Genetic Mapping of Quantitative Trait Loci Affecting Susceptibility to Marek’s Disease Virus Induced Tumors in F₂ Intercross Chickens

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Manuscript received February 3, 1997
Accepted for publication August 7, 1997

ABSTRACT

Marek’s disease (MD) is a lymphoproliferative disease caused by the MD virus (MDV), which costs the poultry industry nearly $1 billion annually. To identify quantitative trait loci (QTL) affecting MD susceptibility, the inbred lines 6 (MD resistant) and 7 (MD susceptible) were mated to create more than 300 F₂ chickens. The F₂ chickens were challenged with MDV (JM strain, moderately virulent) at 1 wk of age and assessed for MD susceptibility. The QTL analysis was divided into three stages. In stage 1, 65 DNA markers selected from the chicken genetic maps were typed on the 40 most MD-susceptible and the 40 most MD-resistant F₂ chickens, and 21 markers residing near suggestive QTL were revealed by analysis of variance (ANOVA). In stage 2, the suggestive markers plus available flanking markers were typed on 272 F₂ chickens, and three suggestive QTL were identified by ANOVA. In stage 3, using the interval mapping program Map Manager and permutation tests, two significant and two suggestive MD QTL were identified on four chromosomal subregions. Three to five loci collected explained between 11 and 23% of the phenotypic variation, or 32–68% of the genetic variance. This study constitutes the first report in the domestic chicken on the mapping of non-major histocompatibility complex QTL affecting MD susceptibility.

Marek’s disease (MD) is a lymphoproliferative disease, caused by a member of the herpesvirus family, that costs the poultry industry nearly $1 billion annually (Purchase 1985). Diseased chickens infected by the Marek’s disease virus (MDV) commonly exhibit paralysis, blindness, and visible lymphoid tumors that result in condemnation of the birds. Although vaccination programs have effectively reduced the incidence of MD, there is evidence that current vaccines do not protect well against some highly pathogenic MDV strains that have emerged in recent years (Witter and Hunt 1993). Also, MD vaccines control rather than eliminate losses from MD because they do not block MDV infection. As a result, MDV is ubiquitous on poultry farms, and all chickens are exposed to the pathogenic agent at 1 day of age.

All these factors point to the need to complement vaccinal protection with alternative methods such as genetic resistance (Spencer et al. 1974; Gavora and Spencer 1979). And even if a specific disease has been controlled through vaccination, genetic resistance is of value because it represents a safeguard against heavy losses in the case of disease outbreaks. When available, genetic resistance to disease probably provides the most reliable, economical, and environmentally sound strategy for disease control.

Genetic resistance to MD had been known for more than 60 years (Calnek 1985). Although genetic resistance is complex, genetic selection for high levels of resistance can be obtained within relatively few generations (Cole 1968). The development of effective vaccines in the late 1960s, however, greatly reduced interest in the genetic control of MD. Ironically, genetically resistant lines were shown to have greater vaccinal immunity and higher egg production than susceptible lines (von Krogh et al. 1972; Spencer et al. 1974; Gavora and Spencer 1979).

Most traits of economic importance in human, animal, and plant species are assumed to be controlled by numerous genes at distinct loci referred to as quantitative trait loci (QTL). Thompson (1961) suggested the use of Mendelian markers to map the genomic position of QTL and to dissect complex quantitative traits at least partly into their underlying Mendelian components. The current availability in many species of highly polymorphic DNA markers allows the development of well-saturated genetic maps and, consequently, the genetic dissection of complex quantitative traits. A further improvement is the development of powerful statistical methods and computer packages that handle linkage analysis between marker loci and QTL for experimen-

The chicken is an ideal animal model for the genetic dissection of complex traits. Because of the high reproductive capacity and relatively short life cycle, several generations of large chicken families can be produced and characterized in a short period of time. Also, inbred lines that display a variety of characteristics can be developed. Consequently, the unique characteristics of the chicken model allow the use of contemporary biometrical techniques that have already proven successful for plant species. Chickens also provide a good model for studying susceptibility to viral diseases in other vertebrates, including humans, because a number of virally induced tumors occur in the domestic fowl (Bishop 1983; Reddy et al. 1988; Watson et al. 1987).

Here, we report the identification of non-major histocompatibility complex (MHC) QTL affecting several components of MD susceptibility. To avoid the confounding effects of MHC genes, which are known to influence MD susceptibility (Bacon 1987), a cosegregation analysis was carried out in F2 intercross chickens bred from the inbred lines 6a and 7a that are identical for class I, II, and IV MHC genes. Using single-marker and interval mapping analyses, five genomic regions affecting MD susceptibility were identified. This study sheds light on the multigenic basis of MD susceptibility in the chicken and provides further evidence that the identification of genes affecting susceptibility to complex diseases is possible with the use of contemporary biometrical and molecular tools. This study constitutes a first report in the domestic chicken on the mapping of non-MHC genes that affect MD susceptibility. Furthermore, it also provides a model for elucidating viral disease susceptibility genes in other vertebrates, including humans.

