HLA-DRB*1501 Associations with Magnetic Resonance Imaging Measures of Grey Matter Pathology in Multiple Sclerosis

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Abstract

Background: The HLA-DRB*1501 haplotype influences the risk of developing multiple sclerosis (MS), but it is not known how it affects grey matter pathology.

Aim: To assess HLA-DRB*1501 effects on magnetic resonance imaging (MRI) cortical grey matter pathology.

Methods: Whole and lesional cortical grey matter volumes, lesional and normal-appearing grey matter magnetization transfer ratio were measured in 85 people with MS and 36 healthy control subjects. HLA-DRB*1501 haplotype was determined by genotyping (rs3135388).

Results: No significant differences were observed in MRI measures between the HLA-DRB*1501 subgroups.

Conclusions: The HLA-DRB*1501 haplotype is not strongly associated with MRI-visible grey matter pathology.

INTRODUCTION

Significant progress has been made understanding the interplay between environmental and genetic factors in people who develop multiple sclerosis (MS). More than 100 gene loci have been found to influence the risk of a person developing MS, however the genetic factor most strongly associated with the development of MS is the polymorphism at the HLA-DRB1 locus, and in particular the HLA-DRB*1501 haplotype (International Multiple Sclerosis Genetics Consortium et al., 2007). HLA-DRB*1501 encodes a component of the histocompatibility complex II cell surface receptor necessary for antigen presentation (Hebbring et al., 2013), and this has been implicated in an aberrant T-cell mediated inflammatory response against myelin (International Multiple Sclerosis Genetics Consortium et al., 2007). Up to 30% of the European population are HLA-DRB*1501 positive and this haplotype is associated with a 2-4 fold risk of developing MS (Dyment et al., 2004). Relatively little is known about whether or not the HLA-DRB*1501 haplotype influences clinical and radiological manifestations of MS. Genotype-phenotype studies have shown that an HLA-DRB*1501 positive haplotype is associated with an earlier age at disease-onset (Masterman et al., 2000). However, associations with neurological and cognitive outcomes are less consistent. While some studies have found greater disability in HLA-DRB*1501 positive individuals (Okuda et al., 2009), other have found no (Barcellos et al., 2003) or less severe effects (Silva et al., 2007).

Only a few studies have looked for associations between genotype and magnetic resonance imaging (MRI) measures. Most have looked at the brain white matter (WM) only, and brain WM lesion loads - as seen on PD/T2-weighted MRI scans - appear to be greater in those who are HLA-DRB*1501 positive in some (Matsuoka et al., 2008;

Okuda et al., 2009) but not all studies (Zivadinov et al., 2003). Brain atrophy may also be greater in HLA-DRB*1501 positive compared with negative MS patients (Zivadinov et al., 2007), although not all studies have shown this (Van der Walt et al., 2011; Zivadinov et al., 2003).

In this exploratory work, we looked for associations between the HLA-DRB*1501 haplotype and MRI measures of cortical grey matter (cGM) pathology.

PARTICIPANTS AND METHODS

Participants

Participants were between 18 and 65 years old, and had a diagnosis of MS based on the McDonald criteria (Polman et al., 2005). The control group had no known neurological disease. MS subtypes were classified using the Lublin-Reingold criteria (Lublin and Reingold, 1996). All participants gave written informed consent. This study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by our local institutional ethics committee. Thirty relapsing-remitting (RR), 30 secondary progressive (SP), 25 primary progressive (PP) MS patients, and 36 healthy controls, were included in this study. In the 30 days prior to scanning no patient had had a relapse or steroid treatment. Participants demographics and patients clinical characteristics are given in Table 1.

Clinical assessments

Expanded Disability Status Scale (EDSS, Kurtzke, 1983), MS Functional Composite (MSFC, Cutter et al., 1999) and Symbol Digit Modalities Test (SDMT, Smith, 1989)

scores were determined in the MS group. MSFC and SDMT scores were also assessed in controls to act as reference values.

Image acquisition

Using a 3T Philips Achieva system (Philips Healthcare, Best, The Netherlands) with a 32-channel head coil and multi-transmit technology, T1-weighted volumetric, PSIR, PD/T2-weighted, fluid attenuated inversion recovery (FLAIR), and MTR were acquired. The acquisition parameters are given in Table 2.

Image processing

Lesion measures

Cortical GM lesions were identified on the PSIR images, using JIM (Version 6.0, Xinapse Systems, Northants). Cortical GM lesions were subdivided into intracortical (lesions confined to GM) and leukocortical (lesions that involved both GM and WM). PD/T2-weighted and FLAIR-scans served as reference. Marking of all lesions was carried out blinded to clinical data under the guidance of an expert neuroradiologist (TY).

