# Common viruses associated with lower pediatric multiple sclerosis risk

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## ABSTRACT

**Background:** Because common viruses are encountered during childhood, pediatric multiple sclerosis (MS) offers a unique opportunity to investigate the influence of these viruses on disease susceptibility and the interactions between seroprevalence and select *HLA* genotypes. We studied seroprevalence for Epstein-Barr virus (EBV), cytomegalovirus (CMV), and herpes simplex virus (HSV) type 1 and *HLA-DRB1*1501/1503* status as predictors of pediatric MS.

**Methods:** This was a retrospective analysis of prospectively collected demographic, clinical, and biologic data in subjects up to 18 years of age with early MS, control subjects seen at the same regional referral pediatric MS clinics, and additional healthy pediatric control subjects.

**Results:** Patients with early pediatric MS (n = 189) and pediatric control subjects (n = 66) were tested. Epstein-Barr nuclear antigen-1 seropositivity was associated with an increased odds of MS (odds ratio [OR] 3.78, 95% confidence interval [CI] 1.52–9.38, $p = 0.004$) in analyses adjusted for age, sex, race, ethnicity, and *HLA-DRB1*1501/1503* status. In multivariate analyses including EBV status, a remote infection with CMV (OR 0.27, 95% CI 0.11–0.67, $p = 0.004$) was associated with a lower risk of developing MS. Although a remote infection with HSV-1 was not associated with an increased odds of MS, a strong interaction was found between HSV-1 status and *HLA-DRB1* in predicting MS ($p < 0.001$). HSV-1 was associated with an increased risk of MS in those without a *DRB1*15* allele (OR 4.11, 95% CI 1.17–14.37, $p = 0.03$), whereas the effect was reversed in those who were *DRB1*15*-positive (OR 0.07, 95% CI 0.02–0.32, $p = 0.001$).

**Conclusions:** These findings suggest that some infections with common viruses may in fact lower MS susceptibility. If this is confirmed, the pathways for risk modification remain to be elucidated.

***Neurology*® 2011;76:1989–1995**

## GLOSSARY

**ADEM** = acute disseminated encephalomyelitis; **CI** = confidence interval; **CIS** = clinically isolated syndrome; **CMV** = cytomegalovirus; **EBNA** = Epstein-Barr nuclear antigen; **EBV** = Epstein-Barr virus; **HSV** = herpes simplex virus; **IgG** = immunoglobulin G; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **OR** = odds ratio; **SNP** = single nucleotide polymorphism; **VCA** = viral capsid antigen.

Both genetic and environmental risk factors contribute to adult-onset multiple sclerosis (MS) susceptibility. The main genetic risk factor across all populations is the *HLA-DRB1* gene,[1] although several non-*HLA* genes of modest effect have been identified recently through genome-wide association studies.[2-6] In addition, substantial evidence has accumulated to suggest that exposure to Epstein-Barr virus (EBV), vitamin D deficiency, or cigarette smoking early in life, perhaps even during pregnancy, increase MS susceptibility.[7,8] These risk factors

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*Study funding:* The Pediatric MS Network, initiated and sponsored by the National Multiple Sclerosis Society, includes the following centers: University of California, San Francisco, Stony Brook, Buffalo, University of Alabama Birmingham, Harvard University, and the Mayo Clinic. Dr. Mowry has a National MS Society Sylvia Lawry Fellowship Award and an NIH K23NS067055. Dr. Waubant is also supported by the Nancy Davis Foundation. Dr. James is supported by NIH RR015577, AI082714, U19AI082714, and AR053483. Dr. Oksenberg is supported by NMSS RG2901D9/1.

*Disclosure:* Author disclosures are provided at the end of the article.

have rarely been studied concomitantly,[9-11] thus limiting our understanding of their respective contributions to or possible interplay in disease susceptibility.

