The *SH2D2A* Gene – Contributions to Our Future Understanding of Multiple Sclerosis

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Abstract

The *SH2D2A* gene encodes the T-cell-specific adapter protein (TSAd), which is involved in multiple signalling pathways, including those of the T-cell receptor, the vascular growth factor receptor 2 and the chemokine receptor CXCR4. *SH2D2A* is preferentially expressed in activated T cells, natural killer (NK) cells and endothelial cells. A major function of TSAd seems to be to modulate Src kinase activity within the cell. The *SH2D2A* gene as well as its gene region have been implicated in genetic susceptibility to autoimmune disease in both humans and mice. In this article, the current status of knowledge of the *SH2D2A* gene and its implications for our future understanding of multiple sclerosis are discussed.

Keywords

SH2D2A, genetic susceptibility genes, multiple sclerosis, autoimmune disease, adapter protein

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The *SH2D2A* gene encodes a cytoplasmic adapter protein, T-cell-specific adapter protein (TSAd), which is expressed in activated T cells as well as in endothelial cells. Several lines of evidence imply a contribution of this gene to susceptibility to the development of multiple sclerosis (MS). In this article we will review current evidence, and point to some important aspects of the function of this gene for our future understanding of MS.

Most Susceptibility Genes in Multiple Sclerosis Contribute with Small Effects

Much effort is currently being put into the identification of susceptibility genes in autoimmune diseases such as MS. These diseases are 'complex' in the sense that many genes as well as environmental factors contribute to the development of disease. Until recently, only genes in the human leukocyte antigen (HLA) class II region had clearly been shown to contribute to the genetic susceptibility of MS,[1] with an odds ratio (OR) of about 2.0.[2] The failure to identify other gene regions harbouring MS-predisposing genes by genome-wide linkage studies in families from various MS populations[3] suggests that the other genes predisposing to MS contribute with only small or moderate effects. Still, suggestive linkage to particular gene regions in MS families has allowed identification of new MS-susceptibility gene candidates. One such gene is the *MHC2TA* gene, which encodes a transcription factor that regulates levels of major histocompatibility complex (MHC) class II expression and that has been found to be associated with MS in humans as well as experimental autoimmune encephalomyelitis (EAE) in the rat.[4] Another example is the protein kinase C alpha gene (*PRKCA*).[5,6] The associated polymorphism in this gene correlates with altered expression levels of PKRCA in monocytes.[6] In line with the notion that MS-susceptibility genes contribute with small effects, high-density genome-wide association studies (GWAS) of large patient materials allow identification of novel susceptibility genes contributing small increases to the risk of developing MS.[7] Two of these are *IL7R* and *IL2RA*, which have now been replicated in several independent studies.[8–10] Although the effect of single genes is only to confer small increases in genetic susceptibility, a combination of two genes may act synergistically and amplify the genetic effect considerably.[11]

Activated Auto-aggressive or Regulatory T Cells Are Major Players in Autoimmune Disease

T cells recognise through their T-cell receptors (TCRs) immunogenic peptides presented by HLA molecules on the surface of antigen presenting cells (APCs) (see *Figure 1*). When the TCR binds the peptide–HLA complex with sufficient affinity, the T cell receives an activation signal through its TCR, which may initiate a T-cell-mediated immune response. Once activated in the lymph node, the T cells migrate into the bloodstream, and may access the central nervous system (CNS) by crossing the blood–brain barrier. Within the CNS, auto-aggressive T cells may become reactivated to proliferate and produce disease-promoting cytokines (see *Figure 1*).[12] Recently, it has been recognised that antigen-specific T-regulatory (T_{reg}) cells confer resistance to organ-specific autoimmunity, and the role of these cells in limiting autoimmune tissue damage has been documented in many disease models, including MS.[13] Activation of T cells is thus a double-edged sword that needs to be tightly regulated.

Genes Controlling T-cell Activation Are Candidate Genes in Autoimmune Disease

It is very likely that HLA molecules confer susceptibility to MS and other autoimmune diseases through presentation of particular disease-promoting peptides to T cells. The sequence of events depicted in *Figure 1* implies that genes involved in controlling T-cell activation, as well as cellular migration and blood–brain barrier function, may all contribute to

the development of MS. This is exemplified by mice that have altered susceptibility to spontaneous or induced experimental autoimmune diseases due to dysregulation of the T-cell activation process,[14,15] chemokine-regulated cellular migration,[16,17] or altered blood–brain barrier function.[18] In the search for novel candidate genes in MS that are expressed in activated T-cells, we previously identified and cloned the TSAd encoded by the *SH2D2A* gene.[19]

The *SH2D2A* Gene Is Located in a Chromosomal Region Regulating Experimental Demyelinating Disease

The *SH2D2A* gene in the mouse is located within *Eae3*, a chromosomal region that has been implicated in genetic susceptibility to EAE in the mouse.[20,21] *Eae3* harbours multiple genes of immunological relevance, including *SH2D2A*. However, as yet the susceptibility gene within *Eae3* has not yet been identified.

