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# Adjacent SNPs in the transcriptional regulatory region of the *FADS2* gene associated with fatty acid and growth traits in chickens

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**ABSTRACT.** Delta-6 fatty acid desaturases are rate-limiting desaturases involved in metabolic processes of fatty acids, and they are encoded by the *FADS2* gene. In the current study, an $F_2$ resource population of Gushi chickens crossed with Anak broilers was used to investigate the genetic effects of the chicken *FADS2*. Two adjacent single nucleotide polymorphisms (SNPs) (g.4290C>G and g.4291C>A) were identified in the transcriptional regulatory region of the *FADS2* gene by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and created restriction site-PCR-RFLP. Associations between the two SNPs with chicken fatty acid contents and growth traits were determined using linkage disequilibrium, haplotype construction, and association analysis. The two SNPs and their haplotype combinations were significantly associated with linoleic

 

acid (C18:2), α-linolenic acid (C18:3), arachidonic acid (C20:4), body weight (BW)2, BW4, BW6, shank girth (SG)4, and breast bone length 4 ($P < 0.05$). These results suggested that the SNPs of the *FADS2* gene affected the content of essential fatty acid in muscle, and played a role in the early-stage growth rate of chickens.

**Key words:** Chicken; *FADS2*; Single nucleotide polymorphisms; Haplotype; Fatty acid; Growth traits

## INTRODUCTION

Delta-6 desaturase catalyzes linoleic acid (LA, C18:2) and α-linolenic acid (ALA, C18:3) to γ-linolenic acid (GLA, C18:3) and stearidonic acid (C18:4), which involves the insertion of a double bond between carbon 6 and 7 by dehydrogenation. It is generally accepted that the delta-6 desaturation step is rate-limiting (Gill and Valivety, 1997; Kohamaet al., 1998; Innis, 2003; Vance and Vance, 2008). Subsequently, GLA (C18:3) and stearidonic acid (C18:4) could be further biosynthesized into the long-chain polyunsaturated fatty acids (LC-PUFAs) arachidonic acid (C20:4), eicosapentaenoic acid (EPA, C20:5), and docosahexaenoic acid (DHA, C22:6), as a result of further desaturase dehydrogenations (delta-5 desaturase) and elongase steps (Gill and Valivety, 1997; Nakamura and Nara, 2004; Baylin et al., 2007). These LC-PUFAs are important components for the maintenance of normal cell function and for increasing the body's resistance of the neonates (Farrellet al., 1988). Delta-6 desaturase is encoded by the delta-6 fatty acid desaturases (FADS) gene, *FADS2*. Studies have found that single nucleotide polymorphisms (SNPs) located on the *FADS* gene cluster contributed to the variability of several PUFAs, such as LA, ALA, and eicosadienoic acids, especially arachidonic acid in serum phospholipids and erythrocyte cell membranes (Malerba et al., 2008). Nwankwo et al. (2003) demonstrated that a nucleotide insertion in the transcriptional regulatory region of the human *FADS2* gene resulted in delta-6 desaturase deficiency and decreased *FADS2* transcription. Further evidence showed that a *FADS2* promoter polymorphism increased promoter activity and facilitated binding of the transcription factor ELK1 (Lattka et al., 2010). Furthermore, an SNP in the *FADS1/FADS2* gene appeared to cause a change in plasma lipid profiles in two genetically similar Asian ethnic groups with distinctive lifestyle differences (Nakayamaet al., 2010). Research related to the *FADS2* gene in poultry is more limited, however. Khang et al. (2006) investigated genetic variation in the *FADS1* and *FADS2* genes among different local Vietnamese chicken breeds, and found no polymorphisms in *FADS2*. This study also identified five SNPs and an association was found between these polymorphisms and the egg yolk fatty acid composition in the *FADS2* gene of Japanese quail. This study therefore suggested *FADS2* as a functional candidate gene for traits related to n-6 and n-3PUFA concentration in eggs, as demonstrated in experimental quail selection lines (Khang et al., 2007). However, it is currently not known whether *FADS2* plays a role in chicken growth and development. The *FADS2* gene contains 12 exons and 11 introns, and was clustered with family member *FADS1* on chromosome 5 in chicken, while in humans it clusters with *FADS1* and *FADS3* on chromosome 11. The first exon and intron are the longest (Cho et al., 1999; Khang et al., 2007; Kang et al., 2010). In the present study, we identified genetic variations in chicken *FADS2* through DNA pool sequencing, polymerase chain reaction-restriction fragment length polymorphism

(PCR-RFLP) and created restriction site (CRS)-PCR-RFLP methods, and analyzed relationships between genetic variations and some growth traits in chickens. The objectives of this study were to identify genetic polymorphisms in the *FADS2* gene and to elucidate their associations with chicken performance traits.