Lesion and normal-appearing mask generation

Registrations were performed using NitfyReg (Modat et al., 2010). The PSIR images (and corresponding lesion masks) were affine registered to the T1-weighted volumetric images. After lesion filling (Chard et al., 2010) the T1-weighted volume image was segmented to give GM and WM tissue probability maps using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). Only voxels with a probability of ≥95% of being

GM or WM were included in subsequent analyses. The Montreal Neurological Institute (MNI)-152 T1-template was segmented using SPM8; cortical GM was manually extracted from the resulting GM mask using tools in the Functional Magnetic Resonance Imaging of the Brain Software Library (FSL, Oxford, UK) and affine registered to each subject's T1-weighted volume image. A cortical NAGM mask was obtained by intersecting the thresholded GM mask for a given subject with the MNI-152 derived cortical GM mask, and then subtracting the intracortical and leukocortical lesion masks from this using tools in FSL.

Magnetisation transfer ratio measures

MT_{on} and MT_{off} images for each subject were affine registered to their T1-weighted volume images before calculation of the MTR. MTR (measured in percent units (pu)) was calculated for each voxel as $MTR=(MT_{off}-MT_{on})/MT_{off}$ where MT_{off} and MT_{on} are the signal intensities without and with the application of the off-resonance pulse. With the MTR map, and lesion and tissue masks aligned with the T1-weighted volumetric images, MTR measures were extracted from each of the masked regions (cortical GM lesions, cortical NAGM).

Brain volume measures

After binarising SPM8 tissue probability masks using a maximum likelihood method, brain parenchymal fraction (BPF) was calculated by dividing the sum of the GM and WM volumes by the total intracranial volume, and similarly GM fraction (GMF) by dividing GM volumes by the total intracranial volume (Chard et al., 2010).

Image processing quality assessment

All images were reviewed for artefacts, e.g. associated with subject motion; every registration step for every image was reviewed for accuracy. The segmented T1-weighted images were reviewed. The registration steps and images of every tenth patient were re-reviewed by DTC for artefacts, registration and segmentation quality. Fifteen MTR scans from patients were rejected due to motion artefacts, and so this analysis included 70 patients and 36 controls.

Genotyping

DNA was extracted locally from blood using the standard Qiagen method and genotyped in the University College London Genomics Microarray Centre, using a custom-designed NeuroX genotype array from Illumina (San Diego, CA, USA). Genotyped data was assembled in GenomeStudio (Module Genotyping v1.9.4) per the manufacturer's suggestion (Illumina). Genotype result of rs3135388 was extracted and A-allele (Top allele) was counted as surrogate marker for HLA-DRB*1501. This has previously been shown to be highly sensitive and specific. Allele A of this single nucleotide polymorphism was shown to be highly associated with MS.(Benešová et al., 2013; International Multiple Sclerosis Genetics Consortium et al., 2007)

Statistics

Continuous data are presented as mean (± standard deviation [SD]). EDSS scores are presented as median (range). A Chi-Square Test was used to compare the disease modifying treatment (DMT), smoking status, gender and disease subtypes between the HLA-groups. General linear models were used to compare normally distributed

measures between HLA-DRB*1501-groups. Lesion volumes, MTR in lesions and cortical NAGM, EDSS, MSFC and SDMT were not normally distributed (all $p<0.05$, Shapiro-Wilk Test). For these measures we assessed between group differences using the Mann-Whitney U Test. A general linear model was used to determine if MRI measures and clinical measures differed between HLA-DRB*1501 groups. As age and gender can affect MRI measures, and as disease duration was significantly different between the HLA-groups, these variables, along with smoking status, previous and current disease modifying treatment (DMT), were all included in the model as covariates. All group comparisons were confirmed by bootstrap analysis (case re-sampling, n=1000). Results were considered significant at a $p<0.05$ level. Where significant at this level, we also report if they remain so after Bonferroni correction. Sample size calculations were based on two group t-tests for differences between independent means. We used SPSS (Version 21, Chicago, IL, USA) for the statistical analysis.

RESULTS

Genotyping

All samples had call rates of > 99.5% and none was excluded due to poor calling. Call

frequency for the rs3135388 was 100% and well-clustered (Figure 1).

In total, 42/85 (49.4%) people with MS carried rs3135388 A allele and were inferred as

HLA-DRB*1501 positive (homozygote A/A n=7, heterozygote A/B n=35).

