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Review

Pharmacogenetics of autoimmune diseases: Research issues in the case of Multiple Sclerosis and the role of IFN-β

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Abstract

Pharmacogenetics of auto-immune diseases is a complex field of application for this relatively new discipline, since we still have a partial knowledge of the biological mechanisms of the disease and of the drugs currently used to treat it. We address a few key issues that emerge when planning a pharmacogenetic investigation in Multiple Sclerosis and that relate to the complexities existing at the biological-genetic level and at the phenotypic characterization.

In fact, we think that a clearer characterization of the clinical phenotype representing the end-point of the investigation together with a critical appraisal of the multi-faceted dimension of the genetic component of either the disease and the pharmacogenetic profile of the drug investigated, will help to design more thorough study and to achieve deeper understanding of the practical results. We will primarily focus our research considerations on the role of Interferon Beta (IFN-β) as a prototypal therapeutic agent in Multiple Sclerosis.

Keywords: Multiple Sclerosis; Pharmacogenetics; Interferon Beta; Complex genetic trait; Phenotype

1. Introduction

Pharmacogenetics is a relatively new area of medical genetics, which focus on the identification and study of the genetic mechanisms involved in controlling both the individual response of a patient to a given medication and the emergence of adverse effects related to a given medication. Pharmacogenetics is rapidly progressing in all areas where the knowledge of the biology of a disease is well established and/or for those drugs that have a well-known mechanism of action at the cellular level, like those used in the treatment of some forms of cancer.

However, for autoimmune disorders, and specifically for Multiple Sclerosis (MS), the situation is quite different, since we still have a partial knowledge of the biological mechanisms of the disease and of the drugs currently used to treat it.

Multiple Sclerosis (MS) is a disorder of the central nervous system with an inflammatory and a neurodegenerative component, and is characterized by a variable clinical pattern, which ultimately leads to a progressive neurological dysfunction. MS can present either as a recurrent disorder where acute phases are interspersed with even long periods of quiescence (Relapsing-Remitting, RR) or as a progressive disorder from the beginning without relapses (Primary Progressive, PP) [1]. The relapsing-remitting form is proportionally more frequent in younger patients and in females, but both forms of the disease ultimately lead to a deficit state with a variable degree of disability. Recent revisions of the classification of MS emphasize a neurodegenerative component characterized by axonal loss, which occurs from the early phases of the disease [2] and is considered responsible of the long-term irreversible disability: the neurodegenerative component thus can be interpreted as a consequence of the inflammatory-immune process or as an

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independent pathogenetic element of the disease. Genetics plays a major role in the development of the disease, even though a still unknown non-genetic component, probably of viral origin, has also a key causal role. Whatever the complexity of the etiology, however, the difficult interplay of the biological-genetic component is critically relevant, since our current abilities to treat the disease occur at this level. Unfortunately, we do not yet have a definitive therapy for MS: among the existing treatments, Interferons-beta-1a and -1b (IFN-β) have been shown to decrease clinical relapses, potentially decrease brain disease events and also slow the progressive disability [3]. However, even for IFN-β, the effect of therapy is only partial and a consistent number of patients are considered poor or no-responder to the treatment: a pivotal issue is that we still do not know how and why IFN-β works in definitively treating some patients, while it fails to be effective in other subjects; the solution to this issue is extremely relevant, since it could help us to design more powerful drugs to treat the disease. The only published reports that have addressed this topic looked at several polymorphisms in the IFN-β receptor genes (*IFNAR1* and *IFNAR2*) [4,5] and in the promoter region of the *IL-10* gene [6], but overall no clear significant differences in the genetic makeup of these genes have been found between patients responder or non-responder to the therapy. This preliminary result suggests to re-evaluate and re-think our general strategy in performing pharmacogenetic studies of MS, pointing to two major sources of complexity, that is the clinical dimension of the responder/non-responder phenotype and the multi-faceted component of the genetic mechanism where the effect is most probably due to the joint role of several rather than just one or two genes.

