Clinical, environmental, and genetic determinants of multiple sclerosis in children with acute demyelination: a prospective national cohort study

Brenda Banwell, Amit Bar-Or, Douglas L Arnold, Dessa Sadovnick, Sridar Narayanan, Melissa McGowan, Julia O’Mahony, Sandra Magalhaes, Heather Hanwell, Reinhold Vieth, Raymond Tellier, Thierry Vincent, Giulio Disanto, George Ebers, Katherine Wambera, Mary B Connolly, Jerome Yager, Jean K Mah, Fran Booth, Guillaume Sebire, David Callen, Brandon Meany, Marie-Emmanuelle Dilenge, Anne Lortie, Daniela Pohl, Asif Doja, Sunita Venketaswaran, Simon Levin, E Athen MacDonald, David Meek, Ellen Wood, Noel Lowry, David Buckley, Conrad Yim, Mark Awuku, Pamela Cooper, François Grand’Maison, J Burke Baird, Virender Bhan, Ruth Ann Marrie

Summary

Background HLA-DRB1*15 genotype, previous infection with Epstein-Barr virus, and vitamin D insufficiency are susceptibility factors for multiple sclerosis, but whether they act synergistically to increase risk is unknown. We aimed to assess the contributions of these risk factors and the effect of established precursors of multiple sclerosis, such as brain lesions on MRI and oligoclonal bands in CSF at the time of incident demyelination, on development of multiple sclerosis in children.

Methods In our prospective national cohort study, we assessed children who presented with incident CNS demyelination to any of the 16 paediatric health-care facilities or seven regional health-care facilities in Canada. We did univariate and multivariable analyses to assess contributions of HLA-DRB1*15, Epstein-Barr virus, vitamin D status, MRI evidence of brain lesions, and CSF oligoclonal bands as determinants of multiple sclerosis. We used classification and regression tree analyses to generate a risk stratification algorithm for clinical use.

Findings Between Sept 1, 2004, and June 30, 2010, we screened 332 children of whom 302 (91%) were eligible and followed-up for a median of 3·14 years (IQR 1·61–4·51). 63 (21%) children were diagnosed with multiple sclerosis after a median of 127 days (99–222). Although the risk of multiple sclerosis was increased with presence of one or more HLA-DRB1*15 alleles (hazard ratio [HR] 2·32, 95% CI 1·25–4·30), reduced serum 25-hydroxyvitamin D concentration (HR per 10 nmol/L decrease 1·11, 1·00–1·25), and previous Epstein-Barr-virus infection (HR 2·04, 0·99–4·20), no interactions between these variables were detected on multivariate analysis. Multiple sclerosis was strongly associated with baseline MRI evidence of one or more brain lesion (HR 37·9, 5·26–273·85) or CSF oligoclonal bands (6·33, 3·35–11·96), suggesting established disease. One patient diagnosed with multiple sclerosis had a normal MRI scan, and therefore sensitivity of an abnormal MRI scan for multiple sclerosis diagnosis was 98·4%.

Interpretation Risk of multiple sclerosis in children can be stratified by presence of HLA-DRB1*15 alleles, remote Epstein-Barr virus infection, and low serum 25-hydroxyvitamin D concentrations. Similar to previous studies in adults, brain lesions detected on MRI and CSF oligoclonal bands in children are probable precursors to the clinical onset of multiple sclerosis. Children with a normal MRI are very likely to have a monosymptomatic illness.

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Introduction HLA-DRB1*15 genotype, remote infection with Epstein-Barr virus, and vitamin D insufficiency are possible predisposing factors to multiple sclerosis,1,2 but have not previously been assessed in one cohort. Such an investigation would allow their interrelations and relative contributions to be established. Because risk of multiple sclerosis is strongly affected by country of residence during childhood,3 the contribution of environmental factors to development of this disease can be uniquely explored in children living in an area of high prevalence who have incident demyelination during the time of risk factor acquisition. In addition to consideration of these predisposing factors, clinical, MRI, and laboratory findings at presentation provide predictive information about the likelihood of subsequent disease—although the relative contribution of these features in prediction of multiple sclerosis outcomes in the paediatric population is less well defined than it is for the adult population. Improved identification of children who are very likely to be diagnosed with multiple sclerosis would justify clinical and MRI monitoring for diagnostic confirmation and would enable prompt initiation of targeted treatment. Conversely, identification of children in whom multiple sclerosis is unlikely would substantially reduce concern for the patients, parents, and care providers. We aimed to assess the contribution of predisposing environmental factors and clinical and laboratory findings on development of multiple sclerosis in a national cohort of children in Canada.
Methods

