A quantitative trait locus for a primary antibody response to keyhole limpet hemocyanin on chicken chromosome 14—Confirmation and candidate gene approach

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ABSTRACT A QTL involved in the primary antibody response toward keyhole limpet hemocyanin (KLH) was detected on chicken chromosome 14 in the experimental population, which was created by crossing commercial White Leghorn and a Polish native chicken breed (green-legged partridgelike). The current QTL location is a validation of previous experiments pointing to the same genomic location for the QTL linked to a primary antibody response to KLH. An experimental population was typed with microsatellite markers distributed over the chicken chromosome 14. Titers of antibodies binding KLH were measured for all individuals by ELISA. Statistical models applied in the Grid QTL Web-based software were used to analyze the data: a half-sib model, a line-cross model, and combined analysis in a linkage disequilibrium and linkage analysis model. Candidate genes that have been proposed were genotyped with SNP located in genes exons. Statistical analyses of single SNP associations were performed pointing out 2 SNP of an axis inhibitor protein (*AXIN1*) gene as significantly associated with the trait of an interest.

Key words: keyhole limpet hemocyanin, native chicken breed, quantitative trait loci confirmation, immune response

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INTRODUCTION

Economic pressure on the modern poultry industry has directed selection process toward production traits (e.g., growth, egg production, and feed conversion). Selection based on production traits might affect immune responsiveness, leaving chickens more susceptible to disease. A key word in modern livestock production is sustainability (Pretty, 2008). Another opportunity in the livestock as in poultry production is disease resistance. Genetic selection toward disease resistance might be directed toward a single antigen or toward more general, overall immune responsiveness. From literature, we know successful examples of both approaches (Lamont et al., 2003).

Resistance to most diseases is likely to be controlled by polygenes. One way to detect genes that underlie immune responsiveness is by QTL mapping. In the past years, QTL mapping in chicken has identified chromosomal regions that contribute to variation in economically important traits. Among them, 83 QTL related to disease resistance were identified (Abasht et al., 2006).

In moving from QTL experimental results to the utilization of QTL in breeding programs, a QTL confirmation step is needed. Confirmation of the QTL is an essential step before attempts are made toward the fine mapping of the QTL and the identification of genes underlying traits of interest (Spelman and Bovenhuis, 1998). Confirmation is an independent validation of a QTL in a separate population. Quantitative trait loci confirmation studies have been performed in cattle (Allan et al., 2009; Awad et al., 2010), pigs (Sławinska et al., 2009), and salmon (Houston et al., 2008; Moen et al., 2009). Several validation studies have been conducted for chicken. They focused on fatness traits (Jennen et al., 2005) and resistance to *Salmonella* (Calenge et al., 2009) and immune response toward nonpathogenic antigens (Siwek et al., 2003).

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Keyhole limpet hemocyanin (**KLH**) is a high-molecular-weight protein antigen collected from the hemolymph of the sea mollusk (*Megathura crenulata*). It is a copper-containing high-molecular-weight protein, which is commonly used as a soluble model protein known to induce a T-helper 2 cell (**Th2**)-like response (Bliss et al., 1996). This antigen has been used in several studies of chicken immunity (Hangalapura et al., 2004; Minozzi et al., 2007, 2008) and QTL studies based on microsatellite markers (Siwek et al., 2003). Keyhole limpet hemocyanin is an antigen that birds do not encounter during their lifetime; therefore, it represents a novel antigen, suitable to measure primary immune responses.

A QTL on GGA14 for primary antibody response toward KLH was initially detected in a population of chickens created by crossing 2 lines initially selected for either high and low primary antibody response toward SRBC (Siwek et al., 2003). Selection was based on the individual antibody titer at 5 d after primary i.m. immunization with SRBC at 37 d of age. Two lines were selected for 16 generations and characterized for their immune traits as described by Bovenhuis et al. (2002). Reciprocal crosses of birds from the 18th generation were made to create the F_1 generation and further on, intercrosses were made to produce an experimental F_2 population.

The second population in which a QTL for primary antibody response toward KLH was detected was the feather pecking (**FP**) population. This population was created from a cross between 2 commercial lines of layers. These lines have been selected for production-related traits but differed consistently in FP behavior: the high and the low FP lines (Rodenburg and Koene, 2001; Buitenhuis et al., 2003).

