Clinical features and viral serologies in children with multiple sclerosis: a multinational observational study

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Summary

Background **The full spectrum of clinical manifestations and outcome, and the potential importance of regional or demographic features or viral triggers in paediatric multiple sclerosis (MS), has yet to be fully characterised. Our aim was to determine some of these characteristics in children with MS.**

Methods **137 children with MS and 96 control participants matched by age and geographical region were recruited in a multinational study. They underwent structured clinical-demographic interviews, review of academic performance, physical examination, disability assessment (MS patients only), and standardised assays for IgG antibodies directed against Epstein-Barr virus, cytomegalovirus, parvovirus B19, varicella zoster virus, and herpes simplex virus.**

Findings **MS was relapsing-remitting at diagnosis in 136 (99%) children. The first MS attack resembled acute disseminated encephalomyelitis (ADEM) in 22 (16%) of the children, most under 10 years old (mean age 7·4 [SD 4·2] years). Children with ADEM-like presentations were significantly younger than were children with polyfocal (11·2 [4·5] years; p<0·0001) or monofocal (12·0 [3·8] years; p=0·0005) presentations. Permanent physical disability (EDSS≥4·0) developed within 5 years in 15 (13%) of the 120 children for whom EDSS score was available. 23 (17%) had impaired academic performance, which was associated with increasing disease duration (p=0·02). Over 108 (86%) of the children with MS, irrespective of geographical residence, were seropositive for remote EBV infection, compared with only 61 (64%) of matched controls (p=0·025, adjusted for multiple comparisons). Children with MS did not differ from controls in seroprevalence of the other childhood viruses studied, nor with respect to month of birth, sibling number, sibling rank, or exposure to young siblings.**

Interpretation **Paediatric MS is a relapsing-remitting disease, with presenting features that vary by age at onset. MS in children might be associated with exposure to EBV, suggesting a possible role for EBV in MS pathobiology.**

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Introduction

Onset of multiple sclerosis (MS) in childhood is being increasingly recognised worldwide.[1–12] To date, there are no multinational studies comparing the demographic features, clinical manifestations, outcomes, and putative viral triggers of MS in children.

In adults with MS, local environmental factors such as viral infections experienced before the age of 15 years have been proposed as important determinants of MS risk.[13–15] Epstein-Barr virus (EBV) is one of the viruses associated with MS in adults,[16–22] and more recently with MS in children.[23,24] However, the causative role of EBV in adult-onset MS is challenged by the inherent delay between early-life exposure to the virus and presentation of MS. Onset of MS in childhood, however, provides a unique opportunity to explore putative viral exposures in patients temporally close to the biological events contributing to disease onset.

Our aim was to compare the clinical features and outcome of paediatric patients with MS enrolled from diverse geographical regions, and to assess whether viral associations exist in paediatric MS, whether they are consistent across regions, and whether seroprevalence differs between children with MS and matched children without MS.

Methods

Patients

Paediatric patients with MS were enrolled from 17 sites across four geographical regions (Canada/northern USA, southern USA, South America, and Europe). Children with MS were matched with non-MS control participants by year of birth and region of enrolment.

Children with a diagnosis of definite MS[25,26] before the age of 18 years were eligible for enrolment. Patients over the age of 18 years were enrolled if their diagnosis (at age <18 years) and ongoing management were still under the supervision of the participating paediatric facility. Children whose first demyelinating episodes resembled acute disseminated encephalomyelitis (ADEM), but who subsequently had recurrent disease typical of MS were included; children with recurrent or multiphasic ADEM episodes,[27,28] as well as children with the clinical diagnosis of neuromyelitis optica,[29] were excluded.

Healthy children were recruited at the time of elective orthopaedic or dental procedures, or emergency room assessment for minor illness. Children with non-demyelinating neurological disorders (epilepsy, cognitive impairment, or migraine) were recruited from neurology clinics. Each control participant was matched by year of

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birth with an MS participant enrolled from the same region, although controls were not enrolled from Europe because of the lack of availability of participants or because of local ethics board regulations.

Written informed consent was obtained from parents or guardians. Oral assent was obtained from all participants. The study was approved by the institutional review boards of all participating institutions.

Procedures

Age, sex, country of birth, country of birth of the parents, immigration history, country of residence, immunisation history, past medical illnesses, medications, family history of neurological and autoimmune disease, and sibling information (number and date of birth) were obtained from both case and control participants by direct interview.

For the children with MS, the clinical features of the first and second demyelinating events, disease course, treatments, academic performance, and current functional status (questionnaire) were recorded. Physical examination findings and expanded disability status scale (EDSS)[30] scores were ascribed on the day of enrolment. We also noted whether the EDSS score obtained was concurrent with an acute MS relapse. Academic performance, as reported by parental interview, was defined as follows: above average (achieves "A" or "greater than 80%" grade point average), normal performance (achieves a "B or C", or "60–80%" grade point average), minimal impairment (unable to achieve a "pass" or ">50%" in several classes), moderate impairment (in a regular classroom, but with direct academic support), or severely impaired (unable to function in a regular school setting).

One investigator (BB) categorised the clinical features of the initial and second attacks for all MS patients. Clinical features were deemed monofocal if they could be localised to a single location within the CNS, polyfocal if features implicated involvement of several CNS locations,[31] or ADEM if a polyfocal presentation was associated with encephalopathy, based on consensus definitions.[28] Categorisation was based solely on clinical features without consideration of lesion distribution on MRI. Fatigue, headache, and fever were considered non-localising.

Optical neuritis, defined as acute or subacute visual loss (as measured using Snellen charts), relative afferent pupillary defect, restricted visual fields, and pain with ocular movement,[32] and transverse myelitis, defined by clinical evidence of a spinal cord syndrome, MRI or CSF evidence of inflammation, and exclusion of spinal cord compression,[33] were also assessed in the children with MS.

A standardised protocol was used to detect viral antibodies. Serum samples were aliquoted into 1 mL cryovials, frozen at –70°C, and shipped on dry ice to the licensed microbiology laboratory at the Hospital for Sick Children (Toronto, Canada), where all samples were processed, blinded to clinical diagnosis. Samples were analysed with standardised ELISA kits for IgG antibodies directed against EBV capsid (EBV-VCA), nuclear (EBV-EBNA), and early antigens (EBV-EA; all from DiaSorin, Stillwater, MN, USA), cytomegalovirus (CMV; Zeus Scientific, Rarigan, NJ, USA), parvovirus B19 (Biotrin International Ltd, Mount Merrion, Ireland); varicella zoster virus (VZV; Zeus Scientific or Dade Behring, Marburg, Germany), and herpes simplex virus (HSV; Adaltis Italia SPA, Via Christoni, Italy). CMV, parvovirus B19, VZV, and HSV serologies were recorded as positive or negative as per the manufacturer's criteria.

