Genetic analysis of SNPs in the MLF2 and TCR-β genes for growth traits in Korean native chickens

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ABSTRACT

The myeloid leukemia factor 2 (MLF2) and T-cell receptor β (TCR-β) genes are associated with the development of resistance towards coccidiosis infection. Five single-nucleotide polymorphisms (SNPs) located on these genes (SNP_892 and intron 7 (10) of MLF2 and SNP_88, 434, and 561 of TCR-β) were identified and considered to be the genetic markers for resistance to coccidiosis. In this study, we investigated the association between these SNPs and the body weight of Korean native chicken (KNC) and the possibility of using these SNPs as genetic markers for improving growth in KNCs. KNC specimens (798) were genotyped using high-resolution melting analysis, and single-marker association tests were performed; body weights of KNC were also measured every 2 weeks. Three SNPs [892 and intron 7 (10) of MLF2 and 88 of TCR-β] had significant associations with body weight in some period of growth of KNC. Furthermore, 2 SNPs (434 and 561) of TCR-β were linked and significantly associated with the overall growth of KNCs. Conclusively, the findings of the present study suggested that SNPs in the MLF2 and TCR-β genes could be used as combinations of genetic markers for selecting high growth performance specimens of KNCs.

Key words: Genetic analysis, Korean native chickens, MLF2, TCR-β, SNP

Myeloid leukemia factor 2 (MLF2) and T-cell receptor β (TCR-β) genes are located at 80–90 cM on chromosome 1 of chicken. Compared to uninfected specimens, expression levels of these genes increased in chickens infected with coccidia parasites. It was then demonstrated that these genes are involved in protective immunity against coccidiosis (Kim et al. 2008).

Furthermore, genetic sequencing analysis for detecting single-nucleotide polymorphisms (SNPs) that affect resistance to coccidiosis infection revealed 12 SNPs in the TCR-β gene (5 non-synonymous SNPs, 5 synonymous SNPs, and 2 untranslated SNPs); 4 SNPs in the MLF2 gene; and 4 SNPs in the lymphotactin gene. Of these SNPs, only 5 SNPs in the MLF2 and TCR-β genes were found to affect resistance to coccidiosis infection (Kim et al. 2010). Association between SNPs (892 and intron 7 (10) of MLF2 and 88, 434, and 561 of TCR-β) and oocyst shedding (associated with resistance to coccidiosis) was identified in commercial broiler chickens. Previously, few studies had also revealed that the genotypes of SNPs in the MLF2 and TCR-β genes also affect the change in body weight due to the persistence of infection. In particular, SNP_892 in the MLF2 gene has a significant association with both resistance parameter, oocyst shedding, and decreases in body weight after infection (the change in body weight after infection was measured as an indicator of resistance to coccidiosis infection or susceptibility of chickens inoculated with oocysts). Intron 7 (10) SNP is correlated with the body weight following infection but had no association with oocyst shedding. (Kim et al. 2010). However, the effects of these SNPs on the growth performance of normal/healthy chickens have not been reported so far.

Korean native chickens (KNCs) are pure breeds of Korea and are considered to have multiple origin including Eurasia and Southeast Asia (Lee et al. 2013). Despite recent increases in the import of cheaper foreign broiler chickens under the FTA system, KNCs are preferred and widely consumed because they are generally known for their excellent flavour and texture. Furthermore, KNC meat contains higher amounts of glycine, alanine, and proline than commercial broiler breeds (Choe et al. 2010, Jung et al. 2011). However, it is difficult to produce KNCs in sufficient amounts because of their lower growth rate and feed efficiency than broilers (Choe et al. 2010, Jeon et al. 2010).

We hypothesized that SNPs in the MLF2 and TCR-β
genes which are known as parasite resistance markers are associated with body weight gain in the KNC population. Therefore, we performed an association study for identifying the effect of SNPs on growth in KNCs using high-resolution melting analysis (HRM).

MATERIALS AND METHODS

DNA samples: KNC specimens used for this study were a mapping population of 798 individuals from the livestock farm of the Gyeongnam National University of Science and Technology in Korea. Blood samples were collected from the wing veins of the specimens. The body weight of each specimen was measured from birth until 20 weeks of age for the association analysis.

