβ F47 with Be hypersensitivity (CBD and BeS together) (12) and the association of DRB1*1301 with CBD (5). Other researchers have found that fibroblasts expressing mutated DP2 molecules at position 69 (DPβE69 of DP2 is mutated to DPβK69) or DR13 molecules at position 71 (DRβE71 of DR13 is mutated to DRβR71) respectively, are not capable of inducing Be-specific proliferation and INF-γ expression by lung CD4+ T-cells (8). Together, these findings suggest that there may be other HLA-related markers besides the DPB1 gene that influence BeS or disease.

Methodology: The cohort was comprised of workers from two beryllium production facilities in Eastern Pennsylvania. Facility A operated from 1958 – 1978 and Facility B operated from 1935 – 2000. The cohort consisted of all workers who worked two or more days at either facility. The methods used to assemble this cohort at Facility A have previously been described, including procedures used to contact participants and conduct medical testing (16). A similar approach was used for Facility B.

Medical screening took place from the fall of 1996 through the summer of 2001. In addition to chest radiographs and blood for beryllium lymphocyte proliferation testing (BeLPT), whole blood was collected and frozen for future genetic testing.

Any individual with two positive BeLPTs and/or a chest radiograph reading of ≥ 1/0 by at least two of the three "B" readers were referred to the University of

Pennsylvania for bronchoscopy. A positive BeLPT was defined as when both the SI and Std Ln SI are greater then normal on at least two of six tests with three different concentrations of $BeSO_4$ at either day five or day seven. Lavage fluid was collected for beryllium lymphocyte proliferation testing and a bronchial biopsy was performed during the bronchoscopy. CBD was defined as two positive BeLPTs (borderline results were considered negative) and/or a positive Be proliferation test in lavage fluid with granuloma on lung biopsy and/or fibrosis on chest radiograph. BeS was defined as two positive Be LPTs (borderline results were considered negative) and/or a positive Be proliferation test in lavage fluid and a normal chest radiograph and the absence of granuloma on lung biopsy if a lung tissue was obtained (Table 1).

Individuals with CBD, probable CBD and BeS were included in the genetic testing. Individuals with equivocal results including possible CBD and possible sensitization were not included. Controls for the genetic testing were selected from individuals with completely normal results including no positive or borderline beryllium lymphocyte proliferation testing and a chest radiograph reading of <1/0 by at least two of the three "B" readers. Two controls for each individual with CBD or sensitization were selected. Controls were matched by facility, gender and year of birth within three years.

At the time of the medical examination all participants were approached about completing one consent form for the medical examination and one for the genetic testing. Fifteen individuals with CBD and 11 with sensitization were not tested because they either did not provide consent or blood for the genetic testing. Ninety-five individuals with equivocal medical testing (i.e. only one positive BeLPT) were not eligible for genetic testing either as cases or controls.

Blood for genetic testing was shipped overnight to the lab and frozen the next day. Genomic DNA was prepared from frozen peripheral whole blood four to ten years

after collection using Qiagen columns (QiaAmp 96 DNA Blood kit). The DPB1 gene (exon 2 and 3) was characterized using a commercially available kit designed to provide high resolution typing using the PCR-SSP method (Pel-Freez Clinical system, Brown Deer, WI, USA). When necessary, due to ambiguities or inconsistent patterns of primer amplifications, sequence-based typing was performed. The DPB1 typing was subsequently confirmed by bi-directional sequencing-based typing of exon 2 using primer (AlleleSEQR HLA-DPB1 SBT kit, Atria Genetics, South San Francisco, CA USA). A new allele was identified for one case, and was named DPB1*1902 (18). DRB1 typing was performed by sequence-based typing. All genetic analyses were performed without knowledge of the status of the sample being from a CBD, BeS or control participant.

Chi-square tests and logistic regression were used to compare the frequency of genetic markers between CBD, sensitization and the control groups. The Bonferroni correction factor that was used for the *p* value of different comparisons is indicated in the text of the "Results" section. Odds ratios (OR) and 95% confidence intervals (C.I.) were calculated using Epi Info™ (www.cdc.gov/epiinfo). Attributable risk was calculated using the formula (OR-1)/OR. Multiple tests regarding the interactive relationship among the different HLA residues, homozygosity and linkage disequilibrium were evaluated by the method of Svejgaard and Ryder (19) using recently developed software(20).
Sixty-five individuals with CBD, 44 with BeS and 288 controls were tested for polymorphisms of the HLA-DPB1 and HLA-DRB1 genes at the allele level.
Results: Sixty-five individuals with CBD, 44 with BeS and 288 controls were tested for polymorphisms of the HLA-DPB1 and HLA-DRB1 genes at the allele level.

Participant demographics are shown in Table 2. The numbers of individuals positive for each of the different DPß1 and DRß1 alleles in control, BeS and CBD cases are shown in Tables 3 and 4, respectively.

Each of the DPB1 alleles in the three groups was evaluated for the presence of E69. Figure 1 shows the prevalence of HLA-DPβE69 by disease status and zygosity. The prevalence of an HLA-DPß1E69 positive allele was 60 of 65 (92.3%) among those with CBD, 35 of 44 (79.5 %) among those with BeS and 111 of 288 (38.5%) among controls. The odds ratio for CBD was 19.14 (95% C.I. 7.10-55.92) $p = 1.8 \times 10^{-16}$ and for BeS subjects was 6.20 (95% C.I. 2.73-14.47) $p = 3.82 \times 10^{-7}$. The respective attributable risk was 95% and 84%. P values were not corrected for multiple comparisons since we were examining a priori whether the specific residue E69 was differentially distributed between the groups. The prevalence of homozygotes and heterozygotes among CBD individuals was not significantly different than the prevalence of homozygotes and heterozygotes among BeS individuals but both were significantly different compared to controls (Figure 1).

All other polymorphic residues of the DPβ chain were evaluated and the distribution of DE55,56 containing alleles in CBD subjects was significantly different compared to controls (CBD: 60 of 65 (92.3%); controls: 177 of 288 (61.5%), OR 7.53 (95% C.I. 2.79-21.99) *pcor* = 7.8×10^{-5} (for the 46 comparisons); while in BeS subjects the distribution of DE55,56 was not significantly different as compared to controls after correction, BeS: 37 of 44 (84.1%); control: 177 of 288 (61.5%); OR 3.31 (95% C.I. 1.36-8.46) *pcor* = 0.16 (for the 46 comparisons). DE55 56 residues were in linkage disequilibrium (LD) with E69 in the CBD, BeS and control groups. When the cohort was stratified for the D55, E56 epitope, E69 was independently distributed and significantly different between the CBD subjects and controls as well as between BeS and controls;

when the cohort was stratified based on E69 there was no difference in the distribution of D55, E56 between the CBD subjects and controls as well as between BeS and controls. Similar analyses and results were found with each of the residues H9, L11, and the DEAV84-87 epitope; these residues were differentially distributed between CBD and controls but not between BeS and controls. The results were the same for V36 except for V36 the distribution was also significantly different between BeS and controls. However, like E55 56 all these residues were in strong LD with E69 and did not demonstrate an independent contribution in either the CBD or BeS groups (results not shown).