Polymorphisms in the *SH2D2A* Gene

There are several potentially functionally relevant polymorphisms within the *SH2D2A* gene. In the *SH2D2A* promoter, there is a highly polymorphic dinucleotide (GA) repeat located -345 basepairs (bp) upstream of the first coding ATG.[22] Genotypes homozygous for short alleles of this GA repeat appear to be associated with reduced expression of TSAd in activated primary CD4+ T cells.[23] In the coding sequence of the *SH2D2A* gene there is a non-synonymous single nucleotide polymorphism (SNP) that results in an amino acid change from serine to asparagine (S52N). The frequency of the minor allele (A) of this SNP (rs926103) has been found to range from 0.125 to 0.767 in different populations, with Africans south of the Sahara displaying the highest and Asians the lowest frequencies. The serine in position 52 is a potential phosphorylation site, located N-terminal to the SH2 domain of TSAd. It has previously been shown that phosphorylation of a serine N-terminal to an SH2 domain may influence the specificity of the SH2 domain for its tyrosine phosphorylated ligands.[24] An intriguing possibility is thus that the S52N polymorphism might affect the function of TSAd through modulation of its SH2 domain specificity.

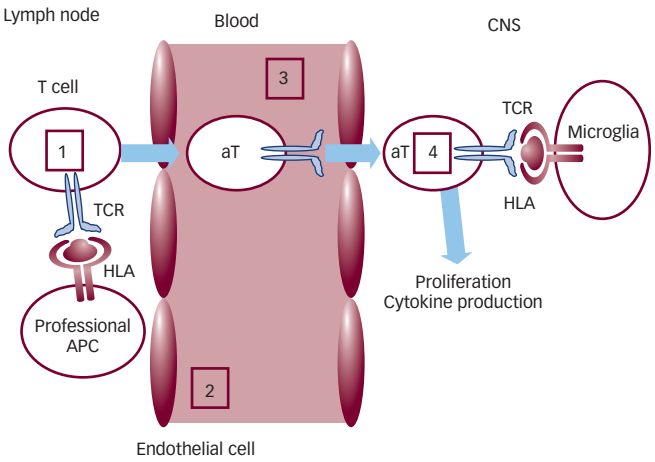
SH2D2A Is Associated with Multiple Sclerosis

Initially, the GA repeat polymorphism in the human *SH2D2A* gene promoter was found to be associated with MS[23] and juvenile rheumatoid arthritis.[25] A further assessment of this association in MS patients from Scandinavian populations revealed that a haplotype of the GA16 repeat allele in the *SH2D2A* promoter and the N52 allele of SNP rs926103 was associated with MS among Norwegians and possibly also among Danes.[26] A meta-analysis of all published gene expression studies in MS patients and controls compared with available linkage data revealed that *SH2D2A* gene expression in peripheral blood lymphocytes has been found to be reduced in MS patients.[27] Thus, alteration in *SH2D2A* gene expression or function may be involved in MS.

Mice Lacking the *SH2D2A* Gene Show Aberration in T-cell and Endothelial Cell Function

Mice with a disrupted *SH2D2A* gene (TSAd knock-out [KO]) appear normal, but T cells from these mice migrate poorly in response to CXCL12/SDF-1α (Berge, submitted) and activated T cells produce less of the T-helper type 1 (Th1) cytokines interleukin 2 (IL-2) and interferon gamma (IFN-γ) than T cells from normal mice.[28] With age, TSAd KO mice spontaneously develop a lupus-like autoimmune disease characterised by the presence of antinuclear antibodies and an apparent failure of activated T cells to undergo apoptosis.[29] Accumulating evidence implicates a failure of myelin-reactive immune cells to undergo apoptosis in the pathological events contributing to MS.[30] Thus, members of the inhibitor of apoptosis (IAP) family of anti-apoptotic genes have been found to be elevated in peripheral blood monocytes and T-cells of patients with aggressive forms of MS.[31] TSAd was recently found to regulate neoangiogenesis in experimental tumours. In mice lacking TSAd, tumours grew more slowly and contained fewer vessels than tumours grown in wild-type mice.[32] Interestingly, neovascularisation may represent a pathological mechanism contributing to sustained autoimmune disease.[33,34] Vascular endothelial growth factor has also been implicated in various autoimmune diseases including MS (reviewed in reference 35). Although mice lacking TSAd display several abnormal features that are also observed in MS patients, it remains to be clarified whether mice lacking *SH2D2A* gene expression have altered susceptibility to EAE.

