Sex-Specific Quantitative Trait Loci Govern Susceptibility to Theiler’s Murine Encephalomyelitis Virus-Induced Demyelination

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ABSTRACT

Susceptibility to Theiler’s murine encephalomyelitis virus-induced demyelination (TMEVD), a mouse model for multiple sclerosis (MS), is genetically controlled. Through a mouse-human comparative mapping approach, identification of candidate susceptibility loci for MS based on the location of TMEVD susceptibility loci may be possible. Composite interval mapping (CIM) identified quantitative trait loci (QTL) controlling TMEVD severity in male and female backcross populations derived from susceptible DBA/2J and resistant BALB/cByJ mice. We report QTL on chromosomes 1, 5, 15, and 16 affecting male mice. In addition, we identified two QTL in female mice located on chromosome 1. Our results support the existence of three linked sex-specific QTL on chromosome 1 with opposing effects on the severity of the clinical signs of TMEV-induced disease in male and female mice.

MULTIPLE sclerosis (MS) is the major demyelinating disease of the central nervous system (CNS) in humans, affecting 0.1% of the North American population, and involves both genetic and environmental factors (Sadovnick and Ebers 1993; Ebers and Sadovnick 1994; Ewing and Bernard 1998; Compston 1999; Kalman and Lublin 1999; Sadovnick 2002). Concordance rates among monozygotic twins are 20–30% while dizygotic twins, full siblings, and nonbiological siblings have concordance rates of ~4% (Ebers et al. 1995; Sadovnick et al. 1996). Although a genetic component to susceptibility has been demonstrated, little is known about the genes that modulate MS. Evidence of an environmental etiology for MS comes primarily from migration studies and geographic distribution data (Ebers and Sadovnick 1994). Migration studies indicate that individuals moving from high-risk areas tend to adopt the low risk of native populations (Ebers and Sadovnick 1994; Compston 1999). Susceptibility to MS is likely the result of complex interactions of environmental triggers on a susceptible genetic background.

Viruses have long been purported to play a role in the etiology of MS. Human herpes virus-6, Epstein-Barr virus, and measles virus have been detected in the brains of MS patients, but no single virus has been associated with all cases (Challoner et al. 1995; Dalgleish 1997). Theiler’s murine encephalomyelitis virus-induced demyelination (TMEVD) is a model for virally triggered MS. TMEV is a murine picornavirus spread in natural and laboratory populations by the fecal/oral route (Miller et al. 1994). Following intracerebral inoculation, the virus establishes a persistent infection of CNS white matter in susceptible strains. CD4+ T cells initiate the disease by infiltrating the CNS and subsequently recruit additional lymphocytes, leading to inflammation and progressive demyelination. Clinical signs become apparent 35–40 days postinoculation and show a progressive course characterized by gait abnormalities, limb spasms, and incontinence (Miller et al. 1994).

H2D and two loci on chromosome 10 have been associated with viral persistence (Clatch et al. 1985; Bureau et al. 1993; Lipton et al. 1995; Bihl et al. 1999). Other loci controlling susceptibility to TMEVD have been identified on chromosomes 3, 6, 11, and 14 (Melvold et al. 1987, 1990; Brahic and Bureau 1998; Bureau et al. 1998; Aubagnac et al. 1999). Recently, we examined the most severely affected animals in a (BALB/cByJ × DBA/2J) × BALB/cByJ backcross by qualitative assessment (Teuscher et al. 1997). We reported that susceptibility to TMEVD was linked to a locus on chromosome 3 between D3Mit29 and D3Mit10 near eae3, a locus associated with susceptibility to experimental allergic encephalomyelitis (EAE), suggesting that a shared susceptibility gene or a cluster of tightly linked genes control susceptibility to both of these demyelinating diseases.