**Materials and Methods**

**Experimental population:** The chicken inbred lines of White Leghorns, line 6 subline 3 (6a) and line 7 subline 2 (7a) were developed at the Avian Disease and Oncology Laboratory (East Lansing, MI; Stone 1975). Although the lines 6a and 7a are identical for the MHC B* haplotype, they differ greatly in MD susceptibility (i.e., 6a is resistant and 7a is highly susceptible; Crittenden 1975; Paderka et al. 1975); lines 6b and 7b are identical at the DNA sequence level for genes of the MHC B-FIV (H. Hunt, personal communication) and B-Lddl (G. Pharr, personal communication). The inbred lines 6a (i.e., MD resistance allele R) and 7a (i.e., MD susceptibility allele r) were crossed as initial parents to produce more than 300 fully pedigreed F1 intercross chickens.

**Assessment of MD susceptibility:** The F2 intercross chickens, along with parental line and F1 control chickens, were produced in five hatch (i.e., ~72 chickens per hatch) for the evaluation of MD susceptibility. Within each hatch, the chickens were randomly assigned into six isolators (i.e., 16 chickens per isolator). The rearing and monitoring of chickens was conducted using standard controlled poultry research conditions. At 1 wk of age, the chickens were challenged with 2000 pfu of JM strain MDV; challenge conditions were empirically determined to maximize differences in disease incidence between the parental lines and the F1 progeny. Chickens that were moribund or survived for 10 wk were killed by CO2 inhalation and necropsied. Nerves and other organs were examined for gross MDV lesions and tumors. Chickens of one hatch did not show acceptable levels of MDV infection in the control birds and were not considered in this study. A few F2 chickens dying from non-MDV-related factors were also discarded. In the end, MD susceptibility data of 272 F2 chickens were considered for the QTL analysis.

Several components of MD susceptibility were evaluated. Viremia (VIR) is the MDV concentration at 2 wk after the MDV challenge (i.e., number of pfu per 10^9 peripheral blood cells; Witter et al. 1969). Tissue (TIS) is the number of different tissues/organs (e.g., vagus [VAG], brachial [BRA], or sciatic [SCI] nerves; or heart [HT], gonad [GON], spleen [SPL], bursa [BUR], lung [LNG], or thymus [THY] visceral) showing gross tumors or lesions at necropsy. Survival (SUR) is the number of days from the chicken MDV challenge to death. Disease (DIS) is the overall phenotypic assessment of each chicken for MD susceptibility (i.e., either resistant or susceptible). Tumor index (TUM) is an index developed using the following scoring system: 0 = alive, healthy, absence of tumors; 1 = single neural lesion; 2 = single visceral lesion or multiple neural lesion or dead with microscopic lesions; 3 = multiple visceral lesion; 4 = single visceral plus multiple neural lesion or multiple visceral plus single neural lesion; 5 = multiple visceral plus multiple neural lesion at necropsy or dead with gross lesions (R. Witter, unpublished results). MD index (MDI) is an empirical pooled index that we developed that assigns weights to each MD trait based on consensus suggestions from experienced MD pathologists and poultry veterinarians; thus, MDI was estimated using standardized MD data (i.e., z = (x − μ)/σ) and the following expression:

\[ \text{MDI} = \log_{10}(\{(\text{VIR*15}) + (\text{TIS*25}) + (\text{DIS*25}) + (\text{SUR*10}) + (\text{TUM*25})\} + 100). \]  

**Marker loci genotyping:** Genomic DNA was extracted from 272 F2 chickens by Southern blotting using TaqI and a F-B-FIV probe (Hunt et al. 1994, Bacon et al. 1996).

**Linkage analysis:** The F2 intercross chickens were genotyped using multipoint analysis of the genotypes at 42 marker loci typed on the entire F2 population (272 chickens) using the program MAPMAKER/EXP, version 3.0 (Lander et al. 1987, Lincoln et al. 1992a). Linkage groups were determined by the results of pairwise comparisons (two-point analysis) with a minimum LOD (log of odds) score of 3.0 for statistical acceptance of linkage and with a recombination fraction of r = 0.32. Following this, three-point analyses were performed for each linkage group comparing three consecutive markers at a time. An LOD value of 3.0 was again used as the linkage criteria for tripiles, while the multipoint analysis using the command "order" generated the most likely order and genetic distances expressed in centimorgans. With the assumption of no interference and a Poisson distribution for crossing over between chromosomes, the recombination fractions were related to genetic distances by means of Haldane's mapping function (Haldane 1919).

