Research Highlights

Highlights from the latest articles in multiple sclerosis

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The future of genetic studies in multiple sclerosis

Evaluation of: Hoppenbrouwers IA, Aulchenko YS, Ebers GC *et al.*: *EVI5* is a risk gene for multiple sclerosis. *Genes Immun.* 9, 334–337 (2008).
– Manuel Comabella

During the last two decades, many investigative groups have dedicated important efforts to identifying the individual genes that confer susceptibility to multiple sclerosis (MS). The main conclusion that has evolved from this work is that the HLA-class II region on chromosome 6p21, specifically the *HLA-DRB1*15* haplotype, contributes by far the most to genetic susceptibility in MS, and results from many MS genetic studies support this association [1]. Unfortunately, despite the evidence that MS is a complex genetic trait with multiple genes contributing to disease susceptibility, genetic studies aiming to identify additional risk genes for MS have been rather disappointing, as many of the candidate genes identified in one study were not confirmed in others. It has not been until recently that additional genes located outside the HLA region have been proposed, although with weaker effects, as solid candidates for MS genetic risk [2]. In particular, three SNPs, two located within the IL-2 receptor-α and one located within the IL-7 receptor-α, were strongly associated with MS. Other SNPs positioned in attractive genes were also found to be associated with the disease. These latest findings have certainly opened new scenarios in MS genetic research.

First, many investigative teams devote substantial efforts to replicating associations of the proposed SNPs with the disease in their local cohorts of cases and controls. The study conducted by Hoppenbrouwers and colleagues is one example [3]. The authors aimed to confirm association of these SNPs in a small cohort of MS patients and controls from a Dutch genetically isolated population. In addition to the HLA locus, two intronic SNPs located in the ecotropic viral integration site 5 (*EVI5*) gene were found to be associated with MS. Interestingly, this association was further validated in a large Canadian MS cohort. *EVI5* is a common site of retroviral integration that might be involved in T-cell disease [4].

Second and last, important work will be needed to identify the causal genetic variants for MS. However, our knowledge on this part is still far from complete.

References

1. Lincoln MR, Montpetit A, Cader MZ *et al.*: A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. *Nat. Genet.* 37(10), 1108–1112 (2005).
2. International Multiple Sclerosis Genetics Consortium; Hafler DA, Compston A, Sawcer S *et al.*: Risk alleles for multiple sclerosis identified by a genomewide study. *N. Engl. J. Med.* 357(9), 851–862 (2007).
3. Hoppenbrouwers IA, Aulchenko YS, Ebers GC *et al.*: *EVI5* is a risk gene for multiple sclerosis. *Genes Immun.* 9, 334–337 (2008).
4. Liao X, Buchberg AM, Jenkins NA, Copeland NG: *Evi-5*, a common site of retroviral integration in AKXD T-cell lymphomas, maps near *Gfi-1* on mouse chromosome 5. *J. Virol.* 69(11), 7132–7137 (1995).

future medicine part of fsg

Is Nogo-A a reliable biomarker for MS?

Evaluation of: Lindsey JW, Crawford MP, Hatfield LM: Soluble Nogo-A in CFS is not a useful biomarker for multiple sclerosis. *Neurology* 71, 35–75 (2008).

– Carmen Espejo

In neurodegenerative diseases, such as MS, failure of damaged axons to regenerate could be the main factor underlying permanent neurological dysfunction. During recent years, Nogo proteins have been identified as potent inhibitors of axonal regeneration [1–3]. More recently, Jurewicz *et al.* have described a small-molecular-weight Nogo-A product as a biomarker for MS [4]. In this study, the authors analyzed, by western blot, cerebrospinal fluid (CSF) samples from 114 patients with MS, 11 clinically isolated syndromes, 115 patients with other neurological diseases and 18 patients with meningo–encephalomyelitis. A soluble 20-kDa Nogo-A product was detected in 96% (110 out of 114) of CSF samples from MS patients, but in none of the control samples. Furthermore, Nogo-A product was also present in all the CSF samples from clinically isolated syndrome patients. However, Nogo-A product was not detected in any of the neuromyelitis optica cases, suggesting that Nogo-A may be a specific biomarker for MS.

Lindsey *et al.* attempted to replicate the aforementioned results, but obtained some controversial data [5]. First, it is important to stress the fact that both groups developed a similar experimental procedure for the western blot and used the same antibody for Nogo-A detection. Even so, Lindsey *et al.* were unable to detect the 20-kDa Nogo-A product described in the previous report. Instead, they found a 25-kDa band in most of the CSF samples from 17 patients with relapsing–remitting MS, 11 patients with primary progressive MS and 12 controls analyzed. When the density of the 25-kDa band was measured with a densitometer, the authors found that the relative density of the band was higher in MS patients than in controls, especially in primary progressive MS. It should be noted that this band was present with the secondary antibody alone, in the absence of the anti-Nogo-A antibody. After some experiments, Lindsey *et al.* suggest that the 25-kDa band may actually correspond to an immunoglobulin light chain band. These authors argue that the immunoglobulin light-chains on the western blot bind both the anti-Nogo and the secondary antibodies, and that the differences between MS patients and controls in the relative density of the band are due to the well-known higher concentration of immunoglobulins observed in MS.

Why have Jurewicz *et al.* not found the band in the absence of anti-Nogo-A antibody or in other neurological diseases?

This report illustrates the necessity of consensus in this kind of work and the variability of the results depending on where they are obtained.