Primer selection and synthesis: Primers used for amplifying fragments of the MLF2 or TCR-β genes from domestic chicken (accession numbers: NM_001030776.2 and M81149.1) were designed using DNASTAR (Madison, WI, USA). Information about the primer sequences is given in Table 1. The genotypes of SNPs were determined by HRM.

PCR reaction and HRM analysis: For HRM analysis, PCR amplification was performed in 20 µl on a Lightcycler®960 instrument (Roche Diagnostics, Indianapolis, IN, USA). The reaction mixture contained 100 ng chicken genomic DNA, 10 pmol of primers, 3 mM Mg²⁺, and 2× Lightcycler®480 High-Resolution Melting Master (Roche Diagnostics, Indianapolis, IN, USA). Amplifications were achieved by PCR: denaturation at 95°C for 1 min, followed by 55 cycles of denaturation at 95°C for 10 sec and then annealing at 54°C or 56°C for 10 sec and extension at 72°C for 15 sec. Following the three-step amplification, HRM was performed as follows: heating to 95°C for 60 sec then cooling to 40°C for 60 sec, a pre-hold step at 65°C for 1 sec, followed by heating to 97°C with 15 fluorescent readings per degree Celsius.

Melting curve acquisition and analysis: The HRM analysis was performed using a Lightcycler®960 instrument. Because the HRM analysis can be used for detecting variations between samples but cannot be used for characterizing the variations, sequencing was initially performed for each studied SNP. To distinguish the genotypes of the unknown samples, sequences of samples that give rise to differently shaped melting curves were identified by sequencing. The curves that were confirmed by sequencing were used as references for the genotypic analysis of the unknown samples in the subsequent HRM analyses. The signal difference between each curve and the reference curves were plotted and automatically clustered into distinct groups of samples having similar melting curves.

Statistical analysis: P<0.05 was considered to be statistically significant. All statistical analyses were performed using Statistical Package for the Social Sciences version 22 software. Analysis of variance was used for the association analysis between genotype and body weight.

RESULTS AND DISCUSSION

The genotypes of 798 KNCs were analyzed for identifying the presence or absence of 5 SNPs [892 and intron 7 (10)] in the MLF2 gene and 88, 434, and 561 in the TCR-β gene. Sequence analysis revealed that heterozygotes and homozygotes have characteristic melting profiles that give rise to differently shaped melting curves. The HRM melting profile was confirmed that the differing profiles corresponded to different sequences. Genotypic frequencies of each SNP are shown in Table 2. Based on the results, two SNPs (434 and 561) were completely co-expressed in the present study.

Kim et al. (2010) had reported that 3 SNPs of TCR-β (88, 434, and 561) were in relatively high LD (r²>0.5) in the F2 population crossed with commercial broilers. In KNCs, SNP_434 and 561 in the TCR-β gene were related to each other, but SNP_88 was independent of the other SNPs. The differences between the results of the present study and previous findings could be attributed to the genetic gap between KNCs and broiler chickens and the relatively high genetic uniformity of KNCs as compared to commercial breeds (Lee et al. 2011). Kong et al. (2006) also reported that the genetic distance between KNC strains and the introduced broiler breeds was high. For this reason, it is quite possible that the results found and obtained in KNCs are different from the commercial broilers reported earlier. Two SNPs [892 and intron 7 (10)] in the MLF2 gene were analyzed in terms of their associations with body weights measured at 11 different time points (at birth and at 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 weeks of age). The results of this analysis are in Table 3. Two SNPs were significantly associated (P<0.05) with body weight. Especially, SNP_892 had a highly significant association with body weight at all time points except at birth and at 2 weeks of age. An association between SNP_intron 7 (10) and body weight was also identified from 6 to 20 weeks, except at 16 weeks (P<0.05).