Participants and study design

In our prospective national cohort study of incident demyelination in children, we obtained comprehensive clinical, laboratory, and MRI data to examine the contribution and interrelations of *HLA-DRB1*<sup>*</sup>15, remote Epstein-Barr-virus infection, and vitamin D status as predisposing factors and clinical features, MRI images, and oligoclonal bands as predictors of multiple sclerosis. We developed a decision tree to aid in counselling regarding multiple sclerosis risk.

All 16 Canadian paediatric health-care facilities and seven additional regional health-care facilities (located >3 h from a paediatric facility) participated in this study, following ethics approval from ethics boards at every site. Children aged younger than 16 years were eligible if they presented to one of the facilities with neurological deficits and MRI findings that were consistent with an acute demyelinating syndrome (defined in webappendix pp 1–2), and were enrolled within 90 days of symptom onset. Guardians of all children and children who were old enough to comprehend the consent process (typically ≥12 years) provided informed written and verbal consent; we obtained assent from younger children.

Procedures

To ensure consistency of the data, site investigators (paediatric neurologists or paediatricians) attended training sessions provided by BB, AB-O, DLA, and DS, and used standardised case report forms to record clinical data. Data were entered centrally by trained staff at The Hospital for Sick Children (Toronto, ON, Canada). One investigator (BB) used a-priori criteria (based on the neurological examination and without reference to neuroimaging features) to delineate whether the clinical features of acute demyelinating syndrome were attributable to one site within the CNS (clinically monofocal disease) or more than one CNS site (clinically polyfocal disease), or whether the child met criteria for acute disseminated encephalomyelitis (polyfocal deficits plus encephalopathy).

Serum and DNA blood samples were obtained up to 90 days before symptom onset, shipped on the day of procurement, and processed centrally with standardised protocols. Serum samples were stored at −80°C until batched analysis, which was done masked to clinical data. We established concentrations of serum 25-hydroxyvitamin D (the biomarker of vitamin D status) with the automated chemiluminescent LIAISON 25-hydroxyvitamin D total assay (DiaSorin, Stillwater, MN, USA).<sup>4</sup> To establish vitamin D status at baseline, we obtained serum 25-hydroxyvitamin D concentrations from participants recruited for clinical reasons.

We detected serum IgG antibodies directed against Epstein-Barr virus capsid antigens, nuclear antigens (EBNA1), and early antigens using standardised ELISA kits (DiaSorin). Remote Epstein-Barr-virus infection was defined by the presence of antibodies against Epstein-Barr virus capsid antigens and EBNA1, and anti-EBNA1 IgG titres were established as previously described.<sup>1</sup> We assayed neuromyelitis optica IgG antibodies by indirect immunofluorescence using primate cerebellar sections with diluted sera (1 in 50) following the manufacturer’s instructions (Instrumentation Laboratory, Lexington, MA, USA), and aquaporin-4 antibodies were quantified using a cell-based assay.<sup>7</sup> Total genomic DNA was extracted from whole blood. We established *HLA-DRB1*<sup>*</sup>15 alleles by use of an allele-specific PCR amplification method.<sup>2</sup> When clinically indicated, lumbar punctures were done and CSF was analysed locally. We recorded total CSF white-blood-cell count, presence of oligoclonal bands (defined as those not present in concurrently processed serum), and the method of oligoclonal band analysis. CSF IgG index was not obtained with sufficient consistency to be included.

For radiological assessment, standardised MRI protocols were optimised at all sites. All participants were offered brain MRI at baseline, 3 months, 6 months, and 12 months, and at a second demyelinating event. We also obtained data from brain and spine scans that were done for clinical reasons.

