Quantitative trait loci associated with the humoral innate immune response in chickens were confirmed in a cross between Green-Legged Partridgelike and White Leghorn

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ABSTRACT Natural antibodies (NA) create a crucial barrier at the initial steps of the innate humoral immune response. The main role of NA in the defense system is to bind the pathogens at early stages of infection. Different pathogens are recognized by the presence of highly conserved antigen determinant [e.g., lipopolysaccharide (LPS) in gram-negative bacteria or lipoteichoic acid (LTA) in gram-positive bacteria]. In chickens, a different genetic background of NA binds LPS and LTA antigens, encoded by different QTL. The main objective of this work was to confirm known QTL associated with LPS and LTA NA. For this purpose a chicken reference population was created by crossing 2 breeds: a commercial layer, White Leghorn, and a Polish indigenous chicken, Green-Legged Partridgelike. The chromosomal regions analyzed harbored to GGA3, GGA5, GGA6, GGA8, GGA9, GGA10, GGA14, GGA15, GGA18, and GGAZ. The data collected consisted of the NA titers binding LPS and LTA (determined by ELISA at 12 wk of age) as well as the genotypes (30 short tandem repeat markers; average of 3 markers/chromosome, collected for generations F_0, F_1, and F_2). The analyses were performed with 3 statistical models (paternal and maternal half-sib, line cross, and linkage analysis and linkage disequilibrium) implemented in GridQTL software (http://www.gridqtl.org.uk/). The QTL study of humoral innate immune response traits resulted in the confirmation of 3 QTL associated with NA titers binding LPS (located on GGA9, GGA18, and GGAZ) and 2 QTL associated with NA titers binding LTA (located on GGA5 and GGA14). A set of candidate genes within the regions of the validated QTL has been proposed.

Key words: quantitative trait loci, lipopolysaccharide, lipoteichoic acid, natural antibody, chicken

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INTRODUCTION

Immunity in vertebrates that ensures their resistance or susceptibility to diseases consists of innate and acquired mechanisms. Innate immunity establishes the first barrier against pathogens and it develops naturally, based mostly on the genetic makeup of the individual. An important part of the humoral innate immunity in vertebrates is the presence of natural antibodies (**NA**), which are immunoglobulins that need no exogenous stimulation of the immune system to be secreted by B-1 cells in large quantities (Bos et al., 1988; Baumgarth et al., 2005). Even though NA express low affinity to pathogens, they are very effective as a first barrier to pathogen invasion, mostly because of their massive presence in the host organism and polyreactivity (i.e., the ability to bind to virtually any antigen in a very short time; Casali and Schettino, 1996; Zhou et al., 2007). Therefore, NA are considered to be a crucial immune barrier at the initial stages of the immune response, before the acquired antibodies are generated (Ochsenbein et al., 1999). The NA bind different highly conserved, homologous epitopes (homotopes); for example, lipopolysaccharide (**LPS**), the molecule found in the outer membrane of gram-negative bacteria, or lipoteichoic acid (**LTA**), which is an ingredient of a cell wall of gram-positive bacteria.

Genetic loci involved in the determination of the NA titers in chickens were initially discovered with a QTL linkage study (Siwek et al., 2006). This study included a whole-genome scan with microsatellite markers and allowed detection of several chromosomal regions encoding innate humoral immune response to environ-

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mental antigens on different chicken chromosomes [i.e., LPS (GGA3, GGA6, GGA8, GGA9, GGA15, GGA18, GGAZ) and LTA (GGA3, GGA5, GGA10, GGA14)]. Confirmation of the QTL is an important step in the workflow of dissecting the genetic background of quantitative traits. Remapping of known QTL regions in independent populations proves the segregation of the given loci and reduces the false-positive results. For this reason the QTL validation studies that use different populations or different marker sets are often reported as follow up studies to initial whole-genome scans, especially in humans (Liu et al., 2003; Tzenova et al., 2004; Cheung et al., 2006), rodents (Fehr et al., 2005; Bennett et al., 2006; Hoopes et al., 2006; Kumar et al., 2007; Radcliffe et al., 2007; Boyle and Gill, 2008), and livestock (Bennewitz et al., 2003; King et al., 2003; Jennen et al., 2005; Jiang et al., 2005; Wattrang et al., 2005; Houston et al., 2008; Sławińska et al., 2009; Siwek et al., 2010).

For a confirmation of the QTL associated with the humoral innate immunity in chickens, a new reference population was set up based on 2 chicken breeds of different origin, selection history, and economic use: Green-Legged Partridgelike (**GP**) and White Leghorn (**WL**). The GP is a dual-purpose Polish chicken, developed at the beginning of 20th century and kept locally in central eastern Poland as a free-range chicken. The GP chicken has been protected from extinction by the means of in situ preservation. The animals in the conservation program were kept in a conservation flock without selection for more than 50 generations. The GP is well adapted to harsh environmental conditions and therefore it is more fit and disease resistant (Cywa-Benko, 2002; Witkowski et al., 2009). The WL is a breed that originated in Italy and was selected for its laying performance. The WL chickens used in our experimental population derived from a pure line (H-33) kept in the conservation flock. The goal of this study was to validate the QTL associated with the NA titers binding LPS and LTA antigens with use of a WL × GP chicken experimental population.

