Journal of Animal and Veterinary Advances 11 (19): 3481-3485, 2012
ISSN: 1680-5593

Associations between Immune Traits and *MHC B-F* Gene in Shandong Indigenous Chickens

[1,2]F.W. Li, [1,2]Y. Lu, [1,2]Q.X. Lei, [1,2]Y. Zhou, [1,2]H.X. Han, [1,2]G.M. Li,
[1,2]B. Wu, [1,2]D.G. Cao and [3]S.B. Wang
[1]Academy of Agricultural Sciences of Shandong Province, Institute of Poultry Science, Jinan, China
[2]Poultry Breeding Engineering Technology Center of Shandong Province, Jinan, China
[3]Bond Breeding Co., Ltd. Shandong, Jinan, China

Abstract: In order to find the relationship between immune traits and the Major Histocompatibility Complex B-F (*MHC B-F*) gene, an immune traits model was established in Wenshang Barred Chicken (LH), Laiwu Black Chicken (LWH) and Jining Bairi Chicken (BR). PCR-SSCP and sequencing methods were used to identified haplotypes in these three Shandong indigenous chickens. As a result, 53 (LH), 52 (LWH) and 54 (BR) Single Nucleotide Polymorphisms (SNPs) were found in the 264 bp of exon 2 in chicken *MHC B-F* gene. The least square analysis showed that 2, 2, 3 SNPs were respectively found significant associations with antibody responses to H5, H9 and ND in LH chickens; 1, 3 and 3 SNPs were respectively found significant associations with antibody responses to H5, H9 and ND in LWH chickens but none with SRBC. In BR chickens, there was association with responses to H5 (2 SNPs), H9 (3 SNPs), ND (3 SNPs) and SRBC (3 SNPs). These results indicate that the genomic region bearing exon 2 of the *MHC B-F* gene has significant effects on antibody responses to SRBC and vaccination against AI and ND.

Key words: Chicken, MHC B-F, PCR-SSCP, immune traits, vaccination

INTRODUCTION

The chicken Major Histocompatibility Complex (MHC) is located on chromosome 16, it plays a key role in disease resistance. The crucial character of the MHC in polymorphism, linkage disequilibrium and haplotypes heredity therefore makes this a promising candidate region for research. The chicken Major Histocompatibility Complex (MHC) is composed by a group of three classes by alloantisera: B-F (Class I), B-L (Class II) and B-G antigen (Kaufman and Lamont, 1996). MHC encoded cell surface glycoprotein antigen has an extremely important role in transplant rejection in the body, antigen presentation, immune response and regulation. Class I antigen (encoded by the *BF* gene) consists of three parts including the α chain, β2-microglobulin and antigenic peptides. The α chain protein are composed of the signal region, extracellular domains (α_1-α_3), transmembrane region, cytoplasmic tail and a peptide-binding cleft formed by folded α_1 and α_2 domains (Kaufman and Lamont, 1996).

Associations between the chicken MHC and immune traits have been reported, particularly antibody production against a variety of antigens such as: *Brucella abortus*, Newcastle Disease Virus (NDV) and Sheep Red Blood Cells (SRBC) (Dunnington *et al*., 1992) and antibody titer to infectious bursal disease virus (Ewald *et al*., 2007). Liu *et al*. (2008) demonstrated that the Cytotoxic T Lymphayte (CTL) response of chickens exposed to infectious bronchitis virus could be evaluated effectively using the prepared MHC-I B-F2*15/peptide tetramer. Qiu (2007) studied the *MHC B-F* gene exons 2 and 3 sequences in two chicken populations which showed a high degree of polymorphism and sequence polymorphism in the amino acid level performance. CAV Lima-Rosa *et al*. (2004) studied 100 local varieties of Brazilian chicken of MHC B-F area the Exon 2 2-4 (a total of 1048 bp), the results obtained 10 mutations in the 23 sequences and compared to the protein sequence with earlier reported, there are many variations sites.