Figure 1: The Sequence of Events from Activation of T cells to Autoimmune Tissue Damage

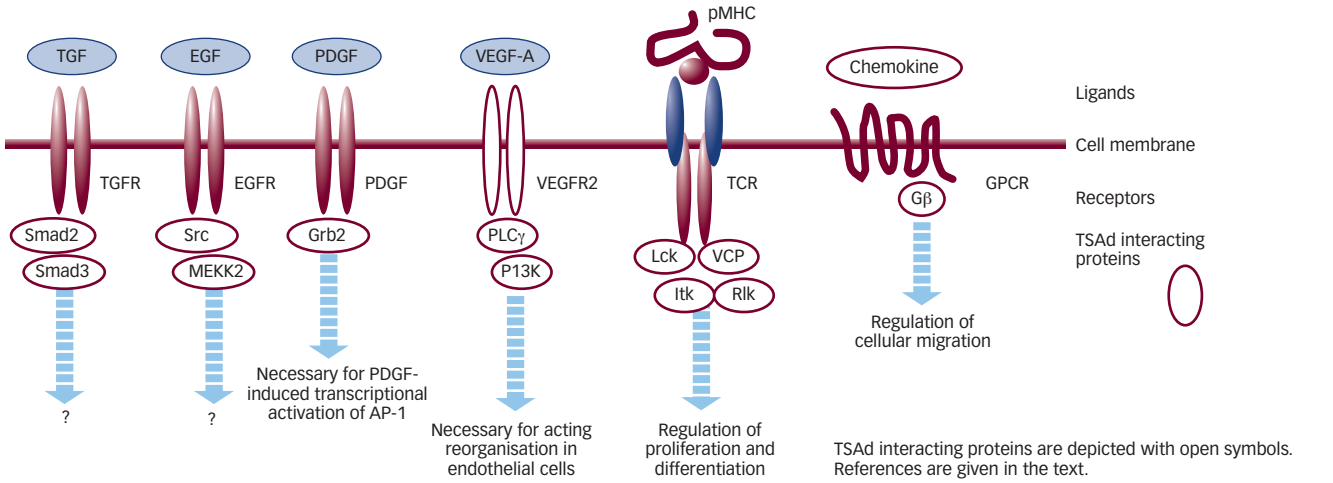


A potentially auto-aggressive T cell is activated by presentation of antigen on a professional antigen presenting cell (APC) in the lymph node (1). The activated T cell migrates into the bloodstream (2) and may cross the blood–brain barrier into the brain (3). In the target tissue the T cell may eventually re-encounter the initial or a very similar peptide–human leukocyte antigen (HLA) complex presented by microglia and thus become re-activated to proliferate and secrete potentially harmful cytokines (4). aT = activated T-cell; CNS = central nervous system; TCR = T-cell receptor.

The *SH2D2A*-encoded Protein TSAd Regulates T-cell Activation and Cellular Migration

Since its discovery in 1998, TSAd has been found to be involved in multiple signalling pathways, e.g. those of the TCR,[28,36–40] vascular endothelial growth factor receptor-2 (VEGFR-2),[32,41] epidermal growth factor receptor (EGFR),[42] platelet-derived growth factor receptor (PDGFR)[43] and transforming growth factor beta receptor (TGFβR)[44] (see *Figure 2*). TSAd participates in these pathways by interacting with a number of different signalling molecules, including members of the Src and Tec kinase family members, i.e. Lck, Src, Itk and Rlk,[36,37,45–47] the serine/threonine kinase MEKK2[42] and the adapter protein Grb2.[43] The role of TSAd as an adapter protein is still not well characterised. TSAd has several binding sites for the Lck SH2 and SH3 domains, and also three tyrosines located within a 25-amino-acid stretch that can be phosphorylated by Lck. This conformation of TSAd may explain how TSAd can modulate Lck activity.[36,45] TSAd binds to Lck and is phosphorylated by Lck through a process whereby the substrate remains bound to the enzyme throughout the phosphorylation cycle.[47] Thus, TSAd has a chaperone-like function for the tyrosine kinase activity of Lck. In this way, TSAd may bring Lck to particular locations within the