## MATERIAL AND METHODS

### Resource populations

In this study, we used an $F_2$ resource family, whose details have been described in a previous study (Han et al., 2011). Seventy $F_1$ animals (7 males, 63 females) were produced from Gushi chickens (24 hens and 2 roosters) and Anak broilers (12 hens and 4 roosters). Four Anak male chickens were mated with 24 Gushi females and 2 Gushi males were mated to 12 Anak females. A total of 860 offspring were produced from the $F_1$ population, which formed the $F_2$ Gushi-Anak resource population. All chickens were reared under the same environmental conditions with free access to feed and water.

### Measurement of phenotypes

Growth traits of chickens were analyzed in this study, along with body weight (BW) and size. The 860 $F_2$ chickens were weighed individually at 0, 2, 4, 6, 8, 10, and 12 weeks respectively, while body size was measured at 0, 4, 8, and 12 weeks, including shank length (SL), shank girth (SG), chest depth (CD), chest breadth (CB), breast bone length (BBL), pectoral angle (PA), pelvis breadth (PB), and body slanting length (BSL). All chickens were slaughtered after 12 weeks. Additionally, the fatty acid constituents of muscle were separated and identified by gas chromatography-mass spectrometry (GC-MS), of which myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), LA (C18:2), ALA (C18:3), eicosadienoic acid (C20:2), arachidonic acid (C20:4), EPA (C20:5), and DHA (C22:6) were detected.

### DNA samples, PCR conditions, and genotyping

Genomic DNA samples were extracted from plasma of $F_2$ resource populations by the phenol-chloroform method. More than 150 DNA samples from $F_2$ individuals were randomly selected to construct the DNA pool, which was sent to Taihegene Biotechnology Co., Ltd (Beijing, China) for SNP screening of the *FADS2* gene.

Two pairs of primers were designed based on the sequencing results and the chicken *FADS2* sequence (GenBank: NW_001471700). Primer P1 (F: 5'-TGAACTGAAGCGTTTGATGG-3', R: 5'-TGGCTTTCTTGGCAATAGG-3') was designed to amplify the 283-bp fragment containing g.4290C>G in the promoter region of the *FADS2* gene. Meanwhile, P2 (F: 5'-ATGGTCAGCCATAGGTCTCCTTT-3', R: 5'-GGCTTCACTCACTGATGTTACTA-3') was designed for the 167-bp fragment covering the g.4291C>A, which was amplified by created restriction site (CRS)-PCR-RFLP. All primers were provided by Sangon Biotech (Shanghai) Co Ltd (Shanghai, China).

The PCR amplification system was performed in a total volume of 25 μL, containing

50 ng DNA, 1 μM each primer, and 12 μL Taq MasterMix (Cwbiotech, Beijing). The PCR was run according to the following program: 5 min at 95°C (initial denaturation), 30 cycles of 94°C for 30 s, 56.5°C (primer P1) or 60°C (primer P2) for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min.

The PCR products were digested by restriction enzyme (*Rsa*I for g.4290C>G and *Spe*I for g.4291C>A) overnight at 37°C. Finally, the digestion products were separated by electrophoresis on 1.5% (g.4290C>G) and 3% (g.4291C>A) agarose gels with ethidium bromide staining. Samples of each genotype were selected and sequenced by Sangon Biotech (Shanghai) Co Ltd. (Shanghai, China).

## Linkage disequilibrium and haplotype

Linkage disequilibrium (LD) between two SNPs was estimated as pairwise $r^2$ and D' values using the SHEsis program (http://analysis.bio-x.cn/myAnalysis.php). Individual haplotypes were inferred by the use of the two SNPs and the PHASE software package version 2.1 (Shi and He, 2005). Haplotypes with frequencies greater than 1% were retained for further consideration.

## Statistical analysis

Phenotypic correlations between genotypes and the selected traits of $F_2$ chickens were calculated using the following linear mixed model in SPSS 17.0:

$$Y_{ijklm} = \mu + G_i + S_j + H_k + f_l + e_{ijklm}$$

where $Y_{ijklm}$ is the dependent variable (analyzed traits), $\mu$ is the overall mean, $G_i$ is the fixed effect of genotype or haplotype, $S_j$ is the fixed effect of sex, $H_k$ is the fixed effect of hatch, $f_l$ is the random effect of family, and $e_{ijklm}$ is the random error.

