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ORIGINAL ARTICLE

Epstein-Barr virus and multiple sclerosis: interaction with HLA

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*Epstein-Barr virus (EBV) infection, history of infectious mononucleosis (IM) and HLA-A and DRB1 have all been proposed as risk factors for multiple sclerosis (MS). Our aim was to analyse possible interactions between antibodies against Epstein-Barr virus nuclear antigen 1 (EBNA1) or EBNA1 fragments, presence of DRB1*15 and absence of A*02. The study population includes newly diagnosed cases and matched controls. Interaction on the additive scale was calculated using attributable proportion due to interaction (AP), which is the proportion of the incidence among individuals exposed to two interacting factors that is attributable to the interaction per se. IM showed association with MS, odds ratio (OR) = 1.89 (1.45–2.48% confidence interval (CI)), as did raised EBNA1 IgG OR = 1.74 (1.38–2.18 95%CI). All EBNA1 fragment IgGs were associated with MS risk. However, EBNA1 fragment 385–420 IgG levels were more strongly associated to MS than total EBNA1 IgG, OR = 3.60 (2.75–4.72 95%CI), and also interacted with both DRB1*15 and absence of A*02, AP 0.60 (0.45–0.76 95%CI) and AP 0.39 (0.18–0.61 95%CI), respectively. The observed interaction between HLA class I and II genotype and reactivity to EBV-related epitopes suggest that the mechanism through which HLA genes influence the risk of MS may, at least in part, involve the immune control of EBV infection.*

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Introduction

Multiple sclerosis (MS) is considered as a complex disease where genetic and environmental factors interact to influence disease susceptibility. To date the strongest genetic factor determining MS risk is the human leukocyte antigen (HLA)-DRB1*15 group of alleles. The HLA-A*02 is also associated to MS, independent of DRB1*15, and has a protective effect.[1] Among environmental factors, available evidence points to Epstein-Barr virus (EBV) infection, sunlight exposure, vitamin D levels and cigarette smoking as the most plausible risk factors for MS.[2] On their own, each risk gene or environmental factor, except HLA-DRB1*15, has small or modest association to the disease. We explore this in relation to EBV-related measures and potent MS-risk genes in a Swedish case–control study, with incident MS cases and matched population-based controls.

MS has been studied in connection with many common viruses, and according to the hygiene hypothesis several infections during early childhood are protective,[3] but if infection occurs later in life there is an increased risk for MS.[4,5] Of note, infectious mononucleosis (IM) and MS share a similar pattern in geographical distribution, socioeconomic status and ethnicity.[6] Compared with controls, MS patients often show elevated levels of antibody reactivity against EBV-related antigens such as viral capsid antigen, Epstein Barr nuclear antigen 1 (EBNA1)[7,8] and several EBNA1 domains, in particular the 385–420 amino-acid domain.[9]

The aim of this study was to investigate the interaction of reported IM and antibody titres toward EBV antigens with HLA-DRB1*15 and A*02 in a large population-based case–control study comprising newly diagnosed MS cases in Sweden.

The interaction between HLA-DRB1*15 or absence of A*02 and EBNA1: 385–420 IgG levels was calculated using departure of additivity of effects criterion, and estimated as attributable proportion due to interaction (AP) with a 95% confidence interval (CI).[10,11] AP is an estimate of the proportion of the cases that are exposed

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to the two interacting factors that is attributable to the interaction itself (that is, beyond the sum of their separate effects). Interaction on the additive scale between two causal factors implies that they are involved in the same sufficient cause leading to disease.

Results

IM showed association to MS when adjusted for sex, age and area of residence, with an odds ratio of 1.89 (1.45–2.48 95%CI) (Table 1).

EBNA1 IgG levels above the median among controls were associated with an increased risk of MS (odds ratio (OR) = 1.74, 1.38–2.18 95% CI). Of the EBNA1 fragments, high titre of IgG antibodies against the 385–420 domain were associated with the highest increase in risk (OR = 3.79, 2.93–4.87 95% CI), but high antibody levels against fragments 61–90, 402–502, 1–89 and 421–450 were also associated with an increased risk (Table 1). These fragments were also significantly associated with disease risk when adjusting for EBNA1 IgG status, showing they had independent effects on risk of MS.

