J Neurol (2005) 252 [Suppl 3]: III/21–III/27
DOI 10.1007/s00415-005-2013-3

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Genomics and proteomics: role in the management of multiple sclerosis

Abstract Epidemiological studies and neuro-imaging have provided important insights into the natural course and prognostic factors of multiple sclerosis (MS), but our ability to predict different courses of the disease, and especially its response to treatment, is still very limited. Pharmacogenetic, pharmacogenomic and proteomic studies aim to assess gene and protein function in disease and promise to help to fill this important gap in our knowledge. Such studies may increase our understanding of disease mechanisms and responses to therapeutic compounds. Large-scale transcriptional expression profiling can be performed using gene chip microarrays; this technology allows screening for differentially expressed genes without having well-defined underlying hypotheses ("discovery-driven research"). To complement the technique, real time reverse transcription and polymerase chain reaction (RT-PCR) can be used for more targeted profiling and provides quantitative data on pre-selected genes. However, to maximise their clinical utility, expression profiling results need to be combined with well-documented clinical and imaging data.

Two forthcoming studies will investigate the long-term effects of early treatment with interferon beta-1b (IFNβ) on the course of MS. The BENEFIT (BEtaseron®/Betaferon® in Newly Emerging MS for Initial Treatment) study will incorporate pharmacogenetic and pharmacogenomic analyses to determine the genetic elements controlling treatment response. BEST-PGx (Betaferon®/Betaseron® in Early relapsing-remitting MS Surveillance Trial – Pharmacogenomics) is an exploratory 2-year study that will investigate the value of RNA expression profiling and pharmacogenetics in predicting treatment response to IFNβ in patients with early relapsing MS. The main goal of BEST-PGx is the identification of differences in gene expression profiles of patients showing differential treatment responses. In addition, this study may reveal new information relevant to the mechanism of action of interferon treatment in MS and also to differences in the underlying pathology of the immune system. These data may help us approach the goal of a really "individualised therapy" with increased efficacy, reduced adverse drug reactions and more efficient use of healthcare resources.

Key words genomics · interferon beta-1b · multiple sclerosis · proteomics · pharmacogenetics

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Introduction

Detailed epidemiological studies and neuro-imaging have provided important insights into the natural course of and prognostic factors for multiple sclerosis (MS). However, MS is a complex, most likely polygenetic disorder, in which several genes interact to result in a heterogeneous pathogenesis; our ability to predict different courses of the disease, and especially its response to treatment, is still very limited. The discovery of new gene targets may be key to developing novel and effective

therapeutic agents for MS. Together with improvements in imaging techniques, genomics and proteomics are important new tools that promise to help in elucidating the pathogenesis of this complex disease.

The need for markers – questions raised in clinical studies

Based on magnetic resonance imaging (MRI) and neuropathological studies in patients with MS, there is evidence that progressive disability is associated with cumulative and irreversible axonal loss [5, 45]. Data also suggest that axonal loss and cerebral atrophy both occur early in the course of the disease, at a time when there is either no disability, or only mild disablement [13, 40]. Further evidence comes from Brex et al. [7] who showed, using natural history studies, a modest correlation between changes in lesion volume in the first 5 years and disability after 14 years in patients presenting with clinically isolated syndrome (CIS) suggestive of MS.

Data from two studies examining early intervention at a diagnosis of CIS – CHAMPS (Controlled High-risk subjects Avonex® MS Prevention Study) and ETOMS (Early Treatment Of MS) – demonstrated the importance of early intervention with interferon beta (IFNβ) as well as the value of MRI as a prognostic marker [1, 2, 12, 24, 27]. The CHAMPS study showed that in patients with high MRI lesion burden and evidence of inflammation, the Kaplan-Meier estimate of the cumulative probability of developing clinically definite MS (CDMS) was 21 % in the IFNβ-1a group, compared with 56 % in the placebo group [2, 24, 27]. A high MRI lesion burden and evidence of inflammation was defined as at least nine T2-hyper-intense lesions and at least one Gd-enhanced lesion on the initial (baseline) MRI scan.

