

# Multiple sclerosis and birth order: a longitudinal cohort study



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## Summary

**Background** Genetic epidemiological studies suggest both genetic and environmental factors have a role in multiple sclerosis (MS). Environmental effects are strongly suggested from geographical gradients, migration data, and discordance rates in twins. In epidemiological studies, risk of MS in offspring of small families and in those with an early birth-order position has been reported and interpreted in the context of the hygiene hypothesis, which is that infections at an early age, introduced by older siblings, are protective. We aimed to study the effect of birth order on MS risk.

**Methods** A longitudinal, population-based sample of individuals with MS and their healthy siblings were identified from the Canadian Collaborative Project on Genetic Susceptibility to MS. Data were grouped according to single (simplex) or multiple (multiplex) siblings with MS in a sibship. Separate analyses were done for each sibship size.

**Findings** We studied 10 995 individuals with MS and 26 336 healthy siblings, and found no relation between MS risk and birth-order position. In simplex sibships of at least seven siblings, slightly more siblings who were born late in the birth order had MS; the same was found for the first-born sibling with MS in a multiplex sibship. Siblings with MS were slightly younger ( $p < 0.0001$ ) than those without MS, contrary to the expected age at onset bias.

**Interpretation** These findings do not support the hygiene hypothesis and could be due to a cohort effect resulting from increasing MS incidence. Birth order has no effect on MS risk in most families, and there is no support for the hypothesis that having older siblings protects against MS.

## Introduction

Consecutive, longitudinal genetic epidemiology studies have been done on a large Canadian population-based sample of patients with multiple sclerosis (MS) and their family members; this research has included studies of twins,<sup>1-4</sup> adoptees,<sup>5</sup> half-siblings,<sup>6,7</sup> conjugal pairs and their offspring,<sup>8</sup> consanguineous matings,<sup>9</sup> and month of birth.<sup>10</sup> Together these studies have contributed to the widely held belief that MS is a complex trait determined by genes and environment.

Despite strong evidence against non-genetic familial transmissibility of MS, other research, typically cross-sectional studies of viral antibody concentrations, suggested various infections are involved. Reports of direct isolation of infectious organisms have not been replicated. Attention continues to be given to the timing of infection, previously recognised as an important factor in paralysis in polio.<sup>11</sup> Skewed birth-order positions within MS sibships could provide some support for such a concept, but only by analogy with known infections and their consequences. To date, birth-order studies have mostly been negative,<sup>12-14</sup> but they might have been underpowered to detect conceptually important effects. Studies of large sibships effectively exclude birth-order effects because in such sibships the likelihood of protective early infection in the later-born siblings should be the greatest.

Allergic rhinitis and immunoglobulin E (skin prick test) studies show consistent associations with birth order.<sup>15,16</sup> The hygiene hypothesis, which states that

exposure to infection early in life leads to protection, can explain these immunoglobulin E mediated allergies—eg, the tendency for later born children or individuals living in rural environments to be protected from disorders such as asthma and atopic dermatitis.<sup>15-18</sup> Attention has thus been given to the age-related timing of environmental exposures. Siblings born late in a birth order are thought to be exposed to infection earlier than those born early in a birth order and to have greater immune stimulation. Early infections are believed to stimulate a natural balance between Th1 and Th2 cytokine responses, which is not skewed towards the Th2-type associated with allergy, thus providing protection against the development of allergy.<sup>19,20</sup> The rarity of MS in less developed countries and the increase of this disorder in people who have migrated from a less developed country to the UK is consistent with such a hypothesis,<sup>21</sup> as are findings for experimental autoimmune encephalomyelitis.<sup>22</sup> By contrast, Sardinian studies report a higher rate of MS in urban rather than rural populations.<sup>23</sup> The hygiene hypothesis was most recently used to explain results from 136 patients with MS in Tasmania (Australia) in whom high exposure to younger infant siblings was protective for MS.<sup>24</sup>

We report a study that investigated predictions of the hygiene hypothesis in MS, specifically whether birth-order position affects MS risk. The study had sufficient power to allow stratification by sibship size and proximity analyses with respect to birth-order position for siblings with MS from multiplex sibships.

