

***HLA-DRB1*1501* influences long term disability progression and tissue damage on
MRI in relapse-onset multiple sclerosis**

WJ Brownlee¹, C Tur¹, A Manole², A Eshaghi¹, F Prados^{1,3}, KA Miszkiel⁵,

CAM Gandini Wheeler-Kingshott^{1,6,7}, H Houlden^{8,9}, O Ciccarelli^{1,9}

¹ NMR Research Unit, Queen Square MS Centre, Department of Neuroinflammation, UCL Institute of Neurology, London, UK

² Salk Institute for Biological Studies, CA, 92037, USA

³ Centre for Medical Image Computing, Department of Medical Physics and Biomedical Engineering, University College London, London, United Kingdom

⁴ e-Health Center, Universitat Oberta de Catalunya, Barcelona, Spain

⁵ Lysholm Department of Neuroradiology, National Hospital for Neurology and Neurosurgery, Queen Square, London, UK

⁵ Brain Connectivity Center, C. Mondino National Neurological Institute, Pavia, Italy

⁷ Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy

⁸ Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK

⁹ NIHR University College London Hospitals Biomedical Research Centre, UK

Key words: clinically isolated syndrome, multiple sclerosis, prognosis, genetics

Corresponding author:

Dr W Brownlee, NMR Research Unit, Queen Square MS Centre, Department of Neuroinflammation, UCL Institute of Neurology, London, UK, w.brownlee@ucl.ac.uk

ABSTRACT

Background: Whether genetic factors influence long-term course of multiple sclerosis (MS) is unresolved.

Objective: To determine the influence of *HLA-DRB1*1501* on long-term disease course in a homogenous cohort of clinically isolated syndrome (CIS) patients.

Methods: 107 patients underwent clinical and MRI assessment at the time of CIS and after 1, 3, 5 and 15 years. *HLA-DRB1*1501* status was determined using Sanger sequencing and tagging of the rs3135388 polymorphism. Linear/Poisson mixed-effects models were used to investigate rates of change in EDSS and MRI measures based on *HLA-DRB1*1501* status.

Results: *HLA-DRB1*1501*-positive (n=52) patients showed a faster rate of disability worsening compared with the *HLA-DRB1*1501*-negative (n=55) patients (annualised change in EDSS 0.14/year vs 0.08/year, $p<0.025$), and a greater annualised change in T2 lesion volume (adjusted difference 0.45ml/year, $p<0.025$), a higher number of gadolinium-enhancing lesions, and a faster rate of brain (adjusted difference -0.12%/year, $p<0.05$) and spinal cord atrophy (adjusted difference -0.22mm²/year, $p<0.05$).

Interpretation These findings provide evidence that the *HLA-DRB1*1501* allele plays a role in MS severity, as measured by long-term disability worsening and a greater extent of inflammatory disease activity and tissue loss. *HLA-DRB1*1501* may provide useful information when considering prognosis and treatment decisions in early relapse-onset MS.

INTRODUCTION

Multiple sclerosis (MS) is an immune-mediated disorder of the central nervous system due to a complex interplay of genetic and environmental factors.¹ More than 200 genetic loci have been identified that influence MS susceptibility, many of which are implicated in immune system processes.² *HLA-DRB1*1501* is the most important known genetic risk factor for MS.³ In Northern European populations, up to half of people with MS carry at least one *HLA-DRB1*1501* allele, and *HLA-DRB1*1501*-positivity is associated with an approximately threefold increase in MS susceptibility.³

The clinical course of MS is a highly variable with significant variation in age at disease onset, relapse rates, MRI findings and disability progression. Genetic factors may be one of the mechanisms responsible for disease course heterogeneity.⁴ *HLA-DRB1*1501* is associated with younger age at disease onset^{3,5-7} but not disease course (relapse or progressive-onset), or disease severity measured using the Expanded Disability Status Scale (EDSS).^{3,5-9} Studies investigating the influence of *HLA-DRB1*1501* on MRI-detected brain and spinal cord pathology in clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS) and primary progressive MS (PPMS) have produced conflicting findings.¹⁰⁻¹⁹ Some studies have found higher T2-hyperintense lesion load^{10,13-15,17}, brain atrophy^{12,13,20}, and microstructural tissue damage^{13,17} in *HLA-DRB1*1501*-positive compared with *HLA-DRB1*1501*-negative patients, but others have not.^{16,18,19} Previous studies have mainly been cross-sectional in nature and included heterogeneous cohorts of patients with varying disease duration, clinical course and exposure to disease-modifying therapies, potentially accounting for these conflicting findings.

