

ORIGINAL ARTICLE

Genetic susceptibility to beryllium: a case–referent study of men and women of working age with sarcoidosis or other chronic lung disease

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ABSTRACT

Objective The study was designed to investigate whether beryllium exposure was related to illness diagnosed as sarcoidosis. Chronic beryllium disease (CBD) and sarcoidosis are clinically and pathologically indistinguishable, with only the presence of beryllium-specific T-lymphocytes identifying CBD. Testing for such cells is not feasible in community studies of sarcoidosis but a second characteristic of CBD, its much greater incidence in those with a glutamic acid residue at position 69 of the *HLA-DPB1* gene (Glu69), provides an alternative approach to answering this question.

Methods Cases of sarcoidosis aged 18–60 years diagnosed in Alberta, Canada, from 1999 to 2005 were approached through their specialist physician, together with age-matched and sex-matched referents with other chronic lung disease. Referents were grouped into chronic obstructive pulmonary disease (COPD), asthma and other lung disease. Participants completed a telephone questionnaire, including industry-specific questionnaires. DNA was extracted from mailed-in mouthwash samples and genotyped for Glu69. Duration of employment in types of work with independently documented beryllium exposure was calculated.

Results DNA was extracted for 655 cases (270 Glu69 positive) and 1382 referents (561 positive). No increase in sarcoidosis was seen with either Glu69 or beryllium exposure (none, <10, ≥10 years) as main effects: longer duration in possible beryllium jobs was related to COPD. In Glu69 positive men with exposure ≥10 years, the trend towards increasing rate of COPD was reversed, and a significant interaction of duration of exposure and Glu69 was detected (OR=4.51 95% CI 1.17 to 17.48).

Conclusions The gene–environment interaction supports the hypothesis that some cases diagnosed as sarcoidosis result from occupational beryllium exposure.

INTRODUCTION

Sarcoidosis is a chronic granulomatous disease with no known cause, thought to represent an exaggerated immunological response to as yet unknown antigens.¹ Both environmental and genetic factors are associated with sarcoidosis.^{2,3} Suggested occupational risk factors include metal working industries (from an ecological study in Switzerland)⁴ and exposure to insecticides.² No clear gene–environmental interaction has been reported for sarcoidosis, contrasting strongly with the highly elevated risk of chronic beryllium disease (CBD) in those with a

What this paper adds

- Sarcoidosis is a chronic granulomatous disease with no known cause but environmental and genetic factors are suspected. Chronic beryllium disease (CBD) is clinically and pathologically indistinguishable from sarcoidosis but its diagnosis requires a positive test for sensitisation to beryllium.
- In those occupationally exposed to beryllium, workers with a glutamic acid in codon 69 (Glu69) in the *HLA-DPB1* gene are at much increased risk of developing CBD.
- If patients (untested for beryllium sensitisation) with possible beryllium exposure at work are at a greater risk of sarcoidosis with the Glu69 variant, this would imply that beryllium contributes to some disease diagnosed as sarcoidosis.
- The finding in the case–referent study reported here of significant gene–environment interaction (Glu69×possible beryllium exposure for 10 years or more) supports the hypothesis that some cases of sarcoidosis result from beryllium exposure in this population.
- The result supports the investigation of further gene–environment interactions in sarcoidosis and the use of Mendelian randomisation approaches in occupational epidemiology.

glutamic acid residue coded for in codon 69 (Glu69) in the *HLA-DPB1* gene.⁵ Given that CBD is clinically and pathologically indistinguishable from sarcoidosis, it is reasonable to hypothesise that genetic variation in the presentation of one or more (as yet unknown) antigens may be causal also in sarcoidosis. The present study was designed to investigate this possibility. This first report addresses a focussed question within this broader thesis: to what extent do cases diagnosed as sarcoidosis result from occupational exposure to beryllium? If CBD is being misdiagnosed as sarcoidosis, a sentinel case reflecting an unrecognised risk in a pool of workers may be missed, together with opportunities for prevention.

Beryllium is a low-molecular-weight metal with properties (light weight, high tensile strength, corrosion resistance) that make it highly attractive,



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either as the metal itself or as an alloy or compound, in a wide range of uses including (as a beryllium-copper bronze) non-spark tools and electrical switch parts and as a metal in nuclear reactors. The health effects of exposure have largely been investigated in the USA in the nuclear industry.⁵ Importantly, no clear dose–response relationship has been found between exposure and CBD, although there is an assumption that sensitisation to beryllium depends on intensity of exposure and perhaps also duration and/or latency.⁶ Although there have been reports of sensitisation to beryllium after exposures of less than 1 year,⁷ progression from sensitisation to CBD may take many years.⁸ In the absence of clear dose–response data (probably reflecting the immunological basis of the disease) and the decision (now disputed) that beryllium is carcinogenic to humans,^{9–10} there is a great deal of uncertainty about a ‘safe’ level of beryllium exposure.¹¹ At present the US Occupational Safety and Health Administration (OSHA) permissible exposure limit is 2 µg/m³, the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit 0.5 µg/m³ and the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value 0.05 µg/m³.

CBD has two characteristics potentially important for investigating its misdiagnosis as sarcoidosis. First, diagnosis of CBD requires pathological changes and evidence of sensitisation to beryllium on a lymphocyte proliferation test (BeLPT). This test has been used clinically to identify patients with CBD misdiagnosed as sarcoidosis, in individual patients¹² and as part of a prospective case series.¹³ The BeLPT, however, is somewhat exacting and costly, and available only from specialist centres. The second significant feature of CBD is the substantial (perhaps 10-fold) increase in risk in those with the Glu69 susceptibility marker, an important (but rare) example of gene–environment interaction at the workplace.^{7–14} In contrast to the exacting handling requirement of blood (or bronchoalveolar lavage fluid) samples for the BeLPT, identification of the Glu69 genetic marker can be made using mailed-in mouthwash or buccal swab samples.¹⁵ This known, and easily identifiable, marker of genetic susceptibility to CBD made possible this study to investigate the misdiagnosis of sarcoidosis: if the genetic susceptibility marker specific to beryllium is more frequent in workers (or subsets of workers) with sarcoidosis, this would support the conclusion (at the population level) that sarcoidosis was, at least in part, attributable to beryllium. Such an approach, sometimes labelled as Mendelian randomisation, has been used to good effect in earlier studies.^{16–17}

The estimation of occupational exposure to beryllium for this study was only possible because of a published analysis of reported beryllium content of dust samples collected during OSHA inspections during 1979–1996 which identified 38 four-digit Standard Industrial Classification (SIC) codes, together with ‘at risk’ tasks in which beryllium had been found in two or more samples at or above the NIOSH recommended standard of 0.5 µg/m³.¹⁸ This report of industries, together with typically exposed jobs within each, reduced the seemingly impossible task of assessing occupational beryllium exposure over many years in widely diverse occupations to one of collecting comprehensive data on specific tasks within previously identified industries.

