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eae36, a Locus on Mouse Chromosome 4, Controls Susceptibility to Experimental Allergic Encephalomyelitis in Older Mice and Mice Immunized in the Winter

Cory Teuscher,*,[1] R. W. Doerge,[†] Parley D. Fillmore[‡] and Elizabeth P. Blankenhorn[§]

**Departments of Medicine and Pathology, University of Vermont, Burlington, Vermont 05405, [†]Departments of Agronomy and Statistics, Purdue University, West Lafayette, Indiana 47907, [‡]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61802 and [§]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, Pennsylvania 19129*

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ABSTRACT

Genetic factors are believed to contribute to multiple sclerosis (MS) susceptibility; however, strong evidence implicating intrinsic and environmental factors in the etiopathogenesis of MS also exists. Susceptibility to experimental allergic encephalomyelitis (EAE), the principal animal model of MS, is also influenced by nongenetic factors, including age and season at immunization. This suggests that age- and season-by-gene interactions exist and that different susceptibility loci may influence disease as a function of the two parameters. In this study, linkage analysis based on genome exclusion mapping was carried out using age and season at immunization restricted cohorts of (B10.S × SJL/J) F_2 intercross mice in an effort to identify such linkages. Significant linkage of EAE to *eae4* and *eae5* was detected with 6- to 12-week-old and summer cohorts. In contrast, significant linkage of EAE to *eae4* and *eae5* was not detected with the >12-week-old and winter/spring populations. Rather, significant linkage to *D4Mit203* at 128.50 Mb on chromosome 4 was detected with animals that were >12 weeks old at the time of immunization or were immunized in the winter. This previously unidentified locus has been designated *eae36*. These results support the existence of age- and season-by-gene-specific interactions in the genetic control of susceptibility to autoimmune inflammatory disease of the central nervous system and suggest that late-onset MS may be immunogenetically distinct.

MULTIPLE sclerosis (MS) is the major inflammatory demyelinating disease of the central nervous system (CNS) in humans. The etiology of MS is unknown but is believed to have an autoimmune basis arising in genetically susceptible individuals as a consequence of an environmental insult (Compston and Coles 2002). Support for a genetic component in MS exists (Dyment *et al.* 2004) and considerable effort has gone into identifying the loci contributing to susceptibility; however, with the exception of linkage and association to *HLA*, there is little consensus and reproducibility across studies (Compston and Sawcer 2003; Herrera and Ebers 2003). These results and the 70–90% discordance rates in identical twins indicate that nongenetic factors also play a strong role in disease risk (Willer *et al.* 2003).

A recent systematic review of potential nongenetic factors in MS indicates that solar ultraviolet radiation and sex hormones are the best candidates (Coo and Aronson 2004). Additionally, parent-of-origin (POO) effects have been reported to influence MS susceptibility and outcome (Hupperts *et al.* 2001). A recent study utilizing a half-sibling approach supports a significant maternal POO effect (2.35% for shared mother and 1.31% for shared father) (Ebers *et al.* 2004). Importantly, the risk for siblings who share only a mother compared with the risk for full siblings was not significantly different (2.34% *vs.* 3.11%, $P = 0.1$), suggesting that the maternal POO effect may be the major component underlying familial aggregation (Giordano and Momigliano-Richiardi 2004). A recent study using 17,874 Canadian patients and 11,502 British patients with MS also revealed a significant association between month of birth and MS risk (Willer *et al.* 2005). This effect was more pronounced in familial cases, implying that gene-environment interactions may underlie the association.

Susceptibility to experimental allergic encephalomyelitis (EAE), the foremost autoimmune model of MS, is also influenced by nongenetic factors. These include both adult (Teuscher *et al.* 1998; Blankenhorn *et al.* 2000; Fillmore *et al.* 2003) and neonatal environmental influences (Dimitrijevic *et al.* 1994; Laban *et al.* 1995a,b). We also recently demonstrated that gender, age, and season at immunization uniquely influence susceptibility to EAE in the mouse (Teuscher *et al.* 2004). Gender was shown to be more important than age and season at immunization in influencing histopathological disease,