Comparing total EBNA1 IgG with the EBNA1: 385–420 IgG showed that the EBNA1: 385–420 IgG levels were more strongly associated to disease, as previously reported.[9] EBNA1: 385–420 IgG and EBNA1: 402–502 IgG showed similar results; when put in the same model EBNA1: 385–420 IgG had an OR = 2.28 (1.58–3.29 95% CI) compared with OR = 1.99 (1.38–2.86 95% CI) for EBNA1: 402–502 IgG. Anti-early antigen IgG levels were not associated to MS risk in our material.

We genotyped the HLA-DRB1 and A locus on the two-digit level, and thus we were only able to distinguish group of alleles, and not specific alleles, such as DRB1*1501. When we refer to either A*02 or DRB1*15 as alleles we mean all suballeles in that particular group. Several HLA alleles showed associations to MS susceptibility; of these only three remained significantly associated with MS when all associated alleles were adjusted for: DRB1*15; OR = 3.19 (2.47–4.12 95% CI), A*02, OR = 0.59 (0.46–0.76 95% CI), and A*03, OR = 1.36 (1.04–1.78 95% CI) (Supplementary Table 1). HLA-DRB1*15 and A*02 were retained for further analyses because they are established as genetic MS risk factors.[1] Analysis among DRB1*15 and A*02 homozygotes, heterozygotes and non-carriers showed no significant differences in estimated risk between homozygotes and heterozygotes for both DRB1*15 and A*02 (Supplementary Table 2).

IM status was analysed for potential interaction effects with the two genetic risk factors, HLA-DRB1*15 positivity and absence of A*02. An interaction effect was seen between IM and DRB1*15 when all cases and controls were included in the analysis, the attributable proportion due to interaction being 0.34 (0.01–0.68 95% CI) (Table 2).

EBNA1 fragment 385–420 IgG showed interaction (AP = 0.60, 0.45–0.76 95% CI), with DRB1*15 in the whole group and also in the HLA-A*02-positive and -negative subgroups (Table 3). This interaction was also observed when stratified by smoking status, AP = 0.46 (0.13–0.79 95% CI) in current smokers and AP = 0.68 (0.51–0.85 95% CI) in never-smokers.

We also observed an interaction effect with absence of A*02 in the same population, AP = 0.39 (0.18–0.61 95% CI) (Table 3). When stratified by smoking status, the AP for current smokers was 0.53 (0.25–0.80 95% CI) and for non-smokers 0.34 (0.04–0.65 95% CI).

Furthermore there was a difference in EBNA1: 385–420 IgG status among cases depending on genotype (Supplementary Table 3) where 84% of all DRB1*15-positive cases had titre above the cut-off (median among controls) compared with 72% among the DRB1*15-negative cases. Similar effects, although more modest, were seen when stratifying for A*02.

We also performed a risk group analysis of the different combinations of our three risk factors, high and low EBNA1: 385–420 IgG levels, DRB1*15 positivity and absence of A*02 (Figure 1). Individuals who did not carry any of these risk factors (DRB1*15 negative, A*02 positive and with low EBNA1: 385–420 IgG titre) were used as reference group. It was found that individuals

Table 1 Infectious mononucleosis and EBNA1 IgG are associated with multiple sclerosis

Risk factor	*Pos. controls*	*%*	*Pos. Cases*	*%*	*OR (95% CI)*	P-*value*
Infectious Mononucleosis (IM)	161/1490	11	114/678	17	1.89 (1.45–2.48)	3×10^{-6}

	Median Controls	*Median Cases*	*%*	*OR (95% CI)*	P-*value*	*OR (95% CI)*[a]	P-*value*[a]
EBNA1 IgG	142.87	150.78	63	1.74 (1.38–2.18)	3×10^{-6}		
EBNA1 IgG: 61–90	9.87	18.11	68	2.19 (1.73–2.77)	6×10^{-11}	2.00 (1.57–2.55)	2×10^{-8}
EBNA1 IgG: 1–89	9.77	20.75	69	2.30 (1.82–2.92)	5×10^{-12}	2.09 (1.64–2.67)	4×10^{-9}
EBNA1 IgG: 385–420	14.17	36.91	79	3.79 (2.93–4.87)	9×10^{-25}	3.60 (2.75–4.72)	2×10^{-20}
EBNA1 IgG: 421–450	12.98	23.33	66	1.91 (1.52–2.41)	5×10^{-8}	1.71 (1.34–2.17)	2×10^{-5}
EBNA1 IgG: 402–502	37.01	62.39	78	3.63 (2.82–4.67)	2×10^{-23}	3.48 (2.65–4.56)	3×10^{-19}
Early antigen IgG	12.08	14.77	54	1.24 (0.99–1.55)	NS		

