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Early-life hygiene-related factors affect risk of central nervous system demyelination and asthma differentially

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Summary

The increasing prevalence of immune-related diseases, including multiple sclerosis, may be partly explained by reduced microbial burden during childhood. Within a multi-centre case–control study population, we examined: (i) the co-morbid immune diseases profile of adults with a first clinical diagnosis of central nervous system demyelination (FCD) and (ii) sibship structure in relation to an autoimmune (FCD) and an allergic (asthma) disease. FCD cases ($n = 282$) were aged 18–59 years; controls ($n = 558$) were matched on age, sex and region. Measures include: history of doctor-diagnosed asthma; sibling profile (number; dates of birth); and regular childcare attendance. FCD cases did not differ from controls with regard to personal or family history of allergy, but had a greater likelihood of chronic fatigue syndrome [odds ratio (OR) = 3·11; 95% confidence interval (CI) 1·11, 8·71]. Having any younger siblings showed reduced odds of FCD (OR = 0·68; 95% CI: 0·49, 0·95) but not asthma (OR = 1·47; 95% CI: 0·91, 2·38). In contrast, an increasing number of older siblings was associated with reduced risk of asthma (P trend = 0·04) but not FCD (P trend = 0·66). Allergies were not over-represented among people presenting with FCD. Sibship characteristics influence both FCD and asthma risk but the underlying mechanisms differ, possibly due to the timing of the putative 'sibling effect'.

Keywords: allergy, asthma, autoimmunity, hygiene, multiple sclerosis, siblings

Introduction

Recent parallel increases in the prevalence of autoimmune and allergic diseases in Western countries are coincident with changes in human ecology brought about by improved sanitation, vaccination and antibiotic usage [1,2]. One pathway linking these trends could be reduced microbial exposure during childhood.

The hygiene hypothesis proposes that having more siblings decreases the risk of allergic disease because siblings are a source of infection; the high load of minor infections in early life assists the correct development of the infant immune system, away from an allergic disease profile. A large number of population-based studies support a protective effect of higher number of siblings (a proxy for repeated exposure to common childhood infections) against development of allergy and asthma (i.e. the 'sibling effect', reviewed in [3]). Indeed, 40% of asthma may be attributable to a lack of infection exposure (based on markers such as sibling number) [4].

The contribution of a hygienic early-life environment to the development of autoimmune disorders is less clear. More than four decades ago, Leibowitz proposed that people with multiple sclerosis (MS) were more likely to have a 'high level of sanitation' in their childhood home [5], but our recent review of the available data [6] indicates only limited support for this hypothesis.

Insights into the early-life determinants of autoimmune and allergic diseases would be enhanced by their contemporaneous evaluation within a single study [7]. Accordingly, we examine, within one study population, the association between markers of a hygienic early-life environment and: (i) a first clinical diagnosis of central nervous system (CNS) demyelination [FCD, including clinically isolated syndrome

(CIS), first diagnosis of primary progressive MS (PPMS), or a second event with an undiagnosed prior CIS and, thus, a new diagnosis of MS [8–11]]; and (ii) asthma in a healthy reference population. Because previous work [12] has shown an interaction between low infant sibling exposure and the human leucocyte antigen-DRB1*1501-DQB1*0602 (HLA-DR15) haplotype on MS risk, we additionally explored the association between having any G allele for HLADR15 and sibling exposure for FCD risk.

Materials and methods

Study population

The Ausimmune Study, an Australian multi-centre population-based case–control study, is well described elsewhere [8]. Cases were aged 18–59 years, resident in one of four study regions and had an incident FCD between November 2003 and December 2006. Of 330 cases notified to the study, 19 (6%) were found subsequently to be ineligible following review by a team of neurologists. A further 29 (9%) declined to participate, leaving 282 participating eligible FCD cases, of whom two-thirds were aged 30–49 years (mean age: 39·79; female: 78%). Of 1118 controls selected initially, 937 (84%) were contacted successfully and, of those contacted, 558 (60%) participated in the study. Controls were selected randomly from the Electoral Roll (compulsory registration for citizens > 18 years) and matched on age (within 2 years), sex and study region; thus, they were aged between 18–61 years (66% aged 30–49 years; mean age = 38·57) and predominantly female (77%). Nine regional Human Research Ethics Committees approved the study and participants gave written informed consent.