The ETOMS study found that a high T2 lesion number at presentation is associated with polysymptomatic events and attack severity [1, 12]. Conversion to CDMS occurred in 43 % of patients with Gd-enhancement compared with 34 % without ($P = 0.085$). When the lack of a Gd-enhanced lesion was substituted by the presence of nine or more T2 lesions, the predictive value increased (41 % vs. 11 %; $P = 0.008$).

The outcomes from CHAMPS and ETOMS pose a series of additional questions that need to be addressed in terms of pinpointing whether dose and frequency of administration are critical in the early phase of MS. There is also a need to determine the long-term effects and impact of early intervention on the underlying pathology. Another important question raised by the data from these two studies is whether all patients presenting with CIS should be treated with disease-modifying therapy. Not all patients with CIS progress in a similar fashion – some will exhibit no further symptoms for many years, while others will deteriorate more rapidly. However, ways of predicting the likely course of disease in a given patient population are currently limited. Initial lesion load on MRI, and antibodies against myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP), as recently suggested by Berger [4], are the "hot candidate markers", but further studies are needed to determine their practical value.

Emerging biotechniques in MS

MS – a highly complex and polygenic disorder – represents a formidable challenge to scientists developing therapies. Identifying the specific genes responsible for the pathogenesis of MS has been problematic not only because of the large number of genes involved but also because of the interactions between these genes and the environment. While several effective therapies for MS exist, individual patient response varies widely. Despite these difficulties, regions of the genome contributing to the susceptibility for MS have been located, and the information obtained from genetic studies may offer the opportunity to define markers for disease susceptibility and prognosis.

Pharmacogenetic and pharmacogenomic analyses aim to identify markers that will predict patient response to therapy and possible tolerability risks. *Pharmacogenetic studies* try to characterise individual genetic differences (at the DNA level) and understand how this diversity causes variability in patients' responses to a specific treatment. *Pharmacogenomics* is the analysis of gene expression (at the RNA level) and aims to identify markers relevant to treatment response.

A number of new, high-throughput techniques have been developed, making it feasible to investigate a vast number of genes and gene products simultaneously; these techniques have significantly advanced the study of various diseases. Although large-scale explorations of gene expression have become routine over recent years, the statistical and mathematical analyses of resulting data is a developing discipline [9, 29, 41].

Comprehensive understanding of gene expression in MS, both during treatment and the course of disease, can help to identify genes that are important in drug response and pathogenesis. However, measuring transcriptional gene expression alone does not provide the complete picture as, for example, post-transcriptional or -translational modifications may process the protein into a different form. Hence, proteomic studies that assess the translation of processed RNA into proteins are a critical accompaniment to genomic technologies (Fig. 1).

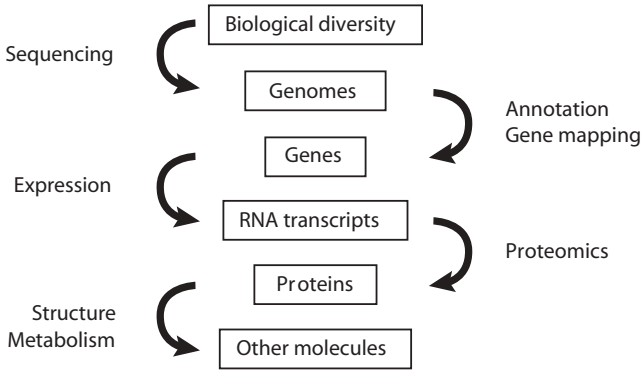

Fig. 1 The steps involved in functional genomics (from [43] with permission of PAREXEL MMS Europe Ltd)

Genetic markers in MS

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation. They occur with a frequency of approximately 1 in 1000 nucleotides, and there are approximately 10 million distributed throughout the human genome. Only approximately 10–30 % of SNPs are located within the coding regions of genes – the vast majority are classed as "perigenic" and are situated in the non-coding and regulatory regions of genes. The abundance of SNPs, coupled with the fact that they are mutationally stable, makes them excellent gene-discovery tools; SNPs associated with a particular phenotype can be used to pinpoint the responsible mutation.