The effect of genotypes or haplotypes of the polymorphisms of the *FADS2* gene on target traits were investigated by least-squares analysis. In cases where the effect of genotypes or haplotypes was significant ($P < 0.05$), the Bonferroni test was used for multiple comparisons of the genotypes, and the additive and dominance effects were estimated using linear regression in SPSS 17.0. The additive effect was estimated as 1, 0, and -1, representing the CC(AA), GC(AC), and GG(CC) genotypes, respectively, whereas the dominance effect was estimated as 1, -1, and 1, representing the CC(AA), GC(AT), and GG(CC) genotypes, respectively.

# RESULTS

## SNP identification and genotyping

DNA sequencing identified two adjacent novel SNPs, g.4290C>G and g.4291C>A, in the transcriptional regulatory region of the *FADS2* gene. For both SNPs, three genotypes were detected through the (CRS)-PCR-RFLP method, which were confirmed by sequencing the homozygote PCR products. For the g.4290C>G site, the frequencies of alleles C and G

were 0.632 and 0.368, respectively. The genotype frequencies of CC, GC, and GG were 0.377, 0.512, and 0.112, respectively. For g.4291C>A, the allele frequencies of C and A were 0.546 and 0.454, respectively, and the genotype frequencies of CC, AC, and AA were 0.230, 0.632, and 0.138, respectively.

## Linkage disequilibrium and haplotype construction

The two SNPs were in strong LD, which was estimated as D' = 0.975, $r^2$ = 0.459. Through estimating SNP haplotype frequencies by using PHASE 2.0, three haplotypes were observed with frequencies > 0.01 among individuals of the $F_2$ resource population, including H1 (C-A), H2 (C-C), and H4 (G-C), with frequencies of 0.450, 0.183, and 0.363, respectively; H3 (G-A) was deleted from further analysis due to its low frequency (0.004).

## Associations of SNPs and chicken performance traits

The g.4290C>G polymorphism showed significant associations with BW2, BW4, BW6, SL4, SG4, BBL4, ALA (C18:3), and arachidonic acid (C20:4) (P < 0.05). Furthermore, chickens with the GC genotype had higher SL4 values. For the other traits, the GG genotype showed significantly higher values than did the GC and CC genotypes. In tests for additive and dominant effects, additive effects were significant (P < 0.05) for BBL4 and ALA (C18:3), and the dominance effect was significant (P < 0.05) for arachidonic acid (C20:4).

Significant associations for the g.4291C>A polymorphism were found with birth body weight (BBW), BW2, BW4, BW6, SG4, BBL4, LA (C18:2), and arachidonic acid (C20:4) (P < 0.05). The mean values of these traits were significantly higher in genotype CC than in genotypes CA and AA. For BW4, BW6, BBL4, and LA (C18:2), the additive effects were significant (P < 0.05), and the dominance effect was significant (P < 0.05) for arachidonic acid (C20:4) (Table 1).