Using predetermined cutoff values based on the manufacturer's criteria, patients were classified as remotely infected if EBV antibodies against both VCA and EBNA1 were detected, recently infected if antibodies against VCA and EA were detected, or EBV-naive if none of the three EBV antibodies were detected. Samples that were seropositive for antibodies against VCA alone were viewed as indeterminate for timing of EBV exposure. Samples positive for VCA, EBNA1, and EA were considered as remotely infected based on the presence of EBNA1 antibodies, since the coexistence of the EA antibodies in remotely infected patients can occur. A second aliquot from EBNA-positive sera was used to determine IgG anti-EBNA1 titres, as per the manufacturer's instructions.

	Canada and northern USA	Southern USA	South America	Europe	All MS participants
Number	75 (55%)	20 (15%)	26 (19%)	16 (12%)	137
Age at enrolment (years)	14·6 (5·8–19·4)	15·7 (6·7–19·6)	11·1 (2·24–17·9)	15·1 (7·8–18·3)	14·1 (2·2–19·6)
Sex ratio (female to male)	1·6:1	2·3:1	1·2:1	1·3:1	1·5:1
Ethnic origin					
White	42 (56%)	4 (20%)	6 (23%)	16 (100%)	68 (50%)
Black	7 (9%)	7 (35%)	0 (0%)	0 (0%)	14 (10%)
Other	26 (35%)	9 (45%)	20 (77%)	0 (0%)	55 (40%)
Country of enrolment	Canada (n=50) USA (n=25)	USA (n=20)	Argentina (n=26)	Russia (n=12) Italy (n=3) Finland (n=1)	

Data are n (%) or mean (range), unless otherwise specified.

Table 1: Demographic features of the paediatric MS participants

Statistical analysis

Descriptive statistics were used to summarise the clinical features of the MS patients. Student's *t*-tests and χ^2 tests were used to examine relations between EBV status (positive for remote infection, or EBV negative), measures of disease severity (relapse rate, EDSS), age at first attack, and sex. For the analyses of academic functioning, analyses of variance (ANOVA) were done to the level of academic functioning as related to age at disease onset and mean duration of illness.

ANOVA was used to compare the age at first attack across the four regions. Following an omnibus significant F-test, the Duncan's multiple range test was used to determine which of the regions differed from one another.

Logistic regression analyses were used to assess: (1) whether outcome features of the initial attack differed between the regions of enrolment, adjusting for age at presentation and sex (ADEM was grouped with polyfocal for these analyses because there were so few children with ADEM); (2) whether age at sampling affected the likelihood of remote EBV infection; (3) whether the outcome of remote EBV infection differed between children with monofocal, polyfocal, or ADEM presentations, adjusting for age at first attack and for region of enrolment; and (4) whether seropositivity for CMV, parvovirus B19, VZV, or HSV differed as a function of region of enrolment, adjusting for age at sampling. *t*-tests were used to determine whether relapse rate or EDSS differed between patients seropositive for remote EBV infection and EBV-negative MS patients. Logistic regression (adjusting for age at sampling) was done to assess whether the outcomes relapse rate and EDSS score were affected by remote infection with both HSV and EBV, remote EBV infection in the absence of previous infection with HSV, or seronegativity for EBV.

Viral titre analyses were done for EBV EBNA1 based on microarray studies that have shown EBNA1 to be the most common EBV protein targeted by antibodies in serum of MS patients.[34] Natural logs of the EBNA1 antibody titres were calculated because titres did not follow a normal distribution, and a Student's *t*-test was

	Findings
MS disease course	
Relapsing-remitting	131 (95%)
Secondary progressive	5 (4%)
Marburg variant	1 (1%)
Method of diagnosis	
Poser*	125 (91%)
International paediatric MS criteria†	133 (97%)
McDonald positive by MRI‡	12 (9%)
Sex	
Female–to–male ratio	1·54:1
Ratio in patients where age at first attack <10 years	1·1:1
Ratio in patients where age at first attack >10 years	1·8:1
Disease characteristics	
Disease duration at time of sample (years)	3·1 (2·3, 0·1–16·5)
EDSS score§	1·5 (0–8·5)
Duration between first and second attack (years)	0·9 (0·04–7·0)
Total number of attacks	3·9 (1–16)
Total number of attacks in first 2 years	2·7 (1–8)
Annualised relapse rate¶	1·2 (0·1–3·9)
Treatment at enrolment||	
Interferon beta-1a (Avonex)	23 (17%)
Interferon beta-1a (Rebif)	24 (18%)
Interferon beta-1b (Betaseron)	16 (12%)
Glatiramer acetate (Copaxone)	12 (9%)
Cyclosphosphamide (Cytoxan)	3 (2%)
Mitoxantrone (Novantrone)	1 (1%)
Azathioprine (Imuran)	4 (3%)
Oral prednisone	7 (5%)
Intravenous methylprednisolone	11 (8%)
None	48 (36%)
Mobility	
Normal	106 (77%)
Limits ambulation/participation because of gait	23 (17%)
Uses cane occasionally	1 (1%)
Uses cane for most of the time	0
Needs cane/support at all times	0
Bilateral support (crutches/walker)	1 (1%)
Wheelchair-intermittently walks for short distance	5 (4%)
Wheelchair-dependent	1 (1%)
Vision	
Normal vision (with corrective lenses)	101 (74%)
Decreased vision (despite corrective lenses)	24 (18%)
Severe visual impairment (requires visual aids)	6 (4%)
Total visual disability (no functional vision)	2 (2%)
Unknown	2 (2%)
Not done	2 (2%)