Identifiable participant characteristics on scans were removed and the anonymous scans were analysed centrally at the McConnell Brain Imaging Centre and Montreal Neurological Institute (Montreal, QC, Canada). Baseline brain MRI scans were scored for the presence or absence of T2 lesions and serial scans were scored for presence of new T2 lesions or gadolinium-enhancing T1 lesions according to criteria for lesion dissemination in time.<sup>8</sup> We assessed spinal cord images for focal lesions or longitudinally extensive lesions that spanned more than three spinal segments.<sup>9</sup>

All participants were examined quarterly in the first year, and once per year thereafter, and at the time of second demyelinating event if applicable. The primary outcome was a diagnosis of multiple sclerosis. Diagnoses of neuromyelitis optica, relapsing demyelination at a single site (optic nerves or spinal cord), and recurrent or multiphasic acute disseminated encephalomyelitis were conferred according to established criteria (see webappendix pp 1–2).

Statistical analysis

We estimated a sample size of 300 participants would be needed on the basis of the assumption that 25% of participants would be diagnosed with multiple sclerosis (<i>β</i>=0.80, <i>α</i>=0.05, seven-to-nine independent variables of interest and a low-to-medium multiple correlation coefficient between variables of 0·2–0·3). Categorical variables are reported as frequency (%) and continuous variables as mean (SD) or median (IQR). For univariate
analyses, we did Kaplan-Meier analysis, χ² tests, Fisher’s exact tests, t tests, and Wilcoxon or Kruskal-Wallis tests as appropriate. For multivariable analysis, we constructed Cox proportional hazards models, where zero time was initial symptom onset and the event of interest was multiple sclerosis (defined at the earliest date of MRI or clinical confirmation). We censored all other participants at the date of database lock for analysis, the last study visit before withdrawal, or loss to follow-up, whichever came first. The proportional hazards assumption was tested using time-dependent covariates and graphical methods.18 We assessed linearity of continuous variables with Martingale residuals and model fit by analysis of residuals. We report unadjusted and adjusted hazard ratios (HRs) and 95% CI as measures of association between multiple sclerosis and age at symptom onset (modelled continuously with age in years), sex, phenotype at presentation, presence or absence of one or more T2 lesions on baseline brain MRI, presence or absence of one or more positive HLA-DRB1*15 alleles, serum 25-hydroxyvitamin D concentration (modelled continuously), and remote Epstein-Barr virus exposure. We report the c statistic for the comparable logistic regression models as measures of model discrimination.

We used classification and regression-tree analysis (CART), which uses a non-parametric binary recursive partitioning method to produce a decision tree that identifies homogeneous subgroups of patients at different risks of a disease state (ie, multiple sclerosis).11 For a continuous variable, CART searches through the full range of values and finds the best cutpoint. The decision tree stratified all patients according to the presence or absence of T2 brain lesions at onset, the age chosen as the optimal cut-point by the CART programme (≤11·85 years or >11·85 years), and clinical presentation with or without acute disseminated encephalomyelitis. Remote Epstein-Barr-virus infection, HLA-DRB1*15 status, and serum 25-hydroxyvitamin D concentration were not retained in the decision tree, which is consistent with baseline clinical and MRI features that suggest a pathobiology already influenced by previous exposure to these prognostic factors. We used the Gini index as our splitting criterion, needing improvements of 0·001 or more to split the nodes. To reduce the risk of overfitting, we used leave-one-out cross-validation. Statistical analyses were done with SAS version 9.2 and PASW version 18.0.

Role of the funding source
This study was funded by the Multiple Sclerosis Society of Canada Scientific Research Foundation. The funding source had no role in study design, data collection, data interpretation, or data analysis, in the writing of the report, or in the decision to submit for publication. BB and RAM had full access to all of the data in the study and BB had final responsibility for the decision to submit for publication.

Results
Between Sept 1, 2004, and June 30, 2010, we enrolled 332 children (figure 1), of whom 302 were eligible and followed up for more than 3 years (table 1). Six (2%) participants withdrew from the study after a median of 93 days (IQR 85–187) of follow-up, and data were censored at time of withdrawal. Of 302 eligible patients with acute demyelinating syndrome, 63 (21%) were diagnosed with multiple sclerosis (24 by MRI evidence of dissemination in time only). Median time to a second clinical event or change in diagnosis was 127 days (IQR 91–222). 50 (79%) of these 63 participants were diagnosed with multiple sclerosis within 365 days of onset. 245 (81%) of 302 participants were treated with corticosteroids, but this did not differ between the 49 (78%) of 63 children diagnosed with multiple sclerosis and the 191 (83%) of 231 children who were not (p=0·17). No participants were treated with disease-modifying therapies before confirmation of their diagnosis of multiple sclerosis.