The association to MS risk for the different variables included in this study. For infectious mononucleosis the number of positive individuals in each group is shown, together with the percentage. The analysis of infectious mononucleosis association was performed on a material consisting of 678 cases and 1490 controls. For all EBV-associated titers the median among the two groups is shown together with the percentage of cases with titer levels over the median among controls. The analysis of the EBV-associated titers was performed on 585 cases and 664 controls. All analyses were adjusted for sex, age and area of residence. EBNA1: 385–420 IgG and EBNA1 402–502 IgG showed similar results; when put in the same model EBNA1: 385–420 had the strongest association to disease, OR = 2.28 (1.58–3.29 95% CI, $P = 1 \times 10^{-5}$) compared to OR = 1.99 (1.38–2.86 95% CI, $P = 3 \times 10^{-4}$) for EBNA1: 402–502 IgG.

[a]Also corrected for EBNA1 IgG status.

Table 2 Interaction analysis of Infectious Mononucleosis (IM) and HLA-DRB1*15 or HLA-A*02 corrected for smoking

*Infectious mononucleosis and DRB1*15*	*No HLA-DRB1*15*				*HLA-DRB1*15*				*AP (95%CI)*	P
	ca	*co*	*OR (95% CI)*	P	*ca*	*co*	*OR (95% CI)*	P		
All cases and controls										
IM no	166	435	1.00 (–)		252	170	3.48 (2.76–4.39)	$<2\times10^{-16}$		
IM yes	34	46	1.80 (1.15–2.81)	0.01	60	21	6.49 (4.01–10.50)	3×10^{-14}	**0.34 (0.01–0.68)**	**0.05**
*In HLA-A*02 negative*										
IM no	99	186	1.00 (–)		133	71	3.72 (2.52–5.48)	4×10^{-11}		
IM yes	22	19	2.40 (1.22–4.74)	0.01	35	10	7.52 (3.49–16.23)	3×10^{-7}	0.32 (−0.23–0.86)	NS
*In HLA-A*02 positive*										
IM no	67	249	1.00 (–)		119	99	4.63 (3.13–6.83)	2×10^{-14}		
IM yes	12	27	1.79 (0.85–3.78)	NS	25	11	8.27 (3.83–17.88)	8×10^{-8}	0.35 (−0.18–0.87)	NS
*Infectious mononucleosis and HLA-A*02*	*HLA-A*2*				*Absence of HLA-A*2*				*AP (95%CI)*	
	ca	*co*	*OR (95% CI)*	P	*ca*	*co*	*OR (95% CI)*	P		
All cases and controls										
IM no	186	348	1.00 (–)		232	257	1.78 (1.36–2.33)	3×10^{-5}		
IM yes	37	38	1.79 (1.06–3.02)	0.03	57	29	3.68 (2.20–6.15)	8×10^{-7}	0.30 (−0.12–0.7)	NS
*In HLA-DRB1*15 negative*										
IM no	67	249	1.00 (–)		99	186	1.98 (1.36–2.86)	4×10^{-5}		
IM yes	12	27	1.69 (0.80–3.57)	NS	22	19	4.22 (2.13–8.35)	4×10^{-4}	0.37 (−0.13–0.87)	NS
*In HLA-DRB1*15 positive*										
IM no	119	99	1.00 (–)		133	71	1.52 (1.01–2.26)	0.04		
IM yes	25	11	1.81 (0.84–3.92)	NS	35	10	2.86 (1.31–6.25)	9×10^{-3}	0.19 (−0.57–0.95)	NS

Adjusted odds ratio with 95% confidence intervals, and associated *P*-values, of developing MS for subjects exposed to different combinations of a genetic factor (DRB1*15 and absence of HLA-A*02) and infectious mononucleosis status among 512 cases and 672 controls. Results shown for all cases and controls with available smoking status (current/none-smokers), and also stratified by HLA-DRB1*15 and A*01 carriage. Interaction measured by attributable proportion (AP) due to interaction, with 95% confidence intervals (CI must not include 0 to be significant). Significant APs can be seen in bold text. All analyses were adjusted for sex, age, smoking status (current(non-smoker), and area of residence, as well as for the other genetic risk factor.

with all three risk factors have a 16-fold higher risk of developing MS than those without these risk factors, OR = 16.03 (9.42–27.30 95% CI).