In the current study, an experimental population was created based on 2 distinct chicken breeds. The first population was an old native polish chicken breed known for its disease resistance [green-legged partridgelike (**ZK**); Cywa-Benko, 2002]. The second breed in this cross was a White Leghorn (**WL**), which is a commercial chicken line selected for production traits. The ZK is a native chicken breed that was recognized as a breed at the end of 19th century (Witkowski et al., 2009). The ZK is known for its resistance to severe climate conditions, excellent hatching and rearing abilities, and disposition for good utilization of pasture (Cywa-Benko, 2002). The idea behind selecting these 2 breeds was to create a contrast between an unselected native breed and therefore (in assumption) having higher disease resistance and a commercial line selected for high production (in assumption) having lower disease resistance.

The goals of the current study were to detect if the 2 chicken breeds, ZK and WL, differed in a primary antibody response to KLH; find out if a QTL on GGA14 for primary antibody response toward KLH can be validated in the current resource population; and select and evaluate candidate genes for a primary antibody response toward KLH.

MATERIALS AND METHODS

Experimental Population

Analysis was carried out in the experimental population, created by crossing 2 breeds of hens: ZK and WL. We assumed that these 2 breeds would differ for primary antibody response toward KLH. Birds were kept on a floor system on a farm at the University of Life Sciences in Lublin. All chickens were vaccinated according to the routine vaccination schedule, which incorporated a vaccine against *Salmonella*, Gumboro disease, bronchitis, bursa of Fabricius disease, and encephalomyelitis. Reproduction of the parental F_0 generation was made by 5 WL roosters, which were mated to 6 to 8 ZK hens each. We obtained 115 individuals in 2 hatches in the F_1 generation, and their phenotypic range was from 8.6 to 16.7 for KLH titers. To create the next generation, F_1 parents were selected based on an antibody response to KLH. A high-responding rooster was mated to low-responding hens and the other way around. All together, 6 roosters were mated to 6 to 9 hens each, which gave 35 dam families. Fertilized eggs were collected and incubated in an automatic incubator. As a result, in the F_2 generation, 506 individuals were obtained in 6 hatches.

DNA Analysis

Genomic DNA, which was used as a matrix for the genotyping of microsatellite markers, was isolated from blood cells. The DNA isolation was performed in the 96-well plates (P-DW-11-C, Axygen Scientific, Union City, CA) using the commercial MasterPure DNA Purification Kit for Blood (MG 711100, Epicentre Biotechnologies, Madison, WI; Miller et al., 1988; Shimizu and Burns, 1995). The DNA extraction procedure recommended by the manufacturer was modified due to the presence of nucleated erythrocytes in the chicken blood.

The PCR reaction was used for the amplification of microsatellite markers using fluorescently labeled oligonucleotides. Seven microsatellite markers located on GGA14 were used for genotyping: MCW0296, ADL0200, ADL0118, MCW0136, MCW0123, ROS005, and MCW0225. Four out of these 7 microsatellite markers were used before to genotype SRBC and FP populations. The reaction was carried out in a volume of 10 μL. Each reaction consisted of a mixture containing 75 m*M* Tris-HCl (pH 8.8), 20 m*M* $(NH_4)_2SO_4$, 0.01% Tween 20, 5 nmol of tetramethylammonium chloride, 2.0 m*M* $MgCl_2$, 20 ng of DNA matrix, 0.25 U of recombinant Taq polymerase (Fermentas, Vilnius, Lithuania), and a pair of specific oligonucleotides for each marker, in an amount from 0.2 to 0.75 pmol each.

After amplification, PCR products were subjected to electrophoresis in 6% denaturating polyacrylamide gel. Fragment length analysis was based on laser fluorescence detection, using an automatic plate sequenator (Li-Cor 4200, Li-Cor Biosciences, Lincoln, NE) together with software designed for genotyping of microsatellite markers (Saga GT, Li-Cor Biosciences).