30 excluded
18 alternative diagnoses
5 isolated small vessel CNS vasculitis
3 brain tumour
2 mitochondrial disease
1 systemic lupus erythematosus
1 infection with Borrelia burgdorferi
1 neuromyelitis optica
1 acute necrotising encephalitis
1 retinitis
1 stroke
1 cerebellitis
1 headache
12 protocol violations
5 enrolment >90 days after onset
4 failure to confirm demyelination
3 aged >16 years

63 multiple sclerosis
39 two or more attacks†
24 MRI dissemination in time only†
1 withdrew after diagnosis

8 relapsing demyelination
2 recurrent optic neuritis‡
2 recurrent transverse myelitis†
2 neuromyelitis optica§
1 ADEM with one non-ADEM event¶
1 ADEM with MRI evidence of dissemination in time||

231 monophasic acute demyelinating syndrome 5 withdrew

Figure 1: Participant characteristics
Children who have an initial event that meets criteria for ADEM have to have two other non-ADEM events to confer a diagnosis of multiple sclerosis.11 ADEM=acute disseminated encephalomyelitis. †Lasting >24 h and within distinct areas of the CNS, or MRI evidence of new lesion accrual on serial MRI. ‡New MRI lesions >5 months after acute demyelination, as per criteria for dissemination in time. ¶Optic neuritis or transverse myelitis in the absence of lesions elsewhere in the CNS and without antibodies to aquaporin-4. §Diagnosed on the basis of optic neuritis, longitudinally extensive (lesion spanning >3 spinal segments) transverse myelitis, or both, and serological evidence of antibodies directed against aquaporin-4. ¶Optic neuritis >3 months after ADEM, but no subsequent non-ADEM events. ||>3 months after ADEM without clinical attacks.

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Female sex and older age at symptom onset were associated with an increased risk of multiple sclerosis. However, although we explored the contribution of race, ancestry, and family history to multiple sclerosis risk, very large populations would be required to fully assess such determinants (table 1). The likelihood of subsequent multiple sclerosis diagnosis differed by phenotype at presentation (table 1, webappendix p 7). Compared with children who presented with polyfocal deficits but no encephalopathy, those children with polyfocal deficits and encephalopathy (ie, acute disseminated encephalomyelitis) were less likely to be diagnosed with multiple sclerosis (HR 0·10; 0·03–0·29). Mean age at onset of acute disseminated encephalomyelitis was 6·46 years (SD 4·2) compared with 10–7 years (4·1) for polyfocal disease (p<0·0001) and 10–6 years (4·1) for monofocal disease (p<0·0001). Mean follow-up in children who had acute disseminated encephalomyelitis at onset was 2·82 years (1·55), compared with 2·82 years (1·55) for polyfocal disease and 3·06 years (1·65) for monofocal disease. Only four (5%) of 77 children with acute disseminated encephalomyelitis were diagnosed with multiple sclerosis (HR for children with vs without acute disseminated encephalomyelitis 0·18, 95% CI 0·07–0·50).

On univariate analysis, the presence of one or more T2 lesions on initial brain MRI was strongly associated with multiple sclerosis (table 2). After adjustment for age at onset, presence of one or more T2 lesions remained associated with increased risk of multiple sclerosis (HR 44·39, 95% CI 6·16–319·98; c statistic 0·85). Only one of the 61 patients diagnosed with multiple sclerosis who underwent baseline MRI had normal brain imaging at onset; the sensitivity of an abnormal MRI for a diagnosis
of multiple sclerosis was 98.4%. Two (2%) of 103 children with normal brain MRI scans at onset had recurrent optic neuritis and two had recurrent transverse myelitis with persistently normal brain MRI scans. Of 223 children who underwent MRI and who were not diagnosed with multiple sclerosis or recurrent non-multiple sclerosis demyelination, 98 had normal brain MRI (43.9% specificity). After exclusion of children with initial presentations that met criteria for acute disseminated encephalomyelitis, 56 of 116 children with one or more lesion on brain MRI were diagnosed with multiple sclerosis (48.3% positive predictive value), corresponding