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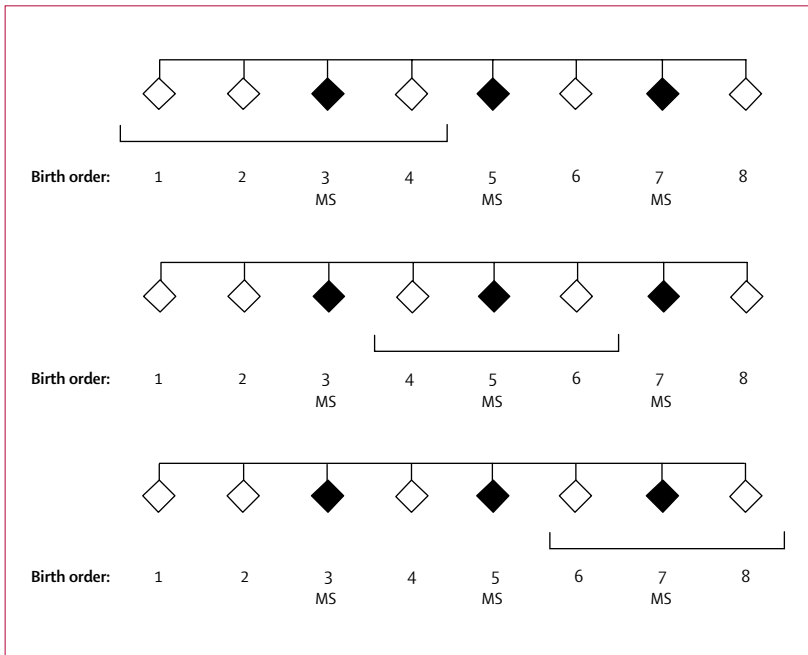
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**Figure:** Possible birth-order positions of three siblings with MS who are in a sibship of eight  
 According to birth-order position, the third sibling is the first sibling with MS. Because the fifth sibling is the second sibling with MS, the first sibling with MS can only have a birth-order position of 1–4. The fifth sibling can only have a birth-order position after the first sibling with MS and up to the third sibling with MS—ie, a position of 4–6. The only possibilities for birth-order position for the third sibling with MS (the seventh sibling) are 6–8.

**Methods**

**Participants**

We analysed data from participants in the Canadian Collaborative Project on Genetic Susceptibility to MS (CCPGSMS);<sup>3–10</sup> strategies, organisation, and ascertainment have been described.<sup>25</sup> We defined sibship as the biological offspring of the same mother and father—ie, individuals who share 50% of their genetic material. Adopted siblings, half-siblings, and step-siblings were excluded for various reasons, including small sample sizes and differences between shared genetics and intrafamilial environment. Index cases with no siblings were included as a comparison group. Ethics approval was obtained from the Clinical Research Ethics Board, Office of Research Services, University of British Columbia (C93-0103).

**Procedures**

We separated the data into two groups, simplex and multiplex, and stratified each group by sibship size. The simplex group included sibships with only one individual with MS (CCPGSMS index case). In the multiplex group, at least two siblings within a sibship had MS; at least one of these individuals was an index case in the CCPGMS. In multiplex sibships, we defined the first sibling affected as the earliest born individual with MS, the second affected as the second earliest born individual with MS, and the third affected as the third earliest born individual with MS.

