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# Association of SNPs in *ODC* and *PRDM16* with Body Weight Traits in Korean Native Chicken

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***ABSTRACT*** Both *ODC* and *PRDM16* genes were known to be associated with body weight traits in chicken. These two genes were located on GGA3 and GGA21, respectively, where the QTLs of body weights are located. Therefore, the objectives of this study were to identify the SNPs in these two genes and their associations with body weight traits in Korean native chicken. Fluidigm Dynamic Array integrated fluidic circuits (IFCs) assay was used to genotype 7 SNPs consisting g.-353C>T, g.2136A>G, g.2524T>C, g.3607C>T SNPs of the *ODC* gene, and g.182216C>T, g.182290A>T, g.182491A>T SNPs of the *PRDM16* gene. Statistical analysis showed that g.2136A>G SNP of the *ODC* was associated with body weight at 20 weeks of age and slaughter weight, and g.3607C>T SNP of the *ODC* was associated with body weight at 2 weeks of age. Association between g.182216C>T SNP of the *PRDM16* and body weight at 12 weeks of age has also been revealed. In addition, g.182491A>T SNP of *PRDM16* has significant correlation with body weight (BW) at 8 weeks, BW at 10 weeks and BW at 14 weeks of age. These results suggested that both *ODC* and PRDM16 could be strong candidate genes for body weight traits in Korean native chicken.

(Key words : body weight, Korean native chicken, *ODC*, *PRDM16*)

## INTRODUCTION

Body weight is one of important growth parameters in poultry industry. In commercial broiler farms, body weight and meat yields become one of major concern to improve the profitability because they affect both effectiveness and efficiency of production cost directly. In recent years, commercial broiler industry has successfully generated chickens with fast growth rate and high feed efficiency, and has also produced various levels of chicken body weight according to consumer preference. On the other hand, the production of chicken meat using native breeds has many problems, such as slow growth rate, low slaughter weight, small body size, and long maintenance period. Consequently, to improve the performance of indigenous chicken breeds has been hampered. In Korea, the effort to conserve native chicken breed has been launched by Korean government since 1994 (Seo et al., 2013). This program is intended to conserve and to improve performance of Korean native chicken (KNC) in order to be accepted in the domestic and international markets.

Nowadays, white meat consumption has been steadily increased in Korea where 90% of them are supplied by import breeds (Park et al., 2012). Recently, conservation program has achieved good results by using DNA marker to discriminate KNC from other breed (Hoque et al., 2012). The KNC has been successfully established into five lines on the basis of plumage colors (Hoque et al., 2011). Using these 5 lines of KNC, the commercial KNC are available in the market. Since Korean consumers generally enjoy the native chickens, undesired traits such as low growth rate and non-uniform performance of native chicken should be solved in order to meet consumers demand. Therefore, selection of economic traits of Korean native chicken is urgently required.

The selection of chicken economic traits based on mole-

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cular markers, which is known as marker assisted selection (MAS) has been achieved great progress in animal breeding and genetics. Molecular genetic approaches such as identification both of QTLs and SNPs are widely applied in agricultural and life science areas. They have been powerful tools to detect favorable alleles for economic traits in chicken. For example, the *ornithine decarboxylase* (*ODC*) gene that is located in 267.4 cM of GGA3 where the QTL region was found by Uemoto et al. (2009), is known as an essential enzyme for cell growth and differentiation (Kern et al., 1999; Pendeville et al., 2001). The variation within the *ODC* gene has been reported having association with growth and carcass traits in an $F_2$ intercross population between Shamo and White Plymouth Rock chickens. In addition, the *PRD1-BF1-RIZ1 homologous domain containing 16* (*PRDM16*) gene has been known to be correlated with growth, fatness and meat quality of Chinese native chicken (Han et al., 2011). This gene controls the cell fate between muscle and brown fat cells. The loss of *PRDM16* from brown fat precursors causes a loss of brown fat characteristics and promotes muscle differentiation (Seale et al., 2008). Therefore, the aim of this study was to evaluate *ODC* and *PRDM16* as candidate genes for body weight in Korean native chicken.

## MATERIALS AND METHODS

### 1. Animals, DNA Extraction and Genotyping

We measured body weight traits in 590 $F_1$ birds from five lines of Korean native chicken [Black: 90, Grey-Brown: 110, Red-Brown: 134, White: 125, and Yellow-Brown: 131] for this study. Growth parameters, body weight traits, were measured and tabulated every two weeks until they were 20 weeks old and slaughtered. They were maintained and reared at the same time and same management under the standard breeding procedures in National Institute of Animal Sciences (NIAS), Korea. For molecular analysis, blood samples were collected by using EDTA containing tube. Then, these blood samples were used to isolate DNA genome according to Miller et al. (1988) standard method. The DNA concentration was measured by using Thermo Scientific NanoDrop (NanoDrop Products, USA), and the DNA concentration was diluted as much 25 ng/μl for each sample. The extracted DNA genome was stored in the refrigerator at −20℃ for other molecular-based analysis. For genotyping, three SNPS of *ODC* gene and four SNPs of *PRDM16* gene have been analyzed by following protocol of Fluidigm® 192.24 SNPtype™ Genotyping Technology (Fluidigm, USA).

