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Calcified Granulomatous Disease: Occupational Associations and Lack of Familial Aggregation

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Abstract

Purpose—The acute host response to histoplasma capsulatum infection varies according to exposure and susceptibility. Late sequelae include calcifications in the lung, thoracic lymphatics,

and spleen. Determinants of calcified granuloma formation are poorly studied and may differ from those affecting acute response. We examined the occupational associations and familial aggregation of radiographic calcified granulomatous disease to characterize the determinants of calcified granuloma formation.

Methods—We analyzed prospectively collected cross-sectional data including computed tomograms from 872 adult members of the Old Order Amish of Lancaster County.

Results—Granulomas were present in 71 % of participants. Granulomas were present in the lung of 57 % of participants, in the hilar or mediastinal lymph nodes of 55 % of participants, and in the spleen of 29 % of participants. No significant differences were observed in the presence of granulomas between men and women. Each year of age was associated with 4 % higher odds of splenic calcifications, and a primary occupation of farming was associated with an 84 % higher odds of splenic calcifications. A compelling pattern of familial aggregation was not observed.

Conclusions—Calcified granulomatous disease does not appear to aggregate in families. Determinants influencing patterns of granulomatous disease include occupation, age, and geographic location.

Keywords

Calcified granuloma; Occupational lung disease; Farming lung disease; Old Order Amish; Amish; Familial aggregation; Histoplasma; Granulomatous lung disease

Introduction

Histoplasma capsulatum is a dimorphic fungus that is commonly found in soil enriched with the droppings of bats or birds, including chickens [1–3]. *Histoplasma* infection occurs through inhalation of spores which replicate in yeast form in the lung and spread through lymphatics to mediastinal nodes. Once the disease extends to the lymphatic system, there is subsequent hematogenous dissemination of the organism to the reticuloendothelial system, most notably the spleen. While occupations and hobbies can result in different rates of exposure to *histoplasma*, acute response to exposure may also vary depending upon features of the host. In affected tissues, *histoplasma* typically elicits a granulomatous host response that calcifies over time. In the Eastern United States, calcified nodules most commonly represent the sequelae of prior exposure to *histoplasmosis*, and are referred to as calcified granulomas [4].

Determinants of calcified granuloma formation are poorly studied and may differ from factors affecting acute response to *histoplasma*. Host characteristics regulating many aspects of the immune response, including granuloma formation, have been shown to have genetic determinants [5–8]. It is conceivable that heritable factors influencing the immune and inflammatory response could result in a differential likelihood of developing calcified granulomas in response to *histoplasma* exposure. For example, genetic differences in the expression of osteopontin could potentially influence both the immune response as well as the calcification process [9]. Osteopontin is involved in granuloma formation [10, 11] and ectopic calcification [9, 12].

We hypothesized that radiologically observed calcified granulomatous disease is associated with occupational farming and also aggregates within families. To examine these hypotheses, we studied a special population—the Old Order Amish (OOA) community of Lancaster County, PA. The Amish are an epidemiologically compelling group for study as they live a relatively homogenous, agrarian lifestyle, and they limit use of modern conveniences such as electricity, telephones, and automobiles [13]. The agrarian lifestyle of the Amish puts them at increased risk of exposure to histoplasma, and characteristics of the Amish community make it well suited for informative studies of familial aggregation. Families average 7–9 siblings, and extended families live either in the same household or nearby. Homogeneity of socioeconomic status and lifestyle in the Amish reduces non-genetic variability and boosts the power to discern genetic components of diseases and related traits. As such, the Amish present a novel epidemiologic opportunity to characterize the determinants of calcified granuloma formation.

Methods

This report is based on 872 Amish individuals who participated in a community survey of cardiovascular health and received an electron beam-computed tomography (CT) scan of the thoracic aorta for the purpose of assessing degree of coronary artery calcification. Study participants were aged 30 years or older and consisted of volunteers who were recruited through word of mouth in the Amish community between 2001 and 2006. The survey was carried out by researchers at the University of Maryland School of Medicine as part of the Amish Complex Disease Program. Recruitment methods and examination procedures have been previously described [14, 15].