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The shared gene hypothesis for non-MHC-linked genes underlying immunopathologically based phenotypes, first proposed by this laboratory (Teuscher 1985; Sudweeks et al. 1993; Meeker et al. 1995), was recently validated with the identification of Bphs, an autoimmune disease susceptibility gene linked to EAE and autoimmune orchitis as histamine receptor H1 (Ma et al. 2002). Interestingly, CD2, a known polymorphic cell surface protein important in T-cell activation, colocalizes with Tmevd2 and eae3 on chromosome 3 (Altevogt et al. 1989; Moseley and Sedlin 1989).

Males and females of the same strain can differ in susceptibility to TMEVD (Kappler et al. 1990; Hill et al. 1998). In addition, a sex effect has been associated with viral persistence in the CNS. Tmevd2 and Tmevd3 were identified on chromosome 10, with males exhibiting a greater viral load than females (Bihl et al. 1999). In this work we used composite interval mapping (CIM; Zeng 1993, 1994) to identify sex-dependent quantitative trait loci (QTL) on chromosomes 1, 5, 15, and 16 in males and two QTL on chromosome 1 in females controlling severity of TMEVD.

MATERIALS AND METHODS

**Animals:** Male and female BALB/cByJ and DBA/2J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). (BALB/cByJ × DBA/2J) × BALB/cByJ backcross mice (BC1) were bred at Northwestern University School of Medicine (Chicago) and the University of North Dakota (Grand Forks, ND). All mice were maintained in polycarbonate cages and received standard mouse chow and water *ad libitum*. Mice used in these studies were maintained according to the guidelines of the Animal Care and Use Committee of the University of North Dakota and Northwestern University, fully accredited by the American Association of Animal Laboratory Care. Of the 170 BC1 animals included in this study, 71 were male and 99 were female.

**Induction of disease:** The BeAn 8386 strain of TMEV was used for disease induction in this study. After plaque purification and titer amplification by serial passage in BHK-21 cells, a working stock was prepared with a titer of $9.7 \times 10^{6}$ PFU/ml. At 7 weeks of age, the mice were anesthetized with methoxyflurane and inoculated in the right cerebral hemisphere with $2.9 \times 10^{6}$ PFU of virus. Control mice were injected with media or were mock infected with BHK lysate in the same DBA/2J or F1 hybrid mice using cohort sizes of 9 male and female mice (see Table 1). In our BC1 population, however, a greater number of males (56 of 71) than females were susceptible to TEMVD (64 of 99, $P = 0.045$). Kappler et al. (1990) have also shown an increase in susceptibility to TMEVD in male mice.

**Evaluation of phenotype:** Following inoculation, the animals were examined independently by two investigators for a period of 13 weeks. Severity of clinical signs was scored on the following basis: 0 for asymptomatic, 1 for moderate (swaying) gait abnormality, and 2 for severe (waddling) gait abnormality. Clinical signs have been previously shown to provide a good correlation with demyelination when compared with histological examination or testing of TMEV-specific delayed-type hypersensitivity responsiveness (McGavern et al. 2000). A quantitative trait value for estimating the overall severity of the disease as a function of time postviral challenge, similar to that used in studies on murine EAE (Butterfield et al. 1998), was calculated by averaging the scores for each animal over the course of the experiment.

**Genotyping and linkage analysis:** Genomic DNA was isolated from liver tissue. PCR-based genotyping using 199 polymorphic microsatellite markers was performed as previously described (Sudweeks et al. 1993; Meeker et al. 1995; Wardell et al. 1995). Microsatellite primers were either purchased from Research Genetics (Huntsville, AL) or synthesized according to the guidelines of the Animal Care and Use Committee of the University (1997). Significant markers are first chosen using a linear regression model with a forward/backward selection procedure in the Smapqtl program of QTL Cartographer. Additional markers, unlinked to the test interval, but with significant effects on the trait, are added to the model to control for the genetic background. In this work, background markers for CIM were chosen using the Smapqtl module of QTL Cartographer in forward/backward selection with an accepting/rejecting significance level of 0.10 using 198 markers (all markers except D15Mit209, which was subsequently added to the analysis). Composite interval mapping was performed using 2-cM increments with a window size of 10 cM, and the 10 most significant background markers selected via Smapqtl as described above were used in our CIM analyses. Tests of significant linkage for QTL are reported as likelihood-ratio test (LRT) statistics. Significance of the linkage between marker loci and putative disease susceptibility gene linked to EAE and autoim-mune microsatellite markers was performed as previously described (McGavern et al. 2000). As such, identification of the genes uniquely controlling susceptibility to TMEVD in males and females may lead to a better understanding of the role of sex-specific QTL in inflammatory diseases of the CNS.