### 2. Statistical Analysis

The effects of SNPs of *ODC* and *PRDM16* on body weight traits of five Korean native chicken lines were calculated statistically by using MINITAB version 14.0 (Minitab Inc., USA). General linear model (GLM) analysis was performed to analyze association between genotypes and phenotypes by following mathematic model:

$$Y_{ijklmno} = \mu + G_i + S_j + B_k + L_l + F_{m(l)} + M_{n(lm)} + \varepsilon_{ijklmno}$$

Where, $Y_{ijklmno}$ is the phenotype of the $o^{th}$ animal, $\mu$ is population mean, $G_i$ is the fixed effect of genotype, $S_j$ is the fixed effect of sex, $B_k$ is the fixed effect of batch, $L_l$ is the fixed effect of line, $F_{m(l)}$ is the random effect of the $m^{th}$ sire nested within the $l^{th}$ line, $M_{n(lm)}$ is the random effect of the $n^{th}$ dam nested within the $l^{th}$ line and $m^{th}$ sire, and $\varepsilon_{ijklmno}$ is the residual error associated with the $o^{th}$ animal. The level of significance for an association was set at $P<0.05$. The Tukey's test was carried out to distinguish between genotypes.

## RESULTS

### 1. Genotype and Allele Frequencies for SNPs in *ODC* and *PRDM16* Gene

Total seven SNPs have been genotyped in this study. In details, four SNPs of the *ODC* and three SNPs of the *PRDM-16* has been investigated. They were considered to genotype according to their position and the results of previous studies. Three SNPs in the *ODC* gene, g.2136A>G, g.2524T>C, and g.3607C>T, were previously reported in NCBI, on the other hand, other selected SNPs were based on the previous studies. Of these, two SNPs namely g.2524T>C SNP of the *ODC* and g.182290A>T SNP of the *PRDM16* were not polymorphic. For the calculation of genotype and allele frequencies, five SNPs were analyzed. Only two genotypes have been found for each SNP (Table 1). In addition, all of them were

minor allele frequencies and in Hardy-Weinberg equilibrium, except for the g.182491A>G SNP of the *PRDM16* which has more than 10% of allele frequency and its Chi-square value was highly significant, indicating this SNP was not in Hardy-Weinberg equilibrium (Table 1).

### 2. The Association of SNPs in *ODC* and *PRDM-16* with Body Weights

The SNPs used in this study are located spread from promoter region to coding region of the gene. One SNP, g.-353C>T SNP in the *ODC* gene, is located in promoter region, and other SNPs are located in exon region. In particularly, g.2136A>G and g.3607C>T SNP in the *ODC* gene are located in exon 3 and exon 7, respectively. Other two SNPs, namely g.182216C>T and g.182491A>G SNPs, are located in exon 9 of the *PRDM16* gene.

Five of the seven SNPs observed in this experiment showed variation in the population. Four of them were associated with body weights in Korean native chicken (Table 2). Both g.2136A>G and g.3607C>T SNPs in the *ODC* gene were significantly associated with body weight at 20 weeks of age (BW20) and slaughter weight (SW), and body weight at 2 weeks of age (BW2), respectively. Birds having AA genotypes for g.2136A>G SNP were significantly higher BW20 and SW than heterozygous AG genotypes, while chicken having CT genotypes for g.3607C>T SNP were heavier BW2 than homozygous one. Furthermore, two SNPs in the *PRDM-16* gene have also been significantly associated with body weight traits. The g.182216C>T SNP was associated with body weight at 12 weeks of age (BW12). In case of g.182491A>G SNP, body weight at 8 weeks of age (BW8), BW10, and BW14 were associated with this SNP. Homozygous CC and AA were consistently heavier body weight than heterozygous one. These results suggested both *ODC* and *PRDM16* may be play an important role in chicken growth, and their variation can be used as molecular marker to select chicken carrying favorable genotypes for body weight in Korean native chicken.

## DISCUSSION

**Table 1.** Genotype and allele frequencies for the SNPs in *ODC* and *PRDM16*

| Location of variation | Gene region | Genotype | Genotype frequency | Allele | Allele frequency | $\chi^2$ (*P*-value) |
|---|---|---|---|---|---|---|
| g.-353C>T | *ODC* Promoter region | CC | 0.90 | C | 0.95 | 1.75 (0.19) |
| | | CT | 0.10 | T | 0.05 | |
| | | TT | 0.00 | | | |
| g.2136A>G | *ODC* Exon 3 | AA | 0.93 | A | 0.96 | 0.89 (0.35) |
| | | AG | 0.07 | G | 0.04 | |
| | | GG | 0.00 | | | |
| g.3607C>T | *ODC* Exon 7 | CC | 0.90 | C | 0.95 | 1.69 (0.19) |
| | | CT | 0.10 | T | 0.05 | |
| | | TT | 0.00 | | | |
| g.182216C>T | *PRDM16* Exon 9 | CC | 0.88 | C | 0.94 | 2.42 (0.12) |
| | | CT | 0.12 | T | 0.06 | |
| | | TT | 0.00 | | | |
| g.182491A>G | *PRDM16* Exon 9 | AA | 0.69 | A | 0.85 | 19.62 ($9\times10^{-6}$) |
| | | AG | 0.31 | G | 0.15 | |
| | | GG | 0.00 | | | |