Here we report on a genotype-phenotype study in a prospectively recruited and well-characterised cohort of CIS patients. We deeply phenotyped patients with up to five MRI scans

up to 15 years after disease onset to investigate the influence of *HLA-DRB1*1501* on MRI measures of inflammation and neurodegeneration, and long-term disability.

METHODS

Patients

We studied 107 CIS patients prospectively recruited between 1995 and 2004. Inclusion criteria were age 16–50 years, a typical CIS syndrome and no previous history of neurological symptoms. All patients were assessed clinically and with MRI within 3 months of onset (mean 48 days), and invited to return for scheduled follow-up after approximately 1, 3, 5 and 15 years (Figure 1), irrespective of clinical status. Blood samples were obtained at the time of the most recent follow-up (n=104) in 2014-2015, or from stored samples obtained at earlier time points in patients who were deceased (n=3). MS was diagnosed using the McDonald 2010 criteria²¹, and disease course was classified as RRMS or secondary progressive MS (SPMS) using the Lublin criteria.²² We assessed disability at each time point using the EDSS.

The study was approved by the institutional research ethics committee (reference number 13/LO/1413). All patients provided written informed consent at the time of study entry and at each subsequent follow-up visit.

MRI acquisition and image-analysis

Participants underwent MRI of the brain and spinal cord at the time of CIS then after ~1, 3 and 5 years, and brain MRI only at 15 years (Figure 1). All scans obtained over the first 5 years of follow-up were done on the same 1.5T Signa Scanner (General Electric, Wisconsin, USA). Proton density (PD)/T2-weighted and post-contrast T1-weighted scans of the brain and whole

spinal cord, plus a dedicated volume acquired inversion prepared fast spoiled gradient echo (FSPGR) scan of the cervical cord were acquired. There was a major hardware and software upgrade during the study period that was considered in statistical analyses. At 15 years brain MRI was done on a 3T Achieva TX scanner (Philips Healthcare, Best, The Netherlands). Details of the MRI acquisition protocol for the study have been reported previously.²³

We identified brain T2-hyperintense lesions on PD/T2-weighted scans and T1-hypointense lesions on 2D spin echo T1-weighted scans at each time point. Brain T2-hyperintense and T1-hypointense lesions were outlined using a semi-automated edge-finding tool (JIM6, Xinapse systems, Aldwinckle, UK). Brain T2-hyperintense lesion volume (T2LV) and T1-hypointense lesion volume (T1LV) were obtained by multiplying lesion area by slice thickness. The number of gadolinium-enhancing lesions at baseline, 1, 3 and 5 years was identified from post-contrast T1-weighted scans. We used post-contrast T1-weighted 2D spin echo scans after filling of T1-hypointense lesions for brain atrophy measures.²⁴ The normalised brain volume (NBV) at baseline was calculated using SIENAX and the percentage brain volume change (PBVC) at each follow-up time point with respect to baseline was calculated using SIENA. For the SIENAX and SIENA analyses brain extraction tool (BET) was used for the skull mask only and the brain tissue mask was computed using Similarity and Truth Estimation for Propagated Segmentations (STEPS), to avoid bias due to cerebrospinal fluid ²⁵.

We identified the number of T2-hyperintense spinal cord lesions on sagittal PD/T2-weighted scans of the whole cord at the time of CIS and after 1, 3 and 5 years. We calculated the upper cervical cord cross-sectional area (UCCA) at the level of C2/C3 from reformatted sagittally-acquired images using an active surface model.²⁶

Genotyping

We extracted genomic DNA from whole blood using standard techniques. Sanger sequencing was used to identify the rs3135388 single nucleotide polymorphism (SNP). The A allele of rs3135388 is strongly associated with *HLA-DRB1*1501* and patients heterozygous or homozygous for the A allele are inferred to be *HLA-DRB1*1501*²⁷. All genotyping was done by a single neurogeneticist blinded to the patient's clinical status and MRI findings.

