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Genomics, proteomics, metabolomics: what is in a word for multiple sclerosis?

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Purpose of review
Multiple sclerosis (MS) is the most common chronic inflammatory neurological disease. Despite major advances the aetiology of this disease it is still not completely understood. In the post-genome era, advances in global screening technologies offer an opportunity to accelerate the search of new pathological pathways and to identify new therapeutic targets. Some recent publications using novel global screening methods at the genome, transcriptome, proteome and metabolome levels are discussed.
Recent findings
The genetic association of susceptibility to MS with loci outside the MHC has been reconfirmed. Evidence of parent-of-origin and seasonal effects on disease susceptibility add further complexity to the genetics of MS. The search for MS susceptibility genes continues using the candidate-gene approach as well as large-scale single-nucleotide-polymorphism association studies and novel cross-species synteny analysis. Genome-wide expression profiling using microarrays produced numerous therapeutic targets and is progressing towards profiling of rare cells. Advances in classical proteomics methods paved the way to new initiatives aiming at determining the proteome of the nervous system in normal and diseased states. Although progress is still slow, array-based methods are making an impact on the MS field.
Summary
The complexity of MS is clearly reflected in the latest findings using global profiling methods. Nevertheless, these new technologies are confirming some of the basic aspects of the disease pathophysiology, i.e. its polygenicity, the central role of neuroinflammation and the emerging neurodenegerative processes. These data are primarily the results of genomic approaches, yet promising attempts are also made using proteomics and metabolomics.

Keywords
genomics, metabolome, microarrays, multiple sclerosis, proteomics

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Abbreviations

EAE experimental autoimmune encephalomyelitis
MS multiple sclerosis
SNP single nucleotide polymorphism

1350-7540

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the nervous system. The disease is characterized by the generation of activated antigen-specific T and B cells, their migration to the nervous system, local reactivation by resident antigen-presenting cells, activation of local glial and microglial cells, secretion of chemokines, cytokines and other inflammatory mediators (for example, complement), and NO, and eventually demyelination of axons throughout the central nervous system. Genetically and histopathologically MS is a heterogeneous disease [1•,2]. Indeed, the genetic dissection of the MS susceptibility trait performed in a variety of populations has led to the identification of several susceptibility regions within the MHC locus and several non-MHC-genetic contributions. Despite considerable efforts the identification of putative susceptibility genes in these regions has so far not been feasible [3].

The new advances in global genome-screening tools such as single nucleotide polymorphism (SNP) microarrays, cDNA and/or oligonucleotide-microarray-based gene-expression profiling are accelerating this process. In particular the combination of genetic SNP association studies and expression profiling is a promising approach. This and the data available from the proteome and metabolome studies are likely to offer not only an improved understanding of the pathophysiology of MS but also develop valuable biomarkers [4•].

Genetics of multiple sclerosis

MS is a complex multifactorial polygenic disease, influenced by age, gender, hormonal and environmental factors. Throughout the years numerous linkage and association studies were conducted and a large number of susceptibility regions identified. Nevertheless, the HLA locus remains the only common and confirmed susceptibility locus for MS. This is not surprising, bearing in mind the heterogeneous nature of the disease,

different diagnostic criteria and the genetic heterogeneity and incomplete penetrance, as well as the environmental factors in the different populations studied. These facts have fostered a new generation of genetic collaborative studies that involving particularly larger numbers of families from different populations. The effort culminated in the genetic analysis of MS in Europeans (GAMES) screening approach [5] and continued with a new generation of genome screens involving larger families and increased marker density [6•,7•].

The search for non-MHC susceptibility genes is likely to benefit from the new microarray-based SNP screening technology. Indeed the first data on the technical feasibility of the method in MS have already been published by the international Multiple Sclerosis Consortium. It promises a dense coverage and accurate genotyping of a large number of well-characterized families within a short period [8•].

