RISK OF DEVELOPING CHRONIC BERYLLIUM DISEASE AND BERYLLIUM SENSITIZATION ASSOCIATED WITH HLA-DPB1 AND DRB1 POLYMORPHISMS AND MAGNITUDE OF BERYLLIUM EXPOSURE

By

Vitri Widyaningsih

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ABSTRACT

RISK OF DEVELOPING CHRONIC BERYLLIUM DISEASE AND BERYLLIUM SENSITIZATION ASSOCIATED WITH HLA-DPB1 AND DRB1 POLYMORPHISMS AND MAGNITUDE OF BERYLLIUM EXPOSURE

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Background: Beryllium exposure is a necessary but not sufficient cause of CBD and BeS. The presence of HLADPB1 Glutamine 69 and other polymorphisms were shown in previous studies to influence disease development. Our goal was to examine genetics and exposure effect in the development of BeS and CBD and progression from BeS to CBD

Methods: DNA-based typing was conducted for all subjects (n=361) consisting of 61 CBD, 41 BeS, and 259 exact matched controls. Exposures were assessed through job history and industrial hygiene records.

Results: Glutamine 69 increased the risk of Chronic Beryllium Disease and Beryllium Sensitization (OR respectively 25.7; 95% CI 6.1-108.5 and 6.4; 95% CI 2.4-17.1). Glutamine 71 had an important role in the development of BeS (OR 2.4; 95% CI 1.1-5.6) and was shown to be protective for CBD among Beryllium sensitized individuals (OR 0.38, 95% CI 0.16-0.87). There was no clear dose-response or interaction between genetics and exposure, but the matched controls without susceptible genes, although having the highest exposure, remained healthy.

Conclusion: Glutamine 69 increased the odds of developing CBD and BeS. Glutamine 71 showed an important role in the development of BeS and possibly reducing the risk of progression to CBD. Further work to explore other polymorphisms is needed to assess exposure-genetic interactions and dose-response associations.

DEDICATION

To my husband Nugroho and our daughter Ardella, without you I'm nothing.

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CHAPTER I

BACKGROUND

I. OVERVIEW

Beryllium is a naturally occurring metal, which was not reported to cause toxicity until after its extensive use in industry.(1,2) Beryllium has been commonly used in aerospace, electronics, and munitions industries.(1) Beryllium toxicity was first reported in the mid-1950s in the form of acute symptoms and a more chronic progressive form.(3) This report was soon followed by the implementation of an occupational exposure limit for beryllium that caused a decrease in the incidence of acute beryllium disease. The chronic form, in terms of sensitization and chronic beryllium disease, however, is still an occupational health problem.(2)

The two major types of chronic beryllium toxicity are: a subclinical form of beryllium disease, beryllium sensitization (BeS), with in vitro proliferation of lymphocytes; and Chronic Beryllium Disease (CBD), a clinical form characterized by shortness of breath, cough and granulomas in the lung.(2,4)

Despite reports from multiple studies conducted on beryllium exposure and beryllium disease, the pathogenesis as well as exposure-disease association is still unclear.(2,4,5) Several recent studies show that low dose exposure to beryllium well below the OSHA permissible exposure limit can cause beryllium disease.(6–10) Several other studies have reported that solubility of beryllium and possible skin exposure influence the development of the disease.(11,12) A host-disease interaction has also been evaluated in studies of genetic susceptibility to beryllium sensitization and CBD .(2, 5, 13)

Although a lot of focus has been directed to beryllium exposure, how the exposure interacts with genetic susceptibility in the development of beryllium disease, is still not well defined.(14–16) This thesis will evaluate the effect of different polymorphisms of HLA-DPB1 and HLA-DRB-1 and their interaction with detailed exposure level measurements comprised of mean, cumulative and peak exposure, to assess the gene-exposure relationship in the development of beryllium sensitization and beryllium disease.

II. AIMS

1. To examine genetics and exposure influence in the development of BeS and CBD
2. To examine genetics and exposure influence in the progression of BeS to CBD

III. HYPOTHESIS

1. The risk of developing CBD and BeS is based on both the occurrence of certain HLA-DPB1 and DRB1 polymorphisms and the magnitude of beryllium exposure
2. The risk of progressing from BeS to CBD is based on both the occurrence of certain HLA-DPB1 and DRB1 polymorphisms and the magnitude of beryllium exposure

CHAPTER II

LITERATURE REVIEW

I. BERYLLIUM

Beryllium is a metal commonly found in the environment.(1,2) It can be found in coal, wood, water, food and stones.(1,2) The general population can be exposed to low levels of beryllium through air, drinking water, and food.(1) Although people are naturally exposed, only at a higher level of exposure, mostly though inhalation in industrial processes, has beryllium been reported to cause disease.(1–3)

Beryllium is commonly used in the manufacturing of aerospace, automotive, energy, defense, medical, and electronics due to its specific characteristics.(1,2) Beryllium is one of the lowest density metals, but one of the most rigid, even more rigid than steel.(1) Exposure in the beryllium processing or manufacturing industries is higher than beryllium exposure in other industries such as aluminum or nuclear facilities.(1–3)

Since cases of acute beryllium toxicity and chronic lung disease due to beryllium were first recognized in the 1950s, an occupational exposure limit to beryllium was implemented by Federal OSHA. (1–3) The OSHA regulations have helped to decrease the incidence of acute beryllium toxicity, although cases of chronic toxicity to beryllium are still reported. (1–3,17)

II. BERYLLIUM TOXICITY

a. Definitions

Beginning in the mid-1950s, it has been known that beryllium can cause different kinds of diseases; acute beryllium toxicity, beryllium sensitization which could be assessed after the development of a blood screening test for beryllium in 1989, and chronic beryllium disease.(3,18)

In 1949, the Atomic Energy Commission (AEC) set an occupational permissible exposure limit (PEL) with a daily 8-hour time-weighted average (TWA) of 2.0 $\mu g/m^3$ but it was not until 1971 that OSHA adopted this standard for industries.(19) After the implementation of the standard, the incidence of acute beryllium disease was controlled and became very rare.(1,8) Beryllium sensitization and chronic beryllium disease, however are still prevalent amongst workers who are exposed, even when exposure is below the permissible limit.(2,5)

Beryllium sensitization is defined as individuals who have positive beryllium lymphocyte proliferation test results (LPTs) without any positive result on the following work up for CBD (chest radiograph, lung biopsy).(18) Chronic Beryllium Disease (CBD) is defined as individuals who had positive beryllium lymphocyte proliferation test results (LPTs) with non-caseating granuloma on lung biopsy or a positive bronchial lavage.(4)

b. **Epidemiology**

Studies conducted in several facilities with beryllium exposure have found a prevalence of 0.3-16.6% for beryllium sensitization (BeS) and 0-7.6% for Chronic Beryllium Disease (CBD).(6,7,20–33) The wide range of prevalence is due to different levels of beryllium exposure, and different ascertainment of cases (previous worker, current worker, or both), as well as the sensitivity and specificity of Beryllium Lymphocyte Proliferation Testing (BeLPT) which probably differs across laboratories.(2,5)

Higher levels of beryllium exposure are often found in beryllium manufacturing industries, but low level exposure in aerospace industries, nuclear facilities, weapons or munitions industries, aluminum industries, beryllium distribution, and mining has also been reported to cause sensitization, and even chronic disease (Table 1). (6, 7, 17–30) Certain work related processes such as machining were reported to cause an increase in the prevalence of beryllium toxicity. (6,34)

In 2004, it was estimated that 134,000 workers in the United States were exposed to beryllium.(13) Cullen et al (1986) and Henneberger (2004) estimate that until the 1980s, up to 800,000 workers in government or industries across the United States were occupationally exposed to beryllium.(13,17)

Schubauer-Berigan and colleagues, in their mortality studies of beryllium workers found that beryllium exposure was related to lung cancer, Chronic Obstructive Pulmonary Disease (COPD), and nervous system and urinary tract cancers independent of cigarette smoking and exposure to other lung carcinogens.(35)

The International Agency for Research on Cancer (IARC) has listed beryllium as a carcinogen, (36) although recent findings for the association between beryllium exposure and lung cancer were not conclusive.(37) Schubauer-Berigan and colleagues found a positive association between beryllium exposure and lung cancer, but Boffetta et al in their review concluded that the causal criteria for an association was not well established.(37,38) The mortality rate for beryllium toxicity as reported by Newman et al in 1996, ranged from 5.8 to 38%. The difference in study design, follow up duration and also type of exposure contributes to this wide range.(4)

c. Factors Affecting Development of Disease

Exposure to beryllium is a necessary cause for development of BeS and CBD.(2,5) However, several studies have evaluated potential factors that increase the risk for beryllium toxicity such as age, gender, race, ethnicity, smoking, respiratory symptoms, spirometric or radiographic abnormalities, but only a few found positive associations.(6,20,26,27) Age was reported as a risk factor for development of BeS and CBD after controlling for duration of exposure.(6) No significant associations between smoking and the disease were reported from these studies.(20,26,27) Kreiss et al also showed that allergic history was not a risk factor for sensitization (20) which was further emphasized by Schuler (2005), who reported that self-reported skin problems associated with exposure to pickling fluids, coolants, or other work, were not related to either beryllium sensitization or CBD. (27)

Table 1. Studies on Prevalence of Beryllium Sensitization and Chronic Beryllium Disease from 1990-2012							
Studies	**Population**	**Sample**	**BeS**		**CBD**		**Detection of CBD**
			N	**%**	**N**	**%**	
Kreiss et al, 1993(20)	Ceramics Company, Colorado	505	9	1.78	9	1.8	Lung biopsy
Kreiss et al, 1993(21)	Rocky Flats Nuclear Plant	890	17	1.9	13	1.8	Lung biopsy
Kreiss et al, 1996(6)	Beryllia Ceramics Plant, Arizona	136	8	5.9	6	4.4	Lung biopsy
Stange et al 1996(7)	Rocky Flats Nuclear Plant	4,397	107	2.43	29	0.7	Biopsy + X-ray
Kreiss et al, 1997(22)	Beryllia Ceramics Plant, Ohio	627	59	9.4	24	3.8	Bronchoscopy
Henneberger, 2001(23)	Beryllia Ceramics Plant, Arizona	151	15	9.9	8	5.3	Biopsy
Deubner et al, 2001(24)	Mining Extraction	75	3	4.0	1	1.3	Biopsy
Newman et al, 2001(25)	Machining	235	22	9.4	13	5.3	Bronchoscopy
Sackett et al, 2004(26)	Nuclear Weapon	2,221	19	0.9	2	0.09	Biopsy
Schuler et al, 2005(27)	Copper Be Alloy Finishing	153	10	6.5	6	3.9	Bronchoscopy
Rosenman et al, 2005(28)	Beryllium Plant, Pennsylvania	577	96	16.6	44	7.6	Biopsy and X-ray
Stanton et al, 2006(29)	Beryllium Alloy Distribution	88	1	1.1	1	1.1	Biopsy
Taiwo et all, 2008(30)	Aluminum Smelter	734	2	0.3	2	0.3	Not specified
Arjomandi, 2010(31)	Nuclear Weapons	1,875	59	3.1	5	0.3	Bronchoscopy
Taiwo et al, 2010(32)	Aluminum Smelter	1,932	9	0.47	2	0.1	Not specified
Mikulski et al, 2011(33)	Munitions Plant	524	8	1.5	0	0	Clinical

d. **Natural History**

Beryllium sensitization has been reported in workers who were exposed to Beryllium even for just a few months.(23,25,27,39,40) Of all sensitized individuals, around 11-31% will develop CBD over the following 4-7 years.(41,42) It is still not clearly defined why some individuals become sensitized, and what factors play a role in the progression to CBD.(4,5,43,44) Several factors have been proposed, such as duration of exposure in which individuals who had longer exposure would be more likely to develop CBD, but this was still inconclusive since there were other studies that reported different findings, and it might also be due to the longer latency period of CBD or a host-related factor.(20,21,23,28)

Several studies recommended a follow up of sensitized individuals to assess the development of CBD ranging from 2-4 years, including pulmonary function testing and X-rays to look for clinical symptoms of CBD. (41,42,45) Among the positive for Beryllium Lymphocyte Proliferation Testing (BeLPT) patients without CBD symptoms, half become BeLPT negative on their follow up testing and some workers did not develop CBD even after being followed for 12 years. (41,42) These results set the foundation for periodic screening for beryllium-exposed individuals to detect beryllium sensitization and CBD.(41,42,45)

e. **Diagnosis**

The transformation of lymphocytes due to beryllium exposure in sensitized individuals enabled screening for the disease in asymptomatic individuals.(4,18) Beryllium sensitization (BeS) can be measured with beryllium-specific lymphocyte

proliferation testing (BeLPT) using white blood cells or broncho-alveolar lavage cells.(2,18) This test is commonly used to conduct screening and surveillance for beryllium-exposed workers.(20,26,45) BeLPT can also characterize workplace risk and evaluate the effectiveness of preventive interventions.(2)

Further examination to identify Chronic Beryllium Disease (CBD) is conducted for those individuals with positive BeLPT results.(20,46) The examination includes bronchoscopy with broncho-alveolar lavage and trans-bronchial biopsy, and a chest radiograph.(44,46) Workers with positive BeLPTs but negative granulomatous lung disease on further examination are at risk for developing CBD in the future.(2)

The gold standard for the diagnosis of CBD includes histologic evidence of granuloma from a lung biopsy and proliferative response of broncho-alveolar cells to beryllium.(46) CBD can also be diagnosed by radiographic findings consistent with granulomas and positive blood proliferative response to beryllium and localization of beryllium inside the granuloma.(46)

f. Treatment

Early detection followed by prompt treatment of CBD can lead to regression and prevent further progression, hence reducing the morbidity and mortality of the disease. (46,47) CBD cannot be cured but is treatable; the goal of the treatment is to reduce morbidity and mortality.(46) Cessation of beryllium exposure and administration of systemic corticosteroids is the current standard management of CBD. (46,47) Regression can be obtained by early corticosteroid intervention.(46,47)

Avoidance of further exposure to beryllium for sensitized individuals is important to prevent progression of disease.(45,46)

Patients with BeS are followed up regularly, to detect any signs of early lung damage.(42,46,47) The examination includes a history and physical examination, a chest radiograph, and pulmonary function tests.(46,47) Patients with early lung damage are given 40 mg of prednisone on alternate days for 6 months.(46,47) The dosage is then tapered by no more than 10 mg every other month unless there is evidence of renewed disease activity which is evaluated by the same examination as used for disease progression.(46,47) The lowest dose of prednisone that prevents disease activity is then maintained.(46,47) It is uncertain whether this treatment has to be continued for the rest of the individual's life.(46,47) However, once pulmonary fibrosis develops, it is not reversible even with corticosteroid treatment.(46,47) Patients who undergo treatment should be monitored using pulmonary function testing and high resolution chest computed tomography.(46,47)

g. Prevention

There is no international exposure standard and different countries are implementing different exposure limits for beryllium (Table 2).(48) Even within the United States, there are differences in the Permissible Exposure Limit (PEL) for beryllium.(48) The current OSHA PEL in the United States is 2.0 $\mu g/m^3$.(49) This level prevents acute beryllium disease but recent studies show that it is not protective enough to prevent BeS and CBD.(7,9,10) Therefore, recommendations have been

made for OSHA to lower the current permissible limit for beryllium exposure to 0.2 $\mu g/m^3$.(50,51) A lower PEL for beryllium per 8-hour shift was implemented by the Department of Energy (DOE) in 1999 (0.2 $\mu g/m^3$), (50) by the State of California in 2004 (0.2 $\mu g/m^3$), (52) and also by the National Institute for Occupational Safety and Health (NIOSH) which recommends a limit of 0.5 $\mu g/m^3$ for 8 hours of occupational exposure.(49) Secondary prevention through periodic medical screening is also recommended for beryllium exposed workers. (6,27)

Table 2. Current Permissible Exposure Limits and Recommendations of Beryllium Levels in Different Countries (48)

Country	**Limit Value (8 hours) in $\mu g/m^3$**	**Limit Value (Short Term) in $\mu g/m^3$**
Australia**	2	
Austria**	2	8
Belgium**	2	10
Canada-Ontario	2	10
Canada-Quebec**	0.15	
Denmark	1	2
France**	2	
Japan*	2	
Latvia	1	
New Zealand	2	
Poland**	0.2	
Singapore	2	
South Korea	2	10
Spain**	0.2	
Sweden	2	
Switzerland	2	
USA – NIOSH*	0.5	0.5
USA – OSHA**	2	5
United Kingdom**	2	