Discussion

We have studied four established risk factors for MS susceptibility, history of IM, anti-EBV antibodies and genetic risk factors (presence of DRB1*15 and absence of A*02) and how they interact to modulate MS risk. We confirmed the effect of both IM and levels of anti-EBNA1 IgG on MS risk, as well as showed that anti-EBNA1: 385–420 IgG is a stronger risk marker than anti-EBNA1 IgG.

We also observed interactions between IM status and DRB1*15 and between anti-EBV antibody titre and DRB1*15. HLA-DRB1*15 and anti-EBNA1 antibodies have been established as statistically independent risk factors for MS in previous studies using smaller study populations than the present study. It was shown that presence of high EBNA1 IgG levels was associated with higher risk for MS among DRB1*15-positive individuals compared with DRB1*15 negative individuals.[12,13] But causal interaction, that is, departure from additivity was not tested in these studies as we have shown here. These interactions have been suggested by previous smaller studies but have not been formally tested.[14,15] The interaction between EBNA1: 385–420 IgG and absence of A*02 is instead a novel finding.

Virtually, all MS patients are seropositive for EBV infection compared with about 95% of healthy controls. The infection usually takes place in early childhood with only mild symptoms. In North America and Europe there is a tendency for delayed infection in early adolescence, possibly due to better hygiene conditions, and 35–50% of these cases develop IM.[16] MS cases have increased antibody levels to specific EBNA1 domains, of which antibodies against the EBNA1: 385–420 domain were associated previously with the highest risk,[9] a finding that we confirm in this investigation.

EBV could be the environmental factor necessary to initiate disease in adults or the increased immunological response to EBV infection in MS could be the consequence of immune dysregulation associated with the disease itself. However, as MS patients do not have increased immune responses to other common viruses, the higher rate of EBV seroprevalence and the higher anti-EBV antibody titre cannot be due to some hyper-immune state in MS patients. The increased risk for MS with higher age at EBV infection,[16] and the results of a large-scale longitudinal epidemiological study showing that MS develops only after EBV seroconversion[17] are

Table 3 Interaction analysis of EBNA1 fragment 385–420 IgG and HLA-DRB1*15 or absence of HLA-A*02

*IgG titer and DRB1*15*	*No HLA-DRB1*15*				*HLA-DRB1*15*				*AP (95%CI)*	P	P*
	ca	*co*	*OR (95% CI)*	P	*ca*	*co*	*OR (95% CI)*	P			
All cases and controls											
385–420 IgG low	50	186	1.00 (–)		40	63	2.42 (1.45–4.02)	0.007			
385–420 IgG high	116	156	2.85 (1.91–4.24)	3×10^{-7}	218	78	10.67 (7.07–16.10)	$<2 \times 10^{-16}$	**0.60 (0.45–0.76)**	$\mathbf{4 \times 10^{-14}}$	$\mathbf{5 \times 10^{-6}}$
*In HLA-A*02 negative*											
385–420 IgG low	28	76	1.00 (–)		21	23	2.66 (1.25–5.66)	0.011			
385–420 IgG high	74	64	3.39 (1.92–6.00)	3×10^{-5}	121	33	11.52 (6.26–21.20)	4×10^{-15}	**0.56 (0.32–0.81)**	$\mathbf{8 \times 10^{-6}}$	$\mathbf{4 \times 10^{-3}}$
*In HLA-A*02 positive*											
385–420 IgG low	22	110	1.00 (–)		19	40	2.40 (1.16–4.95)	0.02			
385–420 IgG high	42	92	2.38 (1.31–4.32)	5×10^{-4}	97	45	11.88 (6.51–21.68)	8×10^{-16}	**0.68 (0.50–0.87)**	$\mathbf{5 \times 10^{-13}}$	$\mathbf{5 \times 10^{-6}}$
*IgG titer and absence of HLA-A*02*	*HLA-A*2*				*Absence of HLA-A*2*				*AP (95%CI)*	P	P*
	ca	*co*	*OR (95% CI)*	P	*ca*	*co*	*OR (95% CI)*	P			
All cases and controls											
385–420 IgG low	40	151	1.00 (–)		49	99	1.77 (1.08–2.89)	0.007			
385–420 IgG high	139	137	3.75 (2.46–5.73)	1×10^{-9}	195	97	7.43 (4.85–11.39)	$<2 \times 10^{-16}$	**0.39 (0.18–0.61)**	$\mathbf{4 \times 10^{-4}}$	$\mathbf{9 \times 10^{-4}}$
*In HLA-DRB1*15 negative*											
385–420 IgG low	22	110	1.00 (–)		28	76	1.78 (0.94–3.40)	0.08			
385–420 IgG high	42	92	2.28 (1.26–4.15)	0.007	74	64	5.97 (3.35–10.64)	2×10^{-9}	**0.49 (0.21–0.76)**	$\mathbf{5 \times 10^{-4}}$	$\mathbf{2 \times 10^{-3}}$
*In HLA-DRB1*15 positive*											
385–420 IgG low	19	40	1.00 (–)		21	23	1.90 (0.83–4.35)	0.131			
385–420 IgG high	97	45	4.91 (2.51–9.62)	4×10^{-6}	121	33	7.83 (3.94–15.55)	5×10^{-9}	0.26 (−0.15–0.67)	NS	