In this study, LH, BR and LWH are famous Shandong indigenous chicken with adaptability, strong disease resistance which might be used as markers for improved immune function and aid in illustrating promiscuous peptide binding of MHC Class I. The purpose of this study is to find molecular markers for immune traits by genetic variation analysis of chicken MHC B-F exon 2 sequences and provide theoretical support for the breeding for disease resistance.

Corresponding Author: D.G. Cao, Poultry Breeding Engineering Technology Center of Shandong Province, Jinan, China

MATERIALS AND METHODS

Experimental animals and vaccination procedure: Chickens (LH, LWH, and BR, 100 of each breed) were from stocks maintained at the Institute of Poultry Science, at the Chinese Academy of Agricultural Sciences of Shandong province. All birds were hatched on the same day, housed on cages. Birds had access to feed (commercial corn-soybean diets meeting the National Research Council's (NRC) requirements) and water *ad libitum*. Peripheral blood was collected from all the chickens on days 134. The sample was collected using 0.5% Ethylene Diamine Tetraacetic Acid (EDTA) as anticoagulant (for DNA isolation) and the remainder was allowed to clot. Serum was obtained from all samples after centrifuging at 3000×g for 5 min. The standard vaccination protocol for all birds is shown in Table 1. All birds were challenged with SRBC at days 128.

Immunological assays: Serum titers of antibodies against AI and Newcastle Disease (ND) viruses were determined by inhibition of agglutination. Serial dilutions (1:2-1:2048) of serum were made in 96 well, V-bottom microtiter plates containing 50 mL of Phosphate Buffered Saline (PBS) in all wells. Antigen (50 μL of NDV or Avian Influenza Virus (AIV), four hemagglutination units, Harbin Veterinary Research Institute, CAAS, China) was added to all of the wells except the last row which served as controls. The Ag-serum mixture was pre-incubated for 10 min at 37°C then a suspension of 1% Specific Pathogen Free (SPF) rooster erythrocytes (50 μL) was added and incubated for 30 min. Appropriate controls were included. The highest dilution of serum (expressed as reciprocal log2 values) causing complete inhibition was considered to be the titer.

Antibody response to challenge with Sheep Red Blood Cells (SRBC): The birds were injected with 1 mL of 25% SRBC diluted in PBS at 128 days of age, the blood sampling on days 134. The SRBC antibody titers were determined by hemagglutination method described by Hudson and Hay (1976).

Genomic sequencing of the Major Histocompatibility Complex (MHC) B-F exon 2: Genomic DNA was extracted from day 134 erythrocytes using the standard procedure (Sambrook *et al*., 1989). All primers used for amplification of exon 2 (encoding α_1 domains) of *B-F* genes were designed using the Oligo 6.0 program and were based on the chicken B-F sequence (GenBank Accession No. M31012), they were obtained from Li Ge Co. Ltd, Jinan. The expected size of the product including the entire exon 2 was 396 bp. The upstream primer (5'-CCC GCCCGTAACC CCA CCC-3') was taken from intron 1 and the downstream primer (5'-AGCCCATCCCACACCCACGG-3') was from intron 2. The PCR reaction was performed in a final volume of 10 μL containing 0.8 μL of genomic DNA (2.5 ng μL^{-1}), 0.2 μL of each primer (20 pmol μL^{-1}), 3.8 μL ddH_2O, 5 μL of 2×MasterMix (Tiangen, Beijing, China). The following PCR cycle condition was used: an initial denaturation at 94°C for 3 min; 30 cycles of 94°C for 45 sec, 70.5°C for 45 sec and 72°C for 45 sec and a final elongation at 72°C for 6 min. The quantity and integrity of the products were examined after electrophoresis on 1% agarose gel and staining with 0.25 μL mL^{-1} ethidium bromide.

Polymerase Chain Reaction Single Strand Confirmation Polymorphism (PCR-SSCP) analysis: About 3 μL of PCR reaction products were denatured at 98°C for 8 min and immediately chilled on ice to prevent reannealing. Denatured PCR products were subject to SSCP analysis in 10% polyacrylamide gels at 150 V and 4°C for 16 h in 0.5×TBE buffer (Oto *et al*., 1993). The bands were visualized after staining with silver.