*) Recommendation limit
**) Standard limit, with legal implication

III. EXPOSURE-DISEASE ASSOCIATION

Exposure to beryllium is a necessary cause for the development of beryllium toxicity, either in the form of acute disease, sensitization or chronic disease.(3) Since the first few cases were reported back in the mid-1950s, several measures have been implemented including the adoption of an occupational exposure limit for beryllium.(34) Several studies, however, reported that higher exposure to beryllium does not always cause disease, and that even low exposure to beryllium can cause disease.(7,9,10,29,31) These studies find that even with exposure lower than the PEL standard by OSHA (7,9,10,27) or minimum opportunistic contact with beryllium can cause sensitization and even the development of CBD.(7,20,53) Schuler et al (2005) found that exposures higher than 0.2 $\mu g/m^3$ were associated with sensitization and CBD and rarely found cases in areas which maintained air exposure lower than this level.(27) This level of 0.2 $\mu g/m^3$ has been adopted as California's 8 hour occupational exposure limit since 2004.(54)

Another mechanism that has been proposed to explain this lack of a clear dose-response relationship is the different solubility of different beryllium compounds and also the different form and particle size which may influence the entry pathway as well as pathogenesis of the disease.(11,24,32) A lower rate of beryllium disease was found in the aluminum smelting environment which might be due to the more soluble form of beryllium although there was also the consistent use of respiratory protection in the population studied.(32) Skin contact, which was proposed as an entry for beryllium exposure, has also been shown to contribute to the development of beryllium disease.(12,55)

Studies have also found that beryllium disease can develop years after cessation of exposure (10,53) Additionally, a longitudinal study that assessed the prevalence of disease after implementation of several measures to control high beryllium exposure found no decrease in sensitization or CBD.(13)

The different measurement methods across different studies, in terms of mean, average, and cumulative exposure, may also influence the variety of results.(51,54) Henneberger studied peak, cumulative and mean exposure of beryllium and found that the prevalence of beryllium disease is greater in the long term worker compared to the short term worker (9.1% vs. 1.4%, p 0.06).(23) However, they were unable to show a distinct difference in association between disease and peak, cumulative, and mean exposure level and beryllium toxicity which might be due to limited statistical power.(23)

There is no clear dose-response relationship between beryllium exposure and beryllium toxicity.(24,41) Several factors that have been proposed to explain this are: different measures of exposure, solubility of beryllium exposure, and multiple pathway entries which include skin contact.(12,13,28) The fact that not everybody who had the same beryllium exposure develops the disease, infers a possible host-agent-environment dynamic, and studies have been conducted to determine if gene susceptibility of exposed individuals is related to the pathophysiology of beryllium toxicity.(5,56)

IV. GENE SUSCEPTIBILITY RELATED TO BERYLLIUM TOXICITY

The development of CBD and BeS is based on a type IV hypersensitivity mechanism which involves activation of T cells and MHC Class II antigens.(5) Richeldi et al were the first to assess several genes related to this mechanism.(56) In their preliminary study, they found that HLA-DP and not HLA-DR and HLA-DQ genes were related to CBD.(56) In their 1993 study they found that 97% of CBD patients had residue Glutamate in position 69 of the HLA-DPB1 gene compared to 30% in unaffected subjects, and proposed the use of HLA-DPB1-Glu69 as a marker for CBD risk.(56,57) Subsequent studies also found that this allele not only influenced development of CBD but also beryllium sensitization.(58,59) Some authors reported that the homozygosity of this allele increased the risk of CBD (58–60), Beryllium sensitization (59,60), as well as CBD severity (61). The odds ratio (OR) for the association of HLA-DPB1Glu69 with CBD regardless of zygosity ranged from 3.7-19.14 (60,62,63) and 3.3-6.9 for sensitization.(60,63)

Further studies also showed that the presence of non-0201 alleles of HLA-DPB1 Glu69 was an important marker for beryllium toxicity.(58,59,61,63) Several other alleles have also been linked to beryllium sensitization (HLA-DRArg74) (62), while DQ-B1-G86 (64), DRB1-S11 (64), DRB1-S13 (61), DQB1-06 (61), were associated with CBD, and DRB-Glu71 (63) and TNF-α-308 (62) were associated with both BeS and CBD. Rosenman et al (2011) proposed that the negative charge contributed by specific polymorphisms in conjunction with DPβ-E69 was associated with CBD and BeS and that this polymorphism was related to how peptides were presented to T cells involved in the pathophysiology of CBD and BeS.(63)

Table 3. Studies on Genetics and Beryllium Toxicity				
Author, Year	**Study Design**	**N Cases**	**N Control**	**Result**
Richeldi et al, 1993 (56)	Case Control	33 CBD	44	HLA-DPB1-0201 increase the risk of CBD ($P < 0.05$) 97% of CBD vs. 30% of controls expressed the HLA-DPBglu69 ($P < 0.001$). Conclusion: HLA-DP has a role in development of CBD Residue 69 can be used as a potential marker of CBD.
Wang et al, 1999 (58)	Case Control	20 CBD	75	Homozygous DPB1Glu69 in both alleles were found more in CBD group (6/20) vs. control group (1/75). Most Glu69 carriers from the control group had a DPB1 allele-0201 (68%), while CBD group had a non-0201 DPB1 Glu69-carrying allele (84%). Conclusion: Specific Glu69-containing alleles and their homozygosity increase the risk of CBD.
Wang et al, 2001 (59)	Case Control on BeS	25 BeS	163	88% of BeS has HLA-DPB1-Glu69, and 24% were homozygous. Conclusion: HLA-DP related to BeS
Saltini et al, 2001 (62)	Case Control on BeS and CBD	23 BeS 22 CBD	93	HLA-DPGlu69 associated with CBD (OR 3.7, p=0.016, 95% CI 1.4–10.0). High TNF-a-308-2 marker associated with both BeS and CBD (OR 7.8, $p < 0.0001$, 95% CI 3.2–19.1), no difference between CBD and BeS. HLA-DRArg74 associated with BeS (OR 3.96, p=0.005, 95% CI 1.5–10.1).
Rossman et al, 2002(64)	Case Control on BeS and CBD	30 BeS 25 CBD	82	HLA-DPB1-E69 was the most important marker for sensitization, and did not differentiate BeS and CBD. A significant association with CBD was observed with HLA-DQB1-G86 (p=0.04), and HLA-DRB1-S11 compared with BeS (p=0.03). Conclusion: HLA-DPB1-E69 is a marker for susceptibility to beryllium sensitization. HLA amino acid epitopes on HLA-DRB1 and -DQB1, in association with or independently of HLA-DPB1-E69 may be associated with progression to CBD. Did not find an association with homozygosity.

Table 3 (cont'd). Studies on Genetics and Beryllium Toxicity				
Author, Year	**Study Design**	**N Cases**	**N Control**	**Result**
Maier et al, 2003 (61)	Case Control on BeS and CBD	50 BeS 104 CBD	125	DPB1 Glu69 gene is associated with CBD and BeS (OR 10.1 for CBD and 9.5 for BeS). The majority of BeS and CBD subjects displayed non-0201 Glu69 alleles. Glu69 homozygosity was highest in CBD, and lowest in control. DRB1-13 and DQB1-06 were associated with CBD in the absence of Glu69. Markers of disease severity were associated with Glu69 homozygosity. Conclusion: DPB1 Glu69 is a marker of sensitization and not specific for disease.
McCanlies et al, 2004 (60)	Case Control on BeS and CBD	64 BeS 90 CBD	730	HLA-DPB1Glu69 was associated with both CBD (OR 9.4; 95% CI 5.4, 16.6) and sensitization (OR 3.3, 95% CI 1.9, 5.9). CBD and BeS were more likely to be homozygous compared to controls (P<0.001). Conclusion: Evaluation of HLA-DPB1 haplotypes, gene–environment and gene–gene interactions will be important for fully understanding the immunogenic nature of BeS and CBD.
Rosenman et al, 2011 (63)	Matched case control on BeS and CBD	44 BeS 65 CBD	288	92.3% CBD have HLA-DPbE69 residue (OR 19.14 (95% CI 7.10 to 55.92) p 1.8310-16) and 79.5% BeS (OR 6.20 (95% CI 2.73 to 14.47) p 3.82310-7) and 38.5% of control. Conclusion: Protective effect of the DPB1-0201 positive haplotype may involve particular polymorphisms outside of the DPB1 gene.
Silveira, 2012 (43)	Case Control	502 Bes/CBD	653	CBD cases were more likely than controls to carry a non-02 E69 allele than 02 Glutamine 60, with odds ratios ranging from 3.1 (2.1–4.5) to 3.9 (2.6–5.9) (p < 0.0001). Conclusion: The less frequent non 02 alleles increase the risk for CBD more than the 0201 alleles.

V. INTERACTION OF GENETIC SUSCEPTIBILITY AND EXPOSURE ON BERYLLIUM TOXICITY

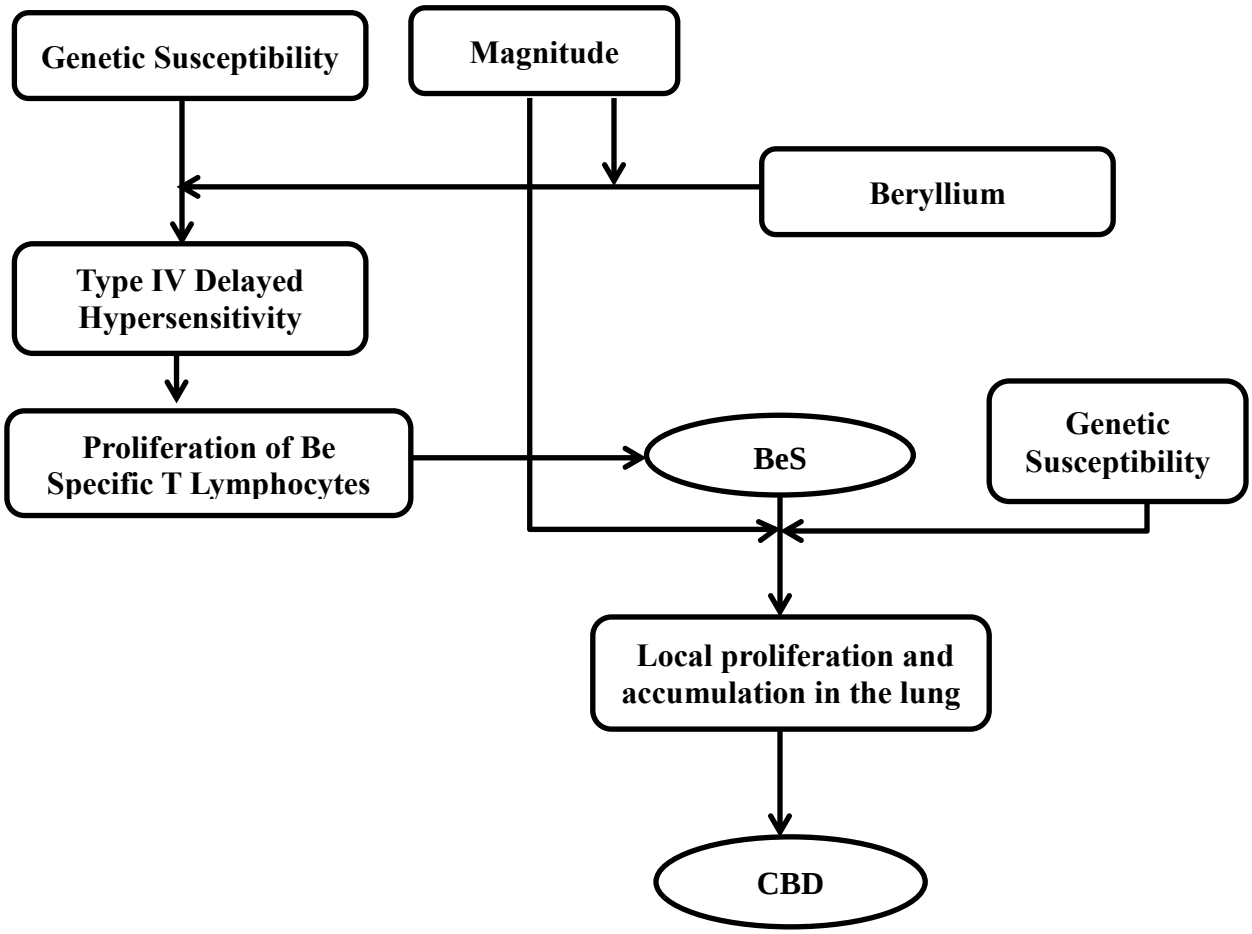
There have been only a few studies that focused on the association of beryllium exposure, genetic susceptibility, and development of beryllium sensitization or CBD.(14–16) The first study was conducted in 1997 by Richeldi et al who reported that 32.3% of a worker population had HLA-DPB1Glu69, and found that the presence of this genetic biomarker was associated with an increased risk of CBD in highly exposed individuals.(14)

Van Dyke et al in their case control studies on beryllium exposed workers found that both exposure and genetic susceptibility had an independent effect on the development of the disease, and found even higher odds ratios in subjects with both genetic susceptibility and exposure.(15,16) They also found that people with homozygosity were at a greater risk of developing the disease.(15,16) These studies used the lifetime average level of exposure of individuals and specifically assessed the HLA-DPB1 Glu69 allele.(15,16) The interaction between different types of exposure measurements, different genetic susceptibilities to beryllium with CBD and BeS, and especially how only a percentage of sensitized individuals go on to develop CBD, however, is still uncertain.(15,16)

Therefore, our study will assess the association of different genetic polymorphisms associated with CBD and BeS and the interaction of peak, average and cumulative beryllium exposure of the individual to better understand the association.

Table 4. Studies on Genetic and Exposure Interaction in Development of Beryllium Toxicity				
Author, Year	**Study Design**	**N Cases**	**N Control**	**Result**
Richeldi et al, 1997 (14)	Case Control on CBD	6 CBD 2 BeS	119	HLA-DPB1Glu69 present in 30% controls, and 83% in CBD (*P* 0.01), and in none in BeS. The presence of the marker was associated with higher prevalence of CBD (HLA-DPB1Glu69-positive machinists 25%; HLA-DPB1Glu69-negative machinists 3.2%, P 0.05). Conclusion: Genetic susceptibility factor adds to the effect of process-related risk factors.
Van Dyke et al, 2011(15)	Case Control	35 BeS 19 CBD	127	Increased odds for BeS and CBD among DPbE69 carrier (OR 6.06, 95% CI 1.96 to 18.7). Exposure of 0.1 mg/m^3 (lifetime weighted average) increased the odds of CBD (OR 3.98, 95% CI 1.43 to 11.0). Those with both risk factors had higher increased odds (OR 24.1, 95% CI 4.77 to 122). Conclusion: DPbE69 carriage and high exposure to beryllium appear to contribute individually to the development of BeS and CBD.
Van Dyke et al, 2011 (16)	Case Control	70 BeS 61 CBD	255	HLA-DPB1-E69 carriage increased odds for CBD (OR, 7.61; 95% CI, 3.66–15.84). Each unit increase in lifetime weighted average exposure increased the odds for CBD (OR, 2.27; 95% CI, 1.26–4.09). Compared with E69-negative genotypes, a heterozygote E69-positive 02 allele increased the odds for BeS (OR, 12.01; 95% CI, 4.28–33.71) and CBD (OR, 3.46; 95% CI, 1.42–8.43). A single non-02 E69 allele further increased the odds for BeS (OR, 29.54; 95% CI, 10.33–84.53) and CBD (OR, 11.97; 95% CI, 5.12–28.00). Conclusion: E69 and beryllium exposure both contribute to the odds of CBD. Non-02 E69 carriers and E69 homozygote at higher odds than those with 02 genotypes.

Figure 1: Conceptual Framework of Exposure and Genetic Interaction in the Development of Sensitization and CBD



CHAPTER III

METHODOLOGY

I. STUDY DESIGN

This thesis analyzed the data from a case control study conducted by Rosenman et al during 1996-2010.(28,63)

II. SAMPLE CHARACTERISTICS

a. Population

The study population consisted of workers in two beryllium processing facilities in eastern Pennsylvania.

b. Sample selection

Using personnel records, 5490 workers who were on the payroll for 2 or more days at either of two beryllium facilities were identified. There were 1349 individuals working between 1958 through 1978 in Plant 1, and 4141 individuals working from 1935 to 2000 in Plant 2.

