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Population structure and *HLA DRB1*1501* in the response of subjects with multiple sclerosis to first-line treatments

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ABSTRACT

Using retrospectively collected outcome data for treatment naïve subjects treated with either glatiramer acetate (GA) ($n=332$) or interferon beta (IFN β) ($n=424$), we replicated the lack of a significant difference in efficacy between these treatments. Further, for both treatments, we observed a decline in the hazard of a relapse over time, which may suggest the existence of subsets of subjects with differential responses to each treatment. The *HLA DRB1*1501* allele explained some of this variation in event-free survival while on GA, and we found suggestive evidence that an *IRF8* polymorphism influences event-free survival in IFN β treated subjects.

1. Introduction

In multiple sclerosis (MS), the two most frequently used first-line disease modifying treatments (DMTs), glatiramer acetate (GA) and interferon beta (IFN β), have been clearly shown to reduce the relapse rate, slow the appearance of new and enhancing lesions on magnetic resonance imaging (MRI), and delay progression of disability in randomized, placebo-controlled trials (Jacobs et al., 1996; The IFNB Multiple Sclerosis Study Group, 1993; PRISMS (Prevention of Relapses and Disability by Interferon beta-1a Subcutaneously in Multiple Sclerosis) Study Group, 1998; Paty and Li, 1993; Johnson et al., 1995; Comi et al., 2001). It is expected that with diminished inflammation in the early phase of the disease, patients treated with these DMTs will benefit in terms of less long-term accumulation of disability, and, indeed, there is some evidence that IFN β slows long-term progression and delays entry to the secondary progressive phase of multiple sclerosis (Trojano et al., 2007).

However, multiple sclerosis is a heterogeneous disease, and patients vary in their response to treatment with either agent. A recent study found that approximately one-third of patients did not have a decrease of annual relapse rate while on IFN β, the more extensively studied of the two DMTs (Waubant et al., 2003). Similarly, a large fraction of subjects treated with GA appear to have little benefit from their treatment (Johnson et al., 1995; Comi et al., 2001; Fusco et al., 2001). Recent comparative studies have also shown that over several clinical and radiographic outcomes, GA appears to be equivalent to various forms of IFN β (Cadavid et al., 2009; Mikol et al., 2008; O'Connor et al., 2009). However, the relapse rate experienced on one of these agents does not appear to predict relapse rate on the other (Gajofatto et al., 2009). Thus, as would be suspected from their known mechanisms of action, the two treatments affect disease activity through different pathways, and the subset of individuals that benefit from treatment may not be the same among GA treated or IFN β-treated subjects.

While there are other existing and emerging treatments for MS, GA and IFN β will probably remain the principal first-line agents for the foreseeable future given their relatively benign adverse event profile when compared to those of other treatments. This same reason also makes them attractive candidates for second-line treatment, particularly if the subset of subjects that are most likely to have a low rate of inflammatory events while on a given DMT can be identified. Here, we explore the heterogeneity of treatment response to DMT using a retrospective analysis of subjects first treated with either GA or IFN β. In both treated subject samples, the distribution of events consistent with inflammatory demyelination suggests the existence of more than one subset of subjects. We go on to support the previously proposed role of the *HLA DRB1*1501* allele in explaining some of the

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variation in GA response. We also explore whether time to a first demyelinating event while on DMT correlates with either (1) an *IRF8* polymorphism that influences the level of gene expression among interferon response genes or (2) an aggregate measure of genetic susceptibility for MS.

2. Materials and methods

2.1. Human subjects

1119 subjects with remitting relapsing multiple sclerosis and DNA samples were selected from 3 sample collections at the Partners MS Center in Boston — the Comprehensive Longitudinal Investigation of MS at the Brigham and Women's Hospital (CLIMB)(Gauthier et al., 2006), MS Genetics Collection, and MS Registry (De Jager et al., 2008) — for retrospective investigation on response to treatment with glatiramer acetate and interferon beta (IFN β-Ia IM, IFN β-Ia SC, and IFN β-Ib SC). 612 of these subjects were selected from the CLIMB study; an additional 469 subjects were selected from the MS Genetics study; and 38 more subjects were taken from the MS Registry. Many subjects participated in more than one study. Patients with primary and secondary progressive forms of the disease were excluded. Other criteria for exclusion included treatment with a DMT for less than 6 months and treatment that began before electronic records were available. Those subjects with periods of treatment >1 year during which information related to disease activity was ambiguous or absent were flagged as having "insufficient information" and excluded from the analysis. 756 subjects met our criteria and were included in our analysis. 723 (95.6%) had relapsing–remitting disease (RRMS) diagnosed by McDonald criteria, 10 (1.3%) had a progressive relapsing form, and 23 (3.0%) had a clinically isolated demyelinating syndrome (CIS), which is treated in the same manner as RRMS.

The replication cohort of MS patients on IFN β therapy, predominantly of northern European heritage, was recruited in Germany by primary-care physicians and neurologists(Hoffmann et al., 2008). All patients were tested for occurrence of anti-IFN β antibodies. All patients included were Ab-negative as determined by ELISA as described previously(Hoffmann et al., 2008).