METHODS

Cases and referents

A case–referent study was designed of patients living in Alberta, Canada from 1 January 1995 to the time of first diagnosis of sarcoidosis (International Classification of Diseases (ICD)-9 code 135; cases) or other chronic respiratory diseases (ICD-9

codes 466, 490–518; referents). Patients with sarcoidosis diagnosed in Alberta aged 18–60 years from 1 January 1999 to 31 December 2005 were eligible as cases if they had evidence of pulmonary involvement. Only new diagnoses of sarcoidosis were included: a case was considered a new diagnosis if a specialist physician recorded a diagnosis of ICD-9 code 135 in the period 1999–2005 and no diagnosis of ICD-9 135 had been recorded by any Alberta physician for this patient in the period 1995–1998. The diagnostic code was not required to be the main (or first) code recorded for that patient visit, and only the code that made the patient eligible for the study was recorded. Up to five potential referents were chosen for each case, individually matched on sex, age (± 2 years) and date of clinic visit (after the case diagnosis but within 12 months). Referents were chosen with ICD-9 codes that indicated lung disease that was chronic or had the potential to become chronic: those with an ICD-9 code of 135 since 1995 were ineligible. Hypersensitivity pneumonitis (ICD-9 code 495) was excluded because of diagnostic overlap with sarcoidosis. A referent was only used once: if there was a match to more than one case, alternative referents were used. Patients with an eligible diagnosis (case or referent) but who were found, on chart review, to have an additional diagnosis of carcinoma or cystic fibrosis were omitted, as were those known to have died.

Identification and recruitment of cases and referents

In Alberta, healthcare is free at the point of delivery and registration with the Alberta Health Care Insurance Plan is mandatory for all residents of the province. Physicians are paid through the scheme and complete administrative billing records that include up to three ICD-9 diagnoses for patient services. Specialist physicians (who have completed a specialist residency recognised by the Royal College of Physicians and Surgeons of Canada) are paid on a different schedule than family physicians and general practitioners. Access to the billing codes on the administrative records was possible only with the agreement of the physician, specifically elicited for this study.

There was no validated algorithm for the diagnosis of sarcoidosis through administrative records and it was decided to maximise the likelihood that a recorded diagnosis of sarcoidosis was truly sarcoidosis by restricting physician recruitment to internal medicine and pulmonary specialists and to review medical files to identify coding errors. No additional requirement (such as biopsy confirmation) was imposed, although the chart review included confirmation of pulmonary involvement. Specialist physicians were approached by the research team to ask for their agreement that the Alberta Ministry of Health (AMH) might identify all eligible cases for which they had recorded a diagnostic code of 135 and also, for the cases identified, up to five eligible referents. The AMH identified cases and referents for all physicians who gave such written consent. The medical files of patients listed were examined to ensure that the condition was consistent with sarcoidosis or, for referents, other chronic lung disease. The physician then signed an information letter asking patients selected to return a signed consent to the study team. Patients who consented were sent a recruitment package.

The preinterview package contained three forms to be completed before the interview as an aide memoire for dates of (1) every residence since birth, (2) all occupations since leaving school and (3) employment in any of the 34 industries (online supplementary appendix 1), of which 18 were a somewhat compressed version of the 38 four-digit industry codes previously identified with exposure to beryllium in OSHA samples,^{18–20}

were industries with possible beryllium exposure but not reported to have OSHA beryllium samples and 6 industries employing large numbers within Alberta of interest to the Province. A collection kit for the mouthwash sample was also included in the prequestionnaire package.

Exposure to beryllium

When participants reported that they had been employed in one of the industries listed in online supplementary appendix 1 the interviewer completed an industry-specific questionnaire designed for this study. All industries recorded on the job history were coded to US SIC four-digit codes¹⁹ and in rare instances where an industry-specific questionnaire was indicated but had not been completed, the job description was scrutinised to determine whether a potentially beryllium-exposed job had been carried out. All periods potentially exposed were classified as (1) 'listed': both industry and job/task within that industry were included in the published listing of OSHA samples with beryllium $\geq 0.5 \mu\text{g}/\text{m}^3$; (2) 'assumed listed' periods as a construction labourer with insufficient information about the work site and (3) 'not-listed', periods of employment with possible beryllium exposure not included in the list of positive OSHA results. Total duration to diagnosis was calculated for each of these three categories. Date first working in such a job was also determined to allow examination of latency.

Determination of Glu69 status

DNA was extracted where possible from each mouthwash sample received using methods adapted from Garcia-Closas *et al.*¹⁵ Samples were thawed at room temperature and then centrifuged at 1500g for 15 min. The supernatant was decanted and the cell pellet was resuspended in Tris-EDTA. The samples were centrifuged a second time at 1500g for 15 min and the supernatant again decanted. The samples were then split into two groups on cell pellet volume, less than or greater than 100 μL . For samples with volumes $<100 \mu\text{L}$, the QIAamp DNA Investigator kit (Qiagen) was used with the following modifications: cell pellets were lysed overnight at 55°C in 90 μL buffer ATL (Qiagen) and 10 μL of proteinase K (20 mg/mL). The remaining steps were carried out on the QIAcube (Qiagen) according to the manufacturer's instructions. For samples with volumes $>100 \mu\text{L}$, the QIAamp DNA Mini kit (Qiagen) was used with the following modifications: cell pellets were lysed overnight at 55°C in 180 μL buffer ATL and 20 μL of proteinase K (20 mg/mL). The remaining steps were carried out on the QIAcube (Qiagen) according to the manufacturer's instructions. All DNA samples were eluted in TE and stored at 4°C.

Genotyping for the Glu69 allele (NM_002121.4 p.Lys98Glu) was performed by sequencing exon 2 of the *HLA-DBP1* gene. Genomic DNA was PCR amplified prior to sequencing using the ABI BigDye Terminator Cycle Sequencing kit (V1.1; Life Technologies) and the ABI 3130 Genetic Analyzer (Life Technologies). Primer sequences and reaction conditions are available on request. Sequence subtraction was performed using Mutation Surveyor software (SoftGenetics).