Measurements

Detailed diagnostic information about the cases was obtained by neurological assessment, including magnetic resonance imaging (MRI) for CNS lesions. Participants self-reported: smoking history; regular attendance at childcare before age 6 years (at least once a week with more than three other children); whether or not they had been breast-fed; date of birth of each sibling and whether or not they were resident in the same house as the subject; highest education level achieved; parity (females only); family history of allergic or autoimmune diseases; and personal medical history. For the latter, participants responded to the question: 'Have you had, or do you have, any of the following conditions?' and age at onset of each condition, including asthma (doctor-diagnosed); hayfever (sneezing, runny or blocked nose without a cold or flu, sometimes accompanied by watery, itchy eyes); eczema (any itchy rash that was coming or going for at least 6 months affecting the folds of the elbows, behind the knees, in front of the ankles, under the buttocks or around the neck, ear or eyes); rheumatoid arthritis (doctor-diagnosed); and chronic fatigue syndrome (severe fatigue for 6 months, not relieved by rest and accompanied by four of the following: new headache, multiple joint pain, muscle pain, unrefreshing sleep, impaired memory or concentration, sore throat, tender cervical or axillary lymph glands, post-exertion malaise) [13–15].

Participants recorded location of residence and pet ownership for every year of life in personal work and residence calendars. Residential locations were classified according to the Australian Standard Geographical Classification (ASGC) [16] as a proxy for exposure to a rural environment.

Genomic DNA was isolated from whole blood or saliva ($n = 725$). Single nucleotide polymorphisms (SNPs) in or near MS-susceptibility genes were genotyped using the SNPline method by KBiosciences (Hoddesdon, Herts, UK).

Statistical analyses

In total, 282 FCD cases and 558 controls participated in the Ausimmune Study; two cases and 19 controls could not be matched to a participating control/case and one case had incomplete data, leaving a total of 279 cases and 539 controls for case–control analysis. Analyses presented here include: (a) a case–control analysis ($n = 818$), restricted to 279 FCD cases and their 539 matched controls with complete data; and (b) a controls-only analysis ($n = 554$), comprising all interviewed controls with allergy data, including those not matched to a participating case.

Allergies with onset after the date of the FCD diagnosis (cases), or corresponding pseudodiagnosis date (controls), were censored. We calculated sibship size, number of older and younger siblings and cumulative exposure to younger infant siblings (aged ≤ 2 years) before the subject was age 6 years (referred to hereafter as 'infant sibling exposure') [17].

Conditional logistic regression provided matched odds ratios (ORs) for FCD. Unconditional logistic regression was used to assess associations among controls. Education and smoking history (ever *versus* never) were retained as covariates in all analyses to ensure that effects were assessed independently of socio-economic status (SES; associated with household size) and past smoking (linked to a higher risk of MS and increased asthma symptoms). For relevant multivariable models, we adjusted for variables associated previously with FCD [9–11]. To examine the possibility that any observed association with sibship size or number of older siblings was due partly to selection bias in controls, results were stratified by level of education (SES). Among cases, proportional hazards modelling was used to examine any effect of sibling contact on age of diagnosis of FCD, adjusting for education and smoking status. Tests for linear trend were conducted by fitting ordered categories of a variable as a single ordinal variable in the models; trend *P*-values were based on the Wald test. Analyses were

conducted using STATA (version 9·2) (StataCorp, College Station, TX, USA, 2006).

Results

Participants were predominantly Caucasian (cases: 97%; controls: 95%); selected characteristics are shown in Table 1. The number of siblings ranged from one to 13, with more than half of participants reporting having either one (cases 20%; controls 20%) or two (cases 32%; controls 33%) siblings. Few cases or controls (5% and 4%, respectively) reported being an 'only child'.

Personal medical history

History of chronic fatigue syndrome, but not allergic disease or rheumatoid arthritis, was associated with increased FCD risk; none of the FCD cases ($n = 11$) reporting chronic fatigue were classified as PPMS (Table 2). FCD cases were no more likely to develop asthma in childhood (i.e. before age 6 years or between ages 6–10 years; data not shown) than matched controls.

Among controls, the prevalence of doctor-diagnosed asthma was 24% (males: 19%; females: 25%). The average age of asthma onset was 16·4 years [standard deviation (s.d.) = 14·9]. Those with eczema or hayfever were much more likely to also report asthma than those without these allergic conditions (Table 2).