[1]*Corresponding author:* Immunobiology Program, C317 Given Medical Bldg., University of Vermont, Burlington, VT 05405.
E-mail: c.teuscher@uvm.edu

and in contrast to CNS lesions, age and season at immunization significantly and independently influence susceptibility to clinical signs and do so equally in both males and females. Moreover, linkage to *eae5*, the *H2*-linked locus controlling susceptibility to clinical disease, was age and season dependent. These data suggest that age- and season-by-gene interactions exist and that different subsets of susceptibility loci may control clinical disease as a function of the two parameters. In this study we carried out linkage analysis based on genome exclusion mapping using phenotyped age and season restricted cohorts of (B10.S × SJL/J) F_2 intercross mice to map such loci. We report that linkage to *eae4*, as with *eae5*, is restricted to 6- to 12-week-old and summer cohorts (Teuscher *et al.* 2004); and the mapping of a previously unidentified locus on chromosome 4, designated *eae36*, controls susceptibility to EAE in F_2 intercross mice that were >12 weeks old at the time of immunization or were immunized in the winter.

MATERIALS AND METHODS

Animals: The mice used in this study comprise 760 genotyped (B10.S/DvTe × SJL/J) F_2 mice used in our earlier studies (Teuscher *et al.* 2004) and an additional cohort of 366 genotyped (B10.S/SgMcdJ × SJL/J) F_2 mice generated at the University of Illinois at Urbana–Champaign (Fillmore *et al.* 2004). Animals were maintained under standard environmental conditions, including controlled temperature, humidity, and a 12-hr light:12-hr dark cycle and in accordance with the Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Additionally, the infectious disease status of the colony was monitored serologically using a standard sentinel program. No change in the serologic profile of the animals was observed over the course of the experiments.

Induction and evaluation of EAE: Mice were immunized for the induction of EAE as described previously (Butterfield *et al.* 1998; Teuscher *et al.* 2004). Briefly, mouse spinal cord homogenate (MSCH) was generated using retired breeder SJL/J mice purchased from either the Jackson Laboratory or the Charles River Laboratory (Wilmington, MA). MSCH–complete Freund's adjuvant (CFA) emulsions were prepared by syringe extrusion using disposable syringes and a 21-gauge double-hub microemulsifying needle. Animals received 0.3 ml of SJL/J MSCH–CFA emulsion via two subcutaneous injections in the posterior right and left flank (2 × 0.15 ml). One week later all mice were similarly injected at two sites on the right and left flanks anterior of the initial injection sites. In this way each animal received a total of 2.0 mg dry weight SJL/J MSCH and 30.0 μg of *Mycobacterium tuberculosis* H37Ra. Animals were monitored for clinical signs of EAE starting at day 10 after injection through day 60. All animals that exhibited any clinical signs greater or equal to a flaccid tail and/or hind leg weakness for 2 or more consecutive days were considered affected.

Linkage analysis: Informative microsatellite primers were either purchased from Research Genetics (Huntsville, AL) or synthesized according to sequences obtained through Mouse Genome Informatics. Polymerase chain reaction parameters for microsatellite typing were as previously described (Butterfield *et al.* 1998). Microsatellite size variants were resolved by electrophoresis on large-format denaturing polyacrylamide gels and visualized by autoradiography on Kodak film (Eastman-Kodak, Rochester, NY).

A chi-square test statistic was employed to test single-marker linkage in 2 × 3 contingency tables at an average marker density of ~10 cM. To accommodate the well-known statistical issues associated with single-marker linkage analysis (Churchill and Doerge 1994), we relied on permutation testing to assess the statistical significance. The phenotypic data were randomized, while holding the genotypic information fixed, and the chi-square test statistic was calculated for each stratified marker (Good 1993). The experimentwise threshold values were calculated as previously described (Churchill and Doerge 1994). Given 1000 permutations of the original data, the 5% experimentwise threshold was used to indicate significant linkage to marker loci.