After completed electrophoresis, the gel image as a tagged image file format (TIFF) file was subjected to automated analysis using a computer program for the genotyping of microsatellite markers (Saga GT, Li-Cor Biosciences). Based on the entered data, which consisted of the gel image and a text file with information about the order of individuals, the panel of analyzed microsatellite loci, and the position of molecular weight markers, the program automatically performed genotyping. Text files with the genotypic data were imported into Microsoft Excel (Microsoft Corp., Redmond, WA), which—based on a tool called The Excel Microsatellite Toolkit (http://animalgenomics.ucd.ie/sdepark/ms-toolkit/; Park, 2001)—was used to assess the initial frequency of alleles. Genotypes, which occurred with low-frequency alleles (<1%) were tested once again. In the next stage, the analysis of the inheritance errors in the genotypic data was performed. To this purpose, a computer program GENOPED ver. 2.1. (http://www.people.cornell.edu/pages/zz19/research/genoped/) was used, which allowed the identification of genotyping incompatibilities of data compiled from a known pedigree. In dubious cases, an offspring genotype was eliminated from further analysis.

Phenotypic Data Collection

Total antibody responses to KLH were measured in individual plasma samples obtained at 7 d after s.c. immunization with 1 mg of KLH (Cal Biochem-Novabiochem Co., La Jolla, CA) in 1 mL of PBS (pH 7.2) at 12 wk of age. Antibody titers to KLH of all birds were measured by an indirect ELISA as described by Sijben et al. (2000).

Titers were expressed as the $\log_2$ values of the highest dilution giving a positive reaction. Antibody titers were measured twice: before KLH immunization (further referred to as KLHd0) and at 7 d after s.c. immunization (further referred to as KLH).

ANOVA

Primary antibody response toward KLH was analyzed by a mixed 3-way ANOVA with the sex and the line as fixed effects, the sire as a random effect, and with all possible interactions. All analyses were done with the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The sire MS error was used to test the overall significance of the effect of the progeny group-lines. The MS error of the interaction between sex and sire within progeny group was used to test the significance of effect of sex and of the interaction between the sex and the progeny group. Least squares means are shown in Table 1 and pairwise comparisons were performed when a main effect was found to be significant.

QTL Analysis

Regression interval mapping was used for QTL detection. Analyses were performed with Web-based GRID QTL software (http://www.gridqtl.org.uk/index.htm; Seaton et al., 2006; Hernández-Sánchez and Knott, 2009). Two different genetic models were used: 1) paternal or maternal half-sib analysis (HSportlets) and 2) line-cross analysis model (F2inbredportlets). In the paternal-maternal half-sib model, no assumptions are made concerning the allele frequencies in the founder lines and number of QTL alleles.

In the line-cross model, the power of QTL detection depends on the assumption of fixation of QTL alleles for the trait of interest in the founder lines. In this model, the alternative alleles at the QTL are traced back to the founder lines.

Linkage Disequilibrium and Linkage Analysis Approach

Multimarker linkage disequilibrium and linkage analysis (**LDLA**) mapping was developed by T. H. E. Meuwissen and M. E. Goddard in 2001. LDLA is a high-resolution gene mapping approach based on sophisticated mixed linear models, applicable to any population structure. Additional to the marker information and pedigree structure this approach uses population history information. Linkage disequilibrium and linkage analysis analyses were done using software available on http://cleopatra.cap.ed.ac.uk/gridsphere/gridsphere (Hernández-Sánchez et al., 2009). Sex and batch were used as covariates. The R method was used to estimate historical identity-by-descent probabilities (Hernández-Sánchez et al., 2006). Two models were used in QTL analysis: an additive model and a dominant model.

Table 1. Analysis of variance, least squares means, and SE for keyhole limpet hemocyanin (KLH) in 4 lines of chicken, the 2 parental lines [green-legged partridgelike (ZK) and White Leghorn (WL)] and generation F_1 and generation F_2

ANOVA	n	KLH
Main effects		
Line		***
Sex		NS
Line × sex		NS
Comparison of least squares means		
WL	5	12.62 ± 0.50^{ab}
ZK	17	12.64 ± 0.92^{ab}
F_1	113	13.55 ± 0.19^{a}
F_2	504	11.37 ± 0.09^{b}

[a,b]Least squares means in the same column and with no common superscript differed significantly ($P \leq 0.05$).

***$P \leq 0.001$.

Table 2. Single nucleotide polymorphism position and localization within candidate gene

Localization	Gene name[1]	SNP identification number	SNP position (bp)	Polymorphism
GGA14	*AXIN*	rs15012401	12806971	G/A
GGA14	*AXIN*	rs15012428	12848125	C/T
GGA14	*TRAP1*	rs13660891	13185119	G/C
GGA14	*TRAP1*	rs15012569	13191491	C/T
GGA14	*MAPK8IP3*	rs13787287	15085929	A/G
GGA14	*MAPK8IP3*	rs16014743	15123215	C/T

[1]*AXIN1* = axis inhibitor protein; *TRAP1* = tumor necrosis factor receptor-associated protein 1; *MAPK8IP3* = mitogen-activated protein kinase 8 interacting protein 3.

