Familial Aggregation and Heritability of Sarcoidosis: A Swedish Nested Case-Control Study

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ONLINE SUPPLEMENT

Supplementary Methods

Sensitivity analysis

We conducted several sensitivity analyses. First, we were concerned that familial aggregation estimates might have been influenced by a more thorough ascertainment of the proband case's relatives due to the history of sarcoidosis in the proband. To address this, we repeated our familial RR analysis and regarded probands to be exposed only if relatives received their diagnosis of sarcoidosis at least a year before the proband. Second, we examined whether the familial RR was biased owing to misclassification of the sarcoidosis definition. As outlined below, we used probabilistic bias analysis methods [1, 2] to re-estimate the familial RRs under predetermined bias assumptions. We also tested a stricter definition for sarcoidosis requiring at least two visits listing sarcoidosis in the National Patient Register.

Last, to examine the robustness of heritability estimates, we calculated the ceiling heritability of sarcoidosis using Falconer's method [3]. Heritability (including potential influence of common environmental effects) equals twice the tetrachoric correlations between probands and first degree relatives [3]. For the calculations, we used a liberal and a strict sarcoidosis prevalence estimate for the Swedish population [4].

Probabilistic bias analysis for sarcoidosis definition misclassification

We followed the methods described by Lash et al. [2] and Bollaerts et al. [1] to test the robustness of the familial relative risk from the main analysis against potential misclassification of our register-based definition used for the ascertainment of probands and relatives. We defined exposure as having ≥1 first degree relative with sarcoidosis and used the numbers of exposed and unexposed cases and controls from the main analyses to calculate the crude odds ratio (interpreted as the familial relative risk):

	Cases	**Controls**
≥1 first degree relative with sarcoidosis	831	1907
No first degree relatives with sarcoidosis	19491	162721

We then assigned probability distributions for bias parameters as follows:

$$\text{Positive Predictive Value (PPV)} \sim \text{Beta}(15,4)$$

$$\text{Negative Predictive Value (NPV)} \sim \text{Uniform}(1)$$

The PPV values centred at 80% and NPV was uniformly defined to be 100% considering the rarity of sarcoidosis in the general population.

We performed Monte Carlo simulations with 10 million repetitions sampling from the above distributions to define the bias parameters. We sequentially misclassified the outcome definition (sarcoidosis in the proband) and the exposure (sarcoidosis in the relative). The final estimate for the familial relative risk represents the 50th percentile of the re-estimated familial relative risks. We used

the residual error from each simulation to calculate 95% simulation confidence intervals (2.5th and 97.5th percentiles). Of note, the resulting simulation confidence intervals did not account for the residual random error from the original analyses.

The crude odds ratio was calculated using the information above to be 3.64**.** Adjusting for sarcoidosis definition misclassification in simulations resulted in a largely similar odds ratio (3.61 [95% simulation confidence interval 3.33–3.93]). The familial relative risk from main analysis estimated from a logistic regression model was 3.73 (95% confidence interval 3.43–4.06).

Supplementary References

1. Bollaerts K, Shinde V, Dos Santos G, et al. Application of Probabilistic Multiple-Bias Analyses to a Cohort- and a Case-Control Study on the Association between Pandemrix and Narcolepsy. *PLoS One* 2016: 11: e0149289.
2. Lash TL, Fox MP, Fink AK. Applying Quantitative Bias Analysis to Epidemiologic Data. Springer-Verlag, New York, 2009.
3. Falconer DS, Mackay TF. Introduction to Quantitative Genetics. 4th ed. Pearson, Harlow, UK, 1996.
4. Arkema EV, Grunewald J, Kullberg S, et al. Sarcoidosis incidence and prevalence: a nationwide register-based assessment in Sweden. *Eur Respir J* 2016: 48: 1690-1699.

Supplementary Tables

Table E1. Distribution of age at inclusion and sex amongst first and second degree relatives of proband cases and controls.

	Mean age at inclusion (SD)		Female, %	
	Relatives of cases	**Relatives of controls**	**Relatives of cases**	**Relatives of controls**
First degree relatives				
All	48.0 (25.2)	48.0 (25.5)	50	50
Parents	74.3 (15.7)	74.2 (15.5)	51	51
Full siblings	45.3 (14.2)	45.0 (14.3)	49	49
Offspring	27.1 (15.9)	26.1 (15.7)	49	49
Second degree relatives				
Half siblings	40.5 (15.8)	39.5 (15.6)	48	49

Table E2. Relative risk of sarcoidosis associated with having one or more first degree relatives with sarcoidosis, stratified by probands' age at inclusion and sex.

	Age 18–49 years at inclusion			Age ≥50 years at inclusion		
	N exposed/N total (%)			N exposed/N total (%)		
	Cases	Controls	RR (95% CI)	Cases	Controls	RR (95% CI)
≥1 first degree relative	438/10 138 (4.3)	975/85 445 (1.1)	3.99 (3.55–4.48)	393/10 184 (3.9)	932/79 183 (1.2)	3.48 (3.08–3.92)
Female proband	177/3765 (4.7)	392/31 667 (1.2)	4.02 (3.34–4.82)	221/5959 (3.7)	535/45 959 (1.2)	3.41 (2.90–4.00)
Male proband	261/6373 (4.1)	583/53 778 (1.1)	3.97 (3.42–4.61)	172/4225 (4.1)	397/33 224 (1.2)	3.57 (2.98–4.29)
≥2 first degree relatives	16/10 138 (0.2)	22/85 445 (<0.1)	6.01 (3.13–11.55)	12/10 184 (0.2)	27/79 183 (<0.1)	3.62 (1.81–7.22)

Table E3. Relative risk of sarcoidosis associated with having a first degree relative with the disease, stratified by kinship and sex of proband and relative.