From various databases, it was determined that as of 12/31/1988, 328 (24.3%) individuals from Plant 1 and 2293 (55.4%) from Plant 2 had died.

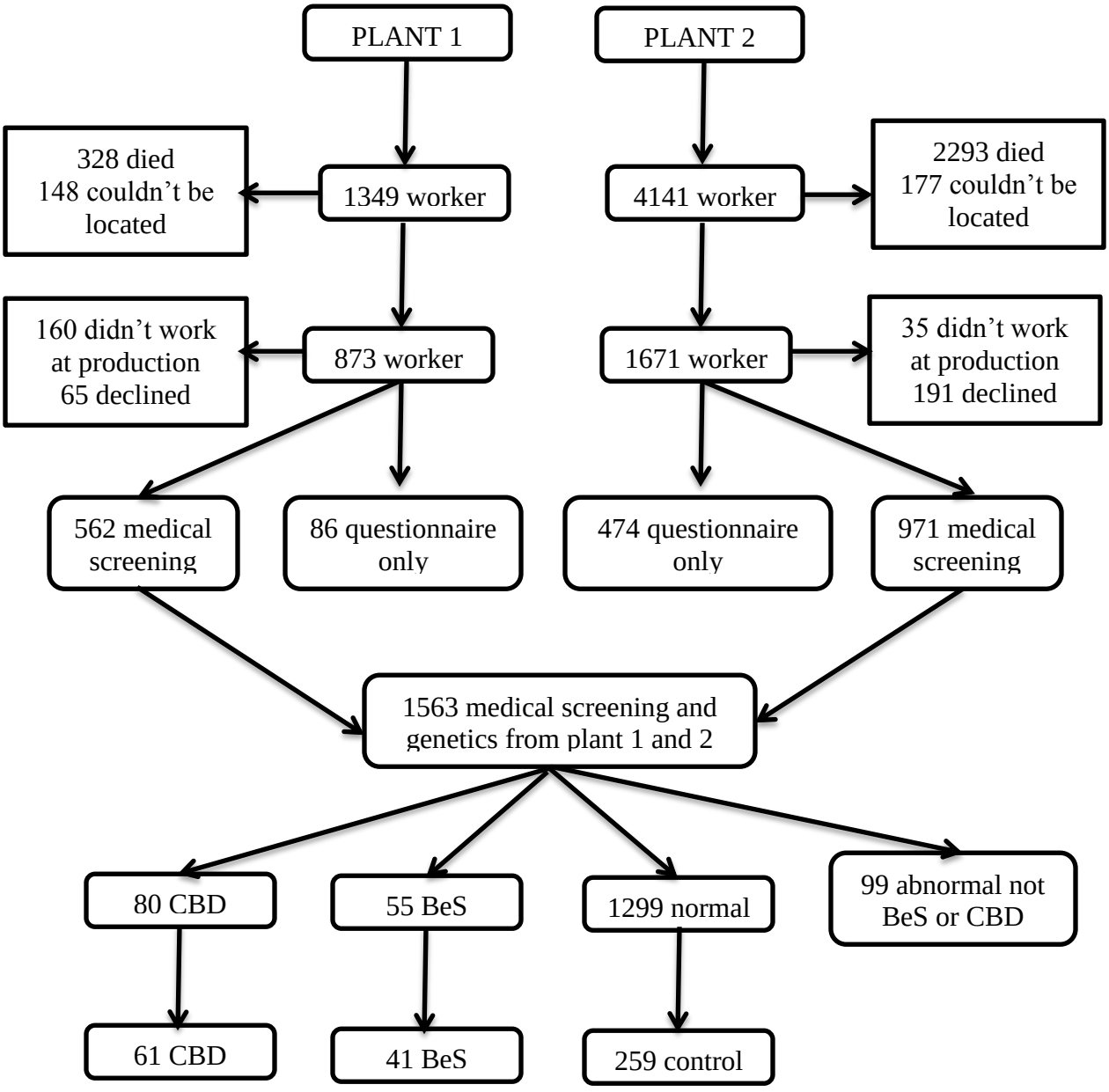
Mailing and follow up phone calls were initiated in 1996 to offer free medical screening for beryllium-related disease to members of the cohorts not known to have died as of 12/31/88.

One hundred forty eight (11%) workers from Plant 1 and 177 (4.3%) workers from Plant 2 could not be located. Among the 873 workers who were located from Plant 1, 160 said that they worked for the company but not in the beryllium production Plant, 65 declined to participate, 86 completed the questionnaire only, and 562 individuals participated in the medical screening.

From Plant 2, 1671 workers were contacted for the medical screening, 35 said that they did not work in the beryllium production Plant, 191 declined to participate, 474 completed the questionnaire only, and 971 participated in the medical screening. Therefore, for both plants a total of 1533 individuals participated in the medical screening, 256 declined and 560 completed a questionnaire only.

The medical screening occurred from 1996-2001. From the medical screening, 80 people met the case definition for CBD and 55 for BeS. Fifteen of these workers with CBD and 11 with BeS either did not provide consent or blood for genetic testing, hence genetic data was available from 65 workers who were diagnosed with CBD and 44 workers who were identified as BeS. A total of 288 individuals who underwent genetic testing and had completely normal medical testing were chosen as controls. Complete data on medical testing, genetics, and exposures, were available for 61 CBD, 41 BeS, and 259 controls that were included in the final analysis.

Figure 2: Study Population and Sample Selection



III. VARIABLES AND MEASUREMENTS

a. Dependent Variables

1. Beryllium Sensitization

Beryllium sensitization was defined as individuals who had two positive beryllium lymphocyte proliferation tests (BeLPTs) without a positive result on the following work up for CBD (chest radiograph and lung biopsy, if performed).

2. Chronic Beryllium Disease

Chronic Beryllium Disease (CBD) was defined as individuals who had two positive beryllium lymphocyte proliferation tests (BeLPTs) or positive BeLPT from a bronchial lavage sample. Probable CBD was characterized as individuals who had two positive blood BeLPTs and positive lavage BeLPT or a positive radiograph.

b. Independent Variables

a. HLA-DPB1 and DRB1 polymorphisms

HLA-DPB1 and HLA-DRB1 polymorphisms were tested by using Polymerase Chain Reaction (PCR) on Oiagen columns from a venous whole blood sample that had been frozen the day after the original blood collection.

b. Exposure to Beryllium

Beryllium exposure was defined as occupational exposure to beryllium that was calculated with regards to duration, level of total exposure (cumulative, mean and peak), and type of exposure.

Exposure data was obtained by calculating the Daily Weighted Average (DWA), Job Exposure Matrix and Task Exposure Matrix and then calculating the mean exposure, peak exposure, and cumulative exposure for each worker. Exposure was also classified to chemical form which consisted of soluble, non-soluble and mixed as well as physical form which consisted of dust, fume, and mixed.(28)

IV. DATA COLLECTION

Individuals who had two positive beryllium lymphocyte proliferation tests (BeLPTs) and/or a chest radiograph reading $\geq 1/0$ in parenchymal profusion determined by at least two of the three physicians certified to interpret chest radiographs for pneumoconiosis were referred for bronchoscopy, the testing of lavage fluid for beryllium lymphocyte proliferation and a trans-bronchial biopsy. Individuals with definite or probable CBD and BeS were classified as cases.

The control group was matched by Plant, gender, and year of birth within 5 years. Two to three controls were chosen for each case. These controls had completely normal results on chest radiograph and BeLPT testing. There were initially an additional 35 individuals with suspected CBD or BeS who had had matched controls selected. After subsequent review it was determined that these 35 individuals did not have CBD or BeS

and no genetic analysis was performed on their blood but the now 70 extra controls did have genetic analyses and were appropriately reassigned to other cases as controls

Data was collected through questionnaire, medical screening, and genetic testing. The questionnaire included data on demographics, a detailed work history to assess beryllium exposure, and also history of previous illness. Medical screening was conducted from 1996-2001 for all workers who were eligible and signed informed consent to allow genetic testing was obtained. Genetic analyses were conducted 4-10 years after collection using Oiagen columns from a venous whole blood sample that was frozen the day after the original blood collection. Beryllium exposure was calculated for each participant by reviewing past sampling data and work processes combined with duration of exposure obtained from each plant's employee work history records. Exposure metrics used were cumulative, peak and average exposure to beryllium. Exposure data also included the type of beryllium, solubility and physical form.

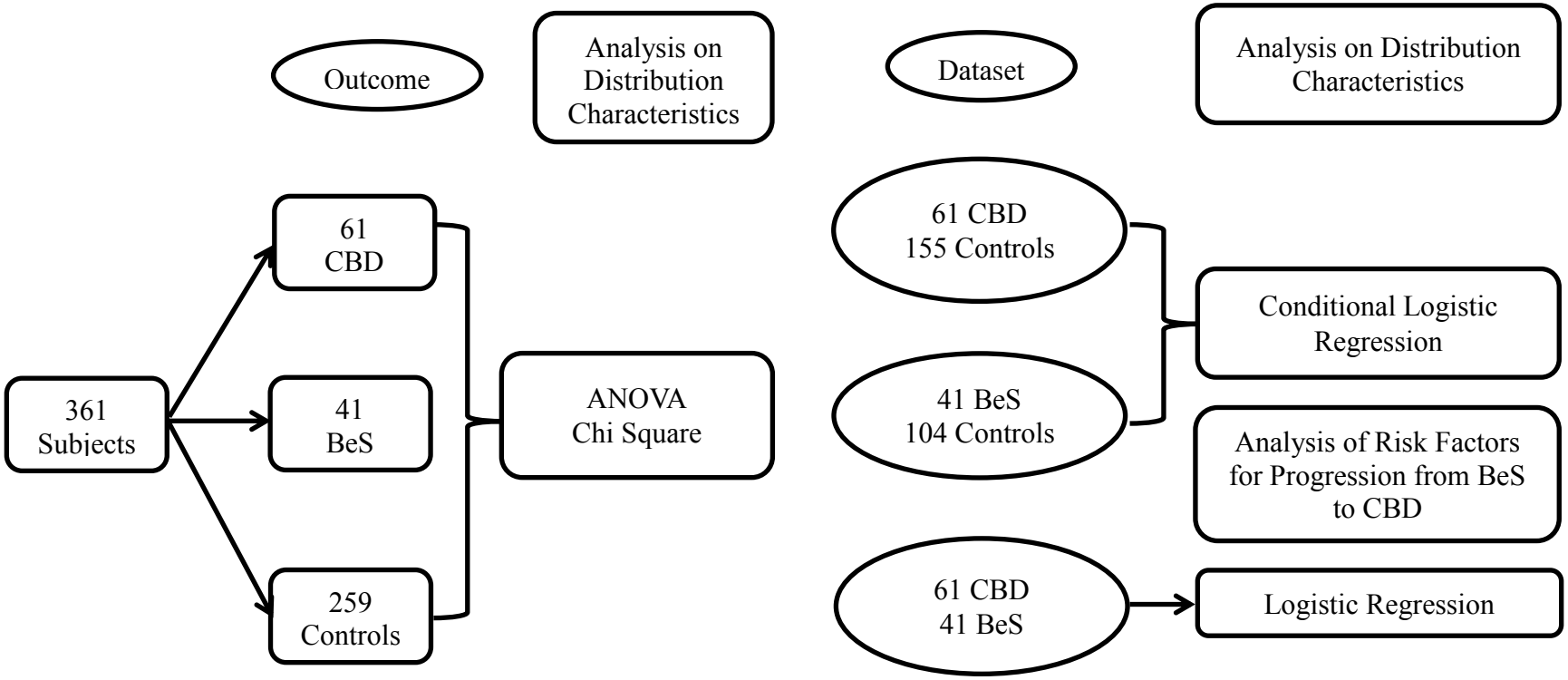
V. DATA ANALYSIS

Data analysis was conducted using SAS 9.3 to assess associations between genetics and exposure with the development of CBD and BeS and progression of BeS to CBD. Analysis included descriptive statistics of demographic, exposure, and genetic distribution, as well as multivariable conditional logistic regression to assess the association of genetics and exposure with CBD and BeS. The steps for data analysis included univariable analysis on beryllium exposure (mean, peak, and cumulative exposure as continuous variables) and genetic factors (categorical) as independent variables with the development of CBD or BeS. Variables that were associated with the development of CBD and BeS on univariable

analysis at $p \leq 0.1$ were then included in multivariable analyses. Possible interactions were also ascertained to assess potential effect modifiers.

Separate analysis was conducted using conditional logistics regression for CBD and their controls (total 216 subjects consisting of 61 CBD cases and 155 controls) and BeS and their controls (total of 145 subjects consisting of 41 BeS and 104 controls). Further analysis was also conducted for CBD and BeS cases only (61 CBD and 41 BeS) using unconditional logistic regression to assess factors influencing the progression from BeS to CBD. Whenever necessary, detailed analyses and multiple comparisons with Bonferroni correction were conducted. Additional analysis for coding exposure, the Hockey Stick method was also used since a proportion of individuals had exposure levels below the limit of detection (close to zero, or even zero level). For analyses where exposure was coded using Hockey Stick method, two variables for exposure were created and entered into the model: 1) individuals with exposure bellow the limits of detection were assigned value of 0, and those that had exposure above the detectable limit were assigned a value of 1, and 2) those with exposure above zero, were assigned their actual exposure levels. All analyses evaluating the effect of exposure, genetics and their interaction on risk of CBD and BeS were repeated using this approach for coding exposure.

Figure 3: Statistical Analysis



CHAPTER IV

RESULTS

I. DEMOGRAPHIC CHARACTERISTICS

We analyzed data from a total of 361 subjects consisting of 61 CBD, 41 BeS, and 259 controls. A comparison of CBD, BeS, and controls regarding their demographic characteristics is shown in Table 5. There was a significant difference in proportion of race (p= 0.0122) and gender (p= 0.0267) between CBD, BeS, and controls. Subjects were mostly male (94.2%) and white (98.1%) and there were more non-white and female individuals in the sensitized group compared to the control and CBD groups. There were no significant difference in plant (p=0.5847) or history of smoking (p=0.8732).

Table 5. 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's method					

II. EXPOSURE CHARACTERISTICS

Individuals working in Plant 2 (operating from 1935-2000) had significantly higher exposure than those working in Plant 1 (operating from 1958-1978), in terms of cumulative exposure, mean exposure and peak exposure (respectively, p = 0.0094; 0.0005; and <.0001) as seen in Table 6.

There was a significant difference in type of exposure, in which individuals working in Plant 1 had higher chemical exposure for mixed, non-soluble, and soluble chemical compared to Plant 2. Although not statistically significant, individuals working in Plant 2 also had higher physical exposures than those in Plant 1.

Table 6. Exposure Characteristics by Plant

Exposure*		**Plant**		**Total Average N=361**	**P value**
		1 N=184	**2 N=177**		
Duration (In Years)		8.63	13.89	10.87	0.1233
Total Exposure					
Cumulative		145.15	2555.88	599.55	**0.0094**
Log Cumulative		4.03	2.47	4.36	**0.0094**
Mean		1.63	23.20	7.47	**0.0005**
Peak		3.45	159.49	24.08	**<.0001**
Type of Exposure					
Chemical					
Mix	Cumulative	40.66	844.06	434.57	**<.0001**
	Mean	0.44	11.78	6.00	**<.0001**
	Peak	1.52	42.95	21.83	**<.0001**
Non soluble	Cumulative	84.61	153.95	118.61	**0.0009**
	Mean	0.95	1.47	1.21	**<.0001**
	Peak	3.01	5.49	4.23	**0.0008**
Soluble	Cumulative	19.89	59.97	39.54	**<.0001**
	Mean	0.24	0.56	0.40	**<.0001**
	Peak	1.09	4.87	2.94	**<.0001**
Physical					
Mix	Cumulative	37.14	276.88	154.68	0.1358
	Mean	0.41	3.24	1.80	0.3776
	Peak	1.47	13.22	7.23	0.1235
Dust	Cumulative	**	309.68	309.68	n/a

Table 6 (cont'd). Exposure Characteristics by Plant					
Exposure*		**Plant**		**Total Average N=361**	**P value**
		1 N=184	**2 N=177**		
	Mean	**	8.29	8.29	n/a
	Peak	3.04	29.84	16.18	0.1065
Fume	Cumulative	16.03	424.04	216.08	0.3727
	Mean	0.20	2.28	1.22	0.0578
	Peak	1.02	19.41	10.04	0.8474
*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure **) Data on cumulative and mean dust levels not available for Plant 1 P Value was obtained from Wilcoxon two sample test comparing 2 plants					

Due to the higher exposure received by workers in Plant 2, we ran separate analyses of exposure by CBD, BeS, and control status within each Plant and found differences of duration between the three groups within each plant, but only found significant differences in Plant 1 for cumulative exposure, and all measures of mixed chemical exposure and mixed physical exposures among the three groups (Table 7).