For the current study, CT scans were evaluated for granulomatous disease in the lung, hilar or mediastinal lymph nodes, and spleen by an experienced physician (RMR). Emphysema scores were calculated using Airway Inspector software (www.airwayinspector.org). Coronary and aortic calcification scores were quantified using the Agatston method, as previously described [14]. All studies were conducted with approval from the Institutional Review Board of the University of Maryland.

The prevalence of calcified granulomas was compared between the Amish and a non-Amish population, using data from the Pittsburgh Lung Screening Study (PLuSS) as the comparator [16, 17]. Both the Amish and the PLuSS samples were drawn from Pennsylvania populations during a similar time period. PLuSS constituted a CT screening study conducted from 2002 to 2005 assessing CT screening for lung cancer. Participants enrolled were between 50 and 79 years of age with a significant tobacco exposure. Calcified pulmonary granulomas were tabulated in PLuSS by an experienced radiologist and have not previously been reported [17].

Statistical Analysis

Data were presented as medians with interquartile range (IQR), and odds ratios (OR) with 95 % confidence intervals (CI). Between-group comparisons applied Wilcoxon rank-sum, Kruskal–Wallis, or Chi-square testing. Granuloma presence was treated as a dichotomous variable for each anatomic location as well as for the presence of granuloma in any location.

Additional analysis included treatment of granulomatous disease as an ordinal variable based on the number of anatomic locations (from 0 to 3) where granulomas were found. We assessed the correlations of granulomas with coronary and aortic calcification both in dichotomous and ordinal terms. As results were similar, we limited presentation to the dichotomous analyses. We used logistic regression to test for associations of granuloma presence with coronary and aortic calcification, adjusted for age and sex. For familial aggregation analysis, we calculated between-pair correlations using the FCOR module in the SAGE statistical program (S.A.G.E. Statistical Analysis for Genetic Epidemiology, version 6.3). With the exception of familial aggregation analysis, all statistical analyses were performed using STATA 11 SE software (StataCorp-LP, College Station, TX). For all analyses, $p < 0.05$ was considered significant.

Results

The study population included 872 Amish participants. Examples of calcified granulomas in each of the three categorized anatomic locations are shown in Fig. 1. Population characteristics, including differences according to the presence of granulomatous disease, are presented in Table 1. Granulomas were present in 71 % of participants. Granulomas were found in the lung in 57 % of participants, in the hilar or mediastinal lymph nodes in 55 % of participants, and in the spleen in 29 % of participants. No significant differences were observed between men and women in the frequency of granulomas observed in the lung (61 vs 55 %, $p = 0.08$), in the hilar or mediastinal lymph nodes (57 vs 53 %, $p = 0.3$), in the spleen (31 vs 27 %, $p = 0.1$), or in any location when considered together (72 vs 70 %, $p = 0.4$). Spirometric data were available in a subset of 272 participants. No differences were found in spirometry or emphysema scores between categories of granuloma. The presence of splenic granulomas was associated with older age (62 years IQR 51–69 vs 56 years IQR 45–65, $p < 0.0001$) and a higher rate of occupational self-reporting of farming/farmer's wife (38.8 vs 25.8 %, $p = 0.002$). In a logistic regression model including both age and occupation, age remained significant (adjusted OR 1.04, 95 % CI 1.02–1.05, $p < 0.001$), as did farming (adjusted OR 1.84, 95 % CI 1.24–2.71, $p = 0.002$). Participants with splenic granulomas were also more likely to have coronary calcification (55.8 vs 43.8 %, $p = 0.008$) and aortic calcification (64.0 vs 47.9 %, $p < 0.001$). In logistic regression models controlling for age, however, these associations were no longer significant: coronary calcification (adjusted OR 0.92, 95 % CI 0.59–1.45, $p = 0.7$); aortic calcification (adjusted OR 1.0, 95 % CI 0.59–1.68, $p = 1.0$). Age-stratified models yielded similar results with no detectable association between splenic calcifications and coronary or aortic calcification after age stratification.