Multiple observations have supported the possibility of multifaceted gene–environment interactions, although only a few have been reported for MS and are unconfirmed.[10,12-15] Interestingly, the strongest genetic risk factor for MS, *HLA-DRB1*, is a coreceptor for EBV entry into B cells.[16] Several studies have failed to find an interaction between these 2 risk factors.[9-11]

One group in whom MS is increasingly being identified is children.[17] The shorter time lag between putative exposures and disease onset in pediatric-onset vs adult-onset MS suggests that children may have a higher genetic variant susceptibility burden or have a greater collection of exposures to environmental risk factors. Studying subjects who develop the disease in childhood may therefore substantially enhance our ability to identify gene–environment interactive risks. Furthermore, if disease susceptibility is in part explained by exposure to common viruses during childhood, it might be easier to detect this effect in children because seroconversion has occurred in most individuals by adulthood. The only environmental factor that has been consistently associated with pediatric-onset MS risk is a remote EBV infection.[18-20]

The primary objectives of this study were to determine whether a remote infection with EBV, cytomegalovirus (CMV), or herpes simplex virus (HSV)–1 is associated with a greater risk of development of MS in children and whether the presence of *HLA-DRB1*1501* or *1503* influences this risk, either independently or in an interactive fashion with these viruses.

## METHODS

**Case patients and control subjects.** This study was approved by the local institutional review boards of all participating centers. All patients and parents signed assent and consent forms for this study. Patients younger than 18 years at the onset of their first MS symptoms were recruited at 6 regional pediatric MS centers sponsored by the National Multiple Sclerosis Society (University of California, San Francisco, SUNY at Stony Brook, SUNY at Buffalo, University of Alabama, Birmingham, Harvard, and Mayo Clinic). The control group included pediatric patients seen at the same clinics during the same period for whom clinically isolated syndrome (CIS) or MS was ruled out and healthy pediatric subjects recruited for a prior study.[21] The majority of the healthy subjects were collected in Oklahoma, but some samples came from Southern California and New York City. All individuals seen at the pediatric MS clinics between January 2006 and May 2008 who consented to be in this study and provided serum or DNA were included. Pediatric MS and CIS were defined according to the operational definitions established by the International Pediatric Multiple Sclerosis Study Group.[17] When several blood samples were available, the first sample collected for a given patient was chosen. Race and ethnicity were defined according to the self-reported race and ethnicity as defined by the NIH.

**Viral studies.** Batched EBV, CMV, and HSV-1 assays (immunoglobulin G [IgG]) were performed blindly at the Oklahoma Medical Research Foundation (J.A.J.) with normalized ELISAs. Viral capsid antigen (VCA) IgG seroprevalence represents remote EBV infection, whereas Epstein-Barr nuclear antigen (EBNA)–1 IgG seropositivity often represents evidence of EBV latency. EBV-VCA, CMV, and HSV-1 antibodies were analyzed by commercially available, standardized ELISAs (Wampole Laboratories, Princeton, NJ) as described previously.[21,22] Quality control requirements included having positive and negative controls, which met predetermined requirements, as well as calibrators, which fell within a known range of reactivity. Results are reported as international standardized ratios with positive responses $\geq$1.1, negative responses $<$0.9, and equivocal responses between 0.91 and 1.09. All equivocal results were retested, and repeated equivocal results were dropped from the analysis. With use of an ELISA with full-length EBNA-1 as an antigen (Biodesign, Carmel NY), samples were tested for anti-EBNA-1 seropositivity and relative concentration as described previously.[21,22] EBNA-1 was diluted in carbonate coating buffer and coated at 1 $\mu$g per each well of a 96-well plate. Serum was diluted at 1:100 and 1:1,000 and incubated with the bound antigen. Specific antibodies were detected using alkaline phosphatase-conjugated antihuman IgG (Jackson ImmunoResearch, West Grove, PA). All ELISAs had positive and negative controls that must meet set quality control measures to include data for analysis. In addition, a positive control was used to standardize assays between plates. Positive anti-EBNA-1 responses were $\geq$4 SD above a panel of controls known to be EBV-seronegative.