Candidate gene analysis: Gene-specific restriction fragment length polymorphisms were generated for nonsynonymous single nucleotide polymorphisms in *Csf3r*, *Pnrc2*, and *Htr1d* (http://www.aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home). A 916-bp *Csf3r* product was PCR amplified with the primers 5′-CCTCTCCAACCCTCCTAACAG-3′ and 5′-CAGATTCAGGCGGATTTCTG-3′. Restriction of the B10.S product with *Apo*I results in 289- and a 627-bp fragments whereas cleaving the SJL/J allele with *Apo*I gives rise to 201-, 289-, and 426-bp fragments. For *Pnrc2*, a 918-bp product was PCR amplified with the primers 5′-GAGCCTAATGCCATCTTGTC-3′ and 5′-CTCTGGGTACTTATCCACTGC-3′. Restriction with *Afl*II gives rise to 386- and 532-bp fragments for SJL/J and 247-, 285-, and 386-bp fragments with the B10.S allele. A 1026-bp *Htr1d* product was PCR amplified (5′-CAGTAGAGTGTCAAAGGCGAG-3′ and 5′-GCGGCCATACAGGATAATG-3′) and the products restricted with *Tsp45*I. The SJL/J allele exhibits two fragments (326 and 700 bp) while the B10.S allele results in three fragments (259, 326, and 441 bp). In all cases, genomic DNA was amplified under standard conditions and the products/fragments were electrophoresed in 2% agarose gels and visualized by ethidium bromide.

RESULTS AND DISCUSSION

Genome exclusion mapping was carried out utilizing 170 informative microsatellite markers and DNA isolated from a cohort of 760 phenotyped (B10.S × SJL/J) F_2 intercross mice (Butterfield *et al.* 1998; Teuscher *et al.* 2004) having a mean age at immunization of 15.3 ± 10.5 weeks. When all animals were included in the analysis, significant linkage to marker loci on chromosomes 7, 16, and 17 was detected (Table 1). Linkage to other marker loci at or above the suggestive level (90–95% level for permutation-derived thresholds) was not detected. These linkages are consistent with prior results based on the incidence of clinical disease and reflect linkage to *eae4*, *eae11*, and *eae5*, respectively (http://www.informatics.jax.org/).

Stratification by age and season at immunization (Teuscher *et al.* 2004) resulted in significant linkage to *eae4* and *eae5* with both the 6- to 12-week-old and summer cohorts (Table 1). Importantly, linkage to these loci was not detected even at the suggestive level with the >12-week-old and winter cohorts, indicating that genetic linkage of disease susceptibility to both *eae4* and *eae5* is age and season dependent; *i.e.*, age and season at immunization are capable of overriding the major genetic checkpoints controlling susceptibility to EAE in

TABLE 1

Linkage of EAE susceptibility to marker loci is a function of age and season at immunization

Marker	Mb[a]	All[b]	6–12 wk[c]	>12 wk[d]	Summer[e]	Winter[f]	*eae* locus
D7Mit85	45.74	21.1; >99%	17.2; 95–99%	4.8; <90%	16.8; 95–99%	4.9; <90%	*eae12*
D7Mit233	62.26	23.0; >99%	19.6; >99%	5.6; <90%	20.7; >99%	3.6; <90%	*eae4*
D16Mit140	69.73	16.0; 95–99%	7.6; <90%	8.5; <90%	10.1; <90%	7.8; <90%	*eae11*
H2-K	31.70						*eae1*
H2-D	32.97						
D17Mit105	38.72	14.3; 90–95%	13.5; <90%	3.0; <90%	15.0; 90–95%	1.5; <90%	*eae5*
D17Mit176	40.15	22.2; >99%	19.8; >99%	5.1; <90%	22.2; >99%	2.6; <90%	
D17Mit51	40.91	15.8; 95–99%	15.8; 95–99%	3.5; <90%	18.4; >99%	1.2; <90%	
D17Mit115	44.99	16.0; 95–99%	11.6; <90%	5.2; <90%	15.8; 90–95%	1.9; <90%	
D17Mit70	50.12	17.1; 95–99%	15.0; 90–95%	3.0; <90%	17.7; 95–99%	1.8; <90%	

Mice were immunized for the induction of EAE and scored as affected and unaffected starting on day 10 (Butterfield *et al.* 1998).

[a] Locations are based on the March 2005 mouse (*Mus musculus*) draft genome data obtained from the Build 34 assembly by NCBI.

[b] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 14.22, 95% = 15.73, 99% = 20.57; $n = 760$, marker number = 153.

[c] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 14.08, 95% = 15.62, 99% = 18.43; $n = 374$, marker number = 170.