Rates of pulmonary granulomas observed in the Amish population were higher than in the non-Amish population from PLuSS (Table 2). For participants aged 50–80 years, pulmonary granulomas were observed in 58.1 % of the Amish, but in only 9.7 % of the non-Amish cohort ($p < 0.0001$). The correlation between age and higher rates of granuloma suggested in the Amish cohort was also apparent in the PLuSS cohort.

We then examined for evidence of familial aggregation (Table 3). The best-represented pairs were among siblings. There were no significant associations with granulomas to suggest

familial aggregation in any of these sibling pairings. Mother–daughter pairs ($n = 79$) correlated significantly for the presence of pulmonary granulomas ($r = 0.30 \pm 0.10$, $p = 0.007$). Father–daughter pairs ($n = 60$) showed trends ($p = 0.1$) for all categories of granuloma, and the correlation was consequently most apparent when examined as an additive ordinal variable ($r = 0.32 \pm 0.11$, $p = 0.001$).

Discussion

In this study, we evaluated the prevalence and heritability of pulmonary granulomatous disease in a well-characterized, prospectively evaluated cross-sectional population of Old Order Amish. Evidence of calcified granulomatous disease in the lung, hilar/mediastinal nodes, or spleen was found in 71 % of participants. In adjusted analysis, each year of age was associated with 4 % higher odds of splenic calcifications, and a primary occupation of farming was associated with 84 % higher odds of splenic calcifications. A compelling pattern of familial aggregation was not observed. The rates of granulomas observed were much higher in the Amish than in the PLoSS cohort. The presence of granulomatous disease was associated with neither vascular calcification, nor differences in lung function.

Over 11 million CT examinations of the thorax are performed each year in the United States [18]. Enthusiasm for thoracic imaging by CT will likely increase due to recent studies and task force recommendations supporting the role of CT in both lung cancer screening as well as for risk stratification of cardiovascular disease [19–21]. In the course of these studies, incidental findings are common and are likely to gain attention with the increased frequency of CT scanning. Small calcified nodules are a common incidental finding on CT examinations of the thorax [22–24] and in the Eastern United States most often represent sequelae of histoplasma exposure [4].

Thoracic manifestations of histoplasma infection on imaging vary depending upon numerous factors including immune status of the patient, degree of antigen exposure, and time between the initial exposure and imaging [25]. In the acute setting, most immune competent patients who are infected with histoplasmosis due to inhalation of spores will be asymptomatic [26]. If a patient is exposed to a large antigen load, symptoms often mimic those of viral- or community-acquired bacterial pneumonia and include fever, chills, dyspnea, and cough. The association between splenic granulomas and both advanced age as well as farming as an occupation raises the possibility that granulomas in this location reflect a greater quantity or duration of exposure.

The sixfold higher rates of calcified granulomas observed in the age-matched Amish compared to the rates observed in the non-Amish sample focused in Pittsburgh warrant some consideration. Rates of histoplasma exposure are known to be higher in rural settings compared to urban, and are particularly high on farms [27, 28]. As even the Amish who do not self-identify as farmers often have experience with farmwork and an agrarian existence [13], it could be that this common lifestyle conveys increased exposure to histoplasmosis. Although both the Amish and PLoSS samples were taken from Pennsylvania, it is informative to consider geographic differences in more detail. Rates of histoplasmin reactivity were studied between 1958 and 1969 in male navy recruits age 17–21. Amongst

these recruits, 606 were from Lancaster county and were found to have a rate of histoplasma reactivity of 25.7 %. This is approximately 6.6 fold higher than the rate of reactivity in histoplasma reactivity (3.9 %) observed in the counties surrounding Pittsburgh [28]. This is remarkably concordant with the sixfold difference in pulmonary granulomas we observed between these groups. It is therefore probable that the primary factor influencing the observed differences in rates is geographic location. This correlation further supports the assertion that these granulomas are a result of histoplasma exposure [4].

Prior studies pertaining to genetic associations with histoplasmosis have been few and limited. One study demonstrated higher gene frequencies of HLA-B22 and HLA-B17 observed in areas of higher rates of histoplasmin skin reactivity [29]. Numbers in this study were limited to fewer than 30 subjects per area, and association with histoplasmosis was speculative. Our study failed to demonstrate familial aggregation, which would suggest that if a genetic predisposition does exist for the development of calcified granulomas after exposure to histoplasma, the effect must be minor. These results were surprising, as we expected familial aggregation to occur by virtue of similar exposures as well as common genetic predispositions.