Based on a recent report suggesting that the totality of the negative charges are important for CBD susceptibility rather the E69 residue alone (20), we evaluated our cohort for the same parameter of negative charges among E69 positive subjects. Alleles that code for -9 or -7 charges were significantly associated with CBD and BeS with the -9 charge being a significantly greater risk then the -7 charge (figure 2). These epitopes were then evaluated as combined total molecular negative charges in categories <-13, vs. -13 to <-16, vs. -16 to -18 between those with CBD, BeS and the controls; the combined negative charge among those who were E69 positive and had CBD was < - 13 for 48 of 58 (82.8%), -13 to -15 for 6 of 58 (10.3%) and -16 to -18 for 4 of 58 (6.9%). Among those who were E69 positive and sensitized it was 26 of 35 (74.3%), 4 of 35 (11.4%), and 5 of 35 (14.3%) and among controls it was 93 of 110 (84.5%), 9 of 110 (8.2%) and 8 of 110 (7.2%). There were no statistical differences for total negative charge (CBD vs Control, X^2= 0.22, p= 0.895; BeS vs. control, X^2= 2.11, p= 0.348; CBD vs. BeS, X^2 = 1.45, p= 0.484).

It has also previously been reported that among E69 positive CBD subjects there is a differential distribution of DPB1 alleles and that, while the DPB1*0201 allele is higher among controls, there are a number of other less common alleles (non-

DPB1*0201 or DPB1*0201 negative), which contain E69 that are more prevalent among those with CBD (21). In our cohort it was also observed that among DPβE69 positive subjects the distribution of DPB1*0201 alleles was decreased in the CBD subjects as compared to control group [controls 75 of 111 (67.6%); CBD subjects 31 of 60 (51.7%)], while in contrast, the non-DPB1*0201 alleles were increased in the CBD group compared to controls [CBD subjects 29 of 60 (48.3%); controls 36 of 111 (32.4%)]. This difference was significant [OR=1.95 (95% C.I. 0.97-3.91) p=0.041,], (Figure 3). No statistical difference was found in the distribution of the DPB1*0201 and non-DPB1*0201 alleles between BeS subjects and controls (figure 3). Among the 20 non-0201 alleles containing HLA-DPβE69 in our study group, ten were more frequent in individuals with CBD (*0202, *0301, *0601, *0901, *1001, *1101, *1401,*1601, *1701, and *7101) and ten were less frequent (*0101, *0401, *0501, *1301,*1901, *2001, *2301, *2901, *4501 and *4601).

To further elucidate the role of the 0201 allele, we stratified our cohort for the DPB1*0201 allele . No significant differential distributions of any DP or DR residues were identified among DPβE69 positive, *0201 positive subjects. However, among DPβE69 positive, 0201 negative subjects we did observe that the epitope DPβ DE55, 56 occurred more often among CBD subjects, 27 of 29 (93.1%) and at a lower frequency among controls, 18 of 36 (50.0%); OR=13.50 (95% C.I. 2.51-96.16) p*cor* = 0.0082. The p value was corrected for the 45 different tests performed, accounting for the 45 polymorphic residues that were evaluated. In the DPB1*0201 positive BeS group we did not identify any residue association that was significant even in the absence of any correction factor. When the group of DPE69 positive DPB1*0201 negative BeS subjects was evaluated it was found that the DPβ DE55, 56 epitope was not increased among BeS subjects. Therefore, the contribution of the DPβ DE55, 56 epitope was evident among

CBD individuals who were DPβE69 positive, 0201 negative, but not in the corresponding group of BeS subjects.

Not all CBD or BeS subjects were positive for the DPβE69 residue. In search of other HLA associations that may further explain susceptibility, we searched for polymorphisms in the DRB1 locus. Among DPβE69-negative individuals with CBD compared to controls, the DRβE71 residue was significantly increased with 5 of 5 (100%) CBD subjects vs. 34 of 177 (19.2%) controls; (OR and C.I.undefined as one cell is zero) *pcor* = 0.045 (for the 123 comparisons). Residue DRβN37 also demonstrated an association with CBD (p = 0.00378) but this difference was not significant upon correction for the 123 tests.

Among BeS subjects who were DPβE69 negative, the DRβE71 residue was significantly increased [9 of 9 (100%) CBD subjects vs. 34 of 177 (19.2%) controls; (OR and C.I.undefined as one cell is zero) *pcor* = 1.1×10^{-4} (for the 123 comparisons)]. Additionally the DRβN37 residue was significantly increased when comparing BeS to controls [9 of 9 (100%) BeS subjects vs. 56 of 177 (31.6%) controls; (OR and C.I.undefined as one cell is zero) *pcor* = 0.0065 (for the 123 comparisons)]. Additionally residue DRβH32 was also differentially distributed between BeS and controls [9 of 9 (100%) BeS subjects vs. 66 of 177 (37.3%) controls; (OR and C.I.undefined as one cell is zero) *pcor* = 0.026 (for the 123 comparisons)]. Residues E71, N37 and H32 in pairs, were in strong LD in both BeS and controls. Neither E71, N37 or H32 were distributed differently among individuals stratified for any of the three and evaluated for the distribution of each of the other two amino acids. It is therefore unclear as to whether any of these three residues is stronger than the other in terms of predisposing to BeS among DPβE69 negative subjects.

Among DPβE69 negative BeS subjects the DRβS11 and DRβS13 were also increased among BeS subjects but neither of these two associations were significant after correction for multiple tests (123 comparisons). Both residues were in strong linkage disequilibrium with DRβE71, in both control and BeS subjects. When the population of alleles was stratified for DRβS11 or DRβS13 the distribution of DRβE71 was significantly increased among BeS subjects as compared to controls (DRßS11: OR 10.54 (95% C.I. 2.43-52.30) p = 0.0031; DRßS13: OR 9.00 (95% C.I. 2.07-44.75) p = 0.004. When the population of alleles was stratified for the DRβE71, neither the DRβS11 nor the DRβS13 residues demonstrated any differential distributions. These results suggest that the DRβE71 residue has a functional role that is more important than that of DRβS11 or DRβS13 in BeS, while the reverse is not so. Table 5 summarizes our analyses of all the DRB1 residues previously reported as being associated with CBD or BeS.

Evaluation of the whole cohort for the distribution of DPβE69 and/or DRβ71 found that 65 of 65 (100%) CBD subjects, 44 of 44 (100%) BeS subjects and 145 of 288 (50.3%) controls were positive for either of these amino acids. Both these associations were highly significant, $p = 7.1 \times 10^{-14}$ and $p = 2.37 \times 10^{-11}$, respectively (OR and C.I.undefined as one cell is zero).

Discussion/Conclusion: Our study cohort is one of the largest involving the characterization of HLA class II molecules in CBD and BeS cases. Members of our cohort had longer latency periods from first and last exposure to beryllium than most previously reported cohorts. It is also one of the most complete, in terms of typing of DRB1 and DPB1 genes at the allele level. The analyses we performed comprehensively examined the role of every single polymorphic residue in both isotypes, as well as a

number of related questions such as the role of homozygosity and relative strength of association or linkage disequilibrium between residues.