Major Histocompatibility Complex (MHC) B-F exon 2 sequencing: Exon 2 sequencing was performed in both directions with the upstream and downstream primers described. After eliminating flanking intronic portions to obtain just exon 2, sequences were compared using the DNASRAT6.0 Software package.

Table 1: Immunization program of the experimental chickens

Age/days	Vaccine	Remarks	Vaccination route
1	Marek's disease vaccine	Marek's disease cryophilic vaccine (CV1988/Rispens)	Subcutaneous injection
7	Newcastle disease (Lasota strain)	Emulsified inactivated vaccine	Subcutaneous injection
	Infectious bronchitis (H120 strain) virus vaccine	Live freeze-dried vaccine	Eye drop and nose drop
14	Infectious bursal disease	Live freeze-dried vaccine	Drinking water
28	Newcastle Disease (ND) virus oil adjuvant vaccine bronchitis (H120 strain) virus vaccine	Live freeze-dried vaccine	Eye drop and nose drop
	avian influenza H5 and H9(AI)	Recombinant emulsified inactivated vaccine (H5N1 and H9N2 subtype)	Subcutaneous injection
63	Infectious bursal disease	Live freeze-dried vaccine	Drinking water
81	Newcastle Disease (ND)	Emulsified inactivated vaccine	Intramuscular injection
93	Avian Influenza H5 and H9(AI)	Emulsified recombinant in activated vaccine	Intramuscular injection
132	Newcastle Disease (ND) and infectious bronchitis (H120 strain) virus vaccine and egg drop syndrome	Emulsified inactivated vaccine	Intramuscular injection

Statistical analyses: All the antibody titers of SRBC, AI and ND were presented as log transformed value and the arithmetic means of these log values were calculated. The difference of antibody titer between breeds and the association between SNP alleles and overall immune traits were analyzed with GLM procedures of SAS (SAS Inst. Inc., Cary NC). The arithmetic mean of AI and ND titer across all sampling days (134) was calculated for each bird and used in the analysis of association.

RESULTS

Comparison of Avian Influenza (AI), Newcastle Disease (ND) and Sheep Red Blood Cells (SRBC) titers among the three breeds: The SRBC, ND, H5 and H9 antibody titers of the experimental populations were shown in Table 2. The antibody titer of SRBC, ND, H5 and H9 in LWH were highest and significant difference with the other two populations ($p<0.01$). Antibody titer of ND were significant difference between the LH chickens and BR chickens ($p<0.01$) no significant difference were found in the other three antibody titers between them.

Comparison between breeds of DNA and protein consensus sequences: Using 100 chickens from each breed, Different bands revealed by PCR-SSCP of the MHC B-F exon 2 indicated the minimal number of allelic forms of this region of the gene and each was identified as being a distinct haplotype. PCR products were sequenced in the Li Ge Co., Ltd. Jinan, 65 SNPs were found in three breeds. The 39 SNPs in all three breeds changes as detailed in Table 3. There were, respectively 53, 52 and 54 nucleotide changes in LH, LWH and BR Chicken, of the total 65 amino acid changes in MHC B-F exon 2 of both breeds, 42 in LH breed, 41 in LWH and 45 in BR.

All the SNPs were analyzed for association with immune traits in the three breeds and the SNPs which had significant effects on immune traits were shown in Table 4 ($p<0.05$). Of 65 SNPs in three breeds, one SNP at loci 166 was associated with SRBC titers in BR ($p<0.05$) five SNPs at loci 69, 99, 144, 149 and 226 were associated with H5 titers ($p<0.05$), eight SNPs at loci 65, 129, 159, 166, 180, 204, 220 and 232 were associated with H9 titers ($p<0.05$) eight SNPs at loci 48, 69, 127, 149, 192, 196, 220 and232 were associated with ND titers ($p<0.05$).