***HLA-DRB1*1501* and *1503* genotyping.** All DNA samples of patients with pediatric-onset MS and control subjects were typed with single nucleotide polymorphisms (SNPs) for the presence of *HLA-DRB1*1501/1503* and the number of copies. *HLA-DRB1* genotyping was performed using a validated gene-specific TaqMan assay designed to identify specifically the presence or absence of *DRB1*1501* or *1503* alleles. An internal positive control (*β-globin*) was included in each well to confirm that the reaction amplified successfully. PCR was performed in a total volume of 10 $\mu$L, containing 20 ng DNA, 1× TaqMan Universal PCR Master Mix (Applied Biosystems), 0.6 $\mu$M *DRB1*1501/1503*-specific primers (forward 5′-ACG TTT CCT GTG GCA GCC TAA-3′ and reverse 5′-TGC ACT GTG AAG CTC TCC ACA A-3′), 0.3 $\mu$M control primers (forward 5′-ACT GGG CAT GTG GAG ACA GAG AA-3′ and reverse 5′-AGG TGA GCC AGG CCA TCA CTA AA-3′), a 0.225 $\mu$M VIC-labeled *DRB1*1501/1503*-specific probe (5′-AAC AGC CAG AAG GAC ATC CTG GAG CA-3′), and a 0.025 $\mu$M 6FAM-labeled control probe (5′-TCT ACC CTT GGA CCC AGA GGT TCT TTG AGT-3′). Amplification was performed in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) with an initial 95°C for 10 minutes, followed by 50 cycles of 95°C for 15 seconds and 62°C

**Table 1** Patients' demographics and rate of seropositivity for various viruses

| | Pediatric MS/CIS (n = 189) | Pediatric control subjects (n = 66) | p Value |
|---|---|---|---|
| Age at disease onset, y, mean ± SD | 12.9 ± 4.0 | NA | NA |
| Age at sampling, y, mean ± SD | 14.9 ± 3.3 | 14.7 ± 4.1 | 0.79 |
| Hispanic ethnicity, % | 31.5 | 14.3 | 0.008 |
| Nonwhite race, % | 25.9 | 18.0 | 0.21 |
| Female, % | 65.6 | 66.7 | 0.73 |
| *DRB1*1501/1503*-positive, % | 46.9 | 32.8 | 0.06 |
| Anti-EBNA-1–positive, % | 88.6 | 54.8 | <0.0001 |
| Anti-VCA–positive, % | 86.8 | 52.5 | <0.0001 |
| Anti-CMV–positive, % | 28.2 | 35.5 | 0.28 |
| Anti-HSV-1–positive, % | 40.3 | 31.7 | 0.23 |

Abbreviations: CIS = clinically isolated syndrome; CMV = cytomegalovirus; EBNA = Epstein-Barr nuclear antigen; HSV = herpes simplex virus; MS = multiple sclerosis; NA = not applicable.

for 1 minute. Results were confirmed with the tagging SNP rs3135391.

**Statistical analysis.** Calculations and statistical analyses were performed using Stata 10.0 statistical software (StataCorp, College Station, TX). Means ± SD or medians (ranges) were used to summarize demographic and clinical data. Proportions were compared with $\chi^2$ tests. Multivariate analysis using logistic regression was performed, adjusted for age, sex, race, ethnicity, and *DRB1* status, to evaluate whether children who were seropositive for exposure to each virus or for *DRB1* were more likely than others to have pediatric-onset MS. We also included EBNA-1 status (positive or negative) as a covariate in the models for HSV-1 and HSV-2 and CMV. Interactions were assessed by generating an interaction term for each virus (positive or negative) and *DRB1* status. Because the sample size was small, we performed within-*DRB1* (positive or negative) group analyses of the effect of viral status when the *p* value for the interaction term was <0.1.

**Table 2** Multivariate analyses of the risk of developing pediatric-onset MS or CIS adjusted for age at blood draw, sex, race, and ethnicity[a]

| | OR | 95% CI | p Value |
|---|---|---|---|
| Anti-EBV VCA–positive | 3.72 | 1.48–8.85 | 0.005 |
| *DRB1*1501/1503*-positive | 3.29 | 1.41–7.68 | 0.006 |
| | | | |
| Anti-EBNA-1–positive | 3.78 | 1.52–9.38 | 0.004 |
| *DRB1*1501/1503*-positive | 2.75 | 1.21–6.27 | 0.02 |
| | | | |
| Anti-CMV–positive | 0.27 | 0.11–0.67 | 0.004 |
| *DRB1*1501/1503*-positive | 2.85 | 1.23–6.63 | 0.01 |
| Anti-EBNA-1–positive | 5.15 | 1.93–13.70 | 0.001 |
| | | | |
| Anti-HSV-1–positive | 0.85 | 0.36–2.03 | 0.72 |
| *DRB1*1501/1503*-positive | 2.72 | 1.19–6.23 | 0.02 |
| Anti-EBNA-1–positive | 4.39 | 1.70–11.34 | 0.002 |

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; CMV = cytomegalovirus; EBNA = Epstein-Barr nuclear antigen; EBV = Epstein-Barr virus; HSV = herpes simplex virus; MS = multiple sclerosis; OR = odds ratio; VCA = viral capsid antigen.
[a] This table presents analyses combining all control subjects (n = 66).