2. Genetic bases of the pharmacogenetics of MS

As in the majority of pharmacogenetics traits and most notably in complex diseases, a set of genes that belong to two major domains, the metabolism of the drug and the mechanism specifically responsible for the medication effect, jointly influence the effect of the treatment. While the genetic control of the metabolism of small molecules is well known, we still do not yet have a definitive and clear knowledge of the metabolism of a protein drug as in MS. Nonetheless, the proper functioning of the complex metabolism and breakdown of, say, IFN-β, will undoubtedly and markedly affect the ultimate outcome of the treatment of MS. At a relatively simple level, we can imagine that the inability to properly metabolize IFN-β could contribute to the development of adverse effects on one side, hence the need to suspend the treatment, and to the inability of carrying a response at the other extreme level. In any case, even when the drug does not have difficulties in eliciting a response at the target level, a genetic flaw at the metabolic level could thwart its therapeutic effect: despite the relevance of the topic, there are no published studies addressing this issue. When we consider the pharmacodynamic component of the drug, we face additional problems. First, it is probably limiting to focus only on the effect of polymorphisms at one gene: expanding the example of the current therapy of MS, we know that IFN-β is involved in at least three different pathways, one regulating the Cytokines (CK), enhancing IL10 and inhibiting IL12 secretion [7,8], a second related to the effect of Toll-like receptors [9], and finally the JAK-STAT pathway [10,11]. A detailed review of these pathways would require a lengthy appraisal of these systems, but here we want just to point up a few considerations. The Toll-like receptor signaling pathway includes more than 60 genes and, among others, IFN-β seems implicated in T-cells stimulation and, even more directly, in controlling for antiviral effects, which may represent an interesting link between the genetic component and the proposed trigger effect of a virus in the emergence of the disease [9]. Via the cytokine receptor system, the IFN-β is also part of the JAK-STAT signaling pathway, composed by more than 20 major genes, with a role in growth, proliferation and immunity mediated by the STAT proteins and CBP through the IFN-receptors *IFNRA1* and *2*. These two pathways are very interesting for their role in the evolution of the immune system, most notably the Toll-like receptor pathway, and for the signaling in response to IFN-inducible genes, modulating or altering biological responses: if some current hypotheses showing a more direct relation of IFN-β with STAT5 are correct, it should be remembered that STAT5 knockout mice exhibit defects in T-cell (and NK cell) proliferation, thus suggesting a pathogenetic correlation with some hypothesis related to the immune dysfunction in MS. Among the three, the Cytokine (CK) pathway is surely the most complex of all and there already are excellent reviews that describe the role of IFN-β within the CK pathway. Here, in addition to the well known role of the cytokines in inflammation and immunity, we want to draw attention to another still poorly studied dimension of cytokines, that is their role in the central nervous system (CNS). The Cytokines in fact are relevant for several ‘‘functions’’ in CNS, since they have been implicated in neuronal differentiation and migration, in acute and chronic neurodegenerative processes, in modification of the synaptic plasticity and in affecting and regulating the metabolism of neurotransmitters. Thus either considering a dysregulation of Cytokines as an etiopathogenetic factor for a given disease, or on the contrary their use as therapeutic agents as for IFN-β in MS, we should be aware of a potential effect as modulators of CNS functions.

The complexity and the current limited knowledge of the different mechanisms of action of interferon therapy in MS pathology suggest us that pharmacogenetics studies on MS needs to be performed through large-scale genome screening and/or large-scale expression studies as well as through interaction modeling and pathway analyses to take into account all the hypothetical genes and proteins potentially involved in the process.

3. Clinical phenotype

Despite a tremendous technological improvement of pharmacogenetics in the last years, it is remarkable how small has been the methodological contribution to define the efficacy

of a given drug and to discuss and compare which is the best study design for pharmacogenetic studies.

We want to add our point of view to partially fill this gap by addressing also a few clinically relevant issues.

