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MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction

Maria Swanberg[1,10], Olle Lidman[1,10], Leonid Padyukov[2], Per Eriksson[3], Eva Åkesson[4], Maja Jagodic[1], Anna Lobell[1], Mohsen Khademi[1], Ola Börjesson[2], Cecilia M Lindgren[5], Pia Lundman[6], Anthony J Brookes[7], Juha Kere[5], Holger Luthman[8], Lars Alfredsson[9], Jan Hillert[4], Lars Klareskog[2], Anders Hamsten[3], Fredrik Piehl[1,10] & Tomas Olsson[1,10]

Antigen presentation to T cells by MHC molecules is essential for adaptive immune responses. To determine the exact position of a gene affecting expression of MHC molecules, we finely mapped a previously defined rat quantitative trait locus regulating MHC class II on microglia in an advanced intercross line. We identified a small interval including the gene MHC class II transactivator (*Mhc2ta*) and, using a map over six inbred strains combined with gene sequencing and expression analysis, two conserved *Mhc2ta* haplotypes segregating with MHC class II levels. In humans, a –168A→G polymorphism in the type III promoter of the MHC class II transactivator (*MHC2TA*) was associated with increased susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction, as well as lower expression of *MHC2TA* after stimulation of leukocytes with interferon-γ. We conclude that polymorphisms in *Mhc2ta* and *MHC2TA* result in differential MHC molecule expression and are associated with susceptibility to common complex diseases with inflammatory components.

Many diseases of both inflammatory and primarily noninflammatory origin have increased expression of MHC class I and II molecules, T-cell infiltration and tissue damage. Further supporting the idea that MHC molecules have a role in disease pathogenesis is the fact that many chronic inflammatory diseases with a complex etiology are influenced by genes in the HLA region, particularly the class II genes. $CD4^+$ T-cell reactivation in a target organ requires re-recognition of antigenic peptides bound to MHC class II molecules on the surface of specialized antigen-presenting cells[1]. Thus, the amount and cellular distribution of MHC class II molecules are suggested to be important factors for adaptive immune responses, including those taking place in the target tissue of an immune attack. The potential impact of MHC on disease motivates researchers to determine the position of any gene polymorphism that affects MHC expression pattern.

Differences in MHC class II molecule expression on central nervous system (CNS) microglia in rat strains, both under normal conditions[2] and after nerve injury[3,4], suggest that genetic polymorphisms affect transcriptional regulation. The underlying genes, however, remain to be identified, and there may be many candidates. Expression of MHC class II molecules can be elicited by a series of inflammatory stimuli that converge on the interferon-γ (IFN-γ) signaling pathway. In addition, other signals such as interleukin-1β, interleukin-4, interleukin-10, transforming growth factor-β and glucocorticoids can act as modulators[5,6]. Intracellular activation leads to the assembly of a transcriptional complex consisting of several different molecules interacting with the promoter elements of class II genes. Polymorphisms in any of these known regulators could cause the observed differences in MHC class II expression in rat strains.

Ultimately, gene polymorphisms in the rat should be examined for their relevance to human disease or physiology. But direct testing of a large number of these candidates in humans with a classical hypothesis-driven approach would be extremely cumbersome. We pursued an alternative approach with unbiased gene mapping using rat models[7,8] to identify candidate genes that can subsequently be tested for association in large human samples. MHC molecules become upregulated in the rat CNS after peripheral nerve trauma[9,10]. Genetic mapping of strain differences in expression of MHC class II molecules after nerve injury (ventral root avulsion, VRA), carried out in a rat F_2 intercross between strains DA ($RT1^{av1}$) and PVG ($RT1^c$)[8], identified a single quantitative-trait locus (QTL), *Vra4*, with significant linkage

[1]Department of Clinical Neuroscience, Neuroimmunology Unit, Karolinska Institutet, Stockholm, Sweden. [2]Department of Medicine, Rheumatology Unit and [3]Atherosclerosis Research Unit, King Gustaf V Research Institute, Karolinska Institutet, Stockholm, Sweden. [4]Department of Neurology, Neurotec, Karolinska Institutet, Huddinge, Sweden. [5]Department of Biosciences at Novum, Karolinska Institutet, Huddinge, Sweden. [6]Department of Medicine, Danderyd's Hospital, Stockholm, Sweden. [7]Center for Genomics and Bioinformatics, Karolinska Institutet, Stockholm, Sweden. [8]Lund University, Department of Clinical Sciences, Malmö, Sweden. [9]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. [10]These authors contributed equally to the work. Correspondence should be addressed to T.O. (tomas.olsson@cmm.ki.se).

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(lod score = 27.4) to expression of MHC class II molecules. Here, using an advanced intercross line (AIL), in which a higher recombination frequency allows more precise positioning[11,12], we finely mapped *Vra4* to a 5.3-Mb chromosome interval that includes the gene *Mhc2ta*. Differences in the transcript levels of *Mhc2ta* and the MHC class II associated invariant chain (*Cd74*) between the two parental strains, as well as in heterogeneous AIL F_8 rats stratified for the *Vra4* max marker; the expression pattern in intra-*Vra4* AIL F_{10} recombinants; and the presence of distinct *Mhc2ta* haplotypes across inbred strains differing in degree of expression of MHC class II molecules are all consistent with the idea that decisive functional polymorphisms in the 5′ flanking region of *Mhc2ta* cause the strain differences in expression of MHC class II molecules.

On the basis of these results, we selected three SNPs in *MHC2TA*, genotyped them in three separate case-control groups and found that a –168A→G polymorphism in the 5′ flanking region of type III *MHC2TA* is associated with increased risk of rheumatoid arthritis, multiple sclerosis and myocardial infarction. The two first diseases have inflammatory pathogenesis, and inflammatory components of atherosclerosis are now widely recognized[13]. The polymorphism was associated with functional differences, as demonstrated by differential MHC molecule transcription upon IFN-γ stimulation of peripheral blood leukocytes *in vivo*. *MHC2TA* is now of interest as a pharmacogenomic marker, drug target and candidate gene for involvement in other human diseases with inflammatory components.

RESULTS

Identification of *Mhc2ta* haplotypes underlying *Vra4*

We finely mapped the MHC class II–regulating locus *Vra4* previously identified in F_2 rats[8] using an F_8 AIL. Dense genotyping throughout *Vra4* resulted in a single sharp peak with a maximum lod of 26.4 at the marker *D10Mgh25* (**Fig. 1**). The MHC class II immunolabeling pattern in F_8 rats stratified for *D10Mgh25* mimicked the respective patterns for the parental strains (**Fig. 2**). The points at which the lod score drops by 1.5 relative to the maximum linkage support in the QTL interval define a genomic region of 5.3 Mb, homologous to mouse chromosome 16A3-B1 and human chromosome 16p13 (**Fig. 1**). This region comprises ~40 genes, among them the key candidate *Mhc2ta*, encoding the class II transactivator, which is a known regulator of MHC class II expression. The marker with the highest lod score, *D10Mgh25*, is located in intron 15 of *Mhc2ta*.