Statistical analysis

Differences in demographic and clinical characteristics between the *HLA-DRB1*1501*-positive and negative groups were investigated using univariable linear regression for continuous variables and Chi-squared test for proportions. To assess differences at baseline and over time in disability and MRI measures between *HLA-DRB1*1501*-positive and *HLA-DRB1*1501*-negative patients, we used mixed-effects models with EDSS or the MRI measure of interest as the dependent variable. We used linear mixed-effects models for all MRI metrics except for gadolinium-enhancing lesion number where Poisson mixed-effects models were used. The independent variables included: time (in years), genotype (*HLA-DRB1*1501*-positive or negative), and an interaction term 'time X genotype' to detect between-group differences in changes over time. Three-way interaction terms ('time X genotype group X age' and 'time X genotype group X sex') were added among the independent variables to test whether the differences in the rates of change between those who were *HLA-DRB1*1501*-positive and *HLA-DRB1*1501*-negative were related to age at onset or sex. For all models we explored age, sex, and a binary indicator of whether the scan had been performed before or after the scanner upgrade as independent variables. Whenever the p-value for the regression coefficient for 'genotype group' was <0.05 we assumed there were significant differences between genetic groups at baseline. Whenever the p-value for the regression coefficient of the interaction term 'time X genotype group' was <0.05 we assumed there were significant

differences between groups in the evolution of disability or MRI measures over time. Similarly, whenever the three-way interactions including either age or sex was significant, we assumed the differences in rates of change between allele groups depended on age or sex, respectively.

All mixed-effects models were random intercept and random slope (for time) models. For the random effects we chose an unstructured covariance structure, allowing a non-zero covariance between random slope and random intercept. These models allowed us to account for repeated measures (within an individual), and, assuming missingness at random, they used all available data points, allowing us to overcome the problem derived from the missing data points and therefore reducing the risk of type II error.

Significance level was set at the level of 0.05. Statistical analyses were carried out in Stata 13.1.

RESULTS

The baseline demographic, clinical and MRI profile of the cohort grouped by *HLA-DRB1*1501* status is shown in Table 1. Fifty-two (49%) patients were *HLA-DRB1*1501*-positive (49 [94%] heterozygous, 3 [6%] homozygous) and 55 (51%) were *HLA-DRB1*1501*-negative. The *HLA-DRB1*1501*-positive patients were younger than the *HLA-DRB1*1501*-negative patients at the time of CIS, although this didn't reach statistical significance. The *HLA-DRB1*1501*-positive patients had a higher T2LV (mean 2.50ml vs 1.14ml, $p<0.001$) and a greater number of gadolinium-enhancing lesions (mean 1.90 vs 0.51, $p<0.001$) at baseline compared with the *HLA-DRB1*1501*-negative patients. These differences remained after excluding patients who remained CIS after 15 years ($n=19$).

Development of multiple sclerosis and disability worsening

MS developed in 47/52 (90%) *HLA-DRB1*1501*-positive patients and 41/55 (75%) *HLA-DRB1*1501*-negative patients (odds ratio=3.21, 95%CI 1.07 to 9.68, $p=0.032$). A similar number of patients in both groups started disease-modifying treatment during follow-up. 14 (27%) *HLA-DRB1*1501*-positive patients initiated first-line therapies (beta interferon $n=8$, glatiramer acetate $n=5$, teriflunomide $n=1$), with 5 (36%) patients escalating to second-line therapies (fingolimod $n=2$, natalizumab $n=3$), whereas 11 (20%) *HLA-DRB1*1501*-negative patients initiated first-line therapies (beta interferon $n=6$, glatiramer acetate $n=4$, dimethyl fumarate $n=1$) and 3 (27%) escalated to second-line therapies (fingolimod $n=2$, alemtuzumab $n=1$).

SPMS developed in a similar proportion of *HLA-DRB1*1501*-positive patients (11/52 [21%]) than *HLA-DRB1*1501*-negative (7/55 [13%]) patients ($p=0.248$); three patients died during follow-up from complications of severe SPMS at 15 years (2 *HLA-DRB1*1501*-positive, 1 *HLA-DRB1*1501*-negative).

EDSS scores increased over time in both groups, but the *HLA-DRB1*1501*-positive patients showed a faster rate of disability worsening compared with the *HLA-DRB1*1501*-negative patients (annualised EDSS change 0.14/year vs 0.08/year, $p<0.025$, Figure 2). The rate of change in EDSS was similar after excluding patients who remained CIS at follow-up (annualised change in EDSS 0.16 vs 0.11, $p<0.05$).

