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Eae19, a New Locus on Rat Chromosome 15 Regulating Experimental Autoimmune Encephalomyelitis

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ABSTRACT

Multiple sclerosis (MS) and its animal model, myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (MOG-EAE), share a complex genetic predisposition with contributions from the major histocompatibility complex class II genes and many other genes. Linkage mapping in F_2 crosses between the susceptible DA rat strain and the resistant ACI or BN rat strains in various models of autoimmune neuroinflammation have repeatedly displayed suggestive linkage to a region on rat chromosome 15. A direct study of this region was undertaken in congenic strains by transferring resistant ACI alleles to the susceptible DA background. Phenotypic analysis demonstrated lower maximal and cumulative EAE scores in the DA.ACI–*D15Rat6-D15Rat71* (C15), DA.ACI–*D15Rat6-D15Rat48, D15Rat126-D15Rat71* (C15R3b), and DA.ACI–*D15Rat23-D15rat71* (C15R4) strains compared to the parental DA rat strain. Linkage analysis was then performed in a (DA × PVG.AV1)F_7 advanced intercross line, resulting in a LOD score of 4.7 for the maximal EAE score phenotype at the peak marker D15Rat71 and a confidence interval of 13 Mb, overlapping with the congenic fragment defined by the C15R3b and the C15R4 strains. Thus, a new MOG-EAE locus with the designation *Eae19* is identified on rat chromosome 15. There are 32 confirmed or predicted genes in the confidence interval, including immune-responsive gene 1 and neuronal ceroid lipofuscinose gene 5. Definition of loci such as *Eae19* enables the characterization of genetically regulated, evolutionary conserved disease pathways in complex neuroinflammatory diseases.

MULTIPLE sclerosis (MS) is a chronic inflammatory demyelinating disease that affects the central nervous system. Susceptibility to MS is based on interactions among several genes and influences by, largely unknown, nongenetic factors (Ebers *et al.* 1986, 1995; Sadovnick *et al.* 1993, 1996; Ebers 1996). The major histocompatibility complex (MHC) has been known to regulate MS since 1972 (Jersild *et al.* 1973; Ebers *et al.* 1986; Olerup and Hillert 1991). So far, very few individual non-MHC genes regulating MS have been identified by whole-genome scans or association studies due to the heterogeneity, polygeneity, and environmental influences in MS (Ebers *et al.* 1996; Haines *et al.* 1996, 2002; Sawcer *et al.* 1996; Kuokkanen *et al.* 1997; Chataway *et al.* 1998; Broadley 2001; Coraddu 2001; Akesson *et al.* 2002; Ban *et al.* 2002). Animal models of MS, such as experimental autoimmune encephalomyelitis (EAE), can circumvent these problems by minimizing the heterogeneity and controlling the environmental conditions (Lucchinetti *et al.* 1996; Lassmann *et al.* 2001). Unbiased identification of genes controlling autoimmune neuroinflammation is important, since such genes represent evolutionarily conserved disease pathways that are prime candidates for therapeutic interventions. A major problem for other approaches in MS, such as studying selected candidate regulatory molecules and cellular subsets, is to determine if the observed deviation is a cause or consequence of disease and if the pathway is involved in disease progression or protection.

EAE induced with myelin oligodendrocyte glycoprotein (MOG) in certain rat strains shares features of MS such as a relapsing/remitting disease course and a prominent demyelination (Adelmann *et al.* 1995; Johns *et al.* 1995). The formation of demyelinated lesions in MOG-EAE depends on both T cells and anti-MOG antibodies (Linington *et al.* 1988). MHC class II genes and multiple other genes influence this response (Weissert *et al.* 1998b; Dahlman *et al.* 1999b; Jagodic *et al.* 2001). An autoimmune response against MOG in MS patients suggests that MOG plays an important role also in the pathogenesis of MS (Sun *et al.* 1991; De Rosbo *et al.* 1993; Wallstrom *et al.* 1998). Thus, MOG-EAE is a relevant model to utilize in studies of mechanisms underlying the development of autoimmune neuroinflammation. The dark Agouti (DA) rat strain is susceptible to MOG-EAE, while the PVG.AV1 and the ACI strains are relatively resistant (Weissert *et al.* 1998b). The DA, PVG.AV1, and ACI rat strains all share the MHC haplo-

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type RT1.AV1 (Hedrich 1990). This allows the establishment of intercrosses and congenic strains specifically aimed at identifying non-MHC loci regulating MOG-EAE.

Previous studies of MOG-EAE, whole-spinal-cord-induced EAE, and experimental autoimmune neuritis (EAN) have found a suggestive linkage (Lander and Kruglyak 1995) to a region on rat chromosome 15. In MOG-EAE and EAN, the suggestive linkage was observed in (DA × ACI)F_2 rats subjected to genome scans with microsatellite markers (Dahlman *et al.* 1999b, 2001). In spinal-cord-induced EAE, a suggestive linkage was observed in (DA × BN)F_2 rats (Dahlman *et al.* 1999a).