[d] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.55, 95% = 14.90, 99% = 16.99; $n = 288$, marker number = 153.

[e] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 14.40, 95% = 15.83, 99% = 18.35; $n = 540$, marker number = 170.

[f] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.87, 95% = 15.52, 99% = 18.91; $n = 220$, marker number = 153.

younger animals and in animals immunized in the summer.

The absence of significant linkage to marker loci with the >12-week-old and winter/spring cohorts suggests that disease susceptibility in older animals and in animals immunized in the winter may be less dependent on genetic factors and thus would not support the concept that polymorphism in different genes controls susceptibility to autoimmune inflammatory disease of the CNS as a function of age and season at immunization. Alternatively, the inability to detect a statistically significant linkage in these two cohorts in part may be due to limited sample size and insufficient power (228 in the >12-week-cohort and 220 in the winter cohort). To address this issue, an independent cohort of 366 mice consisting primarily of animals immunized in the winter and having a mean age of 31.0 ± 12 weeks at immunization was studied. As with the stratified cohorts, significant linkage to *eae4*, *eae11*, and *eae5* was not detected. However, significant linkage of EAE to *D4Mit221*, *D4Mit203*, and *D4Mit204* was detected (Table 2). Thus, age- and season-by-gene-specific interactions in older mice and mice immunized in the winter involve one or more loci on chromosome 4 that do not contribute to age- and season-by-gene-specific interaction in younger mice and in mice immunized in the summer. Linkage of EAE to chromosome 4 markers has not been reported previously; consequently, we have designated this locus *eae36*. Interestingly, *eae36* colocalizes with *Lbw2*, *Lmb1*, *Arvm2*, *Sles2*, *Idd9.1*, and *Idd11*, raising the possibility that this region of chromosome 4 harbors one or more shared autoimmune disease loci (Teuscher 1985; Sudweeks *et al.* 1993; Meeker *et al.* 1995; Ma *et al.* 2002).

Taken together, these data suggest that age- and season-by-gene interactions exist and that different subsets of susceptibility loci appear to control clinical disease as a function of the two parameters. Age-by-gene interactions are of potential significance in the genetics of MS. MS is considered to be primarily a disease of young adults since the age range of disease onset is usually between 20 and 40 years (Poser *et al.* 1982) with a mean age of onset of 27 years (Kurtzke 1993). However, late-onset MS, defined as the first presentation of clinical symptoms after 50, has been reported to be in the range of 1.1–10.0% (Noseworthy *et al.* 1983; Marra 1984; Safran 1989; White *et al.* 1990; Azzimondi *et al.* 1994; Polliack *et al.* 2001; Delalande *et al.* 2002). Importantly, a number of studies (Noseworthy *et al.* 1983; Hooge and Redekop 1992; Azzimondi *et al.* 1994; Trefouret *et al.* 1996; Polliack *et al.* 2001; Delalande *et al.* 2002), albeit not all (White *et al.* 1990), report a poorer prognosis and a more rapid progression to disability in late-onset MS patients compared to younger patients. This suggests that late-onset MS may represent a phenotypically and genotypically distinct subset of patients. In this regard our data support the concept that late-onset autoimmune inflammatory disease of the CNS is in fact immunogenetically distinct from young-adult-onset disease.

TABLE 2

Linkage of EAE susceptibility to marker loci in older F_2 intercross mice immunized in the winter

Marker	Mb[a]	All[b]	>12 wk[c]	Winter[d]	*eae* locus
D4Mit221	122.23	17.8; 95–99%	14.8; 90–95%	13.0; <90%	*eae36*
D4Mit203	128.50	21.6; >99%	18.9; >95–99%	17.6; 95–99%	
D4Mit204	132.29	23.3; >99%	20.9; >99%	21.1; >99%	
D7Mit85	45.74	7.0; <90%	7.3; <90%	7.1; <90%	*eae12*
D7Mit233	62.26	7.0; <90%	7.3; <90%	7.0; <90%	*eae4*
D16Mit140	69.73	1.3; <90%	2.5; <90%	2.2; <90%	*eae11*
H2-K	31.70				*eae1*
H2-D	32.97				
D17Mit105	38.72	0.7; <90%	0.4; <90%	2.3; <90%	*eae5*
D17Mit176	40.15	0.2; <90%	0.3; <90%	1.0; <90%	
D17Mit51	40.91	0.2; <90%	0.4; <90%	0.3; <90%	
D17Mit115	44.99				
D17Mit70	50.12	1.9; <90%	2.6; <90%	0.5; <90%	

Mice were immunized for the induction of EAE and scored as affected and unaffected starting on day 10 (Butterfield *et al.* 1998).