We next used a haplotype-based approach to characterize the linked region responsible for the strain differences in expression of MHC class II molecules. We have phenotypic information on the variable

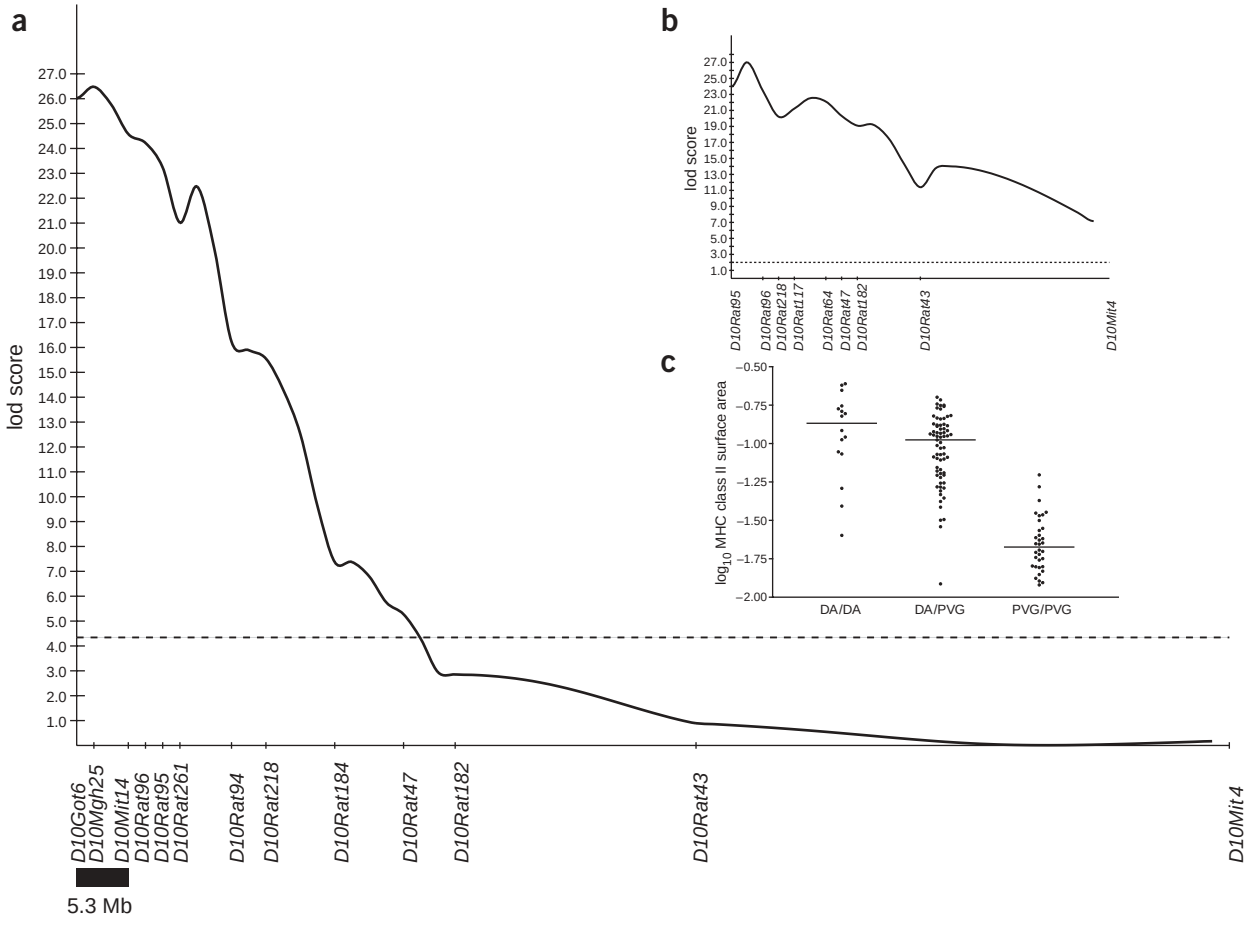

Figure 1 Fine mapping of the *Vra4* locus in the AIL F_8 generation. (**a**) lod score graph showing linkage for expression of MHC class II molecules at the very centromeric end of rat chromosome 10. The lod score calculated in MAPMAKER/QTL was 26.4 with marker *D10Mgh25*. The 1.5 lod-score drop interval corresponds to a physical size of 5.3 Mb. (**b**) lod score graph adapted from ref. 8 showing *Vra4* mapped in an F_2 generation in the same region shown in **a**. Note the enhanced resolution in F_8 compared with F_2 generations. (**c**) Scatter plot showing the genotype-phenotype distribution at *D10Mgh25* in the F_8 population. Expression of MHC class II molecules (log_{10} surface area) was roughly ten times higher in DA/DA rats compared with PVG.1AV1/PVG.1AV1 (PVG/PVG) rats and was intermediate in heterozygous rats.

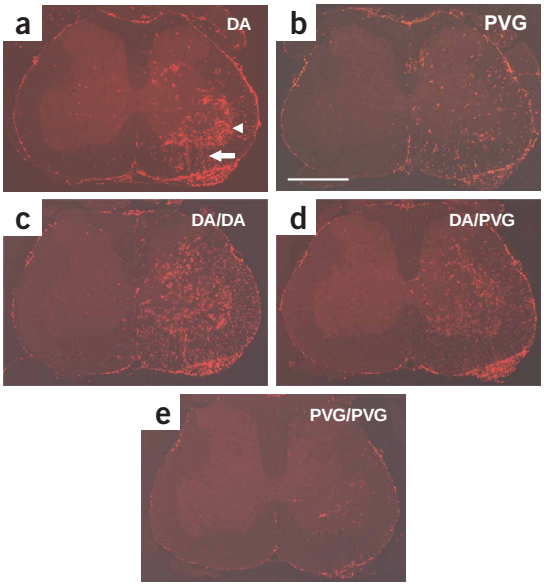

Figure 2 VRA-induced expression of MHC class II molecules in the spinal cord. Micrographs showing spinal cord sections immunostained for OX-6 (MHC class II) in strains DA (**a**) and PVG.1AV1 (PVG; **b**). OX-6–positive cells are present in the gray matter of the ventral horn of the lesioned side (arrowhead in **a**), as well as in the ventral white matter of the spinal cord that contains crossing motor axons (arrow in **a**). Stratification for marker *D10Mgh25* in the AIL F_8 generation showed a distinct genotype-specific pattern (DA/DA, **c**; DA/PVG.1AV1 (DA/PVG), **d**; PVG.1AV1/PVG.1AV1 (PVG/PVG), **e**). Scale bar, 1 mm (**b**).

induction of expression of MHC class II molecules in response to VRA in a panel of six inbred rat strains[3,4]. Strains DA, BN and LEW had strong upregulation of MHC class II molecules in contrast to strains E3, PVG.1AV1 and ACI. We used these six strains to create a microsatellite-based haplotype map of the *Vra4* locus, consisting of 15 microsatellite markers across 5.8 Mb at the very acrocentric end of chromosome 10 (**Fig. 3**). If the same locus governs expression of MHC class II molecules in all these strains, at least two haplotypes should segregate with level of expression of MHC class II molecules. We observed a distinct segregation in strains DA, BN and LEW compared with strains E3, PVG.1AV1 and ACI between markers AU048079 and *D10Mit14*, a region of ~2.7 Mb containing 23 genes annotated in rat or mouse (**Fig. 3**).

We carried out further fine mapping by identifying F_{10} AIL rats with recombinations in *Vra4*. We determined expression of *Cd74*, a marker of MHC class II molecules, in F_{10} rats with and without recombinations in *Vra4* between markers *D10Mgh25* and *D10Mco46* (referred to as the recombination interval). *Cd74* transcript levels were associated with the genotypes in the recombination interval, reducing the number of candidate genes to 13 (data not shown).

We sequenced *Socs1*, *Mhc2ta*, *Nubp1* and *Emp2*, as well as two genes (*Snn* and *Litaf*) flanking the recombination interval, in strains DA and PVG.1AV1 and sequenced polymorphic loci in other strains BN, LEW, E3 and ACI. The sequence-based SNP haplotype map identified three regions segregating the two groups of rats with high and low expression of MHC class II molecules (**Fig. 3**). These regions contain *Nubp1*, *Snn* and *Mhc2ta*, which we subjected to expression analysis because polymorphisms were located to untranslated (*Snn*) and 5′ flanking regions (*Nubp1* and *Mhc2ta*). Expression analysis of *Nubp1* in the spinal cord by RT-PCR showed no difference in transcript levels between naive DA and PVG rats 7 and 14 d after VRA (data not shown). We assessed *Snn* expression in another set of experiments using rat Affymetrix RGU-34A microarrays (M.S. *et al.*, unpublished data). There were no differences in transcript levels of *Snn*, or any of the other genes in the region represented on RGU-34A, in DA versus PVG naive rats 5 or 14 d after VRA. These results leave only *Mhc2ta* as a candidate gene for underlying strain differences in expression of MHC class II molecules.