Loci involved in TMEVD severity were identified using CIM on subpopulations consisting of males and

RESULTS AND DISCUSSION

Gait abnormalities were seen in 120 of the 170 BC1 animals in the study. Significant differences in sex-specific susceptibility were not seen in parental BALB/cByJ, DBA/2J, or F1 hybrid mice using cohort sizes of 9 male and female mice (see Table 1). In our BC1 population, however, a greater number of males (56 of 71) than females were susceptible to TEMVD (64 of 99, $x^2 = 4.03, P = 0.045$). Kappler et al. (1990) have also shown an increase in susceptibility to TMEVD in male mice. Sex-specific effects have also been observed in overall susceptibility to MS (Duquette et al. 1992) as well as in disease subtypes (Runmarker and Anderson 1993). As such, identification of the genes uniquely controlling susceptibility to TMEVD in males and females may lead to a better understanding of the role of sex-specific QTL in inflammatory diseases of the CNS.

Loci involved in TMEVD severity were identified using CIM on subpopulations consisting of males and
TABLE 1
Incidence of symptoms of TMEVD in DBA/2J, BALB/cByJ, (BALB/cByJ × DBA/2J) F<sub>1</sub> hybrid, and (BALB/cByJ × DBA/2J) × BALB/cByJ backcross mice by sex

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Total</th>
<th>Unaffected (clinical score = 0)</th>
<th>Affected* (clinical score = 1 or 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBA/2J</td>
<td>M</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>F</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>M</td>
<td>9</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>BALB/cByJ</td>
<td>F</td>
<td>9</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>M</td>
<td>9</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>F</td>
<td>9</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>BC1</td>
<td>M</td>
<td>71</td>
<td>15</td>
<td>56</td>
</tr>
<tr>
<td>BC1</td>
<td>F</td>
<td>99</td>
<td>35</td>
<td>64</td>
</tr>
</tbody>
</table>

* Animals were considered affected if they displayed either mild (score = 1) or severe (score = 2) abnormalities in gait for three consecutive weekly evaluations or for four out of five consecutive weekly evaluations.

females. In the male population (n = 71), CIM revealed significant loci (α = 0.05) on chromosomes 1, 5, and 15. A QTL on chromosome 1 (Tmevd6) near D1Mit170 at 19.5 cM accounted for 9.5% of the variation in the severity of the clinical signs associated with TMEVD severity (Figure 1, Table 2). (Mouse chromosomes are acrocentric; thus all centimorgan distances are relative to the centromere.) Interestingly, the negative additive effect (−0.26; see Table 2) indicated that the susceptibility allele was derived from the TMEVD-resistant BALB/cByJ. A QTL on chromosome 5 (Tmevd7) at 72 cM, near D5Mit30, accounted for 16.6% of the variation (Figure 1, Table 2) and increased severity at this locus was associated with the DBA/2J allele. On chromosome 15 at 4.7 cM near D15Mit12, a QTL (Tmevd8) accounted for 14.1% of the trait variation. Additionally, in male mice, suggestive linkage (α = 0.10) was found on chromosomes 15 and 16. A QTL on chromosome 15, at 22.2 cM near D15Mit5, accounted for 8.1% of the variation in the trait, while a locus on chromosome 16 near D16Mit50 at 53.5 cM accounted for 8.1% of the variation (Figure 1, Table 2). Interestingly, the QTL identified on chromosome 16 in males colocalizes with eae11, a locus controlling lesion severity and susceptibility to EAE in males (Butterfield et al. 1999). Linkage to this region of chromosome 16 may reflect a hormonally regulated gene or gene complex controlling immunologically mediated demyelination. Independent verification of the suggestive loci on chromosomes 15 and 16 will be required before these QTL will be given TMEVD designations.