Table 7. Exposure Characteristic by Outcome within Each Plant								
Exposure*	**Plant 1**				**Plant 2**			
	CBD N=28	**BeS N=23**	**Control N=133**	**P Value**	**CBD N=33**	**BeS N=18**	**Control N=125**	**P Value**
Duration (Years)	8.73	5.22	9.20	**0.0487**	8.72	5.83	15.34	**0.0090**
Total Exposure								
Cumulative	118.72	77.66	162.39	**0.0366**	437.9	232.28	1357.90	0.3135
Log Cumulative	3.86	3.25	4.20	**0.0366**	4.37	4.06	4.89	0.3135
Mean	1.42	1.53	1.69	0.2851	12.96	16.65	13.25	0.6440
Peak	2.63	2.90	3.71	0.0849	24.13	22.25	54.45	0.5337
Chemical Mix								
Cumulative	20.93	9.51	50.20	**0.0029**	351.5	146.25	1072.75	0.4851
Mean	0.28	0.37	0.48	**0.0248**	12.01	15.03	11.25	0.9544
Peak	1.09	1.09	1.68	**0.0347**	20.42	20.33	52.09	0.4418
Non Soluble								
Cumulative	84.69	60.86	88.70	0.6954	86.17	86.02	181.40	0.1432
Mean	0.94	0.83	0.97	0.7539	1.50	1.77	1.43	0.2013
Peak	2.11	2.60	3.27	0.3259	4.70	3.18	6.02	0.1705

Table 7 (Cont'd). Exposure Characteristic by Outcome within Each Plant								
Exposure*	**Plant 1**				**Plant 2**			
	CBD N=28	**BeS N=23**	**Control N=133**	**P Value**	**CBD N=33**	**BeS N=18**	**Control N=125**	**P Value**
Soluble								
Cumulative	13.10	7.29	23.49	0.2498	0.18	0.00	84.20	0.4226
Mean	0.21	0.33	0.24	0.5181	0.03	0.00	0.78	0.5927
Peak	0.65	1.18	1.17	0.2776	2.72	0.00	6.13	0.5974
Physical Mix								
Cumulative	20.59	7.65	45.72	**0.0023**	74.61	61.49	360.62	0.1031
Mean	0.25	0.32	0.46	**0.0127**	1.58	5.95	3.29	0.6392
Peak	0.99	1.09	1.64	**0.0260**	7.98	11.48	14.84	0.1661
Dust								
Cumulative	**	**	**	n/a	195.5	123.19	366.21	0.2759
Mean	**	**	**	n/a	7.45	10.79	8.15	0.2981
Peak	2.11	2.60	3.32	0.2300	12.38	17.18	36.23	0.2505
Fume								
Cumulative	12.29	6.49	18.47	0.2837	165.5	16.02	550.03	0.2242
Mean	0.18	0.30	0.19	0.4482	4.51	0.07	2.02	0.5128
Peak	0.55	1.16	1.10	0.2238	12.74	1.34	23.74	0.2004

*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure

**) Data on cumulative and mean dust levels not available for Plant 1

P Value was obtained from Kruskal Wallis test comparing CBD, BeS and Controls

For further analysis, exposure was ascertained as cumulative exposure, because it reflects the total exposure received by each subject by taking into account the duration of exposure. A skewed distribution of cumulative exposure was detected, hence for further analysis transformation to log scale for cumulative exposure was used (Appendix 1).

To understand the effect of exposure level on disease outcome, we compared exposure level between Plant 1 and Plant 2 for each category of outcome (Table 8).

Table 8. Comparison of Exposure between Plant 1 and Plant 2 for Chronic Beryllium Disease, Beryllium Sensitization, and Control Individuals									
Exposure*	**CBD**			**BeS**			**Control**		
	Plant 1 N=28	**Plant 2 N=33**	**P value**	**Plant 1 N=23**	**Plant 2 N=18**	**P value**	**Plant 1 N=133**	**Plant 2 N=125**	**P value**
Duration									
Duration in years	8.73	8.72	0.9711	5.22	5.83	0.2263	9.20	15.34	**<.0001**
Total Exposure									
Cumulative	118.72	437.91	0.4053	77.66	232.28	0.2026	162.39	1357.9	**0.0258**
Log Cumulative	3.86	4.37	0.4053	3.25	4.06	0.2026	4.20	4.89	**0.0263**
Mean	1.42	12.96	0.3657	1.53	16.65	0.0640	1.69	13.25	**0.0024**
Peak	2.63	24.13	0.1097	2.90	22.25	0.0568	3.71	54.45	**<.0001**
Type of Exposure									
Chemical									
Mix									
Cumulative	20.93	351.49	**0.0009**	9.51	146.25	**0.0037**	50.20	1072.7	**<.0001**
Mean	0.28	12.01	**0.0002**	0.37	15.03	**0.0079**	0.48	11.25	**<.0001**
Peak	1.09	20.42	**0.0026**	1.09	20.33	**0.0224**	1.68	52.09	**<.0001**
Non Soluble									
Cumulative	84.69	86.17	**0.0379**	60.86	86.02	0.0854	88.70	181.40	**0.0246**
Mean	0.94	1.50	**0.0054**	0.83	1.77	0.1456	0.97	1.43	**0.0013**
Peak	2.11	4.70	**0.0141**	2.60	3.18	0.1607	3.27	6.02	**0.0359**
Soluble									
Cumulative	13.10	0.18	**0.0054**	7.29	0.00	**0.0035**	23.49	84.20	**<.0001**
Mean	0.21	0.03	**0.0072**	0.33	0.00	**0.0035**	0.24	0.78	**<.0001**
Peak	0.65	2.72	**0.0039**	1.18	0.00	**0.0018**	1.17	6.13	**<.0001**
Physical Mix									
Cumulative	20.59	74.61	0.3270	7.65	61.49	0.5838	45.72	360.62	0.2396
Mean	0.25	1.58	0.6778	0.32	5.95	0.9628	0.46	3.29	0.2203
Peak	0.99	7.98	0.3230	1.09	11.48	0.8931	1.64	14.84	0.1640

Table 8 (Cont'd). Comparison of Exposure between Plant 1 and Plant 2 for Chronic Beryllium Disease, Beryllium Sensitization, and Control Individuals									
Exposure*	**CBD**			**BeS**			**Control**		
	Plant 1 N=28	**Plant 2 N=33**	**P value**	**Plant 1 N=23**	**Plant 2 N=18**	**P value**	**Plant 1 N=133**	**Plant 2 N=125**	**P value**
Dust									
Cumulative	**	195.54	n/a	**	123.19	n/a	**	366.21	n/a
Mean	**	7.45	n/a	**	10.79	n/a	**	8.15	n/a
Peak	2.11	12.38	0.6460	2.60	17.18	0.3852	3.32	36.23	**0.0495**
Fume									
Cumulative	12.29	165.51	0.4419	6.49	16.02	0.4172	18.47	550.03	0.2837
Mean	0.18	4.51	0.8519	0.30	0.07	0.1130	0.19	2.02	0.1443
Peak	0.55	12.74	0.4866	1.16	1.34	0.0964	1.10	23.74	0.5413

*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure

**) Data was not obtained from Plant 1 for cumulative and mean physical dust exposure

Comparison was conducted using non parametric Wilcoxon two sample test comparing the level of exposures for each disease category (Chronic Beryllium Disease, Beryllium Sensitization, and controls) between the two plants

Significant differences were found between Plant 1 and Plant 2 for duration, cumulative exposure, peak exposure, and mean exposure in the control group, but not in BeS or CBD (Table 8). Plant 2 had higher cumulative, mean, and peak exposure compared to Plant 1 in control individuals. Controls in Plant 2 also had longer duration of exposure compared to Plant 1. We also found a significant difference of exposure level between Plant 1 and Plant 2 when analyzed within each disease status (Table 8).

Mixed chemical exposure was higher in Plant 2 for all disease categories (CBD, BeS and controls). Non soluble chemical were only significantly different between plant 1 and plant 2 for CBD and control, in which workers in Plant 2 had higher exposure than those working in Plant 1. However, CBD and BeS individuals in Plant 1 had a higher exposure of soluble chemical than those in Plant 2 (Table 8).

When data from the two plants was combined, there was no significant difference of exposure between CBD, BeS, and control individuals, except for duration ($p = 0.0010$), cumulative exposure ($p = 0.0236$), as well as in type of exposures for mixed chemical exposure ($p = 0.0230$) and all measures of mixed physical exposure (p value respectively = 0.0007; 0.0247 and 0.0052 for cumulative, mean, and peak physical exposure) as shown in Table 9.

Table 9. Exposure and Type of Exposure by Outcome

Exposure	**Outcome**			**Total Average**	**P Value**
	CBD N=61	**BeS N=41**	**Control N=259**		
Duration					
Duration in years	8.89	5.49	12.19	10.87	**0.0010**
Total Exposure					
Cumulative	291.40	145.54	743.99	599.55	**0.0236**
Log cumulative exposure	4.13	3.60	4.53	4.36	**0.0236**
Mean	7.66	8.17	7.32	7.47	0.4931
Peak	14.26	11.39	28.40	24.08	0.1480

Table 9 (Cont'd). Exposure and Type of Exposure by Outcome					
Exposure*	**Outcome**			**Total Average**	**P Value**
	CBD N=61	**BeS N=41**	**Control N=259**		
Type of Exposures					
Chemical					
Mix					
Cumulative	199.76	69.54	547.65	434.57	**0.0230**
Mean	6.62	6.81	5.72	6.00	0.2151
Peak	11.54	9.54	26.20	21.83	0.0599
Non Soluble					
Cumulative	85.49	71.91	133.80	118.61	0.1540
Mean	1.24	1.25	1.19	1.21	0.4830
Peak	3.51	2.86	4.61	4.23	0.0594
Soluble					
Cumulative	6.11	4.09	53.03	39.54	0.1461
Mean	0.11	0.19	0.50	0.40	0.3209
Peak	1.77	0.66	3.58	2.94	0.2371
Physical					
Mix					
Cumulative	49.81	31.29	198.92	154.68	**0.0007**
Mean	0.97	2.79	1.84	1.80	**0.0247**
Peak	4.77	5.65	8.06	7.23	**0.0052**
Dust					
Cumulative	195.54	123.19	366.21	309.68	0.2759
Mean	7.45	10.79	8.15	8.29	0.2981
Peak	7.66	9.00	19.33	16.18	0.0627
Fume					
Cumulative	95.18	10.67	277.07	216.08	0.1464
Mean	2.52	0.20	1.08	1.22	0.3790
Peak	7.14	1.24	12.11	10.04	0.1398

*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure
Comparison was conducted by Kruskal Wallis non parametric test

III. GENETIC CHARACTERISTICS FOR BOTH PLANTS AND COMBINED

Table 10 shows that there was no significant difference of genetics distribution between Plant 1 and Plant 2 except for Serine 13 and Serine 11 (Table 10). However, when compared by disease states (Table 11) these two genes showed no significant difference in proportion among CBD, BeS, and controls.

Table 10. Genetics Distribution by Plant

Gene (N, %)	**Plant 1 N = 184**	**Plant 2 N = 177**	**P value**
Glutamine 69			
Positive	95 (51.6)	94 (53.1)	0.7788
Negative	89 (48.4)	83 (46.9)	
Glutamine 71			
Positive	48 (26.1)	48 (27.1)	0.8245
Negative	136 (73.9)	129 (72.9)	
Serine 11			
Positive	141 (76.7)	109 (61.6)	**0.0020**
Negative	43 (23.3)	68 (38.4)	
Serine 13			
Positive	128 (69.6)	102 (57.6)	**0.0184**
Negative	56 (30.4)	75 (42.4)	
Arginine 74			
Positive	37 (20.1)	25 (14.1)	0.1318
Negative	147 (79.9)	152 (85.9)	
Asparagine 37			
Positive	73 (39.7)	59 (33.3)	0.2111
Negative	111 (60.3)	118 (66.7)	
Histidine 32			
Positive	89 (48.4)	73 (41.2)	0.1735
Negative	95 (51.6)	104 (58.8)	
Phenyl alanine 47			
Positive	142 (77.2)	130 (73.4)	0.4114
Negative	42 (22.8)	47 (26.6)	
Tyrosine 26			
Positive	38 (20.7)	27 (15.2)	0.1821
Negative	146 (79.3)	150 (84.8)	
Comparison was conducted using Chi Square			

Glutamine 69 and Glutamine 71 were significantly different between the three groups (respectively, p =<.0001 and 0.0026) as shown in Table 11. We also found a significant difference in the distribution of homozygosity (p value < 0.0001) and non-0201 alleles among glutamine 69 positive individuals (p=0.0167) in which BeS and CBD had a higher proportion of non-0201 alleles compared to controls.

Table 11. Comparison of Gene Distribution between CBD, BeS and Control

Gene (N, %)	**CBD N = 61**	**BeS N = 41**	**Control N=259**	**Total N = 361**	**P value**
Glutamine 69					
Positive	56 (91.8)	32 (78.1)	101 (39.0)	189 (52.4)	**<.0001**
Negative	5 (8.20)	9 (21.9)	158 (61.0)	172 (47.6)	
Glu69 homozygosity					
Homozygous	10 (16.4)	8 (19.5)	17 (6.6)	35 (9.7)	**<.0001**
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)	
Glu69-0201 allele					
Positive	28 (45.9)	17 (41.5)	72 (27.8)	117 (32.4)	**0.0167**
Negative	28 (45.9)	15 (36.6)	29 (11.2)	72 (20.0)	
Glu69 negative	5 (8.20)	9 (21.9)	158 (61.0)	172 (47.6)	
Glutamine 71					
Positive	16 (26.2)	20 (48.8)	60 (23.2)	96 (26.6)	**0.0026**
Negative	45 (73.8)	21 (51.2)	199 (76.8)	265 (73.4)	
Serine 11					
Positive	44 (72.1)	32 (78.1)	174 (67.2)	250 (69.3)	0.3248
Negative	17 (27.9)	9 (21.9)	85 (32.8)	111 (30.7)	
Serine 13					
Positive	38 (62.3)	30 (73.2)	162 (62.6)	230 (63.7)	0.4083
Negative	23 (37.70)	11 (26.8)	97 (37.4)	131 (36.3)	
Arginine 74					
Positive	10 (16.4)	5 (12.2)	47 (18.1)	62 (17.2)	0.6335
Negative	51 (83.6)	36 (87.8)	212 (81.9)	299 (62.8)	
Asparagine 37					
Positive	18 (29.5)	20 (48.8)	94 (36.3)	132 (36.6)	0.1384
Negative	45 (70.5)	21 (51.2)	165 (63.7)	229 (63.4)	
Histidine 32					
Positive	27 (44.3)	21 (51.2)	114 (44.0)	162 (44.9)	0.6860
Negative	34 (55.7)	20 (48.8)	145 (56.0)	199 (55.1)	

Table 11 (Cont'd). Comparison of Gene Distribution between CBD, BeS and Control					
Gene (N, %)	**CBD N = 61**	**BeS N = 41**	**Control N=259**	**Total (N = 361)**	**P value**
Phenyl alanine 47					0.9419
Positive	46 (75.4)	30 (73.2)	196 (75.7)	272 (75.3)	
Negative	15 (24.6)	11 (26.8)	63 (24.3)	89 (24.7)	
Tyrosine 26					0.5114
Positive	10 (16.4)	5 (12.2)	50 (19.3)	65 (18.0)	
Negative	51 (83.6)	36 (87.8)	209 (80.7)	296 (82.0)	
Comparison was conducted using Chi Square					

IV. GENETICS AND EXPOSURE ASSOCIATION WITH CHRONIC BERYLLIUM DISEASE AND BERYLLIUM SENSITIZATION

a. Genetic and Exposure Association with Chronic Beryllium Disease

On univariable conditional logistic regression HLA-DPB1glu69 and allele type were found to have a significant association with the development of CBD relative to glutamine 69 negative individuals (Table 12, complete univariable analysis is shown in Appendix 5). The 0201 negative allele had a greater association with development of CBD compared to 0201 positive.