Of the seven SNPs identified in *Mhc2ta*, the four SNPs located in the 5′ flanking region of isoform III and IV and the two SNPs in exon 1 of isoform I of *Mhc2ta* defined two haplotypes shared by strains DA, LEW and BN and strains PVG.1AV1, E3 and ACI, respectively (**Fig. 3**). The three SNPs in the coding sequence were all synonymous. We determined transcript levels of *Mhc2ta* with real-time RT-PCR in homogenates of the ipsilateral L3 ventral quadrant from naive DA and PVG rats 7 and 14 d after VRA. *Mhc2ta* transcripts were upregulated after injury and were significantly higher in DA rats than in PVG rats both 7 and 14 d after VRA (data not shown). This indicates that the SNPs located in the 5′ flanking region affected transcription of *Mhc2ta*. We also studied transcript levels of *Cd74* in the same material and found that these were similar to those of *Mhc2ta* (data not shown). To determine whether *Mhc2ta* transcript levels were influenced by loci outside *Vra4*, we analyzed its expression in a group of heterogeneous AIL F_8 rats stratified only for haplotype at *D10Mgh25*, the *Vra4* marker with the maximum lod score. Transcripts levels of both *Mhc2ta* and *Cd74* were two to six times higher in rats with DA/DA alleles than in rats with PVG.1AV1/PVG.1AV1 alleles (**Fig. 4a**,**b**). We detected significant differences for all three *Mhc2ta* isoforms (data not shown). There was also a strong correlation in individual AIL F_8 rats between *Mhc2ta* and *Cd74* expression, despite the heterogeneity across the genome (**Fig. 4c**). Thus, the differential expression of MHC class II molecules was mapped by the identification of haplotypes and SNPs to a small QTL spanning the 5′ flanking and 5′ transcribed region of *Mhc2ta*.

To ascertain whether strain differences in expression of MHC class II molecules could also be detected with classical inflammatory stimuli, we injected IFN-γ or LPS unilaterally in the striatum of DA and PVG rats. Seventy-two hours after injection of 50 U IFN-γ, we observed widespread MHC class II labeling throughout the ipsilateral hemisphere in DA rats and substantially less pronounced labeling in PVG rats (**Fig. 5**). The labeled surface area, as determined by image analysis, was 9.1% in DA rats compared with 0.41% in PVG rats. We obtained similar results after injections with LPS (4.4% versus 1.2% labeled surface area, respectively), indicating that the observed strain-dependent MHC II expression is caused by a general MHC class II–regulating pathway common to several types of inflammatory stimuli.

Association of *MHC2TA* with inflammatory diseases

To study the impact of genetic heterogeneity in the human homolog *MHC2TA*, we genotyped SNPs in case-control samples for three human complex diseases with inflammatory components: myocardial infarction (387 cases and 387 controls), multiple sclerosis (548 cases and 528 controls) and rheumatoid arthritis (1,288 cases and 709 controls). In a previous study[14], the substitution –168A→G in the 5′ flanking region of the type III *MHC2TA* promoter was present at an allele frequency of 0.37 (ref. 14). On the basis of literature reports and our own findings in the rat, we selected the SNP —168A→G (rs3087456; SNP1) for genotyping, together with two additional SNPs in *MHC2TA*. We found significant differences in allele frequencies for SNP1 in individuals with myocardial infarction and

rheumatoid arthritis compared with their respective controls (**Table 1**). Analyzed with a dominant model, the presence of G at position −168 was significantly associated with increased risk of myocardial infarction ($P = 0.025$) and rheumatoid arthritis ($P = 0.008$). The odds ratio (OR; and 95% confidence interval, c.i.) for the presence of G in the myocardial infarction and rheumatoid arthritis cohorts were 1.39 (1.04–1.85) and 1.29 (1.07–1.56), respectively. There was no significant difference between individuals with multiple sclerosis and corresponding controls ($P = 0.312$), which were healthy blood donors matched by age and ethnicity but not gender. But individuals with multiple sclerosis significantly differed from all genotyped controls ($P = 0.038$) as well as from the rheumatoid arthritis controls ($P = 0.013$, OR = 1.34, 95% c.i. = 1.07–1.69). In contrast to multiple sclerosis, the rheumatoid arthritis control material was population-based. We observed no significant association for SNP2 (rs4774) or SNP3 (rs2229320plus27bp) in single-marker comparisons (**Supplementary Table 1** online). The association was explained by SNP1 alone and was not significantly improved by haplotype analysis. Therefore, the presence of G at position –168 of the 5′ flanking region of type III *MHC2TA* is associated with disease.

Expression of class II genes correlates with *MHC2TA* genotype

We next investigated whether the *MHC2TA* genotype was also functionally related to differential expression of *MHC2TA* and MHC class II molecules. We established an *ex vivo* stimulation protocol in which we used the key MHC molecule–inducing cytokine, IFN-γ, to stimulate peripheral blood cells (PBCs) and then determined whether *MHC2TA* haplotype variants were associated with differences in the expression of *MHC2TA*, *HLA-DRA*, *HLA-DQA1* and *CD74* (the MHC class II–associated invariant chain). We also analyzed expression of tumor necrosis factor (TNF) as a control for stimulation. The initial protocol was titrated using PBCs from healthy individuals (data not shown). We established PBC cultures from individuals with rheumatoid arthritis with different SNP1 genotypes and stimulated them with increasing concentrations of IFN-γ. Expression of TNF mRNA did not differ significantly between the groups (data not shown). In contrast, expression of *MHC2TA* mRNA was lower in cells from individuals with genotype GG than in cells from individuals with other genotypes at all concentrations of IFN-γ, although these differences became significant only at IFN-γ concentrations of 50 U ml^{-1} (**Fig. 6a**). We observed a similar, but more pronounced, segregation for all the MHC class II transcripts that we studied (**Fig. 6b–d**). Furthermore, there was