Analysis of the female population (n = 99) revealed two QTL influencing the severity of disease symptoms. A significant QTL, Tmevd9, was found on chromosome 1 at 32.8 cM, near D1Mit76, and accounted for 7.6% of the variation (Table 2, Figure 2). This QTL colocalizes...
TABLE 2
Location and effects of QTL controlling severity of symptoms in (BALB/cByJ × DBA/2J) × BALB/cByJ backcross mice

<table>
<thead>
<tr>
<th>QTL designation</th>
<th>Chr.</th>
<th>cM^a</th>
<th>Marker^b</th>
<th>LRT</th>
<th>Additive %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmevd6</td>
<td>1</td>
<td>19.5</td>
<td>D1Mit170</td>
<td>16.21</td>
<td>−0.26</td>
</tr>
<tr>
<td>Tmevd7</td>
<td>5</td>
<td>72</td>
<td>D5Mit30</td>
<td>26.45</td>
<td>0.37</td>
</tr>
<tr>
<td>Tmevd8</td>
<td>15</td>
<td>4.7</td>
<td>D15Mit12</td>
<td>18.34</td>
<td>−0.36</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>22.2</td>
<td>D15Mit5</td>
<td>14.10</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>53.5</td>
<td>D16Mit50</td>
<td>14.06</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tmevd6</td>
<td>1</td>
<td>19.5</td>
<td>D1Mit170</td>
<td>16.21</td>
<td>−0.26</td>
</tr>
<tr>
<td>Tmevd9</td>
<td>1</td>
<td>32.8</td>
<td>D1Mit76</td>
<td>14.61</td>
<td>−0.33</td>
</tr>
</tbody>
</table>

^a Location according to Mouse Genome Informatics (MGI; http://www.informatics.jax.org/).
^b Marker at the peak linkage, determined by permutation threshold (Figures 1 and 2).
^c Likelihood-ratio test statistic.
^d Additive effect of the QTL relative to the BALB/cByJ homozygote. A positive value indicates that the mean trait value for the BALB/cByJ homozygotes is greater than the mean trait value for the heterozygous animals.
^e Percentage variance accounted for by a QTL at the specified location.

with Cd28 and Cd152 (Ctla4), important cell surface molecules in the control of T-cell activation. In contrast to Tmevd6, the DBA2/J allele at this locus decreased disease severity in females.

Additionally, suggestive linkage in females was seen on chromosome 1 at 19.5 cM (D1Mit170), accounting for 6.8% of the experimental variation. This QTL is at the same location as Tmevd6 identified in males. In contrast to males (additive effect = −0.26), the additive effect of Tmevd6 in females was 0.25, indicating that the DBA/2J allele increased severity in females while a BALB/cByJ allele increased severity in males. The presence of a QTL in the same interval of chromosome 1 in male and female populations with opposite additive effects suggests that Tmevd6 may contain two closely linked QTL with opposite effects in males and females. Alternatively, sex hormones may differentially regulate the same QTL in males and females. Interference of the sex-specific QTL at Tmevd6 on chromosome 1 most likely prevented their identification by classical interval mapping since they had effects in opposite directions (Zeng 1993, 1994). Further studies will be necessary to elucidate the position and effects of the Tmevd6 locus in males and females.

In this study, we have shown that sex-specific QTL play a role in susceptibility to TMEVD with QTL on chromosomes 1, 5, 15, and 16 controlling disease severity in males, while two QTL on chromosome 1 influence severity in females. These sex-specific QTL were identified only when the experimental population was stratified by sex and analyzed using CIM. Similar sex-specific QTL have been identified in the genetic control of both clinical and histopathologic EAE, the other major animal model for MS (Butterfield et al. 1999, 2000; Blankenhorn et al. 2000). Additionally, this study demonstrates, in a practical sense, the utility of CIM in detecting multiple linked, sex-specific QTL and that resistant strains of mice may harbor TMEVD susceptibility loci that become relevant only as they interact with susceptibility loci from different strains. This may explain why significantly greater numbers of BCI males were affected with TMEVD while parental DBA/2 and BALB/cByJ mice did not show significant differences in sex-biased susceptibility. A summary of both the sex-specific and non-sex-specific TMEVD-modifying loci identified to date is found in Table 3.