Data are n (%), mean (range), or mean (median, range). *Children were considered as Poser-criteria positive[25] if they experienced two or more demyelinating events, involving distinct areas of the central nervous system, separated by at least 30 days. †Diagnostic criteria for paediatric MS.[28] ‡Children were considered as McDonald MRI-positive[26] if they experienced a single demyelinating event, but showed evolution of new enhancing white matter lesions on MRI scans obtained more than 30 days from an initial scan, or new lesions on T2-weighted images obtained from two or more MRI scans done more than 3 months from an MRI obtained at the time of the initial demyelinating event. §EDSS scores were provided for 129 children. Nine additional children were experiencing a MS-related relapse on the day of scoring and were excluded from the analysis. Removal of these patients from the analysis did not change analyses of EDSS and outcome. Data are median (range). ¶Measured in 71 patients with disease duration >2 years. ||Some children were treated with more than one immunomodulatory or immunosuppressive medication. At the time of sample procurement, one child was receiving intravenous solumedrol for an acute relapse, and ten had received intravenous solumedrol within 30 days of sampling, but had recovered from the recent relapse. Two children were receiving antidepressants, three children were on medication for spasticity, and 12 were receiving anticonvulsant therapies either for seizure management or for dysaesthesias. Four children were on medication for fatigue.

Table 2: **Clinical features of the paediatric MS participants**

used to analyse the mean titres. Titre values from the positive samples (>20 AU/mL) obtained from non-MS children were divided into tertiles.[35] χ^2 tests were used to assess the difference in the frequency distribution of the EBNA1 titres between cases and controls. For matched pairs, a paired *t*-test was used to compare EBNA1 titres.

Conditional logistic regression for a 1:1 matched design was used to assess whether the outcome differed between cases and controls as a function of seropositivity for each of remote EBV infection, CMV, parvovirus B19, VZV, or HSV, adjusting for multiple comparisons with a Bonferroni correction; and seropositivity as defined by a three-level variable combining EBV and HSV as follows: both EBV and HSV; EBV but no HSV; or no EBV. This variable was assessed for its association with MS by use of conditional logistic regression.

Conditional logistic regression was used to test whether any particular month of birth was more associated with MS. December was arbitrarily chosen as the reference month. A matched design could not be used to assess familial sibling relationships, since we excluded participants with half-siblings or adopted siblings for whom sibling co-habitation patterns could not be determined. *t*-tests were used to determine whether cases and controls differed as a function of sibling rank, place in the sibship (oldest or youngest), mean number of siblings, and early life (defined as birth to age 6 years) absolute and cumulative sibling exposure to all siblings, preschool age young siblings (<6 years), or school-age siblings. For early life exposures, absolute sibling exposure was defined as the total number of years that a participant was exposed to one or more siblings; cumulative sibling exposure was defined by the sum of years of exposure to each sibling.

Statistical analyses were done with SAS version 9.1.

	Attack 1	Attack 2
Age at attack (years)	11·0 (1·6–17·9)	11·9 (2·0–18·0)
Monofocal	72 (53%)	87 (64%)
Age (years)	12·0 (12·5, 1·6–17·3)	12·4 (13·3, 2·4–17·6)
Polyfocal	43 (31%)	31 (23%)
Age (years)	11·2 (12·5, 1·9–17·2)	12·2 (12·3, 2·1–18·0)
ADEM	22 (16%)	7 (5%)
Age (years)	7·4 (6·4, 2·1–17·9)	6·7 (7·6, 2·0–10·2)
McDonald positive	12 (9%)	..
Age (years)	10·8 (13·3, 3·2–16·4)	..

Data are n (%), mean (range), or mean (median, range).

Table 3: **Attack history in paediatric patients with MS**

	Number of patients	Disease duration (years)
Above average (receives mostly As in school)	33 (24%)	2·5 (2·1)
Normal (functions well in school)	55 (40%)	2·8 (2·2)
Minimal (struggling but obtains passing grades)	16 (12%)	3·7 (3·4)
Moderate (needs assistance with school work)	18 (13%)	4·7 (3·1)
Severe (unable to function in regular classroom)	5 (4%)	5·4 (6·6)
Too young for school	5 (4%)	1·3 (1·0)
Unknown	3 (2%)	1·7 (1·4)
Not asked	2 (2%)	5·7 (1·6)

Data are n (%) or mean (SD).

Table 4: **Academic performance of paediatric patients with MS**

Role of the funding source

The study sponsor had no role in the design or conduct of this study. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

137 children with MS were enrolled between February, 2003, and June, 2006 (table 1). 12 patients were over the age of 18 years at enrolment. Children enrolled from South America were younger than children enrolled from the other regions (p<0·0001). 96 control participants (47 healthy children, and 49 children with minor neurological disorders) without MS were also enrolled.

The clinical features of the children with MS are shown in table 2. The female to male ratio was lower in the 44 children who experienced their first demyelinating event under age 10 years than for the 93 older children (p=0·03; table 2). Age less than 10 years was selected as an approximation of pre-puberty,[36] although formal Tanner pubertal staging was not done.

The relative proportion of monofocal, polyfocal, or ADEM-like phenotypes at first attack and second attack, and the age of children with each presentation are shown in tables 2 and 3. All but one of the 137 children with MS experienced a relapsing-remitting disease course. The exception was a child (enrolled from Argentina) who experienced a highly aggressive demyelinating phenotype (Marburg variant), characterised by MRI disease progression in the absence of clinical relapses. Five (4%) children developed secondary progressive MS after a median disease duration of 5·8 (range 2·8–10·4) years. No child had primary progressive MS.

Monofocal features at first attack were more common in European patients than in children enrolled from Canada/northern USA (odds ratio [OR] 2·04, 95% CI 0·59–7·04), southern USA (4·4, 1·01–2·0), and South America (10·0, 2·08–50·0). The increased presentation of monofocal features at onset in children enrolled from Europe, and conversely the increased presentation of polyfocal features (including ADEM) in South American children remained significant, even when adjusted for age at presentation (p=0·0095). ADEM was the initial clinical diagnosis in 22 (16%) children. All but four of the 22 children have experienced more than two clinical demyelinating events. In the four children with only two demyelinating events, the second event was monofocal in all four, and occurred more than 3 months after initial presentation. No child met criteria for multiphasic