Prior studies have demonstrated associations between histoplasmosis exposure and occupation. Epidemiologic data of acute histoplasmosis have demonstrated outbreaks associated with occupational exposures involving disruption of soil enriched with histoplasma by virtue of the presence of bat or bird guano. Examples include cave workers exposed to bat guano [30], construction workers exposed to dust contaminated with bird or bat droppings [31], and workers at an industrial facility where bird guano that had collected on the roof was disrupted [32]. Outside of outbreaks, occupational associations with histoplasma exposure have been estimated using skin testing. Such studies have demonstrated higher rates of histoplasmin reactivity associated with various occupations including poultry workers [29, 33], cave tourist-guides [29], guano collectors [29], and farmers [34]. To our knowledge, our study is the first to demonstrate association between the granulomatous sequelae of histoplasma exposure and occupation.

Several limitations of our study deserve mention. The methods did not permit quantification of inter-observer variability between the PLS and the Amish groups. While this could contribute to some differences in observed granuloma rates, calcification is easily discernible on CT scanning (Fig. 1) which facilitates detection and would likely lead to low rates of discordance. The concordance observed between geographic rates of histoplasmin skin reactivity supports this assertion.

The lack of association between the presence of granuloma and observable difference in lung function may suggest that prior exposures leading to granuloma formation do not result in detectable lung function decrements. Confidence in this conclusion is limited by power estimates. Lung function measures were available in only a minority of participants, and the sample was consequently 80 % powered to detect a rather large (~11 %) difference in FEV1 percent predicted. Similarly, lack of association between vascular calcification and granuloma calcification could represent an issue of power, despite availability of these

measures from the entire sample. Nonetheless, the finding suggests that there is not a strong common diathesis shared by granulomatous calcification and vascular calcification.

Conclusions

Calcified granulomatous disease does not appear to aggregate in families. Determinants influencing patterns of granulomatous disease include occupation, age, and geographic location.

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Abbreviations list

CI	Confidence interval
CT	Computed tomography
IQR	Interquartile range
OOA	Old Order Amish
OR	Odds ratio
PLuSS	Pittsburgh Lung Screening Study

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Fig. 1.
Examples of calcified granulomatous disease in the three anatomic locations: **a** Hilar and mediastinal lymphatics; **b** Pulmonary; **c** Splenic

Table 1

Study cohort characteristics by categories of granuloma

	Granuloma absent (n = 250)	Granuloma spleen (n = 252)	Granuloma lung (n = 500)	Granuloma lymph nodes (n = 476)	Any granuloma present (n = 619)	p [†]
Age at evaluation (years)	57 (45–65)	62 (51–69) [‡]	58 (47–67)	58 (47–67)	58 (47–67)	0.1
Male sex	43 %	49 %	47 %	46 %	46 %	0.4
BMI	28 (24–31)	28 (24–31)	28 (24–31)	28 (25–31)	28 (24–31)	0.5
Diabetes mellitus	5.5 %	4.4 %	3.8 %	3.8 %	3.9 %	0.3
Hypertension	18 %	23 %	19 %	18 %	20 %	0.5
Ever smoke tobacco	18 %	24 %	23 %	23 %	22 %	0.2
Farmer/Farmer's Wife (n = 602)	29.2 %	38.8 % [*]	30.9 %	30.9 %	29.3 %	1.0
Cardiovascular measures						
SBP (mmHg)	115 (108–124)	118 (109–130)	116 (109–128)	117 (108–128)	117 (109–128)	0.3
DBP (mmHg)	69 (63–74)	70 (63–78)	70 (63–77)	70 (63–76)	70 (64–77)	0.02
Total cholesterol (mg/dL)	211 (184–233)	212 (190–240)	212 (186–237)	212 (187–238)	212 (186–238)	0.5
Triglycerides (mg/dL)	73 (54–109)	72 (52–100)	73 (54–110)	73 (55–111)	73 (54–110)	0.8
HDL (mg/dL)	55 (45–67)	56 (48–66)	56 (45–65)	55 (47–65)	56 (46–66)	0.5
LDL (mg/dL)	132 (112–157)	139 (114–163)	137 (114–162)	138 (114–163)	137 (114–162)	0.3
Coronary calcification	39.4 %	55.8 % [*]	50.3 %	47.9 %	50.1 %	0.02
Aortic calcification	48.5 %	64.0 % [*]	53.4 %	53.4 %	53.9 %	0.2
Pulmonary measures						
FEV1 % (n = 272)	82.8 (69.1–97.1)	85.8 (66.7–99.4)	83.2 (67.6–97.2)	83.3 (69.2–97.1)	84.5 (67.6–99.0)	0.9
FVC % (n = 272)	87.7 (75.6–97.1)	86.7 (75.5–95.3)	86.6 (76.3–95.2)	86.3 (75.6–95.2)	86.6 (76.8–96.5)	0.7
Emphysema score	0.28 (0.18–0.36)	0.27 (0.18–0.37)	0.27 (0.19–0.38)	0.27 (0.19–0.37)	0.28 (0.19–0.37)	0.9

