Blood gene-expression profiling for preonset identification of multiple sclerosis

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“The use of gene-expression technology in the study of (multiple sclerosis) has provided the ability to identify gene targets that carry the promise of obtaining new insights into many facets of the disease’s pathogenesis.”

Multiple sclerosis (MS) is a complex autoimmune disease of the CNS leading, over time, to significant disability in young adults due to the development of multifocal lesions in the CNS that are characterized by inflammation, demyelination and axonal injury. The etiology of MS is considered to be autoimmune, based on its prominent genetic component [1,2]. The disease is characterized by a relapsing–remitting pattern in 85% of patients; however, the factors that trigger the first acute neurological presentation of MS and give rise to the first spurt are unknown.

One of the major goals in medicine today is to provide tools for the prediction of a future illness in terms of which patients will develop which disease and when. An example already within reach is predictive genetics, which uses genetic testing to estimate the risk in asymptomatic individuals for developing a genetic disease with a high predictive value. However, in the case of a multifactorial heterogeneous disease such as MS, the task is more complicated, since, unlike single gene disorders, MS is also influenced by environmental factors.

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Gene-expression technology involves the intricate unfolding of the genetic information in the DNA that leads to biologic variation based on RNA complexity. The technology established on the simultaneous measurement of the expression levels of tens of thousands of

genes provides a powerful characterization of molecular networks associated with a specific disease and is being extensively used to classify disease subtypes, identify therapeutic targets and draw information related to operating biological pathways [3].

The use of gene-expression technology in the study of MS has provided the ability to identify gene targets that carry the promise of obtaining new insights into many facets of the disease’s pathogenesis. Over the last decade, this has allowed us to correlate gene-expression signatures with disease progression, to assess drug response to treatment and to better understand the mechanisms operating in relapse and in remission. Our laboratory has focused on the use of gene-expression profiling to characterize specific disease-related signatures and clarify operating biological networks within each signature. We have concentrated on three major aspects:

- Diagnosis across MS disease stages and at the predisease stage;
- Prognosis in relation to the time to next relapse and in relation to clinical outcome in terms of disability;
- Treatment response to immunomodulatory drugs.

We first identified a specific peripheral blood gene-expression signature that characterizes relapsing–remitting MS. This signature implicates the underlying processes involved in MS pathogenesis, including T-cell activation and expansion, inflammation and apoptosis. Another transcriptional signature of 721 genes involved in cellular recruitment, epitope spreading and escape from regulatory immune

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surveillance identified MS patients in acute relapse compared with remission. Moreover, the gene signature that differentiated acute relapse from remission was enriched by 55 apoptotic-related genes involved in both the caspase-dependent pathways and T-cell receptor (TCR)-mediated apoptosis, suggesting that the inflammatory process in active MS is targeted by the inhibition of proapoptotic genes and repression of antiapoptotic genes that allow the existence of prolonged abnormal immune responses [4,5].

To predict MS outcome, we performed microarray-based clinical research to analyze peripheral blood gene-expression signatures of MS patients with good and poor clinical outcomes. Using this dichotomizing classification, we identified an outcome-predictive signature of 29 genes biologically related to zinc-ion binding and cytokine activity regulation pathways involved in inflammation and apoptosis [6]. In accordance with this, by analyzing the transcription levels of peripheral blood cells in relapsing–remitting patients, we predicted the time to the next acute relapse [7]. Although it is clear that these studies cannot provide perfect correlations between susceptibility genes and disease phenotypes, they may offer good predictive value. In this regard, we aimed to study subjects in the predisease state of MS, when they were considered to be healthy, to look for early susceptibility expression profiles for related genes and pathways.

The real key to MS is enfolded in the early event(s) that triggers myelin and axonal damage. It is not known how long the disease is in the preactive stage before clinical signs appear, as no data exist in relation to the timeframe of disease activity before symptoms emerge. The use of gene-expression profiling for the preonset identification of MS is a ‘prediction into the past’, similar to revealing the solution in a detective mystery. When Hercule Poirot or Miss Marple, Agatha Christie’s famous detectives, analyze a mystery, they try to look back into the past in order to understand the chain of successive events that led to the crime. In essence, this is the approach we used to explore the early disease-triggering operating mechanisms: understanding the chain of events that operate in the predisease state will enable better understanding of the pathogenesis of MS, and the puzzle could begin to be pieced together to construct the underlying story. In this aspect, similarly to a detective going over a process of deductions and ‘predicting backwards’, we

studied healthy subjects between 6 months and 9 years before they presented with their first neurological event of MS, to capture revealing expression profiles during the predisease state, and, in essence, to identify predictors of MS [8].