Adjusted odds ratio with 95% confidence intervals, and associated *P*-values, of developing MS for subjects exposed to different combinations of a genetic factor (DRB1*15 and absence of HLA-A*02) and an environmental factor, in this case either EBNA1 IgG or any of the five EBNA1 fragment IgGs, compared to subjects carrying none of the risk factors. Results shown for all cases and controls with available smoking status (current/none-smokers), and also stratified by HLA-DRB1*15 and A*01 carriage. Interaction measured by attributable proportion (AP) due to interaction, with 95% confidence intervals (CI must not include 0 to be significant). Statistically significant ($P < 0.05$) interactions are marked in bold. All analyses were adjusted for sex, age, smoking status, and area of residence, as well as for the other genetic risk factor. A total of 424 cases and 483 controls were included in the analysis.
$P^* = P$-value after 100 000 permutations.

evidence that the association between MS and EBV is genuine, and that EBV may be a necessary requirement for adult MS to develop.[16] Following that type of argument, it should be possible to prevent some MS cases by vaccinating against EBV and preventing young individuals from developing IM.[18] However, vaccination against such a common virus might have unpredictable consequences on human health.

Still little is known of how EBV is involved in MS pathology. Molecular mimicry has been suggested as one possible mechanism through which EBV may induce autoimmunity in MS. There are protein sequence similarities between EBNA1 and myelin basic protein which could possibly explain a potential cross-reactivity between myelin basic protein and viral proteins and the interaction between HLA-DRB1*15 and EBNA1 IgG, which we have observed in this investigation.[19] Interestingly, sequence similarities are also found between EBNA1—located within EBNA1: 385–420—and αβ-crystallin, an inhibitor of inflammation.[20] Because MS patients have a strong T-cell-mediated autoimmunity towards αβ-crystallin,[21] it is possible that high anti-EBNA1 antibody levels might lead to increased autoimmunity against αβ-crystallin, and thereby increase inflammation.

Another possibility for EBV involvement in MS is that the virus itself induces a chronic immunopathological response. Our observation of the interaction between HLA class II genotype and reactivity to EBV-related epitopes suggests that the mechanism through which HLA genes influence the risk of MS may, at least in part, involve the immune control of EBV infection. Interestingly, HLA-DR has another role in EBV infection, in addition to antigen presentation. Studies have shown that the virus uses HLA-DR as a co-factor to successfully enter and infect resting B-cells. The viral protein gp42 binds to the polymorphic region of HLA-DR and induces penetration of the cellular membrane, which may suggest that allelic differences within the HLA-DR locus can influence the outcome of EBV infection.[22]

Whether EBV has a role in MS through persistent infection in the CNS is controversial. Serafini *et al*.[23,24] have shown that most B cells infiltrating the MS brain express latent EBV proteins, and that viral reactivation appears restricted to plasma cells in active lesions and in the meningeal B-cell follicles. However, these findings

