nosis, cardiac valves express angiogenic factors that cause neovascularization. This led Yoshioka *et al.* to study the role of antiangiogenic factors in valvular heart disease.

The authors found that deficiency of chondromodulin-1 had no obvious effect in younger mice (8 weeks), but resulted in increased angiogenesis in the cardiac valves of aged mice (90 weeks). Such age-dependent increase in angiogenesis was associated with valve thickening, calcification and turbulent blood flow. This pathology is similar to the clinical findings in the 2–5% of all elderly humans who suffer from aortic stenosis[10]. They carry an 80 percent 5-year risk of progression to heart failure, valve replacement or death[10]. The disease is debilitating, and there currently is no effective therapy other than surgical aortic valve replacement[10].

The authors went on to show that in a mouse model of atherosclerosis (*Apoe*$^{-/-}$mice), chondromodulin-1 was absent in calcified regions of the cardiac valves and its absence was associated with increased density of microvessels[3]. Upregulation of vascular endothelial growth factor (VEGF)-A in the regions undergoing angiogenesis implies that VEGF might have a role in loss of avascularity and acquisition of a vascularized phenotype in aged cardiac valves[3]. *In vitro* angiogenesis assays further confirmed the direct antiangiogenic effect of chondromodulin-1 in this setting[3].

Cardiac valve disease has thus for the first time been linked to a lack of an endogenous angiogenesis inhibitor. But valve disease certainly involves more players.

Yoshioka *et al.* found that inhibition of chondromodulin-1 in conditioned medium from valvular interstitial cells only partially suppressed the net antiangiogenic activity of this medium[3]. This finding is in line with those from other experimental systems indicating that more than one endogenous angiogenesis inhibitor may be needed for the maximum antiangiogenic effect. For example, in the eye, chondromodulin-1 and PEDF (pigment epithelium–derived factor) are present, and so are the precursors for other antiangiogenic factors such as endostatin and tumstatin[4,11].

What's more, in contrast to results presented here, other research groups have found that endostatin and SPARC (two other endogenous inhibitors of angiogenesis) are upregulated in stenotic aortic valves but not in normal ones[12,13]. We are just beginning to unravel the potentially diverse contributions of various antiangiogenic molecules in the biology of cardiac valves.

Initial research into cardiac valve dysfunction was focused on evaluating the stimulators of angiogenesis such as VEGF, fibroblast growth factor and platelet-derived growth factor. However, it is also crucial to understand the flip side of the angiogenic balance[4].

The notion that endogenous inhibitors of angiogenesis have diverse physiological roles in different locations of the body gains further support with the new study[3]. Nonetheless, further genetic and biochemical studies are needed to get a better picture of how these inhibitors function at a mechanistic level to impart specific antiangiogenetic properties.


1. Folkman, J. *Nat. Med.* **1**, 27–31 (1995).
2. Gariano, R.F. & Gardner, T.W. *Nature* **438**, 960–966 (2005).
3. Yoshioka, M. *et al. Nat. Med.* **12**, 1151–1159 (2006).
4. Nyberg, P., Xie, L. & Kalluri, R. *Cancer Res.* **65**, 3967–3979 (2005).
5. Sugimoto, H. *et al. J. Biol. Chem.* **278**, 12605–12608 (2003).
6. Fowble, V.A., Vigorita, V.J., Bryk, E. & Sands, A.K. *Clin. Orthop. Relat. Res.* **450**, 225–230 (2006).
7. Moses, M.A. *et al. Proc. Natl. Acad. Sci. USA* **96**, 2645–2650 (1999).
8. Kusafuka, K. *et al. Am. J. Pathol.* **158**, 1465–1472 (2001).
9. Hayami, T. *et al. FEBS Lett.* **458**, 436–440 (1999).
10. O'Brien, K.D. *Arterioscler. Thromb. Vasc. Biol.* **26**, 1721–1728 (2006).
11. Dawson, D.W. *et al. Science* **285**, 245–248 (1999).
12. Chalajour, F. *et al. Exp. Cell Res.* **298**, 455–464 (2004).
13. Charest, A. *et al. Heart*, published online 18 May 2006 (doi: 10.1136/hrt.2005.086595).




