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Clinical Study

Sex-specific prediction of interferon beta therapy response in relapsing-remitting multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is a demyelinating disorder predominantly affecting young people. Currently, interferon beta (IFNβ) is a common treatment for MS. Despite a large effort in recent years, valid biomarkers with predictive value for clinical outcome and response to therapy are lacking. In order to identify predictive biomarkers of response to IFNβ therapy in relapsing-remitting MS patients, we analyzed expression of 526 immune-related genes with the nCounter Analysis System (NanoString Technologies, Seattle, WA, USA) on total RNA extracted from peripheral blood mononuclear cells of 30 relapsing-remitting MS patients. We used a Wilcoxon signed-rank test to find an association between certain gene expression profiles and clinical responses to IFNβ. We compared the expression profile of patients who responded to IFNβ treatment (n = 16) and non-responsive IFNβ patients (n = 14). The analysis revealed that the expression of eight genes could differentiate between responsive and non-responsive men ($p \leqslant 0.005$). This differentiation was not evident in women. We analyzed results from an additional cohort of 47 treated and untreated patients to validate the results and explore whether this eight gene cluster could also predict treatment response. Analysis of the validation cohort demonstrated that three out of the eight genes remained significant in only the treated men ($p \leqslant 0.05$).

Our findings could be used as a basis for establishing a routine test for objective prediction of IFNβ treatment response in male MS patients.

1. Introduction

Multiple Sclerosis (MS) is a chronic demyelinating autoimmune disease that attacks the central nervous system. It is characterized by inflammation, demyelination and axonal degeneration [1]. Like many other autoimmune diseases, MS is believed to arise from a complex interaction between environmental and genetic factors [2]. MS has a heterogeneous nature which is reflected by variability in clinical course, timing of relapses and rate of disability progression [3].

The available therapies for MS are typically effective during the earlier relapsing stage of the disease. Interferon beta (IFNβ) is one of the most frequently used USA Federal Drug Administration approved drugs for the treatment of MS and is the most commonly utilized therapy to prevent exacerbations in relapsing-remitting multiple sclerosis (RRMS) [4]. IFNβ has been shown to decrease the rate of relapse by approximately 30% [5,6], delay progression of disability and lower the number of active lesions on MRI [7]. It is current practice that MS patients start an effective therapy as early as possible in order to prevent neurological disability.

Although IFNβ is a favorable treatment for MS, it may cause significant adverse effects including injection site reactions, flu-like symptoms, leukopenia, hepatic function disturbances and depression. Two-thirds of treated patients experience at least one of these side effects. Moreover, the clinical response rate to IFNβ is estimated to be around 30–50%. The response rate varies between patients ranging from full response (responders) to complete lack of response (non-responders) [8], however, clear criteria for such classifications are still lacking.

Identification of objective predictive biomarkers for treatment response would be valuable in the clinical management of the disease, preventing unnecessary adverse effects and costs. In recent years, several studies have indicated that gene expression patterns are associated with IFNβ response status but no clinically beneficial predictors have been established [9,10].

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We conducted array-based gene expression analyses involving human immune-related genes in peripheral blood mononuclear cells (PBMC) of responders and non-responders to IFNβ treatment in an effort to identify a biomarker for treatment response.

2. Methods

2.1. Patients

Patients with RRMS (n = 77) were recruited from the MS clinic at Hadassah Medical Center. All participants signed informed consent. These studies were approved by the local Helsinki Committee and the Israeli Institutional Review Board.

The initial study cohort included 30 patients (21 women and nine men) with average age 36 ± 11 years, mean disease duration of 7.7 ± 5 years (average ± standard deviation [SD]) and Kurtzke's Expanded Disability Status Scale (EDSS) of 3 ± 1.1 (average ± SD). Eleven patients were treated with IFNβ1b (Betaferon; Bayer Healthcare, Leverkusen, Germany) and 19 were treated with IFNβ1a (12 with Avonex [Biogen Idec, Cambridge, MA, USA] and seven with Rebif [Merck, Whitehouse Station, NJ, USA]). Sixteen patients were clinically classified as responders (with a good response to IFNβ) and 14 were classified as non-responders.

The validation cohort included 47 patients, including 21 treated patients (10 women and 11 men), of average age 41 ± 10 years, mean disease duration of 8.6 ± 6.3 years (average ± SD) and EDSS of 2.6 ± 1.9 (average ± SD). One patient was treated with IFNβ1b (Betaferon), 20 with IFNβ1a (19 with Avonex and one with Rebif). The validation cohort also included 26 untreated patients (18 women and eight men), of average age 35 ± 7 years, mean disease duration of 5 ± 3.2 years (average ± SD) and EDSS of 3.2 ± 2.2 (average ± SD).

