RAPID COMMUNICATIONS

Fine-Mapping of Coccidia-Resistant Quantitative Trait Loci in Chickens

E.-S. Kim,* Y. H. Hong,† W. Min,‡ and H. S. Lillehoj†[1]

*Department of Animal Sciences, University of Wisconsin-Madison, 53706; †Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, USDA, Beltsville, MD 20705; and ‡College of Veterinary Medicine, Gyeongsang National University, Jinju, Gyeongnam 660-701, South Korea

ABSTRACT Two commercial, pure broiler lines with different susceptibility to coccidiosis were used to fine-map QTL associated with the previously identified marker LEI0101, located at 259 cM on chromosome 1 and shown to be significantly associated with disease resistance. Eight additional microsatellite markers linked to LEI0101 were used for genotyping of F_1 parents and F_2 offspring (n = 314), and their associations with oocyst shedding, as a marker of disease resistance, were determined in birds experimentally infected with *Eimeria maxima.* Single-point analysis of 4 families showed that logarithm of odds (LOD) scores at all marker loci were >0.5, with the exception of marker LEI0071, located at 242 cM (LOD score = 2.45). Multipoint analysis showed a maximum LOD score between LEI0071 and LEI0101 at 254 cM (LOD score = 3.74). Although the LEI0071 marker was mapped near LEI0101 by linkage analysis, the physical location of LEI0071 was not identified. Further studies to determine the physical locations of these and other markers will allow additional application association mapping techniques using single nucleotide polymorphism markers.

Key words: fine-mapping, quantitative trait loci, disease resistance, coccidiosis, chicken

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INTRODUCTION

Coccidiosis is a ubiquitous intestinal protozoan infection of poultry that seriously impairs the growth and feed utilization of animals following intestinal infection by *Eimeria* species. Although tremendous improvements in commercial chicken production traits have been accomplished using classical genetic breeding techniques, selection of commercial poultry stocks with improved disease resistance using similar techniques has been relatively unsuccessful (Gavora, 1990). With the advent of QTL mapping strategies, DNA markers that are associated with disease resistance can be identified in particular genotypes, and this information can subsequently be used in MAS of breeding stocks. Multiple studies have reported QTL mapping of poultry BW, feed efficiency, and other carcass traits, as well as resistance to Marek's disease and antibody production (Vallejo et al., 1998; Xu et al., 1998; Van Kaam et al., 1999a,b; Yonash et al., 1999, 2001; Tatsuda and Fujinaka, 2001; De Koning et al., 2004; Sasaki et al., 2004; McElroy et al., 2005; Schreiweis et al., 2005; Lagarrigue et al., 2006).

In a previous report, 119 microsatellite markers were used in a chicken whole-genome scan to identify QTL associated with resistance to coccidiosis (Zhu et al., 2003). That study identified marker LEI0101, located at 259 cM on chromosome 1, as being significantly associated with disease resistance, as measured by reduced oocyst fecal shedding of birds experimentally infected with *Eimeria maxima.* Flanking markers of this locus were identified from 240 to 280 cM. Because this 40-cM region is approximately equivalent to 50 to 60 Mb, it was too large to identify additional genes or markers correlating with resistance to coccidiosis. Thus, the goal of the current study was to utilize more closely spaced microsatellite markers to perform a fine-map analysis of this region.

MATERIALS AND METHODS

Resource Populations

Two commercial, pure broiler lines showing different susceptibility to coccidiosis were selected as resource populations for QTL fine-mapping, as previously described (Zhu et al., 2003). Both lines have been under selection over 20 generations for a variety of production traits. Chickens were crossed to produce F_1 and F_2 birds. Twelve families were generated from the F_1 population. Their F_2 offspring were inoculated with 1×10^4 sporulated oocysts of *E. maxima,* and oocyst fecal shedding was measured as previously described (Zhu et al., 2003).

Marker Genotyping and QTL Analysis

A total of 314 full-sib offspring and parents were genotyped. Eight microsatellite markers linked to LEI0101

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[1]Corresponding author: hlilleho@anri.barc.usda.gov

Table 1. Microsatellite markers used in this study

Marker	Linkage (cM)	Physical location (Mb)
MCW0058	241	NA[1]
LEI0071	242	NA
MCW0101	248	72.6
MCW0318	251	71.4
MSU0090	253	NA
LEI0101	259	75.3
ADL0251	264	77.4
MCW313	295	81.1
ADL0314	416	87.9

[1]NA = not available.

were analyzed by PCR (Table 1). These markers were selected based on linkage map, physical map, and marker informativeness (Groenen et al., 2000). The PCR reaction was performed as described previously (Zhu et al., 2003). The data were adjusted for hatch, sex, and interaction between hatch and sex. Sequential oligogenic linkage analysis was used to perform QTL linkage analysis (Almasy and Blangero, 1998). This package detects QTL by the variance component linkage method using identify-by-descent probability among markers in sib-families. The threshold of significance was based on the guidelines suggested for genome scans by Lander and Kruglyak (1995), i.e., a logarithm of odds (**LOD**) score of 2.7 for single-point linkage analysis and 3.6 for multipoint linkage analysis.