*Patient demographics across the HLA-DRB*1501 groups*

Age and gender were not significantly different between HLA-DRB*1501 positive and

negative patients (Table 1).

Clinical outcome measures

Measures of physical and cognitive disability were similar across the HLA-DRB*1501

groups (Table 1). Differences between the HLA-DRB*1501 groups did not remain

significant after Bonferroni correction.

MRI outcome measures in MS and healthy controls

Mean BPF, GMF, and cNAGM magnetization transfer ratio (MTR) were significantly

lower in MS than healthy control subjects.

*MRI outcome measures across the HLA-DRB*1501 groups*

Mean BPF was higher in the HLA-DRB*1501 positive than negative MS group, but this

did not remain significant after correction for multiple comparisons. GMF, cGM lesion

volume, MTR in cGM lesions and cNAGM were similar between the HLA-DRB*1501

groups (Table 2).

Sample size calculation

Based on MTR differences between the two HLA-groups observed, sample size calculations estimate 83, 126 and 134 participants would be required in each group to detect a significant difference in cNAGM, cGM and mixed cGM-WM lesions, respectively (power 80%, alpha=0.05).

DISCUSSION

In this study, we investigated associations between HLA-DRB*1501 haplotype, a set of MRI measures sensitive to GM demyelination and neurodegeneration, and neurological and cognitive scores. We found no clear effect of HLA-DRB*1501 haplotype on the radiological or clinical disease burden.

We have been able to find only one study (post-mortem, n=47, 80% SPMS) looking for associations of HLA-DRB*1501 haplotype with the extent of cGM lesions in the motor cortex (DeLuca et al., 2013) and, consistent with our whole brain results, no link was found. Similarly, we have identified a single study looking at GM MTR, and this was in a PPMS cohort (n=41) followed up over 5 years (Tur et al., 2014). Consistent with our findings, baseline GM MTR and GM volumes did not differ between the HLA-DRB*1501 groups, however during follow-up GM MTR declined faster in the HLA-DRB*1501 positive group (Tur et al., 2014).

In the present study, BPF but not GMF appeared to be higher in the HLA-DRB*1501 positive than negative MS group, although the effect was modest (about 1/3 of the difference seen between MS and controls), and when corrected for multiple comparisons the difference was not significant. Previous studies have yielded mixed results, either with greater GM atrophy in the HLA-DRB*1501 positive group (n=41, 34% progressive MS, Zivadinov et al., 2007), or no difference (n=41, 100% PPMS, Tur et al., 2014) These studies included different proportions of progressive MS patients and it is possible that HLA-DRB*1501 may have different effects in RRMS and progressive MS.

The main limitation of this study is the low number of participants, which will have limited sensitivity to associations of MRI measures with HLA-DRB*1501. Power calculations

based on the result of the present study suggest that a substantially larger cohort would be required to demonstrate - if present - significant differences.

While HLA-DRB*1501 is the best studied susceptibility allele in MS, the influence of HLA-regions is complex. Other HLA-variants have been linked with the risk of developing MS and subsequent disease severity, and these may interact with HLA-DRB*1501: For example, Liguori *et al.* found in a longitudinal study of 518 people with MS, that in HLA-DRB1*1501 positive people, HLA-DRB1*10 was associated with a lower rate of WM lesion accrual (Liguori et al., 2011). Because of the limited number of subjects in our cohort we have focused on HLA-DRB1*1501 but future studies are warranted to explore the impact of other polymorphisms on MRI measures in MS. Moreover, ethnicity is an important factor in genetic analyses (Bove and Chitnis, 2014). In the present cohort, >90% of the patients were Caucasian, however, we have not performed a detailed genetic analysis of ethnicity.

In conclusion, we found no evidence that HLA-DRB*1501 is a risk factor for greater MS disease effects in cortical GM, as assessed using a series of MRI measures sensitive to demyelination and neurodegeneration.

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Table 1: Demographics and clinical features of patients and healthy controls.

