DNA Microsatellites Linked to Quantitative Trait Loci Affecting Antibody Response and Survival Rate in Meat-Type Chickens

N. Yonash,*1,2 H. H. Cheng,† J. Hillel,* D. E. Heller,* and A. Cahaner*

*Faculty of Agricultural, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, PO Box 12, Rehovot 76100, Israel; and †USDA Agricultural Research Service, Avian Disease and Oncology Laboratory, 3606 East Mount Hope Road, East Lansing, Michigan 48823

ABSTRACT Selection for immune response parameters may lead to improved general disease resistance. Because disease resistance and immune response are hard-to-measure quantitative traits with low to moderate heritability, they may respond more efficiently to marker-assisted selection (MAS) than to phenotypic selection. To detect DNA markers linked to quantitative trait loci (QTL) associated with immune response, a resource half-sib family of 160 backcross (BC1) and intercross (F2) birds was derived from a cross between two meat-type lines divergently selected for high or low antibody (Ab) response to *Escherichia coli*. By using 25 microsatellite DNA markers covering ~25% of the chicken genome, initial genotyping of 40% of the resource family was followed by complete genotyping of the entire family with four suggestive markers. Three of these markers exhibited significant association with immune response: (1) ADL0146 on Chromosome 2 associated with Ab to SRBC and Newcastle disease virus (NDV), (2) ADL0290 on linkage group 31 affecting Ab to NDV, and (3) ADL0298 on linkage group 34 associated with Ab to *E. coli* and survival. The family was also genotyped with five linked markers from two of the suggested regions, and interval mapping was applied. The results confirmed the significant effects, suggested the location of the QTL, and confirmed the genetic association between immune responses and disease resistance. These findings support the idea of improving poultry immunocompetence by MAS.

(Key words: disease, microsatellite marker, quantitative trait loci, immune response, genetic map)

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INTRODUCTION

In the US alone, where poultry is the third largest agricultural commodity, the cost of disease prevention, vaccination, and medical treatments is estimated at $15 billion per year (USDA, 1997). Ironically, due to the isolation and improved sanitary conditions required for maintaining *Mycoplasma*-free flocks and the limited exposure of nuclear breeding flocks to infectious agents, natural genetic selection for disease resistance may be inhibited. Thus, it is desirable to actively select for resistance to each of the primary diseases. Results from several studies (e.g., Gross et al., 1980; Siegel et al., 1982; Dunnington et al., 1986; Pitcovski et al., 1987; Yamamoto et al., 1991; Leitner et al., 1992; Finard et al., 1993; Yonash et al., 1996) suggest that selection for immune response parameters may efficiently improve disease resistance in commercial flocks.

Immune response in chickens is affected by several genetic factors as well as by the environment (Gavora, 1993) and is a typical quantitative trait, controlled by quantitative trait loci (QTL). Due to the low to medium heritability of the trait and the difficulties associated with reliable measurements, improvement of immune responses by direct phenotypic selection is difficult. Selection of individuals according to genotype for a marker associated with QTL of such traits (marker-assisted selection, MAS) is preferred. Hence, detection of linkages between DNA markers and QTL associated with immune responses is important.