ADEM.[28] Recently proposed diagnostic criteria for paediatric MS require that children presenting with an ADEM-like first demyelinating event experience two non-ADEM attacks to confirm a diagnosis of MS.[28] However, because these new criteria were not available at the time of study enrolment, we elected not to retrospectively exclude these four children. Figure 1 shows the relation between presenting phenotype and age at onset. Children with an ADEM-like presentation were significantly younger (mean age 7·4, SD 4·2 years) than were children with polyfocal (11·2 [4·5], p<0·0001) or monofocal (12·0 [3·8] years, p=0·0005) presentations. The mean age of the monofocal and polyfocal patients did not differ (p=0·3). Most children had little or no physical impairment, as assessed by EDSS score (table 2). 15 (13%) children had EDSS scores greater than or equal to 4·0, five of whom had an EDSS score greater than 6·0 (requirement for ambulatory aid). The EDSS score at enrolment did not differ as a function of age at first demyelinating attack (p=0·25), age at study enrolment (p=0·99), or relapse rate (p=0·33). However, mean disease duration was 5 years for the 15 children with EDSS scores of 4·0 or greater, compared with 2·9 years for the 105 children with lower EDSS scores. Academic performance was negatively affected by longer disease duration (p=0·02; table 4). The mean age at disease onset was younger in the children with severe academic impairment than in children with normal or above average academic performance, although the small number of children in the severely impaired group limited statistical power. Fatigue of sufficient severity to interfere with normal daily activity was reported by 61 (45%) of the children on the day of study enrolment; 82 (60%) reported experiencing such fatigue at some point in their disease course. At the time of enrolment, 88 (64%) of the 137 children were receiving disease-modifying therapy for MS (table 2). Three of the children not on therapy at the time of enrolment had received interferon treatment in the past.

Optic neuritis occurred in 30 (22%) children. 23 of these children experienced optic neuritis as a monofocal event (17 unilateral, five bilateral, one non-specified), four as a component of a polyfocal attack (two unilateral, two bilateral), one child had bilateral optic neuritis concurrent with transverse myelitis, and two children had optic neuritis (one unilateral, one bilateral) in the context of ADEM. Mild optic neuritis may have been missed, and results of visual evoked potentials were not available. Monofocal brainstem or cerebellar symptoms occurred in 25 (18%) children. Transverse myelitis occurred in 31 (23%) children, most of whom experienced it in the contest of polyfocal symptoms or as a component of an ADEM-like attack. Only nine (7%) children presented with monofocal transverse myelitis.

The frequency of EBV, CMV, HSV, parvovirus B19, and VZV seropositivity by region is shown in table 5. CMV and HSV were the only viruses that differed in terms of seropositivity when compared across all four regions. For

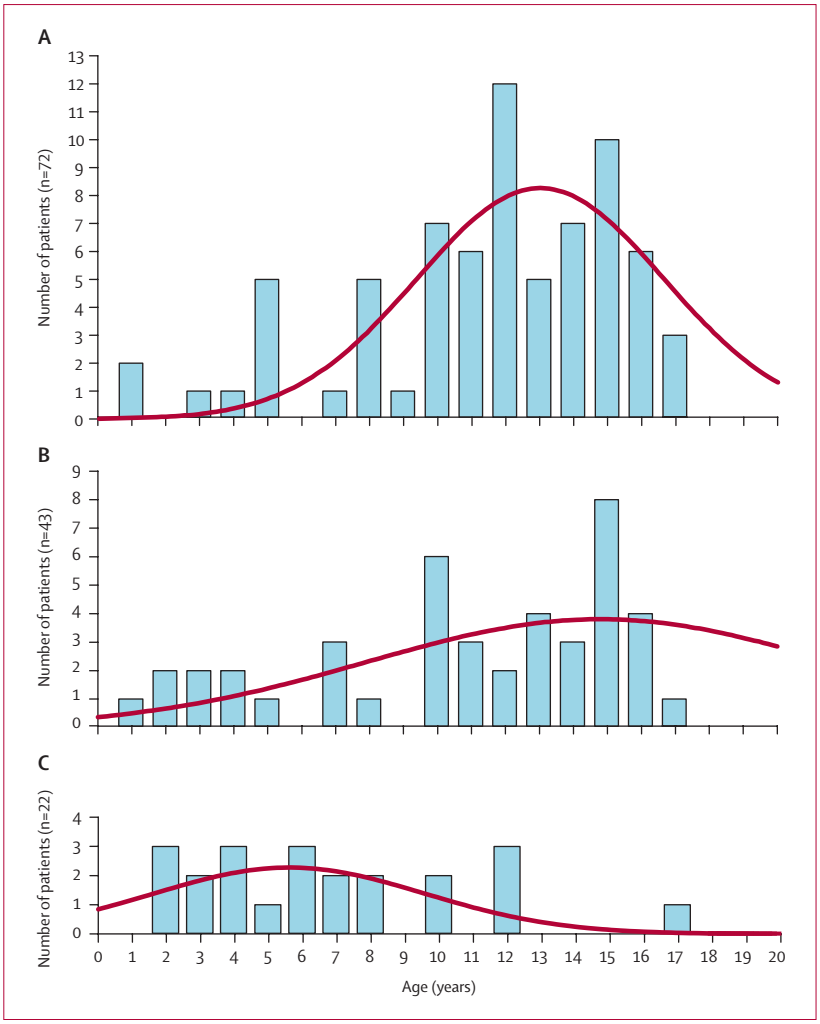

***Figure 1*: Age at first attack, by phenotype**
(A) Monofocal. (B) Polyfocal. (C) Acute disseminated encephalomyelitis. Red line represents the normal curve.

CMV, the OR for South America versus Canada/northern USA was 2·9 (95% CI 1·0–8·0, p=0·04); for South America versus southern USA it was 9·3 (1·8–47·5, p=0·007); and for Europe versus southern USA it was 5·3 (1·1–26·6, p=0·04). For HSV, the OR for South America versus Canada/northern USA was 5·7 (1·9–17·1, p=0·002), for South America versus southern USA was 15·5 (2·8–85·1, p=0·002), and for Europe versus southern USA was 6·9 (1·3–40·0, p=0·02). However, the seropositivity for CMV and HSV was under-represented in children enrolled from the southern USA, and after adjustment for multiple comparisons, the differences in CMV seropositivity remained significant for the comparison of South America with southern USA only (p=0·042), and for HSV for the comparison of South America with Canada/northern USA (p=0·01) and South America with southern USA (p=0·01) only.