Measurement of potential confounders

Information on confounders for sarcoidosis or other chronic lung disease was collected using the questionnaire. This included 'cigarette years' (usual number of cigarettes/day while smoking \times total years smoked to date of diagnosis) for those ever smoking as much as 1 cigarette/day for 12 months, ethnicity self-reported as black, education (completed high school or equivalent), together with sex and age at diagnosis. Sarcoidosis

in a first-degree blood relative (parent, sibling, child), as participant by the patient, was also recorded.

Statistical methods

Distribution of participants was examined by diagnosis (sarcoidosis) and three subgroups of referents (asthma (ICD-9 code 493), chronic obstructive pulmonary disease (COPD; ICD-9 codes 490–492, 496) and other (ICD-9 codes 466, 494, 501–518)). Duration of time spent in each of the 34 industries of interest was examined and those with 50 or more participants ever employed (within sex) identified for more detailed analysis of diagnosis (sarcoidosis, asthma, COPD, other) by duration of employment in the industry. Total duration of employment in jobs potentially exposed to beryllium was calculated and examined by diagnosis, adjusting for potential confounders. Sarcoidosis was then examined in those with and without a Glu69 marker, stratifying for exposure, and a logistic regression analysis carried out (with sarcoidosis against all referents as the dependent variable), including a gene–exposure interaction, and adjusting for potential confounders. Because a relatively large proportion of participants recruited did not form part of a cluster (ie, a case was recruited but not any of the referents or referents were recruited but the case was not) an unconditional regression was used. Sensitivity analyses were carried out to examine the dependence of the interaction effect on the definition of exposure. These included varying the duration of exposure required (with 10 years or more as the initial breakpoint), stratifying for latency and, in separate analyses, excluding periods in 'assumed listed' employment and adding periods in 'not listed' work.

Power

Power calculations are given in online supplementary appendix 2. Briefly, if the prevalence of biologically significant exposure to beryllium was found to be 5% in referents, a gene–environment effect producing a threefold increase in risk could be found with 80% power using 617 cases and 1234 referents assuming (1) Glu69 marker present in 30% of the population, (2) the exposure in the absence of Glu69 confers only a 10% excess odds of the disease and (3) Glu69 in the absence of exposure does not confer risk.

RESULTS

Participation

Six hundred and fifty-two respiratory and internal medicine specialists were identified of whom 467 were approached. Those omitted were either retired or had a practice unlikely to see adult patients with sarcoidosis. Of the 467 physicians approached 254 agreed. The most frequent reasons given by those who refused were that they had not seen any eligible patient with sarcoidosis. At least one eligible case was found for 110 specialists, with a total of 1826 cases and 5935 referents identified in the administrative records for these physicians. An attempt was made to screen the clinical records of all these patients but this was achieved for only 6480 (1629 cases and 5211 referents). Review of the charts eliminated 238 cases and their matched referents (N=1129). Of 1392 cases and 4081 referents approached, questionnaires were completed by 684 cases and 1454 referents. In both cases and referents those who participated were older than those who did not, and among the referents, men were less likely to take part.²⁰ Among the 684 cases, 30% (204) had had a confirmatory lung biopsy and a further 20% (140) biopsy of a lymph node or some other

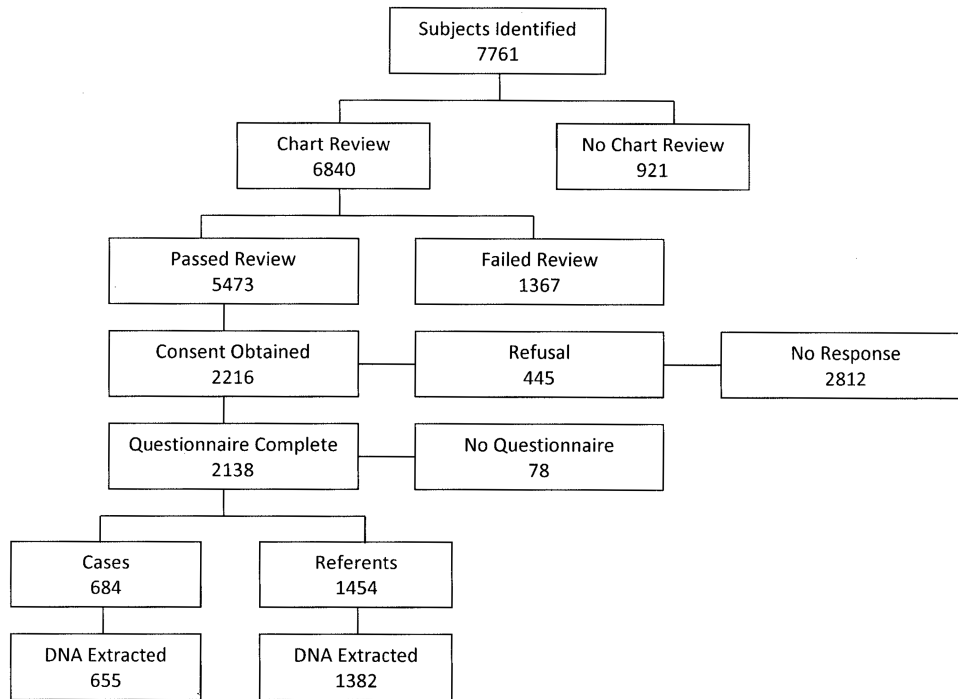


Figure 1 Recruitment flow diagram.

organ.²⁰ Genotyping for Glu69 status was obtained for 2037 (655 cases (378 men) and 1382 referents (692 men); [figure 1](#)).

Description of the sample

Asthma was by far the most frequent referent diagnosis with the only other diagnosis importantly represented being COPD (online supplementary appendix 3). Confounders differed with diagnostic group ([table 1](#)). Those with sarcoidosis were least likely to smoke (but with similar smoking patterns in asthmatics) and had a higher proportion with black ethnicity and self-reported first-degree relatives diagnosed with sarcoidosis. Those with COPD were less likely to have completed high school, more likely to be heavy smokers and had the highest median age. All factors shown in [table 1](#) differed significantly ($p < 0.001$) among the four diagnostic groups.

