Pharmacogenomics of the response to IFN-β in multiple sclerosis: ramifications from the first genome-wide screen

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Evaluation of: Byun E, Caillier SJ, Montalban X et al.: Genome-wide pharmacogenomic analysis of the response to interferon-β therapy in multiple sclerosis. Arch. Neural. 65(3) 337–344 (2008). Specifically, IFN-β is the most widely used disease-modifying therapy for the treatment of multiple sclerosis. The main benefits of the therapy, fewer and less severe relapses as well as delayed disease progression, are seen in only approximately 50% of the patients. Genetic polymorphisms may constitute in-built determinants of individual differences in response to IFN-β. Prior attempts to identify such ‘predictors of response’ were hypothesis-driven in that they were based on preselection of candidate genes associated with Type I interferon pathways. In the present study, the authors performed the first ever nonbiased genome-wide association screen in an attempt to identify response-predictive SNPs. Using a robust four-stage completion strategy coupled to advanced SNP ranking/clustering algorithms, 18 significant SNPs were identified, many of which are located in genes that have never before been linked clearly to Type I interferon biology or therapeutic effects. While this study was not designed per se so as to validate earlier findings, genes arising from previous pharmacogenomic studies were generally not confirmed. This is due to major discrepancies between interstudy sets of used SNPs, but may also reflect differential strategies for ascertainment of response to IFN-β, or simply Type I/II errors. The 100-K SNP screen by Byun et al. hallmarks a new stage of pharmacogenomics research applied to multiple sclerosis treatments. Through the judicious implementation of DNA pooling on SNP microarrays, it vividly demonstrates that informative genome-wide pharmacogenomic screens can be performed at a fraction of the cost of individual microarray genotyping. Although, unquestionably, higher-density SNP screens and further replication studies are needed, this study is instrumental in bringing the concept of personalized medicine a (small) step closer to the multiple sclerosis patient.

In addition, it has generated a flurry of novel information of likely importance in furthering our understanding of Type I interferon biology.

The results from a recent genome-wide pharmacogenomic screen have provided a first glimpse into the complex, polygenic nature underlying the clinical response to IFN-β in multiple sclerosis (MS) [1]. MS is a chronic inflammatory demyelinating disease of the CNS that affects predominantly young female adults (2:3 women:man). IFN-β is the most commonly used disease-modifying treatment and produces significant benefits [2], be it only in a proportion of treated patients (~50%). The course of MS is largely unpredictable, and, as opposed to other diseases, clear quantitative criteria for management of both treatment and response to it are lacking. Identification of (a) marker(s) for IFN-β treatment efficacy would allow provision of the treatment to those patients likely to respond to it (or unlikely to experience adverse effects, such as depression or flu-like symptoms), whilst providing an objective rationale for transferring probable nonresponders to a different treatment method (e.g., dacluzimab or natalizumab). Discovery of potential response biomarkers is currently undertaken by means of diverse, but complementary, strategies, such as, gene-expression profiling [3-5] and proteomics-driven studies [6,7], or SNP screens [8-13]. Of these approaches, the potential for routine implementation of response-predictive SNPs is particularly attractive (no issues with marker fluctuation over time and according to cell type, cost-effectiveness, minimally invasive to the patient, e.g., DNA collection through mouth swab; by contrast, proteomics/transcriptomics methods are more likely to depend on accessible specific cell populations and/or body fluids). For now, it is still not known (and will likely remain so for some time) which strategy or combination of strategies will ultimately deliver the most reliable IFN-β response marker(s).

Keywords: genome, IFN-β, multiple sclerosis, polymorphism

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The area of pharmacogenomics research applied to MS treatments is, as yet, quite young, and studies focusing on potential SNP biomarkers have been limited to the scrutiny of preselected candidate genes thought to be associated with Type I interferon biology (reviewed in [14]). The study by Byun and colleagues [1] constitutes a significant departure from these earlier studies in that it represents the first ever attempt to identify of molecular markers for interferon therapy response heterogeneity through a genome-wide screen.

Summary of methods & results
Multiple sclerosis patients included in the screen were selected from four collaborating centers located in the genetically homogeneous Mediterranean basin [1]. This selection was done purposely in order to minimize population-based allelic differences between patient-recruitment centers. All patients had clinically definite relapsing–remitting MS and were followed up prospectively for 2 or more years after onset of therapy (Rebif®, Betaseron® or Avonex®). Rigid response criteria were established so as to include only extreme clinical-response phenotypes. Responder and nonresponder DNA pools were prepared from groups of approximately 20 subjects each (total of 206 patients included), and each DNA sample was included in three different pools to allow for technical replication on separate Affymetrix GeneChip® 100K arrays. The screening was performed in four stages:

- **Stage 1**: microarray SNP typing on DNA pools;
- **Stage 2**: ranking of top candidate SNPs by p-value significance and linkage disequilibrium clustering, based on the notion that significant SNPs are likely to segregate in haplotypes;
- **Stage 3**: validation of candidate SNPs arising from the pooled-DNA approach through individual genotyping;
- **Stage 4**: increased power for association analysis by inclusion of 81 more response-classified subjects.