To determine if the rat chromosome 15 region indeed is important for the development of MOG-EAE, we transferred this region from the EAE-resistant ACI to the susceptible DA background with a speed congenic approach (Wakeland *et al.* 1997). Linkage mapping was then performed in a (DA × PVG.AV1)F_7 advanced intercross line (AIL) (Darvasi and Soller 1995, 1997; Jagodic *et al.* 2004). An AIL is created by random intercross breeding of two inbred strains for several generations, resulting in genetically unique individuals with a mixture of founder chromosomal fragments. Theoretically, an AIL gives at least a $t/2$-fold reduction in the confidence interval compared to an F_2 cross, given that t, where t is the number of generations, is large enough (Darvasi and Soller 1997; Xiong and Guo 1997). We combine the congenic strain and the AIL analysis to define a new MOG-EAE locus designated *Eae19*.

MATERIALS AND METHODS

Parental rat strains and basic conditions: DA rats were originally obtained from the Zentralinstitut for Versuchstierzucht (Hans Hedrich, Hannover, Germany) and A × C 9935 Irish (ACI) rats were from Harlan Sprague Dawley (Indianapolis). MHC-congenic (RT1.AV1) Piebald-Viral-Glaxo (PVG) rats, PVG.AV1 (also previously referred to as PVG-$RT1^a$), were obtained from Harlan UK (Blackthorn, UK). All the rats were locally bred in the animal facility at the Center for Molecular Medicine, Karolinska Institutet. Eight- to 15-week-old male and female rats were used in the six experiments with congenic rats. Rats were routinely tested for specific pathogens according to a health-monitoring program for rats at the National Veterinary Institute in Uppsala, Sweden. They were kept in a 12-hr light/12-hr dark cycle and housed in polystyrene cages containing aspen wood shavings, with free access to water and autoclaved standard rodent chow. The local ethical committee approved the experiments.

Breeding of the chromosome 15 congenic strains and the advanced intercross line: Speed congenics were generated with a marker-assisted selection technique, mainly as described by Wakeland *et al.* (1997). An ~25-cM fragment of ACI alleles from the D15Rat6 marker to the D15Rat71 marker was transferred to the DA rat background. Initially, (DA × ACI)F_1 rats were backcrossed to DA rats. From the N_2 generation, the rats were genotyped with 70 microsatellite markers outside the congenic region, with a mean distance between markers of 20 cM. One male rat from each generation, having the least amount of remaining donor (ACI) alleles in the genome, was selected for further breeding and mated with several DA female rats. In the N_6 generation, all 70 background markers were fixed as DA homozygous. One further backcross was performed and heterozygous rats for the chromosome 15 region were subsequently intercrossed to produce the congenic strain DA.ACI–*D15Rat6-D15Rat71* (N_7F_1). From the first intercross, offspring rats were genotyped with eight microsatellite markers within the congenic region to detect intraregional recombinations. We selected the full-length congenic strain DA.ACI–*D15Rat6-D15Rat71* (C15) and the recombinant congenic strains DA.ACI–*D15Rat6-D15rat13* (C15R1), and DA.ACI–*D15Rat6-D15Rat48* (C15R3), and DA.ACI–*D15Rat23-D15rat71* (C15R4) for experiments. After the fifth experiment we regenotyped the rats and found that some C15R3 rats shared a region with the C15R4 from D15Rat126 to D15Rat71, so we separated the C15R3 into DA.ACI–*D15Rat6-D15Rat48* (C15R3a) and DA.ACI–*D15Rat6-D15rat48, D15Rat126-D15Rat71* (C15R3b) according to the genotyping results.

The advanced intercross line originated from the DA and the PVG.AV1 rat strains that share the RT1.AV1 MHC haplotype, thus allowing identification of non-MHC genes. One important reason for choosing the DA × PVG strain combination was to permit dense genotyping, since these strains display a high rate of polymorphic microsatellite markers: ~60% (compared to ~10% for the DA × ACI strain combination) according to the Whitehead Institute (http://www-genome.wi.mit.edu/rat/public/). The breeding scheme for the (DA × PVG.AV1) AIL has previously been reported (Jagodic *et al.* 2004). Briefly, to create the F_1 generation, breeding pairs with DA female founders and PVG.AV1 female founders were established. The F_2 generation was produced from seven couples each of F_1 rats with DA and PVG.AV1 as female founders, respectively. The F_3 generation originated from 50 breeding couples with both types of female founders. Random breeding of 50 males and females, consistently avoiding brother-sister mating, produced the subsequent generations. Three F_7 litters were produced from the 50 F_6 breeding couples for the MOG-EAE experiments.

Induction and clinical assessment of MOG-EAE: The rats were anesthetized with sevoflurane and immunized intradermally in the tail base. Each rat received 200-μl inoculums containing 100 μl recombinant rat MOG (rMOG; aa 1–125) in saline emulsified in 100 μl incomplete Freund's adjuvans (Sigma-Aldrich, St. Louis). The dose of rMOG (aa 1–125) was selected upon titration in the susceptible parental DA rats. In the congenic strain experiments, the dose was 13, 20, or 65 μg/rat, depending on the batch of rMOG, and 40 μg/rat for the AIL animals. Animals were weighed and clinical signs of disease were evaluated from day 7 to day 33–40 postimmunization (p.i.). The clinical signs were scored as follows: 1, tail weakness or tail paralysis; 2, hind leg paraparesis (gait disturbance) or hemiparesis; 3, hind leg paraparalysis or hemiparalysis; 4, tetraparalysis, urinary, and/or fecal incontinence. A relapsing/remitting disease course was defined as an improvement in the disease score either from 3 or 4 to 1 or from 2, 3, or 4 to 0, which was maintained for at least 2 consecutive days and followed by an increase in the clinical score of at least 2 points that lasted for at least 2 days.