We confirmed the previously reported association of HLA-DPβE69 residue with both CBD and BeS (3, 4, 5-7). The OR for CBD was 19.14 (95% C.I. 7.10-55.92), while the OR for BeS subjects was 6.20 (95% C.I. 2.73-14.47), with an attributable risk of 95% and 84%, respectively. Although the DPβE69 residue is associated with the development of both CBD and BeS without CBD, it is possible that the risk may be different for these two outcomes. In other studies, the risk for sensitization has been greater (OR of 9.9 for BeS vs. 5.8 for CBD) (4), or the residue conveyed equal risk for both CBD and BeS (19). The differences in ORs for CBD and BeS between studies may simply reflect study differences in confirming CBD, latency since first or last exposure to beryllium, or sample size. In any case, this residue is important for both phenotypes since there is a significant difference when the CBD and BeS subjects are compared to the beryllium-exposed controls.

It has also been suggested that the inheritance of two DPβE69 positive alleles might confer a greater risk for progression to CBD (6). We found no difference in the risk of developing CBD vs. BeS by zygosity and therefore question that homozygosity of DPβE69 is a risk factor that contributes and differentiates CBD from BeS (Figure 1).

Another observation in our study was that the less frequent DPβE69-positive but non-DPB1*0201 alleles are more common in individuals with CBD compared to controls, and that individuals who are DPβE69 positive but do not have evidence of CBD are more likely to have the *0201 allele. This has previously been reported (21) and our results confirm this finding (Figure 3). Furthermore, when our cohort was stratified for DPβE69 positive, DPB1*0201 negative subjects, it was found that the DPβDE55, 56

epitope was associated with CBD but not BeS. It therefore derives that besides the DPβE69 residue, the DPβDE55, 56 epitope is also associated with CBD among non-DPB1*0201 alleles. Note that the DPβDE55, 56 was not increased among DPβE69 positive CBD subjects. Considering that the DPB1*0201 allele is also positive for the DPβDE55, 56 epitope, we hypothesize that either the DPB1*0201 allele itself or other genes besides DPB1 include an element with a protective role that renders this molecule/haplotype to be not associated with CBD among DPβE69 positive subjects. When the whole population was stratified for the DPB1*0201 allele no residue was found to be associated with disease outcome. Therefore another gene on the DPB1*0201 positive haplotype is most likely responsible for influencing disease outcome and not the DPB1*0201 allele itself. This hypothesis sets the stage for further investigation of this group of DPB1*0201 positive individuals in genome-wide association studies for the identification of particular SNP polymorphisms outside of the DPB1 gene, which possibly exert a protective role. This observation of an association of the DPβDE55, 56 epitope in CBD individuals who are DPβE69 positive, DPB1*0201 negative, along with our other finding showing that DPβDE55, 56 is not distributed differently among DPβE69 positive CBD subjects and controls suggests that the DPβDE55, 56 epitope plays a role in disease susceptibility but is secondary to DPβE69. The role of DPβDE55, 56 became evident only after we removed the DPß1*0201 positive subjects from the population. Similar to the results of Snyder (20) we found an increased risk for CBD for individuals with -9 and -7 charge alleles, with the risk being greater for the -9 vs -7 charge alleles and no significant difference in the total molecular charge for both CBD and BeS. . Unlike Snyder we found a similar result for BeS. In addition, our results suggest that other negatively charged HLA epitopes, such as the DPβDE55, 56 epitope,

play a role in CBD susceptibility, provided it is in the absence of a protective influence exerted by the DPB1*0201 haplotype. This in turn suggests an interactive relationship of residues and/or genes, some with susceptibility others with protective roles of different strengths that influence the disease process. The particular location of the DPβD55, which is equivalent to D57 of the DQβ chain, which is known to be associated with other autoimmune diseases, like type 1 diabetes (22) or the DQβRLD55-57 epitope, which is associated with the acute inflammatory demyelinating polyneuropathy, a form of Guillain-Barre syndrome (23) is consistent with the influence of the DPβDE55, 56 epitope on the binding of beryllium or peptide on particular HLA alleles.

Similar to previous investigators we found that 8% of individuals with CBD and 20% with BeS were HLA-DPß1E69 negative while 62.7% of beryllium exposed controls were HLA-DPß1E69 positive (11). Inhibition of Be stimulated T-cell proliferation assays by anti DR monoclonal antibodies in DPβE69 negative subjects argues for a role of the DR molecule in the development of CBD and Be sensitization (12) . Our study found polymorphisms of the DRB1 gene that are relevant. Among DPβE69 negative subjects, DRβE71 was significantly increased in both CBD and BeS subjects compared to controls, while DRβN37 and DRβH32 were significantly increased among BeS but not among CBD subjects. When the three residues were evaluated for strength of association in the BeS group, it was demonstrated that they were all in strong linkage disequilibrium among themselves within BeS and control groups and that neither of the two were more strongly associated with BeS than the other. Whether the DRβN37 and DRβH32 residues are truly associated with BeS and not with CBD needs further evaluation and confirmation in other populations. There were a number of secondary residues such as DRβS11 and DRβS13 that may play a secondary role (Table 5). While the same

observations are not made among CBD subjects, it is possible that beryllium-influenced peptide binding patterns are different between DR molecules with different combinations of key residues and as such, different DR molecules or combinations may affect the ensuing T-cell responses such that they only lead to BeS and not CBD. Peptide binding studies and T-cell functional assays are needed to evaluate their possible contribution in disease development.

The significance of DRβE71 has previously been demonstrated in functional assays where DR molecules with E71 are capable of inducing Be specific proliferation and IFN-γ expression by lung CD4 positive T-cells (8) and DR13 has been shown to be increased among DPβE69 negative subjects with CBD (5). Even though this latter observation does not necessitate the involvement of E71, because only some DR13 alleles are positive for E71 while others are not, it does suggest the involvement of the DRB1 gene as a possible element of susceptibility in individuals who are negative for the DPβE69 residue. Another more recent study did not report on the association of DRβE71 with DPβE69 negative CBD or BeS subjects but did report that DRβN37 was associated with the combined population of both CBD and BeS subjects (12). However, this study attributed the association of DRβN37 with beryllium hypersensitivity to linkage disequilibrium with DRβF47 and not to a direct role of DRβN37 (12). We did not find an association with DRβF47 in our cohort. In our study, in the absence of the DPβE69 residue, only the DRB1 gene with the polymorphisms of DRβE71 was significant, although possibly other DRß1 residues influence the development of CBD and/or BeS. Further population and functional studies should clarify the role of these two residues in the process of sensitization and disease.