Gene ontology analysis of the stage 1 microarray data revealed that top-ranked SNPs with differing allelic distribution between responders and nonresponders were more likely to be found in genes coding for ion channels and signal-transduction pathways, for example, glutamate and γ-aminobutyric acid receptor genes (discussed below). In stage 2, 35 candidate SNPs were selected, which were then typed individually in stage 3. The degree of correlation of allele frequencies between the triplicate pools was very high ($r^2 \geq 0.99$), and pooled frequencies closely matched true frequencies obtained by individual genotyping, attesting to the success and accuracy of the DNA pool microarray strategy. Following stage 4 joint analysis, significant response-associated SNPs were found in a variety of genes, including the heparan sulfate proteoglycan gene glypican 5 and two ECM genes. Interestingly, these genes code for proteins known to be involved in neuronal repair and growth or, respectively, to be targeted for degradation by MMPs in conjunction with extravasation of inflammatory leukocytes through basement membranes. In addition, the authors probed their dataset for SNPs/genes scrutinized in earlier IFN-β pharmacogenomic studies. They generally did not confirm previously reported associations.

Discussion & implications
The study by Byun and colleagues [1] has yielded a wealth of novel information on genes that are potentially involved in the manifestation of clinical responses to IFN-β in MS. Some of these genes were already known to be functionally or biologically linked to interferon activity. However, this is much less the case for a group of genes that surfaced through the gene ontology analysis. Intriguingly, the study by Byun et al. [1] shows top-ranked SNPs that differ between responders and nonresponders in genes encoding several members of two major families of neurotransmitter-gated channels. It is worthwhile to explore the potential functional implications of this finding in a bit more detail.

Extracellular neurotransmitter-gated channels are known to be expressed by oligodendrocytes [15,16]. These types of channel include those activated by glutamate and GABA, which are the main excitatory and inhibitory neurotransmitters of the CNS. The functional significance of the signals mediated by glutamate and GABA receptors are unknown, but it has been suggested that they may control oligodendrogenesis and myelination. Strikingly, sustained activation of glutamate receptors expressed in oligodendrocyte precursors, as well as mature oligodendrocytes, can cause excitotoxicity, a feature that contributes to the progression of white-matter damage in MS and other pathological conditions (Figure 1) (reviewed in [16]). In addition, differences between responders and nonresponders to IFN-β in MS...
Figure 1. SNPs in genes encoding neurotransmitter-gated ion channels and genes regulating glutamate homeostasis may contribute to determining IFN-β responsiveness in multiple sclerosis.

Glutamate and GABA receptors are expressed in oligodendrocyte precursors and mature oligodendrocytes. In normal conditions, glutamate is taken up by glutamate transporters, which prevents overactivation of glutamate receptors and oligodendrocyte damage by excitotoxicity. In multiple sclerosis, increased extracellular levels of glutamate [29] may arise as a consequence of inflammation and impaired expression and/or function of glutamate transporters (illustrated with an X on the glutamate transporter), and can lead to excitotoxic damage of oligodendrocytes and myelin (illustrated in pink). IFN-β, by attenuating the inflammatory response, can exert a regulatory effect on glutamate homeostasis and, thus, limit oligodendrocyte excitotoxicity. Microglial cells have been omitted for simplicity.

EAAT: Excitatory amino acid transporter; GABA-R: GABA receptor; GluR: Glutamate receptor; OPC: Oligodendrocyte progenitor cell.
including genes associated with glutamate homeostasis, such as the excitatory amino acid transporter EAAT2 (SLC1A2) and GAD1 [1]. EAAT2 is a critical regulator of extracellular glutamate levels [17], the function of which is impaired during primary or secondary CNS inflammation, thus leading to oligodendrocyte excitotoxicity [18]. On the other hand, GAD1, also known as GAD67, catalyzes the production of GABA from glutamate and, as a consequence, regulates the shift from excitation to inhibition in brain cells, including oligodendrocytes. Moreover, GAD is known to be a major pancreatic β-cell autoantigen, recognized by humoral and cellular immune components, which plays an important role in the pathogenesis of autoimmune Type 1 diabetes [19,20].