**RESULTS** **Case patient and control subject characteristics.** A total of 189 patients with pediatric-onset MS (n = 161) or CIS (n = 28) at the time of sampling and 66 pediatric control subjects (38 neurologic and 28 healthy controls) provided blood samples for this study. All patients with CIS subsequently developed MRI or clinical activity, thus meeting the criteria for relapsing-remitting MS.[17] Among neurologic control subjects, diagnoses included neuromyelitis optica (NMO) (n = 4), acute disseminated encephalomyelitis (ADEM) (n = 9), bladder cyst (n = 1), sarcoid (n = 1), vasculitis (n = 1), headaches (n = 1), anxiety disorder (n = 1), scleroderma en coup de sabre (n = 2), mitochondrial or metabolic disorders (n = 2), paraneoplastic syndrome (n = 2), low-grade tumor (n = 1), peripheral neuropathy (n = 3), nonspecific white matter changes (n = 1), recurrent optic neuritis with normal CSF and normal MRI (n = 2), transverse myelitis (n = 1), and unknown other neurologic disorders (n = 6). Demographics and *HLA-DRB1*1501* or *1503* status are presented in table 1.

**Remote infection status.** The frequency of remote infections with EBV, CMV, and HSV-1 is reported in table 1 for individuals within each group. The presence of a remote infection with EBV (i.e., VCA-positive or EBNA-1–positive individuals) was strongly associated with increased odds of pediatric MS or CIS independent of age, sex, race, ethnicity, and *HLA-DRB1* status (see table 2 for analyses using all control subjects and tables 3 and 4 for analyses performed separately using neurologic and healthy control subjects). Whereas no apparent association was found for remote infections with HSV-1 after adjustment for *HLA-DRB1* status, race, and ethnicity (odds ratio [OR] 1.02, 95% confidence interval [CI] 0.45–2.30, $p = 0.95$), CMV seroprevalence was associated with a lower risk of developing MS or CIS (OR 0.37, 95% CI 0.16–0.84, $p = 0.02$). The data were similar when neurologic and healthy control subjects were analyzed separately (data not shown).

Because a previous EBV infection has consistently been reported to be associated with increased MS risk, we added EBV status to the models that included other viruses, so we could evaluate the independent effect of those infections. When we added EBNA-1 status to the multivariate models including the other viruses tested, no substantial change in the odds for MS or CIS were seen for the respective viruses studied, whereas the OR associated with EBNA-1–positive status remained elevated (table 2). The data were similar when neurologic and healthy

**Table 3** Multivariate analyses of the risk of developing pediatric-onset MS or CIS adjusted for age at blood draw, sex, race, and ethnicity using neurologic control subjects[a]

| | OR | 95% CI | *p* Value |
|---|---|---|---|
| Anti-EBV VCA–positive | 4.24 | 1.46–12.27 | 0.008 |
| *DRB1*1501/1503*-positive | 2.22 | 0.75–6.54 | 0.15 |
| | | | |
| Anti-EBNA-1–positive | 5.42 | 1.83–16.08 | 0.002 |
| *DRB1*1501/1503*-positive | 1.86 | 0.66–5.27 | 0.24 |
| | | | |
| Anti-CMV–positive | 0.26 | 0.08–0.79 | 0.02 |
| *DRB1*1501/1503*-positive | 2.08 | 0.71–6.06 | 0.18 |
| Anti-EBNA-1–positive | 7.65 | 2.30–25.40 | 0.001 |
| | | | |
| Anti-HSV-1–positive | 1.54 | 0.47–5.09 | 0.48 |
| *DRB1*1501/1503*-positive | 2.01 | 0.69–5.86 | 0.20 |
| Anti-EBNA-1–positive | 5.56 | 1.83–16.85 | 0.002 |

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; CMV = cytomegalovirus; EBNA = Epstein-Barr nuclear antigen; EBV = Epstein-Barr virus; HSV = herpes simplex virus; MS = multiple sclerosis; OR = odds ratio; VCA = viral capsid antigen.
[a] This table presents analyses using neurologic control subjects only (n = 38).

control subjects were analyzed separately (tables 3 and 4, respectively).