References

1. Chen MS, Huber AB, van der Haar ME *et al.*: Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* 403, 434–439 (2000).
2. Prinjha R, Moore SE, Vinson M *et al.*: Inhibitor of neurite outgrowth in humans. *Nature* 403, 383–384 (2000).
3. Grandpre T, Nakamura F, Vartanian T, Strittmatter SM: Identification of the Nogo inhibitor of axon regeneration as a reticulon protein. *Nature* 403, 439–444 (2000).
4. Jurewicz A, Matysiak M, Raine CS, Selmaj K: Soluble Nogo-A, an inhibitor of axonal regeneration, as a biomarker for multiple sclerosis. *Neurology* 68, 283–287 (2007).
5. Lindsey JW, Crawford MP, Hatfield LM: Soluble Nogo-A in CFS is not a useful biomarker for multiple sclerosis. *Neurology* 71, 35–75 (2008).

A role for cyclophosphamide in the treatment of MS?

Evaluation of: Krishnan C, Kaplin AI, Brodsky RA: Reduction of disease activity and disability with high-dose cyclophosphamide in patients with aggressive multiple sclerosis. *Arch. Neurol.* 65(8), 1044–1051 (2008).

– Jaume Sastre-Garriga

Present MS therapies are only partially effective. The need for rescue second-line MS therapies has prompted research in the field of more potent immunosuppressive drugs. Cyclophosphamide is a non-specific immunosuppressant, affecting both T- and B-lymphocytes, which has long been used to treat patients with MS. A recent Cochrane review concluded that the overall effect of cyclophosphamide in the treatment of progressive MS does not support its use in clinical practice [1]. Based on the excellent MRI response in aggressive MS patients to mixed regimens of cyclophosphamide as preparation for hematopoietic stem cell transplantation, the authors of the present study investigated the clinical and radiological efficacy of high doses of cyclophosphamide alone in nine patients with severe MS [2], which was defined as "one clinical exacerbation between 6 and 12 months prior to treatment or a sustained increase in more than one Expanded Disability Status Scale point in the past year and two or more gadolinium-enhancing lesions on each of two pretreatment MRI scans". The authors were able to demonstrate significant reductions in disease activity and disability in most patients, coupled with acceptable safety parameters.

In the era of targeted immunotherapy and pharmacogenomics, this study represents a valuable effort to assess the usefulness of a rather unspecific immunosuppressant. Alhough the suboptimal design and small sample size of

the present work makes it impossible to draw definitive conclusions on the safety and efficacy of high-dose cyclophosphamide in patients with aggressive MS, the study reported here adds to the body of evidence in favor of a role of this drug in the MS armamentarium. There is a clear need for larger, optimally designed clinical trials that help us to define the role of cyclophosphamide in the treatment of patients with MS.

References

1. La Mantia L, Milanese C, Mascoli N, D'Amico R, Weinstock-GuttmanB: Cyclophosphamide for multiple sclerosis. *Cochrane Database Syst. Rev.* (1), CD002819 (2007).
2. Krishnan C, Kaplin AI, Brodsky RA *et al.*: Reduction of disease activity and disability with high-dose cyclophosphamide in patients with aggressive multiple sclerosis. *Arch. Neurol.* 65(8), 1044–1051 (2008).

Future treatments for multiple sclerosis

Evaluation of: Hauser SL, Waubant E, Arnold DL et al.: B-cell depletion with rituximab in relapsing–remitting multiple sclerosis. *N. Engl. J. Med.* 358(7), 676–688 (2008).
– Jordi Rio

Owing to the partial efficacy of the present MS therapies, it is necessary to find alternative treatments for nonresponder patients. Rituximab is a genetically engineered chimeric monoclonal antibody that depletes $CD20^+$ B cells through a combination of cell-mediated and complement-mediated dependent cytotoxic effects and the promotion of apoptosis. Based on the role of autoimmune B cells in the pathogenic mechanism of MS, the authors of the present study investigated the radiological and clinical efficacy of a single course of rituximab in 104 patients with active relapsing–remitting MS in a Phase II double-blind, placebo-controlled clinical trial during 48 weeks [1]. The authors demonstrated MRI and clinical evidence that selective $CD20^+$ B-cell depletion is a potentially effective approach in the treatment of relapsing–remitting MS. As compared with placebo, rituximab significantly reduced the number of gadolinium-enhancing lesions and the number of relapses.

When the present therapeutic approach of MS is mainly focused on T-cell mechanisms of action, the emergence of new therapies searching for other mechanisms is of importance to open the spectrum of therapies in a disease without a definite cure. Although the fundamental mechanism of rituximab on disease activity in MS is not known, the findings of this paper suggest that we need to investigate the role of B cells in the disease process. Despite the positive results of this trial, there are questions that need to be answered, such as those regarding dose and duration and concerns about safety. Recently, deaths in patients receiving rituximab have been documented [2]. With current and future treatments that suppress and manipulate the immune system, there is risk for severe acute infections and reactivation of latent infections. Therefore, it is critical to proceed cautiously when immune system modification strategies are being evaluated, for fear of unleashing undesirable or even fatal diseases. Fortunately, this complication remains a rare event.

References

1. Hauser SL, Waubant E, Arnold DL *et al.*: B-cell depletion with rituximab in relapsing–remitting multiple sclerosis. *N. Engl. J. Med.* 358(7), 676–688 (2008).
2. Berger JR: Progressive multifocal leukoencephalopathy. *Curr. Neurol. Neurosci. Rep.* 7(6), 461–469 (2007).

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