RESULTS

Using single-locus analysis of association with oocyst shedding, the LOD scores at 5 marker loci from 240 cM to 265 cM were >0.5 based on the data from 4 families classified as informative (Figure 1). In contrast, single-point linkage analysis of marker LEI0071 at 242 cM on chromosome 1 gave a LOD score of 2.45, and the marker LEI0101 at 259 cM also was confirmed as significantly associated with disease resistance (LOD score = 2.90). The physical location of LEI0101 was verified at 75.3 Mb on chromosome 1, but the location of LEI0071 was not identified. Multipoint linkage analysis with 1-cM increments based on the 4 selected families revealed a LOD score of 3.74 at 254 cM on chromosome 1 (Figure 2). The maximum LOD point was located between the LEI0071 and LEI0101 markers. When 12 families were used for the multipoint analysis, the maximum LOD score decreased to 1.31 at linkage-map location 247 cM (Figure 2). The F_2 offspring of the largest family was divided into 3 groups based on their genotype (1/1, 1/3, and 3/3) inherited from their parents with marker genotypes of 1/3 and 1/3. The difference between genotypes 1/1 and 3/3 of this locus was significant based on a multiple-comparison test ($P < 0.05$). Allele 3 was potentially linked to the high-oocyst shedding QTL allele.

DISCUSSION

Due to limitations in the available informative polymorphic markers for each family, linkage between markers and traits were evaluated separately for each parent. Marker LEI0071 was highly informative in several families compared with neighboring markers closely linked to LEI0101. The LOD scores were close to 0.0 for less polymorphic markers. In the 4 families selected for the current study, several markers were not informative enough to detect QTL. The remaining 8 families displayed no heritability and were assumed to be noninformative in a previous study (Zhu et al., 2003). The QTL could not be detected using additional flanking markers with the 12 families. This result indicated that genes related to coccidiosis resistance may not be segregated within the 8 noninformative families. Although this chicken population has been under selection for 20 generations, resis-

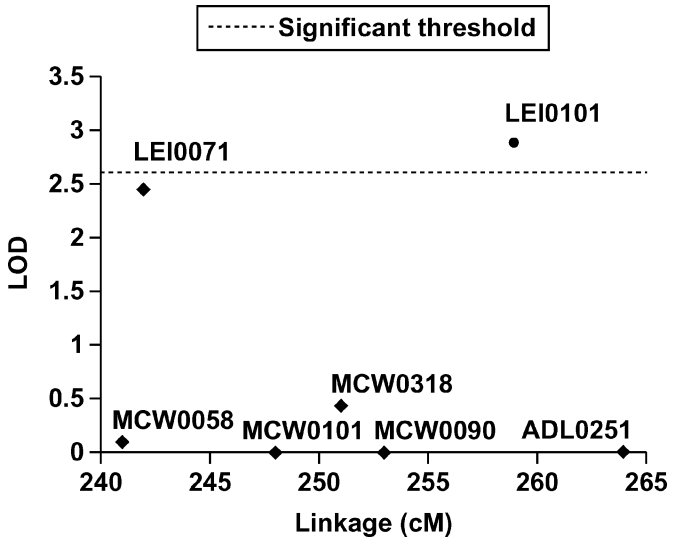

Figure 1. Logarithm of odds (LOD) scores of single-point association analysis of 7 microsatellite markers based on oocyst shedding in 4 selected families.

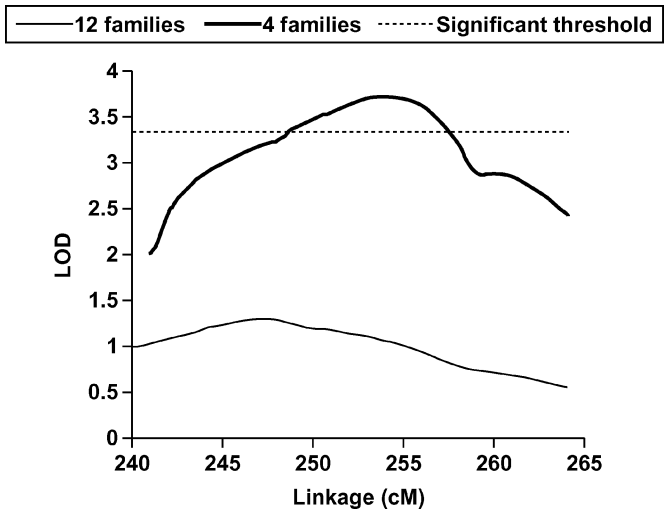

Figure 2. Logarithm of odds (LOD) scores of multipoint linkage analysis based on oocyst shedding in 4 selected families and 12 families.

tance gene(s) might not be selected in some individuals of the resistance line. Also, QTL could have originated from multiple origins of alleles in outbred families.

