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HLA genotype as a marker of multiple sclerosis prognosis: A pilot study

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ABSTRACT

Objective: The identification of a biomarker with prognostic value is an unmet need in multiple sclerosis (MS). The objective of this study was to investigate a possible association of HLA genotype with disease status and progression in MS, based on comprehensive and sensitive clinical and magnetic resonance imaging (MRI) parameters to measure disease effects.
Method: A total of 118 MS patients (79 females, 39 males) underwent HLA typing. Patient MS status was assessed at two time points in a 2-year interval, based on clinical scores (including EDSS, MSSS, T25FW, 9-HPT, SDMT, BVMT, CVLT-II) and MRI evaluations. Quantitative brain MRI values were obtained for whole brain atrophy, FLAIR lesion volume change and number of new lesions using MSmetrix. Predefined HLA patient groups were compared as of disease status and progression. Global assessment was achieved by an overall *t*-statistic and assessment per measurement by a Welch test and/or Mann Whitney *U* test. The effects of multiple covariates, including age, gender and disease duration as well as scan parameters, were also evaluated using a regression analysis.
Results: The HLA-A*02 allele was associated with better outcomes in terms of MSSS, EDSS and new lesion count (Welch test p-value < 0.05). The HLA-B*07 and HLA-B*44 alleles showed a global negative effect on disease status, although none of the measurements reached significance (p-value < 0.05). Results for the HLA-DRB1*15, HLA-DQB1*06 and HLA-B*08 alleles were inconclusive. The influence of the confounding variables on the statistical analysis was limited.

1. Introduction

In the 1970s and 1980s, linkage analyses revealed that different variations in the HLA region affected the risk of developing multiple sclerosis (MS) [1–8]. More recent studies have demonstrated that the genetic risk for MS is dominated by a series of class II risk alleles, while protective signals are mainly driven by class I alleles [9–11]. Among the class II risk alleles, the partially dominant HLA-DRB1*15:01 allele has been shown to have the strongest association with MS, especially in Caucasian populations [9,10,12–15]. HLA class I alleles have been associated with either reduced (HLA-A*02:01, HLA-B*44:02) [16–20] or increased (HLA-A*03, HLA-B*07) susceptibility to MS [18,19,21,22], and the HLA-A*02:01 has been shown to drive the protective signal.

Contradictory results were obtained in various studies investigating the possible associations of HLA genotypes with MS severity, which may be explained by the small sample size of many of these studies, the different classifications for patient ascertainment, the difficulties to evaluate disease severity due to the unpredictable disease course, and the lack of validated method for assessing prognosis [23]. A longitudinal observation in the United States using brain magnetic resonance imaging (MRI) in 518 MS patients, who met the revised International Panel criteria for MS or had a clinically isolated demyelinating syndrome (CIS), has shown that lower brain atrophy and T2-lesion volume were observed in HLA-B*44 positive patients, especially those who were HLA-DRB1*15:01 negative [24].

A prospective cross-sectional study conducted in 505 MS patients in California has shown an association between the HLA-DRB1*15:01 allele and a reduction in *N*-acetyl-aspartate concentrations within normal appearing white matter (WM) via ^{1}HMR spectroscopy, an increase in white matter T2 lesion volume by MRI, a reduction in normalized brain parenchymal volume, and an impairment in cognitive functions measured by Paced Auditory Serial Addition Test (PASAT-3) [25].

Abbreviations: BVMT, Brief Visual Memory Test; CIS, clinically isolated demyelinating syndrome; CSF, cerebrospinal fluid; CVLT-II, California Verbal Learning Test-II; EDSS, Expanded Disability Status Scale; FLAIR, Fluid Attenuated Inversion Recovery; GM, gray matter; MRI, magnetic resonance imaging; MS, multiple sclerosis; MSSS, Multiple Sclerosis Severity Scale; NEDA-3, no evidence of disease activity based on absence of relapses; PASAT-3, Paced Auditory Serial Addition Test; SDMT, Symbol Digit Modalities Test; T25FW, Timed-25-Foot-Walk; WM, white matter; 9-HPT, 9-Hole Peg Test.

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Other studies found a connection between this allele and the main epidemiological (gender, exposure to vitamin D) features of the disease [26,27]. In the Californian study, the HLA-DRB1*15:01 allele was more frequently detected in women and in patients with disease onset at a younger mean age [25]. In two other studies including 729 MS patients from the United Kingdom and 286 Norwegian MS patients, respectively, the presence of HLA-DRB1*15:01 was also associated with younger age at diagnosis and female sex, but there was no association with disease course or outcome and with the presence of oligoclonal bands in the CSF [23,28]. The association between the HLA genotypes and age at onset is probably driven by the HLA-DQB1*06:02–HLA-DRB1*15:01 and the HLA-DQA1*01:01–HLA-DRB1*01:01 haplotypes [9,10,29]. The presence of the HLA-DR2 haplotype (molecular designation HLA-DRB1*15:01, HLA-DQA1*01:02, HLA-DQB1*06:02) also associated with a higher risk of developing clinically definite MS within 5 years in 178 patients with optic neuritis [30]. Moreover, a family-based cohort analysis including 808 MS patients and using the Expanded Disability Status Scale (EDSS) scores to assess severity has shown that HLA-DR2 homozygotes were significantly less frequent in patients with mild or benign MS compared with patients having non-mild MS, suggesting that HLA-DR2 may act as a disease modifier [31]. Several other studies using cerebrospinal fluid (CSF), MRI and clinical outcome measures to assess disease severity failed to confirm the results about the influence of the HLA-DR2 haplotype on the prognosis, like a cohort study in Sweden with a 25-year follow-up in 121 patients with definite or probable MS [32], a study conducted in a large data set of 948 Swedish patients with definite, probable, or possible MS [29], a study in 729 MS patients in the United Kingdom with definite or probable MS [23], or an Italian study in 100 MS patients according to the McDonald diagnostic criteria [33]. A recent study in 85 patients with MS and 36 healthy controls has also shown that HLA-DRB1*15:01 was not strongly associated with MRI-visible gray matter (GM) pathology [34].

