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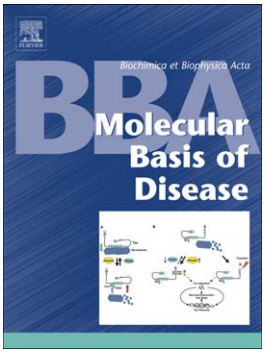

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Genetics of multiple sclerosis

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Introduction

A genetic contribution to MS pathogenesis has been proven ever since the association with the HLA-DR2 locus was discovered more than 30 years ago. The search for additional MS risk genes has been frustrated by a significant lack of reproducibility. This was until recently. In the last two years there have been tremendous breakthroughs in the search for novel risk genes. The main reasons for this progress has been the introduction of novel techniques, improved statistical methods and an awareness that nothing can be solved without extensive international collaboration, including data from high numbers of patients. As anticipated the effects of these novel genes are small. In relation with the old stable giant of the associated HLA locus, the novel dwarfs have woken up, with odds ratios that generally are below 1.4.

A. Current knowledge

The genetic component in MS

Although familial aggregation of MS has long been recognised, systematic age-adjusted recurrence risks for relatives of persons with MS were first published in 1988 (1). Subsequent studies showed that first-, second- and third-degree relatives of patients with MS were more likely to have the disease than the general population. Studies in twins showed a significant excess of concordance in monozygotic as compared to dizygotic twins (2-6). In a study of individuals with MS who were adopted an excess of risk in the genetically related family was found, whereas no excess of risk in the adopting family was found (7). Affected husband and wife couples do not occur more frequently than would be expected by chance. However the risk for their offspring is greater than if just one parent is affected (8). For half-siblings the risk is approximately half the risk for full siblings, regardless whether they were raised together or apart (9, 10). Together these data suggest that living with someone who has MS only increases your risk on MS if you are a relative of that person, in which case your risk increases with relatedness.

Mode of inheritance in MS

Although, as discussed above, data confirm that genetic factors are unequivocally relevant in MS, most MS families contain no more than two or three affected individuals and no clear mode of inheritance can be inferred from segregation analysis. However the nonlinear relationship between familial recurrence risk and

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degree of relatedness, as well as the available genetic data suggest that between 20 and 100 common variants, each increasing risk by only a modest factor of 1.2-1.5, would be sufficient to account for the prevalence and heritability of MS. In addition, it may be possible that rare variants, with allele frequencies below 5%, play a role, especially in multiplex MS families (11).

Maternal versus paternal transmission

Several genetic epidemiological studies in MS families have indicated a skewed maternal transmission of MS (10). (12). However, another study on patients with affected parents, the quite opposite finding of paternal transmission has been presented.(13) (14, 15). The likely explanation for such discrepancies is that more than one mechanism is at stake. Opposing transmission models (maternal versus paternal) in individual families could be diluted by inclusion of many small families.

Parent of origin effects by genetics or epigenetics

A parent-of-origin effect in MS could be the result of several mechanisms. These may operate differently in alternative inheritance models for MS: genetic as well as environmental, or both. One of the possible genetic mechanisms is a threshold effect due to an increased number of penetrant susceptibility genes in a given parental lineage. Another possibility is the involvement of epigenetic mechanisms in disease transmission by the affected parent. Epigenesis means that there are chromatin and DNA modifications affecting gene expression. These modifications are transmitted through cell division, but do not influence the underlying DNA sequence of that person. If epigenesis occur early in life, it can affect the availability of the critical gene product or modify the risk associated with a given gene polymorphism. Epigenetic effects do not have to occur in all cells from one person, they can also operate only in the cells of specific tissues. The threshold and epigenetic mechanisms could also occur in conjunction, especially in complex disorders as MS.