<table>
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<tr>
<th>Table 2: Laboratory and imaging features at presentation: univariate analyses</th>
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<td>≥1 T2 lesion</td>
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<td>Serum 25-hydroxyvitamin D concentrations within 40 days of onset</td>
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<td>≤49.8</td>
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Data are number of positive tests/number of participants for whom a specific investigation was done and available for centralised assessment (%), mean (SD), or median (IQR), unless otherwise stated. ADS=acute demyelinating syndrome. AU=arbitrary units. *For 12 participants, only clinical examination and clinically obtained MRI studies were recorded, and four patients consented to clinical follow-up only, but their characteristics did not differ from other participants, eight children with recurrent demyelination did not meet criteria for multiple sclerosis (figure 1) and are not included in the monophasic ADS or multiple sclerosis columns, but are summarised in webappendix p 6. †Spine MRI was available for 98 participants with clinical transverse myelitis or for whom spinal cord imaging was done as part of their assessment for demyelination; compared with focal transverse myelitis, longitudinally extensive transverse myelitis was associated with a lower risk of MS (hazard ratio 0.17, 95% CI 0.05–0.59), even after adjustment for age (the mean age of children with ≥1 longitudinally extensive spinal lesion was 8.28 years (SD 4.9) compared with 12.1 years (3.8) for children with focal lesions only [p=0.001]). ‡We did CSF analysis with isoelectric focusing in 130 children, with immunofixation in 14, and by protein electrophoresis in 23; in ten children, the method of analysis was not specified, in centres without access to isoelectric focusing methods, the frequency of oligoclonal bands might be underestimated. §We analysed anti-Epstein-Barr-virus nuclear antigen titres for all children with serological evidence of remote Epstein-Barr-virus infection and sufficient serum for analysis. ¶Hazard ratios per 10 nmol/L increase in 25-hydroxyvitamin D concentration.
to a more than 60-fold increased risk (HR 61.23, 95% CI 8.47–442.57). Conversely, of these 116 children without acute disseminated encephalomyelitis, 96 had a normal brain MRI, of whom 95 were not diagnosed with multiple sclerosis (98.9% negative predictive value). Baseline MRI status was a more robust correlate of multiple sclerosis than was clinical phenotype (webappendix p 7).

44 (26%) of 170 children tested had CSF oligoclonal bands (table 2), which were most common in children with polyfocal demyelination (16 [44%] of 36 children), followed by children with monofocal demyelination (22 [27%] of 108 children), and then children with acute disseminated encephalomyelitis (eight [19%] of 42 children, p=0.04). Of those tested, 24 (60%) of 40 children diagnosed with multiple sclerosis had oligoclonal bands compared with 18 (14%) of 123 of those not diagnosed with multiple sclerosis (HR 6.33; 95% CI 3.35–11.96). 24 (57%) of 42 children with positive oligoclonal bands were diagnosed with multiple sclerosis.

Compared with 49 children with negative bands and no lesions on MRI, the 39 children with positive oligoclonal bands and MRI lesions had a much greater likelihood of diagnosis of multiple sclerosis (HR 45.89, 95% CI 6.18–340.58). In 51 children with no lesions on MRI, only two (4%) had positive oligoclonal bands, but neither was diagnosed with multiple sclerosis.

146 (68%) of 216 participants analysed had serum 25-hydroxyvitamin D concentrations of less than 75 nmol/L. Concentrations seemed to be lower in the winter (median 53.8 nmol/L, IQR 37.8–71.2) and spring (59.2, 42.3–74.8) than they were in the summer (71.5, 49.8–89.7) and autumn (68.7, 42.6–87.0), but this was not a significant difference (p=0.06). Risk of multiple sclerosis did not differ by season of presentation of acute demyelinating syndrome.
Age at onset >11·85 years

... seropositive, median anti-EBNA1 titres were higher in those subsequently diagnosed with multiple sclerosis than they were in those with monophasic demyelination (p=0.003), as has been reported in a study of EBNA1 titres in adults with multiple sclerosis.¹⁸ ¹⁹ 97 (35%) of 279 children for whom HLA analysis was done had one or more HLA-DRB1*15 alleles and were at increased risk of multiple sclerosis (table 2).

Our multivariable models showed that HLA-DRB1*15 alleles, remote Epstein-Barr-virus infection, and reduced vitamin D concentrations were independently associated with development of multiple sclerosis (table 3, figure 2), but no interactions between these three predisposing factors were detected. After adjustment for age at onset, the presence of HLA-DRB1*15 alleles remained associated with development of multiple sclerosis, but remote Epstein-Barr-virus infection and reduced vitamin D concentrations did not significantly affect outcomes. Furthermore, in analyses restricted to the more homogeneous population of white participants (table 1) and adjusted for season, remote Epstein-Barr-virus infection, low serum 25-hydroxyvitamin D concentrations, and presence of HLA-DRB1*15 alleles were associated with development of multiple sclerosis. Although the findings need to be interpreted with caution because of the low power of the analysis, table 3 shows the association of the predisposing factors of interest with multiple sclerosis as defined by a second clinical event.