Longitudinal changes in MRI measures

Brain MRI measures

T2LV increased in both groups over time (Table 2 and Figure 3) but the annualised change in T2LV was higher in the *HLA-DRB1*1501*-positive compared with the *HLA-DRB1*1501*-negative patients (adjusted difference 0.45ml/year, 95%CI 0.11 to 0.71, $p<0.025$). The findings were similar for T1LV. The *HLA-DRB1*1501*-positive patients had a higher number of GdE at all time points (Figure 4), compared with those who were *HLA-DRB1*1501*-negative (incident rate ratio [IRR] = 4.45, 95%CI 1.82 to 10.91, $p=0.001$).

Both the *HLA-DRB1*1501*-positive and *HLA-DRB1*1501*-negative patients had evidence of brain atrophy over time (Table 2 and Figure 3). The rate of brain atrophy was significantly faster in *HLA-DRB1*1501*-positive compared with *HLA-DRB1*1501*-negative patients (adjusted difference PBVC -0.12%/year, 95%CI -0.19 to -0.01, $p<0.01$). Given the observed differences in T2 lesion load in *HLA-DRB1*1501*-positive and *HLA-DRB1*1501*-negative patients analyses were repeated after adjusting for T2LV. The results were similar: annualised PBVC was higher in the *HLA-DRB1*1501*-positive compared with the *HLA-DRB1*1501*-negative patients (adjusted difference -0.12%/year, 95%CI -0.20 to -0.02, $p<0.05$).

Spinal cord MRI measures

Spinal cord MRI was available over the first 5 years of follow-up. The number of spinal cord lesions increased over time in both groups with a non-significant trend towards a greater increase in the number of spinal cord lesion number in the *HLA-DRB1*1501*-positive patients (adjusted difference 0.07 lesions/year, 95%CI -0.32 to 0.38, $p>0.05$). Spinal cord was observed in both groups (Table 2), but the *HLA-DRB1*1501*-positive had evidence of faster spinal cord atrophy compared with *HLA-DRB1*1501*-negative patients (adjusted difference -

0.22 mm²/year, 95%CI -0.36 to -0.01, p<0.05). These differences remained even after adjusting for brain T2LV.

Longitudinal changes in MRI measures: Multiple sclerosis patients only

Patients who remained CIS were over-represented in the *HLA-DRB1*1501*-negative group. Analyses were repeated after excluding patients who remained CIS after 15 years (Table 3). In the subgroup of patients with MS at 15 years the findings were similar to the whole cohort: *HLA-DRB1*1501*-positive MS patients had a greater annualised change in T2LV (adjusted difference 0.52ml/year, 95%CI 0.24 to 0.70, p<0.01) and T1LV (adjusted difference 0.17ml/year, 95%CI 0.11 to 0.24, p<0.01), a higher number of gadolinium-enhancing lesions at all time points (IRR = 3.20, 95%CI 1.36 to 7.50, p=0.007) and faster annualised change in PBVC (adjusted difference -0.14%/year, 95%CI -0.26 to -0.11, p<0.01) and UCCA (adjusted difference -0.19 mm²/year, 95%CI -0.34 to -0.13, p<0.05) compared with the *HLA-DRB1*1501*-negative patients.

Interactions of *HLA-DRB1*1501* with age and sex

Three-way interaction terms found no influence of age or sex with *HLA-DRB1*1501* status on longitudinal changes in disability and MRI measures.

DISCUSSION

The extent to which genetic factors influence MS disease course is unresolved. In this prospective, longitudinal study of CIS patients, with serial MRI measurements, we found an association of *HLA-DRB1*1501* with the development of MS, and disability worsening, mediated by a more inflammatory MRI phenotype and a greater extent of neurodegeneration.

*HLA-DRB1*1501* status may provide complementary information to other clinical, MRI and biological factors known to be important in the evolution of relapse-onset MS.^{28,29}