Table 2: Antibody titer of SRBC, ND, H5 and H9 in three breeds

	Antibody titers (Mean)			
Breed	SRBC	ND	H5	H9
LH	6.94 ± 0.15^{B}	10.27 ± 0.175^{A}	5.58 ± 0.24^{B}	5.32 ± 0.23^{B}
BR	7.27 ± 0.15^{B}	9.08 ± 0.175^{B}	6.19 ± 0.24^{B}	5.48 ± 0.23^{B}
LWH	8.00 ± 0.16^{A}	10.38 ± 0.179^{A}	7.60 ± 0.25^{A}	6.89 ± 0.24^{A}

Means (±SD) are each based upon 100 individuals. Within a column, values followed by different uppercase letters are significantly different ($p<0.01$)

Table 3: SNPs in MHC B-F exon 2 and amino acid changes in three indigenous chicken populations

Location and SNP	AA and change	Breed
4 T/C	Leu/Pro	BR
5 C/A	Leu/-	BR/LH/LWH
7 A/T	His/Leu	BR/LH/LWH
12 C/T	Leu/-	BR/LH/LWH
23 C/T	Ile/-	BR/LH/LWH
24 C/T	Gln/Tyr	BR/LH/LWH
25 A/C/G	Gln/Pro/Arg	LH
26 A/T	Gln/His	BR/LH/LWH
28 C/T	Thr/Met	BR/LH/LWH
31 C/G	Ala/Gly	BR/LH/LWH
42 C/T	Pro/Ser	BR/LH/LWH
48 C/T	Pro/Ser	LH/BR
55 A/T/G	Gln/Leu/Arg	BR/LH/LWH
57 C/T	Pro/Ser	LH/BR/LWH
64 T/A	Phe/Tyr	BR/LH/LWH
65 C/G	Phe/Leu	BR/LH/LWH
69 A/G	Thr/Ala	BR/LH/LWH
70 C/T/A	Thr/Ile/Asn	LH/BR/LWH
71 T/C	Thr/-	LH/BR/LWH
72 G/A	Val/Met	LH/BR/LWH
75 G/C	Gly/Arg	LH/BR/LWH
77 G/A	Gly/-	BR/LH
80 C/T	Tyr/-	BR/LH/LWH
95 C/T	Leu/-	LH/LWH
99 G/A	Val/Met	BR/LH/LWH
119 G/T	Ala/-	BR/LWH
126 T/G/A	Tyr/Asp/Asn	BR/LH/LWH
127 A/T/C	Tyr/Phe/Ser	BR/LH/LWH
128 C/T	Tyr/-	BR/LH/LWH
129 G/T	Val/Leu	LH/BR/LWH
135 C/T	Arg/Cys	BR/LWH
141 G/T	Glu/Tyr	LH/BR/LWH
143 G/C	Glu/Tyr	BR/LWH
144 T/C	Trp/Arg	LH/BR/LWH
149 A/G	Ile/Met	BR/LH/LWH
158 G/C	Lys/Asn	BR/LH/LWH
159 G/A	Ala/Thr	BR/LWH
166 A/G	Gln/Arg	BR
179 T/C/A	Asp/-/Glu	BR/LWH
180 G/A	Gly/Arg	BR/LH/LWH
183 C/G	Gln/Glu	LH
192 A/C	Ile/Leu	LH/BR
193 T/C	Ile/Thr	LH
194 C/G	Ile/Met	BR/LH/LWH
195 G/A	Gly/Arg	BR/LH/LWH
196 G/T/C	Gly/Val/Ala	BR/LH/LWH
197 A/C	Gly/-	LH/LWH
200 G/T	Gln/His	BR/LWH
201 G/C	Gly/Arg	BR/LH/LWH
204 A/C	Asn/His	BR
211 A/G	Gln/Arg	BR
216 G/A	Asp/Asn	LH/LWH
218 C/G	Asp/Glu	BR/LH/LWH
219 C/A	Arg/Ser	LWH
220 G/A	Arg/His	BR/LH/LWH
221 C/A	Arg/-	LH/LWH
224 G/T	Glu/Asp	BR/LH
225 A/G	Asn/Asp	BR/LH
226 A/G	Asn/Ser	LH
232 G/A	Gly/Asp	BR/LH/LWH
235 T/C	Ile/Thr	BR/LH/LWH
243 C/G/A	Arg/Gly/Arg	LH/LWH
244 G/A	Arg/Gln	LWH
248 C/A/G	Arg/-	BR/LH/LWH
256 A/G	Gln/Arg	BR/LH/LWH