16 (57%) of 28 children with all three risk factors were diagnosed with multiple sclerosis, compared with only one (5%) of 20 who had no such exposures (webappendix p 8; HR 5·27, 95% CI 1·23–22·6).

We created a decision tree to assist clinicians in stratification of patients with acute demyelinating syndrome with respect to their risk of multiple sclerosis (figure 2). On the basis of brain MRI, age at onset, and clinical phenotype (ie, with or without acute disseminated encephalomyelitis), the decision tree predicted a risk of multiple sclerosis of less than 5% in children with acute disseminated encephalomyelitis or normal brain MRI. For children with a presentation of an acute demyelinating syndrome other than acute disseminated encephalomyelitis and abnormal brain MRI, those older than 11·85 years had a 60-6% predicted risk of multiple sclerosis, whereas those under the age 11·85 years have a predicted risk of 28-1%.

**Discussion**

In our nationwide prospective cohort study of children with incident demyelination, the presence of

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Figure 2: Multiple sclerosis risk stratification algorithm for children presenting with an acute demyelinating syndrome

We generated the algorithm by classification and regression-tree analysis (CART).¹¹ Use of the algorithm provides risk estimates of 1·9–60·6%. The classification accuracy is 83·7%. ADEM=acute disseminated encephalomyelitis.
need replication, but raise the possibility that vitamin D status either modifies the effect of other prognostic factors or that vitamin D status influences the degree of disease activity or time to relapse. Webappendix p 5 summarises data for the presence or absence of every predisposing factor in children diagnosed with multiple sclerosis.

Our approach to the data was to assess predisposing factors as predictors of multiple sclerosis. MRI evidence of brain lesions and CSF oligoclonal bands were also assessed as predictors of multiple sclerosis, but were not regarded as predisposing factors. Our first statistical model of the eventual diagnosis of multiple sclerosis was designed to show an association with the predisposing factors (table 3, webappendix p 8). Because abnormal brain MRI and CSF oligoclonal bands were assumed to show an already established multiple sclerosis biology, the predictive power of these variables should be strong. The good association that we noted between abnormal brain MRI and subsequent diagnosis of multiple sclerosis, and the nearly 99% negative predictive power of a normal brain MRI at initial presentation, coupled with the increased prevalence of oligoclonal bands in children diagnosed with multiple sclerosis (HR 6·33, 95% CI 3·35–11·96) support this assertion and its clinical relevance. Separate models should show associations between the predisposing factors and the presenting clinical and paraclinical characteristics. In a logistic-regression model adjusting for age at onset, remote Epstein-Barr-virus infection was associated with a decreased risk of an acute disseminated encephalomyelitis compared with non-acute disseminated encephalomyelitis presentation (OR 1·91, 0·98–3·73). Finally, predisposing factors might initiate a disease process that is initially subclinical, thus influencing clinical and MRI features and the likelihood of CSF oligoclonal bands at the time of presentation with an acute demyelinating syndrome. If so, we would expect that inclusion of predisposing factors and clinical or paraclinical characteristics in the same model would result in the clinical and paraclinical features being so powerfully associated with the outcome that the contribution of predisposing factors could be obscured. In support of this concept, when MRI was included in the multivariate model, HLA status was no longer retained as a variable (table 3), and when clinical presentation was included (specifically with or without acute disseminated encephalomyelitis), remote Epstein-Barr-virus infection was no longer retained. However, vitamin D status was retained even in the presence of clinical and paraclinical data.