Relation of diagnosis to exposures at work

The distribution of participants who had worked in each of the 34 industries of interest was examined and the relation to diagnosis explored for those industries with 50 or more participants (within sex) having any period of employment (other than exclusively office work). For men there were eight such industries (online supplementary appendix 4); for women there were only three (oil and gas, agriculture and healthcare). For men in some industries (eg, construction) there was a clear increase in COPD with increasing duration of employment (online supplementary appendix 4) but the risk of sarcoidosis increased with duration of employment only in healthcare, where the unadjusted OR for 10 years or greater employment was 2.60 (95% CI 1.04 to 6.53). This reduced to 1.87 (95% CI 0.71 to 1.95) when adjusted for confounders. For women, asthma, but not sarcoidosis, tended to increase with years of work in

Table 1 Description of the sample

	Diagnosis											
	Sarcoidosis		Asthma		COPD		Other		All referents		Total	
	N	Per cent	N	Per cent	N	Per cent	N	Per cent	N	Per cent	N	Per cent
Male	391	57.2	386	46.1	190	52.9	148	57.6	724	49.8	1115	52.2
Completed high school	548	80.1	663	79.1	198	55.2	181	70.4	1042	71.9	1590	74.4
Black ethnicity	15	2.2	3	0.4	2	0.6	0	0.0	5	0.3	20	0.9
First-degree relative with sarcoidosis	36	5.3	7	0.8	2	0.6	4	1.6	13	0.9	49	2.3
Never smoked*	401	58.8	469	56.0	64	18.0	111	43.5	644	44.5	1045	49.1
1–250 cigarette year	185	27.1	200	23.9	62	17.5	48	18.8	310	21.4	495	23.2
250<500 cigarettes year	47	6.9	92	11.0	72	20.3	42	16.5	206	14.2	253	11.9
≥500 cigarettes year	49	7.2	77	9.2	157	44.2	54	21.2	288	19.9	337	15.8
Median age	44.3		44.0		51.4		50.6		47.1		46.2	
N	684	100.0	838	100.0	359	100.0	257	100.0	1454	100.0	2138	100.0

*Eight smokers had missing information on quantity or duration. COPD, chronic obstructive pulmonary disease.

Table 2 Estimated exposure to beryllium dust by diagnosis and sex

Diagnosis	Men						Women					
	Beryllium dust exposure						Beryllium dust exposure					
	None		<10 years		≥10 years		None		<10 years		≥10 years	
N	Per cent	N	Per cent	N	Per cent	N	Per cent	N	Per cent	N	Per cent	
Sarcoidosis	327	36.5	47	29.9	17	27.9	288	28.5	4	36.4	1	100.0
Asthma	324	36.1	47	29.9	15	24.6	446	44.1	6	54.5	0	–
COPD	130	14.5	42	26.8	18	29.5	168	16.6	1	9.1	0	–
Other	116	12.9	21	13.4	11	18.0	109	10.8	0	–	0	–
Total	897	100.0	157	100.0	61	100.0	1011	100.0	11	100.0	1	100.0

COPD, chronic obstructive pulmonary disease.

healthcare: unadjusted OR for employment of 10 years or greater was 1.42 (95% CI 0.98 to 2.06), only slightly reduced to 1.39 (95% CI 0.94 to 2.04) with allowance for confounding.

The distribution of exposure to beryllium (derived from 'listed' or 'assumed listed' employment) is shown by sex and diagnosis in table 2. Only 1.2% of women (12/1023) had ever been in an occupation with possible exposure to beryllium, but in these there was perhaps some trend, with sarcoidosis being seen more in those with some exposure to beryllium dust. Among men nearly 20% (218/1115) had been employed at some point in a job potentially exposed, though only 61 (5.5%) for 10 years or more. Without consideration of genotype, years of exposure to possible beryllium in dust was associated with COPD rather than sarcoidosis (only 14.5% having COPD in those with no beryllium exposure but 29.5% in those exposed for 10 years or more). In a logistic regression analysis, with a diagnosis of COPD as the outcome, the OR for employment in (dusty) jobs with possible beryllium exposure >0 to <10 years was 1.99 (95% CI 1.26 to 3.14) and for 10 years or greater 2.02 (95% CI 1.04 to 3.92), compared with no exposure, having adjusted for cigarette-years of smoking, black ethnicity,

first degree relative with sarcoidosis, completing high school and age at diagnosis.

The proportion of cases and referents with one or more Glu69 positive markers is shown by sex in online supplementary appendix 5: no relation was seen overall between the genetic susceptibility marker and diagnosis ($\chi^2=2.95$; $df=3$; $p=0.4$). Table 3 gives the relation between genotype, beryllium exposure and diagnosis in the 1070 men genotyped: very little influence of Glu69 is seen in those estimated not to be exposed to beryllium ($\chi^2=0.78$; $df=3$; $p=0.9$) or those exposed for <10 years ($\chi^2=4.41$; $df=3$; $p=0.2$) but the distribution of cases with exposure ≥10 years is unlikely to be due to chance ($\chi^2=9.08$; $df=3$; $p=0.03$). Among the 58 men exposed for at least 10 years, half were Glu69 positive and these had a higher rate of sarcoidosis (44.8%) than those with similar estimated exposure but Glu69 negative (13.8%), whose diagnosis was most likely to be COPD. In a logistic regression analysis with a diagnosis of sarcoidosis as the outcome and main effects for genotype, exposure to beryllium (10 years or more) and with allowance for smoking (cigarette-years), completing high school, first degree relative with sarcoidosis, age at diagnosis and black ethnicity, the interaction term for exposure 10 years or longer together with a positive Glu69 marker was significantly elevated (OR=4.51 95% CI 1.16 to 17.48; table 4). Within the Glu69 negative group the proportion of cases of COPD increased steadily with years of exposure to dust possibly containing beryllium (from 14% to 38%) but this effect was importantly modified towards sarcoidosis in the presence of the Glu69 marker (table 3).

Among the 211 men genotyped and exposed to beryllium dust, a majority (126:59.7%) was first exposed more than 20 years before diagnosis with only 18 first exposed <10 years before. When those with latency from first exposure of <10 years were excluded the size of the OR of interaction changed little (increasing to 4.54). Omitting those with <15 years latency increased the OR of interaction to 5.95 (95% CI 1.31 to 27.05). In those with latency ≥20 years, the point estimate of OR increased further, to 11.23 (95% CI 1.17 to 108.18), although with a very wide CI. Other sensitivity analyses, (1) excluding 'assumed listed' exposures, (2) including non-listed exposures or (3) changing the cut off from 10 to 5 years or 15 years, resulted in somewhat smaller (but always positive) estimates of the interaction term (data not shown). No similar interaction with genotype was found for any of the individual industries included in online supplementary appendix 4.