MATERIALS AND METHODS

Experimental Population

Analyses were carried out based on the experimental population created by crossing 2 chicken breeds, GP and WL, as described by Siwek et al. (2010). The animals were reproduced in 3 generations. For reproducing the F_0 generation, 5 flocks were created, each of them consisting of 1 WL male and 5 to 8 GP females. Altogether, 115 F_1 individuals were obtained in 2 hatches. Matings among F_1 progeny were set based on the selection criterion, which was adaptive immune response to keyhole lympet hemocyanine (**KLH**; high vs. low antibody titers) 7 d after immunization with exogenous antigen (i.e., KLH). As a result, 491 F_2 individuals were obtained in 6 hatches over a 4-mo period (June–September 2007). The blood samples for further analysis were collected from nonimmunized animals (d 0) at 12 wk of age. For the blood collection a Vacuette blood collection system (Greiner Bio One, Frickenhausen, Germany) was used. On average, 2 mL of blood was drawn from the wing vein of the chicken into the individual tubes coated with EDTA anticoagulant and centrifuged ($600 \times g$ for 5 min at room temperature) to separate blood cells from serum. Afterward, serum was aspirated to sterile tubes and the samples were stored at −20°C until further analytical procedures.

DNA Extraction and Microsatellite Genotyping

Genomic DNA was isolated from blood cells using MasterPure DNA Purification Kit for Blood (Epicenter Biotechnologies, Madison, WI). The extraction procedure recommended by the manufacturer (Miller et al., 1988) was modified because of the presence of nucleated erythrocytes in the chicken blood (10 μL of the blood cells pellet was used for DNA extraction). The microsatellite markers that flank the known QTL regions linked to LTA and LPS were selected based on the literature data housed by AnimalQTLdb (Hu et al., 2007). Altogether, a set of 30 microsatellite markers was amplified in PCR reactions. In general, the PCR was carried out in a volume of 10 μL. Each reaction contained 75 m*M* Tris-HCl (pH 8.8), 20 m*M* $(NH_4)_2SO_4$, 0.01% Tween 20, 5 nmol of TMACl, 2.0 m*M* $MgCl_2$, 20 ng of DNA template, 0.25 U of recombinant Taq polymerase (Fermentas, Vilnius, Lithuania), and from 0.2 to 0.75 pmol of specific primers. Forward primers were labeled with IRD800 fluorescent dye (MWG, Biotech, Ebersberg, Germany). Reverse primers were unmodified (Genosys, Sigma-Aldrich GmbH, Schnelldorf, Germany). The following markers were amplified in the multiplex PCR reactions: MCW0222, MCW0214, ADL0118 and ADL0345 (first multiplex PCR), MCW0150 and MCW0123 (second multiplex PCR), and LEI0097 and ADL0200 (third multiplex PCR). Temperature programs set on a PCR machine (TGradient, Biometra GmbH, Goettingen, Germany) included 5 min of double-stranded DNA denaturation at 95°C, followed by 35 amplification cycles (DNA denaturation at 95°C for 30 s; primers annealing at 48–52°C for 30 s; and DNA synthesis at 72°C for 30 s) and final DNA synthesis at 72°C for 4 min. Genotypes at 30 microsatellite loci were determined for all individuals by gel electrophoresis performed on a 4200 Li-Cor DNA Analyzer (Li-Cor Biosciences, Lincoln, NE). Alleles were scored with SAGA genotyping software (Li-Cor Biosciences).

Determination of NA Titers by ELISA

Two environmental antigens (LPS and LTA) that bind different NA were applied in ELISA to define the overall humoral innate immune response in chickens. Titers of NA were determined in the serum of 12-wk-

old chickens by ELISA. The assay was performed for 2 homotopes (LPS and LTA) according to the methodology reported by Siwek et al. (2006). Briefly, the titration plates were coated with 4 μg/mL of LPS from *Escherichia coli* (Sigma-Aldrich GmbH) and 10 μg/mL of LTA from *Staphylococcus aureus* (Sigma-Aldrich GmbH). Serum samples were applied on the coated plates. Binding of NA to LPS and LTA antigens was detected by 1:20,000 diluted rabbit antichicken antibody ($IgG_H{}^+{}_L$; Nordic, Tilburg, the Netherlands) conjugated to peroxidase (PO; RAch/IgG $_H{}^+{}_L$/PO). Substrate for enzymatic color reaction was 0.05% H_2O_2 with addition of tetramethylbenzidine. Absorbancies were measured with a multiscan (Labsystems, Helsinki, Finland) at 450 nm. Results were expressed as $\log_2$ of the maximum serum dilution at which a positive color reaction was detected.