Finally, the respective effect of the various viruses studied was analyzed. In that model adjusted for age, sex, race, ethnicity, and *HLA-DRB1* (table 5), a remote infection with EBV and to a lesser extent with HSV-1 was strongly associated with increased odds of MS or CIS, whereas a remote infection with CMV was independently associated with lower odds for MS or CIS.

**Table 4** Multivariate analyses of the risk of developing pediatric-onset MS or CIS adjusted for age at blood draw, sex, race, and ethnicity using healthy control subjects[a]

| | OR | 95% CI | *p* Value |
|---|---|---|---|
| Anti-EBV VCA–positive | 3.41 | 0.93–12.49 | 0.06 |
| *DRB1*1501/1503*-positive | 4.61 | 1.46–14.59 | 0.009 |
| | | | |
| Anti-EBNA-1–positive | 3.07 | 0.76–12.42 | 0.11 |
| *DRB1*1501/1503*-positive | 4.17 | 1.32–13.18 | 0.01 |
| | | | |
| Anti-CMV–positive | 0.21 | 0.06–0.78 | 0.02 |
| *DRB1*1501/1503*-positive | 4.17 | 1.28–13.53 | 0.017 |
| Anti-EBNA-1–positive | 4.95 | 1.06–22.96 | 0.04 |
| | | | |
| Anti-HSV-1–positive | 0.55 | 0.17–1.77 | 0.32 |
| *DRB1*1501/1503*-positive | 3.73 | 1.17–11.90 | 0.03 |
| Anti-EBNA-1–positive | 3.84 | 0.92–16.02 | 0.06 |

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; CMV = cytomegalovirus; EBNA = Epstein-Barr nuclear antigen; EBV = Epstein-Barr virus; HSV = herpes simplex virus; MS = multiple sclerosis; OR = odds ratio; VCA = viral capsid antigen.
[a] This table presents analyses using healthy control subjects only (n = 28).

**Interactions between *HLA-DRB1* and viral status in predicting MS or CIS.** No interaction was detected between antibodies against EBNA-1, VCA, and CMV and against *HLA-DRB1*1501/1503* (data not shown). A strong interaction was detected for HSV-1 and *HLA-DRB1* ($p < 0.001$). HSV-1 positivity was associated with increased risk of MS in *HLA-DRB1*–negative individuals (OR 4.11, 95% CI 1.17–14.37, $p = 0.03$), whereas in the *HLA-DRB1*–positive patients, the direction of the association was reversed (OR 0.07, 95% CI 0.02–0.32, $p = 0.001$). The data were similar when neurologic and healthy control subjects were analyzed separately, although the 95% CIs were wider (data not shown). No confounding was identified between the presence of *DRB1*1501/1503* and viral status.

**DISCUSSION** In addition to finding an association between EBV and pediatric MS risk, our novel observation that a remote CMV infection is independently associated with a lower odds of MS or CIS, even in models including EBV status, suggests that there might be a complex interplay between various viral infections acquired during childhood and MS risk. In a prior adult study, the OR for MS when CMV was positive was 0.8, and although the 95% CI included 1, it was not centered on 1 (0.5–1.2), thus not excluding an association with lower odds of MS.[23] Our findings are preliminary and have to be reproduced in a larger cohort, ideally with longitudinal samples to provide an answer about the timing of various infections.

Although a remote HSV-1 infection was found more frequently in the pediatric MS or CIS group, it was not definitively associated with MS risk after adjustment for age, sex, race, ethnicity, *HLA-DRB1*, and EBNA-1 status. However, HSV-1 did appear to have a strong role in predicting MS or CIS when evaluated separately in *DRB1*-positive and *DRB1*-negative subjects. HSV-1 positivity was associated with greater MS risk in *DRB1*-negative subjects but reduced risk in *DRB1*-positive subjects. A recent study failed to detect a contribution of HSV-1 status (OR 0.97) to MS risk.[10] Like us, the authors found a combined effect of *HLA-DR15* positivity and HSV-1 seronegativity that was almost 5 times higher than expected. Interestingly, in our study, HSV-1 positivity alone was not associated with increased odds, but the association of HSV-1 with MS risk differed strikingly, depending on *DRB1* status. For individuals who were *DRB1*-negative, the OR for MS associated with HSV-1 positivity was 4.1, whereas