Candidate Gene Approach

Candidate genes were selected within the confirmed QTL region on GGA14. Genes were selected in silico, in the region selected by microsatellite markers flanking QTL for KLH. A list of loci associated with specific primary immune response toward KLH was prepared on the basis of the databases: National Center for Biotechnology Information (Wheeler et al., 2005), Kyoto Encyclopedia of Genes and Genomes (Kanehisa and Goto, 2000), and Gene Ontology (Gene Ontology Consortium, 2001).

SNP Selection, Genotyping, and Analysis

For each candidate gene selected within the QTL region, 2 SNP were selected. Selection was done with the BioMart tool from Ensembl (http://www.ensembl.org/index.html). All of the SNP were selected in coding regions of each gene. All together, 6 SNP were selected that were subjected for future genotyping in family members of the resource population. Single nucleotide polymorphism selection, position on the chromosome, and expected polymorphism are presented in Table 2. The SNP were genotyped in 200 individuals of the resource population with the SNaPshot Kit (Applied Biosystems, Carlsbad, CA). Two hundred birds selected for SNP genotyping had the highest information for microsatellite markers, information on their phenotype, and they equally represented all of the families of the resource population. To generate the template for the SNP analysis, PCR amplification was performed in a total volume of 35 μL. Each reaction contained 100 ng of the template DNA, 75 m*M* Tris-HCl (pH 8.8 at 25°C), 20 m*M* $(NH_4)_2SO_4$, 0.01% Tween 20, 1.5 m*M* $MgCl_2$, 200 μ*M* each deoxynucleoside triphosphate, and 1 U of Taq polymerase (Fermentas). The PCR amplification program was as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation (95°C, 1 min), annealing (50 to 52°C, 1 min), and DNA synthesis (72°C, 1 min) followed by a final DNA synthesis (72°C, 10 min). Subsequently, PCR products were checked on the agarose gel. After amplification, post-PCR purification was performed as follows: 5 μL of the PCR product was incubated with 1 U of FAST alkaline phosphatase and 2 U of exoI (Fermentas) for 1 h at 37°C, followed by 15 min at 80°C for enzyme inactivation. Primer extension reactions were carried out in a final volume of 10 μL containing 3 μL exoI-FAST-treated PCR product (5 to 50 ng of DNA) as a template, 2 μL of the SNaPshot Ready Reaction Mix (Applied Biosystems), and 0.2 μ*M* each primer. The following amplification protocol was applied: 35 cycles of 10 s at 96°C, 5 s at 50°C, and 30 s at 60°C. After the extension reaction, PCR products were treated with FAST alkaline phosphatase (1 U per sample) for 1 h at 37°C. For electrophoresis, 1 μL of the purified primer extension reaction products was mixed with 9 μL of HiDi formamide and 0.2 μL of GeneScan-120 LIZ size standard (Applied Biosystems), denatured for 5 min at 95°C, and separated by capillary electrophoresis on an ABI Prism 3130XL genetic analyzer (Applied Biosystems) in a 36-cm-length capillary using the POP7 polymer. Alleles were scored using Gene Mapper 3.7 software (Applied Biosystems).

Statistical Procedure

Single nucleotide polymorphism allele association with a trait of interest was analyzed with Haploview software (http://www.broadinstitute.org/mpg/haploview; Barret et al., 2005). The second part of the analysis was performed with the GLM procedure of SAS (SAS Institute Inc.) Antibody response toward KLH has a skewed, not normal distribution and was therefore log-transformed. The underlying assumption of the experiment is the line-cross model assuming that both parental lines are homozygous but inherit different alleles to their offspring. The F_1 population is then assumed to be all heterozygous and the F_2 generation is informative. Log KLH was modeled as:

$$Y_{ijklm} = sire_i + b \times KLHd0 + SNP1_j + SNP4_k + SNP6_l + e_{ijklm}, \quad [1]$$

where sire is the sire of the F_2 generation bird (i = 1 to 8), KLHd0 is the baseline KLH level, and the 3 SNP account for the genetic variation captured with this analysis (j, k, and l; AA, AB, and BB genotypes, respectively), and e is the residual term of each of the m (m = 200) observations i, j, k, l, and m.