Conventional MRI is highly sensitive to the detection of focal inflammatory pathology in MS. *HLA-DRB1*1501*-positive patients had a higher brain T2 lesion load at presentation and a greater accrual of new T2 lesions over time, and a higher number of gadolinium-enhancing lesions at all time points measured, compared with *HLA-DRB1*1501*-negative patients. These differences were maintained after excluding people who remained CIS, suggesting that the observed differences were not simply due to an over-representation of monophasic CIS patients in the *HLA-DRB1*1501*-negative group. *HLA-DRB1*1501* and other class II human leukocyte antigen alleles are involved in immune processes including antigen presentation and T cell activation that may influence inflammatory disease activity in MS. A more inflammatory phenotype in *HLA-DRB1*1501*-positive patients is supported by other radiological, immunological and pathological studies. Previous MRI studies in patients with CIS^{10,15}, RRMS¹³ and PPMS¹⁷ have reported higher brain T2 lesion load in *HLA-DRB1*1501*-positive patients. Our previous longitudinal study in early PPMS found a higher brain T2LV at all time points in *HLA-DRB1*1501*-positive compared with *HLA-DRB1*1501*-negative patients over 5 years¹⁷, in keeping with the current findings in a larger cohort with early relapse-onset MS. A greater extent of histopathological demyelination has been reported previously in *HLA-DRB1*1501*-positive patients, supporting our findings *in vivo*.

*HLA-DRB1*1501* is known to influence the age at disease onset in MS, and we observed a trend towards younger age in *HLA-DRB1*1501*-positive CIS patients. Age is linked to disease activity in MS: relapses rates and radiological disease activity are greater in children than adults with MS, and relapse rates decline in older adults.³⁰ We found no interaction between

age and *HLA-DRB1*1501* indicating that younger age at disease onset is unlikely to be the explanation for the more inflammatory phenotype observed.

As expected in a cohort of CIS and early RRMS patients, a faster rate of brain atrophy was observed (-0.31 to -0.43%/year) compared with published normative data from healthy controls of a similar age (approximately -0.23%/year).^{31,32} The *HLA-DRB1*1501*-positive patients had a faster rate of brain compared with *HLA-DRB1*1501*-negative patients, even though the *HLA-DRB1*1501*-negative patients tended to be older, suggesting that *HLA-DRB1*1501* influences the rate of brain atrophy, over and above any age-expected change. Other studies have found evidence of greater whole brain atrophy¹³ and subcortical grey matter atrophy²⁰ in *HLA-DRB1*1501*-positive compared with *HLA-DRB1*1501*-negative patients, and greater microstructural tissue damage assessed using ¹H magnetic resonance spectroscopy and magnetization transfer imaging in normal-appearing tissues.^{13,17} The observed differences in brain atrophy and microstructural tissue damage in the *HLA-DRB1*1501*-positive patients may be a consequence of more prominent inflammatory disease, accelerating neurodegeneration in *HLA-DRB1*1501*-positive patients. Focal demyelinating lesions result in significant neuroaxonal loss and early changes in brain T2 lesion load on MRI are associated with the development of later brain atrophy in MS.³³ After adjusting for brain T2 lesion load we found that differences in whole brain and spinal cord atrophy were maintained. However, conventional MRI is insensitive to the detection of diffuse inflammation within normal-appearing tissues and the meninges that may be relevant to tissue damage and disability progression.³⁴ Post-mortem studies have found a greater extent of diffuse parenchymal and meningeal inflammation in *HLA-DRB1*1501*-positive patients^{35,36}, that may contribute to a faster rate of neurodegeneration.

In contrast to previous studies¹⁴ we found no statistically significant association of *HLA-DRB1*1501* status with spinal cord lesions, although on average there was a greater increase in the number of spinal cord lesions over time in *HLA-DRB1*1501*-positive patients. This may reflect that the study was underpowered with regard to spinal cord lesion number (only a third of patients developed new spinal cord lesions during follow-up), or technical factors with spinal cord lesion number quantified using sagittal T2-weighted scans only, which may underestimate the number of cord lesions. It would have been useful to be able to quantify the area (or volume) of spinal cord lesions, but this was not possible using the scans acquired. A previous post-mortem study found that the number of spinal cord lesions was similar in *HLA-DRB1*1501*-positive and negative patients, but the extent of lesions, measured by the area of demyelinating plaques, was significantly greater in patients who were *HLA-DRB1*1501*-positive.³⁵