Restriction fragment length polymorphisms (RFLP) at three loci of the chicken MHC were found to be associated with the immune responses of young broilers (Yonash et al., 1999b). However, they explained only part of the genetic variation, suggesting that non-MHC genes (QTL) may also affect these traits, and linked markers could be detected by a genome-wide search. Maps of the chicken genome (Bumstead and Palyga, 1992; Cheng et al., 1995;...
ChickMap URL, 1999; Poultry Genome Project URL, 1999), built on highly polymorphic genetic markers (DNA microsatellites), allow scanning for markers linked to QTL of interest. However, a prerequisite is the availability of a resource population with sufficient genetic variation to detect linkages between a segregating QTL and a genetic marker. In a genome-wide scan with 420 microsatellite markers, none was found to have a significant effect on chick BW at 23 d of age (Van Kaam et al., 1999a) or BW at 48 d of age (Van Kaam et al., 1999b) despite the high heritability of these traits in the sufficiently large resource population. In that study, both parental lines were similarly selected for high BW, hence the population derived from their cross apparently did not segregate for single QTL with a significant main effect on this trait. However, in another study of Marek’s disease (MD), searching a smaller resource population of egg-type chickens with fewer markers yielded 14 different genomic regions (QTL) associated with the disease (Yonash et al., 1999a). The successful detection of QTL was probably due to the unique population derived from two inbred parental lines that differed in susceptibility to MD. Indeed, most of the successful QTL mapping experiments have been conducted in F₂ or backcross (BC) families derived from crosses between parental populations differing for the traits of interest. In most instances, the parental populations were inbred lines, a viable option for many plant species (e.g., Paterson et al., 1989; Schechert et al., 1999) and mice (e.g., Hilbert et al., 1991; Morris et al., 1999), for which all segregating markers are informative. Farm animals, for which mostly outbred parental populations are available, must differ genetically to allow successful detection of marker-QTL association, as in the crosses between the wild boar and domesticated pig (Andersson et al., 1994), the cross between Meishan and Yorkshire swine (Paszek et al., 1999), or the cross between meat-type and egg-type chickens (Khatib, 1994). Similarly, a population comprising F₂ and BC families, derived from a cross between two meat-type (broiler) lines that were divergently selected for early immune response (Heller et al., 1992; Leitner et al., 1992; Yonash et al., 1996), exhibited large genetic variation that facilitated the successful detection of RFLP markers linked to the selected trait (Yonash et al., 1999b). However, because of the outbred nature of the parental lines, the necessary linkage disequilibrium was obtained only within families (Hillel, 1997).

Resource populations in livestock species are usually characterized by small full-sib families. Even in poultry, maternal productivity is limited compared with most plants. Hence, QTL analysis of animal species is commonly based on analyses of the sire alleles within large half-sib sire families, either by analysis of variance (Soller and Genizi, 1978), chi-square test (Neumann-Sorensen and Robertson, 1961; Gelderman, 1975), regression analysis, or advanced least-squares method based on simultaneous analysis of many linked markers (Knott et al., 1996). The latter method has been successfully used in several studies to detect chromosomal regions carrying QTL (e.g., Andersson et al., 1994; Paszek et al., 1999). The present study utilized a unique resource population, derived from a cross between two divergent lines, to search for linkages between microsatellite DNA markers and QTL associated with immune response in young broilers.

**MATERIALS AND METHODS**

**Experimental Population**

Broiler lines that were divergently selected for high (HC) or low (LC) antibody (Ab) response to *Escherichia coli* vaccine administered subcutaneously at 10 d of age (Heller et al., 1992; Leitner et al., 1992) were crossed after five selection cycles to generate F₁ progeny. A single F₁ male was mated with five F₁ females, five HC females, and one LC female, producing 83 F₂ progeny in three hatches and 77 BC₁ progeny in three additional hatches. These 160 birds were the resource population for this study.

**Phenotypic Measurements**

The chicks were intramuscularly vaccinated with attenuated commercial Newcastle disease virus (NDV) vaccine³ at 7 d of age, with *E. coli* (Leitner et al., 1992) and with 2% SRBC intravenously at 14 d of age. At 21 d of age, serum samples were collected from the chicks, and Ab response to NDV vaccine was determined by hemagglutination-inhibition test as log₂ of the dilution factor (Brugh et al., 1978). The Ab response to SRBC was determined by a hemagglutination test as log₂ of the dilution factor (Siegel and Gross, 1980). The Ab response to *E. coli* vaccine was determined by ELISA (Leitner et al., 1990). The natural log of the antibody titer (Y₁) was calculated as described by Leitner et al., (1992) by using a linear regression equation:

\[
Y_1 = 0.45 (P_i; N) - 0.45
\]

where \(Y_1\) is the antibody titer of sample i, \(P_i\) is the sample’s optical density reading, and N is the optical density of the plate’s low (‘negative’) standard.