2.2. Selection criteria

RRMS patients were considered eligible for inclusion in the study if they suffered from clinically definite RRMS and were treated with IFNβ therapy. Patients must also have suffered from at least two documented relapses over the two years prior to treatment onset. Patients treated with other immunosuppressive medications during the six month period prior to inclusion, patients with other systemic autoimmune diseases, and women who were pregnant or had delivered in the previous three months were not considered eligible. Patients who were unable to sign informed consent were excluded from the study.

2.3. Response assessment

The response assessment was focused on extreme clinical phenotypes to maximize the ability to detect differences. Responders were defined as the patients who had no relapses and no change in the EDSS over a 2 year follow-up period. Non-responders were defined as those who experienced at least two relapses or an increase in EDSS of at least one point during the 2 year follow-up period. Disability data were collected at 3 month intervals. EDSS was defined at each visit by an experienced neurologist from the MS Center. A relapse was defined as a new symptom or worsening of a preexisting symptom attributable to MS activity, confirmed by neurological examination. MRI served as a surrogate marker for detection of subclinical disease activity and response to IFNβ treatment.

2.4. RNA isolation and analysis

A blood sample was drawn from each patient 2 hours before the next IFNβ injection. PBMC were freshly isolated from heparinized blood with Lymphoprep (Axis Shield PoC AS, Oslo, Norway) density gradient centrifugation.

Total RNA was extracted from PBMC using Tri Reagent (Sigma-Aldrich, St Louis, MO, USA) according to the manufacturer's instructions. The quantity and quality of the RNA was tested using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The expression of 526 immune-related human genes was analyzed using the nCounter code set panel (NanoString Technologies, Seattle, WA, USA). This assay is based on direct digital detection of mRNA molecules of interest using target-specific, color-coded probe pairs without the use of reverse transcription or amplification [11].

2.5. Statistical analysis

We analyzed our data with linear correlation discovery (LCD), as described previously [12], and with heatmap analysis [13]. We used non-parametric statistics in order to overcome the lack of knowledge related to the bioclinical attribute distributions and gene expressions. The LCD and the heatmaps were built using R software [14] and R package gplots [15] (Institute for Statistics and Mathematics, Vienna University of Economics and Business, Vienna, Austria).

The initial analysis of the bioclinical data collected for the present research was conducted using the LCD process. LCD is useful for determining the direction and strength of a relationship between the measured expression of each of the 526 immune-related human genes and each of the bioclinical parameters available for every patient in the study [12].

Correlations with $p \leqslant 0.05$ were selected in order to build heatmaps [13]. Heatmaps allow the visual identification of patients with similar gene expression in a number of genes. The gene expression patterns were also examined according to different demographical (sex, age, class) and bioclinical (treatment, disease status, EDSS) parameters, and $p \leqslant 0.05$ was considered significant.

Pathway analysis was performed using Ingenuity Pathway Analysis (Qiagen, Germantown, MD, USA).

3. Results

Analysis of the data obtained by the array-based gene expression of samples extracted from responders and non-responders revealed a significant correlation between the expression of 80 genes and IFNβ treatment response (Wilcoxon signed rank test; $p \leqslant 0.05$). Although the correlation was significant, the heatmap did not show a clear discrimination between responders and non-responders (Fig. 1A).

To establish diagnostic significance, the signature of the gene expression of these 80 genes should differentiate between responders and non-responders. To accomplish that, we examined the influence of other clinical parameters on gene expression and assessed the correlations between gene expression levels and various parameters including age, EDSS, sex and disease duration. In these subgroup analyses, the 80 gene signature was able to discriminate between men responders and non-responders ($p \leqslant 0.05$) (Fig. 1B).

Furthermore, using a more stringent cutoff ($p \leqslant 0.005$) we revealed that a specific group of eight genes are more highly expressed in male responders than in male non-responders and we can discriminate between these two groups (ZAP70, IFNAR2, ZEB1, JAK1, RPL19, EEF1G, MAP4K2, SKI) (Fig. 1C). We used Ingenuity Pathway Analysis and found that four out of the eight discriminating genes (ZAP70, IFNAR2, ZEB1, JAK1) belong to the T lymphocyte differentiation pathway.