The significance of the DRβ residue E71 for developing CBD or BeS is reminiscent of other previous reports on the role of these residues in peptide binding, in T-cell receptor interactions and in other HLA-disease association studies, where the same polymorphic residues have been implicated. Position DRβ71 of pocket 4 is known to be critical for both peptide binding and T-cell interactions. Using transfectants, expressing a number of different DR molecules modified by site directed mutagenesis at specific sites, it was demonstrated that T-cell responses were significantly influenced by pocket 4 residues (including β71) (24). It has been reported that DRß71 influences the charge of the P4 pocket and as such it is a critical determinant of MHC - linked susceptibility to pemphigus vulgaris (25), rheumatoid arthritis (26, 27), multiple sclerosis (28), tuberculoid leprosy (29) and epithelial ovarian carcinoma (30). The residues DRβ71of pocket 4 in pemphigus vulgaris patients carries negative charges (glutamate), while in rheumatoid arthritis carries positive charges (lysine or arginine). The charge of DRβ71 confers selective binding of peptides with a positive charge in the pemphigus vulgaris and with a negative charge in rheumatoid arthritis patients (25). Pocket 4 has also been shown to be critical in the binding of the immunodominant peptide (residues 84-102) of myelin basic protein, a putative auto antigen in multiple sclerosis, to DRB1*1501 in patients with relapsing – remitting multiple sclerosis (31, 32). It has been reported that the presence of alleles encoding DRβ chain containing a negative charged glutamic acid residue at β71 or β74 protects against the development of a relapsing – remitting multiple sclerosis course or increases the susceptibility to a primary progressive course of multiple sclerosis or both (28). Finally in tuberculoid leprosy, DRβ71 has been reported to be associated with the presence of arginine (29). Independent of these immunologically based diseases, the DRβDE70, 71 epitope has also been shown to be associated with protection to epithelial ovarian carcinoma (30).

When the entire cohort was evaluated for the presence of DPβE69 and or DRβE71, it was found that 100% of CBD and 100% of BeS subjects were positive for at least one of the two residues but only about 50% of the control subjects were positive for either of the two residues. The presumed significance of these amino acids at different positions in the HLA-DPB1 and HLA-DRB1 genes is that they change the binding of beryllium or of a peptide bound to beryllium and as such influence the presentation to T-cells and of the immune process that leads to CBD and or sensitization. However while these HLA polymorphisms are necessary for developing CBD and/or BeS, there may not be sufficient. These findings suggest that there may be additional reasons that contribute to disease outcomes including: other genetic polymorphisms that still need to be identified or cumulative, peak or type of exposure (i.e. particle size or physical property) that may interact with genetic variability (36-38).

The observation that almost every beryllium-affected individual is positive for these HLA polymorphisms, while only 50% of controls are positive for the same residues, is extremely important in the context of identifying additional genes involved in the disease process. It appears that these HLA residues are necessary but not sufficient for the disease phenotype. Stratification of our population based on the specific HLA polymorphisms should reveal differences in genome-wide association studies, between control and all the other subjects that will go beyond HLA polymorphisms and will be important for predicting disease susceptibility and/or protection. Current preliminary genome-wide association studies performed in our population suggests that indeed this may be the case.

Initial analyses of beryllium exposure levels which were presented at the Philadelphia conference (See Section G) did not show an interaction between genetic analsysis and Be exposure metrics.

A second limitation is that even though our cohort was relatively large, our sample size was still limited, particularly when stratifying solely by E69 negative CBD or BeS subjects, 5 and 9 subjects respectively, to ensure sufficient statistical power to detect a statistically significant difference. Also, because of the multiple comparisons we performed we used conservative correction factors. Even though a difference was not significant after correction, it is possible that a larger sample size would yield a significant difference. We calculated the power of the observed comparisons between disease groups of polymorphisms using the obtained sample sizes, and found that most of the comparisons had power <0.50. None of the comparisons of diseased vs. sensitized had power exceeding 0.38. For comparing diseased to non-diseased, only the comparisons of either homozygous or heterozygous for E69, non-0201 alleles in E69 positive individuals, N37 in E69 negative individuals, and N32 in E69 negative individuals had power >0.50. For comparing sensitized to non-diseased, only the comparisons of homozygosity, heterozygosity or either for E69, non-0201 alleles in E69 positive individuals, N37 in E69 negative individuals, N37 in E69 positive individuals, H32 in E69 positive individuals, and Ser13 in E69 negative individuals had power >0.50.

The results of this study continue to expand our knowledge about the genetics of beryllium disease. HLA molecules definitively play a significant role in this immunologically mediated disease and identifying the specific HLA components has contributed to the identification of future directions that should be pursued for a more comprehensive understanding of its pathophysiology. Ideally these pursuits will contribute to interventions that will prevent the occurrence of beryllium disease as well as insight into other conditions with similar pathological mechanisms.

Table1. Minimum Criteria For Beryllium Disease Categories

Disease Category	Bronchial Lavage	Biopsy Granuloma	Chest Radiograph	Beryllium LPT
Chronic Beryllium Disease (CBD)	+ BeLPT	Positive	*	*
	Not done	Positive	*	Two + BeLPTs
Probable CBD	+ BeLPT	Not done	Fibrosis any zone	*
	Negative or not done	Not done	Fibrosis any zone	Two + BeLPTs
Possible CBD	Not done	Not done	Lower or mid lobe fibrosis	- BeLPT or single + BeLPT and no re-test
Sensitization	- BeLPT	Negative or not done	Normal	Two + BeLPTs
	+ BeLPT	Negative or not done	Normal	*
	Not done	Negative or not done	Normal	Two + BeLPTs
Possible Sensitization	Negative or not done	Negative or not done	Normal	Single + BeLPT and no retest or - BeLPT retest

* Test result does not affect disease categorization.

Table 2. Demographic Characteristics of Participants

Characteristic	Chronic Beryllium Disease n = 65	Beryllium Sensitization n = 44	Controls n = 288
Age, yr	59.9 ± 10	60.3 ± 9.7	60.2 ± 9.6
Male sex, %	94	83	91
Caucasian, %	100	96	98
Facility A, %	49	57	52
Duration of Work, yrs	9.1 ± 9.8	6.3 ± 9.6	11.6 ± 12.2

Data are presented as mean ± SD.

Table 3. HLA-DPB1* Allele Distribution in Control, Beryllium Sensitization (BeS) and Chronic Beryllium Disease (CBD) Subjects

	Control n = 288		Beryllium Sensitization n = 44		Chronic Beryllium Disease n = 65	
Alleles	n	%	n	%	n	%
DPB1*0101	30	10.42	1	2.27	2	2.56
DPB1*0201	75	26.04	19	43.18	34	43.59
DPB1*0202	2	0.69	-	-	2	2.56
DPB1*0301	45	15.63	6	13.64	11	14.10
DPB1*0401	190	65.97	25	56.82	36	46.15
DPB1*0402	61	21.18	7	15.91	18	23.08
DPB1*0501	12	4.17	3	6.82	3	3.85
DPB1*0601	6	2.08	2	4.55	8	10.26
DPB1*0901	2	0.69	3	6.82	4	5.13
DPB1*1001	5	1.74	5	11.36	8	10.26
DPB1*1101	9	3.13	-	-	2	2.56
DPB1*1301	15	5.21	6	13.64	7	8.97
DPB1*1401	5	1.74	-	-	4	5.13
DPB1*1501	3	1.04	-	-	-	-
DPB1*1601	1	0.35	2	4.55	1	1.28
DPB1*1701	7	2.43	3	6.82	7	8.97
DPB1*1901	4	1.39	1	2.27	2	2.90
DPB1*1902	2	0.69	-	-	-	-
DPB1*2001	4	1.39	-	-	-	-
DPB1*2301	8	2.78	1	2.27	-	-
DPB1*2601	1	0.35	-	-	-	-
DPB1*2901	1	0.35	-	-	-	-
DPB1*4501	1	0.35	-	-	-	-
DPB1*4601	1	0.35	-	-	-	-
DPB1*7101	1	0.35	-	-	1	1.28
DPB1*8801	-	-	1	2.27	-	-