Table 4: Effects of SNPs on the immune traits in three indigenous chicken populations

	Immune trait Antibody titer variation sites (p<0.05)			
Breed	ND	H5	H9	SRBC
LH	48,192,196	99,226	180,232	166
BR	69,220,232	144,149	159,166,204	-
LWH	127,149,220	69	65,129,220	-

DISCUSSION

Comparison of immune function in three breeds: SRBC is a polyvalent non-pathogenic antigen which used to stimulate a humoral immune response which in birds is considered to reflect a generalized ability to produce antibodies (Gross *et al*., 1980). The Parmentier confirmed SRBC antibody titers family resistance to the disease was higher than the low antibody titers family. The mean of SRBC antibody titer in three Shandong indigenous chicken ranged from 6.94-8.00, the results are similar to those of Wu which reported that the mean of Beijing oil chicken and leghorn hens SRBC antibody titer ranged from 6.9-9.1 on 6 days after immunization. In the three breeds, the Laiwu Black chicken SRBC antibody titer was the highest and the ND, H5 and H9 antibody titer were also. Which was same to Parmentier's results But ND antibody titer was inconsistent with the SRBC antibody titers. The ND antibody titer of LH was higher than the BR's ($p<0.05$) the results showed that the response was different on different antigens. Dunnington *et al.* (1989) found that compared to strains with low antibody, the strain with high SRBC antibody levels had higher resistance for infectious diseases (such as Marek, Newcastle, etc.). However, not all diseases have the same performance (such as *E. coli*). Amponsem found that the injection site and dose affect the level of SRBC antibody titers.

Association between immune traits and Single Nucleotide Polymorphisms (SNPs) on exon 2 of the Major Histocompatibility Complex (MHC) *B-F* gene: In the current study, immune performance and polymorphisms in the MHC Class I α_1 domain were compared in three Shandong Indigenous breeds. The results indicated that total 65 SNPs were found and 39 out of 55 sites occurred in all the three breeds. Liu *et al.* (2009) detected the SNPs in exon 2 of *MHC B-F* gene in the 250 Beijing oil chicken and 250 Leghorn leghorn hens, 47 SNPs were found in Beijing oil chickens of MHC B-F exon 2 of 264 bp sequence 43 SNPs were found in Leghorn hens. The results were consistent with Liu's findings and proved that *MHC B-F* gene was rich in polymorphisms.

The correlation analysis between SRBC antibody titers and specific immune antibody (ND, H5 and H9) showed that there were multiple SNPs significantly associated with the H5, H9, ND and SRBC antibody titers. Site 166 in BR were significantly associated with SRBC titers ($p<0.05$) and the site was also associated with H9 antibody titer ($p<0.05$). Site 220 were significantly associated with ND titer in LWH and BR ($p<0.05$) and the site also was significantly associated with H9 antibody titers in LWH chicken ($p<0.05$). Liu found one SNP position at 127 A/T in exon 2 of MHC B-F Which was significantly associated with Salmonella antibody response ($p<0.05$). In the study, the locus 127 was also found in LWH chicken and was significantly associated ND antibody titers ($p<0.05$). Wu (2007) found that multiple SNPs loci of *MHC B-F* gene exon 2 were significantly correlated with AI, ND antibody titers in Beijing oil chickens and leghorn hens. Zhou and Lamont (2003) found that there were molecular markers significantly associated with *Salmonella enteritidis* antibody response in exon 2 and 3 of chicken *MHC B-F* gene.