We investigated the influence of *HLA-DRB1*1501* on long-term disease course, but not other HLA or non-HLA genes known to influence MS susceptibility. Other HLA loci interact with *HLA-DRB1*1501* to amplify (or attenuate) disease susceptibility.³ A previous cross-sectional study calculated the total HLA genetic risk burden by testing for all known HLA alleles that influence susceptibility.²⁰ The HLA allele dose was multiplied by the reported effect size then cumulated. A higher HLA genetic risk burden score was associated with a younger age at disease onset and a greater extent of subcortical grey matter atrophy, although these associations were mainly driven by *HLA-DRB1*1501*. There were too few *HLA-DRB1*1501* homozygous patients (n=3) in this cohort to investigate whether *HLA-DRB1*1501* allele genetic burden influences long-term outcomes. Median EDSS at 15 years was 2.0 in both *HLA-DRB1*1501* homozygous and heterozygous patients but this study is under-powered to detect any difference. We also did not collect information on environmental factors such as vitamin D, Epstein-Barr virus, body mass index and cigarette smoking that may interact with *HLA-DRB1*1501* to influence MS susceptibility³⁷, and potentially disease course. Future studies

should investigate how gene-environment interactions influence MS disease course and disability accrual.

The patients in this cohort were predominantly untreated. There were too few treated patients to assess how *HLA-DRB1*1501* might impact on response to disease-modifying therapies. A more inflammatory phenotype might be associated with a better response to anti-inflammatory treatments. *HLA-DRB1*1501* has been associated with a more favourable treatment response to glatiramer acetate^{38,39}, but not interferon- β ^{38,10}. Further work is required to determine whether *HLA-DRB1*1501* may predict response to treatment.

A number of cross-sectional studies have found no association of *HLA-DRB1*1501* status and MS phenotype, other than age at disease onset^{3,7}. We studied a homogenous cohort of patients followed prospectively from the time of presentation with CIS, who were carefully phenotyped with established MRI measures of inflammation and neurodegeneration. We studied patients longitudinally over a uniquely long follow-up period of 15 years, unlike previous studies that have largely been cross-sectional in nature.^{10,12-14,16,18-20} A potential disadvantage of longitudinal clinical-MRI studies is drop-out of subjects over time. However, retention in this study was high – clinical status and disability was assessed in all patients after ~15 years and over 75% of the cohort had MRI at all five time points. Patients with optic neuritis were over-represented in this cohort, however, the frequency of *HLA-DRB1*1501* positivity is similar in patients with optic neuritis and other CIS types.¹⁵ The high proportion of patients with optic neuritis in our cohort is unlikely to account for the observed differences between *HLA-DRB1*1501*-positive and negative patients.

CONCLUSION

We provide evidence that *HLA-DRB1*1501* influences long-term disease course in relapse-onset MS, potentially mediated by a more inflammatory disease phenotype with faster accrual of T2 brain lesions, a higher number of gadolinium-enhancing lesions, and a faster rate of brain and spinal cord atrophy. *HLA-DRB1*1501* may be one of the factors underlying disease course heterogeneity in patients with early relapse-onset MS.

FUNDING

The study was supported by the National Institute for Health Research (NIHR) University College London Hospitals Biomedical Research Centre, the United Kingdom MS Society and the Neurological Foundation of New Zealand.

CONFLICTS OF INTEREST

Dr Brownlee has received speaker honoraria for educational activities and/or acted as a consultant for Biogen, Janssen, Merck, Novartis, Roche, Sanofi-Genzyme and Viatris, and serves on the editorial board for Multiple Sclerosis Journal.

Dr Tur is currently being funded by a Junior Leader La Caixa Fellowship. The project that gave rise to these results received the support of a fellowship from “la Caixa” Foundation (ID 100010434). The fellowship code is LCF/BQ/PI20/11760008. She has also received the 2021 Merck’s Award for the Investigation in Multiple Sclerosis (Spain) and a grant from Instituto de Salud Carlos III (ISCIII), Spain (grant ID: PI21/01860). In 2015, she received anECTRIMS Post-doctoral Research Fellowship and has received funding from the UK Multiple Sclerosis Society (grant number 77). She has also received speaker honoraria from Roche and Novartis. She serves on the Editorial Board of Neurology and Multiple Sclerosis Journal.

Dr Manole has nothing to disclose.

Dr Eshaghi has received travel support from the National Multiple Sclerosis Society and honorarium from the Journal of Neurology, Neurosurgery and Psychiatry for Editorial Commentaries. He has received research grants from the UK Medical Research Council (MRC), Innovate UK, Biogen, Merck and Roche. He is the founder and equity stake holder in Queen Square Analytics Limited. He serves on the editorial board of Neurology (American Academy of Neurology).

Dr Prados received a Guarantors of Brain fellowship 2017-2020 and is supported by National Institute for Health Research (NIHR), Biomedical Research Centre initiative at University College London Hospitals (UCLH).