Genotyping: A total of 152 clinically affected rats and 162 randomly selected unaffected rats in the (DA × PVG.AV1)F_7 AIL were genotyped. Affected animals were selected on the basis of displaying unambiguous signs of the disease (minimum score 1 for >2 days accompanied with weight loss). Rats in the unaffected group did not display any signs of disease, including a steady increase in weight (Jagodic *et al.* 2004). DNA was extracted from the tail tip according to a standard protocol (Laird *et al.* 1991). The region analyzed in the AIL included the region defined in the full-length congenic C15 strain (Figure 1). This 25-cM (~53 Mb) large region, extending from D15Rat6 to D15Rat71, was first genotyped with 15 microsatellite markers, and then another region from D15Rat71 to D15Rat103 (~15 Mb) near the telomere was

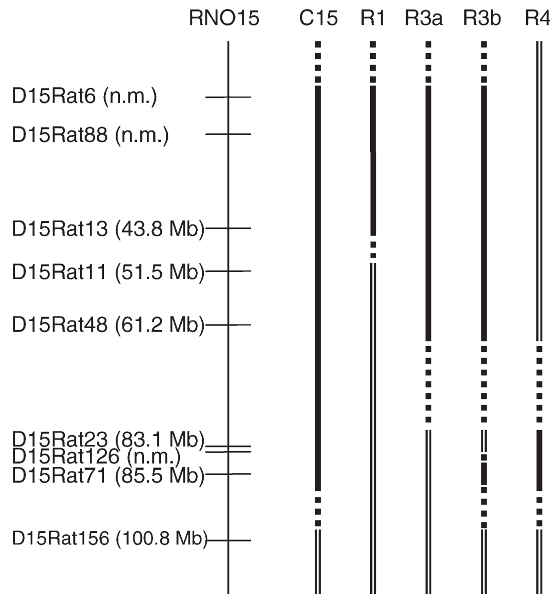

Figure 1.—A schematic of the distal part of rat chromosome 15, aligned with the intervals defined in the congenic strains. The full-length congenic strain DA.ACI–*D15Rat6-D15Rat71* (C15) and the recombinant congenic strains DA.ACI–*D15rat6-D15rat13* (C15R1), DA.ACI–*D15Rat6-D15Rat48* (C15R3a), DA.ACI–*D15Rat6-D15rat48, D15Rat126-D15Rat71* (C15R3b), and DA.ACI–*D15Rat23-D15rat71* (C15R4) are depicted. The thin vertical line represents rat chromosome 15 along with microsatellite markers placed according to positions in megabases derived from the rat genome sequence (http://www.nsembl.org/Rattus_norvegicus/). Markers not mapped to assembly in the current Ensembl database are marked "n.m." and positioned according to the SHRSP × BN version 7 linkage map (http://rgd.mcw.edu/). The thick black vertical lines represent different ACI rat intervals transferred to the DA rat background and the dashed lines represent the interval within which recombination has occurred. The open vertical line represents DA rat background genes.

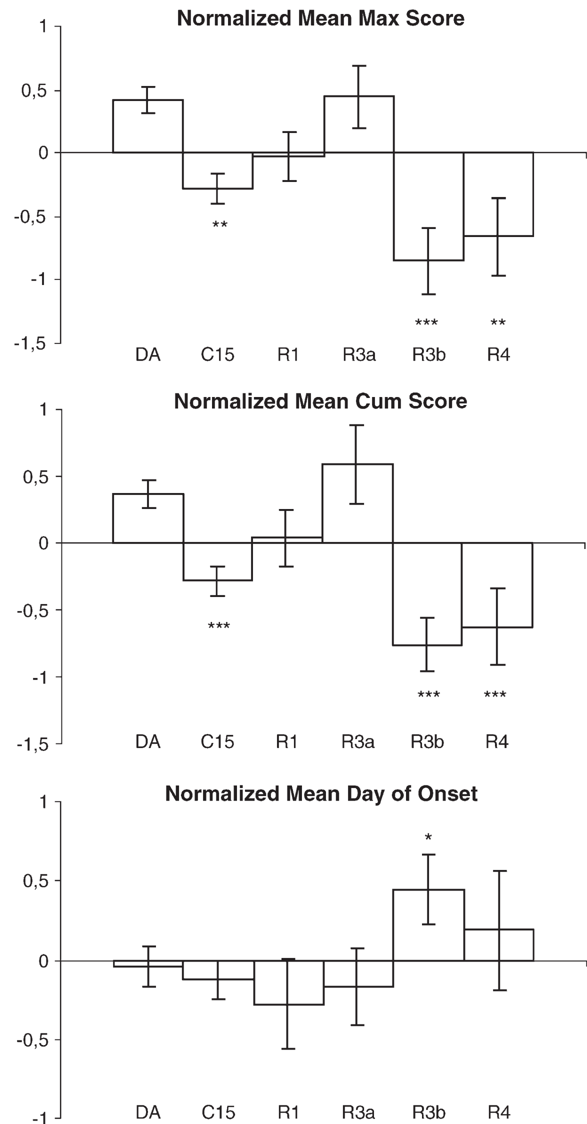

Figure 2.—Combined analysis of the clinical MOG-EAE phenotypes in six separate experiments encompassing the DA (n = 72), C15 (n = 49), C15R1 (n = 23), C15R3a (n = 9), C15R3b (n = 14), and C15R4 (n = 17) strains. Maximum EAE score, cumulative EAE score, and onset day were tested with the Wilcoxon two-sample test after normalization; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Pairwise comparisons were made with the congenic strains and the DA strain. Mean values and SEM for disease onset day after immunization were calculated only for affected rats.