**Statistical analyses:** Before the QTL analysis, the components of MD susceptibility were assessed for significant devia-
QTL Affecting MD Susceptibility

QTL analysis: The analysis to identify QTL affecting MD susceptibility was divided into three stages. Stage 1 involved the identification of potential genomic regions with QTL residing near each marker. Eighty selected F2 chickens corresponding to the upper and lower values of VIR, TUM, and TIS [i.e., selective genotyping (SG)] were typed at 65 loci spaced throughout the chicken genome (Table 1; Cheng et al. 1995). Simple associations between the F2 progeny genotypes at each marker locus and MD susceptibility values were assessed using analysis of variance (ANOVA; SAS 1988). Marker loci producing ANOVA F-test statistic with \( P \leq 0.20 \) were considered suggestive of potential regions with QTL.

Stage 2, the suggestive markers detected in stage 1, plus available flanking markers, were typed on the entire F2 population (i.e., 272 chickens; Table 1). Associations between the F2 chicken genotypes at each locus and MD values were estimated using both parametric and nonparametric (i.e., Kruskal-Wallis) ANOVA (SAS, 1988). Nonparametric approaches of QTL analysis were used for discrete MD traits (e.g., DIS, BRA, SCI, and VAG). At this stage, marker loci producing ANOVA F-test statistic with \( P \leq 0.0016 (x^2 \geq 12.8) \) and \( 0.000052 (x^2 \geq 19.7) \) were considered suggestive and significant, respectively (Lander and Kruglyak 1995).

Stage 3 involved the estimation of approximate position, the expected additive and dominant effects, and the proportion of MD trait variation explained by individual QTL (i.e., \( R^2 \)) using the interval mapping program Map Manager QT, version b8 (Manly 1996). To declare a suggestive or significant QTL, chromosomewise critical threshold levels were estimated by 1000 permutations of our mapping data (Churchill and Doerge 1994).

Type of gene action: Average levels of dominance were estimated using the ratio dominance/additive effects (i.e., \( h^2 \)).

### TABLE 1

<table>
<thead>
<tr>
<th>LG</th>
<th>Stage 1 SGa</th>
<th>No. marker with ( P \leq 0.2b )</th>
<th>No. flank markersc</th>
<th>Stage 2 total No. marker</th>
<th>Expt. totald</th>
<th>Genome coverage (cM)</th>
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<td>339</td>
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<td>Old 2 New 4</td>
<td>9</td>
<td>12</td>
<td>245</td>
</tr>
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<td>1</td>
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</tr>
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<td>5</td>
<td>143</td>
</tr>
<tr>
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<td>100</td>
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<td>3</td>
<td>56</td>
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<td>E52</td>
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<td>—</td>
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<tr>
<td>E53</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>21</td>
<td>8</td>
<td>13</td>
<td>42</td>
<td>78</td>
</tr>
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</table>

LG, linkage group; Ch, chromosome number; E, East Lansing LG.

a Selective genotyping: 40 MD-resistant and 40 MD-susceptible chickens from the tails of the F2 population (\( \sim 30\% \) of the population) were typed at 65 marker loci.

b Locii producing F-test statistic with \( P \leq 0.02 \) at stage 1.

c Old, markers already typed at stage 1; new, markers typed only at stage 2.

d Total no. markers, number of markers typed at stage 1 plus number of markers typed at stage 2.

e Restriction fragment-length polymorphism marker.
In some instances, genetic effects were estimated by using least square means (SAS 1988) and standard expressions of genetic analysis (Falconer 1981). Gene type of action for each QTL was determined on the basis of h and by the criteria of Stuber et al. (1987) with underdominance or recessive h < 0 with additive h = 0–0.20, partial dominance h = 0.21–0.80, dominance h = 0.81–1.20, and overdominance h > 1.20.

**Loci interaction:** Digenic epistatic interactions among marker loci for a given MD susceptibility trait were detected by testing all the possible two-locus interactions (i.e., 861 interactions) among all the marker loci (42 markers) typed on the entire F2 population. Two-factor ANOVAs were performed using the GLM procedure (SAS 1988). To declare the significance of loci interaction on the expression of MD susceptibility, the frequency of observed significant interactions at probability levels of P ≤ 0.05, 0.01, and 0.001 were determined and compared with those frequencies that might be expected just by chance.

**RESULTS**

**Distribution of MD traits:** Almost 50% of the chickens developed MD as expected using the optimized challenge conditions. The frequency distributions of values for VIR, TIS, SUR, TUM, and MDI did not deviate significantly from normality. When these MD traits were transformed (i.e., log_{10} for VIR and SUR; SQR for TIS and TUM) and analyzed, both transformed and nontransformed data provided similar LOD values (data not presented). Therefore, results of nontransformed MD traits are reported here.

As was expected, discrete MD susceptibility traits (e.g., DIS, VAG, BRA, SCI, GON, HT, SPL, BUR, THY, and LNG) deviated significantly from normal distribution. Between 74 and 129 chickens had MD tumors in neural tissue (BRA, SCI, and VAG; data not presented). However, only one to seven chickens showed MD tumors in visceral (GON, HT, and LNG) and lymphoid (SPL, BUR, and THY) tissues. Therefore, nonparametric methods of QTL analysis were performed only for the following discrete MD traits: DIS, BRA, SCI, and VAG. Similarly, when analyzed, both transformed and nontransformed discrete MD traits provided similar LOD values; therefore, results of nontransformed discrete MD traits are reported here.