Taken together, the findings described above indicate that variability in genes of the glutamate and GABA signaling systems may underlie responsiveness to IFN-β by shifting the balance between electrical excitation and inhibition of oligodendrocytes (Figure 1). Indeed, IFN-β can block proinflammatory cytokine-induced inhibition of glutamate uptake and thus regulate tissue excitability in cell culture [21]. If this were the case in MS patients, IFN-β-responders may be amenable to the regulation of glutamate- and GABA-related genes by this cytokine.

Approximately 112 candidate genes have been scrutinized in earlier IFN-β pharmacogenomics studies [8–13]. In particular, the IFNAR1 and IFNAR2 genes have received a lot of attention, with the main outcome of only limited, if any, support for a role in response [8,10,11]. In our group, we sequenced the promoter regions of 100 genes that contain interferon-stimulated response elements, hallmarks of Type I interferon inducibility, in DNA pools of MS patients responding and not responding to IFN-β [10]. We detected 54 polymorphisms located within or close to these interferon-stimulated response elements. Polymorphisms in 15 genes showed allelic distortions between these pools, and four candidate genes were retained following individual genotyping in 94 responders and 68 nonresponders (MX1, IFNAR1, LMP7 and CTSS, the cathepsin S gene).

Byun and colleagues [1] scrutinized their dataset for SNPs in IFNAR1, IFNAR2, MX1, LMP7 and CTSS, the cathepsin S gene. Byun and colleagues [1] scrutinized their dataset for SNPs in IFNAR1, IFNAR2, MX1, LMP7 and CTSS, but could not confirm the earlier findings for a potential role of these genes (with the caveat that the analyzed SNPs were different from those studied before owing to design limitations of the Affymetrix 100K microarray, which is enriched for SNPs clustering around, rather than within, genes, and thus oversamples the intergenic areas [11]). However, CTSS was equally identified as a candidate modifier of the response to glatiramer acetate therapy in MS [22]. Cathepsin S is a lysosomal cystein protease that may be involved in the proteolytic processing of human myelin basic protein, leading to release of immunodominant epitopes [23]. Generally, cysteine proteases are known to be active in regulating antigen presentation by both MHC class II and CD1D [24], and are, as such, of obvious relevance to the etiopathogenesis of autoimmune disorders.

The only gene simultaneously, although weakly, arising as a candidate response modifier from both the Byun [1] and Cunningham [10] studies is the interferon-inducible dsRNA-dependent protein kinase (PRKR). A promoter PRKR CCGn repeat emerged in the pooled DNA stage [10], while an SNP in the neighboring region showed up significantly in the study by Byun et al. [1]. Several reasons may account for this general lack of overlap. It is of relevance to have a closer look at the differences and similarities in the designs of both of these studies, as these may point to some of the major challenges to be addressed if future MS pharmacogenomics studies are to deliver. First, the criteria for the definition of (non)response differ between both studies (Table 1). Therefore, as the (non)responder strata in each of both studies do not represent the same phenotypic trait, eggression of de facto shared pharmacogenetic determinants may have been obscured. Second, Byun and colleagues [1] recruited patients from the Mediterranean region, while the Cunningham study [10] focused on Northern Irish patients. Well-documented differences in northern versus southern European population substructure [25], and in the north–south prevalence [26] and, potentially, genetics of MS [27], together incite the question whether European population substructure may impact upon the distribution or epiphany of genetic IFN-β response determinants. Indeed, subethnic differences in the pharmacogenetics of important polymorphisms have been documented. For example, within a group of asthma patients of Hispanic descent, a strong association was found between greater bronchodilator drug responsiveness and the β2 adrenergic receptor Arg16 genotype in Puerto Ricans, which was absent in Mexicans [28]. Third, as already indicated above, the microarrays used by Byun et al. [1] did not contain the same promoter SNPs as those analyzed in our study [10]. Thus, the response-associated genes of the Cunningham study [10] may have been missed if the contributing SNPs happened not to be part of a
haplotype tagged in the Byun study [1]. Fourth, data collection and analysis in each study was based on a limited number of response-classified patients (<300 and <200 for [1] and [10], respectively). Given the lack of correction for multiple comparisons in both studies, some of the associations found may simply represent false positives, while the power to detect effects conferred by weak response modifiers or SNPs with low minor allele frequency may have been lacking.