Susceptibility genes for MS

The HLA region

The first recognition of an association of MS with genes in the MHC region was in 1972 (16, 17). Until very recently, despite many linkage and association studies, only the HLA-class II region of the HLA-DR2 haplotype on chromosome 6p21 has been shown to be significantly associated with MS. Three candidate risk genes of this haplotype, *HLADRB1*1501* (encoding HLA-DR2b), *HLADRB5*0101* (encoding HLA-DR2a) and *HLADQB1*0602* (encoding HLA-DQ6), are so tightly linked that they are almost invariably inherited together (18). Therefore classic genetic studies failed to discriminate between them. More recently, more powerful genetic studies have implicated *HLADRB1*1501* as the main susceptibility allele in MS (19, 20). The association is strongest in northern Europeans, but is seen in virtually all populations with a notable exception in some Mediterranean populations where MS is associated with DR4. By now it is clear that *HLA DRB1*15* is not the sole risk increasing allele (21).

*HLADRB1*17* increases risk for MS, but to a lesser extent than *HLA-DRB1*15* (21, 22). *HLADRB1*14* has the most dominant role in MS MHC genetics and completely abrogates any risk associated with *HLADRB1*15*, when they are inherited together. The relative risk of getting MS for an individual who carries the *HLA-DRB1*15* allele

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is about 3. *HLADRB1*14* together with *HLADRB1*15* reduces the relative risk to approximately 1 (21). Complex gene-gene interactions, called “epistasis”, also seem to occur in the HLA region. *HLA-DRB1*08* more than doubles the risk associated with a single copy of *HLA-DRB1*15*, whereas on its own, *HLA-DRB1*08* has only a modest effect (21).
In addition, *HLADRB1*01* and *HLADRB1*10* protect against MS but only in the presence of *HLA-DRB1*15* that operate from the other chromosome – that is in *trans* (21, 23, 24), although reports from Sweden suggest that HLADRB1*01 may be protective on its own (25) (figure 1).

Regarding the HLA class I region, it has recently became clear that there are loci that increase the risk for MS (26, 27). Interestingly, the class I region involved in EBV-related infectious mononucleosis also increases the risk for MS (28). This draws attention to common genetic backgrounds between MS and primary EBV infection.

Epigenetic effects in the HLA region have also been observed in MS. Using the large Canadian database, it has been possible to study affected aunt/uncle/niece/nephew (“AUNN”) pairs. It was found that in these pairs, the allele frequencies for *HLA-DRB1*1501* were different between the first and second generations affected. *HLA-DRB1*15* frequency in affected males remained the same over the two generations, whereas affected aunts had significantly lower *HLA-DRB1*15* frequency compared with their affected nieces. They also found that the risk carried by *HLA-DRB1*15* was greater in families with affected second-degree relatives (AUNN: OR 4) when compared to those consisting of only affected first-degree relatives (ASP: OR 2). So HLA contributed to risk in both family types, however the degree of contribution is not the same and is dependent on family structure and of MS status of the relatives of the transmitting individual. This demonstrates heterogeneity of risk among *HLA-DRB1*15* haplotypes based on whether collateral parental relatives are affected. This study implicates gene–environment interactions in susceptibility (29). The distorted transmissions may well be applicable to other non HLA-DRB1*15 alleles, shown to be involved in susceptibility or resistance. The fact that HLA-DRB1*15 frequency remained the same over two generations in affected males in contrast to affected females, suggests that epigenetic modifications play a role in the gender bias in MS.