The effects of the covariates hatch, isolator, sex, and...
weight of chicken were not significant (data not presented); therefore, they were not considered in the models of QTL analysis.

**Phenotypic correlation between components of MD susceptibility:** The phenotypic correlation between components of MDV susceptibility (e.g., VIR, TIS, SUR, TUM, MDI, DIS, BRA, SCI, and VAG) were all significant at \( P \leq 0.0001 \) (data not presented). As was expected, SUR was negatively correlated with the other components of MD susceptibility; VIR showed lower levels of correlation when compared to the other traits.

**Segregation ratios of marker loci:** In stage 1, when using SG, 52 marker loci (80%) fit their expected codominant 1:2:1 segregation ratio (data not presented), and 13 markers (20%) did not fit the expected 1:2:1 segregation ratio. However, in stage 2, all the 42 marker loci (100%) typed on the entire F2 population fit their expected codominant 1:2:1 segregation ratio (data not presented).

### TABLE 2

<table>
<thead>
<tr>
<th>LG</th>
<th>Marker</th>
<th>VIR</th>
<th>SUR</th>
<th>TIS</th>
<th>TUM</th>
<th>MDI</th>
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<td>0.115</td>
<td>0.188</td>
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</table>

| \( P \) values associated with MD traits

- LG, linkage group; Ch, chromosome number; E, East Lansing LG.
- Selective genotyping: 40 MD-resistant and 40 MD susceptible chickens from the tails of the F2 population (~30% of the population) were typed at 65 marker loci.
- Viremia (VIR) is the MDV concentration at 2 wk after the MDV challenge. Tissue (TIS) is the number of different tissue/organisms showing gross MD tumors or lesions at necropsy. Survival (SUR) is the number of days from the chicken MDV challenge to death. Disease (DIS) is the chicken phenotypic assessment for MD susceptibility. Tumor index (TUM) and MD index (MDI) are pooled indexes.

### TABLE 3

| LG | Marker | MD trait | \( P \) | \( \chi^2 \) | \( R^2 \)
<table>
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### Genetic maps and genome coverage:
In general, there was a good agreement in linkage groups and locus order between the \( 63 \times 72 \) F2 chicken genetic maps and those of the reference chicken genetic maps (Cheng et al. 1995; Groenen et al. 1996; Mariani et al. 1996). However, there was some difference in genetic distances between adjacent marker loci. Generally, the genetic distances estimated in this study were slightly larger than those of the East Lansing reference genetic maps (Figure 1), which are based on 52 meiotic events.

Assuming a 3000-cM length of the chicken genome, the genome coverage in this study was ~64% (Table 1). The 78 markers used covered 24 chicken linkage groups and the Z chromosome with an average spacing of 24–30 cM between marker loci.

### QTL analysis:
In stage 1, using SG and ANOVA techniques, 21 markers producing \( F \)-test statistics with \( P \leq 0.05 \).
0.20 were identified (Tables 1 and 2). These suggestive markers were considered as residing near potential QTL affecting MD susceptibility.

In stage 2, the 21 suggestive markers identified at stage 1, plus available flanking markers (42 total markers), were typed on the entire F2 population (Table 1), and three suggestive QTL affecting MD susceptibility were identified using ANOVA techniques (Table 3). A suggestive QTL affecting SUR and MDI was located in chromosome 2 (Ch2; ADL185). Another suggestive QTL affecting the incidence of MD tumors in SCI tissue was located in Ch7 (ADL326). The third suggestive QTL affecting MDI was detected in E16 (ADL240). The phenotypic trait variation explained by these suggestive QTL is between 5 and 7%.

In stage 3, using interval mapping and permutation tests, two significant (Ch2 and Ch8) and two suggestive QTL (Ch4 and Ch7) affecting several components of MD susceptibility were identified on four chromosomal subregions (Table 1; Figure 1). It is very likely that all the QTL detected in this study are pleiotropic; therefore, the suggestive QTL affecting VIR (Ch2) and SUR and TIS (Ch8) are accounted as part of the significant QTL detected on Ch2 and Ch8, respectively.

In Ch2, a significant QTL affecting SUR and MDI was located around the locus ADL185 (Table 4; Figure 1); this QTL is named MD1 (Figure 1). The QTL explains between 6 and 7% of the MD trait variation. The QTL appears to be recessive for MD susceptibility, and the alleles of the MD susceptible parent 72 increase MD susceptibility (Table 5). In addition, a suggestive QTL affecting VIR is located in the interval ADL185-MCW63 (Table 4). The trait variation explained by this suggestive QTL is 3%. This QTL is also recessive for the line 72 allele; however, the alleles of the resistant parent 63 increase VIR levels (Table 5).