### Table 1. Primer information set

| Gene     | SNP name | Primer sequence                         | Product size | Temperature |
|----------|----------|-----------------------------------------|--------------|-------------|
| MLF2     | G892A/SNP_intron 7 (10) | CGAAGAATGAAAAAAGAGCC TGATATGACTGTTGAAATGC | 113 bp       | 56°C        |
|          |          | TAGCTCGTTTTTTAATCGTTTTC GACCTGATCCAGGTTAGGC | 111 bp       | 56°C        |
| TCR-β    | A88G     | GAGTAGTCTCCAGCACAGGA CTTTTATTTATGGTCTGGTTTGA | 197 bp       | 54°C        |

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Table 2. Allele and genotype frequencies of SNPs within MLF2 and TCR-β gene in Korean native chickens

| Gene     | SNP            | Allele 1 | Allele 2 | Allele frequency | Genotype frequency |
|----------|----------------|----------|----------|------------------|--------------------|
| MLF2     | SNP_892        | A        | G        | 0.71             | 1/1 0.46           |
|          | SNP_intron 7 (10) | A       | G        | 0.80             | 1/1 0.66           |
| TCR-β    | SNP_434        | A        | G        | 0.83             | 1/1 0.70           |
|          | SNP_561        | C        | T        | 0.24             | 1/1 0.08           |

Table 3. Association between SNPs of MLF2 gene and growth traits in Korean native chickens

| SNP                  | Genotype | Body weight (S.D.) |
|----------------------|----------|--------------------|
|                      | Birth    | BW2               |
| MLF2_SNP_892 A/A     | 43.92    | 176.57            |
| A/G                  | 43.12    | 172.09            |
|                      | 938.88   | 1231.75           |
|                      | 1772.90  | 1923.75           |
|                      | 1799.76  | 1997.76           |
|                      | 2268.71  | 2169.71           |
|                      | 398.24   | 446.44            |
|                      | 43.12    | 172.09            |
|                      | 938.88   | 1231.75           |
|                      | 1772.90  | 1923.75           |
|                      | 1799.76  | 1997.76           |
|                      | 2268.71  | 2169.71           |
|                      | 398.24   | 446.44            |
| MLF2_SNP_intron 7 (10) A/A | 43.62    | 172.12            |
|                      | 709.64   | 1618.38           |
|                      | 253.29   | 345.75            |
|                      | 381.27   | 473.14            |
|                      | 43.12    | 172.09            |
|                      | 709.64   | 1618.38           |
|                      | 253.29   | 345.75            |
|                      | 381.27   | 473.14            |
| MLF2_SNP_intron 7 (10) A/G | 43.62    | 172.12            |
|                      | 709.64   | 1618.38           |
|                      | 253.29   | 345.75            |
|                      | 381.27   | 473.14            |

Two SNPs in the MLF2 gene [892 and intron 7 (10)] had been reported to be associated with a drop-off in body weight as a resistance parameter for coccidiosis (Kim et al. 2010). These results were demonstrated in a chicken population following infection due to Eimeria maxima oocyst. To use these SNPs as biomarkers for selecting high growth performance, it is essential to verify the effort of SNPs on growth in healthy chickens. In this study, KNCs having A alleles of SNP_892 and intron 7 (10) exhibited higher growth than other genotypes of KNCs. Specimens with the A/A genotype of TCRb_SNP_434 (or the T/T genotype of TCRb_SNP_561) exhibited higher growth than other genotypes of KNCs, and these differences were statistically significant.

In one of the previous studies, it was reported that SNPs (88, 434, and 561) in the TCR-β gene were significantly associated with body weight during the entire growth period of KNCs. Specimens with the A/A genotype of TCRb_SNP_434 (or the T/T genotype of TCRb_SNP_561) exhibited higher growth than other genotypes of KNCs, and these differences were statistically significant.

In a genome-wide association study of the Eimeria maxima response in broiler chickens (Hamziæ et al. 2015), 5 SNPs significantly associated with body weight gain were located on GGA1, 3, and 5. The genomic regions of GGA1 and GGA3 are in the vicinity of the MGAT4C and KCNK3 genes, respectively whereas that of GGA5 is in the upstream region of the THBS1 gene.

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Previous studies of the MLF2 and TCR-β genes investigated the change in body weight in broiler chickens as a parameter associated with resistance to coccidiosis infection (Kim et al. 2010, Hong et al. 2011). The haplotypes including 4 SNPs (892 and 947 in the MLF2 gene, 177 in the TCR-β gene, and 187 in the zyxin genes) were associated with loss of body weight and oocyst shedding in chicken post infection (Hong et al. 2011).
However, the association between the MLF2 and TCR-β SNPs and the growth of coccidiosis non-infected chickens has not been demonstrated so far in any of such previous studies. This study presents a new approach for utilizing a previously known DNA biomarker for dual purposes; viz. for predicting body weight gain by repeated analysis of the association between the markers associated with parasite resistance and for chicken growth.