**Table 1.** Associations of different genotypes within the FADS2 gene with chicken fatty acid and growth traits.

| SNPs | Traits | SNP genotype | | | P | Additive effect | P | Dominance effect | P |
|---|---|---|---|---|---|---|---|---|---|
| | | Means ± SE | | | | B ± SE | | B ± SE | |
| g.4290C>G | | CC | GC | GG | | | | | |
| | BW2 (g) | 121.352 ± 2.499[a] | 122.622 ± 2.423 | 127.378 ± 3.022[b] | 0.037 | -0.048 ± 1.028 | 0.963 | 0.547 ± 0.669 | 0.414 |
| | BW4 (g) | 315.536 ± 7.612[a] | 322.827 ± 7.466 | 334.295 ± 8.683[b] | 0.003 | -2.491 ± 2.488 | 0.317 | 0.075 ± 1.612 | 0.963 |
| | BW6 (g) | 554.757 ± 14.182[a] | 560.482 ± 13.912 | 582.516 ± 16.254[b] | 0.037 | -3.394 ± 4.706 | 0.471 | 2.801 ± 3.056 | 0.360 |
| | SL4 (cm) | 5.472 ± 0.071 | 5.634 ± 0.067[b] | 5.322 ± 0.098[a] | 0.041 | 0.072 ± 0.040 | 0.071 | -0.034 ± 0.026 | 0.193 |
| | SG4 (cm) | 2.683 ± 0.030[a] | 2.700 ± 0.029 | 2.748 ± 0.035[b] | 0.038 | 0.010 ± 0.011 | 0.385 | 0.007 ± 0.007 | 0.360 |
| | BBL4 (cm) | 6.147 ± 0.064[a] | 6.234 ± 0.062 | 6.389 ± 0.080[b] | 0.001 | -0.057 ± 0.027 | 0.040 | -0.008 ± 0.018 | 0.669 |
| | C18-3 | 0.178 ± 0.019[a] | 0.193 ± 0.015 | 0.294 ± 0.033[b] | 0.008 | -0.044 ± 0.017 | 0.012 | 0.006 ± 0.011 | 0.580 |
| | C20-4 | 2.248 ± 0.108 | 2.033 ± 0.086[a] | 2.897 ± 0.191[b] | 0.000 | -0.159 ± 0.103 | 0.126 | 0.183 ± 0.065 | 0.005 |
| g.4291C>A | | AA | AC | CC | | | | | |
| | BBW (g) | 30.068 ± 0.524[a] | 30.576 ± 0.462 | 31.061 ± 0.496[b] | 0.017 | -0.189 ± 0171 | 0.269 | -0.173 ± 0.108 | 0.112 |
| | BW2 (g) | 118.742 ± 2.894[a] | 121.924 ± 2.411[a] | 126.421 ± 2.670[b] | 0.002 | -1.880 ± 1.109 | 0.090 | 0.124 ± 0.702 | 0.860 |
| | BW4 (g) | 311.473 ± 7.920[a] | 320.569 ± 6.896 | 328.985 ± 7.437[b] | 0.006 | -6.105 ± 2.649 | 0.021 | 0.796 ± 1.684 | 0.637 |
| | BW6 (g) | 543.404 ± 15.274[a] | 560.466 ± 13.235 | 570.675 ± 14.277[b] | 0.037 | -10.351 ± 5.072 | 0.042 | 1.785 ± 3.213 | 0.579 |
| | SG4 (cm) | 2.658 ± 0.032[a] | 2.696 ± 0.027 | 2.726 ± 0.030[b] | 0.022 | -0.016 ± 0.012 | 0.180 | 0.002 ± 0.008 | 0.823 |
| | BBL4 (cm) | 6.134 ± 0.071[a] | 6.203 ± 0.055 | 6.312 ± 0.064[b] | 0.009 | -0.066 ± 0.029 | 0.026 | 0.011 ± 0.019 | 0.555 |
| | C18-2 | 2.539 ± 0.193[a] | 2.569 ± 0.091 | 3.032 ± 0.156[b] | 0.030 | -0.285 ± 0.123 | 0.021 | 0.134 ± 0.078 | 0.085 |
| | C20-4 | 2.350 ± 0.170 | 2.053 ± 0.080[a] | 2.541 ± 0.137[b] | 0.006 | -0.158 ± 0.110 | 0.151 | 0.200 ± 0.068 | 0.004 |

[a,b]Means within a row with no common superscript differ significantly (P < 0.05).

## Associations of the haplotypes with chicken growth and performance traits

Six haplotype combinations were found in the $F_2$ resource population. Significant associations were found between the haplotype combinations and LA (C18:2), ALA (C18:3), arachidonic acid (C20:4), BW2, BW4, BW6, SG4, and BBL4 ($P < 0.05$). For most of these, the diplotype of H4H4 (G-C/G-C) showed significantly higher levels than those with other diplotypes (Table 2).

**Table 2.** Associations of different haplotype combinations within *FADS2* gene with chicken fatty acid and growth traits.

| Traits | Haplotype combinations | | | | | | P |
|---|---|---|---|---|---|---|---|
| | Means ± SE | | | | | | |
| | H1H1 | H1H2 | H1H4 | H2H2 | H2H4 | H4H4 | |
| BW2(g) | 118.885 ± 2.912[a] | 122.748 ± 2.665 | 121.626 ± 2.476 | 122.851 ± 5.308 | 127.201 ± 3.100[b] | 127.380 ± 3.039[b] | 0.011 |
| BW4(g) | 311.253 ± 8.294[a] | 320.524 ± 7.827 | 320.971 ± 7.419 | 315.424 ± 12.923 | 332.378 ± 8.681[b] | 333.842 ± 8.601[b] | 0.008 |
| BW6(g) | 542.898 ± 16.218[a] | 562.878 ± 15.136 | 558.612 ± 14.457 | 555.993 ± 25.221 | 572.883 ± 16.906 | 587.333 ± 16.642[b] | 0.019 |
| SG4(cm) | 2.659 ± 0.033 | 2.707 ± 0.031 | 2.693 ± 0.029 | 2.680 ± 0.055 | 2.733 ± 0.035 | 2.746 ± 0.035 | 0.045 |
| BBL4(cm) | 6.133 ± 0.076[a] | 6.178 ± 0.069 | 6.216 ± 0.063 | 6.157 ± 0.133 | 6.332 ± 0.081 | 6.362 ± 0.080[b] | 0.014 |
| C18-2 | 2.539 ± 0.192[a] | 2.453 ± 0.170[a] | 2.624 ± 0.107[a] | 4.221 ± 0.486[b] | 2.972 ± 0.243 | 2.794 ± 0.217 | 0.015 |
| C18-3 | 0.225 ± 0.029 | 0.146 ± 0.026[a] | 0.198 ± 0.016 | 0.140 ± 0.073 | 0.174 ± 0.037 | 0.287 ± 0.033[b] | 0.021 |
| C20-4 | 2.350 ± 0.169 | 2.177 ± 0.150 | 2.008 ± 0.094[a] | 2.024 ± 0.428 | 2.225 ± 0.214 | 2.870 ± 0.191[b] | 0.005 |