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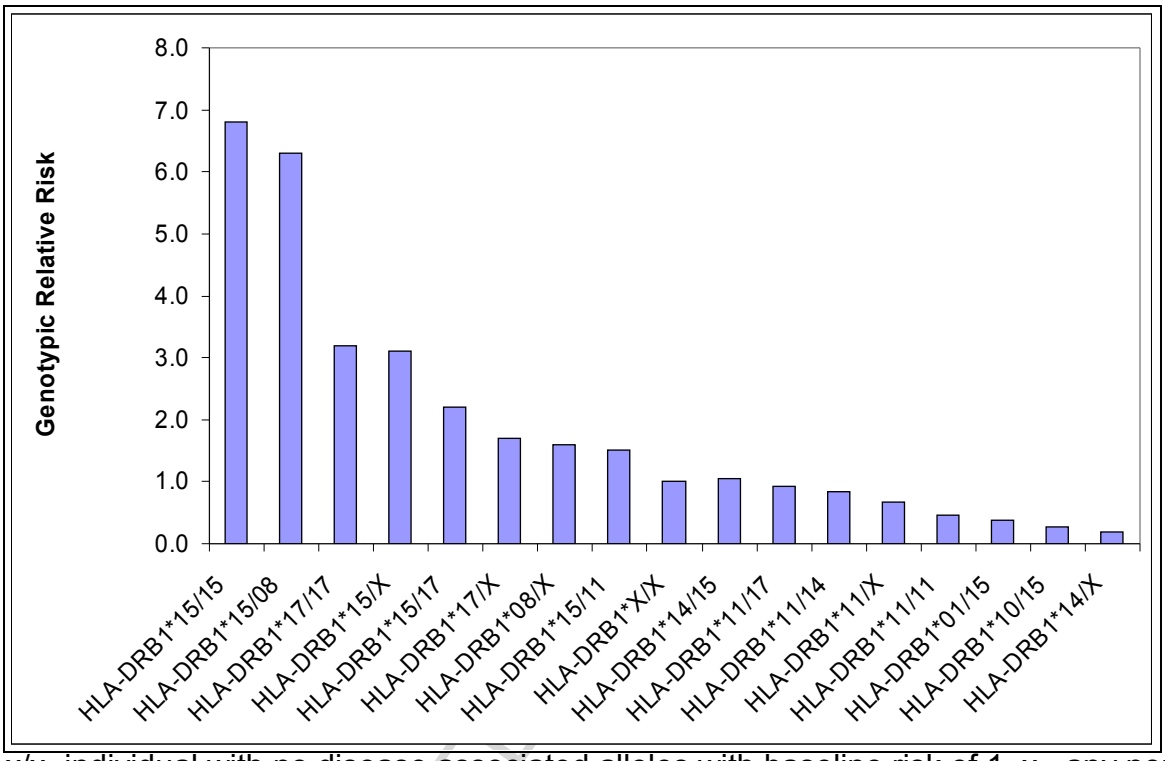
Fig 1: Genotyping relative risks for MS for combinations of alleles at the HLA-DRB1 locus.



x/x=individual with no disease associated alleles with baseline risk of 1, x= any non-disease associated allele.

(figure provided by S. Ramagopalan and G.E. Ebers)

Little is known about the influence of the presumed environmental risk factors for MS and the MS risk genes. Some evidence has recently been provided that vitamin D and infectious mononucleosis (IM) influences the risk associated with *HLA-DRB1*15.* Epidemiological studies provided strong evidence that the geographical distribution of MS risk is the result of environmental factors operating at the population level. Sunlight, specifically through its role in generating active vitamin D, is a likely important environmental factor for the disease (30). It has been shown that dietary vitamin D intake reduces disease risk (31) and that MS patients are deficient in vitamin D (32). Vitamin D has its actions on immune and central nervous system development and function. So, vitamin D could influence MS risk in this way.
Most biological effects of vitamin D are regulated by the vitamin D receptor (VDR). This receptor influences the rate of transcription of vitamin D responsive genes by acting as a ligand activated transcription factor that binds to vitamin D responsive elements (VDREs) in gene promotors. Ramagopalan and colleagues localized by sequence analysis a single MHC vitamin D response element (VDRE) to the promotor region of *HLA-DRB1*. Sequencing of this promotor in chromosomes from *HLA-DRB1* homozygotes showed absolute conservation of this putative VDRE on *HLA-DRB1*15 (24)*. Cells transiently transfected with the *HLA-DRB1*15* gene promoter showed increased expression on stimulation with 1,25-dihydroxyvitamin D3 (P = 0.002) that was lost either on deletion of the VDRE or with the homologous 'VDRE' sequence found in non–MS-associated *HLADRB1* haplotypes. A specific increase in the cell surface expression of *HLA-DRB1* upon addition of vitamin D was

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seen only in *HLA-DRB1*15* bearing cells (24). The biological meaning of this vitamin D induced specific HLA class II expression is not clear. It is not directly in line with the supposed disease suppressing role of vitamin D. Generally spoken, higher class II expression is pathogenic. However, more complex processes may be involved, such as class II induced thymic deletion, and/or the exact timing of vitamin D effects in human life, for example in utero. In any case the above effect is a nice example of gene expression under the influence of environmental factors.