Dr Miszkiel has nothing to disclose.

Prof Gandini Wheeler-Kingshott has nothing to disclose.

Prof Houlden has nothing to disclose.

Prof Ciccarelli receives research funding from the National Institute for Health and Care Research (NIHR) (NIHR RP: RP-2017-08-ST2-004) , National and UK Multiple Sclerosis Societies, Rosetrees trust and NIHR University College London Hospitals (UCLH) Biomedical Research Centre; she has received speaker honoraria from Novartis and Merck during the past 12 months; and is the Deputy Editor of Neurology.

REFERENCES

1. Thompson AJ, Baranzini SE, Geurts J, Hemmer B, Ciccarelli O. Multiple sclerosis. *Lancet*. 2018;391:1622-1636.
2. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. *Science*. 2019;365(6460):eaav7188.
3. Moutsianas L, Jostins L, Beecham AH, et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nature Genetics*. 2015;47:1107-1113.
4. Jokubaitis VG, Zhou Y, Butzkueven H, Taylor BV. Genotype and Phenotype in Multiple Sclerosis—Potential for Disease Course Prediction? *Current Treatment Options in Neurology*. 2018;20:18.

5. Masterman T, Ligers A, Olsson T, Andersson M, Olerup O, Hillert J. HLA-DR15 is associated with lower age at onset in multiple sclerosis. *Annals of Neurology*. 2000;48:211-219.
6. Hensiek AE, Sawcer SJ, Feakes R, et al. HLA-DR 15 is associated with female sex and younger age at diagnosis in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry*. 2002;72:184-187.
7. International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature*. 2011;476(7359):214-219.
8. George MF, Briggs FB, Shao X, et al. Multiple sclerosis risk loci and disease severity in 7,125 individuals from 10 studies. *Neurol Genet*. 2016;2:e87.
9. Crielaard L, Kavaliunas A, Ramanujam R, et al. Factors associated with and long-term outcome of benign multiple sclerosis: a nationwide cohort study. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2019;90:761-767.
10. Hauser SL, Oksenberg JR, Lincoln R, et al. Interaction between HLA-DR2 and abnormal brain MRI in optic neuritis and early MS. Optic Neuritis Study Group. *Neurology*. 2000;54:1859-1861.
11. Zivadinov R, Uxa L, Zacchi T, et al. HLA genotypes and disease severity assessed by magnetic resonance imaging findings in patients with multiple sclerosis. *J Neurol*. 2003;250:1099-1106.
12. Zivadinov R, Uxa L, Bratina A, et al. HLA-DRB1*1501, -DQB1*0301, -DQB1*0302, -DQB1*0602, and -DQB1*0603 alleles are associated with more severe disease outcome on MRI in patients with multiple sclerosis. *International Review of Neurobiology*. 2007;79:521-535.
13. Okuda DT, Srinivasan R, Oksenberg JR, et al. Genotype–Phenotype correlations in multiple sclerosis: HLA genes influence disease severity inferred by 1HMR spectroscopy and MRI measures. *Brain*. 2009;132:250-259.
14. Sombekke MH, Lukas C, Crusius JB, et al. HLA-DRB1*1501 and spinal cord magnetic resonance imaging lesions in multiple sclerosis. *Arch Neurol*. 2009;66:1531-1536.
15. Horakova D, Zivadinov R, Weinstock-Guttman B, et al. HLA DRB1*1501 is only modestly associated with lesion burden at the first demyelinating event. *Journal of Neuroimmunology*. 2011;236:76-80.
16. Van der Walt A, Stankovich J, Bahlo M, et al. Heterogeneity at the HLA-DRB1 allelic variation locus does not influence multiple sclerosis disease severity, brain atrophy or cognition. *Mult Scler*. 2011;17:344-352.
17. Tur C, Ramagopalan S, Altmann DR, et al. HLA-DRB1*15 influences the development of brain tissue damage in early PPMS. *Neurology*. 2014;83:1712-1718.
18. Yaldizli O, Sethi V, Pardini M, et al. HLA-DRB*1501 associations with magnetic resonance imaging measures of grey matter pathology in multiple sclerosis. *Mult Scler Relat Disord*. 2016;7:47-52.
19. Muhlau M, Andlauer TF, Hemmer B. HLA Genetic Risk Burden in Multiple Sclerosis. *JAMA neurology*. 2016;73:1500-1501.
20. Isobe N, Keshavan A, Gourraud PA, et al. Association of HLA Genetic Risk Burden With Disease Phenotypes in Multiple Sclerosis. *JAMA Neurology*. 2016.
21. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Annals of Neurology*. 2011;69(2):292-302.
22. Lublin FD, Reingold SC et al. Defining the clinical course of multiple sclerosis: Results of an international survey. *Neurology*. 1996;46:907-911.
23. Brownlee WJ, Altmann DR, Prados F, et al. Early imaging predictors of long-term outcomes in relapse-onset multiple sclerosis. *Brain*. 2019;142:2276-2287.
24. Prados F, Cardoso MJ, Kanber B, et al. A multi-time-point modality-agnostic patch-based method for lesion filling in multiple sclerosis. *NeuroImage*. 2016;139:376-384.
25. Jorge Cardoso M, Leung K, Modat M, et al. STEPS: Similarity and Truth Estimation for Propagated Segmentations and its application to hippocampal segmentation and brain parcellation. *Medical image analysis*. 2013;17:671-684.