Classical methods for identification of susceptibility genes have continued. As expected, a long list of potential candidate genes showing variable degrees of positive associations were published recently, notably PRKCA, PTPRC, NOS2a and Ncf1, among others [9•,10•,11,12•]. Novel bioinformatic and experimental genetic approaches utilizing the wealth of existing data from animal models and human studies have also been employed. A study by Serrano-Fernandez *et al.* [13•] described a global approach to determine intergenomic consensus of MS and experimental autoimmune encephalomyelitis (EAE) susceptibility regions and to fine-map those quantitative trait loci *in silico* and select particularly promising candidate genes for further analysis. The Olsson group [14•] on the other hand characterized a large number of rats with mixed genomes, advanced intercross lines, and used them to fine-map a susceptibility locus, EAE18, syntenic to the human 17p11 locus.

Although most experts agree that MS is influenced by genes, age and seasonal as well as other environmental factors, the experimental epidemiological evidence is not conclusive. Three new fascinating studies from the Ebers and Teuscher groups are offering both epidemiological and experimental support to those hypotheses. By screening thousands of Canadian MS families and following disease prevalence among half-siblings Ebers and colleagues [15••] showed a clear maternal parent-of-origin effect in MS transmission. Additional evidence suggesting an effect of the month of birth on disease susceptibility, with an increased incidence in those born in May, was also presented [16•]. Experimental evidence supporting the latter was published by Teuscher and colleagues [17••] in the EAE model. They showed increased disease incidence during the summer months as compared to winter and spring [17••].

Expression profiling in multiple sclerosis

During the last few years advances and availability of microarray technology have brought a wealth of genome-wide gene-expression profiling studies to all fields including that of MS. The first experiments were rather restricted by the high costs, technical difficulties and problems in interpretation and analysis of the huge amount of data generated in single experiments such as in the pioneering study of Whitney *et al.* [18].

Most of the technical and analytical problems were solved as new analytical programmes were developed, standardization of methods took place and several publically accessible databanks were set up [19]. This was reflected in the latest gene-expression profiling-based publications. Indeed, gene-expression profiling studies confirmed basic concepts in MS, for example the immune-mediated and neuro-degenerative aspects of disease pathogenesis, identified new pathways and pointed to new targets for therapy [20–22]. Nevertheless, one primary problem with earlier studies was the use of mixed tissues from heterogeneous groups of patients and controls or mixed tissues from EAE animals [23•]. This approach is not without risk as shown recently by Lindberg and colleagues [24•]. Even in the normal-appearing brain tissue they observed a minor degree of inflammation. Therefore, introducing new methods to target particular cell types in a standardized way in large numbers of patients and controls such as laser-dissection microscopy is needed to address these issues [25]. Laser-dissection microscopy and single-cell analysis are already being used to study the T-cell repertoire in MS brain lesions [26•]. The ultimate goal would be to determine the expression profile of all important cell types involved in MS including rare effector and target cells. Profiling of rare cells, including neurons, down to the level of a single cell has been demonstrated recently for the dopaminergic neurons of the retina [27•]. Such an approach could be complemented with *in situ* genotyping of DNA mutations by target-primed, rolling-circle amplification of padlock probes, as shown recently for the brain mitochondria [28•].

On the other hand, therapy monitoring of individual patients is a strong area of interest. The hope is to identify surrogate markers for the responder phenotype [29]. As yet this has to be determined empirically and individually. One recent study is that of Iglesias *et al.* [30•], who identified gene-expression signatures revealing enhanced E2F pathway transcription in peripheral blood of MS patients, which was validated in the EAE model and inversely modulated in interferon-β-treated patients. Extension of these studies to include the kinetics of gene expression after different established and new therapies is starting and likely to continue [31•].

One of the most promising advances in the field however is the genetic analysis of genome-wide variations in gene expression; expression mapping. The first human study was recently conducted by Morley *et al.* [32••] on immortalized B cells from healthy donors. They genotyped 14 large families with more than 2700 SNP markers and correlated the genotypes with the expression of more than 8500 genes. The study showed that there are indeed master regulatory genetic loci of gene expression. It would be of great interest to conduct such studies in MS and correlate those master loci with disease-associated susceptibility regions.