Special consideration needs to be given to children with acute disseminated encephalomyelitis. In our study, this group was younger than children with polyfocal or monofocal acute demyelinating syndrome. Only four children (6%) who were diagnosed with multiple sclerosis had acute disseminated encephalomyelitis. The frequency of multiple sclerosis in children presenting with acute disseminated encephalomyelitis in our study differs from a French study of 132 children with acute disseminated encephalomyelitis, of whom 24 (18%) were diagnosed with multiple sclerosis (mean observation 5·4 years [SD 3·3]). Our requirement of clear documentation of encephalopathy, together with a shorter follow-up than occurred in the French study and the adjudication of acute disseminated encephalomyelitis (and all acute demyelinating syndrome presentations) on the basis of clinical features only (as is permitted in the diagnostic criteria), might have contributed to these differences. Compared with children presenting with polyfocal deficits without encephalopathy, children with polyfocal deficits with encephalopathy (ie, acute disseminated encephalomyelitis) were less likely to be diagnosed with multiple sclerosis, supporting the requirement for encephalopathy as a useful criterion for diagnosis of
monophasic disease. Our decision not to assess MRI features as part of the adjudication of clinical presentation was intended to allow determination of the role of MRI as an independent correlate of multiple sclerosis outcome. Finally, we created a simple decision tree to help clinicians to stratify multiple sclerosis risk on the basis of clinical and MRI information that would be readily available at presentation with acute demyelinating syndrome (figure 2). Although the presence of T2 lesions is straightforward to adjudicate on standard imaging, more complex scoring of MRI scans for lesion dissemination in space4 might have added specificity, but such criteria are not commonly used by paediatric health providers and can be described incorrectly.39 Our proposed decision tree will also help to provide appropriate counselling of families and selection of children for surveillance such as serial MRI.

Our study has several limitations. Although we aimed to enrol all eligible Canadian children, those with mild symptoms might not have reported to paediatric health-care facilities. However, we believe that few children with key symptoms were missed because every Canadian paediatric health-care facility participated. Moreover, for 3 years, we used the reporting system of the Canadian Paediatric Society (CPS) to establish the incidence of acute demyelination in Canadian children.41 In the final year of the CPS study, enrolment at all 23 sites in the present study occurred concurrently, and we obtained data for 98% of children reporting to the CPS.41 Although every effort was made to obtain all clinical, laboratory, and MRI data at all times and according to the rigorous protocol parameters designed for the study, we did not succeed for all participants, reflecting the challenges inherent in such work in the paediatric context. This issue was particularly important when considering spinal fluid analyses. We did not do lumbar punctures for research, but were allowed access to information about CSF oligoclonal bands for patients for whom such testing was done on clinical indication. Therefore, children from whom spinal fluid was obtained differed clinically from those whose clinical status at presentation of acute demyelinating syndrome did not prompt lumbar puncture. We also acknowledge that some of the children presently classified as having monophasic demyelination will ultimately be reclassified as having multiple sclerosis. Apart from the 16 children with brain MRI lesions who are presently classified as having monophasic demyelination, the absence of baseline lesions or new lesions on serial MRI over the first year, and published data indicating a short interval between the first and second attacks (typically <12 months) in paediatric multiple sclerosis,17–24 reduces the likelihood of miscategorization of outcome in our cohort. Moreover, such miscategorization would bias our findings towards the null hypothesis. We used CART to develop our decision tree, which is a data-driven technique and is sensitive to the actual dataset, Thus, assessment of the decision tree in an independent cohort is necessary. Finally, we did not assess all putative predisposing factors, but focused on HLA-DRB1*15, Epstein-Barr virus, and vitamin D status because sufficient evidence exists to implicate these factors as biologically plausible.

The presence of HLA-DRB1*15 alleles is an inherited risk factor for multiple sclerosis, whereas vitamin D insufficiency and exposure to Epstein-Barr virus are acquired environmental risks that might contribute through altering expression of immune-related genes20 or immune behaviour through, for example, viral persistence in human B cells and subsequent virus-influenced immunological responses.14,26,27 T cell and B cell responses to myelin and other antigens are raised in children with acute demyelinating syndrome and confirmed multiple sclerosis,28–30 but studies incorporating knowledge of the HLA-DRB1*15, Epstein-Barr virus, and vitamin D status of the child have not been done. Such studies might yield new insights into how predisposing factors contribute to multiple sclerosis biology.