Statistical Evaluation

The genotypic microsatellite data set was evaluated with the Excel Microsatellite Toolkit (Park, 2001) and checked for the Mendelian inheritance errors between parents and offspring. The setup of the experimental population fitted different statistical approaches for QTL study: 1) regression analysis within paternal and maternal half-sib families (**HS**; Knott et al., 1996), including 6 paternal half-sib families with the average size of 82 offspring/sire and 9 maternal half-sib families with the average size of 33 offspring/dam; 2) line-cross regression analysis (**LC**; Haley and Knott, 1992), including 27 of F_0, 45 of F_1, and 491 of F_2 individuals; and 3) linkage disequilibrium and linkage analysis (**LDLA**; Hernández -Sánchez et al., 2009).

All of the models are implemented in a GridQTL package [Seaton et al., 2006; http://www.gridqtl.org.uk/ (HS and LC) and http://cleopatra.cap.ed.ac.uk/gridsphere/gridsphere (LDLA)]. In both HS and LC models, a simple regression analysis was used to calculate the test statistics for the presence or absence of a single QTL at each centimorgan. Significance thresholds (5 and 1%) were individually calculated for each chromosome and each trait by performing 500 chromosome-wide permutations. Confidence intervals of the QTL were calculated using bootstrap with resampling. Hatch and sex of animals were considered as fixed effects. The LC model also included additive and dominant genetic effects. On the other hand, the LDLA model is based on the combined linkage analysis and linkage disequilibrium. In addition to pedigree and molecular markers it uses population history information and the multiple regression method is implemented to estimate the identical-by-descent status of the individual loci. On the basis of the identical-by-descent matrix, additive or dominant variance at the QTL region was calculated, and it was consecutively analyzed for the presence of additive or dominant effects. The results were tested based on the likelihood ratio test, expressed as the D statistic. The significance threshold for the test statistic D that supported the alternative hypothesis (presence of a single QTL) was 3.8.

RESULTS AND DISCUSSION

Evaluation of Genotypic Data Set for Linkage Analysis

The initial genotypic data set for a QTL study comprised 30 microsatellite markers, but after data evaluation 2 markers (MCW0038 and MCW0136) appeared to be monomorphic across the whole chicken population genotyped and therefore uninformative. Thus, they were excluded from further analyses and the following study was performed on 28 microsatellite markers. The panel of genotypes analyzed varied in terms of polymorphism expressed by the number of alleles, heterozygosity, and polymorphic information content (data not shown). On average, 3.7 alleles per microsatellite marker were obtained. Observed and expected heterozygosity reached similar values (0.47) but with high standard deviations, reaching 0.215 and 0.192, respectively. Mean polymorphic information content for 28 loci was 0.42 ± 0.179. Based on the scale of Botstein et al. (1980), the panel of microsatellite markers was assessed as moderately informative.

LPS and LTA NA Titers

The second component of the data set included NA titers binding LPS and LTA. Table 1 presents the descriptive statistics of both NA titers in F_2 generation. In general, the titers of NA were higher for LTA antigen (5.6 $\log_2$ ELISA titers) when compared with LPS antigen (2.9 $\log_2$ ELISA titers). In both cases standard deviation (1.30 for LPS NA and 1.75 for LTA NA) as well as the differences between minimum and maximum values (7.8 for LPS NA and 11 for LTA NA) were high. High diversity in the phenotypic scores is considered desirable because, according to van Arendonk et al. (1994), the greater diversity within the traits, the greater likelihood of obtaining linkage disequilibrium (**LD**) for genes controlling the phenotype. The NA titers reported here were consistent with the literature (Siwek et al., 2006; Minozzi et al., 2008).

The heritability of the NA in the chicken resource population was moderately high for LTA NA titers

Table 1. Descriptive statistics of lipopolysaccharide (LPS) and lipoteichoic acid (LTA) natural antibody (NA) titers in the blood serum of F_2 generation of White Leghorn × Green-Legged Partridgelike chicken crossbreds

Trait	n	Mean[1]	Minimum[2]	Maximum[3]
LPS	491	2.9 (1.30)	1	8.8
LTA	491	5.6 (1.75)	1	12

[1]Mean value of LPS and LTA NA titers; SD given in parentheses.
[2]Minimum value of LPS and LTA NA titers.
[3]Maximum value of LPS and LTA NA titers.

(0.23 ± 0.11) and low for LPS NA (0.10 ± 0.07). The results of heritability of NA titers in chickens are in concordance with the literature data (Siwek et al., 2006; Wijga et al., 2009) and the general concept that fitness traits (e.g., immune-related traits) express lower heritability when compared with other phenotypic characteristics (referred to as nonfitness traits) because of the higher additive and residual variance (Merilä and Sheldon, 1999). High positive genetic correlation was obtained for LPS and LTA NA (0.713). This result is supported by the literature data (Siwek et al., 2006) and suggests that both quantitative traits are encoded by common genes.

QTL Mapping with HS Model

The general results of the QTL mapping including the location of the QTL in the chicken genome are presented in Table 2. Overall, 10 QTL associated with LPS and LTA NA were mapped to 5 chromosomes with use of 3 statistical methods.