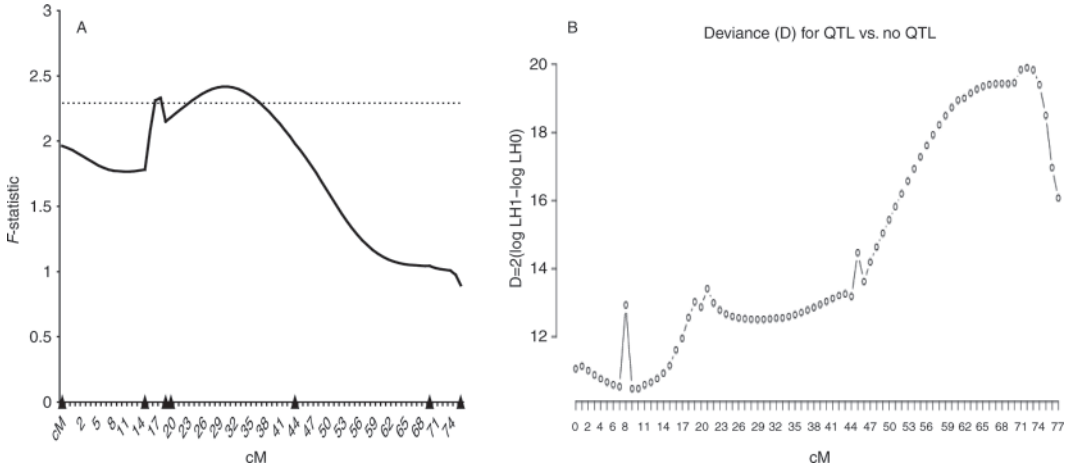

Figure 1. (A) Test statistic for GGA14 with regard to the primary antibody response to keyhole limpet hemocyanin (KLH) under a half-sib analysis model with dam as a common parent for the F_2 cross between green-legged partridgelike and White Leghorn population. The solid curve describes the test statistic and the dotted line indicates threshold. Triangles on the x-axis indicate position of the microsatellite markers. (B) Test statistic for GGA14 with regard to the primary antibody response to KLH under linkage disequilibrium and linkage analysis model for the F_2 cross between green-legged partridgelike and White Leghorn population. The dotted lines describe the test statistic. LH1 = likelihood of hypothesis of linked QTL; LH0 = likelihood of null hypothesis.

RESULTS AND DISCUSSION

Means and ANOVA

The highest average primary antibody response toward KLH was detected in the F_1 generation (13.55) and the lowest value was observed for the F_2 generation (11.37), although it was not significantly different from parental lines. Parental lines had intermediate titer values for primary antibody response toward KLH. There was no significant difference between parental breeds (WL and ZK) in the primary antibody response toward KLH. The basic assumption of the difference in the immune response toward KLH between purebreds selected for production traits (WL) and unselected (ZK) did not come true. An ANOVA and means for KLH titers are presented in Table 1. Not significant heterosis effect was observed in our study in the F_1 generation. Negative heterosis was observed in previous studies on immune traits (Boa-Amponsem et al., 1998; Yang et al., 1998; Campo and Davila, 2002; Minozzi et al., 2007), indicating that crossbreds birds obtained do not seem to show significantly different or higher average immune responses than parental lines. Because crossbreds are known for their better disease resistance compared with purebreds, it might be that negative heterosis for a general immune trait is actually advantageous.

QTL Regression

The QTL detected for the KLH primary antibody response using both half-sib (dam and sire as common parent) and line-cross models are presented in Table 3. For the primary immune response toward KLH, 1 QTL was detected with the half-sib model (dam common parent in the subset of the 19 largest dam families) in a marker bracket MCW0136–MCW0123. The *F*-statistic profile for this QTL is presented in Figure 1A. All of the remaining models, which were used for the QTL regression: half-sib analysis (sire common parent), line-cross analysis additive model and line-cross analysis dominant model, did not provide any evidence of a statistically significant QTL on GGA14. The reason why the experimental data were not explained by the line-cross model might be explained by the lack of difference in the primary antibody response toward KLH in parental breeds (WL and ZK).