mapped with four additional microsatellite markers (Figure 4). The microsatellite markers were obtained from Proligo France SAS (Paris). Polymerase chain reaction (PCR) amplification was performed as previously described with [γ-^{33}P]ATP end labeling of the forward primer (Jacob *et al.* 1995). The PCR products were size fractionated on 6% polyacrylamide gels and visualized by autoradiography. All genotypes were evaluated manually and double checked.

Statistical analysis: Differences in binominal traits (incidence, relapsing/remitting disease, mortality) were tested with the Fisher's exact test. Differences in the maximal score, the cumulative scores, and onset day were tested with the Wilcoxon two-sample test after normalization of the six separate experiments. Normalization was performed by subtracting the mean maximal or cumulative EAE score for the particular experiment from each individual rat's corresponding score and then the sum was divided with the standard deviation for the particular experiment. This allowed all experiments to be analyzed together, despite the variation in the severity of disease in the parental DA rat strain. The JMP 5.1 software (SAS Institute, Cary, NC) was utilized for the analysis above. Linkage analysis in the AIL was performed with the R/qtl software (Broman *et al.* 2003). Permutation tests in the R/qtl software were used to determine the significance levels (Churchill and Doerge 1994). The LOD levels for significant linkage generated with 5000 permutations were 2.3 for the incidence of EAE, 2.0 for the day of onset, 2.9 for the maximum EAE score, and 2.2 for the cumulative EAE score. The confidence interval was defined as a drop of 1 in the LOD score (Lander and Botstein 1989).

RESULTS

A reduced MOG-EAE severity in the C15, C15R3b, and C15R4 strains: Figure 2 gives the mean maximal cumulative score and onset day of the EAE in DA rats

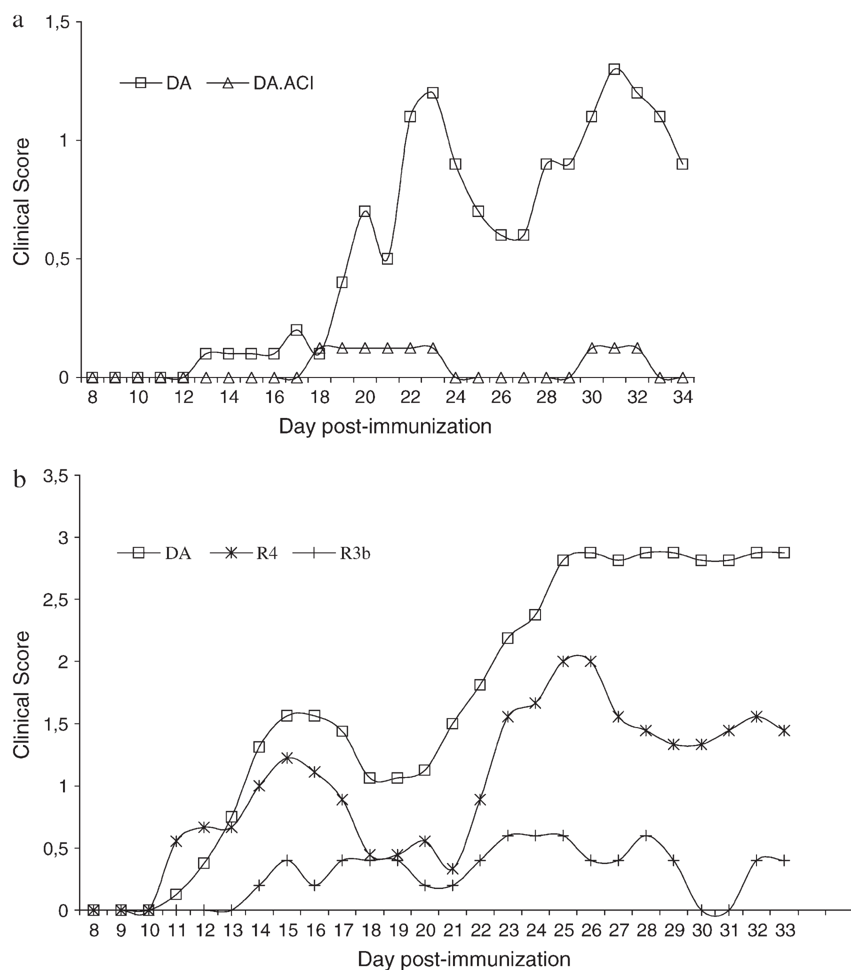

FIGURE 3.—The clinical course of rMOG (aa 1–125)-induced EAE in selected strains and experiments. (a) Experiment 3: a mild disease course in DA rats ($n = 11$) and almost complete protection in C15 rats ($n = 8$). (b) Experiment 5: a severe disease in DA rats ($n = 16$) and reduced disease severity in C15R3b ($n = 5$) and C15R4 ($n = 9$) rats.