Although marker LEI0071 was mapped on chromosome 1 by linkage analysis, its physical location was not confirmed. Physical distance is not proportional to linkage-map distance, and the order of markers in linkage maps may not correlate with physical map locations. Once the exact physical location of LEI0071 is determined, improved analysis to better define the relevant QTL region will be possible. Association mapping, based on population-level linkage disequilibrium between a high density of single nucleotide polymorphic marker haplotypes and the genes of interest, can then be used to reduce the required sample size (Risch and Merikangas, 1996). The proteins encoded by known or predicted genes in and around this genomic region will be good candidate markers. This will allow the localization of specific genes controlling resistance to coccidiosis.

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REFERENCES

Almasy, L., and J. Blangero. 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. Am. J. Hum. Genet. 62:1198–1211.

De Koning, D. J., C. S. Haley, D. Windsor, P. M. Hocking, H. Griffin, A. Morris, J. Vincent, and D. W. Burt. 2004. Segregation of QTL for production traits in commercial meat-type chickens. Genet. Res. 83:211–220.

Gavora, J. S. 1990. Disease genetics. Pages 805–846 in Poultry Breeding and Genetics. R. D. Crawford, ed. Elsevier, New York.

Groenen, M. A., H. H. Cheng, N. Bumstead, B. F. Benkel, W. E. Briles, T. Burke, D. W. Burt, L. B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, A. P. de Leon, M. Soller, H. Takahashi, and A. Vignal. 2000. A consensus linkage map of the chicken genome. Genome Res. 10:137–147.

Lagarrigue, S., F. Pitel, W. Carre, B. Abasht, P. Le Roy, A. Neau, Y. Amigues, M. Sourdioux, J. Simon, L. Cogburn, S. Aggrey, B. Leclercq, A. Vignal, and M. Douaire. 2006. Mapping quantitative trait loci affecting fatness and breast muscle weight in meat-type chicken lines divergently selected on abdominal fatness. Genet. Sel. Evol. 38:85–97.

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

McElroy, J. P., J. C. Dekkers, J. E. Fulton, N. P. O'Sullivan, M. Soller, E. Lipkin, W. Zhang, K. J. Koehler, S. J. Lamont, and H. H. Cheng. 2005. Microsatellite markers associated with resistance to Marek's disease in commercial layer chickens. Poult. Sci. 84:1678–1688.

Risch, N., and K. Merikangas. 1996. The future of genetic studies of complex human diseases. Science 273:1516–1517.

Sasaki, O., S. Odawara, H. Takahashi, K. Nirasawa, Y. Oyamada, R. Yamamoto, K. Ishii, Y. Nagamine, H. Takeda, E. Kobayashi, and T. Furukawa. 2004. Genetic mapping of quantitative trait loci affecting body weight, egg character and egg production in F_2 intercross chickens. Anim. Genet. 35:188–194.

Schreiweis, M. A., P. Y. Hester, and D. E. Moody. 2005. Identification of quantitative trait loci associated with bone traits and body weight in an F_2 resource population of chickens. Genet. Sel. Evol. 37:677–698.

Tatsuda, K., and K. Fujinaka. 2001. Genetic mapping of the QTL affecting body weight in chickens using a F_2 family. Br. Poult. Sci. 42:333–337.

Vallejo, R. L., L. D. Bacon, H. C. Liu, R. L. Witter, M. A. Groenen, J. Hillel, and H. H. Cheng. 1998. Genetic mapping of quantitative trait loci affecting susceptibility to Marek's disease virus induced tumors in F_2 intercross chickens. Genetics 148:349–360.

Van Kaam, J. B., M. A. Groenen, H. Bovenhuis, A. Veenendaal, A. L. Vereijken, and J. A. Van Arendonk. 1999a. Whole genome scan in chickens for quantitative trait loci affecting carcass traits. Poult. Sci. 78:1091–1099.

Van Kaam, J. B., M. A. Groenen, H. Bovenhuis, A. Veenendaal, A. L. Vereijken, and J. A. van Arendonk. 1999b. Whole genome scan in chickens for quantitative trait loci affecting growth and feed efficiency. Poult. Sci. 78:15–23.

Xu, S., N. Yonash, R. L. Vallejo, and H. H. Cheng. 1998. Mapping quantitative trait loci for binary traits using a heterogeneous residual variance model: An application to Marek's disease susceptibility in chickens. Genetica 104:171–178.

Yonash, N., L. D. Bacon, R. L. Witter, and H. H. Cheng. 1999. High resolution mapping and identification of new quantitative trait loci (QTL) affecting susceptibility to Marek's disease. Anim. Genet. 30:126–135.

Yonash, N., H. H. Cheng, J. Hillel, D. E. Heller, and A. Cahaner. 2001. DNA microsatellites linked to quantitative trait loci affecting antibody response and survival rate in meat-type chickens. Poult. Sci. 80:22–28.

Zhu, J. J., H. S. Lillehoj, P. C. Allen, C. P. Van Tassell, T. S. Sonstegard, H. H. Cheng, D. Pollock, M. Sadjadi, W. Min, and M. G. Emara. 2003. Mapping quantitative trait loci associated with resistance to coccidiosis and growth. Poult. Sci. 82:9–16.