Table 4. HLA-DRB1* Allele Distribution in Control, Beryllium Sensitization (BeS) and Chronic Beryllium Disease (CBD) Subjects

	Control n = 288		Beryllium Sensitization n = 44		Chronic Beryllium Disease n = 65	
Alleles	n	%	n	%	n	%
DRB1*0101	45	15.63	5	11.36	16	20.51
DRB1*0102	6	2.08	2	4.55	3	3.85
DRB1*0103	1	0.35	-	-	3	3.85
DRB1*0301	50	17.36	4	9.09	13	16.67
DRB1*0304	-	-	1	2.27	-	-
DRB1*0401	30	10.42	5	11.36	5	6.41
DRB1*0402	6	2.08	3	6.82	1	1.28
DRB1*0403	5	1.74	2	4.55	-	-
DRB1*0404	12	4.17	1	2.27	4	5.13
DRB1*0405	6	2.08	1	2.27	-	-
DRB1*0407	3	1.04	1	2.27	-	-
DRB1*0408	-	-	1	2.27	-	-
DRB1*0701	68	23.61	11	25.00	24	30.77
DRB1*0801	17	5.90	2	4.55	4	5.13
DRB1*0802	1	0.35	1	2.27	-	-
DRB1*0803	1	0.35	-	-	-	-
DRB1*0804	2	0.69	-	-	2	2.56
DRB1*0901	3	1.04	-	-	-	-
DRB1*1001	5	1.74	2	2.67	-	-
DRB1*1101	50	17.36	11	25.00	11	14.10
DRB1*1102	1	0.35	1	2.27	1	1.28
DRB1*1103	3	1.04	-	-	2	2.56
DRB1*1104	22	7.64	3	6.82	9	11.54
DRB1*1109	-	-	-	-	-	-
DRB1*1143	1	0.35	-	-	-	-
DRB1*1201	9	3.13	-	-	6	7.69
DRB1*1301	33	11.46	9	20.45	9	11.54
DRB1*1302	27	9.38	6	13.64	8	10.26
DRB1*1303	15	5.21	2	4.55	2	2.56
DRB1*1305	2	0.69	2	4.55	1	1.28
DRB1*1401	20	6.94	-	-	4	5.13
DRB1*1407	1	0.35	-	-	-	-
DRB1*1501	76	26.39	4	9.09	15	19.23
DRB1*1502	4	1.39	2	4.55	3	3.85
DRB1*1503	2	0.69	1	2.27	-	-
DRB1*1601	13	4.51	3	6.82	2	2.56
DRB1*1602	1	0.35	-	-	1	1.28

Table 5. Results of Analyses of HLA-DRβ With DPβ By Glutamic Status at Position 69 and Disease Status for DRβ Residues Previously Reported in Medical Literature

	DPßE69+		DPßE69-		
DRß Residues	CBD n = 60	BeS n = 35	CBD n = 5	BeS n = 9	Previous Findings From Medical Literature
Arginine 74	NA	NA	NA	NA	E69- only, CBD and S combined[21]
Asparagine 37	NA(-)	NA	NA(+)	+	E69- only, CBD and S combined[21] E69+ and E69- CBD and S combined[4]
Glutamate 71	NA	NA	+	+	E69- only, CBD[8] E69+ and E69- CBD and S combined[4]
Histidine 32	NA(-)	NA	NA	+	E69- only, CBD and S combined[21]
Phenylalanine 47	NA	NA	NA	NA	E69- only, CBD and S combined[21] E69- only, CBD[5]
Serine 11	NA	NA	NA	NA(+)	E69+ and E69- CBD and S combined[4]
Serine 13	NA	NA	NA	NA(+)	E69- only, CBD and S combined[21]
Tyrosine 26	NA	NA	NA	NA	E69- only, CBD and S combined[21]

Definition of abbreviations:

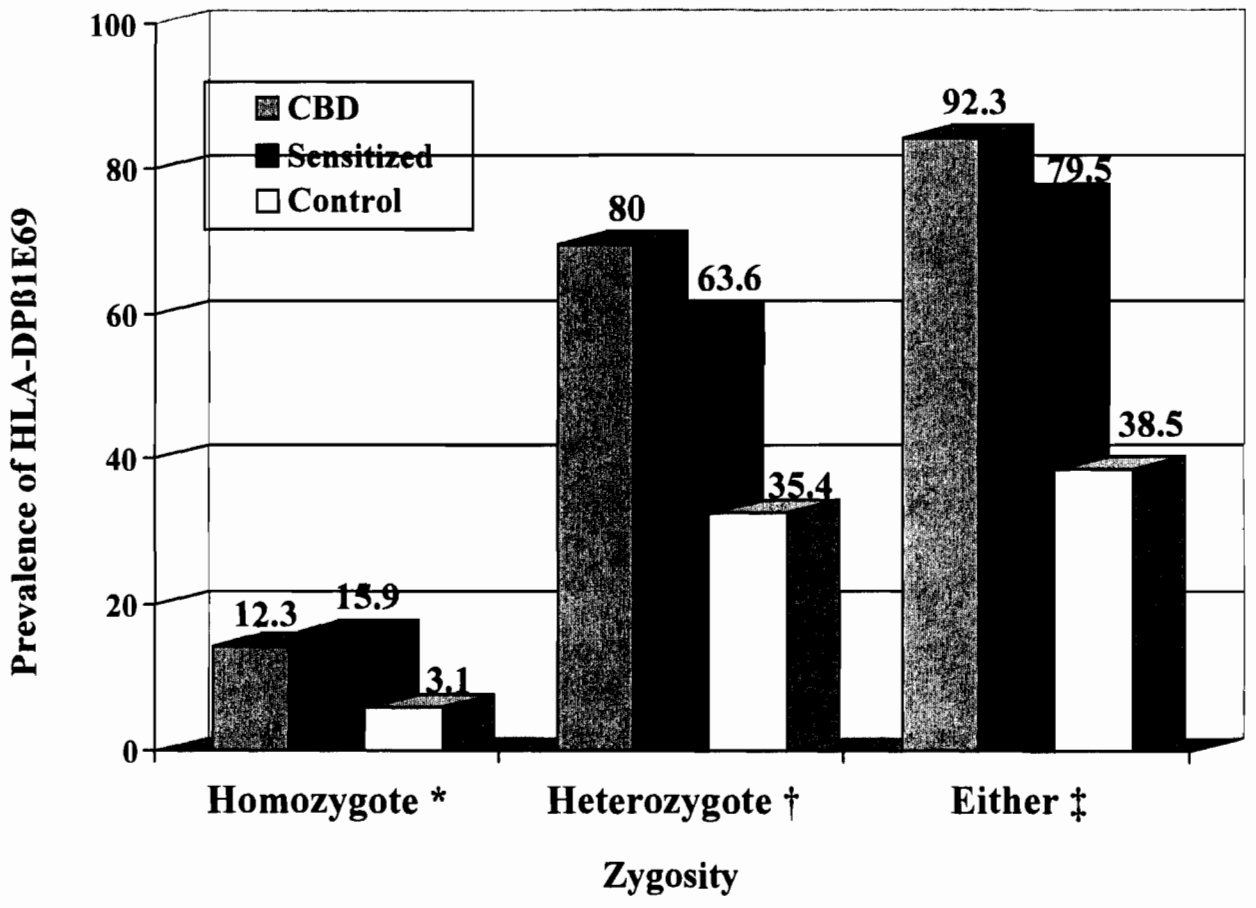
+ = positive statistical association ($p_{cor} < .05$)