Regression analysis with paternal and maternal half-sib model allowed mapping of as much as 6 statistically significant QTL (LPS: GGA5, GGA14, and GGA18; LTA: GGA5, GGA18, and GGAZ). In this model the power of the statistical test is based mostly on the polymorphism of the molecular markers and the heterozygosity of parents (sires or dams) in a QTL region (Weller, 2001). To increase the likelihood of detecting a QTL that segregates within the half-sib family, both sires and dams were genotyped. The number of half-sib families (9 maternal and 6 paternal) was considered sufficient based on the simulation studies conducted by Wu and Jannink (2004), who found the greatest power of QTL mapping when 5 to 10 families were used. Therefore, as mentioned before, segregation of different QTL was detected in maternal and paternal half-sib families. Importantly, 2 QTL (LTA on GGA5 and LPS on GGA18) detected in the initial study by Siwek et al. (2006) were validated in our population with use of the HS model.

QTL Mapping with LC Model

The chicken reference population and experimental setup were designed to meet the criteria of the LC model (Hayes et al., 2005). As discussed above, the lines selected to create a crossbred reference population were genetically distant and they were assumed to differ in the immune response traits analyzed here. However, only 2 significant QTL (LPS on GGA9 and GGAZ) were detected using the LC model. Both QTL validated the previously detected loci (Siwek et al., 2006). The low power of the QTL detection with the LC model was explained by the results of ANOVA as discussed in Siwek et al. (2010), which proved no differences between the parental lines with respect to KLH titers. Keyhole lympet hemocyanine is a highly potent antigen that stimulates the immune response of T helper cell (**Th**)-2 type. The same type of immune response is triggered by LTA homotope (Parmentier et al., 2004). Therefore, the pathways of immune response to KLH and LTA are the same, and lack of differences in primary antibody titers to KLH in founder lines caused no variation in this phenotypic trait in the experimental population. It explains the lack of statistically significant QTL associated with LTA NA in the experimental population described here. On the other hand, LPS homotope has the opposite effect on the immune system to LTA and KLH and it favors Th-1 immune response (Parmentier et al., 2004). As a result, 2 QTL associated with LPS NA were detected here, which supports the statement that the experimental population was well suited for Th-1 immune type (LPS) QTL discovery with LC statistical model, not Th-2 (LTA).

Table 2. Overview of the QTL associated with keyhole lympet hemocyanine, lipopolysaccharide (LPS), and lipoteichoic acid (LTA) natural antibody (NA) levels in chickens

Chromosome	Trait[1]	cM[2]	CI	Marker bracket[3]	Model[4]
GGA5	LPS	79*	73–127	MCW0038–MCW0214	HS dam
GGA5[5]	LTA	103**	79–127	MCW0214–MCW0032	HS dam
GGA9[5]	LPS	19*	0–41	ROS0078–GCT0016	LC a
GGA14	LPS	53*	0–77	ADL118–ROS0005	HS sire
GGA14	LPS	14*	NE[6]	ADL0118–ROS0005	LDLA
GGA14[5]	LTA	49*	NE	MCW0296–ROS0005	LDLA
GGA18[5]	LPS	24*	0–24	MCW0045–MCW0217	HS sire
GGA18	LTA	9*	0–24	MCW0045–MCW0217	HS dam
GGAZ	LTA	44*	20–105	MCW0331–MCW0241	HS sire
GGAZ[5]	LPS	99*	20–105	MCW0331–MCW0241	LC a+d

[1]Phenotypic trait: LPS or LTA NA titers.

[2]cM = relative position of the maximum for QTL.

[3]Microsatellite markers flanking the QTL region.

[4]HS dam = maternal half-sib regression analysis, dam common parent; HS sire = parental half-sib regression analysis, sire common parent; LC a = line-cross regression analysis, additive effects; LDLA = linkage disequilibrium linkage analysis; LC a+d = line-cross regression analysis, additive and dominant effects.

[5]Validated QTL region.

[6]NE = not estimated.

*$P \leq 0.05$; **$P \leq 0.01$.

An advantage of LC model in a QTL analysis is its potential to include the genetic effects into the model (i.e., additive and dominant effects), which point out the interactions between alleles of a given QTL. For the QTL associated with LPS NA titers located on GGA9, the additive effect was negative (−0.4), which means that the QTL allele that was inherited by F_2 offspring encodes for a low level of the phenotypic trait (q allele). Thus, it reduces the level of LPS NA in the filial generation. In the case of the second QTL detected with LC model (LPS on GGAZ) the dominant effects (−0.7) were lower than the additive effects (−0.2), which indicates a lower level of phenotypic trait (LPS NA) in heterozygous individuals.

QTL Mapping with LDLA Model

Because of limitations of the genetic maps resolution used in this study, only 1 chromosome (GGA14) met the criteria for LDLA mapping. The heritability of the QTL effects as calculated in the LDLA model was low and ranged from 0.04 to 0.15, but it was consistent with heritability of the phenotypic traits analyzed here. The QTL on GGA14 associated with LPS showed additive effects (heritability of additive effects, $H_A^2 = 0.13$), whereas QTL linked to LTA NA was inherited in the form of both additive ($H_A^2 = 0.04$) and dominant (heritability of dominant effects, $H_D^2 = 0.15$) effects. Based on our results, GGA14 harbors validated QTL associated with LTA NA.