Statistically significant associations are highlighted in bold

Data are expressed as medians (IQR) or as percentages. FEV1% forced expiratory volume in 1 s percent predicted; FVC% forced vital capacity percent predicted

p values were obtained through Wilcoxon rank-sum or Chi-square test as appropriate.

[†] p value refers to difference between “Any Granuloma” and “Granuloma Absent” categories.[‡] p < 0.0001 compared to participants without splenic granuloma.^{*} p < 0.01 compared to participants without splenic granuloma

Table 2

Rates of pulmonary granulomas between Amish and non-amish cohorts

	<u>PLuSS cohort [16, 17]</u>		<u>Amish cohort</u>		<i>P</i>
	<i>n</i>	%	<i>n</i>	%	
50–59	2,104	8.1	209	60.3	<0.0001
60–69	1,193	11.9	234	56.8	<0.0001
70–79	345	12.2	118	56.8	<0.0001
ALL	3,642	9.7	561	58.1	<0.0001

Table 3

Familial aggregation analysis

	N	Any granuloma		Pulmonary granuloma		Hilar granuloma		Splenic granuloma		Granuloma (ordinal)	
		Corr (SD)	P	Corr (SD)	P	Corr (SD)	P	Corr (SD)	P	Corr (SD)	P
Husband-wife	38	0.32 (0.15)	0.049	0.09 (0.016)	0.6	0.13 (0.16)	0.4	-0.02 (0.16)	0.9	0.11 (0.16)	0.5
Parent-offspring											
Father-son	55	0.04 (0.13)	0.8	0.08 (0.13)	0.5	0.03 (0.13)	0.8	0.24 (0.13)	0.09	0.11 (0.13)	0.4
Father-daughter	60	0.28 (0.12)	0.03	0.26 (0.12)	0.047	0.19 (0.12)	0.1	0.23 (0.13)	0.08	0.32 (0.11)	0.001
Mother-son	53	0.09 (0.13)	0.5	0.06 (0.13)	0.7	0.14 (0.13)	0.3	0.10 (0.14)	0.5	0.13 (0.14)	0.4
Mother-daughter	79	0.17 (0.11)	0.1	0.30 (0.10)	0.007	0.13 (0.11)	0.2	-0.05 (0.12)	0.6	0.03 (0.11)	0.8
Siblings											
Brother-brother	181	-0.03 (0.07)	0.6	-0.08 (0.07)	0.2	-0.05 (0.07)	0.5	0.09 (0.08)	0.26	0.004 (0.08)	0.1
Sister-sister	295	0.04 (0.06)	0.5	0.04 (0.07)	0.5	-0.05 (0.05)	0.3	0.07 (0.07)	0.3	-0.003 (0.06)	1.0
Brother-sister	424	0.07 (0.05)	0.2	0.02 (0.05)	0.7	0.07 (0.04)	0.09	0.09 (0.06)	0.1	0.08 (0.05)	0.1

Statistically significant associations are highlighted in bold

N reflects the number of family pairs, Corr correlation, SD standard deviation