At 21 d of age, all chicks were challenged with pathogenic *E. coli* strain O2:K1. The birds were necropsied at death or 7 d postchallenge and were categorized as 1) affected (dead or sick) or 2) unaffected (healthy).

**Genotyping Procedures**

Microsatellite markers were amplified from DNA of the parents (1 sire and 11 dams) and 160 progeny, as described by Cheng et al. (1995) by using fluorescently labeled primers (courtesy of the US National Animal Genome Research Program Poultry Coordinators J. Dodgson

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³Teva Biology Laboratories, PO Box 27047, Jerusalem, Israel.
and H. Cheng). The PCR products were loaded onto an ABI 377 DNA Sequencer with a 4% polyacrylamide gel. GeneScan 2.1 software was used to determine the alleles. Genome coverage by the selected markers was calculated as possible, the East Lansing chicken genome map that contained, at the initiation of this study, only about 100 publicly available microsatellite markers (Cheng et al., 1995). The 61 microsatellite markers covered 23 of the 43 linkage groups of the East Lansing chicken genome map: eight macrochromosomes, the Z (sex) chromosome, and 14 small linkage groups.

The initial search was conducted in two stages. 1) In order to avoid genotyping with uninformative markers, the sire of the resource population was genotyped for the chosen markers, and those in a heterozygous state were determined. Dams were genotyped for the heterozygous markers. Of the 61 markers, 33 were heterozygous in the sire. For eight of these 33 markers, the two sire alleles were found in most of the dams; hence, they were excluded as uninformative. The remaining 25 informative markers, which represented 14 linkage groups covering about 800 cM (24%) of the genetic map of the chicken, were used in the next step. (2) In order to eliminate genotyping of the entire resource population with markers from regions that were not associated with the traits, initial genotyping used DNA from 40% of the resource population. The birds for this stage were selected according to the E. coli Ab response distribution, those with the lowest 20% and highest 20% of the E. coli Ab phenotypic value within dam families. This stage was selective genotyping only for the E. coli Ab response, but for the other traits, this stage was actually a random-sample genotyping (due to the low phenotypic correlation between the traits).

**QTL Analyses**

The phenotypic data were adjusted for dam and hatch effects by using the model

\[ y_{ijk} = \mu + H_i + D_j + e_{ijk} \]

where \( \mu \) is the grand mean, \( H_i \) is the effect of the ith hatch, \( D_j \) is the effect of the jth dam, and \( e_{ijk} \) represents all remaining effects, including the segregation of the sire alleles among full-sib progeny. The adjusted phenotypic value of the \( y_{ijk} \) progeny was calculated as \( \mu + e_{ijk} \).

For each marker, a bird was scored as 1 when carrying one sire allele and 0 when carrying the other sire allele. In those few cases in which a marker could not be informative because a dam carried the same two alleles as the sire, scoring of heterozygous offspring was based on the assumption that each marker covers 20 cM from each side, while accounting for overlap between markers and for the terminal position (less than 20 cM from the end of a linkage group).

**RESULTS**

**Trait Means, Variances, and Phenotypic Correlation**

Table 1 presents means, SD, and CV of the four traits, after adjustment for hatch and dam effects. The phenotypic correlation between the traits was low (<0.13) and not significant.

**Genotyping and Genomic Coverage**

Sixty-one markers were chosen to represent, as closely as possible, the East Lansing chicken genome map that contained, at the initiation of this study, only about 100 publicly available microsatellite markers (Cheng et al., 1995). The 61 microsatellite markers covered 23 of the 43 linkage groups of the East Lansing chicken genome map: eight macrochromosomes, the Z (sex) chromosome, and 14 small linkage groups.