In general, LDLA is considered a powerful approach in QTL mapping. It uses not only recombination events that are detected in an analyzed reference population but also historical recombination events that occurred in the reference population in the previous generations. The concept of LDLA model derives from the fact that the QTL can result from mutations in one of the ancestor's genomes. This strategy requires the use of markers, which are in LD (Hayes et al., 2005), which usually refers to the markers that are located close to each other (e.g., in chickens, LD extends from 1 to 4 cM; Aerts et al., 2007). But, due to adding information on historical recombinations, LD may also be detected at a distance of several centimorgans, allowing QTL mapping even with use of low-resolution genetic maps. However, to reduce the risk of obtaining false-positive results, LD mapping is accompanied by linkage analysis mapping. The QTL analysis with LDLA model is considered optimal because it uses the strength and precision of LD mapping and it verifies the false-positive results in linkage analysis mapping (Meuwissen et al., 2002).

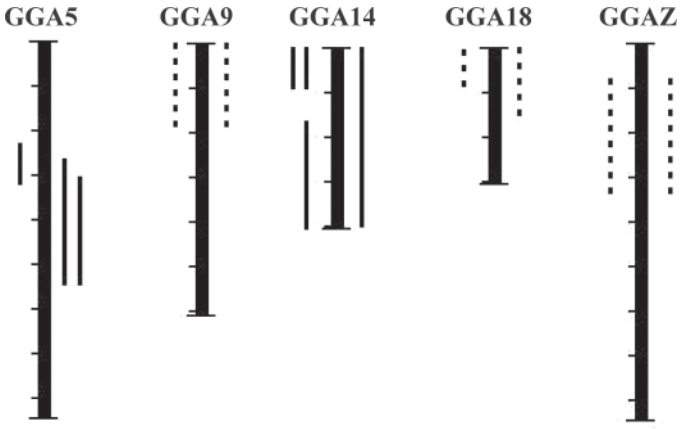

Figure 1. Validated QTL regions associated with lipopolysaccharide (LPS) and lipoteichoic acid (LTA) natural antibodies (NA). The vertical line on the left side of the chromosome represents initial QTL (Siwek et al., 2006) and the vertical line on the right side of each chromosome represents the QTL region validated in this study. The dotted line is LPS QTL and the solid line is LTA QTL.

Validated QTL Associated with LPS and LTA NA

The experiment described here focused on validation of the QTL associated with innate humoral immunity, defined as the titers of natural antibodies to LPS and LTA antigens and detected previously by whole-genome scan (Siwek et al., 2006). The QTL validated in this study are presented in Figure 1. The detailed information is presented in Table 2, in which the validated loci have been indicated. Briefly, out of 14 previously known QTL regions located on 10 chromosomes that were subject to this validation study, 5 QTL regions located on 5 chromosomes altogether were confirmed. They included 3 QTL associated with LPS NA, located on GGA9, GGA18, and GGAZ, and 2 QTL associated with LTA NA, located on GGA5 and GGA14. Interestingly, 2 QTL associated with LPS NA on GGA5 and GGAZ were also confirmed in an across-line SNP association study by Biscarini et al. (2010), who recently reported an association study between single nucleotide mutations in immune-related chromosomal regions and different parameters of innate immunity. The QTL responsible for LPS and LTA NA titers that have been validated in this study contain a set of candidate genes involved in biochemical pathways of humoral innate immunity that has been described elsewhere (Sławińska et al., 2011).

QTL Associated with Health Traits in Chickens

Some of the QTL responsible for LPS and LTA NA titers mapped in our study overlapped with known QTL associated with health traits in chickens. The QTL linked to LPS and LTA NA on GGA5 (CI: 73–127 cM for LPS QTL and 79–127 cM for LTA QTL) overlapped with the QTL responsible for antibody response to SRBC antigen at 68 cM (Siwek et al., 2003a), cloacal bacterial burden after challenge with *Salmonella* Enteritidis at 111 cM (Tilquin et al., 2005), cloacal bacterial burden after challenge with *Salmonella* Typhimurium at 107 cM (Tilquin et al., 2005), time to achieve maximum antibody response to SRBC between 98 and 110 cM (Zhou et al., 2003), and Marek's disease-related