and in congenic C15 and recombinant congenic C15R1, C15R3a, C15R3b, and C15R4 rats pooled from six separate experiments after the normalization. DA, C15R1, and C15R3a rats developed EAE with a high maximal and cumulative EAE score while the C15, C15R3b, and C15R4 rats had less severe MOG-EAE, with lower maximal and cumulative EAE scores ($P < 0.05$–0.001); C15R3b rats had late onset of disease compared to DA rats ($P < 0.05$). The disease incidence, the numbers of rats displaying a relapsing/remitting disease, or a lethal EAE were not significantly different in any of the congenic strains compared to the DA strain (data not shown). However, the power of this analysis was reduced due to the variable expression of EAE in the DA strain, as depicted in Figure 3. In experiment 3 (Figure 3a), there were only mild signs of disease in the DA rats and almost no disease signs in the C15 rats. The disease signs in the DA rats were much more severe in experiment 5, while the R3b and R4 displayed a reduced disease severity (Figure 3b). The overall disease severity was intermediate in experiment 1, mild in experiment 2, and severe in experiments 4 and 6 (data not shown).

Eae19 delineated by linkage mapping in a (DA × PVG.AV1) AIL: A total of 1068 MOG-immunized (DA × PVG.AV1)F_7 rats were monitored 31 days p.i. for clinical signs of EAE. Unambiguous signs of EAE were recorded in 14.8% (158/1068) of the rats. A detailed account of the EAE disease outcome in the F_7(DA × PVG.AV1) AIL rats has been published (JAGODIC *et al.* 2004). All available EAE-affected rats were selected for genotyping ($n = 152$). Randomly selected healthy rats, displaying no signs of EAE and no weight loss, were genotyped in parallel ($n = 162$). The DA × PVG.AV1 strain combination provided a substantially higher degree of polymorphic microsatellite markers than the DA × ACI combination did, as expected. Linkage analysis resolved the C15 region into a significant locus, named *Eae19* (http://ratmap.org/), displaying linkage to several EAE phenotypes. Interestingly, the disease incidence and the day of onset was linked to *Eae19*, indicating that the EAE regulatory effect is not limited to the disease severity, as suggested by the analysis of the congenic strains. The LOD score curves for the different EAE phenotypes are presented in Figure 4. The confidence interval, defined as a drop of 1 in the LOD score, comprises an ∼13-Mb region (D15Mgh4-D15Rat102). The DA allele at the peak marker (D15Rat71) is disease enhancing in an additive fashion. Sequence alignments and map comparisons

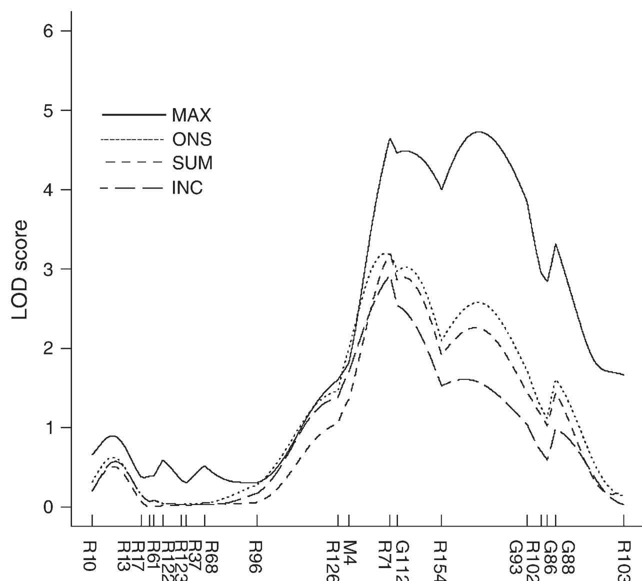

FIGURE 4.—Log-likelihood plot of *Eae19*, identified in the (DA × PVG.AV1)F_7 AIL. *Eae19* displayed significant linkage to all clinical EAE phenotypes: EAE incidence and day of onset and maximum and cumulative EAE scores. The markers in the region are listed on the *x*-axis (R, D15Rat; M, D15Mgh; G, D15Got. *Eae19* is 13 Mb and contains 32 confirmed or predicted genes according to the rat physical map retrieved from http://www.ensembl.org.

revealed that *Eae19* is syntenic to human 13q22.1–q31.2 (Table 1).

DISCUSSION

The definition of *Eae19* in two different strain combinations (DA × ACI and DA × PVG.AV1) strengthens the importance of this locus. Further mapping of genes may also be facilitated by comparisons of genetic polymorphisms among the three different strains, especially since the low polymorphism rate between the DA and the ACI strain may decrease substantially the number of relevant genetic polymorphisms. However, at this stage it is impossible to rule out that *Eae19* is composed of several genes and/or genes that differ between the different strains (MOREL *et al.* 2001; BECANOVIC *et al.* 2004). Definition of subcongenic strains from the C15R4 strain will be performed to further reduce the size of the congenic fragment contributing to relative disease protection. Positional cloning will then be needed to define the exact genes responsible for the EAE-regulating effect of *Eae19*. Successful positional cloning through the definition of smaller and smaller congenic fragments has recently been demonstrated in rat experimental arthritis (OLOFSSON *et al.* 2003).