**Table 2.** Association of SNPs in *ODC* and *PRDM16* with body weight traits in Korean native chicken

| Gene | SNPs | Traits | Genotype (n) | | *P*-value |
|---|---|---|---|---|---|
| | | | AA (546) | AG (44) | |
| *ODC* | g.2136A>G | BW20 | 1,768.20±11.24 | 1,669.10±42.90 | 0.031* |
| | | SW | 1,681.41±11.18 | 1,579.58±42.68 | 0.026* |
| | | | CC (530) | CT (60) | |
| | g.3607C>T | BW2 | 140.36±0.88 | 145.96±2.63 | 0.048* |
| | | | CC (519) | CT (71) | |
| *PRDM16* | g.182216C>T | BW12 | 974.76±7.82 | 922.12±22.09 | 0.027* |
| | | | AA (408) | AG (182) | |
| | g.182491A>T | BW8 | 595.11±5.76 | 573.26±7.97 | 0.031* |
| | | BW10 | 753.10±7.88 | 722.75±10.90 | 0.028* |
| | | BW14 | 1,171.93±10.32 | 1,126.72±14.29 | 0.013* |

Values in Table represent Least Squares Mean (LSM) ±S.E.
Abbreviation: BBW: birth body weight; BW2, BW4, BW6, BW8, BW10, BW12, BW14, BW16, BW18, BW20: body weight at 2 to 20 weeks of age; SW: slaughter weight.
* Superscript in the same row showed significant effects between subject ($P<0.05$).

The *ODC* gene, which is known as a key enzyme in the biosynthesis of polyamines, is regulating factor in stimulating cell proliferation and transformation (Johnson et al., 1995; Kern et al., 1999; Pendeville et al., 2001). Previous studies reported that broiler strain of chicken has over 20-folds higher *ODC* enzyme activity in muscle compared with layer strain (Bulfield et al., 1987). In addition, the high level of mRNA, and enzyme activity of the *ODC* gene were significantly associated with chickens that were genetically-selected for rapid growth (Bulfield et al., 1987; Johnson et al., 1995). Uemoto et al. (2009) published that QTLs was observed at 263~456 cM in GGA3 where the *ODC* gene is located. This location was strongly associated with growth and carcass traits in chicken. Three SNPs and an indel mutation have been found in the promoter region of the *ODC* gene. Two haplotypes, haplotype S and haplotype W, have been constructed based on these variations. The haplotype W, which is derived from White Plymouth Rock (WPR) breed, had positive effects on body weight at 3 to 9 weeks of age and carcass traits (Uemoto et al., 2010). In the present study, two synonymous mutations in exon region of the *ODC* gene were genotyped in KNC. The g.2136A>G SNP (Val-Val) and g.3607C>T SNP (Asp-Asp) have significant effects on BW2, BW20 and SW which is indicated that the *ODC* gene may play important role in chicken growth.

The *PRD1-BF1-RIZ1 homologous domain containing 16* (*PRDM16*) gene, controls the development of brown adipocytes in brown adipose tissue (BAT) and manages bidirectional cell fate switch between skeletal myoblasts and brown fat cells (Seale et al., 2008). An SNP, rs2236518 of the *PRDM16* gene was associated with human body mass index (BMI) in Chinese male (Yue et al., 2013). In rat, enhancement of BAT was associated with lean and healthy phenotypes (Ghorbani et al., 1997). On the other hand, the loss of BAT function was related with obesity and metabolic disorders in mice (Lowell et al., 1993). The *PRDM16* gene is located at 12 cM on chromosome 21 (GGA21). Upstream of the *PRDM-16* gene, located at 17.84 cM, a QTL has been found to be associated with growth in the White Leghorn chicken (Dorshorst et al., 2011). In addition, an association study was performed in Chinese native chicken crossing between Gushi which is representing slow-growing Chinese native chicken and Anka which is representing fast-growing broilers (Han et al., 2011). They reported that three SNPs in exon 6 have

significantly associations with growth, fatness and meat quality. In present study, two SNPs in exon 9 of *PRDM16* were associated with body weight traits in Korean native chicken. Synonymous mutation of g.182216C>T SNP (Ala-Ala) was associated with body weight at 12 weeks of age, while non-synonymous mutation of g.182491A>T (Glu-Val) was associated with BW8, BW10 and BW14.

In conclusion, these finding suggested that the *ODC* gene affected body weights in both early and late stage of age while SNPs of the *PRDM16* also affected body weight in the middle stage of age in KNC. Therefore, they can be applied as molecular markers in KNC for improving growth-related traits. However, in order to use these markers in industrial scale, validating its effect in independent populations is completely required.

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