traits at127 cM (Heifetz et al., 2007). On GGA9, within the CI of the LPS NA QTL validated in our study (0–41 cM), a QTL associated with Marek's disease-related traits was mapped (36.3–42.4 cM; Yonash et al., 1999). Chromosomal location of the QTL associated with LPS NA on GGA14 (0–77 cM) overlapped with several QTL associated with LTA NA (Siwek et al., 2006) and KLH specific antibodies (Siwek et al., 2003b, 2010). On GGA18, a QTL associated with Marek's disease-related traits (23 cM; Heifetz et al., 2007) was located in close proximity to the validated LPS NA QTL (24 cM). Finally, within the CI of the LPS and LTA NA QTL on GGAZ (20–105 cM) an overlap was observed with the QTL associated with antibody response to *Brucella abortus* (86.8–93 cM; Zhou et al., 2003) and Marek's disease-related traits (0–51.6 cM; Heifetz et al., 2007). Most of the QTL associated with chicken health traits that overlapped the chromosomal location of the QTL reported here were responsible for the adaptive immune response. Evidently, a functional correlation between innate and adaptive immune response exists. However, because of the large CI of most of the QTL analyzed, the regions of interest contain many coding and regulatory sequences that affect the given phenotype. Therefore, it is difficult to infer whether the genetic background of those traits is related.

Conclusions

In summary, the QTL study of humoral innate immune response traits carried out in a reference population consisting of GP and WL chicken lines resulted in validation of 5 QTL (located on GGA5, GGA9, GGA14, GGA18, and GGAZ) associated with NA titers binding LPS and LTA environmental antigens. For the validated LPS and LTA QTL the panel of candidate genes was previously proposed. The validated QTL are the best candidate regions for further association study of the SNP located within those candidate genes and the NA titers in chickens.

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REFERENCES

Aerts, J., H. J. Megens, T. Veenendaal, I. Ovcharenko, R. Crooijmans, L. Gordon, L. Stubbs, and M. Groenen. 2007. Extent of linkage disequilibrium in chicken. Cytogenet. Genome Res. 117:338–345.

Baumgarth, N., J. W. Tung, and L. A. Herzenberg. 2005. Inherent specificities in natural antibodies: A key to immune defense against pathogen invasion. Springer Semin. Immunopathol. 26:347–362.

Bennett, B., P. Carosone-Link, N. R. Zahniser, and T. E. Johnson. 2006. Confirmation and fine mapping of ethanol sensitivity quantitative trait loci, and candidate gene testing in the LXS recombinant inbred mice. J. Pharmacol. Exp. Ther. 319:299–307.

Bennewitz, J., N. Reinsch, C. Grohs, H. Levéziel, A. Malafosse, H. Thomsen, N. Xu, C. Looft, C. Kühn, G. A. Brockmann, M. Schwerin, C. Weimann, S. Hiendleder, G. Erhardt, I. Medjugorac, I. Russ, M. Förster, B. Brenig, F. Reinhardt, R. Reents, G. Averdunk, J. Blümel, D. Boichard, and E. Kalm. 2003. Combined analysis of data from two granddaughter designs: A simple strategy for QTL confirmation and increasing experimental power in dairy cattle. Genet. Sel. Evol. 35:319–338.

Biscarini, F., H. Bovenhuis, J. A. M. van Arendonk, H. K. Parmentier, A. P. Jungerius, and J. J. van der Poel. 2010. Across-line SNP association study of innate and adaptive immune response in laying hens. Anim. Genet. 41:26–38.

Bos, N. A., C. G. Meeuwsen, B. S. Wostmann, J. R. Pleasants, and R. Benner. 1988. The influence of exogenous antigenic stimulation on the specificity repertoire of background immunoglobulin-secreting cells of different isotypes. Cell. Immunol. 112:371–380.

Botstein, D., R. L. White, M. Skolnick, and R. W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314–331.

Boyle, A. E., and K. J. Gill. 2008. Confirmation of provisional quantitative trait loci for voluntary alcohol consumption: Genetic analysis in chromosome substitution strains and F2 crosses derived from A/J and C57BL/6J progenitors. Pharmacogenet. Genomics 18:1071–1082.

Casali, P., and E. W. Schettino. 1996. Structure and function of natural antibodies. Curr. Top. Microbiol. Immunol. 210:167–179.

Cheung, C. L., Q. Y. Huang, M. Y. Ng, V. Chan, P. C. Sham, and A. W. Kung. 2006. Confirmation of linkage to chromosome 1q for spine bone mineral density in southern Chinese. Hum. Genet. 120:354–359.

Cywa-Benko, K. 2002. Charakterystyka genetyczna i fenotypowa rodzimych rodow kur objętych programem ochrony biorożnorodności. Rocz. Nauk. Zootech. Rozprawy Habilitacyjne:15.

Fehr, C., R. L. Shirley, J. C. Crabbe, J. K. Belknap, K. J. Buck, and T. J. Phillips. 2005. The syntaxin binding protein 1 gene (*Stxbp1*) is a candidate for an ethanol preference drinking locus on mouse chromosome 2. Alcohol. Clin. Exp. Res. 29:708–720.

Haley, C. S., and S. A. Knott. 1992. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity 69:315–324.

Hayes, B. J., B. P. Kinghorn, and A. Ruvinsky. 2005. Genome scanning for quantitative trait loci. Pages 508–530 in Mammalian Genomics. A. Ruvinsky and J. A. Marshall Graves, ed. CABI, Wallingford, UK.