DISCUSSION

The analysis reported here describes the relation between duration of employment in certain industries and diagnosis of

Table 3 Diagnosis by genotype stratified by beryllium exposure (men)

Beryllium exposure	Genotype (Glu69)					
	Negative		Positive		Total	
	N	Per cent	N	Per cent	N	Per cent
None						
Sarcoidosis	195	37.3	120	35.7	315	36.7
Asthma	185	35.4	128	38.1	313	36.4
COPD	73	14.0	47	14.0	120	14.0
Other	70	13.4	41	12.2	111	12.9
Total	523	100.0	336	100.0	859	100.0
<10 years						
Sarcoidosis	30	34.1	16	24.6	46	30.1
Asthma	22	25.0	24	36.9	46	30.1
COPD	22	25.0	19	29.2	41	26.8
Other	14	15.9	6	9.2	20	13.1
Total	88	100.0	65	100.0	153	100.0
≥10 years						
Sarcoidosis	4	13.8	13	44.8	17	29.3
Asthma	7	24.1	8	27.6	15	25.9
COPD	11	37.9	6	20.7	17	29.3
Other	7	24.1	2	6.9	9	15.5
Total	29	100.0	29	100.0	58	100.0

COPD, chronic obstructive pulmonary disease.

Table 4 Logistic regression analysis of sarcoidosis in men typed on Glu69 (N=1066)*

	Sarcoidosis		All referents		OR**	95% CI
	N	Per cent	N	Per cent		
Smoking (cigarettes year)						
None	238*	63.1	305	44.3	1	–
1<250	85	22.5	137	19.9	0.76	0.55 to 1.07
250<500	28	7.4	98	14.2	0.42	0.26 to 0.67
≥500	26	6.9	149	21.6	0.25	0.15 to 0.41
Ethnicity						
Other	369	97.9	687	99.7	1	–
Black	8	2.1	2	0.3	7.77	1.58 to 38.20
First degree relative with sarcoidosis						
No	354	93.9	685	99.4	1	–
Yes	23	6.1	4	0.6	11.04	3.68 to 33.17
High school completed						
No	71	18.8	206	29.9	1	–
Yes	306	81.2	483	70.1	1.28	0.92 to 1.79
Age at diagnosis	377	–	689	–	0.99	0.98 to 1.00
≥10 years beryllium						
No	360	95.5	648	94.0	1	–
Yes	17	4.5	41	6.0	0.38	0.13 to 1.12
Glu69 positive						
No	228	60.5	409	59.4	1	–
Yes	149	39.5	280	40.6	0.88	0.67 to 1.16
≥10 years beryllium and Glu69 positive						
No	364	96.6	673	97.7	1	–
Yes	13	3.4	16	2.3	4.51	1.16 to 17.48

*Four men with missing smoking quantity.

**Adjusted for all factors in table 4.

chronic lung disease. Specifically it considers the interaction between a genetic marker of susceptibility to beryllium and estimates of potential beryllium exposure at work. It demonstrates such an interaction in men in potentially exposed jobs held over at least 10 years and in doing so is consistent with beryllium being a cause of the disease recorded as sarcoidosis. Clinical confirmation of beryllium in an individual patient would best be carried out by arranging a BeLPT, not easily available in Alberta, but at the population level, the relationship is supported.

This observation, while of considerable interest in demonstrating that such an approach can be informative, does not help with identifying the cause of sarcoidosis in the large majority of cases: the nature of other such gene–environment interactions (with perhaps many alleles specific to many different exposures) remains to be identified. The clear excess of men, who constituted 56.7% of the 1826 sarcoidosis cases of working age in records of participating physicians, is consistent with at least some of the relevant exposures being occupational, but a genome-wide (or at least complete HLA) analysis of alleles in the harvested DNA samples would be needed to make full use of the detailed exposure data to identify further antigens implicated in sarcoidosis.

It is also important to consider the interpretation of negative results from this analysis. First, there was no general increase in risk of sarcoidosis in those with a positive Glu69: the increased risk was found only in those (potentially) exposed to beryllium in dusty jobs. Environmental exposure to beryllium, although detected in air quality monitoring data in Alberta²¹ and in snow-pack near oil sands development,²² appears to be too low to affect risk at the population level: historically, environmental cases of CBD have been reported in the vicinity of large industrial

users and in the family of workers in the USA.^{12 23} Second, even with occupational exposure, the gene–environment interaction was seen only in those with at least 10 years employment in work with the potential for beryllium exposure, and particularly in those with longer latency. While this is a credible pattern, the failure to find an excess of granulomatous pulmonary disease in those with shorter exposure or in Glu69 negative workers should be noted, together with the relatively small numbers in the 10-year plus duration group. If there had not been a clear and biologically plausible prior hypothesis, the significance of the interaction term would have been easily overlooked.

Although the study was designed with referents as ‘all other chronic lung disease’ the selection of cases and referents from the same physician, within the year of first presentation, made it reasonable (although unorthodox) to examine other diagnostic groups within the overall category of chronic lung disease, while recognising that because of the initial design sarcoidosis would appear more frequently than by chance. In this way we considered, for example, the relation of occupation to asthma (in online supplementary appendix 4) or, as reported in more detail here, to COPD. The strong relationship, particularly in those Glu69 negative, between years of exposure to (beryllium containing) dust and COPD was not anticipated. Given that sarcoidosis is consistently found more frequently in non-smokers,¹ and COPD in heavy smokers, this analysis would be particularly susceptible to confounding between smoking and long exposure in dusty (beryllium) jobs. Those with longer exposure in such jobs were indeed more likely to be heavy smokers and residual confounding may in part explain the unexpectedly strong risk of COPD. It is not clear, however, that some such effect could explain the pattern of diagnosis in those with long duration of employment who were Glu69 positive, where sarcoidosis was found more frequently than COPD.