To ensure proper diagnosis and treatment for patients with MS, markers that measure disease activity, prognosis and response to treatment, and that can be used as tools for evaluating clinical trials, are needed. However, the organ affected by MS is not easily accessible using routine sampling methods, making isolation of suitable markers difficult. In addition, candidate markers need to fulfil several requirements: a high degree of reproducibility, be derived from a source suitable for repeated sampling (for example, from blood), and be applicable for use in routine clinical practice. Markers derived from cerebrospinal fluid (CSF), such as an endogenous pentapeptide showing promising results [8], can be reliable indicators of the disease process, but the problem is that CSF is an unsuitable source for repeated sampling. The search for suitable markers is ongoing in both animal models of MS and humans.

Gene expression profiling in MS

Gene expression profiling examines dynamic markers (e. g. RNA and proteins), and the recent completion of the Human Genome Project means that the capacity to perform customised searches for differential gene expression now exists. Gene expression profiling can be performed at the RNA or protein level via analytical platforms called transcriptomics and proteomics, respectively. Current high-throughput methods for simultaneously analysing the transcriptional expression of thousands of genes include subtracted complimentary DNA (cDNA) libraries, serial analysis of gene expression (SAGE), differential display, quantitative reverse transcription and polymerase chain reaction (RT-PCR) and gene microarrays. These techniques have all been successfully applied in MS research; for example, subtracted cDNA libraries have been used to investigate the pathogenesis of lesion development [3, 11], and dysregulation of the inhibitory transcription factor sp3 was determined using the differential display technique [20]. The most powerful and comprehensive techniques, however, are microarrays and RT-PCR.

Microarrays

Microarrays, also referred to as DNA chips, are powerful and versatile tools for genome analysis and exploit the specific binding of complementary single-stranded nucleic acid sequences. Use of gene chip microarrays provides a global approach to RNA expression profiling, as they provide a high gene throughput (some can assess the whole human genome in one experiment) and can be used without well-defined underlying hypotheses. Such "discovery-driven research" generates hypotheses, in contrast to more conventional "hypothesis-driven research", which aims to confirm or reject a pre-existing hypothesis.

cDNA microarrays usually contain double-stranded cDNA sequences of interest that have been synthesised by PCR. Oligonucleotide microarrays, however, are prepared using specific oligonucleotides synthesised directly onto a quartz or silicon wafer using combinatorial chemistry and photolithography [14, 16]. One microarray may contain more than 1 million different oligonucleotides.

Test samples, RNA isolated from the cells or tissue of interest and control samples are labelled with a fluorescent dye and allowed to bind in a quantitative manner to complementary sequences on the microarray. Relative expression levels of the sequences in the test sample can be estimated by comparing the fluorescence intensities, measured by laser scanner, with those of the control sample (see Fig. 2). In the case of MS, this process may involve semi-quantitative screening of the whole genome, using RNA from peripheral blood as the source (whole blood or specific subpopulations of blood cells), or cells from CSF obtained from a relatively small number of patients with MS. In both scenarios, samples from patients who have and have not received various treatments are needed.

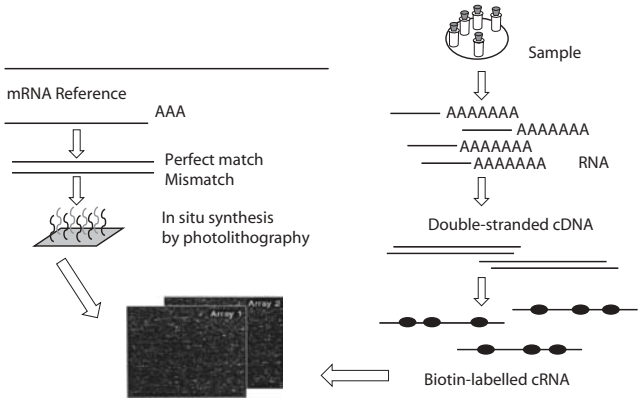

Fig. 2 Schematic overview of oligonucleotide microarray technology (adapted from [39], reproduced with permission of PAREXEL MMS Europe Ltd)