EBV seroprevalence increased as a function of increasing age (mean age at sampling of the 108 EBV-

	Canada and northern USA	Southern USA	South America	Europe	Number of seropositive patients/number of patients with definitive serology* (%)	p value*
EBV (remote)	57 (81%)	13 (93%)	22 (85%)	16 (100%)	108/126 (86%)	0·18
CMV	32 (44%)	3 (20%)	17 (68%)	9 (56%)	61/124 (49%)	0·04
HSV	28 (39%)	3 (21%)	19 (73%)	10 (63%)	60/133 (45%)	0·003
Parvovirus B19	37 (64%)	8 (57%)	13 (54%)	5 (33%)	63/107 (59%)	0·18
VZV	64 (90%)	14 (93%)	19 (79%)	13 (87%)	110/126 (87%)	0·77

Data are number of seropositive patients (%), unless specified otherwise. Patients with insufficient samples or patients with indeterminate results excluded. *For comparison of seroprevalence for each virus across the four regions. Logistic regression was done, adjusting for age at sampling, with Canada/northern USA arbitrarily designated as the reference. Exploratory analysis, unadjusted for multiple comparisons.

Table 5: **Viral seroprevalence by region of enrolment in the participants with MS**

positive MS patients, 14·8 years; mean age at sampling of the 18 EBV-negative MS patients 10·3 years, p<0·0001). Although children presenting with a monofocal attack were 3·64 times (95% CI 1·2–10·9) more likely than children with a polyfocal attack (polyfocal and ADEM grouped together) to have evidence of remote EBV infection (p=0·02), this observation was not significant after adjustment for age at sampling (p=0·17). EBV seroprevalence did not differ as a function of sex (68 [88%] female patients were EBV-positive *vs* 40 [81%] of male MS patients; p=0·41), relapse rate (mean relapse rate in EBV-positive patients 1·18 [SD 0·7] *vs* 1·01 [0·6] in EBV-negative patients; p=0·49), or EDSS score at enrolment (mean EDSS in EBV-positive patients 1·9 [1·6] *vs* 2·06 [1·9] in EBV-negative patients; p=0·79). Co-infection with EBV and HSV, as compared with remote infection with EBV in the absence of HSV, or seronegativity for EBV did not affect EDSS scores (p=0·13) or relapse rates (p=0·09) after adjusting for age at enrolment.

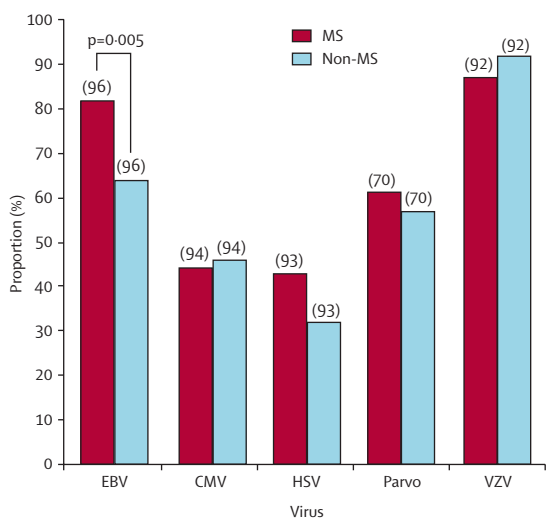

Figure 2: **Seroprevalence of common childhood viruses in children with MS and in control participants**
The number of patients tested for each virus is indicated in the brackets above each bar. Only 96 of the 137 paediatric MS patients were able to be matched with a non-MS control. 12 of the 41 unmatched MS patients did not have serum available for analysis. Viral serology results of the remaining 30 unmatched MS patients (16 enrolled from Europe, nine from South America, and five from Canada/northern USA) revealed the following seropositivities: 97% EBV, 57% CMV, 63% HSV, 48% parvovirus B19, and 90% VZV. Inclusion of these 30 patients would thus only have served to further strengthen the association of remote EBV infection and MS, and would not have affected the findings for the other viruses. CMV=cytomegalovirus. EBV=Epstein-Barr virus. HSV=herpes simplex virus. Parvo=parvovirus B19. VZV=varicella zoster virus.

Figure 2 compares the seroprevalence for remote EBV infection, HSV, CMV, parvovirus B19, and VZV between the 96 participants with MS and their age-matched and geographically matched controls. Children were 2·8 times (95% CI 1·4–5·8) more likely to be in the MS group if they were seropositive for remote EBV (p=0·025 after adjustment for multiple comparisons); sex did not affect this correlation. By contrast, seropositivity for CMV (p=0·88), parvovirus B19 (p=0·75), VZV (p=0·23), and HSV (p=0·1) did not affect the likelihood of being in the MS or non-MS groups. However, co-infection with EBV and HSV was associated with a 3·2 times increased likelihood of being in the MS group (95% CI 1·3–7·4, p=0·02).

EBNA1 titres were compared between the 73 EBNA1 positive patients with MS and 54 EBNA1 positive non-MS controls for whom residual serum was available for analysis. As shown in table 6, the mean EBNA1 titres were significantly higher in EBV-positive MS patients than in EBV-positive controls; 40 (54%) of children with MS had EBNA1 titres in the highest antibody tertile. Of the 73 patients with MS and the 54 controls, 41 were pairs matched by age and region. For these matched pairs, the mean titre in the MS group was significantly higher than that of the matched non-MS participants (187·7 AU [SD 60·05] *vs* 153·22 AU [77·16]; p=0·05).

Comparison of 96 MS patients and 96 non-MS control participants showed no differences in the frequency of a family history of MS (8 [8%] of MS patients *vs* 12 [13%] of non-MS participants; p=0·34), the mean number of siblings per patient in the two groups (p=0·24), sibling rank (p=0·15), or birth order (oldest or youngest) (p=0·38). Children with MS did not differ from non-MS controls in the absolute or cumulative number of years of exposure to all siblings (absolute p=0·88; cumulative p=0·24), preschool age siblings (absolute p=0·78; cumulative p=0·96), or school-age siblings (absolute

	MS (n=73)	Non-MS (n=54)	p value
EBNA1 titre	187·4 (59·5)	152·5 (70·1)	0·006
Low (AU<110)	7 (9·6%)	18 (33%)	0·002*
Moderate (110≤AU≤195)	26 (36%)	18 (33%)	
High (AU>195)	40 (54%)	18 (33%)	

Data are mean (SD) or n (%). *p value, based on a χ^2 test comparing frequency distribution of EBNA1 titres between patients and controls, refers to the relative distribution across the three tertiles.