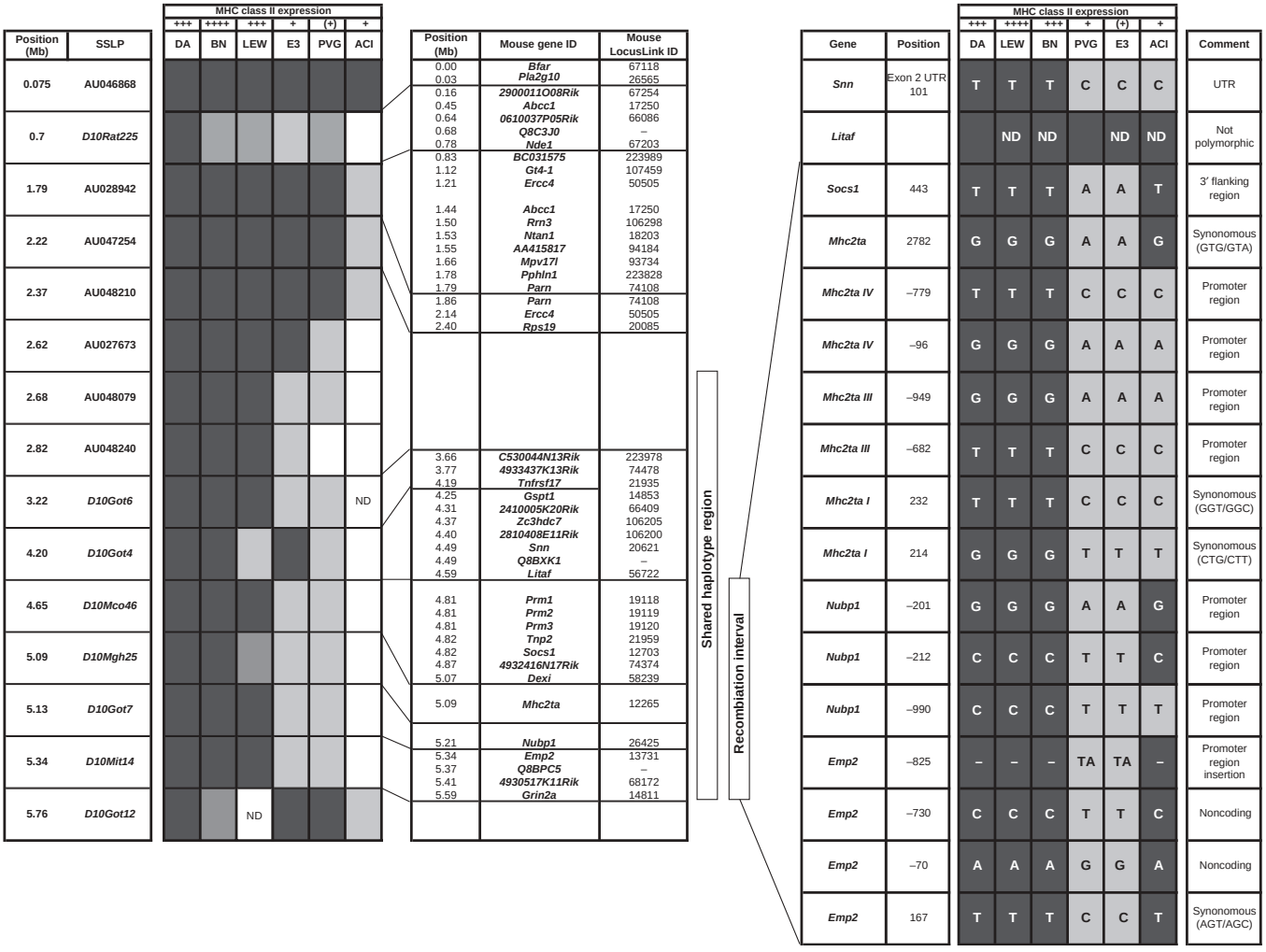

Figure 3 Haplotype map over *Vra4* in rat strains examined for expression of MHC class II molecules. Colors indicate the microsatellite size (left) and SNP allele (right). The shared microsatellite haplotype region is defined by markers AU048079 through *D10Mit14*; the recombination interval, by markers *D10Mco46* through *D10Mit14*. Genes from rat database (release 27.3e.1) with support from mouse database (release 27.33c.1) are included. ND, not determined.

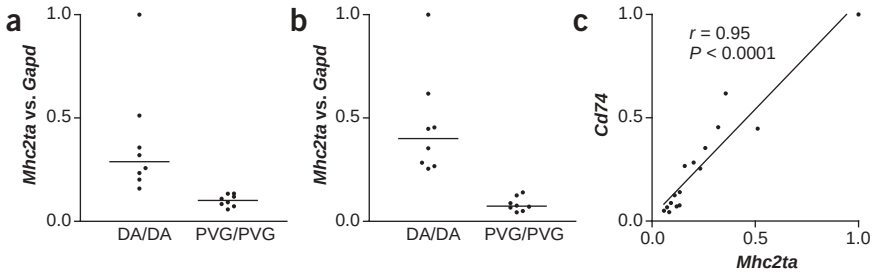

Figure 4 RT-PCR analysis of *Mhc2ta* and *Cd74* transcript levels in ipsilateral L3 segments from F_8 rats 14 d after VRA stratified for marker *D10Mgh25*. (**a**,**b**) DA/DA rats had significantly higher levels of both transcripts than did PVG.1AV1/ PVG.1AV1 (PVG/PVG) rats. (**a**) $P < 0.01$. (**b**) $P < 0.001$. (**c**) A high degree of correlation between levels of *Mhc2ta* and *Cd74* was present in individual F_8 rats.

a highly significant correlation between levels of *MHC2TA* mRNA and the other class II genes in individual samples (**Fig. 7**). These results strongly support the ideas that functional gene polymorphisms in *MHC2TA* are associated with the studied —168A→G substitution and that these have a most significant effect on the transcriptional regulation of MHC class II genes, leading to reduced expression of MHC molecules.

DISCUSSION

Combining findings from rat *in vivo* gene mapping in an AIL, haplotype analysis, gene sequencing and expression analysis with association and functional human case-control studies, we show here that rat strain–dependent differences in expression of MHC class II molecules on microglia are explained by polymorphisms in *Mhc2ta* and that a polymorphism in the human type III *MHC2TA* promoter is associated with low expression of MHC molecules and with susceptibility to three different human diseases with inflammatory components. This is a good example of how mapping of genes that regulate complex phenotypes in experimental rats is relevant for human disease. Of many candidates, we identified *MHC2TA* as relevant for differential expression of MHC molecules and human complex disease. Unlike ordinary association studies in which multiple genes are studied on the basis of preformulated hypotheses or linkage, here we identified *Mhc2ta* and *MHC2TA* using an unbiased approach and characterized a unique functional phenotype. This fact is important when interpreting the degree of statistical significance of association to disease. Although the evidence for an association to differential expression of MHC molecules is solid, the association to disease is of moderate significance and requires replication in larger samples. Larger sample sizes will also allow us to study influences on disease subphenotypes.

In the rat, we cannot completely rule out the possibility of functional polymorphisms outside the sequenced regions or in any of the few other genes, which have no known connection to inflammatory processes, still present in the intrarecombination interval. The fact that extensive differences between parental strains are present also after exposure to classical inflammatory stimuli suggests that the functional gene polymorphism has relevance for inflammatory responses in a broad sense.

MHC2TA was originally identified by cloning of a gene with a null mutation in hereditary MHC class II deficiency (bare lymphocyte syndrome)[15] and is a global regulator of the expression of proteins involved in antigen presentation and processing, including MHC class II and CD74 chains, HLA-DM and MHC class I molecules[16–19]. MHC2TA does not bind DNA itself but rather acts as a platform or chaperone for the assembly of several transcription factors at MHC promoters. It may also have an ancillary or modulatory role in the regulation of the expression of many other genes (*e.g.*, several kinases and phosphatases, transcription factors, cell cycle genes, chromatin remodelers and genes involved in cell signaling)[19]. The activity of MHC2TA is regulated primarily at the transcriptional level[5], which is under the control of four different promoters in humans (pI–pIV) and three promoters in rats (pI, pIII and pIV)[20]. Each promoter is activated in a tissue-specific manner and leads to the generation of different isoforms. A strong chain of evidence links MHC2TA to processes of fundamental importance for the immune system. Therefore, its expression is rate-limiting for constitutive and IFN-γ–induced expression of MHC class II molecules[21].

Transgenic *Mhc2ta*-deficient mice are totally devoid of cells expressing MHC class II molecules except for a subset of thymic epithelial cells[16] and are resistant to experimental autoimmune encephalomyelitis induced not only by active immunization with CNS myelin antigen but also by adoptive transfer of encephalitogenic class II-restricted $CD4^+$ Th1 cells, indicating that CNS antigen presentation is Mhc2ta-dependent[22,23]. More subtle differences may also have immunological effects, as the level of MHC class II molecules modulates immune response[24] and ectopic expression of *Mhc2ta* in transgenic mice leads to preferential differentiation into Th2 cells upon TCR

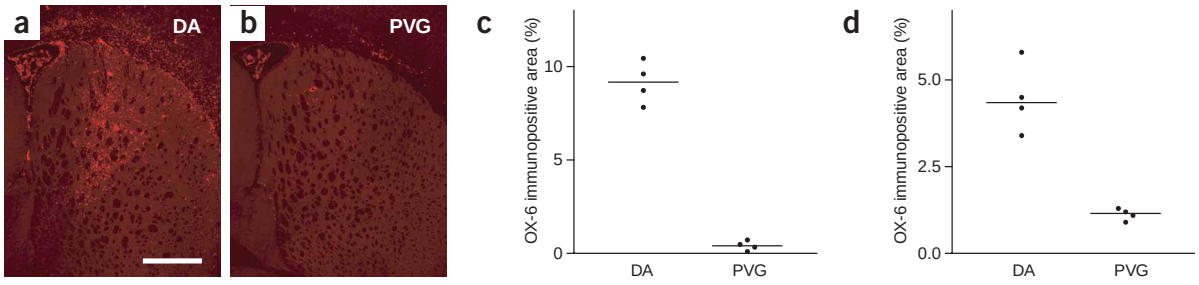

Figure 5 Expression of MHC class II molecules in the striatum after intracerebral injections of IFN-γ or LPS. (**a**,**b**) Micrographs showing IFN-γ–induced OX-6 immunostaining in the left striatum of DA (**a**) and PVG.1AV1 (PVG) (**b**) rats. Scale bar, 1 mm. (**c**) Scatter plot showing the OX-6–labeled surface area in the striatum after IFN-γ injection in DA and PVG.1AV1 (PVG) rats. (**d**) MHC class II levels after LPS injection showed a similar strain-specific pattern. (**c**) $P < 0.0001$. (**d**) $P < 0.001$.