Sibling patterns

In univariate analysis, having any younger siblings was associated with a reduced likelihood of FCD (62% *versus* 71%; $P = 0{\cdot}01$; Table 1). In contrast, those with asthma were significantly less likely to report having any older siblings than those without asthma (50% *versus* 63%; $P = 0{\cdot}009$; Table 1).

After adjustment for age, sex, education and smoking history (Table 3), having younger siblings was associated with reduced FCD risk, but there were no associations between sibling size or older siblings and FCD. In contrast, for asthma, there was no association with exposure to younger siblings, but decreased risk with a greater number of total siblings and older siblings. Cumulative exposure to infant siblings tended to be associated with lower odds of FCD, but a higher asthma risk. These patterns were not materially altered by factors previously linked to FCD in the Ausimmune Study [9–11].

Among controls, stratification by SES (education) revealed similar inverse associations between sibship size and number of older siblings and asthma in all three strata (data not shown), providing reassurance that sibship patterns were not acting as a marker for SES.

The apparent beneficial effect of cumulative infant sibling exposure for MS is reportedly more evident among those with the *HLA-DR15* risk genotype [12]. Here, also, the

Table 1. Characteristics of: (a) FCD cases and controls and (b) controls with and without asthma, in the Ausimmune Study.

Characteristics	Controls* $n = 539$ n (%)	FCD cases* $n = 279$ n (%)	P-value†	Controls no asthma‡ $n = 421$ n (%)	Controls asthma‡ $n = 133$ n (%)	P-value†
Female/male ratio	419/120	214/65		322/99	110/23	
Mean age in years at study interview (s.d.)	38·69 (9·58)	37·75 (9·57)	0·18	38·64 (9·44)	39·19 (9·96)	0·57
Education (tertiary *versus* other)	139 (26·0)	68 (25·4)	0·67	106 (25·3)	35 (26·7)	0·75
Ever smoked (ever *versus* never)	283 (53·0)	171 (62·0)	0·02	215 (51·4)	78 (60·0)	0·09
Breastfed (ever *versus* never)	371 (74·1)	186 (72·1)	0·56	287 (73·0)	88 (73·3)	0·95
Rural residence at birth (yes *versus* no)	84 (17·9)	44 (18·3)	0·90	69 (18·9)	16 (13·9)	0·22
Regular childcare < age 6 years (ever *versus* never)	209 (40·6)	128 (47·8)	0·06	165 (40·8)	51 (41·1)	0·96
Number of siblings (3 or more *versus* less)	220 (41·9)	118 (42·6)	0·85	180 (43·9)	48 (37·2)	0·18
Any older siblings (yes *versus* no)	315 (60·1)	177 (64·8)	0·19	259 (63·3)	65 (50·4)	0·009
Any younger siblings (yes *versus* no)	371 (70·8)	170 (62·3)	0·01	282 (68·9)	99 (76·7)	0·09

Percentages refer to subjects with available data. *Matched FCD cases and controls with complete data. †Derived from two-sample t-test for proportions comparing cases and controls without missing variables for the variable. ‡All interviewed controls with allergy data, including those not matched to a participating case. FCD: a first clinical diagnosis of central nervous system demyelination; s.d.: standard deviation.

Table 2. Association between personal history of allergic and immune-related disorders and: (a) FCD and (b) asthma among controls.

Personal history	Controls* $n = 539$ n (%)	FCD cases* $n = 279$ n (%)	$_{adj}$OR† for FCD (95% CI)	P-value	Controls no asthma‡ $n = 421$ n (%)	Controls asthma‡ $n = 133$ n (%)	$_{adj}$OR§ for asthma (95% CI)	P-value
Asthma¶	127 (23·7)	70 (25·5)	1·15 (0·81–1·64)	0·43	–	–	–	
Eczema¶	103 (19·3)	52 (18·9)	0·99 (0·68–1·43)	0·99	59 (14·1)	50 (37·9)	4·17 (2·61–6·68)	<0·0001
Hayfever¶	236 (44·3)	106 (38·4)	0·84 (0·62–1·14)	0·27	159 (37·9)	90 (69·2)	3·70 (2·37–5·77)	<0·0001
Rheumatoid arthritis	10 (1·9)	4 (1·5)	0·70 (0·21–2·32)	0·56	9 (2·2)	1 (0·7)	0·32 (0·04–2·57)	0·28
Chronic fatigue syndrome	11 (2·1)	11 (4·0)**	3·11 (1·11–8·71)	0·03	7 (1·7)	4 (3·1)	1·73 (0·42–7·15)	0·45