**Table 5** Multivariate model including all available remote viral exposures (adjusted for age at blood draw, sex, race, and ethnicity)

| | OR to develop MS/CIS | 95% CI | *p* Value |
|---|---|---|---|
| Anti-EBNA-1–positive | 5.00 | 1.80–13.90 | 0.002 |
| Anti-CMV–positive | 0.30 | 0.11–0.77 | 0.01 |
| Anti-HSV-1–positive | 2.86 | 0.75–10.89 | 0.12 |
| *DRB1*1501/1503*-positive | 3.00 | 1.27–7.11 | 0.01 |

Abbreviations: CI = confidence interval; CIS = clinically isolated syndrome; CMV = cytomegalovirus; EBNA = Epstein-Barr nuclear antigen; HSV = herpes simplex virus; MS = multiple sclerosis; OR = odds ratio.

the odds of development of MS associated with HSV-1 positivity was reduced in the *DRB1*-positive individuals (OR 0.07).

We report that a remote infection with EBV is a strong risk factor for pediatric MS even after adjustment for age at the time of sampling, *HLA-DRB1*1501* or *1503* status, sex, race, and ethnicity. Anti-EBNA-1 antibody titers were also reported to be a *DRB15*-independent risk factor for adult-onset MS in another study.[9] Finally, unlike what has been reported in adults,[24] the fact that 10% of our patients with pediatric MS or CIS (all of whom subsequently met the criteria for MS) had not yet developed an EBV infection at the time of their blood sample argues that EBV is not necessary for the onset of MS. The frequency of EBV-negative patients with pediatric MS we report is similar to frequencies reported in several pediatric MS cohorts.[19,20]

Distinguishing gene–environment interactions that may reflect biological processes such as molecular mimicry will contribute to the further dissection of disease mechanisms that culminate in MS onset. One of the first studies to suggest that such phenomena take place in MS showed that a T-cell receptor from an patient with MS recognized both a *DRB1*1501*-restricted myelin basic protein and a *DRB5*0101*-restricted EBV peptide.[25] More recently, *HLA-B*4402* was reported to bind poorly to EBV[26] and to be underrepresented in adults with MS.[27] No report of molecular interactions between HSV and *DRB1* are available to understand possible phenomena explaining our epidemiologic observation.

Our study has several limitations. First, viral antibody responses were measured on average 2 (median 1) years after MS onset. Thus, we cannot exclude the possibility that seroconversion may have happened between disease onset and time of sampling. This is an inherent limitation of all studies that do not provide prospective follow-up of very large cohorts before disease development. The design of such a study, particularly as it relates to cost, is prohibitive, and in children is especially difficult because of the difficulty of obtaining blood samples from healthy individuals. However, the facts that one adult nested case-control study reported seroconversion for EBV before MS clinical onset[28] and that our findings hold after adjustment for age suggest that seroconversion occurred before clinical disease onset. Second, although our control group is relatively small, the findings were similar regardless of whether the control subjects were considered together or separately. Whereas the neurologic control cohort included mostly individuals with various neurologic or psychiatric conditions, subjects were enrolled at the same sites through the same referral patterns and during the same period as patients with pediatric-onset MS or CIS. Conversely, the healthy control group was not recruited over the same time period and was enrolled at different sites, although some of the patients were recruited in California and the New York area, as were the majority of our patients with MS and CIS. Most of our neurologic control subjects had other neurologic conditions, some of which are inflammatory in nature such as ADEM and NMO, which could have biased our findings toward the null hypothesis. It is noteworthy that the proportions of individuals positive for EBNA-1, VCA, CMV, and HSV-1 in our pediatric-onset and control groups are comparable with those published previously in children.[20] Finally, the findings presented herein have to be replicated before being considered definitive.

Our findings shed some light on previous reports that higher infant sibling exposure is associated with a lower risk of MS in adults.[10,29] We have now identified specific viruses that may contribute to this phenomenon. Whether a lower rate of infection with viruses that have a protective effect may have contributed to the increased prevalence of MS seen worldwide in past decades is unclear. It is thus important to better determine the timing of protective infections; i.e., are these acquired before or after EBV infection? Identifying risk factors for MS susceptibility and their interactions more clearly will undoubtedly result in individualized preventative strategies in subjects at risk and possibly in the development of new therapeutic agents.