- = negative statistical association ($p_{cor} < .05$)

NA = No association

NA(±) = No statistical association after correction but trend in the direction shown in parentheses

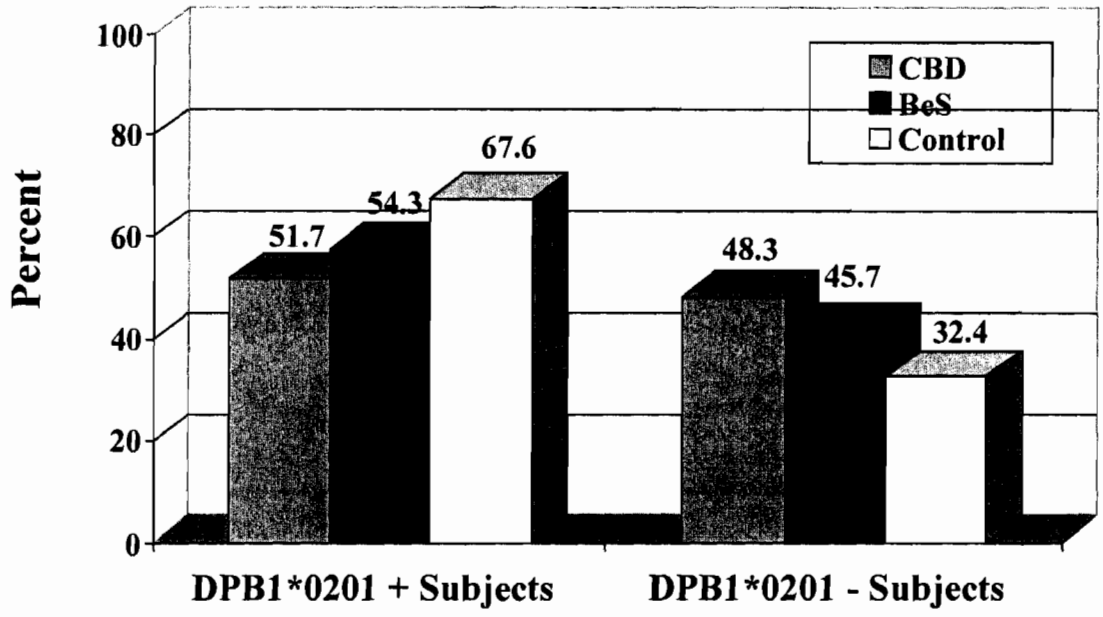
Figure 1. Prevalence of HLA-DPβE69 by Beryllium Disease Category and Zygosity



Number of Cases: Homozygote: CBD=8, BeS=7, Control=9
Heterozygote: CBD=52, BeS=28, Control=102
Either: CBD=60, BeS=35, Control=111

* CBD vs control, $p<0.0001$; BeS vs control, $p<0.0001$; CBD vs BeS, $p=0.88$
† CBD vs control, $p<0.0001$; BeS vs control, $p<0.0001$; CBD vs BeS, $p=0.88$
‡ CBD vs control, $p=1.838 \times 10^{-16}$; BeS vs control, $p=3.8 \times 10^{-7}$; CBD vs BeS, $p=0.08$

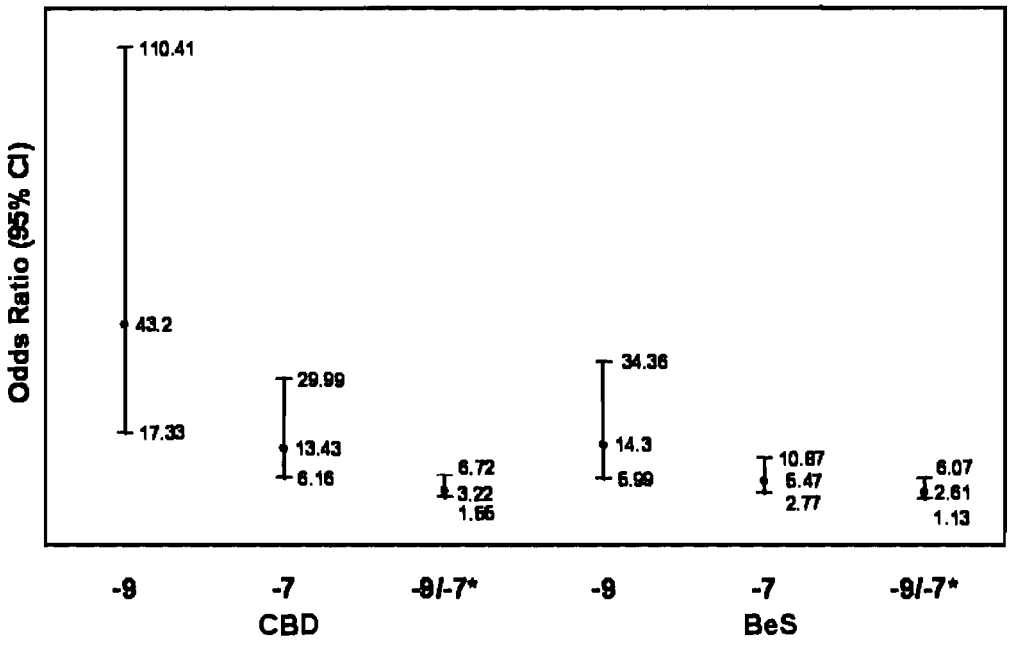
Figure 2. Prevalence of DPB1*0201 + and – Subjects Among HLA-DPβ69 Positive Individuals by Beryllium Disease Category



Number of Cases: DPB1*0201 + Subjects: CBD=31, BeS=19, Control=75
DPB1*0201 - Subjects: CBD=29, BeS=16, Control=36