*Matched FCD cases and controls with complete data. †Matched odds ratios adjusted ($_{adj}$OR) for level of education and ever smoker. ‡All interviewed controls with allergy data, including those not matched to a participating case. §OR adjusted for age, gender, level of education and ever smoker. ¶Prior to FCD onset. **None of these 11 FCD cases were classified as 'primary progressive multiple sclerosis'. FCD: a first clinical diagnosis of central nervous system demyelination; CI: confidence interval.

reduced FCD risk associated with younger siblings was observed among those with the G allele of the *HLA-DR15* maker SNP (rs9271366) (adjusted OR = 0·42, $P = 0·05$), but not among those without it (adjusted OR = 0·92; $P = 0·84$) (test for interaction, $P = 0·405$).

Other hygiene-related measures

There was no association between either FCD or asthma and living in a rural environment, a history of having been breastfed, regular childcare attendance or pet ownership (Table 4).

Family medical history

Compared with their healthy counterparts, FCD cases were four times more likely to report a family history of MS ($P = 0·009$), but there was no association with family history of asthma, hayfever, rheumatoid arthritis, autoimmune thyroiditis or inflammatory bowel disease (Table 5). There were strong and consistent associations between family history of asthma and hayfever in first-degree relatives and the odds of reporting a personal history of asthma, but no association with a family history of any of the autoimmune conditions examined (Table 5).

Age at diagnosis

Among FCD cases, having younger siblings was associated with later age at diagnosis [adjusted hazard ratio = 0·90; 95% confidence interval (CI): 0·83, 0·99 per sibling]. No sibling characteristic was associated with age at asthma onset.

Discussion

At the time of FCD diagnosis, the profile of personal and family allergic disease among cases was similar to that of matched controls, apart from a greater likelihood of prior chronic fatigue syndrome. Sibling exposure demonstrated distinctly different patterns for a presumed autoimmune disease (FCD, as a precursor to MS) and an allergic disease (asthma): having any younger siblings was associated with decreased FCD risk and delayed age of FCD diagnosis, but there was a strong inverse association between sibship size and number of older siblings and asthma.

Strengths of the present study include the large sample and opportunity to examine risk estimates for both FCD and asthma within the same study population, with detailed data on sibling exposure in early life and age at disease diagnosis. Importantly, the prevalence of ever having been diagnosed with asthma was comparable to the 19–21% reported for the Australian population [18], and our focus on allergy incident prior to date of FCD prevented inclusion of diagnoses of asthma, hayfever or eczema resulting from

Table 3. Association between sibling patterns and: (a) first clinical diagnosis (FCD) and (b) asthma among controls.

Sibling patterns	Controls* $n = 539$ n (%)	FCD cases* $n = 279$ n (%)	$_{adj}$ OR† for FCD (95% CI)	*P*-value	Controls no asthma‡ $n = 421$ n (%)	Controls asthma‡ $n = 133$ n (%)	$_{adj}$ OR§ for asthma (95% CI)	*P*-value
Sibling size (continuous)	–	–	0·97 (0·89–1·06)	0·49	–	–	0·84 (0·74–0·97)	0·02
No. older siblings								
None	209 (39·9)	96 (35·2)	Ref		150 (36·7)	64 (49·6)	Ref	
1	144 (27·5)	82 (30·0)	1·15 (0·80–1·67)		117 (28·6)	30 (23·3)	0·61 (0·36–1·03)	
2	95 (18·1)	55 (20·2)	1·12 (0·73–1·71)		76 (18·6)	21 (16·3)	0·70 (0·39–1·26)	
3 or more	76 (14·5)	40 (14·6)	1·09 (0·69–1·71)	0·66	66 (16·1)	14 (10·9)	0·50 (0·25–0·98)	0·04
Any older siblings	315 (60·1)	177 (64·8)	1·13 (0·82–1·54)	0·46	259 (63·3)	65 (50·4)	0·61 (0·40–0·93)	0·02
No. younger siblings								
None	153 (29·2)	103 (37·7)	Ref		127 (31·1)	30 (23·3)	Ref	
1	164 (31·3)	77 (28·2)	0·69 (0·47–1·03)		124 (30·3)	46 (35·7)	1·46 (0·84–2·53)	
2	129 (24·6)	56 (20·5)	0·65 (0·42–1·00)		92 (22·5)	37 (28·7)	1·83 (1·03–3·25)	
3 or more	78 (14·9)	37 (13·6)	0·70 (0·43–1·15)	0·07	66 (16·1)	16 (12·4)	1·02 (0·50–2·05)	0·46
Any younger siblings	371 (70·8)	170 (62·3)	0·68 (0·49–0·95)	0·03	282 (68·9)	99 (76·7)	1·47 (0·91–2·38)	0·12
Infant sibling exposure¶								
<1 years	222 (41·2)	133 (47·7)	Ref		186 (44·2)	44 (33·1)	Ref	
1–<3 years	216 (40·1)	103 (36·9)	0·85 (0·61–1·18)		155 (36·8)	64 (48·1)	1·70 (1·07–2·71)	
3–<5 years	76 (14·1)	35 (12·5)	0·82 (0·51–1·31)		62 (14·7)	17 (12·8)	1·30 (0·68–2·49)	
≥5 years	25 (4·6)	8 (2·9)	0·51 (0·23–1·17)	0·10	18 (4·3)	8 (6·0)	2·04 (0·81–5·13)	0·09