Finally, class II interactions may exist with EBV induced infectious mononucleosis (IM). Epidemiological and serological studies also showed that infection with Epstein-Barr virus is associated with higher risk of MS (33, 34). In particular, primary EBV infection, manifesting as infectious mononucleosis (IM) seems to be associated with increased MS risk. In a Danish study, IM-naïve individuals, *DRB1*15* carried a 2.4-fold (95% confidence interval [CI], 2.0-3.0) increased MS risk, whereas persons with IM history, *DRB1*15* was associated with a 7.0-fold (95% CI, 3.3-15.4) increased MS risk. Thus, the MS risk conferred by *HLA-DRB1*15* was 2.9 (95% CI, 1.3-6.5)-fold stronger in the presence than in the absence of a history with IM (33).

Non-HLA MS risk SNPs that reached genome wide significance

There are different reasons why until very recently no other risk genes than the HLA risk gene have been detected. First the attributable risk of other risk alleles is very small. Furthermore the populations studied worldwide are quite heterogeneous and a major factor clearly has been the lack of power statistical.

To overcome the problem of statistical power, the International MS Genetics Consortium (IMSGC) performed a GWA (genome-wide association) study using 500,000 SNP arrays in 931 families. Subsequently they performed a combined analysis of more than 12000 samples.
The results revealed the existence of 16 other non-MHC susceptibility SNPs in 13 gene loci of modest effect. However only the non-MHC SNP in the *IL2RA* gene achieved genome-wide significant association with susceptibility to MS ($p=2,96 \times 10^{-8}$) (table 1) (35). For a third gene, *IL7R*, convincing functional support was obtained (36, 37). Later genome-wide significance was established in a joint analysis of 11,019 cases and 13,616 controls (38).
It is of note that the three risk sequence-allelic variants are all common variants (that is, with a risk allele frequency of > 10% and <90%). It is likely that in MS epistasis and epigenetics occur and result in direct biological pathways involved in MS.

Follow-up studies and further genome-wide analyses have now provided genome-wide significant support for several non-HLA MS risk genes (39), amongst which are *IL7R, IL2R, CLEC16A (40-42), CD58 (42), CD226 (40), KIF1B (43), KIF21B (44, 45), CD6 (46), IRF8 (46), TNFRSF1A (46), two* loci on chromosome 12 and 20, which have been suspected to relate to the genes *CD40* and *CYP27B1(47, 48), TYK2 and STAT3 (49)* . Strikingly most of these molecules are associated with immune function. It should be noted that the neuronal *KIF1B* gene could not be replicated as an MS risk gene by a large consortium (50, 51).

Function of some different identified risk variants

In most situations it is a SNP in or around the gene that is found to be associated and the real genetic variation associated with MS remains unknown. In addition, for all of

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these genes much remains to be elucidated on the exact place in physiology or in evolution of disease. Nevertheless, it is of most interest that most of these new MS susceptibility loci appear to have an important place in the immune system. Below we briefly discuss what is known about the function of the different identified risk variants. It should be acknowledged here that this research field has a highly dynamic character, and it is virtually impossible to cover all available knowledge of completely validated and robust risk loci at a certain timepoint. Also, one has to realise that most loci have been identified at the SNP level. Direct proof of the gene involved is lacking in most cases. We here present a selection of what we consider the current most interesting and robust MS risk loci, and focus on the functional roles of the likely genes involved.

MHC class II
molecules are highly polymorphic cell-surface glycoproteins that present antigen to CD4+ T helper cells and are integral to successful maintenance of self-tolerance by the immune system and the adaptive immune response to invading pathogens (52). Each HLADRB1 allele forms, by the presence of defined amino acid anchors, a number of specific pockets comprising a peptide-binding groove (53). The binding affinities for disease-related peptides may thus be different for different HLADRB1 molecules as determined by their protein sequence.
Subsequently this may influence composition of T-cell repertoires and so this can ultimately results in *HLA-DRB1* alleles having varying effects on disease risk However protein sequence analysis failed to provide unequivocal support for this hypothesis. This suggests that other risk factors are present on *HLA-DRB1* haplotypes (54), and this perhaps detracts away from antigen presentation as the sole mechanism of the MHC association in MS (55).