**Marker-Trait Analyses**

**Initial Genotyping.** Four markers were found to be associated (at a significance level of < 0.1) with the measured traits. These markers were: 1) ADL0146 on chromosome 2 (Ch.2) associated with Ab to SRBC and NDV; 2) ADL0258 on Ch.8 associated with Ab to SRBC and NDV; 3) ADL0290 in linkage group 31 (E31) associated with Ab to E. coli and NDV; and 4) ADL0298 on Ch.5 associated with Ab to E. coli and rate of survival of E. coli challenge.
**Complete Genotyping.** The four markers were used for complete genotyping of all progeny in the sire family; results are presented in Table 2. Five associations were significant ($P < 0.05$): ADL0146 with Ab to SRBC and NDV, ADL0290 with Ab to NDV, and ADL0298 with Ab and to *E. coli* survival. The magnitude of the significant effects (i.e., the differences between carriers of the two different sire alleles) equaled about 0.35 SD for Ab responses and 0.24 SD for survival rate (Table 2). Small, nonsignificant effects were found in the complete genotyping for ADL0258 on Ch.8, suggesting that the initial genotyping had yielded false-positive results for this marker. The amount of phenotypic variation explained by genotyping had yielded false-positive results for this marker. Their values and significance, obtained from the complete genotyping data, are presented in Table 2. The Ch.2 region exhibited a significant association with Ab to SRBC (all four markers) and Ab to NDV (three markers) (Table 3). Thus, it appears that a QTL affecting Ab response to NDV is located on E31; apparently close to MCW0217. Unfortunately, no linked microsatellite markers are currently available on Ch.5, therefore, it was impossible to verify the results by using the flanking markers of this locus.

**Linked Markers.** According to the updated East Lansing and Wageningen chicken maps (ChickMap URL, 1999), five and four microsatellite markers are linked (within the range of ±30 cM) to ADL0146 on Ch.2 and to ADL0290 on E31, respectively. No marker is linked to ADL0298 on Ch.5. Five of these linked markers were informative in the present study, i.e., heterozygous in the sire. These markers, as well as the three initial ones, are presented in Table 3, along with the size of their alleles and the map distances between them. The 11 dams and the entire resource population were genotyped with these eight informative markers. Based on actual recombination between markers, the linkage order was verified, and the percentages of recombination were similar to the distances in the chicken genome map (Table 3).

The effect of each marker on each trait, an expression of their association, was calculated separately (single-marker analysis). Their values and significance, obtained from the complete genotyping data, are presented in Table 3. The Ch.2 region exhibited a significant association with Ab to SRBC (all four markers) and Ab to NDV (three markers) (Table 3). Thus, it appears that a QTL affecting Ab response to SRBC and NDV is located on Ch.2, close to ADL0146. The E31 region exhibited a significant association only with Ab to NDV (two markers) (Table 3). It appears that a QTL affecting Ab response to NDV is located on E31; apparently close to MCW0217. Unfortunately, no linked microsatellite markers are currently available on Ch.5, therefore, it was impossible to verify the results by using the flanking markers of this locus.

Interval mapping of the two chromosomal regions (Ch.2 and E31) confirmed the effects of these QTL as well as their location within the QTL, i.e., near ADL0146 on Ch.2 and near MCW0217 on E31 (data not shown).

**Discussion**

Identification of QTL affecting a certain quantitative trait requires maximum segregation in these QTL, and in potentially linked markers, and linkage disequilibrium to ensure their cosegregation. Therefore, the ideal resource population is one that is derived from two inbred parental lines genetically differing for the trait of interest and in the marker alleles. Indeed, use of such an optimal population of egg-type chickens yielded very successful results (Yonash et al., 1999a); however, such a population is very rare in livestock. In the present study, the first requirement was fully met: the resource population was derived from a cross between parental lines divergently selected on Ab response, and the F1 sire was heterozygous for the markers used. The cross between divergently selected lines indeed resulted in a highly variable resource population (CV values ranged between 38 and 61%, Table 1). Recently, Van Kaam et al., (1999a,b) searched the chicken genome with 420 microsatellite markers and did not find any QTL significantly associated with BW in a resource population comprising hundreds of genotyped parents and thousands of phenotyped offspring. This result, i.e., no significant QTL for a highly heritable trait, may be due to the choice of parental lines used to generate the resource population of the Van Kaam et al. study. Both were commercial dam lines that had been similarly selected for high BW; hence they probably did not differ in any QTL with a major effect on BW. This result

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**Table 2.** The effects1 and significance2 of microsatellite markers on antibody response to *Escherichia coli*, SRBC, and Newcastle disease virus (NDV) and on survival rate following pathogenic *E. coli* challenge, estimated from genotyping analyses of the entire resource population3.