Table 1 Genotype and allele frequencies for SNP1 (rs3087456)

	Codominant model					Allele frequencies					G dominant model					
	AA	AG	GG	P^a	P^b	A	G	MAF	P^a	P^b	AA	AG+GG	P^a	OR (95% c.i.)[c]	P^b	OR (95% c.i.)[d]
MI	199	160	17	0.038	0.002	558	194	0.258	0.102	0.014	199	177	0.025	1.39 (1.04–1.85)	0.002	1.44 (1.15–1.80)
MI controls	236	130	21			602	172	0.222			236	151				
MS	295	191	34	0.51	0.095	781	259	0.249	0.249	0.028	295	225	0.311	1.14 (0.89–1.46)	0.038	1.24 (1.01–1.51)
MS controls	304	177	27			785	231	0.227			304	204				
RA	728	451	83	0.022	0.047	1907	617	0.244	0.005	0.012	728	534	0.008	1.29 (1.07–1.56)	0.024	1.19 (1.02–1.38)
RA controls	449	221	34			1,119	289	0.205			449	255				
All controls	989	528	82			2,506	692	0.216			989	610				

[a]χ^2 test for comparison with controls for this group. [b]χ^2 test for comparison with all controls included in the study. [c]OR for dominant model with 95% c.i. for comparison with controls for this group. [d]OR for dominant model with 95% c.i. for comparison with all controls included in the study. MAF, minor allele frequency; MI, myocardial infarction; MS, multiple sclerosis; RA, rheumatoid arthritis.

activation[25]. In addition, several infectious pathogens target IFN-γ–induced expression of *MHC2TA* to escape the immune system[26].

Despite the wealth of data concerning the role of MHC2TA in the transcriptional regulation of immune-related proteins, involvement in the regulation of susceptibility to immune-related human disease has not previously been documented to our knowledge. This may be due to the fact that the genes contributing to complex diseases have modest impacts requiring large case-control studies to obtain sufficient statistical power[27]. This may be why some studies in small clinical samples did not show association of *MHC2TA* with autoimmune diseases[28–30]. On the basis of these results, it has been hypothesized that differences in the level of expression of MHC class II molecules stem from polymorphisms in the MHC class II promoters proper and that functional polymorphisms in *MHC2TA* would have too broad a spectrum of effects to be advantageous for an individual[29].

Our results conflict with this view as they identify two functional rat haplotypes in *Mhc2ta* shared by strains that express MHC class II molecules at low and high levels, respectively. In addition, the fact that expression of MHC class II molecules differs between strains after both mechanical injury and classic inflammatory stimuli argues for a direct effect on class II transcription, in concordance with the described properties of Mhc2ta. Functional polymorphisms in *MHC2TA* and thereby differential expression of MHC class II molecules could affect many inflammatory conditions, such as autoimmune responses. This idea is strengthened by the finding of an association of *MHC2TA* polymorphisms with susceptibility to three human diseases with known inflammatory components. *In vitro* stimulation experiments suggest that the risk allele is associated with reduced induction of MHC class II genes by inflammatory stimuli. If the effect on disease is mediated through the differential induction of MHC molecules, either high expression of MHC molecules may protect against disease or, alternatively, low expression of MHC molecules may promote disease. In the latter case, the pathogenic mechanism may be related to a less efficient presentation of antigens to protective, regulatory T cells. Studies suggest that such T cells exist in all three categories of diseases studied here: nervous tissue–specific autoimmune T cells may, under certain conditions, be involved in repair rather than damage, perhaps through the production of neurotrophins[31,32]; regulatory $CD4^+CD25^+$ T cells have been characterized in rheumatoid arthritis[33]; and $CD4^+$ T cells may be antiatherogenic in experimental atherosclerosis[34].

A further speculation along this line is that low levels of expression of MHC2TA may characterize separate disease entities within the clinically categorized syndromes. Our genetic analysis gave the highest degree of association using a dominant model. In contrast, the outcome of the *in vitro* stimulation experiment suggested a recessive effect. Disease susceptibility is a much more complex feature than the phenotype studied *in vitro*. Furthermore, because MHC2TA also regulates the transcription of a number of non-MHC genes, the involved pathways may differ[19]. Even if the increase in disease susceptibility conferred by the risk allele is relatively modest, the outcome is concordant in the samples we studied. Samples from individuals with multiple sclerosis were associated with disease when compared with the pooled control material as well as the population-based controls from the rheumatoid arthritis study, but not when compared with controls recruited from healthy blood donors. This discordance may reflect the fact that healthy blood donors, based on a number of exclusion criteria

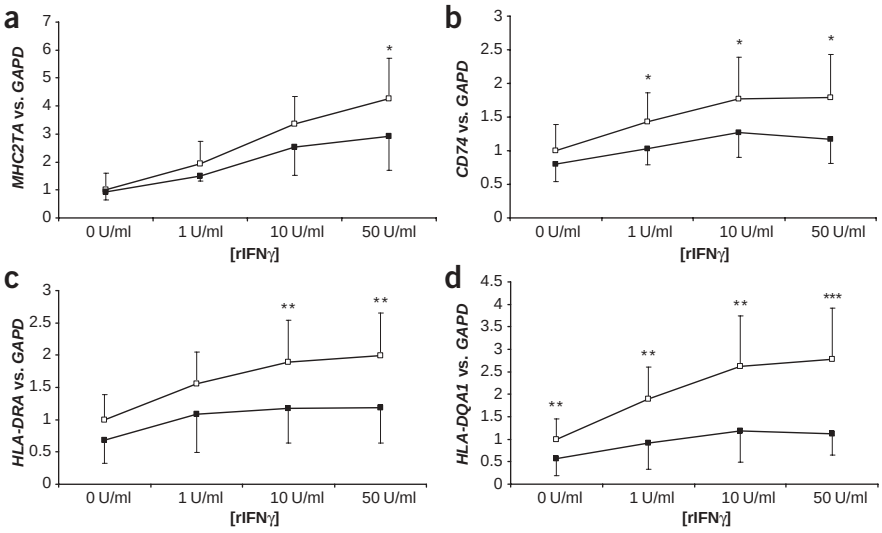

Figure 6 Effect of −168A→G polymorphism on class II associated transcript levels. Expression of *MHC2TA* (**a**), *CD74* (**b**), *HLA-DRA* (**c**) and *HLA-DQA1* (**d**) as dose-response to recombinant IFN-γ (rIFNγ) in human PBCs from homozygous –168G/G (filled squares, $n = 7$) or from –168A/G or –168A/A (open squares, $n = 37$) individuals. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

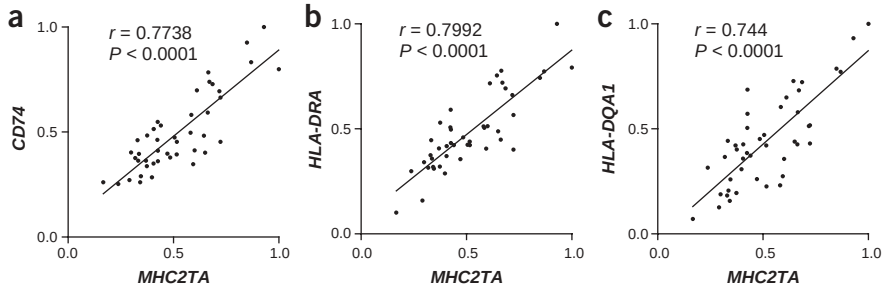

Figure 7 Correlation between MHC2TA and MHC class II associated transcripts in human PBMCs stimulated with 50 U ml^{-1} recombinant IFN-γ (rIFNγ). (**a**) CD74. (**b**) HLA-DRA. (**c**) HLA-DQA1.

such as chronic illness and certain medications, do not have the same allele frequencies as the general population.

The samples we studied comprise three different diseases with relatively high prevalence in the population, and the genetic heterogeneity in *MHC2TA* may therefore have a large effect on morbidity, like other genes involved in common complex diseases[27]. To the best of our knowledge, this is the first example of a gene with an effect on both classical autoimmune disorders and cardiovascular disease. In this context, links do exist between autoimmunity and cardiovascular morbidity[35]. Statins (3-hydroxy-3methylglutaryl-CoA reductase inhibitors) are widely used for prevention of cardiovascular disease. Their therapeutic effect is not confined to lowering cholesterol, as they also have anti-inflammatory effects, including downregulation of *MHC2TA* expression[36]. A statin was shown to ameliorate experimental autoimmune disease concomitant with lowering of *MHC2TA* mRNA levels[37]. Ongoing clinical trials now investigate the therapeutic potential of statins in several autoimmune diseases.