**Panel 1: Analysis of mean age of siblings with and without MS accounting for sibship size**

**Calculation of effect size**

Effect size  $d = (X_1 - X_2) / S_p$

where:

$X_1$  = Mean age of the siblings without MS

$X_2$  = Mean age of the siblings with MS

$S_p^2 = [(N_1 - 1) * S_1^2 + (N_2 - 1) * S_2^2] / (N_1 + N_2 - 2)$  where:

$N_1$  = Number of siblings without MS

$N_2$  = Number of siblings with MS

$S_1^2$  = Variance of the siblings without MS

$S_2^2$  = Variance of siblings with MS

Weighted mean  $d = \sum [N_i d_i] / \sum N_i$  and Associated

variance  $S_d^2 = \sum [N_i (d_i - d)^2] / \sum N_i$

where:

$N_i$  = Number of siblings in sibship size  $i$

$d_i$  = Effect size in sibship size  $i$

**Observed versus expected birth-order position**

$t = (\text{observed average} - \text{expected average}) / SE(\text{mean})$

$df = N - 1$

We assigned birth-order position chronologically, starting with the first-born individual who was assigned a birth-order of one, irrespective of whether they had MS. Therefore the birth-order position of people with MS in simplex sibships could be random. When at least two siblings had MS, the analyses were more complex and required “conditioned” sibship sizes (figure). In multiplex sibships, birth-order position is not random throughout the sibship.

**Panel 2: Analysis of expected mean difference in birth order**

Sum of all differences/total number of differences

$$= \sum_{i=1}^{n-1} i * (n-i) / \sum_{i=1}^{n-1} i$$

$$= \frac{(n^3 - n) / 6}{(n^2 - n) / 2}$$

$$= (n + 1) / 3$$

where  $n$  = sibship

For example, if the sibship size was 7, there were 21 subtractions for a total of 56:

- 7–1=6 7–2=5 7–3=4 7–4=3 7–5=2 7–6=1
- 6–1=5 6–2=4 6–3=3 6–4=2 6–5=1
- 5–1=4 5–2=3 5–3=2 5–4=1
- 4–1=3 4–2=2 4–3=1
- 3–1=2 3–2=1
- 2–1=1

The expected mean difference in birth-order positions between siblings in a sibship of 7 would be 56/21=2.67.

We compared the mean ages of siblings with and without MS, stratified by sibship size, to assess whether those with MS were older than those without MS—ie, whether any findings for birth order might be confounded by an association between MS and an “age of risk”. Expected birth-order position was calculated for each sibship size within simplex and multiplex groups, and compared with observed birth-order position, up to and including sibships with three individuals with MS. The sample size was too small to allow expansion of the analyses to the four (or more) individuals with MS in a sibship.

### Statistical analysis

Mean ages of individuals with and without MS were compared by meta-analysis,<sup>26–28</sup> taking into account variability by sibship size (panel 1). We calculated the effect size, and then the weighted mean and variances. The Z test was used to compare the mean ages of individuals with and without MS. The relation between mean ages and sibship size was assessed with the Spearman correlation coefficient. The observed and expected mean birth-order position was compared with the *t* test; we controlled for sibship size in the simplex group and for conditioned sibship size in the multiplex group.

For the simplex group, calculation of the observed mean birth-order position took into account the actual birth-order position per sibship, by sibship size, for all index cases included in this study. For example, for sibship size three, there were 2226 sibships (index cases). Therefore, the observed birth-order position was the sum of all index case birth-order positions divided by 2226. The expected birth-order position, by sibship size, was calculated accounting for the sum of all the birth orders per sibship, by sibship size, divided by the number of options.

For the multiplex group, observed and expected birth-order positions were calculated as for the simplex group except that the “conditioned” sibship size was used (figure). We compared the difference between birth-order position for the first two siblings with MS in multiplex sibships. The expected mean difference in birth order was calculated (panel 2).

### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and final responsibility for the decision to submit for publication.