Proteomics and multiple sclerosis

The proteome is the total sum of proteins in a particular cell, tissue or organ in a healthy or diseased state. Therefore, profiling the proteome of a disease like MS is the next fundamental step to understand its pathophysiology. As with RNA-expression profiling sequencing of the human and murine genomes has had a positive impact on proteome research. The mainstay of proteome analysis is the combination of two-dimensional gel electrophoresis and mass spectrometry. However, additional new methods for protein sequencing and identifying post-translational modifications of proteins are also commonly used. So far most of the studies in MS have focused on the identification of the proteome of cerebrospinal fluid at different stages of the disease. Recent studies identified new proteins that appear to be MS-specific; for example, Autotaxin or Tetranectin [33]. Other studies focused on refining methods to be able to profile the cerebrospinal fluid proteome of a single individual, a difficult task when bearing in mind that most of the cerebrospinal fluid proteins are abundant plasma proteins [34•,35•]. The long-term aim, however, is to map the proteome of different brain areas of different disease stages. Indeed, an initiative initiated by the human proteome organization HUPO, in the form of the new Human Brain Proteome Project, is leading the way [36,37•]. A few fascinating studies during the last few years in mouse models have been successful. Zabel *et al.* [38] and Klose *et al.* [39] identified hundreds of polymorphic brain proteins from common strains of mice and genetically mapped them and the areas that influence their expression.

Traditional methods to identify new antigenic determinants of MS continued with some success. Using sera from MS patients and control Lefranc, Almeras and colleagues [40,41•] identified new MS-related antigens. Novel techniques of protein and antibody microarrays have also been applied to EAE and are being used in other autoimmune diseases and their animal models [42]. A recent example is the use of murine antigen arrays to predict which mice that are at risk from developing diabetes with 82% specificity [43•]. These technologies are likely to contribute to the development of biomarkers for MS, as well as to global analysis of antibody repertoires, repertoire shift and new antigens [44•]. New protein arrays targeting signalling pathways and arrays that are able to identify active enzymes have also been developed recently and can be applied to MS in the near future [45].

The metabolome and multiple sclerosis

The metabolome is the collection of small molecules, metabolites, present in a cell, tissue or an organism. The systematic high-throughput identification and quantification of those metabolites constitutes the field of metabolomics. The metabolome is the final avenue to explore gene function and cellular processes. Improper degradation of cellular proteins may contribute to and/or serve as a biomarker for disease [46•]. As in the case of proteomics there are methods available to identify globally the metabolome by mass spectrometry, nuclear magnetic resonance spectroscopy and electrochemical arrays [46•,47]. These methods can assign substrates to enzymes, or effects of an inflammatory mediator to metabolites, etc. [48]. Metabolome-based studies in MS and other neurological diseases are limited. However, their potential can be seen in examples such as that of Griffin *et al.* [49•] who followed the effect of cytokine-induced neuropathology on the rat urine metabolome by high-resolution proton nuclear magnetic resonance spectroscopy. In MS as well as in motoneuron disorders and Alzheimer's disease studies are underway to define new metabolic biomarkers for those diseases and MS.

Conclusion

New global profiling technologies promise to solve long-standing issues in MS research such as to identify new disease-promoting factors, to revise simplified views of disease pathways and to discover new targets for therapeutic intervention. Additionally, as a much broader panel of immunotherapies, other than interferon-β and glatiramer acetate, will soon become available – Natalizumab, Leflunomide, Laquinimod or FTY 720 – the potential to identify new surrogate markers for prediction of the disease course and monitoring of therapeutic efficacy will be a major area of progress.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1 • Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 2004; 55:458–468.
A study demonstrating complex heterogeneous neuropathological findings in MS lesions.