This study has important strengths. First, it is sufficiently large to address the question of gene–environment interaction (at least for relatively common genetic markers and exposures). Second, it has detailed occupational histories, allowing us to compute duration of employment by industry and task. Third, in the present analysis, the estimate of beryllium exposure was performed using an independent exposure assessment based on quantitative measures and so avoiding ‘professional judgment’ as a criterion of likely exposure. While there might be differences in occupational practices between Alberta and the US workplaces sampled by OSHA, these are neither likely to be substantial, nor to result in a falsely positive gene–environment interaction. It is of interest that potential beryllium exposures not reported by Henneberger *et al*¹⁸ did not add to the gene–environment interaction. This might be because beryllium was not, in fact, present (OSHA samples existed but were not positive) or that there were no OSHA samples because the jobs were not dusty. Newman and Newman²⁴ recently suggested that occupational sarcoidosis may result from the presence of an antigen (which here would be beryllium) and contemporaneous dust exposures causing inflammation. The results reported here would be consistent with that hypothesis, with the antigen being presented to the immune system more efficiently by those who were Glu69 positive and inflammation being a consequence of the dusty work environment. If that were the case, beryllium sensitisation might not be a pre-requisite and, if negative, the disease may be correctly diagnosed as sarcoidosis.

Use of a job-exposure-like approach was the only feasible way to assess possible beryllium exposure in this population, spread over a Canadian province that is geographically vast and with many years of potential exposure. Its use, without direct

measurements of exposure, precludes investigating misclassification. The estimate that only 5% of men in this study were exposed for 10 years or more was consistent with our initial power calculations for 'biologically significant' exposure but we cannot be sure this is accurate. If we assume that sensitisation to beryllium may have occurred in some of these men, it may be that longer exposure increases the likelihood of a beryllium exposure sufficiently high to cause sensitisation, with (given the presumed low exposure overall) a relatively indolent progression to CBD/sarcoidosis.

As in the present analysis, previous studies have not found a consistent relation between the Glu69 marker and sarcoidosis. An early study in the UK found a strong relation with 26 of 41 cases (63.4%) positive but only 26/76 controls (38.2%) but a further and larger study from the same group found only a small increase in cases.^{25 26} Similarly in the ACCESS study of biopsy confirmed sarcoidosis in the USA, in which 474 case–reference pairs were assessed, the Glu69 variant was found somewhat less frequently in sarcoidosis cases.²⁷ The hypothesis under test here, that exposure was important particularly in the presence of the susceptibility marker, and conversely that the genetic susceptibility marker was only important in the presence of exposure, could not be examined in these earlier studies that did not estimate beryllium exposure. Where such exposure is somewhat rare, the effect of such an interaction may be too small to be noticeably reflected in the main effect, as was recently demonstrated in a theoretical calculation, under these same assumptions.²⁸ The relationship of CBD to the Glu69 allele is one of the very few gene–environment effects in the occupational literature in which the size of the interaction is potentially of importance in advising workers.²⁹ The present study suggests that preventive intervention targeting beryllium exposures should not ignore industries where exposure is presumed to be low or infrequent, and that a high index of suspicion be maintained where sarcoidosis is diagnosed in dusty workplaces where beryllium may be present.

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Contributors NC, JB, IB and AS designed the project with LS and NY developing and executing the algorithms for identifying the patients. JS visited the offices of all collaborating physicians and confirmed patient eligibility in discussion with JB. NC, JB and IG devised and executed the exposure assessment. JP carried out the genotyping with the assistance of JT who extracted DNA from mouthwash samples. NC and JS carried out the data analysis. All authors commented on the draft report and have seen and agreed the final version.

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Competing interests None.

Patient consent Obtained.

Ethics approval Health ethics boards of the University of Alberta and the University of Calgary.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Additional data would be made available to researchers interested in collaboration after discussion of an agreed protocol.

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Appendix 1: Check list of industries with industry specific questionnaire

Industry	
Mining	<input type="checkbox"/> Yes <input type="checkbox"/> No
Oil and Gas	<input type="checkbox"/> Yes <input type="checkbox"/> No
Construction	<input type="checkbox"/> Yes <input type="checkbox"/> No
Pulp and paper industry	<input type="checkbox"/> Yes <input type="checkbox"/> No
Chemical industry	<input type="checkbox"/> Yes <input type="checkbox"/> No
Ceramics, stone, clay, glass or concrete	<input type="checkbox"/> Yes <input type="checkbox"/> No
Metal industry	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of heating equipment	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of industrial machinery	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of computer equipment	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of semi-conductors and related devices	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of electrical equipment	<input type="checkbox"/> Yes <input type="checkbox"/> No
Repairing of electrical equipment	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of automotive electrical equipment	<input type="checkbox"/> Yes <input type="checkbox"/> No
Repairing/rebuilding of automotive electrical equipment	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing/rebuilding of non-electrical vehicle parts	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing/rebuilding of truck trailers	<input type="checkbox"/> Yes <input type="checkbox"/> No
Ship building	<input type="checkbox"/> Yes <input type="checkbox"/> No
Ship repair	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of parts/components for guided missiles or space vehicles	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of dental equipment/supplies	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of jewelry/precious metal goods	<input type="checkbox"/> Yes <input type="checkbox"/> No
Repairing of jewelry/precious metal goods	<input type="checkbox"/> Yes <input type="checkbox"/> No
Metal working	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing of nuclear weapons	<input type="checkbox"/> Yes <input type="checkbox"/> No
Recycling	<input type="checkbox"/> Yes <input type="checkbox"/> No
Manufacturing fluorescent lamps	<input type="checkbox"/> Yes <input type="checkbox"/> No
Armed forces	<input type="checkbox"/> Yes <input type="checkbox"/> No
Lumber/wood products	<input type="checkbox"/> Yes <input type="checkbox"/> No
Farming or other agricultural work	<input type="checkbox"/> Yes <input type="checkbox"/> No
Firefighting	<input type="checkbox"/> Yes <input type="checkbox"/> No
Slaughterhouse or meat-packing	<input type="checkbox"/> Yes <input type="checkbox"/> No
Animal handler/veterinarian	<input type="checkbox"/> Yes <input type="checkbox"/> No
Health care sector	<input type="checkbox"/> Yes <input type="checkbox"/> No

Appendix 2: Sample size needed to detect a gene-environment interaction

The number of cases needed to detect a gene environment interaction is determined by the anticipated size of the associated odds ratio. Using all information available from published studies on the relation of Glu⁶⁹ to sarcoidosis, and the risks of CBD in the presence of beryllium and the variant form, the estimate obtained for the odds ratio of interaction is 6.74. Given a real uncertainty about the weight that can be put on this estimate we calculated sample size using estimates for the gene environment interaction that span this value (3, 6 and 9). A study of 617 cases and 1234 referents would be able to detect a gene-environment interaction odds ratio of 3, given 5% of referents “exposed” to beryllium. In the 1:2 designs, the interaction odds ratio of 6 is detected with 660 cases even if only 1.5% of referents “exposed” to beryllium (not shown in the table).