In the Italian study, a higher T2 lesion load was associated with the presence of HLA-DRB1*04 and HLA-B*07, and a higher T1 lesion load with the presence of HLA-B*07 and HLA-DRB1*12. Moreover, brain parenchymal fraction was predicted by the presence of HLA-DRB1*12 [33]. In a previous study using EDSS to evaluate the level of disability in 163 sporadic MS patients, the HLA-DRB1*01 allele was detected in MS patients with a benign disease course and was missing in malignant MS patients, suggesting that this allele acts as a modifier of disease progression [35]. In contrast, unfavorable outcomes have been reported with regards to the HLA-DR1 haplotype in the Swedish cohort study with a 25-year follow-up [32]. This finding was not confirmed by the Swedish study conducted in the large data set, showing that none of the HLA-DRB1 alleles influences course or outcome in MS [29] nor by another study including 304 MS patients, showing that no single allele from the HLA-DR region was associated with either a good or poor prognosis [36].

The objective of this study was to further investigate a possible association of HLA genotype with disease status and progression in MS, by using comprehensive and sensitive clinical and MRI parameters to measure disease effects. This was the first study applying various clinical (physical and cognitive) scales and using a validated scanner-independent software to extract whole brain atrophy, lesion volume changes, and the number of new lesions between two time points from MRI measurements.

2. Materials and methods

2.1. Data set

Out of the 118 MS patients included in the study, 79 were females (67%) and 39 males (33%). The cohort consisted of 104 relapsing-remitting MS, 8 secondary progressive MS and 6 primary progressive MS. The average age of the patients was 43.2 ± 11.2 years (range: 19.6–66.9 years). The average disease duration was 11.8 ± 7.1 years (range: 0.7–38.8 years), and the average EDSS score was 2.4 ± 1.4 years (range: 1–6.5). The vast majority (93%) of MS patients were treated, of whom 44.5% were using first-line medication (interferon-beta, glatiramer acetate, teriflunomide, dimethylfumarate) and 55.5% second-line medication (natalizumab, fingolimod, alemtuzumab). An overview of the patient information is also provided in Table 1.

This study was approved by our institutional review board and written informed consent was obtained from all participants (reference P2013/098/B406201316929).

HLA typing was performed on DNA extracted from peripheral blood mononuclear cells by low- to intermediate-resolution polymerase chain reaction using sequence-specific oligonucleotides. Reverse dot-blotting was carried out on a nylon membrane containing immobilised sequence-specific oligonucleotide probes used for the typing of HLA class I (HLA-A*02, HLA-B*07, HLA-B*44) and HLA class II (HLA-DRB1*15, HLA-DRB1*04, HLA-DRB1*07, HLA-DQB1*06) alleles (INNO-LiPA®, Fujirebio).

The evaluation of the patients was done at 2 time points with an interval of 2 years (respectively in 2013 and 2015) and was based on both clinical scores and MRI scans (Table 2). Clinical scores included the EDSS, the MSSS (MS Severity Scale), the T25FW (Timed-25-Foot-Walk), the 9-HPT (9-Hole Peg Test), the SDMT (Symbol Digit Modalities Test), the BVMT (Brief Visual Memory Test) and the CVLT-II (California Verbal Learning Test-II). During the 2-year follow-up period, disease evolution was described by the extracted NEDA-3 (no evidence of disease activity based on absence of relapses, EDSS progression, and new T2 or gadolinium-enhancing lesions), the 24-week confirmed SDMT progression, and the EDSS plus. SDMT progression was defined as ≥20% of minimum threshold deterioration, and EDSS plus as progression on ≥1 of 3 components (EDSS, T25FW, and/or 9-HPT) confirmed ≥24 weeks apart and ≥20% of minimum threshold change for T25FW and 9-HPT [37–44]. Brain MRI scans were available from clinical routine, and included a FLAIR (Fluid Attenuated Inversion Recovery) sequence and a T1-weighted turbo field echo sequence (pre- or post-gadolinium injection). The majority of patients were scanned on a Philips Healthcare MR system (Achieva or Intera), but field strengths differed between scans, i.e. 1.5 T or 3 T. One scan showed insufficient image quality and was therefore excluded from the analysis.

2.2. Quantitative MRI measurements

All MRI scans were analyzed using MSmetrix [45,46], a scanner-independent software developed by icometrix, to extract whole brain atrophy, lesion volume changes, and the number of new lesions between both time points.

In a first step, the algorithm was iterated until convergence between (1) the segmentation of healthy tissue (WM, GM, CSF) on T1 lesion filled images, (2) the FLAIR lesion segmentation estimated using the knowledge of healthy tissue segmentations. In a second step, the baseline and follow-up scans were analyzed simultaneously by a Jacobian integration approach using the segmentations of the first step as input. This longitudinal step was performed to guarantee consistency between the segmentations of the individual time points, resulting in measurements for brain atrophy and FLAIR lesion change.

Table 1
Description of the patient population.

Patient count	118
Age (years)	43.2 ± 11.2 (min. 19.6–max. 66.9)
Disease duration (years)	11.8 ± 7.1 (min. 0.7–max. 38.8)
EDSS scores	2.4 ± 1.4 years (min. 1–max. 6.5)
Gender	39 males (33%) - 79 females (67%)
MS types	104 RR-MS, 8 SP-MS, 6 PP-MS
Treatment	93% of the MS patients of which 44.5% first line medication and 55.5% second line medication