Contributors
BB and RAM were responsible for the core design and content of the report and had access to all aspects of the data. AB-O provided comprehensive editorial and content review. BB, AB-O, RAM, DLA, and DS served as the principal investigators of the Canadian Paediatric Demyelinating Disease study, were responsible for obtaining the grant funding, reviewed all aspects of the data, and provided in-depth edits to the final report. SN was involved in the neuroimaging analyses. MMG, SM, and JO’M have participated in data analyses in their capacity as study staff. GE and GD were responsible for the genetic HLA analyses, TV did the neuroradiology optica assays, and RT did the viral studies. HH did the vitamin D assays as a component of her doctoral work, with RV and BB as supervisors. BB, KW, MBC, JY, JKM, FB, GS, DC, BM, M-ED, AL, DP, AD, SV, SL, EAMD, DM, EW, NL, DB, CY, MA, PC, FG’M, JBB, and VB were site investigators, were responsible for enrolment of participants at their sites, and have reviewed and approved the content of the manuscript.

Conflicts of interest
BB, AB-O, DLA, DS, and RAM serve as lead investigators and funds from the study grant (Multiple Sclerosis Scientific Research Foundation) have supported work done at their institution. None of the investigators receives personal salary support from the study sponsor. Funds from the study grant have supported travel for presentations at national and international meetings. BB has received speaker’s honoraria from Merck-Serono, Biogen-IDEC, Bayer Healthcare, and Teva Neuroscience, and serves as an adviser on paediatric therapies for Biogen-IDEC, Merck-Serono, and Genzyme. BB and AB-O are supported by the Multiple Sclerosis Society of Canada (MSSC) and the Canadian Multiple Sclerosis Scientific Research Foundation, and by a New Emerging Team Grant in Autoimmunity supported by the Canadian Institutes of Health Research and MSSC. AB-O has received consultancy fees from Bayhill Therapeutics, Biogen-IDEC, BioMS, Diogenix, Eli-Lilly, Genentech, GlaxoSmithKline, Guthy-Jackson/GGF, Merck-Serono, Novartis, Roche, Teva Neuroscience, and Wyeth. AB-O has received speaker’s honoraria from Bayer, Bayhill Therapeutics, Biogen-IDEC, BioMS, Diogenix, Eli-Lilly, Genentech, GlaxoSmithKline, Guthy-Jackson/GGF, Merck-Serono, Novartis, Ono, Roche, Teva Neuroscience, and Wyeth. AB-O has funding from the US National Institutes of Health, Canadian Institutes of Health Research, the Canadian Multiple Sclerosis Society, Biogen-IDEC, and Teva Neuroscience for research unrelated to the present project. DLA has served as a speaker at meetings or as a consultant for Bayer, Biogen,
Elan, GlaxoSmithKline, Roche, and Teva Neuroscience. DLA receives financial remuneration and stock options from NeuroRx Research. DS has received speaker’s honoraria from Biogen, Merck-Serono, Teva Neuroscience, and Bayer and has grant support (unrelated to the present grant funding) from Canadian Institutes of Health Research, the Multiple Sclerosis Society of Canada and the Canadian Multiple Sclerosis Research Foundation. SN has received consultancy fees or speaker’s honoraria from Teva Neuroscience and NeuroRx Research. MMG, SM, and JO’M served as administrative staff and received salary support from the funding agency (Multiple Sclerosis Scientific Research Foundation). HH received a doctoral salary award from the Canadian Institute of Health Research. RV is a paid consultant for Ortho Clinical Diagnostics and Merck Serono, holds a grant from Dairy Farmers of Canada, receives payment in connection to a patent for a vitamin D supplement, has received payment for lectures including service on speaker’s bureaus from Merck, Carlsson Laboratories, and DiaSorin. GD has received funding from the Multiple Sclerosis Society of Canada. FG’M has received consultancy fees from Biogen-IDEC, Teva Neuroscience, and Novartis, travel reimbursement from Teva Neuroscience and Biogen-IDEC, payment for lectures by Teva Neuroscience, and payment for manuscript preparation (unrelated to the present project) by EMD Serono and for educational presentations Novartis. RAM has funding from the Canadian Institute of Health Research, the Multiple Sclerosis Society of Canada, Manitoba Health Research Council, HSC Foundation, Public Health Agency of Canada, Rx&D Research Foundation, and Sanofi-Aventis for research unrelated to the present project, RT, TV, and GE report no disclosures. KW, MBC, JY, KM, FB, GS, DC, BM, M-ED, AL, DP, AD, SV, SL, EAMD, DM, EW, NLI, DB, CY, MA, PC, FG’M, JBB, and VB served as site investigators and received funds to their institution for study costs. None of the site investigators received personal salary support and no reports any other disclosures.

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References