In Ch4, a suggestive QTL affecting TIS and MDI was identified in the interval ADL331-ADL144 (Table 4; Figure 1); this QTL is named MD2 (Figure 1). The MD trait variation explained by the QTL is 3%. Although the estimated position is identical for both traits, the QTL mode of inheritance defined by these MD traits is different; for TIS, the alleles of the susceptible parent 72 increase MD resistance in an overdominant fashion, while for MDI, the alleles of the susceptible parent 72 increase MD susceptibility in a dominant fashion.

In Ch7, a suggestive QTL affecting VIR, TIS, SUR, TUM, and MDI was identified in the interval ADL180-ADL326 (Table 4; Figure 1); this QTL is named MD3. The suggestive QTL explains between 3 and 4% of the MD trait variation. This suggestive QTL appears to be recessive for the line 72 allele, which increases MD susceptibility (Table 5).
In Ch8, a significant QTL affecting TUM and MDI was identified in the vicinity of the locus ADL258 (Table 4; Figure 1); this QTL is named MD5. The variance explained by the QTL is ~5%. This QTL was partially dominant for the line 72 allele, which increases MD resistance (Table 5). In addition, a suggestive QTL affecting TIS and SUR was identified near the locus ADL258 (Table 4). The trait variation explained by this suggestive QTL was 2-4%. For both suggestive QTL, the line 72 allele increases MD resistance, however, the mode of inheritance is partially dominant for TIS while recessive for SUR (Table 5).

In linkage group E16, the suggestive QTL affecting MDI in the vicinity of the locus ADL240 (Table 3; Figure 1) is named MD6. This QTL is overdominant, and the line 72 allele increases MD susceptibility (Table 5). The total MD trait variation explained the detected QTL is presented in Table 6. For MDI, five QTL explained 23% of the phenotypic trait variation, or 67.6% of the genetic variance; this assumes a heritability \( h^2 \) of 0.34 for MD susceptibility. For SUR and TIS, three QTL explained between 11 and 13% of the phenotypic variation, or 32.3 and 38.2% of the genetic variance, respectively. For VIR and TUM, two QTL explained 6 and 9% of the phenotypic variation, or 17.6 and 26.5% of the genetic variance, respectively.

**Effect of loci interaction on MD susceptibility:** The frequency of significant two-locus interactions observed at a probability of \( P \leq 0.001 \) exceeds significantly those expected by chance for MDI, VIR, SUR, and TUM (Table 7). From the significant two-locus interactions for MDI, two highly significant interactions involving significant QTL affecting MDI were detected. First, the QTL in linkage group Ch4 (ADL144, \( R^2 = 3.0 \); Table 6) and marker ADL289 on E27 (\( R^2 = 2.4 \); Table 3) accounted for 9.5% of the MDI variation in a two-locus model (\( P = 0.0083 \); data not presented). Second, the flanking markers of MD4, ADL258 (\( R^2 = 5.0 \); Table 6) and ADL322 (\( R^2 = 1.0 \); data not presented) accounted for 12.1% of the MDI variation in a two-locus model (\( P = 0.0053 \); data not presented).

**DISCUSSION**

**Genome coverage and limitations of this QTL search:** Microsatellite markers offer many advantageous characteristics for genome mapping and the search of genes (i.e., QTL) affecting susceptibility to complex diseases in domestic species, including humans. With this in mind, with one exception, only microsatellite markers were used in the mapping of QTL affecting MD susceptibility in the chicken. Unfortunately, a chicken genetic map saturated with microsatellite markers was not available at the start of this study. However, a total of more than 700 microsatellite loci are now mapped on three chicken genetic maps (H. Cheng, unpublished results; Grøen et al. 1996), and efforts are underway to consolidate all the maps. The sparse coverage of the

<table>
<thead>
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<th>LG</th>
<th>Marker interval</th>
<th>MD trait</th>
<th>( \mu_{BR} )</th>
<th>( \mu_{TR} )</th>
<th>( \mu_{VR} )</th>
<th>( \mu_{VR} )</th>
<th>( a(d_{a})^{-} )</th>
<th>( d(d_{a})^{-} )</th>
<th>GM (^c)</th>
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<tr>
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<td>ADL185-MCW63</td>
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<td>-1.15 (0.07)</td>
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<td>VIR</td>
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<td>2.1</td>
<td>2.1</td>
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<td>49.5</td>
<td>3.14 (0.18)</td>
<td>-0.15 (0.01)</td>
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<td>0.30 (0.40)</td>
<td>1.42 (1.94)</td>
<td>over</td>
<td></td>
</tr>
</tbody>
</table>

LG, linkage group; Ch, chromosome number; E, East Lansing LG; Viremia (VIR), the MDV concentration at 2 wk after the MDV challenge; Tissue (TIS), the number of different tissue/ organs (e.g., vagus (VAG), brachial (BRA), or sciatic (SCI) nerves) showing MD tumors or lesions; Survival (SUR), the number of days from the chicken MDV challenge to death; Disease (DIS), the chicken phenotypic assessment for MD susceptibility; Tumor index (TUM) and MD index (MDI), pooled indexes.