RT-PCR

Genes that are differentially expressed in treated groups compared with control groups can be analysed in more detail and in larger numbers of patients using quantitative real-time RT-PCR; this technique can analyse hundreds of genes in a more quantitative fashion than microarrays. These investigations provide information on the time course of the changes in expression of the genes of interest during treatment, relative to baseline. Cell biology and DNA studies, as well as protein analysis, can be undertaken in parallel in these samples. Markers that appear to predict responses to therapy can then be subjected to enzyme-linked immunosorbent assay (ELISA) and further proteomic testing in a larger population of patients. The rationale for this method of analysis has been described by Martin et al. [34].

Proteomic techniques

Proteomics is an area of research that examines global changes in protein expression. Technologies such as 2-dimensional (2-D) gel electrophoresis + mass-spectrometry, bead-capture and micro-ELISA are proteomic techniques currently employed successfully for drug discovery and biomarker identification, mainly in the field of oncology [10, 25].

Protein arrays, an emerging class of proteomic technologies, have great potential for acquiring information about post-translational protein modifications that reflect the activity state of signal pathways and networks involved in disease progression or remission [31]. Such arrays have already been used for auto-antibody profiling in autoimmune diseases, including MS [18, 23, 38]. Specialised "myelin proteome" arrays, containing hundreds of proteins and peptides derived from the myelin sheath, have been developed to study autoimmune responses in MS, but extensive validation is needed before they could be used in routine clinical practice.

Clinical applications

Pharmacogenomics

In MS, the first published application of microarray technology used brain samples from a patient with primary progressive MS [47]. In that study, 62 differentially expressed genes were identified. To date, several large-scale expression profiling studies on MS lesions and normal appearing white matter (NAWM) have been performed and have revealed a complex pattern of genes related to both inflammatory processes and unbalanced immune response regulation [3, 11, 19, 30, 32, 35, 44, 47–48]. Microarray analysis of MS lesions obtained at autopsy has also helped to elucidate MS pathology. One study revealed up-regulation of granulocyte colony-stimulating factor expression in acute but not chronic lesions [32]. In contrast, immunoglobulin E receptor expression was up-regulated in chronic silent lesions compared with acute lesions. These two genes were targeted for therapy using the experimental autoimmune encephalomyelitis mouse as a model of MS, and the results corroborated the microarray studies on MS lesions [32].

Microarray technologies have been used to identify prognostic and predictive markers for MS as well as monitor treatment response. In a study of relapsing-remitting MS (RRMS) using cDNA arrays from more than 4000 known human genes, 34 genes were found to be expressed at significantly different levels in MS patients compared with control patients [37]. Based on the expression profiles of 6500–7500 genes, Bomprezzi et al. [6] could distinguish between samples from RRMS patients and control individuals, and identify altered, disease-relevant pathways in MS.

No definite treatment-response profile has been identified in MS, but some effects of IFNβ and glatiramer acetate on gene expression have been described [22, 28]. A comparison of gene expression profiles using cDNA microarrays from patients with RRMS before and after IFNβ-1b treatment demonstrated significant alterations in the expression of 21 genes (from a total of 1263) in response to treatment. Nine of these genes contained IFN-responsive promoter elements [28]. In general, the effects of IFNβ on the gene expression profile are complex and appear to influence many biological processes, including cell migration, matrix degradation, cell cycle control and cytokine and chemokine regulation. For example, Wandinger et al. [46] found that IFNβ-1b up-regulated expression of the chemokine receptor CCR5 and the interleukin (IL)-12 receptor beta$_2$ chain; it also modulated the expression of a number of other genes that may be relevant to MS.

Proteomics

Proteomic techniques have been applied to clinical samples from patients with MS, such as CSF [15, 21]. Using 2-D gel electrophoresis followed by liquid chromatography and mass spectrometry, 65 different proteins were identified, 18 of which had not been reported previously [15]. However, the relevance of these proteins to MS needs further evaluation.