	Healthy Controls	MS group HLA-DRB*1501		P†
		Positive	Negative	
N	36	42/85 (49.4%)	43/85 (50.6%)	
Females	19/36 (52.8%)	27/42 (64.3%)	25/43 (58.1%)	0.658
Age (years)	41.5±12.8	50.7±10.9	49.2±10.9	0.648
MS subtype				0.922
- RRMS	NA	15/42 (35.7%)	15/43 (34.9%)	
- SPMS	NA	14/42 (33.3%)	16/43 (37.2%)	
- PPMS	NA	13/42 (31.0%)	12/43 (27.9%)	
Ever smoked	14/36 (38.9%)	33/42 (78.6%)	12/43 (27.9%)	0.012
- Smoking years	5.0±9.6	15.3±13.8	11.4±15.0	0.209
Age at disease onset (years)	NA	31.1±11.4	34.8±10.0	0.115
Disease duration (years)	NA	19.3 (11.0)	13.6 (9.8)	0.036
EDSS, median (range)	NA	5.0 (1-8)	5.0 (1-6.5)	0.641
SDMT	61.8±9.5	47.79±10.85	43.66±11.98	0.034
MSFC (z score)	0.35±0.26	-11.44±21.96	-10.65±19.91	0.265
Current DMT	NA	31/42 (73.8%)	28/43 (65.1%)	0.482

Previous DMT	NA	15/42 (35.7%)	16/43 (37.2%)	0.886

Mean±standard deviation unless stated otherwise.

†Unadjusted p-values refer to the comparison between HLA-DRB*1501 positive and negative MS groups. There was no significant difference when adjusted for multiple comparisons (Bonferroni).

Abbreviations: Expanded Disability Status Scale (EDSS), MS Functional Composite (MSFC, z-score) and Symbol Digit Modalities Test (SDMT) scores were determined in the MS group. MS = multiple sclerosis, RR = relapsing-remitting, SP = secondary-progressive, PP = primary-progressive, DMT Disease modifying treatment, NA not available.

Table 2: MRI acquisition parameter. All images were aligned to the anterior posterior commissure line.

	T1	PSIR	PD/T2	FLAIR	MTR [¶]
Dimension	3D	2D	2D	2D	3D
Acquisition plane	Sagittal	Axial-oblique	Axial-oblique	Axial-oblique	Sagittal
Resolution (mm^3)	1x1x1	0.5x0.5x2	1x1x3	1x1x3	1x1x1
FOV (mm^2)	256x256	240x180	240x180	240x180	256x256
Matrix	256x256	480x360	240x180	240x180	256x256
TR (ms)	6.9	7301	3500	8000	6.4
TE (ms)	3.1	13	19/85	125	2.7/4.3
TI (ms)	824	400	-	2400	-
Slices	180	75	50	50	180
SENSE	2	-	1.7	1.3	-
Time (min:sec)	6:32	11:26	04:01	03:44	26:00

[¶]For the magnetization transfer ratio (MTR), a 3D slab-selective fast field echo sequence with two echoes was used. A turbo field echo readout was used, with an echo train length of four and turbo field echo shot interval of 32.5 ms giving a total time between successive MT pulses of 51.9 ms, and scan time of approximately 26 minutes. The two echoes were averaged (thereby increasing the signal to noise ratio) for both the MT_{on} and MT_{off} data used to calculate the MTR. Sinc-Gaussian shaped MT pulses with flip angle of 360° and 16 ms duration but in total, the time between MT pulses was 51.9 ms (including the MT pulse duration and gradients).

Abbreviations: D = dimensional, TR = repetition time, TE = echo time, TI = inversion time, SENSE = sensitivity encoding factor, FOV = field of view, FLAIR = fluid attenuated inversion recovery, MTR = magnetization transfer ratio, PSIR = phase-sensitive inversion recovery, PD = proton-density.

Table 3: MRI measures of cortical grey matter pathology and fractional volumes

MRI measures	Healthy controls	MS group HLA-DRB*1501		P†
		Positive	Negative	
Brain parenchymal fraction (%)	82.2±1.6	80.2±1.8	79.5±2.2	0.038
Grey matter fraction (%)	47.7±1.2	47.2±1.1	47.1±1.6	0.570
Intracortical lesion volume, ml	0	0.448±0.308	0.461±0.524	0.958
Leukocortical lesion volume, ml	0	0.464±0.488	0.653±0.923	0.274
MTR in cortical normal-appearing grey matter	32.6±0.8	31.3±1.3	30.9±1.2	0.160
MTR in intracortical lesions	NA	30.7±2.0	30.1±2.4	0.189
MTR in leukocortical lesions	NA	29.0±3.1	28.2±3.3	0.206

† P-values for comparison of HLA-DRB*1501 positive vs. negative group, adjusted for gender, age, disease duration, current and previous disease modifying treatment and smoking status (general linear model, confirmed by bootstrap analysis, case sampling n=1000).

There was no significant difference between the two HLA-DRB*1501 groups when adjusted for multiple comparisons (Bonferroni).

Figure 1: HLA-DRB*1501 genotyping. Call frequency for the rs3135388 was 100% and well-clustered. All samples had call rates of > 99.5% and none was excluded due to poor calling.