Table 12. Factors Significantly Associated with CBD on Univariable Analysis						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P value**
Glutamine 69	3.2970	0.7278	27.03	6.49	112.56	**<.0001**
Glutamine 69 allele						
0201 negative	1.3668	0.3170	35.02	7.96	154.01	**<.0001**
0201 positive	0.8224	0.3164	20.32	4.63	89.24	**0.0094**

Multivariable conditional logistic regression was then conducted, by including several variables that had a marginally significant association with CBD and also checked for biologically plausible interactions. From multivariable logistic regression, HLADPB1-glu69 was found to be the only significant factor related to the development of CBD after

adjusting for log cumulative exposure (Table 13) with an Odds Ratio of 27.52 (95% CI 6.56-115.35). There was no significant interaction found in the analysis.

Table 13. Multivariable Conditional Logistic Regression for the Development of CBD						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Glutamine 69						
Glutamine 69	3.3147	0.7312	27.52	6.56	115.35	**<.0001**
Log cumulative exposure	0.0231	0.0872	1.02	0.86	1.21	0.7915
By allele type						
Glutamine 69 (0201 -)	3.5861	0.7596	36.09	8.15	159.95	**<.0001**
Glutamine 69 (0201+)	3.0234	0.7571	20.56	4.66	90.69	**<.0001**
Log cumulative exposure	0.0356	0.0887	1.04	0.87	1.23	0.6879
Glutamine 69 negative	Ref					
Comparison						
Glutamine 69 (0201- vs. 0201+)	0.5697	0.4109	1.75	0.78	3.92	0.1714

When analyzed based on allele type, we found that subjects with non-0201 alleles had higher OR compared to those with 0201 alleles (respectively, OR 36.09 95% CI 8.15-159.95; OR 20.56 95% CI 4.66-90.69), although when contrasted, the difference between non-0201 alleles and 0201 alleles was not significant (p=0.1714).

b. Genetic and Exposure Interaction with Beryllium Sensitization

The same procedure was conducted for BeS and controls (total of 145 subjects, 41 BeS and 104 controls). HLA-DPB1glu69 and allele type had a significant association with the development of BeS as shown in table 14 (complete univariable analysis is shown in Appendix 7).

Table 14. Factors Significantly Associated with BeS on Univariable Analysis						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P value**
Glutamine 69	1.8430	0.4650	6.32	2.54	15.71	**<.0001**
Glutamine 71	0.9992	0.3842	2.72	1.28	5.77	**0.0093**
Glutamine 69 allele						**< .0001**
0201 positive	0.0787	0.2944	4.49	1.70	11.83	0.7893
0201 negative	1.3433	0.3946	15.88	4.25	59.35	**0.0007**

Multivariable conditional logistic regression was then conducted by including several variables that had a marginally significant association with BeS (Appendix 7) and also biologically plausible interaction in the model. From multivariable logistic regression, glutamine 69 and glutamine 71 were found as significant factors related to the development of BeS after adjusting for other variables in the model with OR respectively 7.08 95% CI 2.59-19.35 and 0R 2.54 95% CI 1.06-6.12 (Table 15). There was no significant interaction found on the analysis.

Table 15. Multivariable Conditional Logistic Regression for the Development of BeS						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Glutamine 69						
Glutamine 69	1.9565	0.5134	7.08	2.59	19.35	**0.0001**
Glutamine 71	0.9337	0.4479	2.54	1.06	6.12	**0.0371**
Log cumulative exposure	-0.2286	0.1230	0.80	0.63	1.01	**0.0632**
By allele						
Glutamine 69 (0201 -)	3.1966	0.7875	24.45	5.22	114.45	**<.0001**
Glutamine 69 (0201+)	1.5676	0.5696	4.80	1.57	14.64	**0.0059**
Glutamine 71	1.0005	0.4657	2.72	1.09	6.78	**0.0317**
Log cumulative exposure	-0.2949	0.1378	0.75	0.57	0.98	**0.0324**
Comparison						
Glutamine 69 (0201 – vs. 0201 +)	1.5486	0.6485	4.71	1.32	16.77	**0.0170**

When analyzed based on allele type, we found that subjects with non-0201 alleles had higher OR compared to those with 0201 alleles (respectively, OR 24.45 95% CI 5.22-114.45; OR 4.80 95% CI 1.57-14.64). This difference was significant when contrasted between non-0201 alleles and 0201 alleles (p=0.0170).

V. PROGRESSION OF CBD FROM BERYLLIUM SENSITIZATION

It is still uncertain what factors influence the progression of BeS to CBD. Unconditional logistic regression was conducted to assess this association only in individuals with CBD (61 subjects) and BeS (41 subjects). From univariable logistic regression we found Glutamine71 as a significant predictor blocking the progression to CBD (Table 16).

Table 16. Factors Which Significantly Differentiate CBD and BeS on Univariable Analysis

Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Glu69	1.1474	0.6002	3.15	0.97	10.21	**0.0559**
Glutamine71	-0.9852	0.4270	0.37	0.16	0.86	**0.0210**
Allele						
0201 -	1.2119	0.6430	3.36	0.95	11.85	**0.0595**
0201 +	1.0867	0.6369	2.97	0.85	10.33	0.0880

From multivariable logistic regression, only Glutamine 71 continued to be a factor related to reducing the risk of progression to CBD after adjusting for log cumulative exposure as seen in Model 2 of Table 17 (OR = 0.38 95% CI 0.16-0.87). There was no significant interaction found in the analysis.

Table 17. Unconditional Multivariable Logistic Regression Analysis on Progression from BeS to CBD						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Model 1						
Intercepts	-0.2625	0.8506				0.7577
0201 +	0.4931	0.7232	1.64	0.40	6.76	0.4953
0201 -	0.4912	0.7857	1.63	0.35	7.62	0.5319
Glutamine 71	-0.7948	0.5244	0.45	0.16	1.26	0.1296
Log Cum Exposure	0.1372	0.1119	1.15	0.92	1.43	0.2204
Model 2						
Intercepts	0.1979	0.4967				0.6902
Glutamine 71	-0.9812	0.4309	0.38	0.16	0.87	**0.0228**
Log Cum Exposure	0.1455	0.1106	1.16	0.93	1.44	0.1884

VI. EXPOSURE CHRACTERISTICS BY GENETICS AND DISEASE STATUS

From univariable and multivariable conditional logistic regression analysis, the amount of exposure was shown to have no significant association with the development of CBD and BeS although exposure is a necessary cause for beryllium-related toxicity. Glutamine 69 was consistently shown to be the significant factor related to the development of CBD and BeS. Approximately 39% of control individuals who tested positive for HLA-DPB1Glu69 gene, however, were not sensitized and remained disease free. Hence, another analysis was conducted to assess the exposure by HLA-DPB1glu69 status, to see whether control individuals who were HLA-DPB1 positive had lower exposure, and therefore did not develop beryllium toxicity.

From Table 18 we can see that there was a difference although not statistically significant in cumulative exposure among control subjects, who were glu69 positive and glu69 negative (p= 0.4106). The result also shows that control individuals with the non-0201 allele have higher exposure than those with the 0201 allele, which infers that this allele cannot explain why these individuals remain healthy.

In CBD and BeS individuals however, the cumulative exposure is higher in those positive for Glutamine 69 compared to individuals with glutamine 69 negative, but these individuals were all positive for glutamine 71 which from our previous reports was shown to be a risk factor in development of CBD and BeS in the absence of glu69.(63) (Table 18). When comparing based on alleles type, individuals carrying 0201 allele had higher exposure compared to those with non-0201 alleles, inferring that individuals who are 0201 negative are more likely to get CBD or BeS, even with less exposure, although the difference is not significant (Table 18).

Table 18. Comparison of Cumulative, Log Cumulative, Mean, and Peak Exposure between CBD, BeS, and Control Groups based on HLA-DPB1Glu69 presence and allele type

Outcome	Exposure	N	Glu69	Mean	P value
Control					
By gene	Cumulative	101	Positive	312.17	0.4061
		158	Negative	1020.03	
	Log cumexp	101	Positive	4.27	0.4061
		158	Negative	4.70	
	Mean	101	Positive	6.83	0.7197
		158	Negative	7.62	
	Peak	101	Positive	11.84	0.4500
		158	Negative	38.98	
By allele	Cumulative	29	0201 negative	407.15	0.4106
		72	0201 positive	273.91	
		158	Negative	1020.03	
	Log cumexp	29	0201 negative	4.60	0.4106
		72	0201 positive	4.14	
		158	Negative	4.70	
	Mean	29	0201 negative	8.15	0.6000
		72	0201 positive	6.30	
		158	Negative	7.62	
	Peak	29	0201 negative	13.27	0.3339
		72	0201 positive	11.26	
		158	Negative	38.98	
CBD					
By gene	Cumulative	56	Positive	307.65	0.9685
		5	Negative	109.36	
	Log cumexp	56	Positive	4.15	0.9685

Table 18 (Cont'd). Comparison of Cumulative, Log Cumulative, Mean, and Peak Exposure between CBD, BeS, and Control based on HLA-DPB1Glu69 presence and allele type					
Outcome	**Exposure**	**N**	**Glu69**	**Mean**	**P value**
		5	Negative	3.94	
	Mean	56	Positive	8.16	0.7524
		5	Negative	2.10	
	Peak	56	Positive	15.28	0.7030
		5	Negative	2.92	
By allele	Cumulative	28	0201 negative	176.31	0.1849
		28	0201 positive	438.99	
		5	Negative	109.36	
	Log cumexp	28	0201 negative	3.69	0.1849
		28	0201 positive	4.61	
		5	Negative	3.94	
	Mean	28	0201 negative	10.27	0.9469
		28	0201 positive	6.05	
		5	Negative	2.10	
	Peak	28	0201 negative	18.43	0.5815
		28	0201 positive	12.12	
		5	Negative	2.92	
BeS					
By gene	Cumulative	56	Positive	166.95	0.3060
		5	Negative	69.40	
	Log cumexp	56	Positive	3.76	0.3060
		5	Negative	3.03	
	Mean	56	Positive	9.43	0.1807
		5	Negative	3.67	
	Peak	56	Positive	13.42	0.1705
		5	Negative	4.20	
By allele	Cumulative	15	0201 negative	132.27	0.3147
		17	0201 positive	197.55	
		9	Negative	69.40	
	Log Cumexp	15	0201 negative	4.03	0.3147
		17	0201 positive	3.52	
		9	Negative	3.03	
	Mean	15	0201 negative	12.91	0.3274
		17	0201 positive	6.36	
		9	Negative	3.67	
	Peak	15	0201 negative	14.68	0.3736
		17	0201 positive	12.31	
		9	Negative	4.20	
Comparison was conducted by Wilcoxon two sample test and Kruskal Wallis non parametric test					

VII. EXPOSURE CHARACTERISTICS IN INDIVIDUALS WITH GLUTAMINE 69

To see whether there was a different level of exposure among those who were susceptible to developing CBD or BeS, we compared exposure levels among the 189 subjects who were positive for glutamine 69 (Table 19). We found no significant difference in cumulative, mean, and peak exposure as well as type of exposure between CBD, BeS, and controls in glutamine 69 positive individuals. The only significant difference we found was in duration of exposure, in which the controls had a longer exposure followed by CBD and BeS (p=0.0217). However, we observed a trend of dose-response in peak exposure in which subjects with CBD had higher exposure than BeS and controls (respectively, peak exposure 15.28, 13.42, 11.84) although the difference was not significant (p=0.6162).

Table 19. Comparison of Magnitude and Type of Exposures between CBD, BeS, and Control Groups based on Individuals with Glutamine 69

Exposure		**Outcome**			**Total Average N=189**	**P Value**
		CBD N=56	**BeS N=32**	**Control N=101**		
Total Exposure						
Duration		9.18	5.08	11.51	9.73	**0.0217**
Cumulative		307.65	166.95	312.17	286.24	0.3329
Log Cumulative exposure		4.15	3.76	4.27	4.15	0.3329
Mean		8.16	9.43	6.83	7.67	0.4739
Peak		15.28	13.42	11.84	13.12	0.6162
Chemical						
Mix	Cumulative	215.31	78.12	175.27	170.69	0.4746
	Mean	7.10	7.84	5.37	6.30	0.7408
	Peak	12.45	11.25	10.05	10.96	0.6204
Non Soluble	Cumulative	91.20	84.56	114.29	102.41	0.5331
	Mean	1.32	1.53	1.36	1.38	0.2453
	Peak	3.69	3.49	3.44	3.52	0.0662
Soluble	Cumulative	1.10	4.28	18.53	10.95	0.3369
	Mean	0.07	0.16	0.12	0.11	0.3962
	Peak	1.87	0.65	0.58	0.98	0.3896
Physical						
Mix	Cumulative	53.06	31.86	116.65	83.45	0.0947
	Mean	0.96	2.73	2.42	2.04	0.1743

Table 19 (Cont'd). Comparison of Magnitude and Type of Exposures between CBD, BeS, and Control Groups based on Individuals with Glutamine 69						
Exposure		**Outcome**			**Total Average N=189**	**P Value**
		CBD N=56	**BeS N=32**	**Control N=101**		
	Peak	5.10	6.33	5.25	5.39	0.2497
Dust	Cumulative	208.16	184.61	198.71	200.03	0.5315
	Mean	7.93	16.17	6.62	8.27	**0.0117**
	Peak	8.21	11.36	8.16	8.72	**0.0497**
Fume	Cumulative	97.05	9.96	34.71	48.99	0.8933
	Mean	2.69	0.15	0.57	1.13	0.7378
	Peak	7.70	1.33	3.22	4.23	0.8763
*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure Comparison was obtained using Kruskal Wallis non parametric test						

VIII. ANALYSIS OF THE EFFECT OF TYPE OF EXPOSURE IN THE DEVELOPMENT OF CBD AND BES USING THE HOCEKY STICK APPROACH FOR CODING EXPOSURE

Based on previous reports, the type of beryllium exposure has been associated with the development of CBD and BeS.(5,11) To test this hypothesis, we ran analyses on exposures that showed marginally significant differences with the Kruskal Wallis test in previous section based on Table 19. However, as noted previously, proportion of individuals had exposure levels below the limit of detection (close to zero, or even zero level).

To assess the effect of exposures that contained zero or minimal exposure, we used the hockey stick method, which assigns a categorical variable for individuals exposed higher than the detectable level and lower than the detectable level (zero values), and a continuous variable for individuals that had higher than the detectable level. The categorical variable compared individuals with lower than detectable levels to individuals

with detectable levels of exposure, while log scale showed the effect of detectable levels of exposure on the development of CBD. From the analyses, type of exposure shows no significant association with development of CBD (Table 20). These types of analyses were repeated for assessing the effect of exposure on the development of CBD, BeS, and progression from BeS and CBD, as well as repeating the analyses for glu69 positive only.

a. Hockey Stick Analyses for CBD

Table 20. Conditional Logistic Regression for the Development of CBD by Type of Exposure with Hockey Stick Analysis

Variable	Coefficient	Standard Error	OR	95% Confidence Interval		P Value
Mean Mixed Chemical						
Glutamine 69	3.2945	0.7306	26.96	6.44	112.90	**<.0001**
Mean mixed chemical	-0.1658	0.4171	0.85	0.37	1.92	0.691
Log mixed chemical	0.0251	0.1124	1.03	0.82	1.28	0.8236
Peak Mixed Chemical						
Glutamine 69	3.2744	0.7288	26.43	6.33	110.26	**<.0001**
Peak Mixed Chemical	-0.0779	0.4812	0.93	0.36	2.38	0.8713
Log peak mixed chemical	-0.0558	0.1544	0.95	0.70	1.28	0.718
Cumulative Soluble						
Glutamine 69	3.3435	0.746	28.32	6.56	122.20	**<.0001**
Chemical Soluble	-0.72	1.0561	0.49	0.06	3.86	0.4954
Log cum soluble	-0.0274	0.2896	0.97	0.55	1.72	0.9247
Peak Soluble						
Glutamine 69	3.3953	0.7573	29.82	6.76	131.58	**<.0001**
Peak Soluble	-0.5268	0.7586	0.59	0.13	2.61	0.4874
Log Peak Soluble	-0.3155	0.5137	0.73	0.27	2.00	0.5391

To have better understanding on the effect of exposure on the susceptible individuals, we run for only those that were glu69 positive. Similar results were also found as seen on table 21. There was no significant effect of different types of exposures on the development of Chronic Beryllium Disease.