3.1. Definition of drug-responder

We can distinguish two different aims of pharmacogenetic studies: the identification of the polymorphisms associated with the development of adverse events to a given drug, and of the polymorphisms associated with an efficacy of the drug. The definition of adverse event, especially if serious or life threatening, is usually consistent and similar across studies.

On the contrary, there is more discrepancy on the definition of what is meant for the efficacy of a given drug, and the ways to measure efficacy clearly varied across different trials.

How did investigations address this issue in MS?

It is well known that in MS there are some disease-modifying drugs, such as IFN, with partial efficacy estimated around 30% of reduction of clinical relapses and around 70% of reduction of magnetic resonance activity [3].

As shown in Table 1, the definition of "responder" is highly variable across studies, sometimes based on clinical criteria, and sometimes on clinical and Magnetic Resonance Imaging (MRI) criteria. MRI are more sensitive than clinical criteria, but we know that there is a low concordance between MRI and clinical data, especially in relationship to the progression of disability, leading to the so-called clinical-MRI paradox [12]. Consequently, MRI can be considered as a surrogate outcome measure, but should not be used instead of clinical criteria [13]. The papers by Trojano [14], Levya [4], Villoslada [15] and Waubant [16] used only clinical criteria, while Sturzebecher [17], Petzold [18], and Rudick [19] used a combination of clinical and MRI criteria. More specifically, Waubant [16] studied 337 patients enrolled in the Lyon European Database for Multiple Sclerosis (EDMUS), and defined responders those who have shown a reduction of the relapse rate on IFN-β (taken for at least 6 months) compared to the previous 1 and 2 years. Trojano [14] and Levya [4] too used clinical criteria to define the efficacy of the drug, but they defined their patients as responders and non-responders based on the reduction of relapse rate and disability progression, however classified in different ways. Villoslada [15] mentioned two different types of criteria, a first more stringent, defined as an absence of relapses and a stable Expanded Disability Status Scale (EDSS) [20], and a second less stringent, defined as a reduction of at least one third of relapse rate. Sturzebecher [17] gave a definition of drug-responder using a combination of clinical, namely a clinically stable disease, and MRI criteria, using a previous definition from Stone [21]. In another paper [19], Rudick applied a logistic regression model and ROC curves in a cohort of patients enrolled in a placebo-controlled clinical trial of IFN-β1a to select the best predictors of drug responsiveness, which were identified as absence of enhancing lesions (criteria 1), less than 2 new T2 lesions developed (criteria 2), or no relapses (criteria 3).

Table 1
Definition of responders across different studies

Study	Number of patients	Responders definition: MRI criteria	Responders definition: clinical criteria − relapse rate (R-R)	Responders definition: clinical criteria − progression of disability	Proportion of responders
Sturzebecher 2003 [17]	10	>60% decrease of gd-enhancing lesions +		"Stable disease"	60%
Waubant 2003 [16]	337 (IFN for at least 6 months)		Lower R-R than 1 or 2 years before treatment		69% (RR); 68% (SP with relapses)
Rudick 2004 [19]	172	Criteria 2: No gd-enhancing lesions in year 1 and 2. Criteria. 3: ≤2 new T2 lesions at year 2 vs. baseline	Criteria 1: No relapse in 2 years		Criteria 1: 68%. Criteria 2: 75%. Criteria 3: 75%.
Petzold 2004 [18]	30	No gd-lesion (when available) +	<2 relapses +	EDSS stable or increased ≤0.5 points	63%
Trojano 2003 [14]	540 (1-year f-up); 378 (2-year f-up)		Criteria 1: No relapses	Criteria 2: No sustained and confirmed increase of ≥1 EDSS score	Criteria 1: 50% (year 1); 30% (year 2). Criteria 2: 96 % (year 1); 90 % (year 2)
Villoslada 2004 [15]	202 (IFN for 2 years)		Criteria 1: No relapse Criteria 2: Decrease of R-R >30% versus 2 years before treatment	Criteria 1: No increase of EDSS	Not reported
Levya 2005 [4]	147		R-R equal or lower than before treatment +	No increase of EDSS after the first year of treatment	70.7%

It is clear that this variability in defining who is a "responder" patient, associated with the lack of rigorous and consistent clinical/MRI criteria introduce a high degree of inconsistencies across the various studies making any direct comparison impossible, and at the same time increases the importance and utility of surrogate markers, such as some hematological markers, able to measure the therapeutic response. For example, there are interesting data on the assessment and titer of autoantibodies against interferons [22–24], which is higher across poor and non-responder patients, but still their predictive utility is not strong enough, thus requiring for new markers of clinical efficacy.