\( a(d_{a})^{-} \), Additive effect of QTL (additive effect in phenotypic standard deviations).

\( d(d_{a})^{-} \), Dominant effect of the QTL (dominant effect in phenotypic standard deviations).

GM, Genetic model; dom, dominant; pdom, partial dominance; over, overdominant; rec, recessive. The GM denomination was determined using the criteria of Stuber et al. (1987): underdominance or recessive \( h < 0 \), additive \( h = 0-0.20 \), partial dominance \( h = 0.21-0.80 \), dominance \( h = 0.81-1.20 \), and overdominance \( h > 1.20 \).
The confidence intervals for resolving the location of our QTL ranges from 30 cM for MD1 ($R^2 = 7.0$) to 125 cM for MD5 ($R^2 = 2.0$).

**Mapping QTL affecting MD susceptibility:** This study constitutes a first report on the mapping of QTL (i.e., non-MHC genes) affecting MD susceptibility in the domestic chicken using contemporary biometrical and molecular genetic tools. We identified five chromosomal subregions of the chicken containing QTL that were affecting several components of MD susceptibility (Figure 1). Two significant QTL affecting several components of MD susceptibility were located in the linkage groups Ch2 and Ch8, and three suggestive QTL affecting several components of MD susceptibility were located in the linkage groups Ch4, Ch7, and E16. From the components of MD susceptibility, it appears that MDI, SUR, and TIS are the most informative traits for QTL mapping (Table 6). VIR was slightly less informative, and this could result from the high variability observed in MD viremia levels.

From the total number of genes underlying polygenic inheritance to disease resistance, only a few are predicted to be responsible for a significant portion of the genetic variation (i.e., 60–70%), while the rest of the variation may be attributable to a large number of genes with small effect (Gavora 1992). The fact that MDI, SUR, and TIS explained 32–68% of the MD genetic variation suggests that some other major MD QTL were undetected in this study because of limitations in the genome coverage.

The standardized additive gene substitution effects ($a_{gw}$) for the QTL detected in this study were between 0.01 and 1.05 phenotypic standard deviations (Table 5). In our experiment, it is unlikely that QTL with $a_{gw} > 1.0$ were undetected in this analysis for the regions surveyed. It is also likely that the power of QTL detec-
tion achieved in this mapping experiment was reason-
ably high because QTL of relative small $\alpha_{\text{crit}}$ effects were still detected.

In general, the QTL affecting MD susceptibility were
of medium to large effects, and all of them displayed a
nonadditive mode of inheritance (i.e., most of the QTL
were recessive with a few exhibiting partial dominance,
dominance, and overdominance; Table 5). However, it
should be considered that if multiple MD QTL are
linked, possible cancellation of additive effects and ag-
gregation of dominance effects, pseudo-overdomi-
nance can be created (Cockerham and Zeng 1996).

A reduced power of single-marker analysis (i.e.,
ANOVA techniques) in detecting QTL-marker associa-
tions in comparison to the interval mapping methods
was observed (i.e., ANOVA techniques detected only
three suggestive QTL). The reduced resolution in de-
tecting QTL by ANOVA procedures was caused by the
length of intervals scanned (i.e., >30 cM). In contrast,
the interval mapping method allowed us to identify two
significant and two suggestive QTL affecting several
components of MD susceptibility. These results concur
with theoretical studies that report higher efficiencies
using interval mapping methods in detecting QTL
compared to single-marker analysis when the scanned
intervals are >30 cM (Knot and Haley 1992; Rebai et
al. 1995).

Though not presented here, the interval mapping
programs MAPMAKER/QTL (Lincoln et al. 1992b)
and QTL Cartographer (Basten et al. 1996) were also
used in the hope of confirming our described results.
MAPMAKER/QTL identified MD1, MD2, MD3, and
MD5 as significant QTL for the same traits; in addition,
significant (LOD $\geq$ 4.3) QTL for VIR (Ch3 between
ADL131 and MCW169) and MDI (E27, ADL289) were
found. QTL Cartographer was more selective and re-
vealed only M D1, MD4, and MD5 as significant ($\alpha =$
0.05) QTL; in addition, significant QTL were detected
for MDI (E27, ADL289) and VIR (E41, ADL149). Un-
fortunately, unreasonable estimates of chromosome-
wide critical threshold levels and $R^2$ were uncovered,
possibly resulting from the large intervals or single
marker linkage groups. This dilemma is being investi-
gated further by genotyping more markers in the re-
region as they become available, followed by statistical
analysis.

**Genetic architecture of MD susceptibility:** Heritability
estimates for MD susceptibility are usually $>0.60$ when
the effects of MHC and non-MHC genes are con-
founded, and if highly virulent MDV strains are used
(Friars et al. 1972; Gavora et al. 1979; Ameli et al.
1992). In contrast, Ameli et al. (1992) reported herita-
bility estimates <$0.34 when using unselected White
Leghorn chickens and moderately virulent MDV
strains. In the present study, the effects of the B haplo-
type MHC genes on the expression of MD susceptibility
are nonexistent because the inbred lines 63 and 72 used
to generate the F2 mapping population were identical
for the MHC class I, II, and IV B haplotype genes. Thus,
even though using F2 crosses of selected strains, we
expect that the heritability for MD resistance was $\sim$0.34 in
this mapping experiment, similar to that reported by
Ameli et al. (1992), because only the effects of non-
MHC genes were measured, and a moderately virulent
MDV strain was used.