Table 21. Conditional Logistic Regression for the Development of CBD by Type of Exposure with Hockey Stick Analysis in Glutamine 69 Positive Individuals						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Mean Mixed Chemical						
Mean mixed chemical	0.1521	0.4562	1.16	0.48	2.85	0.7387
Log mixed chemical	-0.0303	0.1180	0.97	0.77	1.22	0.7972
Peak Mixed Chemical						
Peak mixed chemical	0.3304	0.5298	1.39	0.49	3.93	0.5330
Log Peak mixed chemical	-0.1079	0.1669	0.90	0.65	1.25	0.5180
Chemical Soluble						
Chemical Soluble	1.5400	1.2311	4.66	0.42	52.09	0.2110
Log cum soluble	-0.7568	0.4558	0.47	0.19	1.15	0.0968
Peak Soluble						
Peak Soluble	0.0902	0.8185	1.09	0.22	5.44	0.9123
Log Peak Soluble	-0.8516	0.7934	0.43	0.09	2.02	0.2831

b. Hockey Stick Analysis for BeS

Similar analyses were conducted for factors associated with the development of BeS (Table 22). Only log mixed chemical exposure showed a significant exposure influence on the development of BeS (OR 1.5 with 95% CI 1.1-2.2, p =0.0171), which means that increases in mean mixed chemical exposure among people exposed at higher than the detectable limit, also increased the likelihood for the development of sensitization.

Table 22. Conditional Logistic Regression for the Development of BeS by Type of Exposure with Hockey Stick Analysis						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Mean Mixed Chemical						
Glu71	1.2711	0.4968	3.56	1.35	9.44	**0.0105**
Glu69	2.1322	0.5587	8.43	2.82	25.21	**0.0001**
Mean mixed chemical	-0.6448	0.4730	0.52	0.21	1.33	0.1728
Log mixed chemical	0.4255	0.1785	1.53	1.08	2.17	**0.0171**
Peak Mixed Chemical						
Glu71	1.1331	0.4702	3.11	1.24	7.80	**0.0160**
Glu69	2.0218	0.5225	7.55	2.71	21.03	**0.0001**
Peak Mixed Chemical	-0.6992	0.5458	0.50	0.17	1.45	0.2001
Log peak mixed chemical	0.2285	0.2009	1.26	0.85	1.86	0.2554

Table 22 (Cont'd). Conditional Logistic Regression for the Development of BeS by Type of Exposure with Hockey Stick Analysis						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Cumulative Soluble						
Glu71	1.1615	0.4682	3.19	1.28	8.00	**0.0131**
Glu69	1.9452	0.5073	7.00	2.59	18.91	**0.0001**
Chemical Soluble	1.4402	1.0612	4.22	0.53	33.79	0.1748
Log cum soluble	-0.5472	0.3559	0.58	0.29	1.16	0.1241
Peak Soluble						
Glu71	1.1166	0.4528	3.05	1.26	7.42	**0.0137**
Glu69	2.0222	0.5156	7.55	2.75	20.75	**<.0001**
Peak Soluble	0.9557	1.3997	2.60	0.17	40.41	0.4948
Log Peak Soluble	-0.5571	1.3520	0.57	0.04	8.11	0.6803

However, among the susceptible individuals (those with Glu69 positive), the association is not observed (Table 24). There was no significant effect of different types of exposures in the development of BeS among individuals with the glutamine 69 polymorphism.

Table 23. Conditional Logistic Regression for the Development of BeS by Type of Exposure with Hockey Stick Analysis among Glutamine 69 Positive Individuals						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Mean Mixed Chemical						
Glutamine 71	-0.2126	0.6417	0.81	0.23	2.84	0.7404
Mean mixed chemical	-0.9344	0.6337	0.39	0.11	1.36	0.1404
Log mixed chemical	0.2803	0.2161	1.32	0.87	2.02	0.1946
Peak Mixed Chemical						
Glu71	-0.2548	0.6173	0.78	0.23	2.60	0.6798
Peak Mixed Chemical	-1.1842	0.7817	0.31	0.07	1.42	0.1298
Log peak mixed chemical	0.2415	0.2819	1.27	0.73	2.21	0.3917
Chemical Soluble						
Glu71	-0.4032	0.6359	0.67	0.19	2.32	0.5260
Chemical Soluble	0.4066	1.3436	1.50	0.11	20.91	0.7622
Log cum soluble	-0.6738	0.5344	0.51	0.18	1.45	0.2074
Peak Soluble						
Glu71	-0.4053	0.6130	0.67	0.20	2.22	0.5085
Peak Soluble	0.8233	3.1929	2.28	0.00	1189.61	0.7965
Log Peak Soluble	-1.5991	3.4192	0.20	0.00	164.44	0.6400

c. Hockey Stick Analyses on Progression of BeS to CBD

Analyses were also conducted to evaluate the influence of these exposures on the development of CBD from BeS. No significant association was found between exposures and CBD vs. BeS (Table 24).

Table 24. Unconditional Logistic Regression Comparing CBD and BeS by Type of Exposure with Hockey Stick Analysis

Variable	Coefficient	Standard Error	OR	95% Confidence Interval		P Value
Mean Mixed Chemical						
Intercepts	0.5548	0.3702				0.1340
Glutamine 71	-0.9265	0.4341	0.40	0.17	0.93	0.0328
Mean mixed chemical	0.3958	0.4313	1.49	0.64	3.46	0.3587
Log mixed chemical	-0.1452	0.1297	0.86	0.67	1.12	0.2628
Peak Mixed Chemical						
Intercepts	0.6212	0.3730				0.0959
Glu71	-0.9832	0.4346	0.37	0.16	0.88	0.0237
Peak Mixed Chemical	0.3830	0.4941	1.47	0.56	3.86	0.4383
Log peak mixed chemical	-0.0925	0.1678	0.91	0.66	1.27	0.5814
Chemical Soluble						
Intercepts	0.9835	0.3085				0.0014
Glu71	-1.1802	0.4498	0.31	0.13	0.74	0.0087
Chemical Soluble	-1.3302	0.8847	0.26	0.05	1.50	0.1327
Log cum soluble	0.2456	0.3013	1.28	0.71	2.31	0.4150
Peak Soluble						
Intercepts	0.9813	0.3055				0.0013
Glu71	-1.1184	0.4426	0.33	0.14	0.78	0.0115
Peak Soluble	-1.0305	0.7326	0.36	0.08	1.50	0.1596
Log Peak Soluble	0.1629	0.4965	1.18	0.44	3.11	0.7428

We conducted similar analysis in individuals with glutamine 69 positive and found similar result as seen in Tale 25. There were no significant association found between the type of exposure and development of CBD compared to BeS on subjects positive for Glutamine 69 (Table 25).

Table 25. Unconditional Logistic Regression Comparing CBD and BeS by Type of Exposure with Hockey Stick Analysis among Glutamine 69 Positive Individuals						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
Mean Mixed Chemical						
Intercepts	0.4445	0.3822				0.2448
Glutamine 71	-0.6531	0.5139	0.52	0.19	1.42	0.2037
Mean mixed chemical	0.6029	0.4732	1.83	0.72	4.62	0.2027
Log mixed chemical	-0.1800	0.1385	0.84	0.64	1.10	0.1939
Peak Mixed Chemical						
Intercepts	0.4600	0.3827				0.2293
Glu71	-0.6985	0.5136	0.50	0.18	1.36	0.1738
Peak Mixed Chemical	0.8010	0.5628	2.23	0.74	6.71	0.1547
Log peak mixed chemical	-0.1767	0.1816	0.84	0.59	1.20	0.3305
Chemical Soluble						
Intercepts	1.0015	0.3130				0.0014
Glu71	-0.9636	0.5243	0.38	0.14	1.07	0.0661
Chemical Soluble	-1.0539	0.8634	0.35	0.06	1.89	0.2222
Log cum soluble	0.0581	0.3350	1.06	0.55	2.04	0.8622
Peak Soluble						
Intercepts	1.0008	0.3129				0.0014
Glu71	-0.9617	0.5241	0.38	0.14	1.07	0.0665
Peak Soluble	-1.0844	0.7570	0.34	0.08	1.49	0.1520
Log Peak Soluble	0.1427	0.4913	1.15	0.44	3.02	0.7715

CHAPTER V

DISCUSSION

Our results show the importance of glutamine 69 and glutamine 71 in the development of beryllium toxicity. Although there was no clear dose-response association of beryllium exposure for the development of CBD or BeS, we observed a trend of increasing peak levels and the prevalence of CBD and BeS in individuals with glutamine 69, although this difference was not statistically significant. There was no interaction between genetics and exposure levels observed in our analyses, but we did find that despite having the highest exposure, individuals without either the glutamine 69 or glutamine 71 polymorphisms remained healthy.

Our analysis was conducted on one of the largest cohorts available for studying the effect of beryllium exposure and genetics, and consisted of 361 subjects. We found a significant difference in the proportion of individuals with glutamine 69 among CBD, BeS and controls (p value <0.0001) in which this gene were present in 91.8% of CBD cases and 78.1% of Bes compared to 39.0% in controls (Table 11). This result is consistent with previous studies that also showed a higher proportion of glutamine 69 positive individuals in CBD cases (56) followed by BeS, and the lowest proportion of glutamine 69 positive was found in controls.(58)

We also found significant differences in homozygosity (p <0.0001) between CBD, BeS, and controls. Approximately 17.8% (10/56) of CBD and 28.1% (9/32) of BeS cases were homozygous compared to 16.8% (17/101) of controls. This higher proportion of homozygosity in BeS cases compared to controls is consistent with previous results.(58, 59)

The importance of non-0201 alleles in the development of CBD and BeS had also been previously reported.(58,59) In our study, a higher proportion of 0201 negative alleles were found

in CBD and BeS cases compared to controls. Among those with HLA-DPB1Glu69, the highest proportion of non-0201 carrying individuals were found in CBD (50%) compared to BeS (46.8%) and controls (28.1%). This is also consistent with previous studies conducted by Wang et al who reported a higher proportion of non-0201 allele carriage among CBD, and control individuals (proportion respectively 84% and 32%).(58,59)

The importance of glutamine 71 in beryllium toxicity, in accordance with a previous report on this cohort, was reconfirmed in this analysis.(63) The significantly higher proportion of glutamine 71 carrying individuals was found in BeS cases (48.8%) compared to 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 glutamine 69 or glutamine 71 polymorphisms, compared to only 32.4% of controls. However, when we assessed the importance of glutamine 71 in the development of CBD and BeS among the HLA-DPB1Glu69 negative individuals we found no significant association (p value 0.9984**)** (Appendix 4).

Consistent with a previous report on this cohort, the highest level of exposure was found in control individuals, followed by CBD and BeS.(28) On pairwise comparison, we found a significant difference between BeS and controls for cumulative mixed chemical exposure, peak mixed chemical exposure, and cumulative mixed physical exposure (Appendix 2). There were no significant differences in total exposure between CBD, BeS and controls, except for duration of work and cumulative exposure, (p value 0.0010; 0.0236; respectively). For different type of exposures we found significant difference for mixed chemical exposure ($p = 0.0230$) and all measures of mixed physical exposure (Table 9). The highest levels were found in controls, followed by CBD and BeS (Table 9). On pairwise comparison, the significant differences were

only found among BeS and controls for duration, cumulative mixed physical exposure, and peak mixed physical exposure (Appendix 3).

Observing higher exposure in controls, it is unlikely that a policy to move diseased workers from exposed areas is responsible for the higher level of exposures that were found in controls, because the diagnosis of CBD and BeS was generally made many years or even decades after the worker had left the Plant and the exposure had ceased. Following the findings from the previous report on this cohort, we also hypothesized that host factors, i.e. genetic susceptibility might explain these findings.(28)

To assess the importance of genetic susceptibility in the development of CBD and BeS we ran separate conditional logistic regression analyses on CBD and their controls and on BeS and their controls. Univariable conditional logistic regression showed a significant association of glutamine 69 with the development of CBD (Table 12 and Appendix 5) with a crude odds ratio of 27.0 (95% CI 6.5-112.6).

When considering allele type we found that, consistent with the previous report, non-0201 carriage has a higher risk for developing CBD (OR for non-0201 carrier was 35.02 and 95% CI 7.96-154.01, OR for 0201 carrier was 20.32 and 95% CI 4.63 – 89.24) as shown on table 12. This result is consistent with previous findings that showed the importance of glutamine 69 in the development of CBD especially the non-0201 carriage.(60,62,63) We did not find a significant effect of other demographic, genetics, or exposure variables with the development of CBD. When adjusted for log cumulative exposure in the model, the OR for glutamine 69 was 27.52 (95% CI 6.56-115.35).

Univariable analysis of BeS and their controls (41 BeS and 104 controls) found that in addition to HLA-DPB1glu69, glutamine 71 also showed a significant association (respectively

OR for glutamine 69 is 6.32 with 95% CI 2.54-15.71; OR for glutamine 71 2.72 with 95% CI 1.28-5.77). In multivariable regression analyses adjusted for log cumulative exposure, glutamine 69 and glutamine 71 remain the two significant predictors with Odds Ratios of 7.08 (95% CI 2.59-19.35) and 2.54 (95% CI 1.06-6.12), respectively. A previous study has also linked glutamine 69 as a significant predictor for BeS (15,16,59) but to the best of our knowledge glutamine 71 significance was only recently reported from the previous study of this same study population from two facilities in eastern Pennsylvania.(63)

When considering allele type, we found that individuals with non-0201 alleles had higher OR compared to those with 0201 alleles (OR 24.45; 95% CI 5.22-114.45 and OR 4.79; 95% CI 1.57-14.64 respectively). Contrasting non-0201 carriage with 0201 carriage, we found a significant difference on OR (p=0.0170) where individuals who carry non-0201 alleles had higher risk compared to those with 0201 alleles (OR4.71; 95% CI = 1.32-16.77). This result corroborates Van Dyke et al who also reported a higher odds ratio for non-0201 carriers compared to 0201-carriers.(15,16)

We did not observe a dose-response association and genetic-exposure interaction in the development of CBD and BeS such as that which was reported by Van Dyke et al.(15) Our analyses showed that exposure did not have a significant association with development of beryllium toxicity on univariable and multivariable models, and did not show a significant interaction with genetic characteristics on our multivariable model. When we categorized exposure into quartiles, similar to how analyses were conducted by Van Dyke et al, we still did not find a significant association (Appendix 8). Further analyses using algorithms to define individuals who possibly had high cumulative exposure but never had high peak exposure, or

between individuals with high peak exposure but who maintained a lower cumulative exposure found no significant associations (Appendix 9).

We explored whether different exposure measurement categorization methods between our study and the Van Dyke et al study might explain the differences in results between our study and Van Dyke et al. Our exposure metrics were based on job personnel records and collected well before medical examinations were conducted to determine disease status, which would have minimized recall bias. For this cohort, we calculated and assigned cumulative, mean, and peak exposure for each individual based on their actual job history from company records and actual exposure data from workplace industrial hygiene reports where beryllium exposures in the plant were measured over different time periods. In contrast, Van Dyke assigned exposures based on personal interview of job history after completion of the medical examination which might introduce bias and potential exposure misclassification.(15,28) Although it is unlikely that study methodology greatly influenced these differences, it is important to note that we also used a different control selection method compared to Van Dyke. We assigned controls through exact matching based on gender, Plant, and year of birth, while Van Dyke and colleagues assigned controls through frequency matching based on gender, race, work status, and decade of hire.(15,16)

Van Dyke et al also reported finding a genetic-exposure interaction in the development of CBD and BeS.(15) Although we found no significant association of exposure with disease development and no significant genetic and exposure interactions on our multivariable model, we did find HLA-DPB1Glu69 has a role in explaining why exposure is higher in controls compared to cases. Our analysis showed that individuals who were negative for HLA-DPB1Glu69 had a significantly higher cumulative exposure than controls that carried the HLA-

DPB1Glu69 gene, although this finding was not statistically significant. This result showed that the absence of this susceptibility gene is protective for beryllium toxicity despite the significantly higher cumulative exposure.