IL7R encodes for the receptor for interleukin-7, which is expressed on T and B-lymphocytes. The signal transduced by this receptor is crucial for lymphocyte survival and immune homeostasis. There are indications that the exonic IL7R SNP associated with MS leads to less membrane expressed but more soluble receptor protein, therefore influencing receptor signalling (37).

IL2RA encodes for the alpha chain of the interleukin-2 receptor (IL2R). The involvement of the IL2R chain in the pathogenesis of MS might also be related to the important role that the IL2–IL2R pathway plays in adaptive immune functions .

For the *CLEC16A* protein still little is known regarding its function in humans. However, it is very likely that it does have a function in immune function. It is a member of the C-type lectin receptor family, of which members have described to provide signals for a decision between tolerance and immunity. They can bind bacterial products as well as endogenous ligands and their signal can counteract the signal of Toll-like receptors, therewith influencing T-helper cell function. Our group previously implied a role for C-type lectin receptors in MS pathogenesis, and discussed a link with infections (56).

CD226 is a transmembrane receptor of the immunoglobulin receptor family and is expressed on natural killer cells, T cells, monocytes and subsets of B cells and platelets and is implicated in T cell and NK cell mediated cytotoxicity and platelet activation. CD226 recognizes on most cell types, including neurons, endothelial cells

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and fibroblasts, expressed poliovirus receptor and nectin-2 (57). Interaction of CD226 with poliovirus receptor on endothelial cells is involved in endothelial transmigration of leukocytes (58). In autoimmune encephalomyelitis, an experimental murine model for multiple sclerosis, anti-CD226 treatment results in reduced severity and delayed onset of the disease. This could be the result of altered T-cell activation, altered monocyte extravasation or altered natural killer cell responsiveness towards target cells (59).

CD58 encodes a ligand for the T-cell specific CD2 membrane molecule, an adhesion molecule that transduces important signals for T-cell proliferation and differentiation (60).

CD6 is a lymphocyte receptor that belongs to the scavenger receptor cysteine-rich superfamily. Its expression has also been reported on certain regions of the brain. CD6 contributes to either positive or negative modulation of the activation and differentiation signals for lymphocytes (61, 62). The soluble form of CD6 may also function as a pattern recognition receptor and affects the serum level of TNFα in this context in mice (63).

IRF8 is a member of the interferon regulatory factor (IRF) family, which are specifically expressed in immune cells. The IRF8 locus contains an important transcription factor involved in responses to type I interferons (IFNα and β). Upregulation of interferon responses has been noted in peripheral blood of a subset of untreated MS patients (64, 65). However the role of interferons in the onset of MS is still unclear. Results of a recently performed expression study show that the susceptibility allele near *IRF8* is associated with higher mRNA expression of interferon-response pathway genes in subjects with MS (46).

TNFRSF1A encodes the p55 receptor for tumor necrosis factor alpha (TNFα). Genetic and functional studies suggest that dysregulation of the TNFα pathway has a role in the onset of MS. Diminished TNFα activity seems to be associated with onset of CNS inflammatory lesions in clinical studies (66).

CD40 is a member of the tumor necrosis factor receptor superfamily and is expressed as a co-stimulatory molecule on B cells, dendritic cells, macrophages and microglia. Together with its ligand CD40L (CD154), which is expressed on activated $CD4^+$ T-helper cells, CD40 is an important regulator of both humoral and cellular immunity (67).

CYP27B1 encodes the enzyme 25-hydroxy-vitamin D-1 alpha hydroxylase, which hydroxylates 25-hydroxyvitamin D into the bioactive form (1,25-dihydroxyvitamin D (1,25(OH)2D). This bioactive form regulates through the vitamin D receptor (VDR) the calcium metabolism. It also has important immune functions modulating innate as well as adaptive immunity and tolerance (68) and B-cell homeostasis (69). Furthermore it can direct activated T cells toward a T helper type 2 anti-inflammatory phenotype and induce dendritic cells with tolerogenic properties (70). Epidemiological data suggest a link between vitamin D deficiency and increased incidence of MS and other autoimmune diseases (31, 71, 72).