### Results

We studied 10 995 individuals with MS and 26 336 individuals without MS, who were siblings of those with the disorder. In the simplex group there were 9381 index cases and 24 014 siblings without MS. Sibship size for

Sibship size	Number		Mean age (years)	
	Individuals with MS	Healthy individuals	Individuals with MS	Healthy individuals
1	1008	0	48.01	..
2	2318	2318	45.40	45.07
3	2226	4452	45.34	45.12
4	1573	4719	46.79	46.61
5	931	3724	48.46	48.37
6	505	2525	48.94	49.21
7	302	1812	49.09	49.10
8	196	1372	50.53	51.88
9	112	896	54.39	54.25
10	90	810	52.14	53.18
11	46	460	51.41	54.21
12	25	275	53.00	54.00
13	18	216	50.78	52.31
14	13	169	57.85	59.97
15	9	126	50.44	53.66
16	6	90	54.83	59.49
17	2	32	52.50	61.50
19	1	18	48.00	49.28
Total	9381	24 014	46.88*	48.21*

\*Z=8.81, p<0.0001

**Table 1: Number and mean age of individuals with MS and healthy individuals by sibship size in the simplex group**

this group, including the index case, ranged from one to 19 (table 1). 8863 index cases (95%) were in sibships ranging from one individual to seven siblings.

The multiplex group consisted of 769 sibships, and sibship size ranged from two to 15 (table 2). There were two additional sibships of 17 and 21. 694 (90%) sibships were sized from two to eight and included 1447 siblings with MS and 1692 siblings without MS.

For simplex sibships, the Spearman coefficient for the correlation between sibship size and mean age of

Sibship size	Number		Mean age (years)	
	Siblings with MS	Siblings without MS	Siblings with MS	Siblings without MS
2	152	..	46.54	..
3	313	149	46.48	46.58
4	286	258	47.66	46.70
5	274	391	48.38	47.94
6	194	346	49.61	50.03
7	138	310	53.17	53.36
8	90	238	51.84	52.06
9	56	178	53.95	54.21
10	43	147	52.95	54.47
11	31	123	55.74	55.05
12	12	48	63.17	65.13
13	14	64	51.71	49.56
14	5	23	58.40	60.04
15	2	13	54.00	50.69
17	2	15	48.00	50.73
18–20	0	0	..	..
21	2	19	46.50	51.47
Total	1614	2322	49.09*	51.03*

\*Z=4.97, p<0.0001

**Table 2: Number and mean age of siblings with MS and siblings without MS by sibship size in the multiplex group**

Sibship size	Number of sibships	Observed mean birth-order position	Expected mean birth-order position	SE	t statistic	p
1	1008	1.00	1.00	0	..	..
2	2318	1.49	1.50	0.01	-0.96	0.17
3	2226	1.99	2.00	0.02	-0.58	0.28
4	1573	2.50	2.50	0.03	0.00	0.50
5	931	2.98	3.00	0.05	-0.44	0.33
6	505	3.59	3.50	0.07	1.22	0.11
7	302	3.98	4.00	0.11	-0.18	0.43
8	196	4.98	4.50	0.17	2.88	0.002
9	112	4.89	5.00	0.24	-0.46	0.32
10	90	5.94	5.50	0.31	1.40	0.08
11	46	7.11	6.00	0.45	2.47	0.01
12	25	6.56	6.50	0.80	0.08	0.47
13	18	8.06	7.00	0.79	1.35	0.10
14	13	8.92	7.50	0.97	1.46	0.08
15	9	10.00	8.00	1.07	1.87	0.05
16	6	11.00	8.50	2.21	1.13	0.15
17	0	..	..	..	..	..
18	2	15.00	9.00	1.00	..	..
19	1	11.00	10.00	..	..	..
≥7	820	5.13	4.83	0.10	3.06	0.001
≥11	120	7.91	6.78	0.34	3.32	0.001

Table 3: Observed versus expected birth-order position for individuals with MS by sibship size in simplex group