Our findings suggest that the development of the MS trait occurs early in life, while the transition to established disease may take place years later. During the latent phase, until the emergence of the first clinical symptoms, a gradual unfolding of aberrant immunological mechanisms occurs. This process is associated with a time-dependent epitope spreading as a result of various environmental and genetic factors [9]. To understand these operating pathological mechanisms, which work subclinically for a prolonged period of time, we studied healthy subjects that later developed MS (MS-to-be) and compared their gene expression profiles with matched healthy subjects that remained MS-free. We applied an analytical strategy designed to detect even modest coordinated changes within the expression of functionally related genes. The observed dysregulation of the signaling pathways represented within the 1051 highly variable genes that differentiated between MS-to-be and MS-free subjects point towards the activation of TCR signaling that triggers the *Cbl* and *MAPK* cascade in concert with downstream synergic overexpression of *NFAT* and *MEF2B*. However, this activation failed to augment the expected expression of the nuclear receptor gene family members *NR4A1*, *NR2F1*, *VDR* and *MEF2B*, and resulted in impaired apoptotic machinery, a mechanism that has been shown to be impaired in MS.

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The inability of the immune system to eliminate self-reactive lymphocytes is translated to ‘unwillingness’ of autoreactive T cells to undergo apoptosis. In relapsing–remitting MS patients, multiple cellular defects linked with resistance to cell death were reported, including impaired stabilization of p53, aberrant CD95/Fas expression on CD4⁺ and CD8⁺ T cells and dysregulated expression of BCL-2 family proteins known to be involved in the promotion of cellular resistance to apoptosis

and the prolonging of T-cell survival [10–12]. Comparisons between the operating gene module networks of MS-to-be subjects and those of MS patients during the first clinical attack demonstrated the evolution of altered regulation of nuclear receptor-dependent apoptosis. Our findings define a silent MS trait that is associated with suppressed expression of the nuclear receptor network and inhibited apoptosis of activated T cells. When TCR activation occurs after an environmental event such as infection, it is not followed by the expected apoptotic process. In subjects with the MS trait, failure to appropriately activate the apoptotic machinery results in the development of MS.

In the future, the identification of MS susceptibility genes has the potential to impact the evaluation of patients with increased risk for developing the disease. The lag between alterations in gene expression and the appearance of the first neurological demyelinating event could provide a screening tool for early diagnosis many years before clinical neurologic presentation. Moreover, the recognition of pathways that mediate early disease-triggering processes may affect future therapeutic interventions. Further research could provide a better understanding of early MS pathogenesis, and eventually, elucidating the signaling pathways during the latent onset of MS may enable the utilization of therapeutic strategies to limit the development of the clinical disease. Future potential drugs may block these specific signaling pathways, particularly those that mediate TCR activation and those that promote coordinated assembly of the nuclear receptor gene family network. This paradigm shift could eventually lead to controlled eradication of the disease.

Future perspective

The high-throughput molecular technologies and the related gene-expression signatures identified in MS research could help to better define the differences between MS and non-MS patients, both with and without white

matter involvement. I speculate that 10 years from now, studies that will include larger series of MS samples selected using homogeneous criteria will allow the simultaneous analysis of several thousands of molecules at the proteomic level with comparative genomics at the RNA level and multiplex quantitative reverse transcriptase-PCR. I expect that the objective of identifying and defining an aberrant molecular portrait of patients with MS will be reached, and that the personal portrait, when compared with the portraits of other MS patients, will allow a better understanding of the operating molecular events that are associated with clinical MS activity and progression to disability. These personalized gene expression profiles will enable a different treatment strategy to be developed. This strategy will be based on the characterization of prognostic gene signatures and the identification of predictive markers. We will have a deeper comprehension of the molecular mechanisms regulated by these molecules and intriguing and promising perspectives into their possible use in therapy. Focusing on the biological effects of these mechanisms and the target genes that they regulate, we will be able to modulate their expression and design very potent drugs that will maximize clinical efficacy. These target-designed drugs will serve as powerful tools to directly suppress the abnormal processes that lead to demyelination and axonal loss in a time-dependent manner, and will eventually lead to a change in the course of MS to a benign disease.

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