*Matched FCD cases and controls with complete data. †Matched odds ratios adjusted ($_{adj}$OR) for level of education and ever smoker. ‡All interviewed controls with allergy data, including those not matched to a participating case. §OR adjusted for age, gender, level of education and ever smoker. ¶Cumulative exposure to infant siblings (aged ≤ 2 years) before the subject was aged 6 years. FCD: a first clinical diagnosis of central nervous system demyelination; CI: confidence interval.

Table 4. Association between other hygiene-related exposures and: (a) FCD and (b) asthma among controls.

Hygiene-related exposures	Controls* $n = 539$ n (%)	FCD cases* $n = 279$ n (%)	$_{adj}$ OR† for FCD (95% CI)	*P*-value	Controls no asthma‡ $n = 421$ n (%)	Controls asthma‡ $n = 133$ n (%)	$_{adj}$ OR§ for asthma (95% CI)	*P*-value
Rural residence at birth								
Major city	236 (50·2)	117 (48·6)	Ref		185 (50·7)	61 (53·0)	Ref	
Inner regional	150 (31·9)	80 (33·2)	0·90 (0·61–1·35)		111 (30·4)	38 (33·0)	1·10 (0·68–1·79)	
Outer regional/remote	84 (17·9)	44 (18·3)	0·90 (0·55–1·48)	0·60	69 (18·9)	16 (14·0)	0·60 (0·31–1·17)	0·25
Rural residence at interview								
Major city	304 (57·0)	148 (53·4)	Ref		235 (56·4)	78 (60·5)	Ref	
Inner regional	184 (34·5)	100 (36·1)	0·83 (0·51–1·35)		145 (34·8)	43 (33·3)	0·83 (0·53–1·30)	
Outer regional/remote	45 (8·5)	29 (10·5)	0·87 (0·42–1·80)	0·89	37 (8·9)	8 (6·2)	0·49 (0·20–1·22)	0·12
Breastfed								
No	130 (24·1)	72 (25·8)	Ref		106 (25·2)	32 (24·1)	Ref	
Yes	371 (68·8)	186 (66·7)	0·82 (0·57–1·19)	0·30	287 (68·2)	88 (66·2)	0·99 (0·62–1·61)	0·99
Don't know	38 (7·1)	21 (7·5)			28 (6·7)	13 (9·8)		
Regular childcare < age 6 years								
No	306 (59·4)	140 (52·2)	Ref		239 (59·2)	73 (58·9)	Ref	
<1 year	70 (13·6)	44 (16·4)	1·52 (0·96–2·41)		54 (13·4)	18 (14·5)	1·34 (0·73–2·46)	
1–<3 years	86 (16·7)	55 (20·5)	1·57 (1·00–2·43)		73 (18·1)	16 (12·9)	0·79 (0·42–1·48)	
≥3 years	53 (10·3)	29 (10·8)	1·22 (0·71–2·08)	0·11	38 (9·4)	17 (13·7)	1·78 (0·91–3·51)	0·42
Pet ownership < age 1 year								
No	280 (52·0)	131 (47·0)	Ref		218 (51·8)	74 (55·6)	Ref	
Yes	259 (48·0)	148 (53·0)	1·15 (0·85–1·54)	0·37	203 (48·2)	59 (44·4)	0·88 (0·58–1·34)	0·56