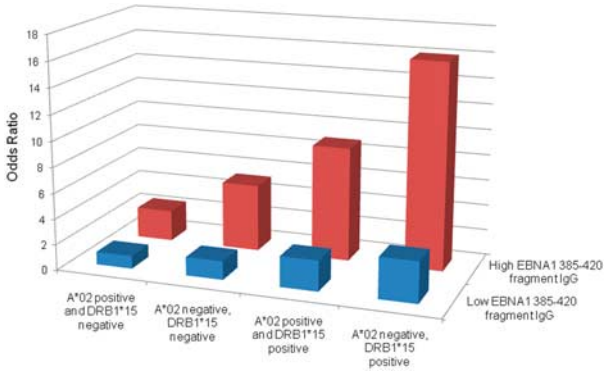

Figure 1 Odds ratios for different combinations of risk factors (HLA-DRB1*15, absence of A*02, and EBNA1: 385-420 IgG) among MS patients and controls.

A three-dimensional representation of the increase in risk, represented by odds ratio, for MS for each combination of the three studied risk factors, EBNA1: 385-420 IgG level, DRB1*15 and absence of A*02. The group with no risk factor was used as the reference group

EBNA: 385–420 IgG	*A*02*	*DRB1*15*	*Cases*	*Controls*	*OR*	*95% CI*	*P-value*
–	–	–	31	135	1.00	(reference group)	
–	+	–	35	102	1.44	0.82–2.54	0.21
–	–	+	30	55	2.37	1.27–4.41	7×10^{-3}
–	+	+	25	35	3.18	1.58–6.42	1×10^{-3}
+	–	–	68	123	2.42	1.46–4.03	1×10^{-3}
+	+	–	103	87	5.23	3.19–8.56	6×10^{-11}
+	–	+	119	61	8.88	5.31–14.85	9×10^{-17}
+	+	+	161	45	16.03	9.42–27.30	2×10^{-24}

have not been replicated to date, probably due to inter-study technical differences.[25] Recently, it was shown that EBV can infect human brain microvascular cells *in vitro* and cause an increase in pro-inflammatory cytokine production.[26]

HLA-B*07 and A*02 have been studied with regard to their association to MS T1 and T2 lesion volumes, disability score and anti-EBV viral capsid antigen IgG. B*07 was associated with increased lesion volumes, tendency to increased viral capsid antigen IgG levels and increased disability. A*02 on the other hand showed protective effects, being associated with lower disability and a trend toward lower viral capsid antigen IgG levels.[27] Similarly, we found a small reduction in frequency of EBNA1: 385–420 IgG among A*02 positive compared with A*02-negative patients (Supplementary Table 3). Microsatellite markers in or near the HLA-A locus have been associated with a higher risk of IM after primary EBV infection.[28] HLA-A*02 is also associated with a lower risk for EBV-related Hodgkin lymphoma, while A*01 is associated with an increased risk for Hodgkin lymphoma. Analysis of the four-digit level HLA typing showed that the most common suballele, A*0201, was driving the protective effect.[29]

Limitations of this study include misclassification of reported IM in the questionnaire. There is a risk for misclassification as not all IM cases are caused by EBV, and primary cytomegalovirus infections can give similar symptoms.[30] The recruitment process might itself introduce a selection bias in that not all patients seek medical care for MS symptoms. Considering the structure of the Swedish healthcare system it is likely that all MS patients are referred to neurological units, making them eligible to be part of the study. Previous reports on this study have participation rates of 93% for MS cases and 73% for controls.[31] The presence of non-respondents may introduce selection bias. However, it is highly unlikely that the demonstrated interaction between EBNA1: 385–420 IgG and DRB1*15/A*02 is affected to a large extent by such a bias. If so, the bias would depend both on genotype and anti-EBNA antibody reactivity. A confirmation of our results in an independent material would give further support to our findings and is important.

The HLA-complex has a strong linkage disequilibrium (LD),[32] and it is possible that the associations of DRB1*15 and A*02 are secondary to another genetic variant within high LD. However, DRB1*15 and A*02 have effects on MS risk, which are independent of each other.[1]

In conclusion, in this population-based case–control study we have demonstrated a statistically significant interaction between EBNA1: 385–420 IgG and presence of DRB1*15, and between EBNA1: 385–420 IgG and absence of A*02. Individuals with presence of all three risk factors: increased EBNA1: 385–420 IgG levels, presence of DRB1*15 and absence of A*02 had a 16-fold risk increase for MS compared with individuals with none of these risk factors. These findings are consistent with the hypothesis that part of the HLA class I and II gene influences in MS are related to differences in the immune response against EBV.