Heifetz, E. M., J. E. Fulton, N. P. O'Sullivan, J. A. Arthur, J. Wang, J. C. Dekkers, and M. Soller. 2007. Mapping quantitative trait loci affecting susceptibility to Marek's disease virus in a backcross population of layer chickens. Genetics 177:2417–2431.

Hernández-Sánchez, J., J. A. Grunchec, and S. Knott. 2009. A web application to perform linkage disequilibrium and linkage analyses on a computational grid. Bioinformatics 25:1377–1383.

Hoopes, R. R. Jr., F. A. Middleton, S. Sen, P. A. Hueber, R. Reid, D. A. Bushinsky, and S. J. Scheinman. 2006. Isolation and confirmation of a calcium excretion quantitative trait locus on chromosome 1 in genetic hypercalciuric stone-forming congenic rats. J. Am. Soc. Nephrol. 17:1292–1304.

Houston, R. D., A. Gheyas, A. Hamilton, D. R. Guy, A. E. Tinch, J. B. Taggart, B. J. McAndrew, C. S. Haley, and S. C. Bishop. 2008. Detection and confirmation of a major QTL affecting resistance to infectious pancreatic necrosis (IPN) in Atlantic salmon (*Salmo salar*). Dev. Biol. (Basel) 132:199–204.

Hu, Z. L., E. R. Fritz, and J. M. Reecy. 2007. AnimalQTLdb: A livestock QTL database tool set for positional QTL information mining and beyond. Nucleic Acids Res. 35:D604–D609.

Jennen, D. G., A. L. Vereijken, H. Bovenhuis, R. M. Crooijmans, J. J. van der Poel, and M. A. Groenen. 2005. Confirmation of quantitative trait loci affecting fatness in chickens. Genet. Sel. Evol. 37:215–228.

Jiang, Z., S. De, M. D. Garcia, K. B. Griffin, X. L. Wu, Q. Xiao, J. J. Michal, B. S. Sharma, and G. B. Jansen. 2005. An independent confirmation of a quantitative trait locus for milk yield and

composition traits on bovine chromosome 26. J. Anim. Breed. Genet. 122:281–284.

King, A. H., Z. Jiang, J. P. Gibson, C. S. Haley, and A. L. Archibald. 2003. Mapping quantitative trait loci affecting female reproductive traits on porcine chromosome 8. Biol. Reprod. 68:2172–2179.

Knott, S. A., J. M. Elsen, and C. S. Haley. 1996. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. Theor. Appl. Genet. 93:71–80.

Kumar, K. G., A. C. Poole, B. York, J. Volaufova, A. Zuberi, and B. K. Richards. 2007. Quantitative trait loci for carbohydrate and total energy intake on mouse chromosome 17: Congenic strain confirmation and candidate gene analyses (*Glo1, Glp1r*). Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R207–R216.

Liu, Y. Z., F. H. Xu, H. Shen, H. Deng, Y. J. Liu, L. J. Zhao, V. Dvornyk, T. Conway, J. L. Li, Q. Y. Huang, K. M. Davies, R. R. Recker, and H. W. Deng. 2003. Confirmation linkage study in support of the X chromosome harboring a QTL underlying human height variation. J. Med. Genet. 40:825–831.

Merilä, J., and B. C. Sheldon. 1999. Genetic architecture of fitness and nonfitness traits: Empirical patterns and development of ideas. Heredity 83:103–109.

Meuwissen, T. H. E., A. Karlsen, S. Lien, I. Olsaker, and M. E. Goddard. 2002. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics 161:373–379.

Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:1215.

Minozzi, G., H. K. Parmentier, S. Mignon-Grasteau, M. G. B. Nieuwland, B. Bed'hom, D. Gourichon, F. Minvielle, and M.-H. Pinard-van der Laan. 2008. Correlated effects of selection for immunity in White Leghorn chicken lines on natural antibodies and specific antibody responses to KLH and *M. butyricum*. BCM Genet. 9:5.

Ochsenbein, A. F., T. Fehr, C. Lutz, M. Suter, F. Brombacher, H. Hengartner, and R. M. Zinkernagel. 1999. Control of early viral and bacterial distribution and disease by natural antibodies. Science 286:2156–2159.

Park, S. D. E. 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. PhD Diss. University of Dublin. UK.

Parmentier, H. K., W. J. A. Van Den Kieboom, M. G. B. Nieuwland, G. De Vries Reilingh, B. N. Hangalapura, H. F. J. Savelkoul, and A. Lammers. 2004. Differential effects of lipopolysaccharide and lipoteichoic acid on the primary antibody response to keyhole limpet hemocyanin of chickens selected for high or low antibody responses to sheep red blood cells. Poult. Sci. 83:1133–1139.

Radcliffe, R. A., P. Bludeau, X. S. Deng, V. G. Erwin, and R. A. Deitrich. 2007. Short-term selection for acute ethanol tolerance and sensitization from an F2 population derived from the high and low alcohol-sensitive selectively bred rat lines. Alcohol 41:557–566.