*Matched FCD cases and controls with complete data. †Matched odds ratios adjusted ($_{adj}$OR) for level of education and ever smoker. ‡All interviewed controls with allergy data, including those not matched to a participating case. §OR adjusted for age, gender, level of education and ever smoker. FCD: a first clinical diagnosis of central nervous system demyelination; CI: confidence interval.

Table 5. Association between family history of allergic and autoimmune disorders and: (a) FCD and (b) asthma among controls.

Family history	Controls* $n = 539$ n (%)	FCD cases* $n = 279$ n (%)	$_{adj}$OR† for FCD (95% CI)	*P*-value	Controls no asthma‡ $n = 421$ n (%)	Controls asthma‡ $n = 133$ n (%)	$_{adj}$OR§ for asthma (95% CI)	*P*-value
Asthma: mother	75 (14·8)	36 (13·8)	0·92 (0·59–1·43)	0·71	49 (12·2)	30 (25·9)	2·81 (1·65–4·77)	<0·0001
Asthma: father	51 (10·3)	26 (10·4)	0·94 (0·56–1·57)	0·82	31 (8·0)	21 (18·3)	2·42 (1·31–4·48)	0·005
Asthma: sibling¶	147 (29·8)	61 (23·9)	0·77 (0·53–1·12)	0·17	94 (24·4)	54 (45·0)	3·02 (1·90–4·79)	<0·0001
Hayfever: mother	145 (30·0)	73 (28·2)	0·96 (0·67–1·38)	0·84	106 (27·5)	41 (37·6)	1·76 (1·10–2·80)	0·02
Hayfever: father	90 (19·3)	44 (18·3)	0·97 (0·63–1·49)	0·89	57 (15·3)	33 (31·1)	2·55 (1·52–4·29)	<0·0001
Hayfever: sibling¶	207 (43·6)	95 (37·9)	0·78 (0·55–1·10)	0·16	151 (40·3)	62 (55·9)	2·29 (1·45–3·64)	<0·0001
Rheumatoid arthritis**	84 (17·7)	40 (16·3)	0·88 (0·56–1·40)	0·60	64 (17·1)	23 (20·2)	1·04 (0·60–1·84)	0·88
Multiple sclerosis**	5 (1·0)	11 (4·3)	4·15 (1·42–12·2)	0·009	4 (1·1)	2 (1·7)	2·26 (0·37–13·82)	0·38
Autoimmune thyroiditis**	20 (4·2)	9 (3·7)	0·91 (0·38–2·20)	0·83	16 (4·2)	4 (3·4)	0·76 (0·25–2·34)	0·63
Inflammatory bowel disease**	35 (7·2)	23 (9·0)	1·49 (0·84–2·66)	0·17	26 (6·8)	10 (8·3)	1·42 (0·65–3·07)	0·38

*Matched FCD cases and controls with complete data. †Matched odds ratios adjusted ($_{adj}$OR) for level of education and ever smoker. ‡All interviewed controls with allergy data, including those not matched to a participating case. §OR adjusted for age, gender, level of education and ever smoker. ¶Additionally adjusted for sibship size. **Mother, father and/or sibling had history of disorder. FCD: a first clinical diagnosis of central nervous system demyelination; CI: confidence interval.

increased medical contact or from disease- or treatment-related changes in immune activity. In this retrospective study, recall bias may have affected the ascertainment of self-reported early-life exposures, such as pet ownership, although this is likely to have been non-differential between cases and controls. Nonetheless, number of siblings and their dates of birth are likely to be recalled with some accuracy.