The results of this study have important implications for the interpretation of the clinical outcome of these studies, as the risk allele is associated with less induction of MHC class II genes in response to inflammatory stimuli. It would be advantageous to relate pharmacogenetically the clinical effect of statins to *MHC2TA* haplotype in these studies. Such a pharmacogenetic application may also be important for treatment guidelines and gene-environment interaction studies. For example, a genotype-specific interaction between HLA type and smoking was recently shown to influence the risk of rheumatoid arthritis[38]. Such prospectively collected case-control materials with extensive exposure information will enable a similar approach for the gene studied here.

In conclusion, we show here that rat strain–dependent differences in expression of MHC class II molecules are explained by polymorphisms in *Mhc2ta* and that an A→G substitution in the 5′ flanking region of type III promoter of *MHC2TA* is associated with lower induction of class II genes and susceptibility to inflammatory disease. Our results encourage association studies in other human diseases with inflammatory components, as well as detailed functional studies to unravel the mechanisms for the effect of *MHC2TA* on disease.

METHODS

Rats and nerve lesions. Strain DA(RT1^{av1}) was originally provided by H. Hedrich (Medizinische Hochschule, Hannover, Germany), and we obtained strain PVG.1AV1 from Harlan UK, Ltd. These two rat strains possess the same MHC complexes; any phenotypic differences in crosses between them will stem from non-MHC genes. We bred the rats used for experiments at our in-house breeding facility under specific pathogen-free and climate-controlled conditions with 12-h light/dark cycles, housed them in polystyrene cages containing wood shavings and fed them standard rodent chow and water *ad libitum*. The AIL was founded by male DA and female PVG.1AV1 rats, and vice versa. We carried out consecutive crossings of offspring from 50 breeding pairs for eight (F_8) or ten (F_{10}) generations. The two AIL experiments consisted of 24 parental rats and 126 male rats in the F_8 generation and 23 parental rats and 186 male rats in the F_{10} generation.

We subjected all rats to unilateral avulsion of the left L3–L5 ventral roots under standardized conditions and in deep isoflurane anesthesia at an age of 6–8 and 8–10 weeks for F_8 and F_{10} rats, respectively. They had a mean postoperative survival time of 14 d (± 2 h). We included unoperated controls. For expression analysis we used female DA and PVG.RT1^c (Scanbur BK) rats. We sampled tissues from naive rats and 7 or 14 d after VRA. We killed rats with CO_2 and perfused them with cold phosphate-buffered saline (PBS). We made serial transverse sections (14 µm) from the L4 segment of the spinal cord with a cryostat. We used ipsilateral quadrants of the L3 segment for semiquantitative PCR. All experiments in this study were approved by the local Ethical Committee for animal experimentation (Stockholms Norra Djurförsöksetiska Nämnd).

Immunohistochemistry and image analysis. The protocol used for immunohistochemistry has been described in detail[4]. We used antigen to rat Ia (MHC class II; clone OX-6, mouse IgG1, Serotec) in the first antibody step and biotin-conjugated donkey antibody to mouse and Cy3-conjugated streptavidin (Jackson ImmunoResearch) in the successive steps. We tested the specificity of the staining in control slides by incubation with an unrelated mouse antibody of the IgG1 isotype. We measured MHC class II immunofluorescence using a computer-based image analysis system[4]. We recorded measurements blindly on coded slides, with identical computer and camera settings for all sections. Each reading was taken from a rectangle of size 0.24 mm × 0.38 mm placed in the ventrolateral part of the ventral horn or the striatum, with a total of ten different spinal cord sections or four different brain sections from each rat analyzed.

Expression analysis. We carried out real-time RT-PCR on rat spinal cord as described[31]. We isolated total RNA from homogenized tissues using Qiagen total RNA extraction kit. Each spinal cord sample consisted of the ipsilateral ventral quadrant from the L3 segment. We exposed RNA samples to DNase digestion before cDNA synthesis. We carried out reverse transcription with 10 µl of total RNA, random hexamer primers (0.1 µg; Gibco BRL) and Superscript Reverse Transcriptase (200 U; Gibco BRL). For TaqMan PCR, we amplified cDNA on an ABI PRISM 7700 Sequence Detection System (Perkin Elmer) with a two-step PCR protocol (95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min). *Mhc2ta*, *Cd74* and *Gapd* primers and probes are given in **Supplementary Table 2** online. We determined the expression of Mhc2ta isoforms I, III and IV and Nubp1 by Qiagen QuantiTect SYBR green (Qiagen) in accordance with the manufacturer's instructions and amplified them using an ABI prism 7700 Sequence Detection System (Perkin Elmer). We assessed primer specificity by analyzing amplicon dissociation curves in each sample. Primers and probes are given in **Supplementary Table 2** online. For *Gapd*, we used the same primers that we used for TaqMan PCR. We isolated RNA and synthesized cDNA from human peripheral blood mononuclear cells (PBMCs) as described[39] for *GAPD*, *MHC2TA*, *TNF*, *HLA-DQA1*, *HLA-DRA* and *CD74*. Primers and probes are given in **Supplementary Table 2** online. We quantified relative transcript levels using the standard curve method and normalized target transcript levels to the corresponding level of *GAPD* transcripts in each sample. We used samples without template or template where the reverse transcription step had been omitted as controls for unspecific contamination and amplification of genomic DNA, respectively.

Genotyping. We extracted genomic DNA from rat tail tips using a standard protocol. We amplified microsatellites from genomic DNA from in-house–bred DA, PVG.1AV1, LEW, ACI and BN rats. DNA from E3 rats was provided by R. Holmdahl (University of Lund, Lund, Sweden). We selected PCR primers for polymorphic simple sequence-length polymorphisms from available Internet

databases (Rat Genome Database, Center for Genomic Research, Whitehead Institute/MIT and UniSTS at National Center for Biotechnology Information). We purchased primers from PROLIGO. We labeled one primer in each pair with [γ-^{33}P]ATP (PerkinElmer), amplified genomic DNA with a standard PCR protocol and separated the amplified fragments on 6% polyacrylamide gels. Genotypes were recorded manually from autoradiographic films independently by two investigators. We included DNA from DA and PVG.1AV1 rats for every marker. We mapped the simple sequence-length polymorphism marker order using the MAPMAKER/EXP[40] computer program.

We obtained data on physical position of rat microsatellite markers, annotated or predicted genes and homologous genes in the mouse from the Ensembl Genome Browser release 27.3e.1 and 27.33c.1, respectively. Homologs of mouse *Pphln1* and *Rps19* were predicted for *Vra4*, although these are located outside the homologous mouse region 16A3-B1.

Sequencing. We amplified sequencing templates on a PTC-225 (MJ research) thermal cycler using a standard three-step PCR protocol with primers purchased from PROLIGO. We carried out the sequencing reaction using the BigDye terminator (Applied Biosystems) and separated and recorded the products on an ABI 3100 (Applied Biosystems). We analyzed DNA sequences using Vector NTI software (InforMax). We sequenced the complete coding sequence and 1 kb upstream of *Mhc2ta* in each of the three isoforms' 5′ flanking regions. We sequenced the other genes as follows: *Nubp1* (1,000 bp of 5′ flanking region and coding sequence), *Soc1* (900 bp of 5′ flanking region, coding sequence and 150 bp of 3′ flanking region), *Emp2* (975 bp of 5′ flanking region, coding sequence and 220 bp of 3′ flanking region), *Litaf* (coding sequence except exon 1), *SNN* (400 bp of 5′ flanking region and coding sequence with 80 bp of the 5′ untranslated region and 250 bp of the 3′ untranslated region).

***In vivo* stimulation experiments.** We administered stereotactic intracerebral injections in the striatum (coordinates: anterior-posterior, −0.3; medial-lateral, +2.5; dorso-ventral, −4.3) to isoflurane-anesthetized 8- to 10-week-old male DA and PVG.RT1^{c} (Scanbur BK) rats using a Hamilton syringe (type 701 RN, gauge 26s). The total injection volume was 2 µl over 2 min, containing a total of 50 U IFN-γ, 5 µg LPS or vehicle alone (fluorophore-labeled dextran in PBS). We removed brains and kept them at −70 °C until sectioning them at 14 µm. We collected sections from the appropriate positions in the brain. We dissected LPS-stimulated spleen from PBS-perfused rats 3 h after intraperitoneal administration of LPS (5 mg per kg body weight).