Table 6: **Comparison of EBNA1 titres between MS and non-MS participants**

p=0·28; cumulative p=0·21). Cases and controls did not differ in terms of month of birth (p=0·3).

Discussion

Our results suggest that the clinical features of paediatric MS at presentation differ by region of enrolment and by age at presentation. We have also shown an association between remote EBV infection and MS in children; this association seems to be consistent across several diverse geographical regions. Factors previously linked to MS in adults—eg, reduced exposure to young siblings early in life and being born in May—do not seem to have significant effects on MS in children.

Interpretation of the relative frequency of clinical features at MS onset from the available literature is challenged by variable categorisation. More than half the children in this study who were eventually diagnosed with MS presented with monofocal symptoms, about a third with polyfocal features, and 16% with a clinical presentation indistinguishable at onset from ADEM. Of interest, ADEM seemed to occur more commonly in younger children, whereas monofocal symptoms, reminiscent of the typical features of adult-onset MS, were more represented in adolescents. Whether the effect of age on clinical manifestations of the first demyelinating event represents a differential capacity of the maturing nervous system for widespread CNS involvement or age-dependent immunological capacity for multifocal targeting of the CNS remains to be more fully explored.

We used a consistent clinical definition to characterise the first clinical attack as ADEM, with the requirement for polyfocal features and encephalopathy.[28] With a similar clinical definition, ADEM was also the first attack phenotype of 29% of 168 children ultimately diagnosed with relapsing-remitting MS reported from France.[1] About 30% of adults with ADEM will experience relapses leading to the diagnosis of MS.[37] Thus, clinical features consistent with ADEM at presentation do not exclude the future diagnosis of MS in children or adults. In a recent study, anti-myelin oligodendroglial protein antibodies were detected in serum from adults and children with monophasic ADEM, a finding rarely detected in patients with relapsing-remitting MS.[38] Development of biomarkers capable of distinguishing ADEM from the first attack of MS would be of great clinical relevance.

The relative proportion of children with monofocal compared with polyfocal/ADEM-like presentations differed by region. Children enrolled from South America were more likely to experience an ADEM-like presentation, whereas European patients were more likely to have a monofocal presentation. These results raise the possibility that regional environmental or genetic factors could affect clinical manifestations of acute demyelination. However, one should note that the South American patients were enrolled from a single, large regional referral centre, and future studies involving many facilities are required to confirm whether an ADEM-like first demyelinating event is more likely in South American children.

In addition to categorisation of first attack features, specific clinical presentations were also reviewed. The low frequency of children presenting with monofocal transverse myelitis was similar to that noted in a series of acute demyelination in children reported from France,[1] suggesting that isolated transverse myelitis is an infrequent presentation of MS in children.

MS in children is a relapsing-remitting disease. None of the 137 paediatric MS patients experienced a primary progressive MS course; by contrast, such disease occurs in an estimated 10–15% in adults.[39] Primary progressive disease is more common in older adult MS patients, raising the possibility that age contributes to the progressive deterioration characteristic of such a disease course. Whether the young age of paediatric-onset MS patients provides some protection from these processes remains to be determined. Also distinct from adult MS is a lower female-to-male ratio in children. This distinction was particularly pronounced for children presenting under age 10 years; the ratio in older children and adolescents was more similar to the 2·0:1 ratio reported in adults from similar demographic regions.[40] The age-related increase in female preponderance at puberty could suggest a pathogenic role for female sex hormones, a protective role of male sex hormones, or age-related sex differences in the expression of genes involved in immune regulation.

After a mean disease duration of only 5 years, 15 (13%) children with MS showed fixed neurological deficits that limited their ambulation (EDSS≥4·0), similar to that reported by Mikaeloff and colleagues,[2] who documented EDSS scores of 4 or greater in 30 (15%) of 197 children with MS enrolled in the French KIDSEP study after a median observation of 4·8 years (from second demyelinating event). Academic performance was negatively affected by increasing disease duration. However, assessment of academic performance on the basis of academic grades alone could underestimate the deleterious effects of MS on cognitive capacity and academic potential; furthermore, data on academic performance before the onset of MS was not available. The full effect of MS on cognitive development in children requires detailed and longitudinal neuropsychological assessments.[41,42]

Less than two-thirds of the MS patients were receiving immunomodulatory or immunosuppressive treatment at the time of study enrolment. The proportion of children receiving these therapies did not differ between geographical regions. Prospective studies of immunomodulatory therapies in children are required to elucidate issues such as access to treatment, rationale for treatment initiation, and assessment of efficacy. Increasing recognition of the safety and tolerability of MS-immunomodulatory therapies in children provides the necessary platform for these future studies.[43–50]

Over 80% of the children with MS, irrespective of geographical residence, were seropositive for remote EBV infection. Seropositivity for EBV was associated with an almost three times increased likelihood of MS compared with age-matched and region-matched controls. Children with MS also had significantly higher EBV-EBNA1 titres, and were more likely to have EBV titres in the highest tertile, than controls. The higher EBV titres could represent heightened immunological responses to EBV, as has been suggested in a similar analysis comparing EBNA1 titres in EBV-positive adult MS patients and controls.[35] Studies of T-cell proliferation to HLA-restricted EBV antigens in EBV-positive children with MS compared with EBV-positive healthy children are now underway. Increased EBV titres might be due to episodic viral reactivation, a process that has been postulated to occur with greater frequency in MS patients than in individuals without MS, especially in association with MS relapses.[51] PCR analysis of EBV DNA in saliva or plasma is required to ascertain lytic cycle occurrence, and to correlate this with clinical disease activity. Of note, to minimise potential cross-reactivity with self-antigens, we specifically selected an EBNA-1 EIA assay that relies on synthetic peptides, and excluded the Gly-Ala repetitive sequence known to cross-react with self epitopes from collagen, keratin, and actin.[52–54]

It has been postulated that previous infection with other herpes viruses could affect the immunological response to EBV. We analysed the effect of co-infection of EBV and HSV, since it has been suggested that individuals infected with HSV could be at lower risk of MS.[55] However, we found that children seropositive for both EBV and HSV were more likely to have MS, as compared with EBV-positive, HSV-negative children. Exposure to many viruses before infection with EBV is more likely to occur in families with several small children, and it has been suggested that individuals exposed to siblings early in life could have a lower risk of MS.[56] However, we could not detect any difference in the number of siblings, or in exposure to younger siblings, between MS and non-MS participants.