As expected, traits likely to be important in deter-
mining the susceptibility to MD (i.e., VIR, TIS, SUR,
TUM, MDI, DIS, BRA, SCI, and VAG) showed highly
significant correlation ($P \leq 0.001$) among each other.
These associations suggest that MD QTL reported here
may be pleiotropic or that several QTL may be closely
linked. In addition, the facts that QTL located in Ch2,
Ch4, Ch7, and Ch8 control two or more MD traits, and
that most of the QTL have similar position and modes
of inheritance (Table 5), suggest further that pleiotro-
ic or closely linked QTL might be controlling MD sus-
cceptibility. At this time, the resolution of QTL mapping
experiments is still not sufficient to discriminate
whether or not a single gene or several closely linked
genes underlie an identified QTL. For example, one
gene displaying overdominance cannot be discrimi-
nated from two closely linked dominant genes, as
shown by Cockerham and Zeng (1996).

Epistasis, or interaction between loci, implies that
the genotype at one locus has an effect on the contribu-
tion at another locus, implying that the genes are act-
ing on the same or a related biological pathway. The
converse of epistasis is genetic heterogeneity, in which
two or more loci are independent causes of disease and
act via separate biological pathways (Cornell and
Todd 1995). In this study, we could not rule out the
role that epistasis might play in determining the ex-
pression of MD susceptibility because we found sugges-
tions of significant interlocus interaction, especially
for MDI levels (Table 7). Among these significant interac-
tions, we observed two that involved significant QTL af-
fecting MDI ($P = 0.0053$ and 0.0083). In each of the
two two QTL models, the nonadditive epistatic compo-
nent was responsible for an increase of 4.1% (from 5.4
to 9.5%) and 6.1% (from 6 to 12.1%) of the explained
MDI phenotypic variation. These results support the
hypothesis that several genes interact to determine MD
susceptibility. This suggestion of epistasis in disease
resistance concurs with what is known about commercial
production traits, where both epistasis and dominance
effects are important (Fairfull et al. 1987). Once more,
QTL mapping research has proven its utility in
investigating this source of nonadditive genetic varia-
tion.

In summary, two significant medium-to-large effect
QTL and three suggestive QTL that affect MD suscepti-
bility were identified (Table 5), which were localized to
five genomic regions of the chicken (Figure 1). In addi-
tion, evidence was found for interlocus interactions
that might be important in determining the expression of MD susceptibility (Table 7). Thus, these results suggest that MD susceptibility resembles a typical polygenic trait that is controlled by a number of genetic factors of nonadditive mode of inheritance and their interactions. In this regard, our findings are in agreement with the tentative oligogenic nature of MD susceptibility proposed earlier by Stone (1975) and Gavora et al. (1979).

**Permutation tests in the search of QTL:** Chicken chromosomes are numerous (2n = 78) and are classified as macrochromosomes (i.e., Ch1-Ch8 and ChZ) or microchromosomes according to size. The microchromosomes present several problems for gene mapping. Microchromosomes are frequently associated with a single chiasma, and in 50% of microchromosomes, this single chiasma is located in the telomeric region. Such microchromosomes are not expected to exhibit recombination over most of their length and will be detected as single-point linkage groups (Rodionov et al. 1992). This limits the use of interval mapping in most of the microchromosomes and justifies the use of dummy markers to facilitate the use of interval mapping in the microchromosomes with a single marker.

In comparison with other species, considering the distinct genetic characteristics of macrochromosomes and microchromosomes of the chicken, we cannot use the same critical threshold levels to declare significant QTL in both types of chromosomes. In addition, every QTL mapping experiment has unique characteristics. Thus, it would be more appropriate to develop empirical critical threshold levels to declare significant QTL by permuting or shuffling the experimental data. Therefore, in this QTL mapping experiment, specific chromosomewise critical threshold levels were determined using our experimental data and permutation tests (Churchill and Doerge 1994).

Although computationally demanding, critical chromosomewise threshold levels were determined using 1000 permutations of our data (Table 4). Using these empirical threshold levels, QTL just arising from chance associations could be detected and eliminated.

**Marker-assisted selection in poultry breeding for MD resistance:** In poultry research and industry, chickens are raised under relatively standard, controlled conditions that minimize the environmental component and increase the accuracy of phenotypic evaluations. The relatively large effect QTL identified here should therefore be detectable in other poultry experiments having experimental conditions more or less similar to this mapping study, provided these QTL are segregating in the genetic stocks analyzed.