Linkage and statistical analysis. We carried out linkage analysis using MAPMAKER/QTL[41]. We subjected image analysis data for expression of MHC class II molecules to log (base 10) transformation to obtain normally distributed data. We carried out rat strain comparisons by Students' *t*-test (IFN-γ and LPS injections). We applied nonparametric statistics (Mann-Whitney) for analysis of mRNA expression using GraphPad Prism 3.0 (GraphPad Software Inc.). We analyzed correlation between expression of *Mhc2ta* or MHC2TA and *Cd74* or CD74 in rats and human PBCs by Spearman rank test. We compared genotypes and allele frequencies using the χ^2 test or Fisher exact test and considered differences to be significant at $P < 0.05$ (StatView, version 5.0, SAS Institute Inc.). *P* values in **Table 1** and **Supplementary Table 1** online were not adjusted for multiple comparisons. We estimated haplotype frequencies by the EM algorithm using the Arlequin software 2.000 (ref. 42) or by Bayesian methods with PHASE 2.1 (ref. 43).

Clinical material. The multiple sclerosis cohort consisted of 548 subjects (413 females and 135 males; mean age 43.9 y, median age 44.0 y, range 13–80 y) originating from Sweden or other Nordic countries fulfilling the McDonald criteria of definite multiple sclerosis[44] recruited by neurologists at the Karolinska University Hospital Huddinge and Solna sites (tertiary referral centers in Stockholm). The corresponding control group consisted of 528 blood donors (217 females and 311 males; mean age 42.7 y, median age 43.0 y, range 18–70 y) residing in the Stockholm area and originating from Sweden or other Nordic countries. Sixty-one percent of the individuals with multiple sclerosis and 29% of the controls carried *HLA-DRB1**1501.

The rheumatoid arthritis cohort consisted of 1,288 subjects (919 females and 369 males; mean age 51.6 y, median age 54.0 y, range 18–70 y; 805 rheumatoid factor–positive, 416 rheumatoid factor–negative, data missing for 67). Newly diagnosed individuals with rheumatoid arthritis were identified in several rheumatology units in Sweden and included in the study if their symptoms fulfilled the ACR criteria of 1987 (ref. 45). The individuals in the corresponding control group ($n = 709$; 522 females, 187 males; mean age 53.4 y, median age 56.0 y, range 18–70 y) were matched by age, sex and residential area. The recruitment of affected individuals and controls was described previously in connection with EIRA study[46]. Ninety-seven percent of the study population was of self-reported Caucasian origin. Seventy-three percent of the individuals with rheumatoid arthritis and 53% of the controls carried *HLA-DRB1**01, *04 or *10.

The myocardial infarction cohort consisted of 387 subjects (68 females and 319 males; mean age 52.5 y, median age 54.0 y, range 49–57 y) recruited from all patients less than 60 y of age who were admitted for acute myocardial infarction to the coronary care units of the three hospitals in the northern part of Stockholm (Danderyd Hospital, Karolinska Hospital and Norrtälje Hospital) during January 1996–December 2000. Recruitment, exclusion criteria and basic characteristics of participants have been described[47]. Ninety-nine percent of the myocardial infarction study group was of self-reported Caucasian origin. The participation rate of eligible patients was 76%. The corresponding control group (69 females and 318 males; mean age 53.0 y, median age 54.0 y, range 49–57 y) comprised sex- and age-matched healthy persons recruited from the general population of the same county.

In the final association analysis, we compared genotype frequencies between the specific disease group and the respective control. The allele and genotype frequencies did not differ statistically between the different control groups. We could therefore also compare each disease with all controls combined to increase statistical power. The human studies were done in agreement with the Declaration of Helsinki, and the protocols were approved by the local ethics committees. Informed consent was obtained from all individuals.

SNP genotyping. On the basis of available information in public databases (National Center for Biotechnology Information), we initially selected seven SNPs in *MHC2TA*. Three of these had to be excluded (two were monomorphic and one assay did not perform robustly (success rate <85%) in our optimizations). We initially typed the remaining four SNPs (rs3087456, rs4774, rs2229320plus27bp and rs2228238) in 186 individuals with multiple sclerosis and 186 of the corresponding controls. SNP rs2228238 was redundant for haplotype analysis and was therefore not analyzed further. We typed the remaining three SNPs in all three populations and their respective control groups: SNP1: rs3087456 (–168A→G from translation start or –155 from transcription initiation), CCTCCC(C/T)ACACCTCT; SNP2: rs2229320plus 27bp (nt 2,536 from coding sequence, exon 11), CAGCTGCT(G/T)GAGCT GCT; and SNP3: rs4774 (nt 1,614 from coding sequence, exon 11), CCTA GACG(G/C)CTTCGAGG. We used a 5′ nuclease assay to genotype SNP1 in individuals with myocardial infarction and rheumatoid arthritis. We genotyped SNP2 by DASH[48] in individuals with myocardial infarction. We used MALDI-TOF[49] to type all three SNPs in individuals with multiple sclerosis and controls as well as to type SNP2 and SNP3 in individuals with rheumatoid arthritis and SNP3 in individuals with myocardial infarction. All analyzed markers were in Hardy-Weinberg equilibrium.

***In vitro* stimulation of PBCs.** We obtained peripheral blood samples from individuals with rheumatoid arthritis who were homozygous ($n = 7$ for GG, $n = 25$ for AA) or heterozygous ($n = 12$) with respect to SNP1. There were no differences in type of therapy between selected groups of donors. We sampled peripheral blood in cell preparation tubes containing sodium citrate (Vacutainer CPT, Becton Dickinson). We separated PBCs by density-gradient centrifugation. We collected interphase cells and washed twice them with Dulbecco's PBS. We assessed the proportion of viable cells with trypan blue. More than 95% of the cells excluded trypan blue in each preparation. We evaluated the stimulation protocol in initial experiments using PBCs from three healthy subjects. Immediately after preparation, we added 1, 10 or 50 U of recombinant human IFN-γ (Preprotech) or medium alone to cultures of 5×10^5 cells each. After 6 h of culturing, we pelleted the cells and lysed them for RNA extraction.

URLs. The Rat Genome Database is available at http://rgd.mcw.edu/. The Center for Genomic Research at the Whitehead Institute/MIT is available at http://www.broad.mit.edu/rat/public/. UniSTS, LocusLink and the National