The mechanisms underlying sex-specific QTL are unknown but may arise as a result of sex hormone regulation of the polymorphic genes underlying these QTL or interactions between mitochondrially or Y-chromosomal genes.
TABLE 3
Summary of TMEVD-modifying loci identified in mice to date

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chr.</th>
<th>Location (cM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tmevd1</td>
<td>6</td>
<td>22</td>
<td>Melvold et al. (1987)</td>
</tr>
<tr>
<td>Tmevd2</td>
<td>3</td>
<td>46</td>
<td>Bureau et al. (1998)</td>
</tr>
<tr>
<td>Tmevd3</td>
<td>14</td>
<td>12.5</td>
<td>Bureau et al. (1998)</td>
</tr>
<tr>
<td>Tmevd4</td>
<td>14</td>
<td>39.5</td>
<td>Aubagnac et al. (1999)</td>
</tr>
<tr>
<td>Tmevd5</td>
<td>11</td>
<td>60</td>
<td>This report</td>
</tr>
<tr>
<td>Tmevd6</td>
<td>1</td>
<td>19.5</td>
<td>This report</td>
</tr>
<tr>
<td>Tmevd7</td>
<td>5</td>
<td>72</td>
<td>This report</td>
</tr>
<tr>
<td>Tmevd8</td>
<td>15</td>
<td>4.7</td>
<td>This report</td>
</tr>
<tr>
<td>Tmevd9</td>
<td>1</td>
<td>32.8</td>
<td>This report</td>
</tr>
<tr>
<td>Tmevp1</td>
<td>17</td>
<td>19.1 (H2D)</td>
<td>Clatch et al. (1985)</td>
</tr>
<tr>
<td>Tmevp2</td>
<td>10</td>
<td>51.5–62</td>
<td>Bihl et al. (1999)</td>
</tr>
<tr>
<td>Tmevp3</td>
<td>10</td>
<td>62–70</td>
<td>Bihl et al. (1999)</td>
</tr>
</tbody>
</table>

* Tmevd loci are defined as susceptibility loci for TMEV-induced demyelination. Tmevp loci are defined as loci controlling viral persistence.

*Location according to MGI (http://www.informatics.jax.org/).

Some-linked genes. The role of sex hormones in the sexual dimorphism observed in immune responsiveness as well as in immunopathologically based diseases has been well documented (Grossman et al. 1991; Da Silva 1999) as has the regulation of immunologically relevant genes such as cytokines (Cutolo 2002). With respect to potential interactions with mitochondrially linked genes, evidence suggests that mutations in mitochondrially encoded genes contribute to an MS-like syndrome, Leber’s hereditary optic neuropathy, which occurs primarily in women (Wissinger et al. 1997). Such mutations, however, do not appear to be a risk factor for MS per se (Reynier et al. 1999). Additionally, a number of immunologically relevant genes are known to be on the X chromosome (http://www.informatics.jax.org/searches/linkmap.cgi). The precedence for Y-chromosome-linked genes that influence immune responses is best exemplified by Yaa, a Y chromosome-encoded gene that interacts with autosomal susceptibility loci to accelerate the development of spontaneous lupus and lymphoproliferation in male mice (Murphy and Roths 1979). A similar male-specific form of hereditary lupus is seen in humans (Lahita et al. 1983). Although the mice used in this study do not possess the classically defined accelerator allele at the Yaa locus, Yaa nevertheless establishes a precedent for the existence of Y-chromosome-linked genes affecting autoimmune disease. Thus, the existence of sex-specific QTL may be responsible for confounding the interpretation of human MS genetic data or masking the presence of susceptibility loci. In light of our findings, MS genetic studies, as well as studies using animal models, should account for the potential sex-specific genetic differences in disease.

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