Conditioned sibship size*	Number of sibships	Observed mean birth-order position	Expected mean birth-order position	SE	t statistic	p
<b>First individual with MS</b>						
1	196	1.00	1.00	0	..	..
2	205	1.53	1.50	0.03	0.86	0.67
3	137	2.13	2.00	0.07	1.87	0.03
4	100	2.58	2.50	0.12	0.69	0.25
5	63	3.19	3.00	0.18	1.07	0.14
6	27	4.00	3.50	0.31	1.61	0.06
7	19	4.84	4.00	0.49	1.73	0.05
8	8	4.75	4.50	0.65	0.39	0.35
9	7	6.71	5.00	0.61	2.82	0.02
10	4	6.50	5.50	1.44	0.69	0.27
13	2	6.50	7.00	1.50	-0.33	0.40
14-16	0	..	..	..	..	..
17	1	13.00	9.00	..	..	..
≥7	41	5.58	4.68	0.38	2.38	0.01
<b>Second individual with MS†</b>						
1	209	1.00	1.00	..	..	..
2	194	1.52	1.50	0.04	0.56	0.29
3	154	1.82	2.00	0.07	-2.62	0.005
4	89	2.47	2.50	0.12	-0.26	0.40
5	59	2.86	3.00	0.18	-0.80	0.21
6	26	3.58	3.50	0.28	0.29	0.39
7	13	2.92	4.00	0.64	-1.70	0.06
8	12	4.33	4.50	0.69	-0.25	0.40
9	8	4.63	5.00	0.89	-0.42	0.34
10	1	1.00	5.50	..	..	..
11	1	2.00	6.00	..	..	..
12	3	4.33	6.50	0.33	-6.51	0.01
≥7	38	3.76	4.66	0.38	-2.37	0.01

\*First individual with MS=number of siblings before the second affected; second individual with MS=number of siblings after first affected in sibship up to end of sibship or up to but excluding third affected in sibship. †Observed mean birth-order position=position within the conditioned sibship size.

Table 4: Observed compared with expected birth-order position for individuals with MS by conditioned sibship size in multiplex group

individuals with MS was 0.62 (p=0.004) and between sibship size and mean age of individuals without MS was 0.78 (p<0.01). When the data were analysed according to sibship size, the overall effect size was d=0.0308 (S<sub>d</sub>=0.0557). These results suggest that ages of affected and unaffected sibs were comparable within each sibship size. However, regardless of sibship size, the index cases (mean 46.88 years [SD 11.64]) were younger than siblings without MS (48.21 years [12.69]; Z=8.81, p<0.0001).

For multiplex sibships, the mean ages of the siblings with and without MS, stratified by sibship size, were similar up to 11 siblings. Analysis according to sibship size showed that the mean age of affected and unaffected siblings within each sibship size was similar (d=0.058, S<sub>d</sub>=0.0819). Overall, however, the siblings with MS (mean 49.09 years [11.45]) were younger than those without MS (51.03 years [12.44]; Z=4.97, p<0.0001). The correlation between sibship size and mean age for siblings with MS was r=0.31 (p=0.13) and for siblings without MS was r=0.45 (p=0.046).

The effect of birth-order position on risk of MS was assessed for each group. In the simplex group, observed and expected birth-order positions were compared for 15 sibship sizes (table 3). Mean observed birth order was higher than expected for sibships of size 8, 11, and 15 (p<0.05), 20% (3 of 15) of the sibships, and did not show any trend. In the multiplex sibships, the birth-order position of the first sibling with MS within a conditioned sibship was compared with expected birth order; the same was done for the second sibling with MS (figure, table 4). For sibships with seven or more siblings, the first individual with MS was born later than expected (simplex: t=3.06, p=0.001; multiplex: t=2.38, p=0.01).

Analysis of ten sibship sizes for the first sibling with MS showed that the observed birth order was higher than expected in sibship sizes three and eight (20%). Analysis of nine sibship sizes for the second sibling with MS showed that the observed birth order was higher than the expected birth order in sibship sizes three and 12 (22%). The same analysis was done for 70 siblings with MS who were the third in their sibship to develop MS; observed and expected birth order were not different (data not shown). Analysis of the difference in birth order between the first and second individual with MS in a sibship for 12 sibship sizes found that half were closer in birth-order position than expected: sibship sizes 4, 5, 8, 10, 11, 12, and 13 (table 5). We also analysed the half-sibling data but found no relation between the presence of MS and sibship size (data not shown).