Number of sets for gene-environment interaction for beryllium

Prevalence of beryllium exposure in general population	Odds ratio of sarcoidosis among exposed population with susceptible genotype	Number of cases to achieve power 80%	
		1 control/case	2 controls/case
0.01	3	4235	2837
0.03	3	1460	986
0.05	3	906	617
0.10	3	495	343
0.01	6	1545	976
0.03	6	536	344
0.05	6	335	218
0.10	6	187	126
0.01	9	1032	633
0.03	9	360	225
0.05	9	226	144
0.10	9	128	86

* Following assumption were made in the sample size calculations: level of significance = 5%; prevalence of sarcoidosis in Canada =30 per 100,000; frequency of *HLA-DPB1* Glu⁶⁹ allele (susceptible genotype) in the general population = 30%; odds ratio of sarcoidosis among unexposed population with susceptible genotype =1.00; odds ratio sarcoidosis among exposed population with wild type genotype =1.10, implying marginal odds ratio of exposure of ~2.0 when the interaction odds ratio=3; unmatched analysis by logistic regression.

Appendix 3: Referent diagnoses (ICD code) and final group

ICD 9 codes	N	Group
Acute bronchitis and bronchiolitis, 466	8	Other
Bronchitis, not specified as acute or chronic, 490	44	COPD
Chronic bronchitis, 491	95	COPD
Emphysema, 492	123	COPD
Asthma, 493	838	Asthma
Bronchiectasis, 494	32	Other
Chronic airway obstruction, not elsewhere classified, 496	97	COPD
Asbestosis, 501	4	Other
Pneumoconiosis due to other silica or silicates, 502	1	Other
Pneumonitis due to solids and liquids, 507	1	Other
Empyema, 510	10	Other
Pleurisy, 511	62	Other
Pneumothorax, 512	8	Other
Abscess of lung and mediastinum, 513	2	Other
Pulmonary congestion and hypostasis, 514	1	Other
Post-inflammatory pulmonary fibrosis, 515	30	Other
Other alveolar and parietoalveolar pneumonopathy, 516	34	Other
Lung involvement in conditions classified elsewhere, 517	7	Other
Other disease of lung, 518	57	Other
Total	1454	

Appendix 4:

Diagnoses by years employed by Glu 69: industry with 50+ employed (Men)

	Years Exposed	Glu 69	Sarcoidosis		Asthma		COPD		Other		Total		p(x ²)
			N	%	N	%	N	%	N	%	N	%	
Oil and Gas	None	-	165	35.3	154	32.9	81	17.3	68	14.5	468	100.0	0.37
		+	112	35.3	118	37.2	53	16.7	34	10.7	3.7	100.0	
		Total	277	35.3	272	34.6	134	17.1	102	13.0	785	100.0	
	<10	-	40	38.5	41	39.4	15	14.4	8	7.7	104	100.0	0.45
		+	22	29.7	29	39.2	13	17.6	10	13.5	74	100.0	
		Total	62	34.8	70	39.3	28	15.7	18	10.1	178	100.0	
	≥10	-	24	35.3	19	27.9	10	14.7	15	22.1	68	100.0	0.37
		+	15	38.5	13	33.3	6	15.4	5	12.8	39	100.0	
		Total	39	36.4	32	29.9	16	15.0	20	18.7	107	100.0	
Construction	None	-	150	36.3	147	35.6	59	14.3	57	13.8	413	100.0	0.80
		+	100	36.0	108	38.8	36	12.9	34	12.2	278	100.0	
		Total	250	36.2	255	36.9	95	13.7	91	13.2	691	100.0	
	<10	-	59	36.0	50	30.5	31	18.9	24	14.6	164	100.0	0.20
		+	33	31.4	40	38.1	24	22.9	8	7.6	105	100.0	
		Total	92	34.2	90	33.5	55	20.4	32	11.9	269	100.0	
	≥10	-	20	31.7	17	27.0	16	25.4	10	15.9	63	100.0	0.99
		+	16	34.0	12	25.5	12	25.5	7	14.9	47	100.0	
		Total	36	32.7	29	26.4	28	25.5	17	15.5	110	100.0	
Chemical Industry	None	-	216	35.9	206	34.2	97	16.1	83	13.8	602	100.0	0.59
		+	138	35.0	145	36.8	67	17.0	44	11.2	394	100.0	
		Total	354	35.5	351	35.2	164	16.5	127	12.8	996	100.0	
	<10	-	11	36.7	8	26.7	5	16.7	6	20.0	30	100.0	0.45
		+	8	26.7	14	46.7	4	13.3	4	13.3	30	100.0	
		Total	19	31.7	22	36.7	9	15.0	10	16.7	60	100.0	
	≥10	-	2	25.0	0	0.0	4	50.0	2	25.0	8	100.0	0.38
		+	3	50.0	1	16.7	1	16.7	1	16.7	6	100.0	
		Total	5	35.7	1	7.1	5	35.7	3	21.4	14	100.0	