[a] Locations are based on the March 2005 mouse (*Mus musculus*) draft genome data obtained from the Build 34 assembly by NCBI.

[b] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 14.05, 95% = 15.88, 99% = 19.43; $n = 366$, marker number = 125.

[c] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.47, 95% = 14.50, 99% = 20.70; $n = 337$, marker number = 125.

[d] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.56, 95% = 15.21, 99% = 18.48; $n = 250$, marker number = 125.

The mechanisms underlying the age and season effects on the genetic control of susceptibility to EAE are unknown. However, they may be acting at the level of the CNS, the immune system, or both since seasonal variation in the plasticity of the structure and function of the adult brain (Tramontin and Brenowitz 2000) and expression of immunological factors (Haus and Smolensky 1999) is well documented. Similar age-associated changes in immune function also exist. Peripheral immunological functions decline with age (Miller 1996), whereas within the brain immunologically important cells such as astrocytes (Goss *et al.* 1991; O'Callaghan and Miller 1991; Nichols *et al.* 1993; Morgan *et al.* 1997) and microglia (Mattiace *et al.* 1990; Peters *et al.* 1991; Ogura *et al.* 1994; Rozovsky *et al.* 1998; Sheffield and Berman 1998; Sheng *et al.* 1998) exhibit increased activation with age. Age-related changes associated with microglial activation include increased phagocytic activity (Dickson *et al.* 1990), expression of MHC class II genes (Mattiace *et al.* 1990; Sheffield and Berman 1998), and secretion of proinflammatory cytokines (Takao *et al.* 1996; Sheng *et al.* 1998; Nanamiya *et al.* 2000). Additionally, increased expression of cytokine and chemokine genes and their receptors occurs within the aging cortex, midbrain, hippocampus, and cerebellum (Felzien *et al.* 2001; Terao *et al.* 2002).

Although MHC class I and II alleles do not segregate in this cross, and therefore cannot underlie *eae1* or *eae5* (Butterfield *et al.* 1998), they nevertheless serve to illustrate a scenario whereby environmental factors influencing the expression of structurally polymorphic disease susceptibility genes can exhibit gene-by-environment-dependent genetic linkage. Genetic resistance due to suboptimal affinity of a peptide ligand for MHC alleles may effectively be overcome by age-related increases in cell-surface density. Consequently, genetic linkage to MHC alleles will be detected only when antigen recognition is primarily a function of peptide–MHC affinity and not of the density of peptide–MHC complexes on the surface of antigen-presenting cells. Importantly, genetic linkage to any structural polymorphism whose functional difference can be overcome by increased expression of either the receptor or the ligand has the potential to exhibit similar behavior in genetic linkage studies.

The Mouse Phenome Database (http://www.aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home) was used to search for nonsynonymous SNPs in immunologically relevant candidate receptor–ligand systems linked to *eae36* (±5 cM of *D4Mit204* or from 118 to 137 Mb). Using this approach, we identified three receptors (Table 3). The first was colony-stimulating factor 3 receptor (*Csf3r*). The SJL/J and C57BL/10 *Csf3r* alleles are distinguished by an N379S polymorphism (rs13477964). Functionally, colony stimulating factor 3 (Csf3) has been shown to elicit long-lived protective effects on both clinical and histopathologic EAE (Lock *et al.* 2002; Zavala *et al.* 2002] and treatment of lupus-prone MRL-*lpr*/*lpr* (Zavala *et al.* 1999) and NOD mice (Kared *et al.* 2005)

TABLE 3

Linkage of EAE susceptibility to *Csf3r*, *Pnrc2*, and *Htr1d* SNP markers in older F_2 intercross mice and mice immunized in the winter

Marker	Mb[a]	All[b]	>12 wk[c]	Winter[d]
D4Mit221	122.23	17.8; 95–99%	14.8; 90–95%	13.0; <90%
Csf3r	125.05	18.8; 95–99%	15.7; 95–99%	15.5; 95–99%
D4Mit203	128.50	21.7; >99%	18.9; >99%	17.6; >99%
D4Mit204	132.29	23.4; >99%	20.9; >99%	21.1; >99%
Pnrc2	134.75	19.6; >99%	18.5; >99%	20.4; >99%
Htr1d	135.30	19.8; >99%	18.9; >99%	21.0; >99%

Mice were immunized for the induction of EAE and scored as affected and unaffected starting on day 10 (Butterfield *et al.* 1998).