We further investigated whether non-0201 alleles of HLA-DPB1glu69 influence this association. Our analysis showed that control individuals with non-0201 alleles had higher exposures than those with 0201 alleles. This result shows that non-0201 alleles, which are associated with increased susceptibility for beryllium toxicity, cannot explain why these control individuals would remain healthy. If non-0201 alleles had an important role, the average exposure would have been expected to be lower in non-0201 carriers than those with 0201 carriers. We did not find any significant difference of exposure between non-0201 and 0201 carrying individuals among CBD and BeS (Table 18).

Although we did not observe a clear dose-response association in our multivariable model, our analyses showed that among those positive for glutamine 69 there was a trend suggesting a dose-response in regards to peak exposure although the trend was not statistically significant.

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.(5,11)

We found significant differences in cumulative mixed chemical exposure and cumulative, mean, as well as peak mixed physical exposure, but the highest exposures were found in controls, followed by CBD and BeS (Table 8). These analyses however, were conducted among all individuals regardless of beryllium susceptibility. To have better comparable groups by taking genetic susceptibility into account, we analyzed the difference of exposure level and exposure type between CBD, BeS and control groups only in individuals with Glutamine 69 (Table 19).

We found no significant difference; however, for some exposures i.e. peak exposure, peak chemical mix, and peak soluble, we did find that CBD individuals had the highest level of exposure compared to BeS and controls, which would suggest a dose-response association (Table 19).

With further analyses using the hockey stick method for coding exposure, we only found a significant effect of log mixed chemical exposure in the development of BeS but did not find any significant association for other types of exposure (Table 22). This suggests that for people who had a detectable level of exposure, an increase in mean mixed chemical exposure will increase the likelihood of the development of BeS (OR 1.5 95% CI 1.1-2.2; p value = 0.0171). We did not observe this effect when analyses were run only for susceptible individuals (Table 23).

Our study also aimed to evaluate factors that influenced the development of CBD from BeS. Using unconditional logistic regression we found that the glutamine 71 gene has a protective effect on the development of CBD (OR 0.38, 95% CI 0.16-0.087), adjusted for log cumulative exposure. This result should be interpreted cautiously and further research is still needed to determine whether the presence of glutamine 71 is truly protective against CBD in sensitized individuals, and what mechanism is involved.

Previous studies have proposed that duration of exposure or genetic factors, including homozygosity of HLA-DPB1Glu69 (61), are linked to this progression.(20,21,23,28) In our study, we found that homozygosity or exposure was not a predictor for disease severity or the development of CBD (Appendix 7).

There have only been a few studies on the interaction of genetics and exposure on the development of beryllium toxicity. The strength of our study is the availability of specific

measurements of beryllium exposure, which included cumulative, mean, and peak exposure as well as different types (i.e., chemical, physical) of beryllium exposures. We also assessed several genes potentially involved in the mechanism of beryllium toxicity, using a relatively large study population with an exact matched case-control design.

A possible limitation of our study includes potential measurement error of exposure which could influence our results, as either over- or under-estimating exposures for various jobs or departments over time. It is unlikely that recall bias of job history would influence our imputation and assigned exposure measurements, because we used personnel records to assess job history and these records were accessed independently of case determination (medical examination). One further potential exposure bias was that our study did not assess the possibility of exposure pathways beyond airway exposure. In particular, skin exposure was not able to be assessed for this study.

Another limitation of our study was the potential differences between the two facilities. Our analyses showed that Plant 2 had significantly higher cumulative, mean, and peak exposure (Table 6) compared to Plant 1 (p value 0.0094, 0.0005, and <0.0001 respectively). The mean exposure in Plant 2 (23.20 $\mu g/m^3$) was also higher than the permissible limit of 2 $\mu g/m^3$ (49). A significant difference was also seen in the type of exposure, in which Plant 2 had significantly higher chemical exposure compared to Plant 1 but there was no significant difference in physical exposure (Table 6). This difference in type of exposure suggests that each Plant had a different industrial environment. Different lengths of time during which each Plant was in operation as well as decade of operation between the two facilities might explain this difference. Plant 2 was open longer and earlier, from 1935-2000, while Plant 1 was open from 1958 to 1978.(63) The

longer duration and earlier starting of operations at Plant 2 might contribute to the different exposure characteristics and exposure levels found between the two facilities. Further, the recommended level of beryllium exposure for workplaces was not implemented until after cases of acute beryllium toxicity were reported in late 1950s.(3) Despite these differences, our study design did match cases with controls within each plant, so any results would presumably control for confounding due to which plant an individual worked.

Our findings on the importance of genetic susceptibility of HLA-DPB1Glu69 in the development of CBD and BeS corroborate previous reports. In addition, we also found that glutamine 71 is important in the development of BeS and decreasing the risk of progressing from BeS to CBD. Our results also showed that either one or both genes were present in all cases of BeS and CBD. The significance of glutamine 71 in the development of beryllium toxicity especially in the absence of glutamine 69, as well as the role of this gene in decreasing the risk of progressing from CBD to BeS, warrants further research.

Our results also imply that there are other factors contributing to the development of CBD and BeS as well as progression of CBD from BeS other than the magnitude and type of exposure, as well as glutamine 69 and glutamine 71 polymorphisms. These factors might influence the interaction of exposure and genetics, which might explain the lack of a dose-response effect of beryllium even in susceptible individuals in our cohort. It has been proposed that it is not only the genetic susceptibility that plays an important role in the development of beryllium toxicity, but also the local environment of the epitopes.(63) Studying the local environment of additional polymorphisms might be important to understand exposure-genetic interaction in the development of beryllium toxicity.

CHAPTER VI

CONCLUSION

From our study we found that HLA-DPB1Glu69 increased the risk of Chronic Beryllium Disease (adjusted OR 27.52; 95% CI 6.56-115.35). Individuals with HLA-DPB1Glu69 non-0201 alleles, had a higher OR compared to those with 0201 alleles (adjusted OR 36.09 95% CI 8.15-159.95 and 20.56 95% CI 4.66-90.69 respectively). HLA-DPB1Glu69 was also significantly associated with development of Beryllium Sensitization (adjusted OR 7.08; 95% CI 2.59-19.35), and among these beryllium sensitized workers, the individuals with non-0201 alleles also had higher ORs compared to 0201 alleles (adjusted OR 24.45; 95% CI 5.22-114.45 and 4.80; 95% CI 1.57-14.64 respectively).

In addition to Glutamine 69, Glutamine 71 also significantly increased the risk of development of Beryllium Sensitization (adjusted OR 2.54; 95% CI 1.06-6.12). However, Glutamine 71 was protective for CBD among BeS and CBD subjects (adjusted OR 0.38, 95% CI 0.16-0.87). Further study is needed to examine the role of Glutamine 71 in the development of CBD and BeS and in the progression to CBD from BeS.

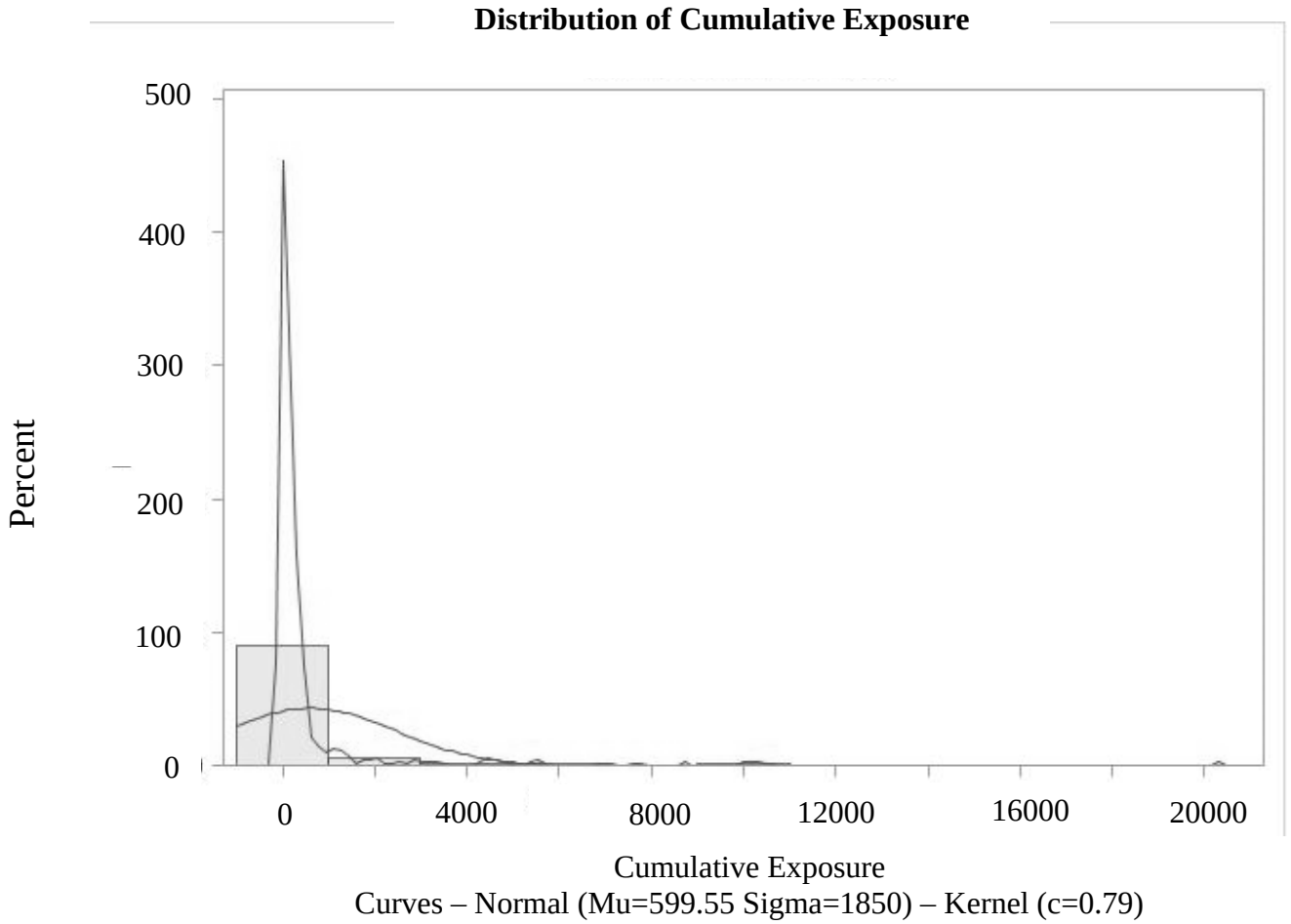
Although our results show no clear dose-response association between the magnitude and type of exposure and beryllium toxicity, we found that the control individuals with the highest exposure are those who do not have the HLA-DPB1Glu69 polymorphism and presumably remain healthy because they are not genetically susceptible. Further work to explore other polymorphisms for an exposure genetic interaction is needed to determine if controlling for these additional polymorphisms will elucidate a dose-response.

APPENDICES

Appendix 1. **Distribution of Cumulative Exposure and Log Cumulative Exposure**

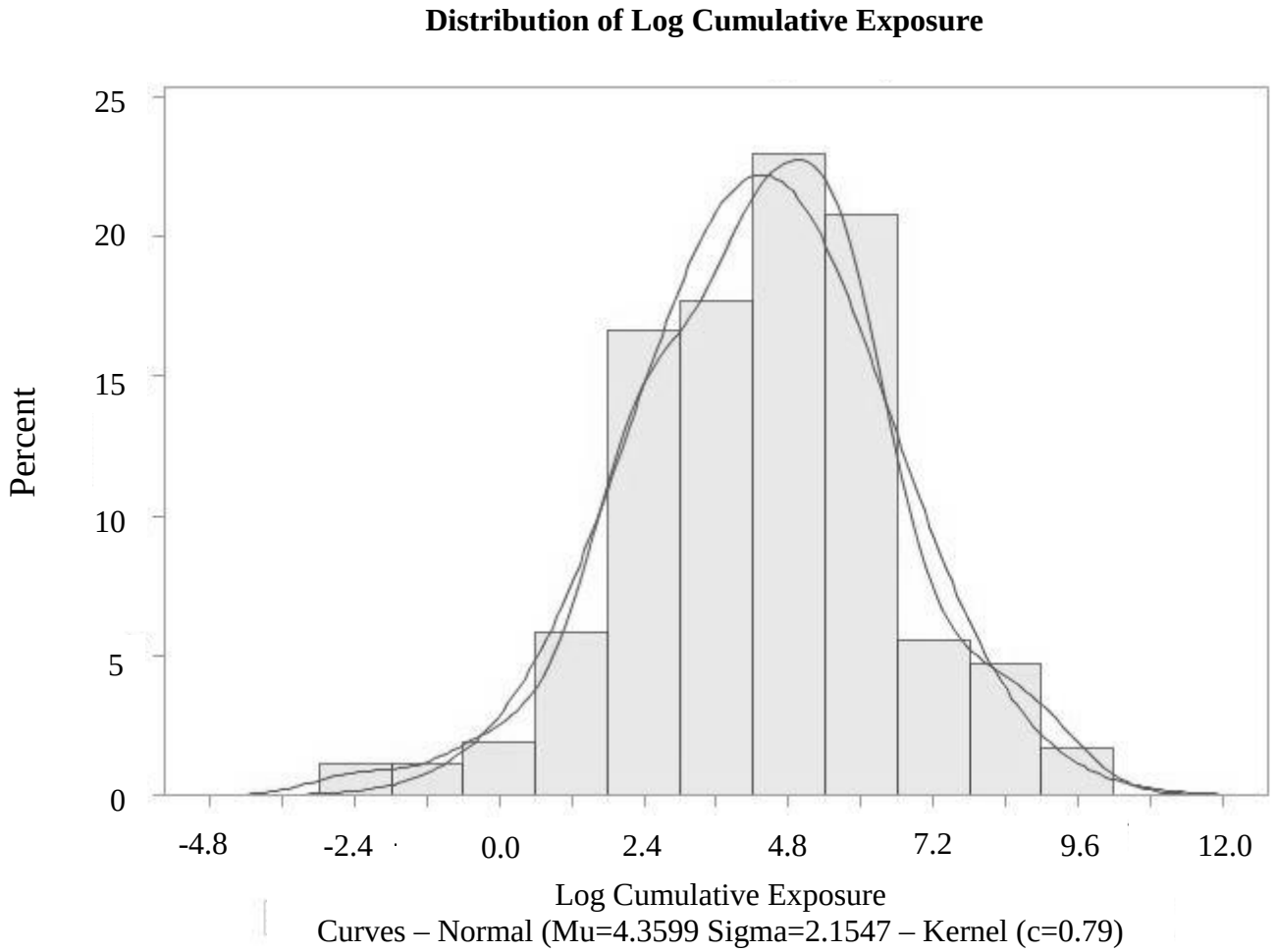
a. Distribution of Cumulative Exposure

Figure 4. Distribution of Cumulative Exposure



b. Distribution of Log Cumulative Exposure

Figure 5. Distribution of Log Cumulative Exposure



Appendix 2. Wilcoxon Two Sample Test for Difference of Exposure in Plant 1

Table 26. Wilcoxon Two Sample Test for Difference of Exposure in Plant 1

Exposure*	Average Level			P Value		
	CBD N=28	BeS N=23	Control N=133	CBD vs. BeS	CBD vs. Control	BeS vs. Control
Duration						
In Years	8.73	5.22	9.20	0.3203	0.4886	0.0202
Total Exposure						
Cumulative	118.72	77.66	162.39	0.2764	0.3066	0.0256
Log Cumulative	3.86	3.25	4.20	0.2764	0.3066	0.0256
Mean	1.42	1.53	1.69	0.7548	0.3872	0.1910
Peak	2.63	2.90	3.71	0.9773	0.2826	0.0407
Type of Exposures						
Chemical Mix						
Cumulative	20.93	9.51	50.20	0.5689	0.0663	0.0030**
Mean	0.28	0.37	0.48	0.9059	0.0698	0.0567
Peak	1.09	1.09	1.68	0.8987	0.2270	0.0127**
Physical Mix						
Cumulative	20.59	7.65	45.72	0.6313	0.0531	0.0024**
Mean	0.25	0.32	0.46	0.9652	0.0451	0.0327
Peak	0.99	1.09	1.64	0.9315	0.1305	0.0181

*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure
Comparison was obtained using Kruskal Wallis non parametric test
**) with Bonferroni correction for multiple comparison in Wilcoxon two sample test, difference of mean is significant at < 0.0167 level

Appendix 3. Wilcoxon Two Sample Test for Difference of Exposure in All Subjects

Table 27. Wilcoxon Two Sample Test for Difference of Exposure in All Subjects

Exposure*	Exposure Level			P Value		
	CBD N=61	BeS N=41	Control N=259	CBD vs. BeS	CBD vs. Control	BeS vs. Control
Duration (Years)	8.89	5.49	12.19	0.1017	0.0922	0.0009**
Cumulative	291.40	145.54	743.99	0.2932	0.1636	0.0187
Log cumexp	4.13	3.60	4.53	0.2932	0.1636	0.0187
Chemical Mix						
Cumulative	199.76	69.54	547.65	0.2904	0.1111	0.0375
Physical mix						
Cumulative	49.81	31.29	198.92	0.3678	0.0193	0.0020**
Mean	0.97	2.79	1.84	0.7452	0.1188	0.0209
Peak	4.77	5.65	8.06	0.6463	0.0479	0.0071**

*) Total exposures and different type of exposures were each measured in µg-year/m^3 unit for cumulative exposure, in µg/m^3 for mean exposure, and in µg/m^3 for peak exposure
Comparison was obtained using Kruskal Wallis non parametric test
**) with Bonferroni correction for multiple comparison in Wilcoxon two sample test, difference of mean is significant at < 0.0167 level

Appendix 4. Proportion of Glutamine 69 and Glutamine 71 Positive Individuals by Disease State and the Importance of Glutamine 71 in the Absence of Glutamine 69

a. Proportion of Glutamine 69 and Glutamine 71 Positive Individuals by Disease State

Table 28. Proportion of Glutamine 69 and Glutamine 71 Positive Individuals by Disease State

Outcome (N, %)	Glutamine 69 and Glutamine 71			
	Negative	Either	Both	Total
CBD	0 (0)	50 (81.97)	11 (18.03)	61
BeS	0 (0)	30 (73.17)	11 (26.83)	41
Control	128 (49.4)	101 (39.0)	30 (11.6)	259
Total	128 (35.5_	181 (50.2)	52 (14.3)	361

b. The Importance of Glutamine 71 in the Absence of Glutamine 69

Testing the hypothesis that Glutamine 71 influences the development of CBD and BeS in the absence of Glutamine 69, the convergence is not reached for CBD and control and CBD and BeS.