A possible problem with gene mapping in congenic strains is the presence of contaminating fragments of DNA from the donor strain, contributing to differences in the disease phenotype between the congenic strains and the parental strains that wrongly would be interpreted as genetically localized to the congenic fragment. The speed congenic strategy applied in the present study is a way to improve the control of contaminating fragments as well as to speed up the process of generating congenic strains. Mapping of the disease expression in recombinant congenic strains is another way to rule out significant contributions from genes outside the investigated congenic fragment. The lack of clinical effects in the C15R1 and C15R3a congenic strains strongly argues against any significant contributions from contaminating ACI DNA fragments outside *Eae19*, since those strains are expected to share possible contaminating fragments with the C15R3b and the C15R4 strains. Another issue in the analysis of EAE QTL is the stability of the models and the observed genetic effects. In the present study, there were clear differences in the six different experiments regarding disease severity in the parental DA rat strain (Figure 3, a and b) as well as a variable difference between the full-length C15 and the DA strain. Differences in the disease expression in parental/control strains are possible to minimize by applying strict protocols for immunization and environmental monitoring, but in practice it is very difficult to obtain completely stable conditions in EAE. It may also be argued that repeating experiments with different

TABLE 1

Position and LOD scores for *Eae19* in the (DA × PVG.AV1)F_7 advanced intercross line

QTL	LOD score[a] Inc	Ons	Max	Cum	Peak marker	Marker interval	CI[b] (Mb)	Syntenic human region[c]
Eae19	2.9	3.2	4.7	3.2	D15Rat71	D15Mgh4–D15Rat102	13	13q22.1–q31.2

[a] LOD scores and thresholds for significance based on 5000 permutations were generated with R/qtl. Significance threshold: 2.3 for Inc (Incidence of EAE); 2.0 for Ons (day of onset), 2.9 for Max (Maximum EAE score) and 2.2 for Cum (Cumulative EAE score).

[b] Confidence intervals (CI) defined as a drop of 1 in the LOD score and the closest corresponding microsatellite markers are reported.

[c] Synteny data derived from http://www.ensembl.org/.

disease severity maximizes the possibility to detect weak genetic effects. It is highly likely that most genetic effects in MOG-EAE (and MS) are relatively weak and/or present only in certain disease subphenotypes (Morel *et al.* 2000). This may help to explain the relative lack of progress in QTL mapping in EAE since the first whole-genome scan was published in 1995 (Sundvall *et al.* 1995).

Linkage mapping in the (DA × PVG.AV1)F_7 AIL localized *Eae19* within a 13-Mb confidence interval, which overlaps with the congenic fragment defined by C15R4. The linkage analysis in the AIL both increased the LOD score and decreased the confidence interval of *Eae19* compared to previous F_2 analysis. This region contains only 32 confirmed and predicted genes, including genes such as immune-responsive gene 1 and neuronal ceroid lipofuscinose gene 5. A current list of genes mapped to the interval can be retrieved at http://www.ensembl.org. There were few obvious candidate genes, which may be explained by the presence of yet-unmapped genes, by the presence of regulatory elements altering the expression of genes mapping outside *Eae19*, or by complex interactions. *Eae19* also overlaps with adjuvant-induced arthritis QTL 4 (*Aia4*) (Kawahito *et al.* 1998), serum cholesterol level QTL 1 (Kato *et al.* 2000), blood pressure QTL cluster 12 (Stoll *et al.* 2000), and gastric cancer susceptibility QTL 1 (Ushijima *et al.* 2000). *Aia4* may be the most interesting of these QTL, since we previously demonstrated EAE regulatory effects in rat strains congenic for arthritis-regulating QTL (Becanovic *et al.* 2003). It is highly likely that some genetically regulated disease mechanisms are shared between arthritis and EAE (Becker *et al.* 1998). Given the emerging evidence for the importance of immune mechanisms in cardiovascular diseases and cancer, a shared genetic regulation with these conditions, as suggested by the overlapping QTL, is another intriguing possibility. However, due to the large numbers of QTL described and the usual size of the confidence intervals, a certain degree of overlap among QTL is expected by chance. *Eae19* is syntenic to the human chromosome 13q22.1–q31.2 that has not shown evidence of linkage to MS. An explanation, in addition to the lack of power to exclude gene regions in human linkage and association studies, is that pathways but not the regulating genes are shared between the animal model and the human disease.

In conclusion, a new EAE-regulating locus on rat chromosome 15, *Eae19*, is mapped in congenic and recombinant congenic strains in combination with linkage analysis in an AIL. Further dissection of *Eae19* is possible by the creation of congenic strains with increasingly smaller congenic fragments. The identification of genetic polymorphisms regulating autoimmune neuroinflammation will reveal disease-relevant mechanistic pathways and thereby provide new targets for therapeutic interventions.

LITERATURE CITED

Adelmann, M., J. Wood, I. Benzel, P. Fiori, H. Lassmann *et al.*, 1995 The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. J. Neuroimmunol. **63:** 17–27.

Akesson, E., A. Oturai, A. Svejgaard, P. Holmans, A. Compston *et al.*, 2002 A genome-wide screen for linkage in Nordic sib-pairs with multiple sclerosis. Genes Immun. **3:** 279–285.

