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

SO-YOUNG CHOI[1], NARESH KUMAR SINGH[2], SONG-YI YANG[3], MIN-WOOK HONG[4], DONG KEE JEONG[5], KYUNGBAEK LEE[6], YEONG HO HONG[7] and SUNG-JIN LEE[8]

College of Animal Life Sciences, Kangwon National University, Chuncheon 24341 Korea

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ABSTRACT

The myeloid leukemia factor 2 (*MLF2*) and T-cell receptor β (*TCR*-β) genes are associated 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. Further, 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

Present address: [2]Department of Veterinary Surgery and Radiology, Faculty of Veterinary and Animal Sciences, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi; [5]Department of Animal Biotechnology, Jeju National University, Jeju, Korea; [6,7]Department of Animal Science and Technology, Chung-Ang University, Anseong, Korea.

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 Mg^{2+}, 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 ($r^2>0.5$) 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 TCGATATGACTGGTGAATGC	113 bp	56°C
TCR-β	A88G	TAGTCTGCTTTTTAACTGTGTTTC GACTGCATCTCAGGGTAGC	111 bp	56°C
TCR-β	A434T /C561T	GAGTAGTTCTCAGCACAGGA CCTTTATTTTATGTCTGGTTTTGA	197 bp	54°C

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		
				1	2	1/1	1/2	2/2
MLF2	SNP_892	A	G	0.71	0.29	0.46	0.48	0.05
	SNP_intron 7 (10)	A	G	0.80	0.20	0.66	0.29	0.05
TCR-β	SNP_88	A	G	0.83	0.17	0.70	0.25	0.05
	SNP_434	A	G	0.76	0.24	0.60	0.32	0.08
	SNP_561	C	T	0.24	0.76	0.08	0.32	0.60

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

SNP	Genotype	Body weight (S.D.)										
		Birth	BW2*	BW4	BW6	BW8	BW10	BW12	BW14	BW16	BW18	BW20
MLF2_SNP_892	A/A	43.92	176.37	411.21	722.10	997.67	1287.74	1635.78	1845.06	2019.05	2171.40	2318.39
		(6.74)	(37.62)	(77.10)	(145.97)	(198.91)	(238.92)	(297.73)	(344.10)	(374.65)	(399.84)	(459.74)
	A/G	43.12	172.09	393.49	684.50	938.88	1231.75	1572.77	1772.90	1923.75	2076.65	2202.95
		(6.14)	(29.67)	(69.55)	(135.59)	(183.68)	(218.70)	(286.88)	(328.49)	(348.33)	(388.75)	(441.06)
	G/G	43.62	172.12	410.76	709.64	979.81	1268.71	1618.38	1799.76	1997.76	2169.71	2286.71
		(4.83)	(45.24)	(85.57)	(152.19)	(211.75)	(248.63)	(345.75)	(381.27)	(436.75)	(473.14)	(514.47)
	p-value	NS#	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05	P<0.01	P<0.01	P<0.01
MLF2_SNP_ intron 7 (10)	A/A	43.66	175.00	405.67	712.34	981.33	1275.06	1620.40	1824.50	1990.93	2146.40	2280.13
		(6.24)	(33.52)	(72.94)	(139.97)	(190.18)	(222.66)	(291.18)	(333.97)	(356.19)	(390.51)	(442.44)
	A/G	43.40	174.07	399.80	692.35	951.81	1237.15	1585.82	1785.23	1944.23	2104.75	2246.86
		(6.91)	(35.52)	(76.93)	(145.05)	(198.41)	(242.93)	(302.37)	(350.04)	(387.01)	(418.46)	(485.69)
	G/G	42.45	162.50	379.40	648.29	894.83	1188.17	1503.95	1693.48	1881.52	1974.36	2095.14
		(4.83)	(39.44)	(76.84)	(144.85)	(204.18)	(253.29)	(311.60)	(338.41)	(398.24)	(405.04)	(446.66)
	p-value	NS	NS	NS	P<0.01	P<0.01	P<0.05	P<0.05	P<0.05	NS	P<0.05	P<0.05

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

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 effect 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 those with other allele groups. These markers were shown to have statistical significance. These results may support the hypothesis that these SNPs could be used as genetic markers for selecting high growth performance in KNCs.