Pharmacogenomics

Genomic studies have generated useful data highlighting the potential pathogenetic mechanisms involved in MS, and allow better insights into the mode of action and efficacy of various treatments. However, expression profiling results need to be combined with well-documented clinical and imaging data to maximise interpretation.

Genetic information must be associated with clinical data on treatment response; these data can only be generated from therapeutic trials with regular clinical and MRI monitoring that include pharmacogenomic analyses in their design. One study of a patient with MS treated with IFNβ-1b detected altered gene expression using a cDNA microarray derived from peripheral blood mononuclear cells (PBMCs); these changes were correlated with MRI changes [46]. In another study, Sturzebecher et al. [42] made an initial attempt to correlate gene expression changes in response to treatment with treatment response as assessed by MRI. This group compared the gene expression profiles, using cDNA microarrays, of patients who responded to IFNβ-1b with those who either initially responded and then became non-responders, or who were not responsive to treatment. The results of the study, albeit based on limited numbers of patients, showed that there were definite differences in the gene expression profiles of treatment-responsive and non-treatment-responsive patients.

Therapeutic studies with IFNβ-1b

Two studies with IFNβ-1b, BENEFIT (BEtaferon®/Betaseron® in Newly Emerging MS For Initial Treatment) and BEST-PGx (Betaferon®/Betaseron® in Early RRMS Surveillance Trial – Pharmacogenomics), have been designed to examine the long-term effects of early treatment on the course of MS.

BENEFIT

The BENEFIT (BEtaseron®/Betaferon®) study is the first to examine the effects of conventional, high-dose, high-frequency IFNβ-1b administration in patients with CIS and MRI evidence of disease, in terms of the time to a second clinical event and, hence, a diagnosis of CDMS [17]. BENEFIT will also examine the long-term benefits of treatment beyond effects on the second clinical event. BENEFIT consists of a 2-year, randomised, double-blind, placebo-controlled, parallel-group study and a 3-year, open-label, follow-up study. In this study, a total of 487 patients have been randomised to either IFNβ-1b 250 μg (8 MIU) subcutaneously every other day, or placebo in a ratio of 5:3. Patients fulfilling the two primary endpoints (time to diagnosis of MS using the McDonald et al. [33] criteria and time to diagnosis of CDMS using the Poser criteria [36]) will be offered treatment with IFNβ-1b in the open-label part of the study. In addition, all patients completing the 2-year study without fulfilling the primary endpoints will also be offered open-label IFNβ-1b treatment. The open-label study will compare the efficacy of treatment initiated after the first clinical event with that initiated after conversion to CDMS.

Exploratory analysis of molecular prognostic factors will be undertaken. This will include expression profiling and pharmacogenetic analyses (the latter being optional and requiring separate informed consent). Blood samples will be taken from these patients at all scheduled visits, and samples for RNA analysis will be obtained at pre-defined points during the initial 2-year study and the follow-up study.

BEST-PGx

BEST-PGx is an exploratory, 2-year, investigator-led, observational study investigating the value of RNA expression profiling (and also pharmacogenetics in one subgroup) for predicting treatment response to IFNβ in patients with early RRMS [26]. The main goal of BEST-PGx is to identify differences in gene expression profiles in patients showing differential treatment responses. In addition, this study may reveal new information relevant to the mechanism of action of IFNβ treatment in MS, but also to differences in the underlying pathology of the immune system in different subgroups of patients.

Conclusions

The pharmacogenomic outcomes of the BENEFIT and BEST-PGx studies will allow some progress towards individualised medicine for patients and, in the process, offer insights into the effective integration of these new technologies into the current clinical trial paradigm.

It is hoped that pharmacogenomics will ultimately allow profiling of patients with MS according to their course of disease and their likely response to a particu-

lar treatment. The definition of molecular markers for MS will possibly allow detailed prediction of the need of a patient for therapy, and a patient's response to relevant aspects of pharmacotherapy, including response to treatment and adverse effects. Using this information, it should be possible to provide individualised therapy to ensure that a patient obtains the most appropriate treatment from the outset. This approach is expected to increase overall treatment efficacy, reduce adverse drug reactions and allow more efficient use of healthcare resources.

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