Infection with EBV is not a requisite component of MS pathogenesis, since 14% of the 126 children with MS for whom serology was definitive were seronegative. Exploration of other viral exposures is clearly required. Seroprevalence for the other common childhood viruses studied (CMV, HSV, parvovirus B19, and VZV) did not differ between participants with MS and non-MS controls, although, because all controls were enrolled from Canada, the USA, or Argentina, the data comparing children with MS to non-MS controls can not be assumed to represent children living in Europe. Many other infectious agents have been analysed in adult-onset MS. For example, studies of human herpes virus 6 and *Chlamydia pneumoniae* have yielded conflicting results in adults.[57,58] A study of *C pneumoniae* in children with MS postulated that increased intrathecal synthesis of antibodies against *C pneumoniae* occurred as a component of a polyclonal response, rather than as a specific infectious association.[59] Clearly, EBV-negative children with MS warrant further study, although this does not negate the importance of EBV in MS pathogenesis. However, our confirmation of the association of remote EBV infection and paediatric MS provides impetus for more in-depth study of viral–host interactions in MS. Furthermore, future collaborative studies using emerging national surveillance programmes will further increase knowledge of this important neurological illness in childhood.

Contributors

BB was the principal investigator, and was responsible for the centralized study database and the analyses of data. LK and AB-O were co-principal investigators, and provided in-depth critical review of the manuscript. JK was the study manager, and assisted in data organization and analysis. DS provided support for statistical analyses. RT was responsible for the viral analyses. BB, LK, ST, JN, AnB, AlB, OB, EW, JKM, CS, MK, MRB, MR, MaryR, JH, BW-G, EAY, KF, MF, MI, MS, VB, and M-ED were on-site investigators responsible for local recruitment and acquisition of serum samples. All authors reviewed and provided comments on the final manuscript.

Conflicts of interest

We have no conflicts of interest.

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References

1 Mikaeloff Y, Suissa S, Vallee L, et al. First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability. *J Pediatr* 2004; **144**: 246–52.
2 Mikaeloff Y, Caridade G, Assi S, Suissa S, Tardieu M. Prognostic factors for early severity in a childhood multiple sclerosis cohort. *Pediatrics* 2006; **118**: 1133–39.
3 Ghezzi A, Deplano V, Faroni J, et al. Multiple sclerosis in childhood: clinical features of 149 cases. *Mult Scler* 1997; **3**: 43–46.
4 Ghezzi A, Pozzilli C, Liguori M, et al. Prospective study of multiple sclerosis with early onset. *Mult Scler* 2002; **8**: 115–18.
5 Banwell BL. Pediatric multiple sclerosis. *Curr Neurol Neurosci Rep* 2004; **4**: 245–52.
6 Simone IL, Carrara D, Tortorella C, et al. Course and prognosis in early-onset MS: comparison with adult-onset forms. *Neurology* 2002; **59**: 1922–28.
7 Belopitova L, Guergueltcheva PV, Bojinova V. Definite and suspected multiple sclerosis in children: long-term follow- up and magnetic resonance imaging findings. *J Child Neurol* 2001; **16**(5): 317–24.
8 Chiemchanya S, Visudhiphan P. Multiple sclerosis in children: a report of 17 Thai pediatric patients. *J Med Assoc Thai* 1993; **76** (suppl 2): 28–33.

9 Deryck O, Ketelaer P, Dubois B. Clinical characteristics and long term prognosis in early onset multiple sclerosis. *J Neurol* 2006; **253**: 720–23.

10 Guilhoto LM, Osorio CA, Machado LR, et al. Pediatric multiple sclerosis report of 14 cases. *Brain Dev* 1995; **17**: 9–12.

11 Mattyus A, Veres E. Multiple sclerosis in childhood: long term katamnestic investigations. *Acta Paediatr Hung* 1985; **26**: 193–204.

12 Shiraishi K, Higuchi Y, Ozawa K, Hao Q, Saida T. Clinical course and prognosis of 27 patients with childhood onset multiple sclerosis in Japan. *Brain Dev* 2005; **27**: 224–27.

13 Pugliatti M, Sotgiu S, Rosati G. The worldwide prevalence of multiple sclerosis. *Clin Neurol Neurosurg* 2002; **104**: 182–91.

14 Dean G, Elian M. Age at immigration to England of Asian and Caribbean immigrants and the risk of developing multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1997; **63**: 565–68.

15 Pugliatti M, Riise T, Sotgiu MA, et al. Evidence of early childhood as the susceptibility period in multiple sclerosis: space-time cluster analysis in a Sardinian population. *Am J Epidemiol* 2006; **164**: 326–33.

16 Ascherio A, Munch M. Epstein-Barr virus and multiple sclerosis. *Epidemiology* 2000; **11**: 220–24.

17 Ascherio A, Munger KL, Lennette ET, et al. Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. *JAMA* 2001; **286**: 3083–88.

18 Buljevac D, van Doornum GJ, Flach HZ, et al. Epstein-Barr virus and disease activity in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2005; **76**: 1377–81.

19 Bray PF, Bloomer LC, Salmon VC, Bagley MH, Larsen PD. Epstein-Barr virus infection and antibody synthesis in patients with multiple sclerosis. *Arch Neurol* 1983; **40**: 406–08.

20 Larsen PD, Bloomer LC, Bray PF. Epstein-Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. *Neurology* 1985; **35**: 435–38.

21 Delorenze GN, Munger KL, Lennette ET, Orentreich N, Vogelman JH, Ascherio A. Epstein-Barr virus and multiple sclerosis: evidence of association from a prospective study with long-term follow-up. *Arch Neurol* 2006; **63**: 839–44 .

22 Haahr S, Hollsberg P. Multiple sclerosis is linked to Epstein-Barr virus infection. *Rev Med Virol* 2006; **16**: 297–310.

23 Alotaibi S, Kennedy J, Tellier R, Stephens D, Banwell B. Epstein-Barr virus in pediatric multiple sclerosis. *JAMA* 2004; **291**: 1875–79.

24 Pohl D, Krone B, Rostasy K, et al. High seroprevalence of Epstein-Barr virus in children with multiple sclerosis. *Neurology* 2006; **67**: 2063–65.