Seaton, G., J. Hernandez, J. A. Grunchec, I. White, J. Allen, D. J. De Koning, W. Wei, D. Berry, C. Haley, and S. Knott. 2006. GridQTL: A Grid Portal for QTL Mapping of Compute Intensive Datasets. Proc. 8th World Congress on Genetics Applied to Livestock Production, Belo Horizonte, Brazil.

Siwek, M., A. J. Buitenhuis, S. J. Cornelissen, M. G. Nieuwland, H. Bovenhuis, R. P. Crooijmans, M. A. Groenen, G. de Vries-Reilingh, H. K. Parmentier, and J. J. van der Poel. 2003b. Detection of different quantitative trait loci for antibody responses to keyhole lympet hemocyanin and *Mycobacterium butyricum* in two unrelated populations of laying hens. Poult. Sci. 82:1845–1852.

Siwek, M., B. Buitenhuis, S. Cornelissen, M. Nieuwland, E. F. Knol, R. Crooijmans, M. Groenen, H. Parmentier, and J. van der Poel. 2006. Detection of QTL for innate: Non-specific antibody levels binding LPS and LTA in two independent populations of laying hens. Dev. Comp. Immunol. 30:659–666.

Siwek, M., S. J. Cornelissen, M. G. Nieuwland, A. J. Buitenhuis, H. Bovenhuis, R. P. Crooijmans, M. A. Groenen, G. de Vries-Reilingh, H. K. Parmentier, and J. J. van der Poel. 2003a. Detection of QTL for immune response to sheep red blood cells in laying hens. Anim. Genet. 34:422–428.

Siwek, M., A. Sławińska, M. Nieuwland, A. Witkowski, G. Zięba, G. Minozzi, E. F. Knol, and M. Bednarczyk. 2010. A quantitative trait locus for a primary antibody response to keyhole limpet hemocyanin on chicken chromosome 14—Confirmation and candidate gene approach. Poult. Sci. 89:1850–1857.

Sławińska, A., M. Siwek, E. F. Knol, D. T. Roelofs-Prins, H. J. van Wijk, B. Dibbits, and M. Bednarczyk. 2009. Validation of the QTL on SSC4 for meat and carcass quality traits in a commercial crossbred pig population. J. Anim. Breed. Genet. 126:43–51.

Sławińska, A., A. Witkowski, M. Bednarczyk, and M. Siwek. 2011. In silico analysis of candidate genes associated with humoral innate immune response in chicken. BCM Proceed. 5(Suppl. 4):S36.

Tilquin, P., P. A. Barrow, J. Marly, F. Pitel, F. Plisson-Petit, P. Velge, A. Vignal, P. V. Baret, N. Bumstead, and C. Beaumont. 2005. A genome scan for quantitative trait loci affecting the *Salmonella* carrier-state in the chicken. Genet. Sel. Evol. 37:539–561.

Tzenova, J., B. J. Kaplan, T. L. Petryshen, and L. L. Field. 2004. Confirmation of a dyslexia susceptibility locus on chromosome 1p34-p36 in a set of 100 Canadian families. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 127:117–124.

van Arendonk, J. A. M., H. Bovenhuis, S. van der Beek, and A. F. Groen. 1994. Detection and exploitation of markers linked to quantitative traits in farm animals. Pages 193–200 in Proc. 5th World Congress on Genetics Applied to Livestock Production. C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, B. W. Kennedy, and E. B. Burnside, ed. University of Guelph, Guelph, ON, Canada.

Wattrang, E., M. Almqvist, A. Johansson, C. Fossum, P. Wallgren, G. Pielberg, L. Andersson, and I. Edfors-Lilja. 2005. Confirmation of QTL on porcine chromosomes 1 and 8 influencing leukocyte numbers, haematological parameters and leukocyte function. Anim. Genet. 36:337–345.

Weller, J. I. 2001. Experimental designs to detect QTL, generation of linkage disequilibrium. Pages 48–73 in Quantitative Trait Loci Analysis in Animals. J. I. Weller, ed. CABI, Wallingford, UK.

Wijga, S., H. K. Parmentier, M. G. Nieuwland, and H. Bovenhuis. 2009. Genetic parameters for levels of natural antibodies in chicken lines divergently selected for specific antibody response. Poult. Sci. 88:1805–1810.

Witkowski, A., K. Kasperczyk, A. Sławińska, and M. Bednarczyk. 2009. The breed of the month: Green-legged Partridgelike. An old, native, Polish hen. GlobalDiv Newsl. 9:12–15.

Wu, X. L., and J. L. Jannink. 2004. Optimal sampling of a population to determine QTL location, variance, and allelic number. Theor. Appl. Genet. 108:1434–1442.

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.

Zhou, H., H. Li, and S. J. Lamont. 2003. Genetic markers associated with antibody response kinetics in adult chickens. Poult. Sci. 82:699–708.

Zhou, Z.-H., Y. Zhang, Y.-F. Hu, L. M. Wahl, J. O. Cisar, and A. L. Notkins. 2007. The broad antibacterial activity of the natural antibody repertoire is due to polyreactive antibodies. Cell Host Microbe 1:51–61.