Future perspective
Deciphering the pharmacogenomics of IFN-β treatment in MS has proved to be a challenge imposed upon the molecular biology, neuroimmunology and biocomputing research communities. Not only is MS a complex genetic trait, but the manifestation of clinical benefits in response to IFN-β seems to be dependent on genetic variation in multiple gene loci [1,10,14]. Thus, low penetrance, gene–gene and/or gene–environment interactions, as well as limited statistical power may, together, jeopardize the systematic identification of treatment-response genes. In addition, there is considerable variation in the range of clinical and paraclinical criteria (expanded disability status scale, MRI, relapse frequency, neutralizing antibodies and so on) currently used to denote the MS patients’ response status (see, for example, [3]). In the absence of an objective and easily quantifiable response (bio)marker, standardization of response criteria, based on international consensual definitions, will be of crucial importance in order to facilitate meaningful performance and comparison of MS drug treatment pharmacogenomics studies. Current IFN-β pharmacogenomics studies typically include only those patients either responding (very) well or not (at all) to the therapy, and patients with an intermediate-response profile are excluded from the screen. Implementation of ‘extreme’ response criteria is likely to maximize the separation distance of response phenotypes, and hopefully, of underlying genetic factors, but necessarily implies that the proportion of patients that can be included in the study is small. The study by Byun and colleagues exemplifies this approach: in total, only 285 response-classified patients could be recruited from four collaborating centers [1]. Therefore, well-organized intercenter and international consortia are the sine qua non of sufficiently powered MS pharmacogenomics studies. A more pronounced commitment of pharmaceutical companies to make samples of their clinical trials available to pharmacogenomic studies is crucial. Only on the condition that the above requirements are met is it likely that pharmacogenomics research in MS will yield data that can be validated in a purposeful manner and that, ultimately, may be of translational relevance. In the framework of such improved *mise en scène*, the use of 500K–1000K SNP arrays logically will provide better coverage of the genome at higher density and, in conjunction with adequate bioinformatics approaches and extensive clinical and demographic data, will allow for exhaustive analysis of gene interactions. In conclusion, the study by Byun and colleagues [1] embodies a significant step forward in our understanding of not only the genetics of IFN-β response heterogeneity, but also of both the limitations inherent to the current settings of MS pharmacogenomics research and the challenges that lie ahead.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

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<tr>
<th>Study</th>
<th>Responder</th>
<th>Nonresponder</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Byun et al. (2008)</td>
<td>No relapses and</td>
<td>At least two relapses or</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td>No increase in EDSS</td>
<td>An increase in EDSS of at least 1 point</td>
<td></td>
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<tr>
<td>Cunningham et al. (2005)</td>
<td>Relapse rate reduced by a third and</td>
<td>Relapse rate is the same or increases</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td>No sustained progression in EDSS (≥1 point if baseline EDSS &lt;5.5) or ≥0.5 points if baseline EDSS ≥5.5</td>
<td></td>
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*In both studies, patients were followed for 2 years after initiation of treatment. In Cunningham et al. [10], response status was not judged in the first 6–9 months of treatment, the rationale being that treatment may not be effective until after this period. EDSS: Expanded disability status scale.

Table 1. Clinical IFN-β responder and nonresponder phenotypes*.
**Executive summary**

**Study design**

- DNA samples from a total of 206 IFN-β-response-classified multiple sclerosis (MS) patients from four collaborating centers were screened using the Affymetrix 100K GeneChip® Array (DNA-pooling approach).

**Analyses performed**

- Stage 1: microarray SNP typing on DNA pools.
- Stage 2: ranking of top candidate SNPs by p-value significance and linkage disequilibrium clustering.
- Stage 3: validation of candidate SNPs arising from the pooled DNA approach through individual genotyping.
- Stage 4: increased power for association analysis by inclusion of 81 more response-classified subjects.

**Results**

- Stage 1: Gene ontology analysis revealed response-associated top-ranked SNPs in genes coding for ion channels and signal transduction pathways.
- Stage 2: 35 candidate SNPs selected and typed individually.
- Stage 3: successful validation of association between response and candidate SNPs.
- Stage 4: joint analysis yields 18 significant SNPs.

**Implications & perspectives**

- Response to IFN-β in MS behaves as a complex genetic trait.
- Several genes and gene families arising from the study were not clearly known before to be functionally linked to interferon activity.
- Little overlap with earlier studies potentially owing to differences in selected SNPs and study design.
- Increased power and consensus on drug response in MS is needed to facilitate further studies.

**Bibliography**

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.


- Review of genes potentially involved in bringing about response to type I interferon therapy (IFN-β in MS and IFN-α in hepatitis).


Summarizes current knowledge on the role of glutamate signaling in glial cell death. This is of relevance to the findings by Byun and colleagues [1], who identified neurotransmitter-gated channel genes as one of the main gene ontology groups associated with response to IFN-β.