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STAT3 encodes for a transcription factor that is involved in multiple pathways and functions, including the Jak-STAT pathway, neuron axonal guidance, apoptosis, activation of immune responses and Th17 cell differentiation (73). Mouse studies have shown that the targeted deletion of STAT3 in CD4+ T-cells prevents the development of experimental autoimmune encephalomyelitis (74). Moreover, increased phosphorylated STAT3 was reported in T cells of patients evolving from a clinically isolated syndrome to defined MS and in relapsing patients (75).

TYK2 or tyrosine kinase 2 is a type I interferon signalling enzyme linked to Th1 cell function. Of note is the fact that TYK2 can activate STAT3 via tyrosine phophorylation (76), therewith linking two MS risk genes in a common pathway. A very low frequency genetic variant of the gene variant has been found associated with MS in white and but also African American individuals (77-79). It is the first rare genetic variant robustly found to enhance the risk for the disease.

KIF1B and *KIF21B*, encode for a kinesin superfamily member that responsible for axonal transport of mitochondria and synaptic vesicle precursors. KIF1B has an ATP ase binding domain and is enriched in motor neurons. Recently dysregulation of ATP-ases and mitochondrial mislocalization has been shown to play a role in several neurodegenerative diseases. There is now increasing evidence that neurodegenerative processes, besides immunologic processes, are important in MS pathology. Irreversible axonal loss is an important mechanism in the development of permanent neurological symptoms. Though primary demyelination may underlie early axonal loss, further progression of neurodegeneration occurs when the compensatory capacity of the CNS is exceeded and the threshold of axonal loss is reached. Mechanisms proposed for this loss of nerve fibres include mitochondrial dysfunction, reduced ATP production and altered expression of sodium channels. *KIF1B* knockout mice have been reported to have CNS abnormalities such as atrophy (80). It should be noted however, that any putative functional role of kinesins in immune cells has been largely left unexplored.

Rare variants associated with MS susceptibility

Large case-control GWAS primarily expose common variants contributing to disease pathogenesis with modest effects. Since the new MS risk loci identified so far, explain less than 1 % of MS risk and HLA 15-40%, other loci remain to be identified for a more comprehensive understanding of the genetic susceptibility of MS. Alternative strategies are needed to identify less common, possibly more penetrant variants. Relatively rare variants with at least moderate penetrance most probably give rise to a higher familial occurrence of MS and so also to a higher occurrence of MS in a genetic isolate with a founder effect. Southern Ostrobothnia in Finland is a genetic isolate with an exceptionally high prevalence and familial occurrence of MS (81-83).

In this isolate the largest pedigree of MS cases could be constructed. In Finnish MS families enriched with cases from this isolate, linkage of MS to chromosome 5p had been detected. The *IL7R* gene is located in the chromosome 5p region. The 5p locus was studied further in the Finnish isolate and monitored for haplotype sharing. This analysis revealed only modest association at *IL7R*, whereas most significant association was found with one haplotype covering the C7-FLJ40243 locus. The identified risk haplotype (~4% in the general European population) is relatively rare,

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but it has obviously become enriched in the southern Ostrobothnian high-risk MS region (12% of cases). It contains complement component 7 (C7), again an important factor in the innate immune system (84). These results exemplify the power of population isolates in the identification of rare disease alleles .

Identifying MS risk genes results in a better understanding of MS pathogenesis?

Still many more markers tested in GWAS have shown association with MS, although not genome-wide significant. Because of the exceedingly large multiple testing involved in these studies, very few exceed the genome-wide significant threshold and those that do not are generally neglected. In many cases where loci with small but measurable genetic effects are involved, it is possible that these loci represent false negatives. It could be possible that meaningful combinations of genes, showing only modest evidence of association in GWAS, can be identified if they belong to the same biological pathway or mechanism.