The QTL for primary antibody response toward KLH has been previously detected in an experimental population created from 2 chicken lines divergently selected

Table 3. Regression analysis of the QTL

Model	Marker bracket	Position (cM)	F^1	$P_{0.05}{}^2$
Half sib dam	MCW0136–MCW0123	31	2.41	2.20
Half sib sire	ROS0005–MCW0225	65	2.46	3.00
Line-cross additive	MCW0136–MCW0123	21	0.59	6.93
Line-cross dominant	MCW0123–MCW0225	58	1.13	5.06

[1]*F*-statistics at the QTL position.

[2]Significance threshold 5%, obtained by 10.000 permutations chromosome-wide.

for a high and low primary antibody response toward SRBC (Siwek et al., 2003). In the SRBC population, QTL evidence was located at the distal part of the chromosome 14 (60 cM) and it was detected with the paternal half-sib model. First validation of this QTL location has been performed in the FP population. The QTL for a primary antibody response toward KLH has been detected in the FP population with paternal half-sib model in a proximal part of the chromosome. The QTL detected on GGA14 in the current study is the second independent confirmation of that chromosomal location in relation to the primary antibody response to KLH. In the SRBC population, which was a primary experimental population for this QTL, similar QTL position (60 cM) has been discovered with 2 statistical models: paternal half-sib model and line-cross model. Two models were used in all 3 populations: half-sib analysis model and line-cross model. Hence, the last model could be successfully applied in the SRBC population only. Data sets from 2 other populations, FP and ZK-WL, were not explained by the line-cross model. This fact might be explained by the selection of the SRBC population for a primary antibody response to SRBC. Both SRBC antigen and KLH antigen are T-cell-dependent antigens. Moreover, the difference in the primary antibody response toward KLH was present in SRBC founder lines (unpublished data) only. The KLH antibody level has not been measured in the FP founder lines. In the current experimental cross, as it was already mentioned, there was no difference in KLH titers in parental breeds.

We detected a QTL on GGA14 with the half-sib analysis model but we did not detect it with the line-cross model. The power of the analysis in the line-cross model depends on the fixation of the QTL alleles in the founder lines. As long as the founder lines are not fixed for different alleles, a QTL line-cross model is not a suitable one. We can observe that in the case of the current reference population. A similar conclusion could be drawn from the analysis of FP data, in which QTL on GGA14 for a primary antibody response was detected with the half-sib analysis model only. However, the use of both models (half-sib analysis model and line-cross analysis model) allows investigating different assumptions about QTL genotype in founder populations. The half-sib model is more general with no assumption about the number and frequency of QTL alleles in founder populations and probably more realistic for many QTL. The line-cross model is robust in an ideal situation when different alleles are fixed in the founder lines, even though it tends to underestimate QTL effects in such situations (Wimmers et al., 2006).

LDLA Analysis

In the next step of the QTL analysis, a LDLA approach was performed. A statistically significant QTL on GGA14 was detected for a primary antibody response to KLH with the LDLA approach. The *F*-statistic profile for this QTL is presented in Figure 1B. The LDLA analysis in this case combines paternal effects with maternal effects. In the QTL detected with a maternal half-sib model in a regression analysis, the confidence interval is large and covers the better part of the chromosome. Therefore, a very precise QTL peak location cannot be pointed out for that analysis. In LDLA, combined analysis peak position is moved toward the distal part of the chromosome as it was in the original analysis in the SRBC population (Siwek et al., 2003).

Candidate Gene Approach and SNP Association

Only 3 out of 6 SNP that were genotyped in the candidate genes turned out to be informative [RS15012401 (axis inhibitor protein, ***AXIN1***), RS15012428 (*AXIN1*), RS13660891 (tumor necrosis factor receptor-associated protein 1, ***TRAP1***)]. The 3 other SNP were monomorphic. The D' analysis performed by Haploview software for the informative SNP is presented in Figure 2. Case-control analysis of a single SNP association done by Haploview software did not prove any statistically significant association between the SNP alleles and the trait of interest. However, SAS analysis performed on the SNP data showed that SNP RS15012428 (*AXIN1*) was heterozygous for all sires used, as expected under the line-cross model. Model [1] explained 25% of the variation in KLH, with sire and regression on KLHd0 significant at, respectively, $P = 0.01$ and $P = 0.03$. The SNP RS13660891 (*TRAP1*) was not significant, but SNP RS15012401 (*AXIN1*) ($P = 0.03$) and SNP RS15012428 (*AXIN1*) ($P = 0.005$) were found significant. Birds with AA, AB, and BB had least squares means of 2.35, 2.54, and 2.50, respectively, on the log-transformed scale, with AA being significantly different from AB and BB, suggesting dominance for B. The SNP RS15012401 (*AXIN1*) yielded 2.57 (AA), 2.42 (AB), and 2.38 (BB) on the log-transformed scale, with AA being significantly different from AB and BB.