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

## DISCUSSION

This study was the first to evaluate associations between genetic variants in the transcriptional regulatory region of the *FADS2* gene with fatty acids in a poultry resource family. In this study, two adjacent novel SNPs were found in the transcriptional regulatory region of the *FADS2* gene. We evaluated whether SNPs in the *FADS2* gene were associated with chicken performance traits. Furthermore, owing to the two SNPs being in close LD, we constructed the haplotypes and then analyzed whether the haplotype combinations showed similar results. The results revealed that the two SNPs and their haplotype combinations showed significant associations with some performance traits, such as LA (C18:2), ALA (C18:3), arachidonic acid (C20:4), BW2, BW4, BW6, SG4, and BBL4.

Until now, the majority of the research concerning *FADS2* has focused on humans, which demonstrated that the *FADS1/FADS2* loci were associated with plasma triglyceride, HDL-C, and LDL-C concentrations in populations with European ancestry (Kathiresan et al., 2008; Aulchenko et al., 2009). Polymorphisms of *FADS1/FADS2* showed strongest associations with fatty acid levels such as LA (C18:2), g-linolenic acid (C18:3), eicosadienoic acid (C20:2), arachidonic acid (C20:4), ALA (C18:3), dihomo-g-linolenic acid (C20:3), adrenic acid (C22:4), eicosapentaenoic acid (C20:5), docosapentaenoic acid (C22:5), among others in human serum phospholipids (Schaeffer et al., 2006). Similar results were demonstrated in the present study on poultry. The two adjacent novel SNPs of the *FADS2* gene, as well as their corresponding haplotypes, were most highly associated with LA (C18:2), ALA (C18:3), and arachidonic acid (C20:4), which are essential fatty acids of the chicken. LA (C18:2) and ALA (C18:3) cannot be biosynthesized *de novo*, and are instead derived from the diet, and subsequently cannot be bioconverted to longer and more unsaturated LC-PUFAs. Further-

more, arachidonic acid (C20:4) was found to be the major metabolite of dietary LA (C18:2) (Poureslami et al., 2010). Merino et al. (2011) reported that genetic variation in the *FADS* gene cluster could alter desaturase activity in human subjects of Caucasian and Asian descent. In the present study, the data was insufficient to determine whether the two adjacent mutations could result in enzyme activity; therefore, the mechanism that caused the observed changes in fatty acid content require further validation.

In poultry, five SNPs were identified in Japanese quail and an association was found between these polymorphisms and the egg yolk fatty acid composition (Khang et al., 2007). However, reports investigating the associations between growth traits and SNPs of the *FADS2* gene in poultry are scarce. The current study provides strong evidence that the adjacent SNPs and their haplotype combinations are associated with BW2, BW4, BW6, SG4, and BBL4. Therefore, the SNPs were associated with the early-stage growth rate of chickens, especially at 4 weeks. In the later growth stage, this advantage became negligible ($P > 0.05$).

In chicken breeding practices, some chickens are selected on the basis of precocity and their ability to reach their market live weight at an early age. The combination of age, low kinetic activity, and high feed intake results in a higher fat accumulation in muscles (Dal et al., 2012). However, in our study, the fast growth rate did not cause significant accumulation of fat in muscles (data not shown).This trait combination would be beneficial for the selection and breeding of high quality broiler chicken species. We speculated that this mutation caused the appearance of the aforementioned fatty acids and its accumulation in the muscle, which would affect the early-stage growth rate of chickens; however, the precise mechanism requires further investigation.

In conclusion, two adjacent SNPs were found in the transcriptional regulatory region of the *FADS2* gene. Both SNPs showed significant associations with body weight and fatty acid traits. Therefore, these results support the hypothesis that the *FADS2* gene plays an important role in the early-stage growth rate of chickens. The limitations of the present study should be considered when interpreting these results and the specific desaturase activity should be measured in future related studies.

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