Figure 2: Receptors and Pathways in Which TSAd Has Been Implicated as a Participating Protein



cell, without the risk that Lck simultaneously phosphorylates bystander proteins in their vicinity. The SH2 domain of TSAd could provide the necessary localisation information for the TSAd–Lck complex. However, as yet only VEGFR-2 and valocin-containing protein (VCP) are known to bind to the TSAd SH2 domain.[32,39] It is highly likely that additional targets for the TSAd SH2 domain exist within T cells that may point to the more precise role of TSAd in temporarily controlling Lck activity, but these remain to be determined. Recently, TSAd was also found to be involved in signalling from G-protein coupled receptors (GPCR)[48] (Berge, submitted). Park and colleagues cloned the Gβ subunit of the heterotrimeric G protein complex as a novel binding partner for TSAd and showed that TSAd is recruited to Gβ upon chemokine receptor signalling.[48] Through its interaction with Itk, we have found that TSAd promotes Itk activation and thereby regulates chemokine (i.e. CXCL12)-mediated T-cell migration by inducing actin cytoskeleton rearrangements (Berge, submitted). Taken together, attenuated TSAd expression may thus potentially influence T-cell activation, T-cell migration, and endothelial cell function, all of which could contribute to the pathogenesis of autoimmune disease (see *Figure 1*).

Epistatic Interaction Between Susceptibility Genes in Multiple Sclerosis and Possibility of Novel Immune Therapies

The association of *SH2D2A* with MS is an example among many, most of which are probably yet to be determined, where a gene provides a moderate increase in risk of disease susceptibility. The mechanism by which these genes contribute to disease may be manifold, and it may be very difficult to pinpoint the exact mode of disease promotion. As the example of TSAd illustrates, a given protein may influence multiple pathways, all of which may have relevance for the given disease. Such pleiotropic function of a protein involved in disease susceptibility also increases the likelihood that certain combinations of genes and gene products present in a given individual may determine whether a particular gene will contribute to disease risk or not. Consider the hypothetical situation where the T-cell-regulatory function of TSAd promotes development of autoimmune disease, whereas the effect of TSAd on endothelial cell function protects against disease. Under normal conditions the protective effect of TSAd via endothelial cells could be dominant over the disease-promoting effect via T cells. However, in combination with variants of particular genes that regulate T-cell activation but not endothelial cell function, certain variants of TSAd may yield a high risk of disease. It must be stressed that at present it is totally unknown whether epistatic interactions exist between TSAd variants and other MS susceptibility genes. The distribution of SH2D2A variants is the same in HLA-DR2-positive and -negative individuals.[23,26] However, epistasis between genes is relevant for MS, as combinations of HLA-DR2 with particular alleles of less effect within or outside of the HLA complex may yield a considerable increase in disease risk,[49] or decrease the severity of the (experimental) disease.[50]

Conclusion

In conclusion, identification of susceptibility genes will have low power in identifying people at risk of developing MS. However, their identification, the characterisation of their function and their possible interactions with other susceptibility genes will point to pathways that are putative targets for novel immune therapies in MS. ■

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MS-ID Consensus Meeting, 14 May 2009, Brussels

The MS-ID Project is co-funded under the public health programme by the European Commission

EMSP: Improving the quality of life of more than 500.000 European patients with multiple sclerosis

EMSP launched the MS-ID Project in 2007 to enhance equity of treatment, access thereto and quality of services for EU citizens affected by MS.

At the MS-ID Consensus Meeting of 14 May, the results of various tools and initiatives were presented:

• The **European MS Register**, the establishment of a transnational MS data collection system in Europe. A pilot, focusing on the major areas of discrepancy, has been developed and tested in countries with extremely different conditions with regards to their treatment of MS. The results of this pilot show that a European Register is feasible if certain conditions are fulfilled…

• The **Code of Good Practice in MS**, an instrument outlining briefly the issues of fundamental importance to people affected by MS, and providing a practical framework describing the best approaches in relation to treatment, research, employment and empowerment of people affected by MS. The Code of Good Practice in MS is a reference document continuously updated and improved aiming at the best quality of life possible for people with MS…

• The **MS Barometer**, a benchmarking tool with scores allocated to the given answers. The aim is to have the best score awarded as recognition of the effectiveness of policies in place optimising the situation of people with MS. The higher the score, the better the disease management, level of support and quality of life of people with MS in a particular country…

The MS-ID Consensus Meeting was held in the framework of the EMSP Annual Congress, which took place in Brussels from 13 to 15 May 2009.

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