# Multiple sclerosis: putting two and two together

John Trowsdale

**A variant gene for antigen presentation is associated with tissue damage in multiple sclerosis. The damaging effects are now shown to be dampened by an allele of a second, neighboring gene.**

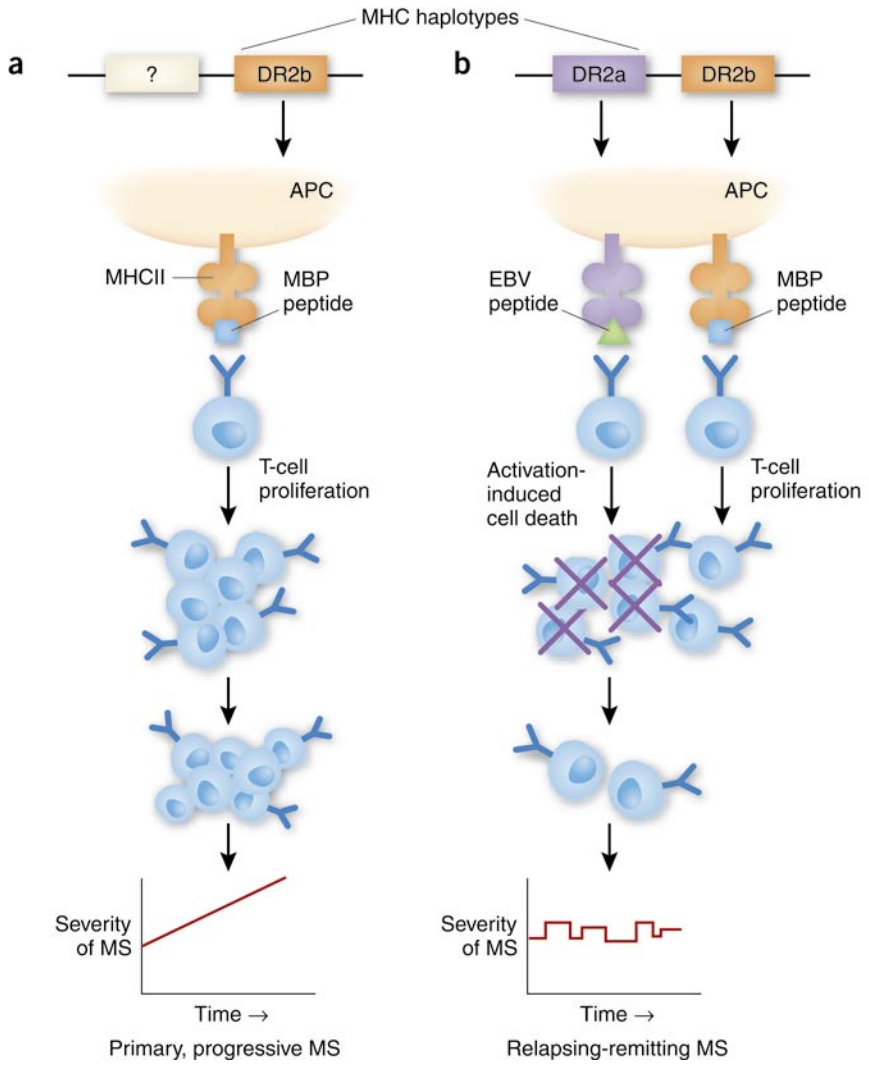
Autoimmune diseases affect about 5 percent of people. Apart from occasional small insights into these conditions, progress into understanding and treating them is frustratingly slow. Genetics provides the main hope, and teams studying the most prevalent types, such as type 1 diabetes and systemic lupus erythematosus, can boast the results of whole-genome screens—identifying dozens of candidate genes, variants of which subtly affect the immune network.

A similar dogged approach to multiple sclerosis has had disappointing results, and the handful of early hits have not been confirmed[1]. There is still only one major gene locus definitively associated with multiple sclerosis, the class II region of the major histocompatibility complex (MHC). It seems sensible, therefore, to glean what information we can from this association. The problem is that the class II region is notoriously difficult to analyze. Undaunted, groups from Denmark and Oxford have suggested a way forward, by placing human class II genes, individually and together, into mice[2].

Multiple sclerosis is a chronic demyelinating disease, inflammatory in character, of the central nervous system. A mouse model, experimental autoimmune encephalomyelitis (EAE), appears to share many of the characteristics of the human disease. EAE may be induced by immunization with myelin proteins, and symptoms can be transferred to naive mice solely by T cells. The severity of the disease is associated with different alleles of the polymorphic MHC class II genes of both species. In humans the main association is with the DR2 haplotype[3]. MHC haplotypes encompass a bewildering array of genes affecting immune responses, but the main candidate region for multiple sclerosis susceptibility comprises alleles of two neighboring genes, *DR2a* and *DR2b*, that are always inherited together.