## AUTHOR CONTRIBUTIONS

E.W. designed the study and coordinated analysis. E.W. and J.M. coordinated data collection. E.W. and E.M.M. interpreted the findings. E.M.M. performed statistical analyses. L.K., T.C., E.A.Y., N.K., J.N., D.C., J.S., A.B., M.M., M.G., B.W.-G., and M.R. contributed demographic, clinical, and biological samples to this study. J.R.O. provided genotyping of all samples. J.A.J. performed all the viral assays and provided healthy control samples. E.W., E.M.M., L.K., T.C., E.A.Y., N.K., J.N., D.C., J.S., A.B., M.M., M.G., B.W.-G., M.R., J.R.O., and J.A.J. participated in writing of the manuscript.

## DISCLOSURE

Dr. Waubant serves on scientific advisory boards for the NIH and Actelion Pharmaceuticals Ltd; has received speaker honoraria from Teva Pharmaceutical Industries Ltd.; has served as a consultant for Actelion Pharmaceuticals Ltd., Roche, and sanofi-aventis; has received free drugs for ongoing trials from sanofi-aventis and Biogen Idec; and receives research support from the NIH, the National MS Society, and the Nancy Davis Foundation. Dr. Mowry receives research support from the NIH, the National NS Society, and Partners MS Center. Dr. Krupp has served on scientific advisory boards for Acorda Therapeutics Inc., Genentech, Inc., Pfizer Inc, Novartis, and sanofi-aventis; has received funding for travel from Acorda Therapeutics Inc., Bayer Schering Pharma, Biogen Idec, EMD Serono, Inc., Genentech, Inc., Teva Pharmaceutical Industries Ltd., Novartis, and sanofi-aventis; has received honoraria from Acorda Therapeutics Inc., Bayer Schering Pharma, EMD Serono, Inc., Biogen Idec, Teva Pharmaceutical Industries Ltd., GlaxoSmithKline, the MS Association of America, and the France Foundation; has received royalties for publication of *Fatigue in Multiple Sclerosis* (Demos, 2005); serves as consultant for Leerink Swan, Gerson Lehrman Group, and Guidepoint Global; serves on speakers' bureaus for Bayer Schering Pharma, Biogen Idec, Teva Pharmaceutical Industries Ltd., and EMD Serono, Inc.; receives research support from Biogen Idec, EMD Serono, Inc., Teva Pharmaceutical Industries Ltd., Novartis, Genentech, Inc., Acorda Therapeutics Inc., BioMS Medical, the NIH (HD38107-02 [PI]), the National MS Society, the Lourie Foundation, and the Slomo and Cindy Silvian Foundation; has received license fee payments for a questionnaire she developed from the following companies: Eli Lilly and Company, MedImmune, Vertex Pharmaceuticals, Wyeth, ZymoGenetics, EPI-Q, Novartis, Genzyme Corporation, Tibotec Therapeutics, Genentech, Inc., Roche, sanofi-aventis, Johnson & Johnson, Janssen, and ER Squibb & Sons; and has served as an expert witness in medico-legal cases. Dr. Chitnis has served as a consultant for Biogen Idec, Teva Pharmaceutical Industries Ltd., and EMD Serono, Inc.; and receives research support from EMD Serono, Inc., the NIH/NINDS, and the National MS Society; and her spouse is employed by Novartis. Dr. Yeh has received research support from the NIH, the Jog for the Jake Foundation, the Children's Guild Foundation, and the National MS Society. Dr. Kuntz has received research support from the National MS Society, the Spinal Muscular Atrophy Foundation, Cooperative International Neuromuscular Research Group/U.S. Department of Education, and the NIH. Dr. Ness has served as a consultant for Merck Serono and receives research support from the NIH/NINDS, EMD Serono, Inc., and the National MS Society. Dr. Chabas has received honoraria from Teva Pharmaceutical Industries Ltd., EMD Serono, Inc., and Pfizer Inc; has received license fee payments and may accrue revenue on office of technology licensing Stanford Docket 501–085 (issued 5/6/2003): Osteopontin Related Compositions and Methods; and receives research support from the National MS Society and the Nancy Davis Foundation. Dr. Strober, J. McDonald, and Dr. Belman report no disclosures. M. Milazzo has received honoraria from Teva Pharmaceutical Industries Ltd. Dr. Gorman receives research support from the National MS Society. 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*Received November 3, 2010. Accepted in final form February 18, 2011.*

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