Ban, M., G. Stewart, B. Bennetts, R. Heard, R. Simmons *et al.*, 2002 A genome screen for linkage in Australian sibling-pairs with multiple sclerosis. Genes Immun. **3:** 464–469.

Becanovic, K., L. Backdahl, E. Wallstrom, F. Aboul-Enein, H. Lassmann *et al.*, 2003 Paradoxical effects of arthritis-regulating chromosome 4 regions on myelin oligodendrocyte glycoprotein-induced encephalomyelitis in congenic rats. Eur. J. Immunol. **33:** 1907–1916.

Becanovic, K., M. Jagodic, E. Wallstrom and T. Olsson, 2004 Current gene mapping strategies in experimental models of multiple sclerosis. Scand. J. Immunol. **60:** 39–51.

Becker, K. G., R. M. Simon, J. E. Bailey-Wilson, B. Freidlin, W. E. Biddison *et al.*, 1998 Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc. Natl. Acad. Sci. USA **95:** 9979–9984.

Broadley, S., 2001 A genome screen for multiple sclerosis in Italian families. Gene Immun. **2:** 205–210.

Broman, K., H. Wu, S. Sen and G. Churchill, 2003 R/qtl: QTL mapping in experimental crosses. Bioinformatics **1:** 889–890.

Chataway, J., R. Feakes, F. Coraddu, J. Gray, J. Deans *et al.*, 1998 The genetics of multiple sclerosis: principles, background and updated results of the United Kingdom systematic genome screen. Brain **121:** 1869–1887.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Coraddu, F., 2001 A genome screen for multiple sclerosis in Sardinian multiplex families. Eur. J. Hum. Genet. **9:** 621–626.

Dahlman, I., L. Jacobsson, A. Glaser, J. C. Lorentzen, M. Andersson *et al.*, 1999a 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.

Dahlman, I., E. Wallstrom, R. Weissert, M. Storch, B. Kornek *et al.*, 1999b Linkage analysis of myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in the rat identifies a locus controlling demyelination on chromosome 18. Hum. Mol. Genet. **8:** 2183–2190.

Dahlman, I., E. Wallstrom, H. Jiao, H. Luthman, T. Olsson *et al.*, 2001 Polygenic control of autoimmune peripheral nerve inflammation in rat. J. Neuroimmunol. **119:** 166–174.

Darvasi, A., and M. Soller, 1995 Advanced intercross lines: an experimental population for fine genetic mapping. Genetics **141:** 1199–1207.

Darvasi, A., and M. Soller, 1997 A simple method to calculate resolving power and confidence interval of QTL map location. Behav. Genet. **27:** 125–132.

De Rosbo, N. K., R. Milo, M. B. Lees, D. Burger, C. C. Bernard *et al.*, 1993 Reactivity to myelin antigens in multiple sclerosis. Peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. J. Clin. Invest. **92:** 2602–2608.

Ebers, G. C., 1996 Genetic epidemiology of multiple sclerosis. Curr. Opin. Neurol. **9:** 155–158.

Ebers, G. C., D. E. Bulman, A. D. Sadovnick, D. W. Paty, S. Warren *et al.*, 1986 A population-based study of multiple sclerosis in twins. N. Engl. J. Med. **315:** 1638–1642.

Ebers, G. C., A. D. Sadovnick, N. J. Risch and C. C. S. Group, 1995 A genetic basis for familial aggregation in multiple sclerosis. Nature **377:** 150–151.

Ebers, G. C., K. Kukay, D. E. Bulman, A. D. Sadovnick, G. Rice *et al.*, 1996 A full genome search in multiple sclerosis. Nat. Genet. **13:** 472–476.

Haines, J. L., M. Ter-Minassian, A. Bazyk, J. F. Gusella, D. J. Kim *et al.*, 1996 A complete genomic screen for multiple sclerosis underscores a role for the major histocompatibility complex. Nat. Genet. **13:** 469–471.

Haines, J., Y. Bradford, M. Garcia, A. Reed, E. Neumeister *et al.*,

2002 Multiple susceptibility loci for multiple sclerosis. Hum. Mol. Genet. **11:** 2251–2256.

Hedrich, H. J., 1990 *Genetic Monitoring of Inbred Strains of Rats*. Gustav Fischer Verlag, New York.

Jacob, H. J., D. M. Brown, R. K. Bunker, M. J. Daly, V. J. Dzau *et al.*, 1995 A genetic linkage map of the laboratory rat, Rattus norvegicus. Nat. Genet. **9:** 63–69.

Jagodic, M., B. Kornek, R. Weissert, H. Lassmann, T. Olsson *et al.*, 2001 Congenic mapping confirms a locus on rat chromosome 10 conferring strong protection against myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. Immunogenetics **53:** 410–415.

Jagodic, M., K. Becanovic, J. R. Sheng, X. Wu, L. Backdahl *et al.*, 2004 An advanced intercross line resolves Eae18 into two narrow quantitative trait loci syntenic to multiple sclerosis candidate loci. J. Immunol. **173:** 1366–1373.

Jersild, C., T. Fog, G. S. Hansen, M. Thomsen, A. Svejgaard *et al.*, 1973 Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. Lancet **2:** 1221–1225.