A consistent and reproducible definition of drug efficacy and "responder" across studies is extremely important for study design and the interpretation of data. As a matter of fact, the dimension of the sample is estimated on the expected proportion of variability explained by certain gene polymorphisms, thus depending on the definition of responder, and the statistical results can substantially differ if the definition of outcome measures changes. Therefore, a consensus on the best definition of treatment response is an absolute need.

Moreover, the efficacy of the drug, or the definition of responders, has to be decided before, instead of after, the planning of a pharmacogenetic study, to avoid post-hoc analyses and "fishing" expeditions, without controlling for multiple testing. The existence of clearly defined criteria would surely help to solve even this problem.

3.2. Outcome measure for drug efficacy

Every drug can have different targets, and its efficacy can be measured using different outcome measures.

There are clear-cut outcome measures, such as death, against which the treatment is given to patients. However, in most of the situations the target of the drug is different. In MS, among the clinical and MRI outcomes used to study the disease, the relapse rate and the number of enhancing lesions reflect the inflammatory component of MS, while the disability progression, as assessed with the EDSS and the number of new T2 lesions, partially reflects the neurodegenerative component of MS.

To further increase the complexity of the disease, the relationship between the inflammatory and the neurodegenerative component is not clear; as a consequence, a drug able to reduce the relapse rate does not always reduce the disability progression. The neurodegenerative component is considered the primary determinant of the neurological deficits in MS patients and therefore, testing the efficacy of a drug by its efficacy in reducing the progression of disability seems more appropriate, using a consensus definition of a sustained and confirmed worsening equal to at least 1.0 point if the baseline EDSS is lower than 5.5 and of 0.5 if the EDSS is equal or greater than 5.5. However, the progression of disability is difficult to measure in the short time, as it needs to be confirmed at 3 or 6 months, and it is also poorly sensitive. Moreover, there are limitations related to the EDSS scale, which measures a mixture of impairment and disability and is strongly weighted towards locomotor disability and poorly related to upper limb deficits and cognitive problems.

3.3. Study design

Most of the pharmacogenetic investigations are designed as retrospective case-control rather than prospective follow-up studies. However, a prospective design allows not only to better evaluate the efficacy of the drug, but also to better define the potential risk factors that should be identified and examined before beginning the specific pharmacogenetic study. Moreover, prospective studies might allow the adjustments of doses or the switch to a different immunomodulant drug as a function of the genotype.

In conclusion, a pharmacogenetic study of MS is not an easy task, as expected, but a simple and careful consideration of few key points can help improving the quality of the investigation. First and foremost, it is essential to establish consistent criteria that define the efficacy of the drug: for example, we can categorize two different levels of responders, in agreement with Villoslada [15], namely full responders (no relapses and no confirmed disability progression when on-treatment) and partial responder (reduction of relapse rate of one third compared with the previous 2 years) contrasted to non-responders (>2 relapses or confirmed disability progression). MRI criteria can be used, but as a surrogate secondary and not as a primary outcome measure. The correct follow-up should be a minimum of 2 years of treatment, and it is highly warranted to perform studies on an intention-to-treat basis, which could take into account also patients who interrupted the treatment because of different reasons (such as adverse events), avoiding selection bias.

Second, it should always be remembered that the definition of responder must be established before the beginning of the study, since a lot of critical parameters of the study itself, like the estimation of the required sample size, are depending upon such a definition Third, a proper causal relationship between the administration of a drug and a relevant clinical outcome can be recognized only with a prospective follow-up design.

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