Disease classification was more precise for FCD than for asthma: for example, the data did not allow differentiation between allergic and non-allergic asthma. However, doctor-diagnosed asthma was recorded, reducing the bias associated with self-report [19]. Moreover, the highly statistically significant findings between: (i) FCD and family history of MS, (ii) asthma and related atopic diseases (hayfever, eczema) and (iii) asthma and history of asthma and hayfever in first-degree relatives provides evidence of construct validity. Some bias may have arisen from the differentially low participation rate in contacted controls (60%) compared to cases (94%). There was no appreciable association between SES and FCD in our data, which argues against major bias by variation in case–control SES or variables that correlate with it [20]. In fact, in this study, there is some evidence that the lack of SES differences between FCD cases and controls reflects control participation patterns: as is commonly reported in studies with less than full participation, control subjects were more highly educated than the general population (e.g. Brisbane study region: 39·7% *versus* 18·7% had completed university) [21]. The apparent absence of an effect of hygiene-related factors (such as childcare attendance) on the development of asthma is inconsistent with findings based on precise measures of microbial exposure [22]; however, these self-reported early-life exposures are probably subject to non-differential recall error, providing a possible explanation for our null results.

For FCD, the downward trend in risk associated with having younger siblings has been reported in previous studies of MS [17,23]. Here, number of older siblings was unrelated to FCD, but this has not been observed consistently [23–26]. In these data, higher younger sibship size was related to later FCD diagnosis and higher infant sibling contact has also been shown to be associated significantly with later MS onset [17].

Our findings are coherent with studies showing an inverse association between older siblings and asthma [27–29], although others have found no such difference [30,31]. Some of the heterogeneity across studies examining sibling characteristics and asthma probably reflects ambiguities over the definition of asthma [32]. Indeed, the 'sibling effect' appears most consistent for hayfever and for clinical markers of allergy, such as skin prick test positivity and immunoglobulin E reactivity [3,31,33].

The mechanisms by which family structure could protect against allergic sensitization are unclear. Our findings suggest that the timing and nature of sibling-linked immu-

nomodulation may differ across immune-mediated diseases. A protective effect exerted by older siblings could be a function of the number of the mother's previous pregnancies, characterizing immune development *in utero* rather than postnatal exposures [34]. It would also be consistent with a window of immunomodulation in very early life or immunomodulation by infectious agents not necessarily restricted to the infant period. In contrast, any effect of infant younger siblings must operate when the participant is already an older child. This calls into question whether a different developmental window is operating by chronological age or whether subsequent repeated exposure and boosting of an established immune response is important. Alternatively, younger siblings could represent exposure to specific infectious agents commonly occurring at a very early age, such as enterovirus, or transmission involving salivary contact [17]. Such immune priming is likely to differ by genetic predisposition. In these data, a protective effect of younger siblings was particularly evident among those with a high MS risk *HLA-DR15* genotype; however, this does not explain the stronger apparent benefit of infant younger siblings reported previously [12], compared to the pattern reported here, because the proportion of *HLA-DR15* high-risk cases was similar in both studies. Further work on gene–environment interactions within the Ausimmune Study, including for non-HLA genes, is underway.

FCD and asthma were not co-associated within individuals and there was no association between FCD and family history of allergy or between asthma and autoimmune disorders. These findings are consistent with the overall body of work on MS and asthma [6], but at odds with the antagonistic T helper type 1 (Th1)–Th2 interpretation of immune responses [35]. However, MS, long considered to reflect Th1 over-activity [36], is now thought to also involve up-regulation of Th17 cells and a defect in thymic natural T regulatory cells [37]. There is also emerging evidence that T cells in MS patients have an altered specificity against Epstein–Barr nuclear antigen 1 (EBNA1) compared to controls. Thus, the specificity of such immune responses may depend not only upon initial, but also repeated, microbial exposure induced by younger siblings.

Our finding of a strong association between chronic fatigue syndrome and FCD is not new. Chronic fatigue syndrome is a frequent manifestation of MS [38], suggesting that similar autoimmune factors underlie these disorders. It does, however, highlight that early CNS demyelinating disease is an important differential diagnosis to consider among people who present with chronic fatigue syndrome.

Conclusion

Whereas higher contact with younger and infant siblings was associated with decreased FCD risk and later age of FCD diagnosis, a protective effect for asthma was exerted by older siblings. These patterns suggest that, while sibship characteristics influence both FCD and asthma risk, the underlying mechanisms differ, possibly due to the timing of the putative 'sibling effect'. The lack of co-existence of FCD and asthma in individuals, corroborated by patterns in family history of autoimmune and allergic diseases, challenges the hypothesis of a common aetiology.

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Disclosure

The authors declare that they have no conflicts of interest or any relevant financial interest in any company or institution that might benefit from this publication.

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