In comparison to other poultry production traits, disease resistance is an ideal candidate for the application of marker-assisted selection (MAS). The use of DNA markers linked to major MD QTL for developing MD-resistant chickens represents an ideal approach because it does not require exposure of the breeding stock to the pathogens (Gavora and Spencer 1978). However, the effectiveness of MAS relative to selection based on challenge with the pathogen depends on the proportion of additive genetic variance explained by the markers and the heritability of the disease trait (Gavora and Spencer 1978; Smith 1967). In the present study, we found that when using three to five loci associated with QTL affecting MD susceptibility, the proportion of additive genetic variance explained by these loci is higher than the MD heritability (Table 6), assuming individual heritability of 0.34 for MD incidence when using nonselected control White Leghorn chickens and moderately virulent MDV strains (Amel i et al. 1992).

The use of MAS for sexing is not new in the poultry industry, and the practice has made a considerable economic impact. The same concepts could apply in the use of QTL detected in the present study to breed chickens for MD resistance. The use of medium-to-large effect QTL in MAS for developing MD-resistant chickens can be implemented by the breeder, and it could supplement current vaccinal control. Before using these QTL in commercial populations, however, we should (1) reconfirm the linkage or association of detected QTL here in other advanced generations and under different genetic backgrounds and (2) saturate the genomic regions displaying MD QTL with more DNA markers and identify the most tightly linked markers or attempt to identify MD candidate genes. Experiments are underway to address both of these issues.

In commercial poultry breeding, the final product is a crossbred animal, and all parental lines used in the cross are improved simultaneously. The development of superior hybrids for production traits and MD resistance can be achieved by complementing MAS with standard breeding schemes. The fact that most of the MD QTL identified in this study are dominant for MD resistance could facilitate their use in the following stepwise breeding scheme: (1) develop hybrid lines carrying favorable alleles at three to four MD QTL chromosomal regions by mating MD-resistant and elite breeders for production traits; (2) select elite parental lines enhanced for MD resistance that have elite breeders for production traits; (3) select elite breeders within each useful hybrid parental line that carry favorable MD QTL alleles; and (4) produce MD-resistant, commercial hybrid chickens by mating the elite breeders from each hybrid line enhanced for MD resistance. Within the crossbred population, linkage disequilibrium between marker and QTL will exist, which will allow the use of MAS to identify single breeds with the best allelic combination for MD resistance.

In theory, it is expected that a sound integration of
molecular marker technology and conventional breeding approaches, provided that the marker-QTL association is determined in the target population, should increase the efficiency of developing commercial MD-resistant chickens. Although there are few experimental reports of successful use of MAS in applied animal breeding programs of domestic species, Steuber (1995) has shown that marker-facilitated backcrossing can be used successfully for introgressing desired alleles at multiple loci for manipulation of complexly inherited traits in maize.

In summary, based on these results and other practical considerations, the use of MAS in the breeding for MD-resistant chickens is largely justified because of the following: (1) the existence of medium to large effect QTL affecting MD susceptibility, (2) most of these QTL are dominant for MD resistance (i.e., QTL recessive for MD susceptibility), (3) a relatively few number of QTL explain about two thirds of the genetic variance for MD susceptibility, (4) the apparent control of MD susceptibility by nonadditive genetic factors suggests that additional genetic improvement for MD resistance can be achieved only by MAS (i.e., MAS better utilizes nonadditive genetic variation), and (5) direct selection by challenging chickens with MDV is difficult and costly.

Future prospects: Genetic mapping studies of genetic factors affecting complex diseases only provides relatively wide chromosomal regions where the actually relevant genes are localized. Through a combination of high-resolution QTL mapping, positional candidate gene, targeted marker development, and subsequent strategies of positional cloning, further characterization of chromosomal regions of interest will eventually allow the precise chromosomal localization and identification of the MD susceptibility genes. In this study, the localization of medium-to-large effect QTL affecting MD susceptibility to five chromosomal regions raises the following possibilities: (1) the fine mapping of these MD QTL regions; (2) the detection of these QTL in advanced generations and different genetic backgrounds; (3) the use of well-characterized major QTL in marker-assisted selection of poultry for MD resistance; (4) the functional characterization of these QTL influencing MD [recombinant congeneric strains for the major MD QTL regions are under development (Bacon et al. 1996)]; and (5) the systematic survey and rest of candidate genes for MD susceptibility through comparative gene mapping. Eventually, the use of adequate experimental designs could lead to the actual cloning of the relevant gene(s).

We appreciate the advice and guidance provided by Lyman Crittenden in this work. R.L.V. is grateful to Mark Daly, Chris Basten, Zhao-Bang Zeng, and Chris Haley for helpful discussion and suggestions in the QTL analysis, and also to Bruce Weir, Eugene Eisen, and Wanda Collins for their continued support. We thank Mary Hutcheson, Evelyn Young, Michel Sturgeon, Bobby Okimoto, and Lenny Provencher for excellent technical support. This research was supported in part by funding from the USDA NRICGP (grant 94-37205-1223 to H.C.C.).

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