Materials and methods

Case identification and selection of controls

This study is based on the Epidemiological Investigation of Multiple Sclerosis (EIMS), an on-going case–control study performed in geographically defined parts of Sweden, comprising the general population aged 16–70 years. The present report analysed newly diagnosed cases and controls included between April 2005 and June 2008. In all, 35 hospital-based neurology units recruited cases to the study. For each case two controls were randomly selected from the national population register with consideration taken to age (predetermined 5-year-age groups), gender and residential area. All cases who did not fulfil the MacDonald Criteria ($n = 169$) were excluded. Participants of non-Scandinavian ancestry (115 cases and 181 controls) were also excluded to prevent bias caused by differences in LD-structure and allele frequency within the HLA. Participants were asked to provide a blood sample and to answer a questionnaire, covering environmental exposures, including if they had ever had IM. All participants provided written informed consent, and the the study was approved by the Regional Ethical Review Board at Karolinska Institutet.

EBNA1 and early antigen antibodies titre measurements

EBNA1 IgG and early antigen IgG were measured with ELISA kits from Biotest, Dreieich, Germany, according to the manufacturer's instructions.[8] EBNA1 fragments and antibody reactivity towards them were produced and analysed as described.[9] The antibody activity of each

sample was expressed in absorbance units, AU, as a percentage of the absorbance of the positive control.[8]

DNA extraction and HLA genotyping
DNA was extracted from blood using standard methods. HLA-A and DRB1 were genotyped with sequence-specific primers[33] using OLERUP SSP HLA Kits (Qiagen, Hilden, Germany). Individuals were classified as a carrier or non-carrier of a specific allele.

Definition of exposures
IM status was recorded as either reported infection or no infection. IgG antibody levels were dichotomised based on the median among controls. Smoking status was either a current or non-smoker. Subjects who smoked regularly during the inclusion year were defined as current smokers.

Age was categorised into eight intervals; 12–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49 and 50–70 years of age at diagnosis. Area of residence was defined as which one of the 21 Swedish counties the participant lived in, dichotomised as rural or urban counties. Three counties, Skåne, Stockholm and Västra Götalands county, in which there are mostly densely populated areas, were defined as urban areas, the rest were defined as rural counties.

For 68 individuals who answered the questionnaire IM status was unknown. For 678 cases and 1490 controls we had information on IM status and data on sex, age and area of residence. EBNA1 titre were determined in 585 cases and 664 controls. A total of 570 cases and 643 controls were HLA-genotyped and had their anti-EBNA1 reactivity measured.

Statistical analysis
Logistic regression analysis was performed using the free software R (version 2.41) and PASW 18 (SPSS Inc., Chicago, IL, USA). Interaction on the additive scale was calculated using AP due to interaction.[34] Rate ratio was estimated by the OR with 95% CI. All analyses were adjusted for age, gender and area of residence, unless stated otherwise. In the interaction analysis we also adjusted for smoking status (current/non-smokers).

Conflict of interest

Ms Sundqvist has received research support from the Swedish Association for Persons with Neurological Disabilities. Professor Hillert has received unrestricted research support from BiogenIdec, MerckSerono and Bayer Schering. Professor Alfredsson receives research support from the Swedish Medical Research Council (Dnr 521-2009-2596) and Swedish Council for Working life and Social Research (Dnr 2009-0650). Professor Tomas Olsson has received grant support for MS research from unrestricted grant support from Biogen-Idec, Bayer, SanofiAventis and Merck, and also lecture fees and/or advisory board consultancies for the same companies. Other authors declare no conflict of interest.

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Author contributions: ES did HLA genotyping and the statistical analysis, provided figures and wrote the paper. PS provided reagents and performed the anti-EBV antibody measurements. ML did HLA genotyping. AKH collected clinical information on the EIMS participants. FA contributed to writing of the paper. IK supervised HLA genotyping and the statistical analysis. JH, LA and TO are responsible for the EIMS study and the design of the study. All authors contributed to the final paper.

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Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gene)