### Discussion

Study of the mean ages of siblings with and without MS found that those with MS were younger than those without MS, suggesting that any differences between the groups were not attributable to an age of risk. For all

sibship sizes in both simplex and multiplex sibships, the mean ages for siblings without MS were above the 24–25 year peak ages of risk for MS. However, individuals with MS could be slightly under-represented in this study because MS is known to lead to a modest number of premature deaths.<sup>29</sup> Nevertheless, participants in the CCPGMS encompass a broad age range. Our findings do not support the prediction of the hygiene hypothesis—ie, that siblings with MS are more likely to be born early in the birth order of siblings. In the multiplex sibships, where the numbers were an order of magnitude smaller than the simplex sibships, the difference in age between siblings with and without MS was not different from the simplex cases.

Among the sibship sizes of at least seven, those with MS in the simplex group and the first affected in the multiplex group had a later birth-order position than expected. In large multiplex sibships, the second sibling with MS was closer than expected in birth-order position to the first sibling with MS. Heterogeneity in multiplex sibships could explain these findings. The close birth-order position of siblings with MS might be a weak unspecified cohort, and the age of the second sibling with MS could reflect a tendency for siblings with MS to be older than those without MS. The larger than average spread in sibling ages within very large pedigrees might be prone to confounding by differences in age of onset or by parental age. An effect of birth-order position in large families with children born over long periods (eg, approaching or exceeding two decades) could result from an increasing population risk of MS because the more recent births (later birth-order positions) would be at high risk. We think this is the most plausible explanation for the finding that siblings with MS are born in a later birth-order position than expected in both simplex and multiplex families, together with an age of onset bias in the opposite direction. The modest decrease in longevity in MS is an unlikely explanation of the results because the multiplex families show the same effect proportionate to the simplex families. In multiplex families, the possible bias from premature death of siblings with MS is mostly accounted for by the survival of one sibling with MS, especially given the birth-order distribution, which would have led to ascertainment of the small number of early MS-related deaths. In any case, our findings are marginal and might reflect one or a combination of small effects, statistical artifact, or heterogeneity within the populations.

A recent study suggested that the risk is not attributable to birth order but to the amount that “at risk” children up to age 6 years are exposed to their infant siblings.<sup>24</sup> 136 cases and twice as many controls were studied, but the analysis was not corrected for multiple comparisons. Assuming birth-order position is the main partial surrogate for exposure to infant siblings, the inability to detect an effect in our study,

	Number of sibships	Observed mean difference in birth-order position	Expected mean difference in birth-order position	SE	t statistic	p
<b>Sibship size</b>						
2	76	1.00	1.00	0.00	..	..
3	154	1.32	1.33	0.04	-0.31	0.38
4	136	1.49	1.67	0.06	-2.96	0.002
5	133	1.86	2.00	0.08	-1.73	0.04
6	90	2.18	2.33	0.13	-1.15	0.13
7	64	2.48	2.67	0.17	-1.11	0.14
8	41	2.39	3.00	0.23	-2.70	0.01
9	26	3.15	3.33	0.33	-0.53	0.30
10	19	2.58	3.67	0.46	-2.37	0.01
11	14	2.71	4.00	0.59	-2.19	0.02
12	5	2.80	4.33	0.80	-1.91	0.06
13	6	2.50	4.67	0.50	-4.34	0.004
14	2	6.00	5.00	3.00	0.33	0.40
15	1	5.00	5.33	..	..	..
16	0	..	..	..	..	..
17	1	6.00	6.00	..	..	..
18	0	..	..	..	..	..
19	0	..	..	..	..	..
20	0	..	..	..	..	..
21	1	5.00	7.33	..	..	..
≥7	180	2.68	3.24	0.12	-4.54	<0.0001

**Table 5: Difference in birth-order position between the first and second siblings with MS in multiplex families**

which is almost two orders of magnitude larger, makes this concept doubtful.