CONCLUSION

This study has clearly shown that genomic variation in exon 2 of the *MHC B-F* gene in chickens has significant effects on antibody responses to vaccination against SRBC, AIV and NDV. Total 65 mutations 53 (LH) 52 (LWH) and 54 (BR) were detected in the 264 bp of exon 2 in three Shandong Indigenous breeds and 39 out of 65 mutations occurred in all the three breeds. Mutation rate reached 0.2462. Multiple SNPs were significantly correlated with SRBC, ND, H5 and H9 antibody titers in three Shandong Indigenous chicken breeds, 53 (LH) 52 (LWH) and 54 (BR) single nucleotide polymorphisms (SNPs) were found in the 264 bp of exon 2 in chicken *MHC B-F* gene. The 2, 2, 3 SNPs were respectively found significant associations with antibody responses to H5, H9 and ND in LH chickens; 1, 3 and 3 SNPs were respectively found significant associations with antibody responses to H5, H9 and ND in LWH chickens but none with SRBC. In BR chickens, there was association with responses to H5 (2 SNPs), H9 (3 SNPs), ND (3 SNPs) and SRBC (1 SNPs).

ACKNOWLEDGEMENTS

The current research was supported by the fund from: Chinese Shandong Province Nature Fund (ZR2009DM004) and Chinese Shandong province high-tech sterile seeds of the seed project topic (2008LZ015).

REFERENCES

Dunnington, E.A., A. Martin, R.W. Briles, W.E. Briles, W.B. Gross and P.B. Siegel, 1989. Antibody responses to sheep erythrocytes for White Leghorn chickens differing in haplotypes of the major histocompatibility complex (B). Anim. Genet., 20: 213-216.

Dunnington, E.A., C.T. Larsen, W.B. Gross and P.B. Siegel, 1992. Antibody responses to combinations of antigens in white Leghorn chickens of different background genomes and major histocompatibility complex genotypes. Poult. Sci., 71: 1801-1806.

Ewald, S.J., X. Ye, S. Avendano, S. McLeod, S.J. Lamont and J.C. Dekkers, 2007. Associations of BF2 alleles with antibody titres and production traits in commercial pure line broiler chickens. Anim. Genet., 38: 174-176.

Gross, W.B., P.B. Siegel, R.W. Hall, C.H. Domermuth and R.T. DuBoise, 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. 2. Resistance to infectious diseases. Poult. Sci., 59: 205-210.

Hudson, L. and F.C. Hay, 1976. Haemagglutination in Practical Immunology. Blackwell Scientific, London, pp: 125-130.

Kaufman, J. and S. Lamont, 1996. The Chicken Major Histocompatability Complex. In: The Major Histocompatibility Region in Domestic Animal Species, Schook, L.B. and S.J. Lamont (Eds.). CRC Press, Boca Raton FL., pp: 35-64.

Lima-Rosa, C.A.V., C.W. Canal, A.F. Streck, L.B. Freitas, A. Delgado-Canedo, S.L. Bonatto and S. Salzano, 2004. B-F DNA sequence variability in Brazilian (blue egg Caipira) chicken. Anim. Genet., 35: 278-284.

Liu, G., Q. Wang, T. Tong, Y. Xiao, Y. Bai, S. Liu and D. Wu, 2008. Construction and functional test of a chicken MHC-I (BF2*15)/peptide tetramer. Vet. Immunol. Immunopathol., 122: 1-7.

Liu, L.B., C.M. Wu, J. Wen, J.L. Chen, M.Q. Zheng and G.P. Zhao, 2009. Association of SNPs in exon 2 of the MHC B-F gene with immune traits in two distinct chicken populations: Chinese Beijing-You and White Leghorn. Acta Agric. Scand. Anim. Sci., 59: 4-11.

Oto, M., S. Miyake and Y. Yuasa, 1993. Optimization of nonradioisotopic single strand conformation polymorphism analysis with a conventional minislab gel electrophoresis apparatus. Anal. Biochem., 213: 19-22.

Qiu, M.H., 2007. The chicken MHC the BF gene exons 2 and 3 of the population genetic structure and phylogenetic relationship. M.Sc. Thesis, Sichuan Agricultural University.

Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, USA., ISBN-13: 9780879695774, Pages: 397.

Wu, C.M., 2007. Study of molecular markers with immune traits of chickens of MHC BF gene SNPs. M.Sc. Thesis, Yangzhou University.

Zhou, H.J. and S.J. Lamont, 2003. Chicken MHC class I and II gene effects on antibody response kinetics in adult chickens. Immunogenetics, 55: 133-140.