Systems biology

In systems biology an integrative approach of available molecular, physiological, clinical, genetic, gene expression, proteomic and/ or biological information is used with the ultimate goal to identify pathways involved in the pathogenesis of diseases. Thereby real-world systems are represented as networks that interconnect different entities. In this way certain properties can emerge that cannot be derived from the individual analysis of each of their components (85).

Baranzini and co-workers have recently put current information on modest genetic effects into a model linked to a human protein network. They aimed to identify sub-networks containing a higher proportion of genes associated with MS than expected by chance. Indeed interesting sub-networks of genes from several immunologic pathways involved in MS susceptibility were identified. Part of them were already known, but also novel neural pathways not identified earlier were uncovered (86).

MS and autoimmune diseases

Intriguing is the fact that most of the above immune related SNPs have overlapping associations with autoimmune conditions such as rheumatoid arthritis, inflammatory bowel disease, SLE, type I diabetes and thyroid autoimmunity (see table 1).
This suggests that in MS and other autoimmune diseases, common pathways are involved in pathogenesis. If different autoimmune disorders share susceptibility genes, you would expect relatives to be not only at greater risk for MS than the general population, but also at greater risk of other autoimmune disorders. Several studies have investigated the rate of autoimmune diseases in MS patients and their biological relatives. The results have been conflicting, which may relate to differences in methodologies and populations studied.

It is striking to note that some immunomodulatory treatments in MS can trigger other AID. The results of a phase II clinical trial with the lymphocyte-depleting humanized monoclonal antibody alemtuzumab (Campath-1H) show that it is highly effective in the treatment of early relapsing-remitting MS (87). A single pulse of treatment results in rapid, profound and prolonged lymphopenia. Especially CD4+ T cells recover slowly, remaining depleted for at least 5 years (88). However, 30 % of patients developed autoimmunity months to years after pulsed treatment with alemtuzumab.

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Most people develop thyroid autoimmunity, mainly Graves' disease, and a few idiopathic thrombocytopenic purpura (ITP) and in a very few other blood components are targetted. In addition 5,5 % of patients develop sustained non-thyroid autoantibodies without clinical disease (88). While the association between lymphopenia and autoimmunity is well recognized, most lymphopenic subjects do not develop autoimmunity. It has been shown that autoimmunity arose in those patients greater T-cell apoptosis and cell cycling in response to treatment. This phenomenon is driven by higher levels of IL-21. Patients who went on to develop autoimmunity had more than 2-fold greater levels of serum IL-21 than the patients who did not. The production of IL-21 seemed to be genetically determined (87).
The recent indication of a kinesin gene involved in MS, draws for the first time attention to genetic processes in the target organ, the CNS (43). Kinesins are responsible for transport of cargo, such as neurotransmitters and mitochondria, and may be related to several other neurodegenerative conditions. Tissue damage in MS may be a result of a disturbed balance between self-protective axonal transport and immune attacks.

Table 1
Some MS risk genes overlapping with risk genes for other autoimmune diseases

Gene	Chr	function	Disease	Ref
IL7R	5	Homeostasis of the memory T-cell pool,	T1D	(89)
IL2R	10	Regulation of T-cells	T1D, Graves' disease	(90, 91)
CLEC16A	16	Provides signals for decisions between tolerance and immunity	Addison's disease, T1D	(40, 92)
CD226		Adhesion and co-stimulation T-cells	T1D, RA, Graves'disease, crohn's disease	
CYP27B1	12	Hydroxylates 25-hydroxyvitamin D into the bioactive form	T1D	(93)
TNFRSF1A	12	Influences the TNFα pathway	Tumor necrosis factor associated periodic syndrome (TRAPS)	(94)
CD40	20	Regulator of humoral and cellular immunity	RA, Graves' disease	(95, 96)
CD58	1	Influences T-cell proliferation and differentiation	RA	(97)
STAT3	17	involved in multiple pathways and functions, including the Jak-STAT pathway, neuron axonal guidance, apoptosis, activation of immune responses and Th17 cell differentiation	CD, UC, hyper-IgE syndrome	(98-101)