	Years Exposed	Glu 69	Sarcoidosis		Asthma		COPD		Other		Total		p(x ²)
			N	%	N	%	N	%	N	%	N	%	
Metal Industry	None	-	224	36.5	206	33.6	99	16.1	85	13.8	614	100.0	0.64
		+	140	34.7	148	36.6	68	16.8	48	11.9	404	100.0	
		Total	364	35.8	354	34.8	167	16.4	133	13.1	1018	100.0	
	<10	-	3	17.6	7	41.2	4	23.5	3	17.6	17	100.0	0.20
		+	5	29.4	10	58.8	2	11.8	0	0.0	17	100.0	
		Total	8	23.5	17	50.0	6	17.6	3	8.8	34	100.0	
	≥10	-	2	22.2	1	11.1	3	33.3	3	33.3	8	100.0	0.53
		+	4	44.4	2	22.2	2	22.2	1	11.1	8	100.0	
		Total	6	33.3	3	16.7	5	27.8	4	22.2	18	100.0	
Armed Forces	None	-	219	36.0	206	33.8	97	15.9	87	14.3	609	100.0	0.36
		+	142	35.0	156	38.4	62	15.3	46	11.3	406	100.0	
		Total	361	35.6	362	35.7	159	15.7	133	13.1	1015	100.0	
	<10	-	6	28.6	5	23.8	6	28.6	4	19.0	21	100.0	0.77
		+	7	29.2	4	16.7	10	41.7	3	12.5	24	100.0	
		Total	13	28.9	9	20.0	16	35.6	7	15.6	45	100.0	
	≥10	-	4	40.0	3	30.0	3	30.0	0	0.0	10	100.0	-
		+	0	0.0	0	0.0	0	0.0	0	0.0	0	100.0	
		Total	4	40.0	3	30.0	3	30.0	0	0.0	10	100.0	
Lumber and Wood	None	-	197	34.7	193	34.0	93	16.4	85	15.0	568	100.0	0.19
		+	138	35.7	146	37.7	63	16.3	40	10.3	387	100.0	
		Total	335	35.1	339	35.5	156	16.3	125	13.1	955	100.0	
	<10	-	22	39.3	16	28.6	12	21.4	6	10.7	56	100.0	0.32
		+	9	26.5	11	32.4	6	17.6	8	23.5	34	100.0	
		Total	31	34.4	27	30.0	18	20.0	14	15.6	90	100.0	
	≥10	-	10	62.5	5	31.2	1	6.2	0	0.0	16	100.0	0.10
		+	2	22.2	3	33.3	3	33.3	1	11.1	9	100.0	
		Total	12	48.0	8	32.0	4	16.0	1	4.0	25	100.0	

	Years Exposed	Glu 69	Sarcoidosis		Asthma		COPD		Other		Total		p(x ²)
			N	%	N	%	N	%	N	%	N	%	
Agriculture	None	-	189	36.0	176	33.5	85	16.2	75	14.3	525	100.0	0.47
		+	123	34.6	135	37.9	57	16.0	41	11.5	356	100.0	
		Total	312	35.4	311	35.3	142	16.1	116	13.2	881	100.0	
	<10	-	20	31.2	22	34.4	14	21.9	8	12.5	64	100.0	0.77
		+	18	40.0	12	26.7	10	22.2	5	11.1	45	100.0	
		Total	38	34.9	34	31.2	24	22.0	13	11.9	109	100.0	
	≥10	-	20	39.2	16	31.4	7	13.7	8	15.7	51	100.0	0.54
		+	8	27.6	13	44.8	5	17.2	3	10.3	29	100.0	
		Total	28	35.0	29	36.2	12	15.0	11	13.8	80	100.0	
Health Care	None	-	216	35.5	201	33.0	102	16.7	90	14.8	609	100.0	0.31
		+	135	33.8	150	37.5	69	17.2	46	11.5	400	100.0	
		Total	351	34.8	351	34.8	171	16.9	136	13.5	1009	100.0	
	<10	-	9	39.1	12	52.2	1	4.3	1	4.3	23	100.0	0.27
		+	7	36.8	6	31.6	3	15.8	3	15.8	19	100.0	
		Total	16	38.1	18	42.9	4	9.5	4	9.5	42	100.0	
	≥10	-	4	50.0	1	12.5	3	37.5	0	0.0	8	100.0	0.07
		+	7	63.6	4	36.4	0	0.0	0	0.0	11	100.0	
		Total	11	57.9	5	26.3	3	15.8	0	0.0	19	100.0	

Diagnoses by years employed by Glu 69: industry with 50+ employed (Women)

	Years Exposed	Glu 69	Sarcoidosis		Asthma		COPD		Other		Total		p(x ²)
			N	%	N	%	N	%	N	%	N	%	
Oil and Gas	None	-	142	27.1	230	43.9	93	17.7	59	11.3	524	100.0	0.59
		+	115	30.2	170	44.6	59	15.5	37	9.7	381	100.0	
		Total	257	28.4	440	44.2	152	16.8	96	10.6	905	100.0	
	<10	-	13	38.2	14	41.2	3	8.8	4	11.8	34	100.0	0.90
		+	4	28.6	6	42.9	2	14.3	2	14.3	14	100.0	
		Total	17	35.4	20	41.7	5	10.4	6	12.5	48	100.0	
	≥10	-	1	12.5	4	50.0	2	25.0	1	12.5	8	100.0	0.53
		+	2	33.3	3	50.0	0	0.0	1	16.7	6	100.0	
		Total	3	21.4	7	50.0	2	14.3	2	14.3	14	100.0	
Agriculture	None	-	148	28.1	229	43.5	91	17.3	59	11.2	527	100.0	0.87
		+	111	29.8	165	44.2	59	15.8	38	10.2	373	100.0	
		Total	259	28.8	394	43.8	150	16.7	97	10.8	900	100.0	
	<10	-	3	13.6	12	54.5	5	22.7	2	9.1	22	100.0	0.17
		+	7	38.9	9	50.0	2	11.1	0	0.0	18	100.0	
		Total	10	25.0	21	52.5	7	17.5	2	5.0	40	100.0	
	≥10	-	5	29.4	7	41.2	2	11.8	3	17.6	17	100.0	0.73
		+	3	30.0	5	50.0	0	0.0	2	20.0	10	100.0	
		Total	8	29.6	12	44.4	2	7.4	5	18.5	27	100.0	
Health Care	None	-	125	30.4	175	42.6	69	16.8	42	10.2	411	100.0	0.97
		+	90	30.9	125	43.0	45	15.5	31	10.7	291	100.0	
		Total	215	30.6	300	42.7	114	16.2	73	10.4	702	100.0	
	<10	-	18	21.2	36	42.4	17	20.0	14	16.5	85	100.0	0.09
		+	17	34.0	23	46.0	8	16.0	2	4.0	50	100.0	
		Total	35	25.9	59	43.7	25	18.5	16	11.9	135	100.0	
	≥10	-	13	18.6	37	52.9	12	17.1	8	11.4	70	100.0	0.88
		+	14	23.3	31	51.7	8	13.3	7	11.7	60	100.0	
		Total	27	20.8	68	52.3	20	15.4	15	11.5	130	100.0	

Appendix 5: Genotype (Glu69 marker) by diagnosis and sex

Diagnosis	Men			Women		
	N	Glu 69 positive	%	N	Glu 69 positive	%
Sarcoidosis	378	149	39.4	277	121	43.7
Asthma	374	160	42.8	427	179	41.9
COPD	178	72	40.4	159	61	38.4
Other	140	49	35.0	104	40	38.5
Total	1070	430	40.2	967	401	41.5