Environmental factors affect MS risk independent of the effect of genes.<sup>10,30</sup> Findings from twin studies challenged the belief that MS is due to a persistent infection and suggested that climate, diet, or both accounted directly or indirectly for the environmental effect in MS.<sup>2</sup> Subsequent studies of adoptees,<sup>5</sup> half-siblings,<sup>6,7</sup> and conjugal pairs<sup>8</sup> have independently corroborated the view that environmental factors act across the broad population. Despite a 30–50 times increase in risk for first-degree relatives of patients with MS,<sup>5</sup> these large studies have not shown any trends for increased risk attributable to common family environment or cohabitation. Nevertheless, it can be argued that these studies have not conclusively excluded the possibility that birth-order position, sibship size, and attendant subtle differences in environmental exposures and the timing of ubiquitous infections have a role in MS risk.

The concept of a common infection as modified by host factors causing disease remains viable in MS. This could be difficult to distinguish from non-infectious ubiquitous exposures by epidemiological methods and requires a central role for host factors. A recent study suggested a potential role for EBV infection at an early age with earlier conversion of serology characterising cases of MS in childhood.<sup>31</sup> By contrast, however, Levin and colleagues<sup>32</sup> described an age-dependent relation between infection with EBV and MS, but only for individuals aged 25 years and older. Prospective studies from Denmark<sup>33</sup> have shown no difference between early



life viral infections in adults with MS and controls and are definitive in our view.

Changes from expected birth-order position have also been attributed to factors including parental age, socioeconomic status, maternal nutrition, and the emergence of maternal–fetal incompatibility with serial pregnancies. Birth-order studies in MS have been inconclusive or negative,<sup>12–14</sup> but may have been underpowered to detect conceptually important effects. A recent paper by Montgomery and co-workers<sup>34</sup> reported an association of MS risk with younger siblings; the odds ratio for people with three or more younger siblings to develop MS compared with those with none was 0.80 (95% CI 0.70–0.96). However, this effect was smaller when the data were adjusted for paternal age, and the data need replication in an independent case-control study.

The design of our study is not case-control, and this might introduce bias and invalidity.<sup>35,36</sup> Berglin<sup>37</sup> commented that differences between observed and expected birth order could be the result of death or physical removal of participants from the area of the study. However, the unique structure of the database for the CCPGMS enabled inclusion of every live-born member of each sibship, whether alive or dead at the time of the study.<sup>13</sup> Therefore we think that the study presented here results in a representative sample of a model population, and could eliminate biases of a case-control design. We believe that individuals without MS who have siblings with MS are better controls than individuals from families without MS.

We found that siblings with MS from simplex sibships were younger than their healthy siblings and, like the first sibling with MS in multiplex sibships, showed no tendency for earlier birth order. Furthermore among the large families, in which the size of any effect would be most clear, the birth-order results did not support the hygiene hypothesis. Analysis of half-sibling data found no relation between the presence of MS and sibship size.

This study does not support the prediction of the hygiene hypothesis, which suggests that people with MS would be born earlier than expected within their sibships. However, a more general protective effect of poor hygiene might not differentiate among birth-order positions. The possibility that the overall MS risk is increasing is suggested by the higher birth order than expected among large pedigrees, but this is speculative. The data presented here cast no doubt on the importance of environmental factors to MS risk, and suggest that environmental risks for MS must be accounted for by factors that do not affect birth-order position. Similar studies in polio, in which the nature and epidemiology of infection are known, show a U-shaped pattern of birth-order-related susceptibility, with high risk in the oldest and the youngest children in sibships.<sup>38</sup> This pattern may reflect epidemic infection and group effects related to family size and crowding,

as well as introduction of risk by younger children. Our data show no analogy with polio. In combination with extensive data from half-siblings,<sup>6,7</sup> adoptees,<sup>5</sup> conjugal pairs, step-siblings,<sup>39</sup> and consanguineous matings,<sup>9</sup> birth-order studies suggest that environmental effects in MS act on susceptible hosts at a broad population level.

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#### Authors' contributions

ADS and GCE designed and coordinated the study and interpreted the statistical analyses. IMLY did the statistical analyses. All authors wrote and edited the paper.

#### Conflicts of interest

We have no conflicts of interest.

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