For BeS and Control

Table 29. Effect of Glutamine 71 in the Absence of Glutamine 69

Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Glutamine 71	1	20.5517	10261.2	0.0000	0.9984

Appendix 5. Univariable Conditional Logistic Regression for Development of CBD

Table 30. Univariable Conditional Logistic Regression for Development of CBD						
Variable	**LR test**	**Score T**	**Wald test**	**OR**	**95%CI**	
					L	U
Smoking (Current vs. never)	0.9794	0.9792	0.9792	1.022	0.395	2.645
Ex vs. never				0.966	0.484	1.927
Unknown vs. never				0.834	0.198	3.525
Race (Black vs. white)	0.2813	0.4733	0.9999	<0.001	<0.001	>999.9
No answer vs. white				<0.001	<0.001	>999.9
Age	0.4609	0.4933	0.4974	1.086	0.884	1.334
Glu69	<0.001	<0.0001	<0.0001	27.030	6.491	112.561
Ser13	0.4203	0.4224	0.4233	1.180	0.647	2.151
Tyr26	0.4424	0.4462	0.4494	0.705	0.320	1.550
His32	0.4781	0.4774	0.4780	1.171	0.652	2.105
Arg74	0.6760	0.6782	0.6764	0.803	0.362	1.783
Ser11	0.1628	0.1689	0.1718	1.433	0.750	2.738
Phe47	0.9857	0.9857	0.9857	0.915	0.461	1.816
Asp37	0.4938	0.4965	0.4971	0.760	0.404	1.427
Glu71	0.5515	0.5477	0.5483	1.281	0.663	2.476
Homozygosity (heterozygous)	<0.0001	<0.0001	<0.0001	26.519	6.325	111.193
Homozygous				30.267	5.809	157.687
Peak exposure	0.1655	0.3604	0.3118	0.994	0.983	1.005
Cum exposure	0.0377	0.0947	0.1083	1.000	0.999	1.000
Log CEMEX	0.3299	0.3265	0.3286	0.930	0.804	1.076
Mean Exposure	0.9514	0.9512	0.9513	1.000	0.983	1.018
Cum Chemical mix	0.0581	0.1455	0.1385	1.000	0.999	1.000
Mean chemical mix	0.8789	0.8796	0.8796	0.999	0.982	1.016
Peak Chemical mix	0.1609	0.3650	0.2983	0.994	0.982	1.005
Cum Chemical N	0.4356	0.4739	0.4944	1.000	0.998	1.001
Mean Chemical NS	0.6883	0.6814	0.6851	1.019	0.930	1.118
Peak Chemical NS	0.4544	0.4617	0.4738	0.987	0.954	1.021
Cum Chemical sol	0.0603	0.2023	0.3101	0.996	0.988	1.004
Mean Chemical sol	0.2428	0.3049	0.3481	0.766	0.440	1.336
Peak Chemical sol	0.2443	0.2873	0.3260	0.989	0.968	1.011
Cum physical mix	0.0074	0.1070	0.0562	0.998	0.996	1.000
Mean physical mix	0.2550	0.3256	0.3815	0.973	0.916	1.034
Peak physical mix	0.1285	0.1493	0.1641	0.987	0.969	1.005
Cum physical dust	0.3337	0.3660	0.3822	1.000	0.999	1.000
Mean physical dust	0.8061	0.8078	0.8081	0.997	0.973	1.022
Peak physical dust	0.0505	0.3196	0.1421	0.987	0.969	1.004
Cum physical fume	0.2948	0.4224	0.4581	1.000	0.999	1.000
Mean physical fume	0.3687	0.3338	0.3742	1.012	0.985	1.040
Peak physical fume	0.6675	0.6712	0.6721	0.997	0.984	1.011

Appendix 6. Univariable Conditional Logistic Regression for Development of BeS

Table 31. Univariable Conditional Logistic Regression for Development of BeS

Variable	LR test	Score test	Wald test	OR	95%CI	
					L	U
Smoking (Current vs. never)	0.3305	0.3806	0.4237	0.698	0.213	2.282
Ex vs. never				0.626	0.270	1.450
Unknown vs. never				0.204	0.024	1.711
Race (Black vs. white)	0.2222	0.1969	0.8868	2.000	0.125	31.97
No answer vs. white				1.060	0.926	1.213
Age	0.3161	0.3678	0.3994			
Glu69	<0.0001	<0.0001	<0.0001	6.315	2.539	15.71
Ser13	0.5260	0.5290	0.5297	1.292	0.581	2.872
Tyr26	0.5764	0.5831	0.5842	0.746	0.260	2.134
His32	0.8622	0.8623	0.8623	0.942	0.477	1.860
Arg74	0.5764	0.5831	0.5842	0.746	0.260	2.134
Ser11	0.3651	0.3704	0.3722	1.490	0.620	3.579
Phe47	0.9443	0.9443	0.9443	1.030	0.452	2.346
Asp37	0.3652	0.3631	0.3645	1.387	0.684	2.811
Glu71	0.0316	0.0297	0.0331	2.231	1.067	4.666
Homozygous (heterozygous)	<0.0001	<0.0001	<0.0001	5.614	2.153	14.63
Homozygous				8.987	2.600	31.06
Peak exposure	0.2033	0.3175	0.3810	0.993	0.978	1.008
Cum exposure	0.0070	0.0766	0.1068	0.999	0.997	1.000
Log Cum exposure	0.0081	0.0086	0.0118	0.756	0.608	0.940
Mean Exposure	0.6608	0.6560	0.6577	1.006	0.980	1.033
Cum Chemical mix	0.0251	0.1198	0.2658	0.999	0.997	1.001
Mean chemical mix	0.4825	0.4782	0.4847	1.009	0.983	1.036
Peak Chemical mix	0.9969	0.9969	0.9699	0.994	0.979	1.008
Cum Chemical NS	0.1577	0.2529	0.2493	0.999	0.996	1.001
Mean Chemical NS	0.8734	0.8759	0.8762	0.992	0.891	1.104
Peak Chemical NS	0.6766	0.6818	0.6835	0.951	0.871	1.037
Cum Chemical sol	0.0510	0.2907	0.2621	0.985	0.960	1.011
Mean Chemical sol	0.5014	0.5862	0.6954	0.961	0.787	1.173
Peak Chemical sol	0.0145	0.2530	0.1379	0.949	0.730	1.233
Cum physical mix	0.0623	0.2267	0.2750	0.998	0.993	1.002
Mean physical mix	0.2184	0.2094	0.3098	1.034	0.969	1.103
Peak physical mix	0.2140	0.3235	0.3854	1.000	0.979	1.022
Cum physical dust	0.1513	0.2147	0.2570	0.999	0.998	1.001
Mean physical dust	0.8720	0.8720	0.8721	1.002	0.977	1.028
Peak physical dust	0.1749	0.2792	0.2551	0.996	0.978	1.015
Cum physical fume	0.0139	0.1502	0.2490	0.994	0.984	1.004
Mean physical fume	0.0633	0.1473	0.2446	0.801	0.552	1.164
Peak physical fume	0.4268	0.5432	0.6931	0.945	0.877	1.018

Appendix 7. Univariable Unconditional Logistic Regression for Progression of BeS to CBD

Table 32. Univariable Unconditional Logistic Regression Comparing CBD and BeS			
Variable	**LR test**	**Score test**	**Wald test**
Smoking status	0.7535	0.7606	0.7662
Race	0.1615	0.2274	0.9997
Age	0.7632	0.7632	0.7632
Glu69	0.0562	0.0542	0.0625
Ser13	0.2185	0.2220	0.2242
Tyr26	0.5216	0.5257	0.5272
His32	0.5663	0.5662	0.5664
Arg74	0.5216	0.5257	0.5272
Ser11	0.4568	0.4597	0.4607
Phe47	0.8544	0.8542	0.8542
Asp37	0.0618	0.0612	0.0632
Glu71yn	0.0257	0.0253	0.0270
Homozygous	0.0944	0.0922	0.1043
Peak exposure	0.5646	0.5696	0.5720
Cumulative exposure	0.1573	0.2038	0.2524
Mean exposure	0.8658	0.8664	0.8665
Cumulative chemical mix	0.1263	0.1987	0.2558
Mean Chemical mix	0.9942	0.9942	0.9942
Peak chemical mix	0.8231	0.8222	0.8225
Cumulative chemical non soluble	0.7046	0.7091	0.7114
Mean Chemical non soluble	0.9734	0.9735	0.9736
Peak chemical non soluble	0.7707	0.7696	0.7700
Cumulative chemical soluble	0.7261	0.7339	0.7396
Mean Chemical soluble	0.4355	0.4311	0.4410
Peak chemical soluble	0.0761	0.1351	0.2550
Cumulative physical mix	0.3953	0.4138	0.4281
Mean physical mix	0.2846	0.2929	0.3664
Peak physical mix	0.6679	0.6709	0.6721
Cumulative physical dust	0.4720	0.4902	0.5010
Mean physical dust	0.5921	0.5874	0.5900
Peak physical dust	0.7404	0.7468	0.7510
Cumulative physical fume	0.0716	0.1558	0.3349
Mean physical fume	0.2128	0.3680	0.5437
Peak physical fume	0.0761	0.1351	0.2550

Appendix 8. Development of CBD and BeS by Exposure Quartiles and Genetics

a. Descriptive Analysis of Log Total Exposure

Table 33. Descriptive Analysis of Log Total Exposure

Exposure	Mean	25th Pctl	Median	75th Pctl	Minimum	Maximum
Logcumexp	4.36	2.78	4.44	5.76	-2.63	9.92
Logmeanexp	0.62	-0.23	0.46	1.34	-3.82	4.88
Logpeakexp	1.39	0.30	1.20	2.08	-3.61	7.57

b. Multivariable Conditional Logistic Regression Using Quartiles of Log Exposure

Table 34. Multivariable Conditional Logistic Regression Using Quartiles of Log Exposure

Variable	Coefficient	Standard Error	OR	95% Confidence Interval		P Value
Development of CBD						
Glutamine 69	3.2800	0.7297	26.58	6.36	111.06	<.0001
logcumexpcat 1 vs. 0	-0.0817	0.5467	0.92	0.32	2.69	0.8813
logcumexpcat 2 vs. 0	0.1355	0.5218	1.15	0.41	3.18	0.7951
logcumexpcat 3 vs. 0	-0.2983	0.5434	0.74	0.26	2.15	0.5830
Development of BeS						
Glutamine 69	2.0587	0.5369	7.84	2.74	22.44	0.0001
Glutamine 71	0.9504	0.4500	2.59	1.07	6.25	0.0347
logcumexpcat 1 vs. 0	-0.4438	0.7317	0.64	0.15	2.69	0.5442
logcumexpcat 2 vs. 0	-0.1960	0.6002	0.82	0.25	2.67	0.7440
logcumexpcat 3 vs. 0	-1.2120	0.7370	0.30	0.07	1.26	0.1001
Progression of CBD from BeS						
Intercept	0.4927	0.4079				0.2270
Glutamine 71	-0.9724	0.4299	0.38	0.16	0.88	0.0237
logcumexpcat 1 vs. 0	0.4325	0.5523	1.54	0.52	4.55	0.4336
logcumexpcat 2 vs. 0	0.1895	0.5441	1.21	0.42	3.51	0.7276
logcumexpcat 3 vs. 0	0.6339	0.6684	1.89	0.51	6.99	0.3429

Appendix 9. Algorithm of Level of Exposure (Cumulative and Peak) by Median Value

Table 35. Multivariable Conditional Logistic Regression Using Algorithm of Exposure						
Variable	**Coefficient**	**Standard Error**	**OR**	**95% Confidence Interval**		**P Value**
CBD and Control						
Glutamine 69	3.5721	0.7786	35.59	7.74	163.71	<.0001
logcumpeakcat3 1 vs. 0	2.6282	1.7921	13.85	0.41	464.40	0.1425
logcumpeakcat3 2 vs. 0	-0.034	0.6435	0.97	0.27	3.41	0.9576
logcumpeakcat3 3 vs. 0	0.0200	0.8083	1.02	0.21	4.97	0.9802
logcumpeakcat3 4 vs. 0	0.9753	0.6680	2.65	0.72	9.82	0.1443
logcumpeakcat3 5 vs. 0	-0.756	0.7425	0.47	0.11	2.01	0.3086
logcumpeakcat3 6 vs. 0	0.4616	0.9357	1.59	0.25	9.93	0.6218
logcumpeakcat3 7 vs. 0	-0.509	0.6491	0.60	0.17	2.15	0.4332
BeS and Control						
Glutamine 69	2.2418	0.5910	9.41	2.96	29.97	0.0001
Glutamine 71	1.0497	0.4754	2.86	1.13	7.25	0.0272
logcumpeakcat3 1 vs. 0	0.4400	1.2889	1.55	0.12	19.42	0.7328
logcumpeakcat3 2 vs. 0	0.3501	0.9119	1.42	0.24	8.48	0.7011
logcumpeakcat3 3 vs. 0	-0.843	0.9878	0.43	0.06	2.98	0.3934
logcumpeakcat3 4 vs. 0	-0.844	0.9571	0.43	0.07	2.81	0.3776
logcumpeakcat3 5 vs. 0	0.3887	0.7444	1.48	0.34	6.35	0.6016
logcumpeakcat3 6 vs. 0	-0.686	1.5123	0.50	0.03	9.76	0.6501
logcumpeakcat3 7 vs. 0	-1.115	0.8952	0.33	0.06	1.89	0.2126
CBD and BeS						
Intercept	0.5943	0.4454				0.1821
Glutamine 71	-1.131	0.4572	0.32	0.13	0.79	0.0134
logcumpeakcat3 1 vs. 0	-0.323	1.1093	0.72	0.08	6.37	0.7711
logcumpeakcat3 2 vs. 0	0.4588	0.6421	1.58	0.45	5.57	0.4749
logcumpeakcat3 3 vs. 0	0.2377	0.8355	1.27	0.25	6.52	0.7761
logcumpeakcat3 4 vs. 0	1.1500	0.8065	3.16	0.65	15.34	0.1539
logcumpeakcat3 5 vs. 0	-0.518	0.6636	0.60	0.16	2.19	0.4346
logcumpeakcat3 6 vs. 0	0.8427	1.2555	2.32	0.20	27.21	0.5021
logcumpeakcat3 7 vs. 0	0.5171	0.7539	1.68	0.38	7.35	0.4927

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