26. Horsfield MA, Sala S, Neema M, et al. Rapid semi-automatic segmentation of the spinal cord from magnetic resonance images: application in multiple sclerosis. *NeuroImage*. 2010;50:446-455.
27. Benesova Y, Vasku A, Stourac P, Hladikova M, Fiala A, Bednarik J. Association of HLA-DRB1*1501 tagging rs3135388 gene polymorphism with multiple sclerosis. *Journal of Neuroimmunology*. 2013;255:92-96.
28. Tintore M, Rovira A, Rio J, et al. Defining high, medium and low impact prognostic factors for developing multiple sclerosis. *Brain*. 2015 138:1863-74
29. Brownlee WJ, Altmann DR, Alves Da Mota P, et al. Association of asymptomatic spinal cord lesions and atrophy with disability 5 years after a clinically isolated syndrome. *Mult Scler*. 2017;23:665-674.
30. Dahlke F, Arnold DL, Aarden P, et al. Characterisation of MS phenotypes across the age span using a novel data set integrating 34 clinical trials (NO.MS cohort): Age is a key contributor to presentation. *Mult Scler*. 2021 *online ahead of print*
31. De Stefano N, Stromillo ML, Giorgio A, et al. Establishing pathological cut-offs of brain atrophy rates in multiple sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry* 2016;87:93-99
32. Battaglini M, Gentile G, Luchetti L, et al. Lifespan normative data on rates of brain volume changes. *Neurobiology of Aging* 2019; 81:30-37.
33. Chard DT, Brex PA, Ciccarelli O, et al. The longitudinal relation between brain lesion load and atrophy in multiple sclerosis: a 14 year follow up study. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2003;74:1551-1554.
34. Kutzelnigg A, Lucchinetti CF, Stadelmann C, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain*. 2005;128:2705-2712.
35. DeLuca GC, Alterman R, Martin JL, et al. Casting light on multiple sclerosis heterogeneity: the role of HLA-DRB1 on spinal cord pathology. *Brain*. 2013;136:1025-1034.
36. Yates RL, Esiri MM, Palace J, Mittal A, DeLuca GC. The influence of HLA-DRB1*15 on motor cortical pathology in multiple sclerosis. *Neuropathology and Applied Neurobiology*. 2015;41:371-384.
37. Hedstrom AK, Hillert J, Brenner N, et al. DRB1-environment interactions in multiple sclerosis etiology: results from two Swedish case-control studies. *Journal of Neurology, Neurosurgery, and psychiatry*. 2021;92:717-722
38. Gross R, Healy BC, Cepok S, et al. Population structure and HLA DRB1 1501 in the response of subjects with multiple sclerosis to first-line treatments. *Journal of Neuroimmunology*. 2011;233:168-174.
39. Dhib-Jalbut S, Valenzuela RM, Ito K, Kaufman M, Ann Picone M, Buyske S. HLA DR and DQ alleles and haplotypes associated with clinical response to glatiramer acetate in multiple sclerosis. *Mult Scler Relat Disord*. 2013;2:340-348.
40. Comabella M, Fernandez-Arquero M, Rio J, et al. HLA class I and II alleles and response to treatment with interferon-beta in relapsing-remitting multiple sclerosis. *Journal of Neuroimmunology*. 2009;210:116-119.