Johns, T. G., N. Kerlero de Rosbo, K. K. Menon, S. Abo, M. F. Gonzales *et al.*, 1995 Myelin oligodendrocyte glycoprotein induces a demyelinating encephalomyelitis resembling multiple sclerosis. J. Immunol. **154:** 5536–5541.

Kato, N., T. Tamada, T. Nabika, K. Ueno, T. Gotoda *et al.*, 2000 Identification of quantitative trait loci for serum cholesterol levels in stroke-prone spontaneously hypertensive rats. Arterioscler. Thromb. Vasc. Biol. **20:** 223–229.

Kawahito, Y., G. W. Cannon, P. S. Gulko, E. F. Remmers, R. E. Longman *et al.*, 1998 Localization of quantitative trait loci regulating adjuvant-induced arthritis in rats: evidence for genetic factors common to multiple autoimmune diseases. J. Immunol. **161:** 4411–4419.

Kuokkanen, S., M. Gschwend, J. D. Rioux, M. J. Daly, J. D. Terwilliger *et al.*, 1997 Genomewide scan of multiple sclerosis in Finnish multiplex families. Am. J. Hum. Genet. **61:** 1379–1387.

Laird, P. W., A. Zijderveld, K. Linders, M. A. Rudnicki, R. Jaenisch *et al.*, 1991 Simplified mammalian DNA isolation procedure. Nucleic Acids Res. **19:** 4293.

Lander, E. S., and D. Botstein, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

Lander, E. S., and L. Kruglyak, 1995 Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. **11:** 241–247.

Lassmann, H., W. Bruck and C. Lucchinetti, 2001 Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. Trends Mol. Med. **7:** 115–121.

Linington, C., M. Bradl, H. Lassmann, C. Brunner and K. Vass, 1988 Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. Am. J. Pathol. **130:** 443–454.

Lucchinetti, C. F., W. Bruck, M. Rodriguez and H. Lassmann, 1996 Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. Brain Pathol. **6:** 259–274.

Morel, L., B. P. Croker, K. R. Blenman, C. Mohan, G. Huang *et al.*, 2000 Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. Proc. Natl. Acad. Sci. USA **97:** 6670–6675.

Morel, L., K. R. Blenman, B. P. Croker and E. K. Wakeland, 2001 The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. Proc. Natl. Acad. Sci. USA **98:** 1787–1792.

Olerup, O., and J. Hillert, 1991 HLA class II-associated genetic susceptibility in multiple sclerosis: a critical evaluation. Tissue Antigens **38:** 1–15.

Olofsson, P., J. Holmberg, J. Tordsson, S. Lu, B. Akerstrom *et al.*, 2003 Positional identification of Ncf1 as a gene that regulates arthritis severity in rats. Nat. Genet. **33:** 25–32.

Sadovnick, A. D., H. Armstrong, G. P. Rice, D. Bulman, L. Hashimoto *et al.*, 1993 A population-based study of multiple sclerosis in twins: update. Ann. Neurol. **33:** 281–285.

Sadovnick, A. D., G. C. Ebers, D. A. Dyment, N. J. Risch, D. Bulman *et al.*, 1996 Evidence for genetic basis of multiple sclerosis. Lancet **347:** 1728–1730.

Sawcer, S., H. B. Jones, R. Feakes, J. Gray, N. Smaldon *et al.*, 1996 A genome screen in multiple sclerosis reveals susceptibility loci on chromosome 6p21 and 17q22. Nat. Genet. **13:** 464–468.

Stoll, M., A. E. Kwitek-Black, A. W. J. Cowley, E. L. Harris, S. B. Harrap *et al.*, 2000 New target regions for human hypertension via comparative genomics. Genome Res. **10** (4): 473–482.

Sun, J., H. Link, T. Olsson, B. G. Xiao, G. Andersson *et al.*, 1991 T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis. J. Immunol. **146:** 1490–1495.

Sundvall, M., J. Jirholt, H. T. Yang, L. Jansson, A. Engstrom *et al.*, 1995 Identification of murine loci associated with susceptibility to chronic experimental autoimmune encephalomyelitis. Nat. Genet. **10:** 313–317.

Ushijima, T., M. Yamamoto, M. Suzui, T. Kuramoto, Y. Yoshida *et al.*, 2000 Chromosomal mapping of genes controlling development, histological grade, depth of invasion, and size of rat stomach carcinomas. Cancer Res. **60:** 1092–1096.

Wakeland, E., L. Morel, K. Achey, M. Yui and J. Longmate, 1997 Speed congenics: a classic technique in the fast lane (relatively speaking). Immunol. Today **18:** 472–477.

Wallstrom, E., M. Khademi, M. Andersson, R. Weissert, C. Linington *et al.*, 1998 Increased reactivity to myelin oligodendrocyte glycoprotein peptides and epitope mapping in HLA DR2(15)+ multiple sclerosis. Eur. J. Immunol. **28:** 3329–3335.

Weissert, R., E. Wallstrom, M. K. Storch, A. Stefferl, J. Lorentzen *et al.*, 1998b MHC haplotype-dependent regulation of MOG-induced EAE in rats. J. Clin. Invest. **102:** 1265–1273.

Xiong, M., and S. Guo, 1997 Fine-scale mapping of quantitative trait loci using historical recombinations. Genetics **145:** 1201–1218.

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