<table>
<thead>
<tr>
<th>Linkage group</th>
<th>Marker</th>
<th>Size of sire alleles</th>
<th>Trait</th>
<th>n5</th>
<th>Effect</th>
<th>$R^2$ (%)</th>
<th>$P(t)^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch.2</td>
<td>ADL0146</td>
<td>152/164</td>
<td>SRBC</td>
<td>156</td>
<td>0.38</td>
<td>4.4</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NDV</td>
<td>157</td>
<td>0.39</td>
<td>3.8</td>
<td>0.014</td>
</tr>
<tr>
<td>Ch.8</td>
<td>ADL0258</td>
<td>162/169</td>
<td>SRBC</td>
<td>156</td>
<td>0.02</td>
<td>0.0</td>
<td>0.889</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NDV</td>
<td>157</td>
<td>0.11</td>
<td>0.3</td>
<td>0.494</td>
</tr>
<tr>
<td>Ch.5</td>
<td>ADL0298</td>
<td>102/106</td>
<td><em>E. coli</em></td>
<td>155</td>
<td>0.34</td>
<td>4.4</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Survival, %</td>
<td>145</td>
<td>0.24</td>
<td>2.8</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NDV</td>
<td>155</td>
<td>0.30</td>
<td>2.7</td>
<td>0.043</td>
</tr>
</tbody>
</table>

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1 Differences between carriers of each of the sire alleles, given in phenotypic SD.
2 Significance of the effect: *t*-test for antibody level; chi-square test for survival rate.
3 Resource population = the F1 + backcross (BC1) family.
4 Ch. = chromosome; E31 = linkage group 31.
5 Number of birds genotyped with the particular marker that were informative for the analysis of the sire alleles.
may support the conclusion of Andersson et al. (1994) that in order to increase the chance (and statistical power) of detecting a QTL, most of the genetic variation in the resource population should result from genetic differences between, and not within, the parental populations. Indeed, the parental lines in the present study were genetically different for the particular traits of interest.

To achieve the second requirement (linkage disequilibrium) in outbred lines, the analysis must be conducted within a full-sib family. Many hatches are needed to produce a sufficient number of full-sib broilers for marker-QTL linkage analysis. This approach may introduce an additional source of environmental noise that may bias the analysis, especially for a typical quantitative trait with low to moderate heritability. In this study, we resolved this dilemma by crossing a single sire with 11 different dams to produce a reasonable number of half-sib progeny. However, this procedure restricted the linkage analyses, because it is not practical to analyze all the alleles (for a particular marker) expected in a group of dams from an outbred line.

The statistical approaches of simple-regression analysis were used, and the linkage analysis was similar to the method employed for the daughter design in cattle (Weller et al., 1990) within a single sire family. With only three or four informative markers within a given chromosomal region found to be associated with the QTL (i.e. on Ch.2 and E31), the information obtained by applying interval mapping (MapMaker/QTL version 3.0b) did not reveal any significant differences over the simple, single-marker comparisons (data not shown).

In identifying marker-QTL associations, the incidence of “false positive” linkage increases when liberal significance threshold levels are used. On the other hand, highly stringent thresholds may increase the risk that QTL will go unreported (“false negative”). Lander and Kruglyak (1995) emphasized the need for genome-wide threshold values in order to avoid a large number of “false positives” for which the \( P \) values for significant and suggestive linkages are between 0.0001 and 0.00001 and between 0.001 and 0.0001, respectively. A few publications (e.g., Curtis, 1996) object to the use of these apparently high and conservative threshold levels and suggest that the results be published as a matter of negotiation and discussion. The \( P \) values reported here are relaxed compared to those suggested by Lander and Kruglyak (1995), because the experimental design in this study (family of half-sibs) has limited statistical power. Therefore, in order to avoid a very important QTL, we decided to consider the critical \( P \) value to be 0.05.