The results of the association analysis between SNPs in the *TCR*-β gene and body weight at birth through 20 weeks of age are shown in Table 4. A statistically significant association between SNP_88 and the body weight from 4 to 20 weeks of age was detected in KNCs (P<0.05). There were also non-significant and small differences that were found between the body weights of each of the genotype groups (A/A, A/G, and G/G) at the birth and early stage of egg-laying. However, the body weight of the A/A groups became distinguished from that of the G/G group at later time points.

In this study, 2 SNPs in the *TCR*-β gene (434 and 561) were completely linked. These SNPs 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 one of the previous studies, it was reported that SNPs (88, 434, and 561) in the *TCR*-β gene were significantly associated with only oocyst shedding (Kim *et al.* 2010). These SNPs have not been shown to be associated with body weight in broiler chickens. However, in our study on KNCs, these SNPs were found to be a potential genetic marker for enhanced growth performance.

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.

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).

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

SNP	Genotype	Body weight (S.D.)										
		Birth	BW[1] 2	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
		(6.95)	(34.37)	(68.96)	(132.99)	(181.18)	(221.58)	(281.63)	(323.89)	(349.56)	(384.37)	(438.28)
	A/G	43.51	170.92	393.20	685.84	942.11	1228.03	1555.44	1745.93	1915.70	2071.29	2210.57
		(4.69)	(35.10)	(85.12)	(162.46)	(221.05)	(252.15)	(324.42)	(368.06)	(395.81)	(430.18)	(482.43)
	G/G	43.56	165.95	378.56	657.39	912.37	1192.15	1526.59	1704.44	1876.10	2024.10	2114.49
		(5.07)	(31.76)	(84.27)	(150.21)	(211.76)	(233.08)	(319.21)	(363.89)	(437.63)	(443.76)	(535.31)
	p-value	NS[2]	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05
TCR-β_SNP_434	(T/T)	43.69	180.41	420.75	739.55	1020.11	1316.98	1681.79	1891.20	2052.81	2216.42	2367.79
(*TCR*-β_	A/A	(6.57)	(36.71)	(70.59)	(135.44)	(183.03)	(217.76)	(279.65)	(325.10)	(346.92)	(379.52)	(434.67)
SNP_561)	A/G	42.62	160.38	365.41	629.15	864.49	1146.68	1451.92	1639.55	1812.31	1942.31	2043.49
	(T/C)	(6.15)	(25.84)	(65.42)	(122.89)	(170.37)	(210.13)	(258.74)	(298.54)	(344.90)	(370.40)	(401.27)
	G/G	45.78	180.66	413.39	723.44	989.78	1275.50	1625.38	1828.47	1993.81	2166.77	2316.84
	(C/C)	(5.05)	(31.84)	(82.84)	(154.31)	(204.80)	(248.66)	(329.83)	(364.65)	(412.18)	(433.57)	(524.99)
	p-value	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

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

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.

Hamziæ E, Buitenhuis 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.

Hong Y H, Kim E S and Lillehoj H S. 2011. Identification of 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.

Kong H S, Oh J D, Lee J H, Jo K J, Sang B D, Choi C H, Kim S D, Lee S J, Yeon S H, Jeon G J and Lee H K. 2006. Genetic variation and relationships of Korean native chickens and foreign breeds using 15 microsatellite markers. *Asian Australasian Journal of Animal Sciences* **19**(11): 1546–50.

Hoque M R, Seo D W, Jo C, Gotoh T and Lee J H. 2013. Reconstruction of phylogenetic relationships of Korean chickens using mitochondrial D loop sequences. *Journal of the Faculty of Agriculture, Kyushu University* **58**(2): 287–93.

Lee P Y, Yeon S H, Kim J H, Ko Y G, Son J K, Lee H H and Cho C Y. 2011. Genetic composition of Korean native chicken populations - national scale molecular genetic evaluation based on microsatellite markers. *Korean Journal of Poultry Science* **38**(2): 81–87.