The *AXIN1* gene (gene identification number 395786) activates the c-Jun N-terminal kinase (**JNK**) cascade. The JNK proteins are ubiquitously expressed, evolutionarily conserved mitogen-activated protein kinases that are involved in stress responses. It has been found that *AXIN1* serves as a scaffold protein for mitogen-activated protein kinase activation and it further determined the structural requirement for this activation. The *AXIN* gene forms a complex with mitogen-activated protein kinase kinase kinase 1 (**MEKK1**) through a novel domain that we call the MEKK1-interacting domain (Zhang et al., 1999). Dimerization-oligomerization of *AXIN* through its C-terminus is required for JNK activation, although MEKK1 is capable of binding C-terminus-deleted monomeric *AXIN*.

During Th2 differentiation, MEKK1 phosphorylation on Thr^{1381} is present. Phosphorylation of MEKK1 at

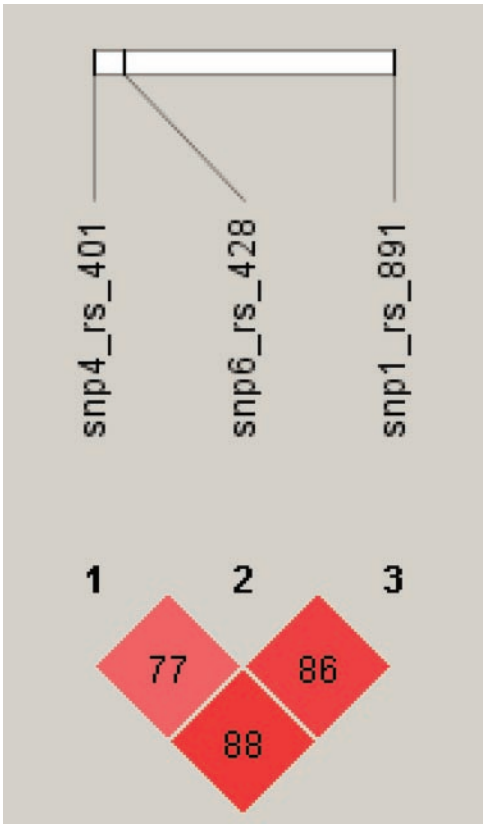

Figure 2. D' analysis performed by Haploview software (http://www.broadinstitute.org/mpg/haploview; Barrett et al., 2005). Color version available in the online PDF.

the residue Thr^{1381} is critical for the activation of the kinase. This residue becomes phosphorylated after either T-cell receptor costimulation of $CD4^+$ T-cell differentiation under Th2 conditions. The MEKK1 kinase domain and Itch kinase domain are important for interleukin 4 and interleukin 5 cytokine expression under Th2 conditions (Enzler et al., 2009).

Both the significant SNP in the *AXIN1* gene as well as the statistically significant QTL for KLH on GGA14 have been detected in the subset of the experimental population. The line-cross model was the only one that was applied to the entire population and did not yield a significant QTL. The prerequisite for a line-cross model was therefore not fulfilled, but situation came close. That means that even if the entire population did not show evidence of a QTL for KLH on GGA14 with the line-cross analysis model, it was sufficient alleles segregating in the sib families to detect a QTL and to find significant SNP in *AXIN1.*

In summary, the results of a QTL experiment aiming at the confirmation of QTL related to a primary antibody response toward KLH were presented in the experimental population obtained from a commercial WL and a polish native chicken breed (ZK). The study confirmed presence of a QTL region on chicken chromosome 14. Positional candidate genes were suggested. The SAS analysis pointed out 2 SNP of the *AXIN1* gene being significantly associated with primary antibody response toward KLH in the experimental population.

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