To reduce the amount of laboratory work, initial genotyping was applied for preliminary identification of suggestive markers. The initial genotyping was conducted by genotyping 40% of the resource population. The birds for the initial genotyping were selected as for selective genotyping according to the \( E. \) coli Ab response distribution, those with the lowest 20% and highest 20% of the \( E. \) coli Ab phenotypic value within a dam family. Therefore, this phase was used as selective genotyping for the \( E. \) coli Ab response, but for the other traits this phase was actually a random-sample genotyping, because of the low phenotypic correlation between them. One would expect a potential loss in statistical power, because somewhat random individuals were used. Indeed, the effects of ADL0258 (Ch.8) on SRBC and NDV, found in the initial genotyping, were not confirmed by the data obtained by complete genotyping. On the other hand, the effects of ADL0146 (Ch.2) on SRBC and NDV and ADL0290 (E31) on Ab to NDV were detected first in the initial genotyping and then were confirmed by complete genotyping of these as well as of the linked markers that were used in the second stage. Vallejo et al. (1997) reported five QTL affecting susceptibility to MD in chickens on chromosomes 2, 4, 7, and 8 and linkage group E16. Recently, Yonash et al.,
(1999a) reported a total of 14 QTL affecting MD following higher-resolution mapping of the MD population. Although markers from these five regions were included in the present study, none was associated with Ab response to E. coli, NDV, or SRBC. Thus the QTL found for resistance to MD appear to have no effect on Ab response of young broilers to E. coli, NDV, or SRBC. However, it must be clear that although both studies deal with immune response, the resource populations were derived from totally different stocks (egg-type vs. meat-type), and immune response was determined at different ages. In the MD study, Ab levels were determined in adult egg-type chickens in which the genetic variation in Ab is usually due to segregation in different gene(s), each responsible for the immune response to a specific antigen. In the present study, Ab levels were determined in young meat-type birds, in which the selection apparently acted on genes responsible for genetic variation in the early maturation and activation of the humoral immune system. Therefore, association of different QTL in each of these studies is expected.

Significant effects of DNA markers exceeded one-half of the phenotypic standard deviation (0.5 × SD) in most studies with livestock species, whereas several significant effects in the present study had values of less than 0.5 × SD (Table 3), probably due to the large SD values (relative to the mean) in a family derived from a cross between divergent lines (i.e., CV values ranged between 38 and 61%, Table 1). In the MD study (Yonash et al., 1999a), in which the resource population was also derived from extremely divergent lines, the CV values of the different traits ranged between 57 and 67% (unpublished data) in agreement with the high variation found in the resource population used in the present study.

In summary, despite limited statistical power and relatively low genome coverage, several microsatellite markers were identified to be associated with QTL for immune response traits in young broilers. None of these markers were linked to the MHC, supporting the suggestion that something other than the MHC also affects the immune response (Pinard et al., 1993; Yonash et al., 1999b). It is suggested that the QTL on Ch.2 is associated with Ab response to SRBC and NDV. The QTL on E31 appears to affect Ab response to NDV and a QTL on Ch.5 was associated with both Ab response and resistance to E. coli. Both findings, i.e., QTL with a general effect on Ab response to several antigens, and the association between Ab response to E. coli and resistance to this pathogen, are in agreement with the correlated responses found in the selected parental lines. These lines were also found to differ in Ab response to NDV and SRBC and resistance to E. coli (Heller et al., 1992; Leitner et al., 1992). The QTL with common effects provide the chromosomal basis for the genetic correlation previously estimated by methods of quantitative genetics (Heller et al., 1992). The findings of the present study, once verified (e.g., in independent resource populations), suggest that the reported markers could be used for MAS to improve Ab response of young broiler chicks to two important pathogens (E. coli and NDV) and their survival rate when challenged by E. coli and even to improve general early and high humoral immune response to any antigen.

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REFERENCES