[a] Locations are based on the March 2005 mouse (*Mus musculus*) draft genome data obtained from the Build 34 assembly by NCBI.

[b] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.48, 95% = 15.16, 99% = 19.50; $n = 366$, marker number = 127.

[c] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.77, 95% = 15.01, 99% = 18.27; $n = 337$, marker number = 127.

[d] Experimentwise critical values for significance levels with 1000 permutations of the linkage data: 90% = 13.81, 95% = 15.51, 99% = 17.59; $n = 243$, marker number = 127.

with Csf3 induces substantial protection from disease. The second candidate in this region is proline-rich nuclear receptor coactivator 2 (*Pnrc2*). The SJL/J and C57BL/10 alleles of *Pnrc2* are distinguished by an L41H substitution (rs13478000). Pnrc2 interacts with nuclear receptors using a proline-rich sequence to modulate their transcriptional activity. Importantly, Pnrc2 interacts with a number of orphan receptors in a ligand-independent manner and with the estrogen, glucocorticoid, progesterone, thyroid, retinoic acid, and retinoid X receptors in a ligand-dependent manner (Zhou and Chen 2001). Interestingly, *Pnrc1/B4-2*, the first proline-rich nuclear receptor coregulatory protein identified, was isolated from a natural killer (NK) minus T-cell subtractive library, suggesting that this newly identified class of nuclear receptor coregulatory proteins may play a significant role in nuclear receptor transcriptional activity in cells of the innate immune system (Chen *et al.* 1995). NK cells have also been shown to regulate CD4 T-cell responses and the outcome of B-cell-mediated autoimmune responses (Shi *et al.* 2000; Dowdell *et al.* 2003). Finally, 5-hydroxytryptamine (5-HT/serotonin) receptor 1D (*Htr1d*) is found in this interval. The SJL/J and C57BL/10 alleles of *Htr1d* are distinguished by an A22V substitution (rs13478001). 5-HT is an indolamine that interacts with multiple receptors mediating a wide range of physiological functions. These include neurotransmitter (Aghajanian and Sanders-Bush 2002), immunomodulatory (Mossner and Lesch 1998), and vascular functions, including regulation of brain microcirculation and blood brain barrier permeability (Cohen *et al.* 1996). In this regard, SJL/J mice, the prototypical EAE susceptible strain, exhibit hypersensitivity to the vasoactive effects of 5-HT (Linthicum and Frelinger 1982), a phenotype that can be blocked by the 5-HT_1 receptor antagonist methiothepin mesylate (Khare *et al.* 2000). In addition, C67BL/6 mice lacking the 5-HT transporter (*Slc6a4*) purportedly develop attenuated clinical and histopathologic EAE as compared to wild-type mice. This difference is associated with reduced production of INFγ by neuroantigen-specific splenocytes and is sexually dimorphic in that it is more pronounced in female mice (Hofstetter *et al.* 2005).

In terms of candidate genes, phenotypes controlled by Csf3/Csf3r and the serotonin/5-HT_1 receptor class exhibit seasonal- (Brewerton 1989; Smaaland *et al.* 2002) and age- (Meltzer *et al.* 1998; Berkahn and Keating 2004) related variations. Similarly, age- and season-dependent fluctuations in nuclear hormone levels are well documented. Under limiting conditions, a *Pnrc2* polymorphism could significantly impact gene expression by influencing the transcriptional activity of multiple nuclear hormone receptors, and thus all three candidate receptors could qualify for the *eae36* culprit gene. Clearly, the identification of the polymorphism underlying *eae36* will aid in delineating the mechanisms underlying age- and season-by-gene-specific interaction in autoimmune inflammatory demyelinating diseases of the CNS.

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