2.2. Data collection

Information was collected from the electronic Longitudinal Medical Record at the Brigham and Women's Hospital and the Partners Healthcare Multiple Sclerosis clinical research database. Information on DMT treatment type, start and stop dates for each treatment, and dates of events were recorded. The type of event was also recorded. The primary outcome measure was time to first event, with event defined as: (1) a clinical relapse consisting of one or more new neurological symptoms or the reappearance of previous symptoms lasting at least 24 h, (2) a change in the T2 hyperintense lesion burden or the presence of any gadolinium-enhancing lesion on magnetic resonance imaging (MRI) as assessed by the clinical neuroradiologist, or (3) an increase in the Expanded Disability Status Scale (EDSS) measure of clinical disability by 1 point, sustained over a 6-month period. Following treatment initiation, a 6-month window was established before an event was recorded to allow time for the medication to become effective. MRIs were performed, on average, once a year per clinical routine; additional MRIs were obtained at the discretion of each treating neurologist based on the clinical course of a given subject.

2.3. Statistical analysis

The baseline characteristics of the treatment groups were compared using a Wilcoxon test or Fisher's exact test as appropriate. For our primary analysis, we also compared the time to first event in patients initially treated with GA to time to first event in patients initially treated with IFN β using a log rank test. Additional events that occurred on each treatment were not included in our analysis. In secondary analyses, we divided IFN β into low-dose (Avonex) and high-dose (Betaseron and Rebif), and we compared the time to first event across the three treatment groups. A multivariate Cox proportional hazards model was also fit to control for baseline confounding by age at onset, gender and disease duration, and reported p-values are based on a Wald test. In addition, the hazard of an event was estimated in each treatment group using a smooth function provided by Allison(Allison, 1995) to determine if the likelihood of an event changed over the course of observation. Additional analyses focused on the behavior of subjects who switched treatments from GA to a form of IFN β, and vice versa. The time to first event on each treatment was compared using a Cox model accounting for the correlation between the times within patients by using the robust variance estimate (Therneau and Patricia, 2000). To evaluate the effect of MS susceptibility loci on time to event in each treatment group, a Cox model for time to event was used assuming an allelic model (additive effect of each allele) and a genotypic model (different effect for each genotype) adjusting for baseline confounders. No corrections for multiple comparisons were completed for the p-values from the genotypic model. In addition, the effect of a genetic risk score on survival was investigated in each treatment group separately using a Cox model. Details for the calculation of an individual's genetic risk score is presented elsewhere (De Jager et al., 2009a); in short, it is the sum of an individual's risk alleles at sixteen susceptibility loci, weighted by the natural log of the odds ratio of each susceptibility allele. The effect of MS susceptibility loci was validated in our replication cohort using the same model controlling for age and gender. All statistical analysis was completed in the statistical package R (Team, 2007) (http://www.R-project.org) and the survival library was used (survival: survival analysis, including penalized likelihood. R package version 2.35-7. http://CRAN.R-project.org/package=survival).

3. Results

3.1. Baseline characteristics of treatment groups

The GA treated group did not differ significantly from the IFN β-treated group in most baseline characteristics (Table 1). However, IFN β-treated patients had a shorter mean disease duration prior to treatment initiation compared to GA treated patients ($p_{GA\ vs\ low\text{-}dose\ IFN\beta} = 0.018$ and $p_{GA\ vs\ high\text{-}dose\ IFN\beta} = 0.0016$). In addition, the low-dose IFN β and high-dose IFN β groups both had higher EDSS scores at baseline than the GA group ($p_{GA\ vs\ low\text{-}dose\ IFN\beta} = 0.0054$ and $p_{GA\ vs\ high\text{-}dose\ IFN\beta} < 0.0001$).

The primary outcome measure of our analysis is the duration of time between treatment onset and the first event consistent with disease activity. Three types of events were recorded to capture disease activity: clinical relapses, the appearance of new T2 hyperintense or enhancing lesions on MRI, and an increase in EDSS of 1 sustained for at least 6 months. As expected, the two treatment groups (GA and IFN β, Fig. 1) were not significantly different in terms of time to first event in univariate analysis ($p = 0.21$; hazard ratio [HR] = 1.12, 95% CI 0.94–1.33) or in multivariate analysis controlling for age at symptom onset, disease duration at treatment initiation and gender ($p = 0.48$; HR = 1.07, 95% CI 0.89–1.27). In the subset of subjects in which EDSS prior to treatment was available ($n = 399$), adding a covariate for baseline EDSS did not alter our results. In terms of type of first event, the proportion of patients experiencing a relapse as the first event was slightly higher in the IFN β group, and the proportion experiencing an MRI event was slightly higher in the GA group (Table 1). However, these differences were not statistically significant. We also compared time to first relapse between the GA and total IFN β treatment groups (Fig. 1). Although patients treated with GA had a longer time to first clinical relapse in univariate analyses ($p = 0.026$; HR = 1.28, 95% CI 1.03–1.59), this effect was no