The results obtained in the present study on the effect of SNPs in the MLF2 and TCR-β genes on KNC growth suggested that the genes and their genetic variations could be used as a candidate gene / genetic marker, respectively, for genetic selection to increase the growth performance of KNCs.

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REFERENCES

Choe S H, Nam K, Jung S, Kim B, Yun H and Jo C. 2010. Differences in the quality characteristics between commercial korean native chickens and broilers. Korean Journal of Animal Science 30(1): 13–19.

Hamzie E, Buitenhuys B, Hérault F, Hawken R, Abrahamsen M S, Servin B, Elsen J M, Laan M H P and Bed'Hom B. 2015. Genome-wide association study and biological pathway analysis of the Eimeria maxima response in broilers. Genetics Selection Evolution 47(1): 91

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Table 4. Association between SNPs of TCR-β gene and growth traits in Korean native chickens

| SNP       | Genotype | Body weight (S.D.) | p-value |
|-----------|----------|--------------------|---------|
|           |          | Birth  | BW2  | BW4  | BW6  | BW8  | BW10 | BW12 | BW14 | BW16 | BW18 | BW20 |
| TCR-β_SNP_88 | A/A      | 43.52  | 175.78 | 407.69 | 712.74 | 981.67 | 1275.73 | 1627.30 | 1835.11 | 1998.59 | 2151.93 | 2289.31 |
|           | A/G      | 43.51  | 170.92 | 393.20 | 685.84 | 942.11 | 1228.03 | 1555.44 | 1745.93 | 1915.70 | 2071.29 | 2210.57 |
|           | G/G      | 45.36  | 161.68 | 378.15 | 717.39 | 912.37 | 1192.15 | 1526.59 | 1704.44 | 1809.76 | 2004.10 | 2114.49 |
| TCR-β_SNPs | A/A      | 43.61  | 180.41 | 420.75 | 713.20 | 983.75 | 1228.03 | 1555.44 | 1745.93 | 1915.70 | 2071.29 | 2210.57 |
|           | (T/T)    | 43.69  | 180.75 | 419.46 | 712.94 | 982.11 | 1228.03 | 1555.44 | 1745.93 | 1915.70 | 2071.29 | 2210.57 |
|           | (C/C)    | 45.78  | 178.66 | 413.39 | 723.39 | 989.78 | 1275.50 | 1625.38 | 1828.47 | 1993.81 | 2166.77 | 2316.84 |
|           | (G/G)    | 45.56  | 169.55 | 385.15 | 709.39 | 902.37 | 1192.15 | 1705.59 | 1804.44 | 1876.10 | 2024.10 | 2114.49 |
| p-value   | NS      | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 | p<0.01 |

1BW means body weight at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 weeks of the age; 2NS means non-significance.

parental line specific effects of MLF2 on resistance to coccidiosis in chickens. BioMed Central 5(4): S21.

Jeon H J, Choe J H, Jung Y K, Kruk Z A, Lim D G and Jo C R. 2010. Comparison of the chemical composition, textural characteristics, and sensory properties of North and South Korean native chickens and commercial broilers. Korean Journal for Food Science of Animal Resources 30(2): 171–78.

Jung Y, Jeon H J, Jung S, Choe J H, Lee J H, Heo K N, Kang B S and Jo C. 2011. Comparison of quality traits of thigh meat from Korean native chickens and broilers. Korean Journal Food Science of Animal Resources 31(5): 684–92.

Kim C H, Lillehoj H S, Bliss T W, Keeler C L, Hong Y H, Park D W, Yamage M, Min W and Lillehoj E P. 2008. Construction and application of an avian intestinal intraepithelial lymphocyte cDNA microarray (AVIELA) for gene expression profiling during Eimeria maxima infection. Veterinary Immunology and Immunopathology 124(3): 341–54.

Kim E S, Hong Y H, and Lillehoj H S. 2010. Genetic effects analysis of myeloid leukemia factor 2 and T cell receptor-β on resistance to coccidiosis in chickens. Poultry Science 89(1): 20–27.

REFERENCES