Center for Biotechnology Information are available at http://www.ncbi.nlm.nih.gov/. The Ensembl Genome Browser is available at http://www.ensembl.org/. The HUGO Gene Nomenclature Committee is available at http://www.gene.ucl.ac.uk/nomenclature/.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Flügel, A. *et al.* Migratory activity and functional changes of green fluorescent effector cells before and during experimental autoimmune encephalomyelitis. *Immunity* **14**, 547–560 (2001).
2. Sedgwick, J.D., Schwender, S., Gregersen, R., Dorries, R. & ter Meulen, V. Resident macrophages (ramified microglia) of the adult brown Norway rat central nervous system are constitutively major histocompatibility complex class II positive. *J. Exp. Med* **177**, 1145–1152 (1993).
3. Piehl, F. *et al.* Non-MHC gene regulation of nerve root injury-induced spinal cord inflammation and neuron death. *J. Neuroimmunol.* **101**, 87–97 (1999).
4. Lundberg, C., Lidman, O., Holmdahl, R., Olsson, T. & Piehl, F. Neurodegeneration and glial activation patterns after mechanical nerve injury are differentially regulated by non-MHC genes in congenic inbred rat strains. *J. Comp. Neurol.* **431**, 75–87 (2001).
5. Ting, J.P. & Trowsdale, J. Genetic control of MHC class II expression. *Cell* **109**, 21–33 (2002).
6. Boss, J.M. & Jensen, P.E. Transcriptional regulation of the MHC class II antigen presentation pathway. *Curr. Opin. Immunol.* **15**, 105–111 (2003).
7. Dahlman, I. *et al.* Genome-wide linkage analysis of chronic relapsing experimental autoimmune encephalomyelitis in the rat identifies a major susceptibility locus on chromosome 9. *J. Immunol.* **162**, 2581–2588 (1999).
8. Lidman, O. *et al.* Discrete gene loci regulate neurodegeneration, lymphocyte infiltration, and major histocompatibility complex class II expression in the CNS. *J. Neurosci.* **23**, 9817–9823 (2003).
9. Maehlen, J., Schroder, H., Klareskog, L., Olsson, T. & Kristensson, K. Axotomy induces MHC class I antigen expression on rat nerve cells. *Neurosci. Lett.* **92**, 8–13 (1988).
10. Streit, W.J., Graeber, M.B. & Kreutzberg, G.W. Peripheral nerve lesion produces increased levels of major histocompatibility complex antigens in the central nervous system. *J. Neuroimmunol.* **21**, 117–123 (1989).
11. Darvasi, A. & Soller, M. Advanced intercross lines, an experimental population for fine genetic mapping. *Genetics* **141**, 1199–1207 (1995).
12. Jagodic, M. *et al.* An advanced intercross line resolves Eae18 into two narrow quantitative trait loci syntenic to multiple sclerosis candidate loci. *J. Immunol.* **173**, 1366–1373 (2004).
13. Libby, P. Inflammation in atherosclerosis. *Nature* **420**, 868–874 (2002).
14. Patarroyo, J.C. *et al.* Single nucleotide polymorphisms in MHC2TA, the gene encoding the MHC class II transactivator (CIITA). *Genes Immun.* **3**, 34–37 (2002).
15. Steimle, V., Otten, L.A., Zufferey, M. & Mach, B. Complementation cloning of an MHC class II transactivator mutated in hereditary MHC class II deficiency (or bare lymphocyte syndrome). *Cell* **75**, 135–146 (1993).
16. Chang, C.H., Guerder, S., Hong, S.C., van Ewijk, W. & Flavell, R.A. Mice lacking the MHC class II transactivator (CIITA) show tissue-specific impairment of MHC class II expression. *Immunity* **4**, 167–178 (1996).
17. Chang, C.H. & Flavell, R.A. Class II transactivator regulates the expression of multiple genes involved in antigen presentation. *J. Exp. Med.* **181**, 765–767 (1995).
18. Kern, I., Steimle, V., Siegrist, C.A. & Mach, B. The two novel MHC class II transactivators RFX5 and CIITA both control expression of HLA-DM genes. *Int. Immunol.* **7**, 1295–1299 (1995).
19. Nagarajan, U.M., Bushey, A. & Boss, J.M. Modulation of gene expression by the MHC class II transactivator. *J. Immunol.* **169**, 5078–5088 (2002).
20. Muhlethaler-Mottet, A., Otten, L.A., Steimle, V. & Mach, B. Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. *EMBO J.* **16**, 2851–2860 (1997).
21. Otten, L.A., Steimle, V., Bontron, S. & Mach, B. Quantitative control of MHC class II expression by the transactivator CIITA. *Eur. J. Immunol.* **28**, 473–478 (1998).
22. Stüve, O. *et al.* The role of the MHC class II transactivator in class II expression and antigen presentation by astrocytes and in susceptibility to central nervous system autoimmune disease. *J. Immunol.* **169**, 6720–6732 (2002).
23. Tompkins, S.M. *et al.* De novo central nervous system processing of myelin antigen is required for the initiation of experimental autoimmune encephalomyelitis. *J. Immunol.* **168**, 4173–4183 (2002).
24. Baumgart, M., Moos, V., Schuhbauer, D. & Muller, B. Differential expression of major histocompatibility complex class II genes on murine macrophages associated with T cell cytokine profile and protective/suppressive effects. *Proc. Natl. Acad. Sci. USA* **95**, 6936–6940 (1998).
25. Otten, L.A. *et al.* Deregulated MHC class II transactivator expression leads to a strong Th2 bias in CD4+ T lymphocytes. *J. Immunol.* **170**, 1150–1157 (2003).
26. Hegde, N.R., Chevalier, M.S. & Johnson, D.C. Viral inhibition of MHC class II antigen presentation. *Trends Immunol.* **24**, 278–285 (2003).
27. Ueda, H. *et al.* Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* **423**, 506–511 (2003).
28. Sartoris, S. *et al.* Analysis of CIITA encoding AIR-1 gene promoters in insulin-dependent diabetes mellitus and rheumatoid arthritis patients from the northeast of Italy: absence of sequence variability. *Hum. Immunol.* **61**, 599–604 (2000).
29. Janitz, M., Reiners-Schramm, L., Muhlethaler-Mottet, A., Rosowski, M. & Lauster, R. Analysis of the sequence polymorphism within class II transactivator gene promoters. *Exp. Clin. Immunogenet.* **18**, 199–205 (2001).
30. Rasmussen, H.B., Kelly, M.A. & Clausen, J. Genetic susceptibility to multiple sclerosis: detection of polymorphic nucleotides and an intron in the 3′ untranslated region of the major histocompatibility complex class II transactivator gene. *Hum. Immunol.* **62**, 371–377 (2001).
31. Hammarberg, H. *et al.* Neuroprotection by encephalomyelitis: rescue of mechanically injured neurons and neurotrophin production by CNS-infiltrating T and natural killer cells. *J. Neurosci.* **20**, 5283–5291 (2000).
32. Olsson, T., Lidman, O. & Piehl, F. Harm or heal - divergent effects of autoimmune neuroinflammation? *Trends Immunol.* **24**, 5–6 (2003).
33. Cao, D. *et al.* Isolation and functional characterization of regulatory CD25brightCD4+ T cells from the target organ of patients with rheumatoid arthritis. *Eur. J. Immunol.* **33**, 215–223 (2003).
34. Mallat, Z. *et al.* Induction of a regulatory T cell type 1 response reduces the development of atherosclerosis in apolipoprotein E-knockout mice. *Circulation* **108**, 1232–1237 (2003).
35. Turesson, C., Jarenros, A. & Jacobsson, L. Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study. *Ann. Rheum. Dis.* **63**, 952–955 (2004).
36. Kwak, B., Mulhaupt, F., Myit, S. & Mach, F. Statins as a newly recognized type of immunomodulator. *Nat. Med.* **6**, 1399–1402 (2000).
37. Youssef, S. *et al.* The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* **420**, 78–84 (2002).
38. Padyukov, L.C.S., Stolt, P., Alfredsson, L. & Klareskog, L. The EIRA study group. A gene-environment interaction between smoking and shared epitope genes in HLA-DRB1 provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum.* **50**, 3085–3092 (2004).
39. Khademi, M. *et al.* T Cell Ig- and mucin-domain-containing molecule-3 (TIM-3) and TIM-1 molecules are differentially expressed on human Th1 and Th2 cells and in cerebrospinal fluid-derived mononuclear cells in multiple sclerosis. *J. Immunol.* **172**, 7169–7176 (2004).
40. Lander, E.S. *et al.* MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**, 174–181 (1987).
41. Lander, E.S. & Botstein, D. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199 (1989).
42. Schneider, S., Roessli, D. & Excoffier, L. *Arlequin ver. 2000: A Software for Population Genetics Data Analysis* (Genetics and Biometrics Laboratory, University of Geneva, Switzerland, 2000).
43. Stephens, M. & Donnelly, P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet.* **73**, 1162–1169 (2003).
44. McDonald, W.I. *et al.* Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann. Neurol.* **50**, 121–127 (2001).
45. Arnett, F.C. *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* **31**, 315–324 (1988).
46. Stolt, P. *et al.* Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann. Rheum. Dis.* **62**, 835–841 (2003).
47. Eriksson, P. *et al.* Human evidence that the cystatin C gene is implicated in focal progression of coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **24**, 551–557 (2004).
48. Jobs, M., Howell, W.M., Strömqvist, L., Mayr, T. & Brookes, A.J. DASH-2 flexible, low-cost, and high-throughput SNP genotyping by dynamic allele-specific hybridization on membrane arrays. *Genome Res.* **13**, 916–924 (2003).
49. Jurinke, C., van den Boom, D., Cantor, C.R. & Koster, H. Automated genotyping using the DNA MassArray technology. *Methods Mol. Biol.* **187**, 179–192 (2002).