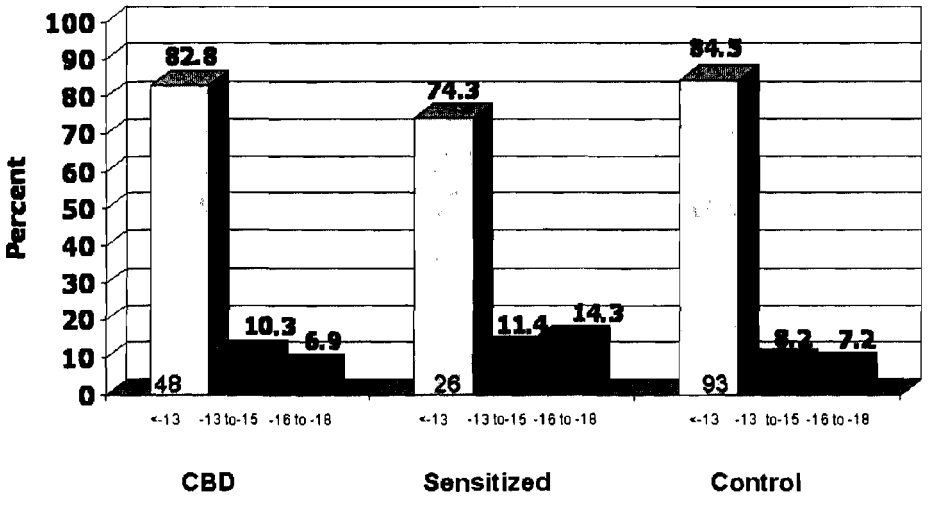
p=0.048

Figure 3. Odds Ratios of Alleles With -9 or -7 Charge Among Individuals With CBD or BeS Who Are E^{69} Positive Versus Controls Who Are E^{69} Negative



*Comparison is -9 vs -7 charge alleles among E^{69} positive individuals

Figure 4. Combined Negative Charge by Individuals for HLA-DPB1 Gene by CBD, Sensitization and Controls Status Among E^{69} Positive Individuals



References:

1. Newman LS, Mroz MM, Balkissoon R, Maier LA. Beryllium sensitization progresses to chronic beryllium disease: a longitudinal study of disease risk. *Am J Respir Crit Care Med* 2005;171:54-60.

2. Sterner JH, Eisenbud M. Epidemiology of beryllium intoxication. *Arch Ind Hyg Occup Med* 1951;4:123-151.

3. Richeldi L, Sorrentino R, Saltini C. HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science* 1993;262:242-244.

4. Rossman MD, Stubbs J, Lee CW, Argyris E, Magira E, Monos D. Human leukocyte antigen class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *Am J Respir Crit Care Med* 2002;165:788-94.

5. Maier LA, McGrath DS, Sato H, Lympany P, Welsh K, du Bois R, Silveira L, Fontenot AP, Sawyer RT, Wilcox E, Newman LS. Influence of MHC class II in susceptibility to beryllium sensitization and chronic beryllium disease. *J Immunol* 2003;171:6910-6918.

6. McCanlies EC, Ensey JS, Schuler CR, Kreiss K, Weston A. The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. *Am J Ind Med* 2004;46:95-103.

7. Wang Z, Farris GM, Newman LS, Shou Y, Maier LA, Smith HN, Marrone BL. Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology* 2001;165:27-38.

8. Bill JR, Mack DG, Falta MT, Maier LA, Sullivan AK, Joslin FG, Martin AK, Freed BM, Kotzin BL, Fontenot AP. Beryllium presentation to CD4+ T cells is dependent on a single amino acid residue of the MHC class II beta-chain. *J Immunol* 2005;175:7029-37.

9. Fontenot AP, Torres M, Marshall WH, Newman LS, Kotzin BL. Berylliun presentation to CD4+ T cells underlies disease-susceptibility HLA-DP alleles in chronic beryllium disease. *Proc Natl Acad Sci USA* 2000;97:12717-12722.

10. Lombardi G, Germain C, Uren J, Fiorillo MT, du Bois RM, Jones-Williams W, Saltini C, Sorrentino R, Lechler R. HLA-DP allele-specific T cell responses to beryllium account for DP-associated susceptibility to chronic beryllium disease. *J Immunol* 2001;166(5):3549-3555.

11. McCanlies EC, Kreiss K, Andrew M, Weston A. HLA-DPB1 and chronic beryllium disease: a HUGE review. *Am J Epidemiol* 2003;157:388-398.

12. Amicosante M, Berretta F, Rossman M, Butler RH, Rogliani P, Berg-Loonen E, Saltini C. Identification of HLA-DRPB^{Phe47} as the susceptibility marker of hypersensitivity to beryllium in individuals lacking the berylliosis-associated supratypic marker HLA-DPB^{E69}. *Respir Res* 2005;6:94.

13. Sato H, Spagnolo P, Silveira L, Welsh KI, du Bois RM, Newman LS, Maier LA. BTNL2 allele associations with chronic beryllium disease in HLA-DPβ1*Glu 69- negative individuals. *Tissue Antigens* 2007;70:480-486.

14. Jonth AC, Silveira L, Fingerlin TE, Sato H, Luby JC, Welsh KI, Rose CS, Newman LS, du Bois RM, Maier LA. TGF-β1 variants in chronic beryllium disease and sarcoidosis. *J Immunol* 2007;179:4255-4262.

15. Sato H, Silveira L, Fingerlin T, Dockstader K, Gillespie M, Lagan AL, Lympany P, Sawyer RT, du Bois RM, Welsh KI, Maier LA. TNF polymorphism and bronchoalveolar lavage cell TNF-α levels in chronic beryllium disease and beryllium sensitization. *J Allergy Clin Immunol* 2007;119:687-696.

16. Rosenman K, Hertzberg V, Rice C, Reilly MJ, Aronchick J, Parker JE, Regovich J, Rossman M. Chronic beryllium disease and sensitization at a beryllium processing facility. *Environ Health Perspect* 2005;113:1366-1372.

17. Rosenman KD, Monos D, Hertzberg VS, Reilly MJ, Rice C, Rossman M. Genetic susceptibility to beryllium toxicity. Proceedings of the American Thoracic Society International Conference; 2007 May 18-23; San Francisco, CA; 175:C18,A564.

18. Dapprich J, Magira E, Samonte MA, Rosenman K, Monos D. Identification of a novel HLA-DPB1 allele (DPB1*1902) by haplotype-specific extraction and nucleotide sequencing. *Tissue Antigens* 2007;69:282-284.

19. Svejgaard, A and Ryder LP. HLA and disease associations: detecting the strongest association. *Tissue Antigens* 1994; 43:18-27.

20. Kanterakisa S, Magiraa E, Rosenman KD, Rossman M, Talsaniaa K, Monos DS. SKDM human leukocyte antigen (HLA) tool: Acomprehensive HLA and disease associations analysis software. Human Immunology 2008; 69:522-525.

21. Snyder JA, Demchuk E, McCanlies EC, Schuler CR, Kreiss K, Andrew ME, Frye BL, Ensey JS, Stanton ML, Weston A. Impact of negatively charged patches on the surface of MHC class II antigen – presenting proteins on risk of chronic beryllium disease. *J R Soc Interface.* (In press)

22. Wang Z, White PS, Petrovic M, Tatum OL, Newman LS, Maier LA, Marrone BL. Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and - DPA1 alleles. *J Immunol* 1999;163:1647-1653.

23. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M. Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study. *Proc Natl Acad Sci USA* 1988; 85:8111-8115.