Sex of proband/ sex of relative	N exposed/N total (%)		RR (95% CI)
	Cases	Controls	
Parents			
Female/Female	63/6186 (1.0)	163/52 536 (0.3)	3.30 (2.56–4.27)
Female/Male	51/5853 (0.9)	112/49 708 (0.2)	3.91 (2.91–5.25)
Male/Female	98/8577 (1.1)	227/72 996 (0.3)	3.74 (3.04–4.61)
Male/Male	59/8219 (0.7)	136/69 814 (0.2)	3.61 (2.74–4.75)
Full siblings			
Female/Female	62/4903 (1.3)	131/42 221 (0.3)	4.79 (3.15–7.28)
Female/Male	80/5180 (1.5)	172/43 585 (0.4)	3.97 (2.75–5.74)
Male/Female	82/6900 (1.2)	156/57 513 (0.3)	4.55 (2.13–6.63)
Male/Male	106/7121 (1.5)	237/60 670 (0.4)	3.35 (2.54–4.42)
Offspring			
Female/Female	61/8908 (0.7)	149/69 143 (0.2)	2.76 (2.00–3.81)
Female/Male	93/9085 (1.0)	225/72 538 (0.3)	3.16 (2.41–4.15)
Male/Female	50/7520 (0.7)	92/59 955 (0.2)	3.77 (2.52–5.64)
Male/Male	58/7684 (0.8)	157/63 029 (0.3)	3.02 (2.21–4.14)

Table E4. Relative risk of sarcoidosis associated with having relatives diagnosed with sarcoidosis at least a year before the proband case.

	Cases		Controls		
	N exposed/ N total	% exposed	N exposed/ N total	% exposed	RR (95% CI)
First degree relatives					
≥1 relative*	454/20 094	2.3	997/161 667	0.6	3.89 (3.47–4.36)
≥2 relatives*	12/20 094	0.1	12/161 667	<0.1	8.09 (3.61–18.13)
Parents†	212/28 835	0.7	454/243 939	0.2	3.96 (3.41–4.60)
Full siblings†	180/23 699	0.8	362/199 486	0.2	4.39 (3.46–5.58)
Offspring†	77/23 761	0.3	193/181 114	0.1	3.20 (2.42–4.23)
Second degree relatives					
Half siblings†	23/6935	0.3	21/13 297	0.2	1.89 (0.88–4.06)

* For these analyses, an indicator for having ≥1 or ≥2 first degree relatives with sarcoidosis was created for each case and control.

† For these analyses, each proband-relative relationship contributed a unique observation in the dataset. The confidence intervals were adjusted for autocorrelation arising from family clustering using robust estimates of the variance.

Table E5. Relative risk of sarcoidosis associated with having relatives with the disease using a stricter definition for sarcoidosis ascertainment (≥2 ICD-coded visits in the National Patient Register, 1964–2013).

	Cases		Controls		
	N exposed/ N total	% exposed	N exposed/ N total	% exposed	RR (95% CI)
First degree relatives					
≥1 relative*	482/14 936	3.2	1045/120 068	0.9	3.86 (3.46–4.32)
≥2 relatives*	10/14 936	0.1	19/120 068	<0.1	4.22 (1.94–9.22)
Parents†	147/22 647	0.7	312/188 961	0.2	3.99 (3.33–4.78)
Full siblings†	202/18 690	1.1	397/156 057	0.3	4.26 (3.42–5.30)
Offspring†	143/22 696	0.6	355/182 051	0.2	3.08 (2.52–3.76)
Second degree relatives					
Half siblings†	28/6017	0.5	33/12 021	0.3	1.30 (0.79–2.15)

* For these analyses, an indicator for having ≥1 or ≥2 first degree relatives with sarcoidosis was created for each case and control.

† For these analyses, each proband-relative relationship contributed a unique observation in the dataset. The confidence intervals were adjusted for autocorrelation arising from family clustering using robust estimates of the variance.

Table E6. Heritability estimates for sarcoidosis using Falconer's methods based on a liability-threshold model for the disease. Liberal and strict estimates of sarcoidosis prevalence in our population obtained from Arkema et al., 2016 were used to account for the case-control study design.

	Cases with relatives with sarcoidosis	Cases with relatives without sarcoidosis	Familial relative risk*	Liberal prevalence assumption		Strict prevalence assumption	
				Sarcoidosis prevalence per 100,000	Heritability (95% CI)	Sarcoidosis prevalence per 100,000	Heritability (95% CI)
≥1 first degree relative	831	19 491	3.73	160	35% (33–37)	64	32% (30–34)
Female/Female†	186	8637	3.56	141	31% (27–35)	55	28% (24–32)
Female/Male†	217	8527	3.58	141	31% (27–35)	55	29% (26–32)
Male/Female†	228	9853	4.10	179	35% (31–39)	73	32% (29–35)
Male/Male†	220	9723	3.49	179	31% (27–35)	73	29% (26–32)
Parents	271	28 564	3.68	160	29% (26–32)	64	27% (24–30)
Full siblings	330	23 774	4.08	160	33% (30–36)	64	30% (27–32)
Offspring	262	32 935	3.23	160	26% (23–29)	64	24% (21–27)

* Estimates obtained from the main analysis in this study.

† Sex of proband/sex of relative.