Gregersen *et al.* asked which gene product, DR2a or DR2b, is responsible for multiple sclerosis. Introducing each of these genes into mice individually could address this question, but first it was necessary to

John Trowsdale is in the Division of Immunology, Department of Pathology, Tennis Court Road, Cambridge CB21QP, UK.
e-mail: jt233@cam.ac.uk




Kim Caesar

**Figure 1** Proposed model for epistatic interaction between DR2a and DR2b in multiple sclerosis (modified from ref. 1). (**a**) Individuals expressing DR2b in the absence of DR2a on the surface of antigen-presenting cells (APCs) bind myelin antigens such as MBP and activate immune responses. This results in proliferation of autoreactive T cells, leading to severe disease. DR2a and DR2b are generally inherited together on a haplotype. Indeed, individuals carrying only DR2b remain to be identified and studied for disease status. A direct prediction of the model is that they would be susceptible to a form of primary progressive multiple sclerosis. (**b**) When DR2a is inherited with DR2b, the 2a molecules bind a 'self' peptide, such as that derived from an EBV antigen. These molecules lead to partial deletion of the autoreactive T cells by activation-induced cell death. The decreased frequency of these cells limits their destructive capability, resulting in delayed onset and relapsing-remitting characteristics.

engineer the animals to express appropriate human T cells. Mice that do not express endogenous T-cell antigen receptors (TCRs) were made to express TCR genes, cloned from humans with multiple sclerosis, that recognize the multiple sclerosis–relevant autoantigen myelin basic protein (MBP) presented by DR2 molecules.

The mice expressing this human Hy TCR as well as the MHC class II molecule DR2b spontaneously developed severe EAE. When the DR2a gene was introduced along with DR2b, the disease severity was reduced and its onset was delayed, with disease features suggestive of the 'relapsing and remitting' human form of multiple sclerosis. The interpretation was that DR2a moderated the severe clinical phenotype imparted by DR2b.

The effect was traced to lower frequencies of Hy T cells circulating in the *DR2a DR2b* animals compared to the singly transgenic *DR2b* mice. In fact, other experiments in the current study showed that the DR2a molecules induced deletion of the Hy T cells, in a tolerizing process that is believed to take place in the circulation (as opposed to the thymus, where central T cell tolerance develops). The effect may be explained by the fact that the Hy TCR, in addition to recognizing MBP, cross-reacts with a self antigen presented by DR2a. The experiments demonstrated an epistatic effect of DR2a, which partially deletes autoreactive T cells, over DR2b—thereby creating a less severe clinical phenotype.

What is the reason for the persistence, over millions of years of evolution, of a molecule such as DR2b that elicits highly autoaggressive responses? Perhaps this molecule provides a vital function in controlling an infectious pathogen. The presence of DR2a alongside may keep self-harm to a minimum, like keeping an aggressive dog on a collar and chain. Occasional savagery may be an unfortunate consequence of evolutionary pressure for life-saving immune responses to infection. The prevalence of the DR2 haplotype, which about a quarter of us carry—rising to 65% in those with multiple sclerosis—shows that this genetic compromise is advantageous, although clearly not for sufferers of multiple sclerosis. But for some the situation could be worse: although they remain to be identified, individuals carrying 2b without 2a may fare more poorly.

It is worth pointing out that the 'self' antigen that seems to tolerize the Hy T cells in multiple sclerosis patients could be derived from Epstein-Barr virus (EBV), as a peptide from this virus has been shown to stimulate the Hy clone when presented by the DR2a molecule. The infection history of an individual is suspected to play a role in tolerizing to some antigens, although viruses have also been proposed as triggers of autoimmunity.

As well as providing a functional model, which is now available for further testing, the data speak to the complexity of MHC genetics. Many combinations of alleles at MHC genes rarely become separated over long periods of evolution, in some cases tens of millions of years—in other words they are in linkage disequilibrium. Many common extant DR haplotypes, particularly DR2, are ancient[4]. One way of interpreting the success of these haplotypes is to suppose that these highly polymorphic genes, some of which comprise over 700 alleles, are honed to work together as a team, a set of alleles, on a particular haplotype[5]. Very rarely, adjacent polymorphic alleles are separated by recombination—perhaps the genetic equivalent of using a poodle's flimsy collar and chain to restrain a rottweiler.

The MHC contains many polymorphic

genes, which affect antigen processing and presentation. It is therefore appropriate that such genes are usually inherited as a coordinated set, given that both class I and class II gene products, many of which are encoded in the MHC, play on the same pool of T cell receptors.

Incidentally, many other regions of the genome also appear to be in linkage disequilibrium, indicating that subtle epistatic effects at linked loci may be widespread. The order of genes in the human genome appears to us fairly random, but this may be illusory. New gene pairings are forged and subjected to selection as species are formed, and pairing of genes in different species may be advantageous rather than simply fortuitous.

We may be beginning to understand how DR2a keeps the DR2b rottweiler in check and how obedience training of T cells by exposure to antigen may be beneficial. But how, in treating multiple sclerosis, do we throw the aggressive DR2b dog a juicy bone? Strategies designed to target and eliminate specific T cells might provide a lead.