T1D: type I diabetes, RA: rheumatoid arthritis, CD: Crohn's disease, UC: ulcerative colitis

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B. Near future

The next step in MS genetics will be to identify the real disease-mediating variants in the recently discovered genetic risk loci. One of the major tools will be DNA sequencing around the current SNPs, or even at the whole genome level.
The identification of the exact disease susceptibility genes does not necessarily define the pathway involved in disease development, or the molecular pathway the gene product is involved in. To determine this, functional studies are necessary (39). To begin to understand the actions of the disease-associated variants, their potential effects could first be analysed in cells and tissues from healthy controls that may or may not have the predisposing genotype. Next, functional and expression analyses can be done, as the newly discovered genetic variants may be located in either known or putative regulatory elements, or coding regions. The molecular pathways of the genetic variants involved should of course be analysed in-depth. The simultaneous quantitative analyses of gene expression will be required as more risk genes become known. And of course the genetic variation on a genome-wide basis in a large number of individuals will provide a more thorough understanding of the molecular networks that are perturbed by the disease associated genetic variants (102).
Obtaining immune cells for such functional studies is much more easy than accessing human CNS cells or tissue. Recent developments in the field of induced pluripotent stem cells (iPS cells) may change this situation. Those cells can now be generated from fibroblasts (103-105), keratinocytes (38) and blood progenitor cells (106) and are likely to revolutionize the generation of *in vitro* disease models. These models may allow for functional and expression studies of cells from healthy as well as MS individuals.
However, variation in gene expression is also affected by factors as epigenetic modification, factors that are not readily identified by SNP or DNA sequencing studies. Epigenetic modifications are markers of how environmental factors may influence gene expression and may be very important in linking genetics to environment. New tools for the mapping of epigenetic modifications are becoming possible. The identification of epigenetic processes in demyelination indicates that investigating dysregulated posttranslational modifications in MS could result in a better understanding of its pathogenesis
Furthermore the network based approach in system biology can result in better knowledge of pathogenesis of MS and finally to better therapies. The genomic portrait of an individual may allow a predictive and personalized approach to therapy. In the near future this process will probably be aided by powerful computers, with information technologies that will manage the available information from the patient's tests, the patient's medical history and genetic history and ever evolving scientific databases.

Gene-environment interactions
A challenging element of future studies will be the characterization of the environmental factors that interact with genetic factors to finally cause the disease. As already described an interaction between vitamin D and *HLA-DRB1*15* has been found even as an interaction between EBV and *HLA-DRB1*15.*

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Besides EBV and vitamin D numerous bacterial and viral infections have been described as potential candidates for MS, as they are often associated with relapses of MS. However no single infection has consistently been associated with disease (107). It is unclear how so many different infections could have a role in the pathogenesis of the disease and how they might interact with genetic factors, unless their role is non-specific, such as in creating inflammation or damaging the blood-brain barrier.
A better understanding of the function of identified risk genes and their role in pathogenesis of MS may not only explain how suspected environmental factors interact with such genetic factors to cause disease but could also result in the identification of additional environmental risk factors.

Novel genes in animal models
Work in animal models can also result in a more comprehensive understanding of the effects of individual genetic variations on disease development. The experimental autoimmune encephalomyelitis (EAE) is a commonly used mouse model for MS. Through this model relevant molecular pathways in MS have already been dissected (108). This has led to the development of new therapies, most notably natalizumab (Tysabri), an α4 integrin-specific antibody that blocks T-cell entry into the CNS (109). The roles of predisposing genetic variants in the pathogenesis of MS can be investigated further by the development of a humanized form of the EAE model in which the animals express transgenes derived from MS patients. Initially individual disease-associated risk genes should be introduced , where after mouse lines expressing individual candidate genes can be cross-bred to allow for pairwise or higher order comparisons of their contributions to disease pathogenesis. Humanized mice have already been used to analyse the varying effects of different HLA genes in MS and their conclusions are supported by complementary genetic data from human populations. However, so far none of the newly identified non-MHC genetic risk variants has been analysed in animal models for MS, but this will become possible in the near future (110).

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