25 Poser CM, Paty DW, Scheinberg L, et al. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. *Ann Neurol* 1983; **13**: 227–31.

26 McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol* 2001; **50**: 121–27.

27 Tenembaum S, Chamoles N, Fejerman N. Acute disseminated encephalomyelitis: a long-term follow-up study of 84 pediatric patients. *Neurology* 2002; **59**: 1224–31.

28 Krupp L, Banwell B, Tenembaum S, for the International Pediatric MS Study Group. Consensus definitions proposed for pediatric multiple sclerosis. *Neurology* 2007; **68**: S7–12.

29 Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006; **66**: 1485–89.

30 Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983; **33**: 1444–52.

31 Uitdehaag BM, Kappos L, Bauer L, et al. Discrepancies in the interpretation of clinical symptoms and signs in the diagnosis of multiple sclerosis. A proposal for standardization. *Mult Scler* 2005; **11**: 227–31.

32 Lucchinetti CF, Kiers L, O'Duffy A, et al. Risk factors for developing multiple sclerosis after childhood optic neuritis. *Neurology* 1997; **49**: 1413–18.

33 Transverse Myelitis Consortium Working Group. Proposed diagnostic criteria and nosology of acute transverse myelitis. *Neurology* 2002; **59**: 499–505.

34 Cepok S, Zhou D, Srivastava R, et al. Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis. *J Clin Invest* 2005; **115**: 1352–60.

35 Sundstrom P, Juto P, Wadell G, et al. An altered immune response to Epstein-Barr virus in multiple sclerosis: a prospective study. *Neurology* 2004; **62**: 2277–82.

36 Gluckman PD, Hanson MA. Evolution, development and timing of puberty. *Trends Endocrinol Metab* 2006; **17**: 7–12.

37 Schwarz S, Mohr A, Knauth M, Wildemann B, Storch-Hagenlocher B. Acute disseminated encephalomyelitis: a follow-up study of 40 adult patients. *Neurology* 2001; **56**: 1313–18.

38 O'Connor KC, McLaughlin KA, De Jager PL, et al. Self-antigen tetramers discriminate between myelin autoantibodies to native or denatured protein. *Nat Med* 2007; **13**: 211–17.

39 O'Connor P. Key issues in the diagnosis and treatment of multiple sclerosis. An overview. *Neurology* 2002; **59** (6 suppl 3)**:** S1–33.

40 Bar-Or A, Smith D. Epidemiology of multiple sclerosis. In: Batchelor T, Cudcowicz M, eds. Textbook of neuroepidemiology. Woburn, MA, USA. Butterworth Heinemann, 2001.

41 Banwell BL, Anderson PE. The cognitive burden of multiple sclerosis in children. *Neurology* 2005; **64**: 891–94.

42 MacAllister WS, Belman AL, Milazzo M, et al. Cognitive functioning in children and adolescents with multiple sclerosis. *Neurology* 2005; **64**: 1422–25.

43 Mikaeloff Y, Moreau T, Debouverie M, et al. Interferon-beta treatment in patients with childhood-onset multiple sclerosis. *J Pediatr* 2001; **139**: 443–46.

44 Banwell B, Reder AT, Krupp L, et al. Safety and tolerability of interferon beta-1b in pediatric multiple sclerosis. *Neurology* 2006; **66**: 472–76.

45 Waubant E, Hietpas J, Stewart T, et al. Interferon beta-1a in children with multiple sclerosis is well tolerated. *Neuropediatrics* 2001; **32**: 211–13.

46 Etheridge LJ, Beverley DW, Ferrie C, McManus E. The use of interferon beta in relapsing-remitting multiple sclerosis. *Arch Dis Child* 2004; **89**: 789–91.

47 Kornek B, Bernert G, Balassy C, Geldner J, Prayer D, Feucht M. Glatiramer acetate treatment in patients with childhood and juvenile onset multiple sclerosis. *Neuropediatrics* 2003; **34**: 120–26.

48 Ghezzi A, Amato MP, Capobianco M, et al. Disease-modifying drugs in childhood-juvenile multiple sclerosis: results of an Italian co-operative study. *Mult Scler* 2005; **11**: 420–24.

49 Pohl D, Rostasy K, Gartner J, Hanefeld F. Treatment of early onset multiple sclerosis with subcutaneous interferon beta-1a. *Neurology* 2005; **64**: 888–90.

50 Tenenbaum S, Martin S, Fejeman N. Disease-modifying therapies in childhood and juvenile multiple sclerosis. *Mult Scler* 2001; **7:** S57.

51 Wandinger K-P, Jabs W, Siekhaus S, et al. Association between clinical disease activity and Epstein-Barr virus reactivation in MS. *Neurology* 2000; **55**: 178–84. 2000.

52 Baboonian C, Venables PJ, Williams DG, Williams RO, Maini RN. Cross reaction of antibodies to a glycine/alanine repeat sequence of Epstein-Barr virus nuclear antigen-1 with collagen, cytokeratin, and actin. *Ann Rheum Dis* 1991; **50**: 772–75.

53 Levitskaya J, Coram M, Levitsky V, et al. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* 1995; **375**: 685–88.

54 Rhodes G, Carson DA, Valbracht J, Houghten R, Vaughan JH. Human immune responses to synthetic peptides from the Epstein-Barr nuclear antigen. *J Immunol* 1985; **134**: 211–16.

55 Martin JR. Herpes simplex virus types 1 and 2 and multiple sclerosis. *Lancet* 1981; **2**: 777–81.

56 Ponsonby AL, van dM, I, Dwyer T, et al. Exposure to infant siblings during early life and risk of multiple sclerosis. *JAMA* 2005; **293**: 463–69.

57 Tejada-Simon MV, Zang YC, Hong J, Rivera VM, Zhang JZ. Cross-reactivity with myelin basic protein and human herpesvirus-6 in multiple sclerosis. *Ann Neurol* 2003; **53**: 189–97.

58 Swanborg RH, Whittum-Hudson JA, Hudson AP. Infectious agents and multiple sclerosis—are *Chlamydia pneumoniae* and human herpes virus 6 involved? *J Neuroimmunol* 2003; **136**: 1–8.

59 Rostasy K, Reiber H, Pohl D, et al. *Chlamydia pneumoniae* in children with MS: frequency and quantity of intrathecal antibodies. *Neurology* 2003; **61**: 125–28.