1. Sospedra, M. & Martin, R. *Annu. Rev. Immunol.* **23**, 683–747 (2005).
2. Gregersen, J.W. *et al. Nature* doi: 10.1038/nature 05133
3. Lincoln, M.R. *et al. Nat. Genet.* **37**, 1108–1112 (2005).
4. Raymond, C.K. *et al. Genome Res.* **15**, 1250–1257(2005).
5. Trowsdale, J. *Genome Biol.* **3**, 3 (2002).

# Alzheimer disease: presenilin springs a leak

Sam Gandy, Mark K Doeven & Bert Poolman

**Presenilins are thought to contribute to Alzheimer disease through a protein cleavage reaction that produces neurotoxic amyloid-β peptides. A new function for presenilins now comes to light—controlling the leakage of calcium out of the endoplasmic reticulum. Is this a serious challenge to the 'amyloid hypothesis' of Alzheimer disease?**

Several proteins are believed to be essential for the pathology of Alzheimer disease. Two of these proteins—presenilins—are involved in a cleavage reaction that generates key protein fragments, known collectively as amyloid-β peptides. According to this "amyloid hypothesis"[1], the clumping in the brain of one especially sticky form of amyloid-β peptide, amyloid-β42, is thought to define the final common pathway toward all forms of Alzheimer disease.

These molecular events have been recognized for years, but now a twist emerges. In a recent issue of *Cell*, Tu *et al.*[2] challenge the prevailing view of presenilin biology. They provide evidence that presenilins, in addition to their cleavage function, also form ion channels responsible for a normal trickle of calcium—known as the 'calcium leak current'—out of a storage depot in the endoplasmic reticulum and into the cytoplasm.

It's not clear whether alterations in calcium handling contribute to the pathology of the disease, although the new findings are in sync with previous studies hinting that this could be the case[3,4]. Also unclear is the relationship of altered calcium handling to the biology or pathology of amyloid-β. But the new findings should provoke a flurry of follow-on experiments evaluating whether or not the calcium leak data fit into the amyloid hypothesis.

Presenilin-1 and presenilin-2 are highly homologous, polytopic membrane proteins that were discovered about ten years ago through standard positional cloning strategies aimed at identifying the genetic bases of the most common forms of early-onset familial Alzheimer disease[1,5]. Each presenilin was later found to form a protein-conducting pore, lined by the active site of the proteolytic activity that generates the C termini of amyloid-β peptides, an activity informally known as 'γ-secretase'.

Cleverly, order is maintained between the secretase function of presenilin and its apparent role in calcium handling: it is the endoplasmic reticulum–localized, unprocessed zymogen form of presenilin that acts as a calcium channel. The protease function of presenilin is only revealed in later compartments (such as the *trans*-Golgi network, endosome and plasma membrane) after the assembly of N- and C-terminal presenilin fragments together with three essential partners (nicastrin, Aph1 and Pen2) necessary to form the minimal functional γ-secretase (**Fig. 1**) (refs. 1,5).

Dual-function pores conducting both proteins and ions are not unprecedented, as such activities were characterized in the early 1990s by Simon and Blobel[6]. This is the first time, however, that presenilins have been formally proposed to play roles in both protein processing and ion conductance. It is also true that membrane proteins have in the past been artifactually identified as ion channels when, in fact, their main physiological function is the transport of some other substance (for example, the misidentification of P-glycoprotein as a chloride channel[7]).

Tu *et al.*[2] suggest that pathogenic missense mutations in the presenilin-1 gene cause loss of the normal endoplasmic reticulum calcium leak current, so that endoplasmic reticulum calcium levels are elevated in the resting state whereas cytosolic levels are normal or even low. Then, when cells are exposed to some stimulus that causes endoplasmic reticulum calcium channels to open, those excess endoplasmic reticulum calcium stores are disgorged into the cytosol. Excessive cytosolic calcium is a well-known mediator of neuronal death, as has long been appreciated in studies of excitotoxicity.

The authors also examine a mutant presenilin informally known as PS1Δ9 because it lacks the ninth exon. They report that this channel leaks calcium out of the endoplasmic reticulum to an excessive extent under basal conditions. PS1Δ9 is unusual in some other respects as well: it is active as a protease without being cleaved into N- and C-terminal fragments (NTF and CTF), and patients with this mutant protein have especially large deposits of amyloid-β (ref.

Sam Gandy is at the Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA. Mark K. Doeven is at the Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, USA, and the Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747 AG Groningen, The Netherlands. Bert Poolman is at the Department of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9747 AG Groningen, The Netherlands.
e-mail: samgandy@earthlink.net