24. Magira EE, Papaioakim M, Nachamkin I, Asbury AK, Li CY, Ho TW, Griffin JW, McKhann GM, Monos DS. Differential distribution of HLA-DQ beta/DR beta epitopes in the two forms of Guillain-Barré syndrome, acute motor axonal neuropathy and acute inflammatory demyelinating polyneuropathy (AIDP): identification of DQ beta epitopes associated with susceptibility to and protection from AIDP. *J Immunol* 2003; 170:3074-3080.

25. Fu XT, Bono CP, Woulfe SL, Swearingen C, Summers NL, Sinigaglia F, Sette A, Schwartz BD, Karr RW. Pocket 4 of the HLA –DR (alpha,beta 1*0401) molecule is a major determinant of T cells recognition of peptide. *J Exp Med* 1995; 181:915-926.

26. Wucherpfennig KW, Yu B, Bhol K, Monos DS, Argyris E, Karr RW, Ahmed AR, Strominger JL. Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity: charged residues of a single MHC binding pocket confer selective presentation of self-peptides in pemphigus vulgaris. *Proc Natl Acad Sci USA* 1995; 92:11935-11939.

27. Zanelli E, Breedveld FC, de Vries RR. HLA class II association with rheumatoid arthritis: facts and interpretations. *Hum Immunol* 2000; 61:1254-1261.

28. Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis: an approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987; 30:1205-1213.

29. Greer JM, Pender MP. The presence of glutamic acid at positions 71 or 74 in pocket 4 of the HLA-DRbeta1 chain is associated with the clinical course of multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2005; 76:656–662.

30. Zerva L, Cizman B, Mehra NK, Alahari SK, Murali R, Zmijewski CM, Kamoun M, Monos DS. Arginine at positions 13 or 70-71 in pocket 4 of HLA-DRB1 alleles is associated with susceptibility to tuberculoid leprosy. *J Exp Med* 1996; 183:829-836.

31. Monos DS, Pappas J, Magira EE, Gaughan J, Aplenc R, Sakkas L, Freedman R, Reveille JD, Platsoucas CD. Identification of HLA-DQalpha and -DRbeta residues associated with susceptibility and protection to epithelial ovarian cancer. *Hum Immunol* 2005; 66:554-562.

32. Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, Gran B, Eaton J, Antel J, Frank JA, McFarland HF, Martin R. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med* 2000; 6:1167–1175.

33. Smith KJ, Pyrdol J, Gauthier L, Wiley DC, Wucherpfennig KW. Crystal structure of HLA-DR2 (DRA*0101, DRB1*1501) complexed with a peptide from human myelin basic protein. *J Exp Med* 1998; 188:1511–1520.

34. Matsuki K, Grumet FC, Lin X, Gelb M, Guilleminault C, Dement WC, Mignot E. DQ (rather than DR) gene marks susceptibility to narcolepsy. *Lancet* 1992; 339:1052.

35. Siebold C, Hansen BE, Wyer JR, Harlos K, Esnouf RE, Svejgaard A, Bell JI, Strominger JL, Jones EY, Fugger L. Crystal structure of HLA-DQ0602 that protects against type 1 diabetes and confers strong susceptibility to narcolepsy. *Proc Natl Acad Sci USA* 2004; 101:1999-2004.

36. Taheri S, Zeitzer JM, Mignot E. The role of hypocretins (orexins) in sleep regulation and narcolepsy. *Annu Rev Neurosci* 2002; 25:283-313.

37. Richeldi L, Kreiss K, Mroz MM, Zhen B, Tartoni P, Saltini C. Interaction of genetic and exposure factors in the prevalence of berylliosis. *Am J Ind Med* 1997; 32:337-340.

38. Schuler CR, Kent MS, Deubner DC, Berakis MT, McCawley M, Henneberger PK, Rossman MD, Kreiss K. Process-related risk of beryllium sensitization and disease in a copper-beryllium alloy facility. *Am J Ind Med* 2005; 47:195-205.

39. Tinkle SS, Antonini JM, Rich BA, Roberts JR, Salmen R, DePree K, Adkins EJ. Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ Health Perspect* 2003;111:1202-1208.

40. Stange AW, Furman FJ, Hilmas DE. The beryllium lymphocyte proliferation test: relevant issues in beryllium health surveillance. *Am J Ind Med* 2004; 46:453-462.

41. Newman LS, Mroz MM, Maier LA, Daniloff EM, Balkissoon R. Efficacy of serial medical surveillance for chronic beryllium disease in a beryllium machining plant. *J Occup Environ Med* 2001; 43:231-237.

G. Publications/Presentations

Kanterakisa S, Magiraa E, Rosenman KD, Rossman M, Talsaniaa K, Monos DS. SKDM human leukocyte antigen (HLA) tool: A comprehensive HLA and disease associations analysis software. Human Immunology 2008; 69:522-525.

Dapprich J, Magira E, Samonte M, Rosenman KD, Monos D. Identification of a Novel HLA-DPB1 Allele (DPB1*1902) by Haplotype Specific Extraction and Nucleotide Sequencing. Tissue Antigens 2007; 69:282-284.

Rosenman, K D, Rossman M D, Hertzberg V, Reilly M, Rice Ca, Kanterakis S, Monos DS. HLA Class II Polymorphisms Associated with Genetic Susceptibility to Beryllium Toxicity (Submitted for publication)

Rosenman KD, Monos D, Hertzberg VS, Reilly MJ, Rice C, Rossman M. Genetic Susceptibility to Beryllium Toxicity. 2007 American Thoracic Society International Conference, San Francisco, California, 5/18-5/23, 2007. Proceeding of the American Thoracic Soc 2007; 175: C18, A564.

Rosenman KD, Monos D, Hertzberg VS, Reilly MJ, Rice C, Rossman M. Results of Surveillance in Former Beryllium Workers. 3rd International Conference on Beryllium. Philadelphia, Pennsylvania, 10/17/07.

Rosenman KD, Monos D, Hertzberg VS, Reilly MJ, Rice C, Rossman M. Gene-Environmental Interaction. 3rd International Conference on Beryllium. Philadelphia, Pennsylvania, 10/18/07.

H. Inclusion of Gender and Minority Study subjects

Study Title: Genetic/Exposure Interaction in beryllium Disease

Total Enrollment: 432 **Protocol Number:**

Grant Number: 5 R01 OH 007495

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
	Sex/Gender			
Ethnic Category	**Females**	**Males**	**Unknown or Not Reported**	**Total**
Hispanic or Latino				**
Not Hispanic or Latino	41	391		432
Unknown (individuals not reporting ethnicity)				
Ethnic Category: Total of All Subjects*	41	391		432 *
Racial Categories				
American Indian/Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White	41	391		432
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of All Subjects*	41	391		432 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	**Females**	**Males**	**Unknown or Not Reported**	**Total**
American Indian or Alaska Native				
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White				
More Than One Race				
Unknown or Not Reported				
Racial Categories: Total of Hispanics or Latinos**				**

I. Inclusion of Children

No children were included. This was a cohort of workers and former workers all over 21 years of age.

J. Materials Available for Other Investigators

Software was developed to conduct genetic analysis and is available for free at http://sourceforge.net/projects/skdm