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Genome-wide association study of meat quality traits in chicken

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ABSTRACT. Meat quality traits are very important in the poultry industry. To identify single nucleotide polymorphisms (SNPs) and candidate genes affecting meat quality traits, a genome-wide association study was performed using the Illumina chicken 60K SNP beadchip in Jinghai yellow chicken. Four meat quality traits were measured. Two SNPs reached 5% Bonferroni genome-wide significance (P < 1.8E-6) and 7 SNPs reached "suggestive" genome-wide significance (P < 3.59E-6) with meat quality. These SNPs were located nearby or in 7 candidate genes, including *CBLN2*, *HPGDS*, *SETD2*, and *ANKRD46*, among others. A total of 5650 haplotpyes were established and only 1 was found to be associated with fat content in leg muscle. These results indicate that the 9 SNPs and

7 genes are important candidate markers and may influence meat quality traits in chicken.

Key words: Chicken; Genome-wide association study; Haplotype; Meat quality traits

INTRODUCTION

Meat quality traits have an important influence on the poultry industry as the flavor of meat is determined by meat quality traits. Many local chicken breeds in China are well-known for their delicious meat. Remarkable advances in the study of meat quality traits have been achieved in China, and numerous genes and quantitative trait loci have been found to be associated with meat quality traits (Abasht and Lamont, 2007; Atzmon et al., 2008; Fang et al., 2010; Tang et al., 2011).

Polymerase chain reaction-restriction fragment length polymorphism and polymerase chain reaction-single-strand conformation polymorphism are widely used to identify SNPs associated with different traits in chicken. Genome-wide association studies (GWAS) have become one of the most commonly used strategies for identifying genes affecting complex traits in humans and other animals. In chickens, some major loci associated with growth (Gu et al., 2011; Xie et al., 2012), egg production (Liu et al., 2011; Wolc et al., 2012), rumpless and ear-tufted traits (Noorai et al., 2012), body composition and meat quality (Liu et al., 2013), resistance to Marek's disease, and the immune response to Newcastle disease virus were identified by GWAS (Li et al., 2013; Luo et al., 2013).

In the present study, to identify SNPs and candidate genes that are significantly associated with meat quality traits, a genome-wide association study was conducted using the 60K SNP beadchip in Jinghai yellow chicken. We sought to identify new candidate genes and region and lay a foundation for marker-assisted selection of Jinghai yellow chicken.

MATERIAL AND METHODS

Experimental population and phenotypic measurements

The population examined in this study included 212 female Jinghai yellow chickens from 19 half-siblings from the same batch. These birds were hatched on the same day and reared in the pens. Birds had access to feed (commercial corn-soybean diets meeting the National Research Council's requirements) and water *ad libitum*. All chickens were in good health. Blood samples were collected when the chickens were slaughtered at 66 weeks of age. Samples of breast muscle and leg muscle were taken to detect the fat content in breast muscle and leg muscle (FLM) as well as the protein content in breast muscle and leg muscle. The descriptive statistics of the traits above are shown in Table 1. The date was transformed using Johnson transformation to follow the standard normal distribution in Minitap (v16).

Genotyping and quality control

DNAs were extracted using the Dzup Genomic DNA Isolation Reagent (Blood) kit

from Sangon Biotech (Shanghai, China). The concentration and quality of genomic DNA was quantified by spectrophotometry and then stored at -20°C. DNA samples were sent to DNA Landmark (Québec, Canada) for genotyping using the 60K SNP beadchip.

Plink (v1.07) was used for quality control of the data (Purcell et al., 2007). The samples with low call rate (<90%) and SNPs showing low call frequency (<95%), low Hardy-Weinberg equilibrium (<1.0E-6), and low minor allele frequency (<3%) were rejected. Finally, 200 of the 212 samples and 46,665 SNPs were examined in this genome-wide association study. The distribution of SNPs after quality control is shown in Table 2.

Table 1. Descriptive statistics of carcass and meat quality traits.

Traits	Max	Min	Average	SD	Best-fit	Normal distribution
FBM (%)	2.8	0.8	1.3	0.3	0.65	Yes
FLM (%)	4.9	2.0	3.4	0.6	0.52	Yes
PBM (%)	27.4	16.8	24.7	1.3	0.56	Yes
PLM (%)	25.4	16.5	21.7	1.0	0.68	Yes

Table 2. Basic statistics of small nucleotide polymorphisms after quality control.

Chr	Physical map (Mb)	No. of SNPs	SNP density[1]
1	200.95	7244	27.74
2	154.79	5466	28.32
3	113.62	4165	27.28
4	94.20	3396	27.74
5	62.23	2192	28.39
6	35.84	1729	20.73
7	38.30	1829	20.94
8	30.56	1350	22.64
9	24.02	1187	20.24
10	22.42	1302	17.22
11	21.87	1241	17.62
12	20.46	1403	14.58
13	18.27	1186	15.41
14	15.76	1018	15.52
15	12.93	1029	12.56
16	0.42	11	37.99
17	10.61	846	12.55
18	10.85	857	12.66
19	9.90	830	11.93
20	13.92	1484	9.38
21	6.86	766	8.95
22	3.90	300	12.99
23	6.02	601	10.02
24	6.38	743	8.58
25	2.02	168	12.00
26	5.07	640	7.82
27	4.83	479	10.07
28	4.47	560	7.99
LGE22C19W28_E50C23	0.88	109	8.10
LGE64	0.018	3	6.00
z	74.58	1942	38.40
0	0.00	589	0.00
Total	1026.948	46665	22.04

[1]Unit of SNP density was Kb/SNP. Chr = chromosome.

Population structure

Statistics analysis

The population structure of the experimental population was evaluated by multidimensional scaling analysis using the Plink program. The steps were as follows: linkage disequilibrium based on SNP pruning of autosomes was applied with an $r^2 > 0.2$, and 12,877 independent SNPs were obtained. Next, pair-wise identity-by-state distances of the SNPs and multidimensional scaling analysis components were calculated. The multidimensional scaling analysis diagram was plotted using the R (2.15.1) program (Wang et al., 2009). Principal component analysis (PCA) was performed using the genome-wide complex trait analysis software v1.24. PCA1 and PCA2 were used in models to the reduce population stratification effect (Wall et al., 2003; Wang et al., 2009; Ramos et al., 2009). Haplotype analysis was carried out using the PLINK program based on 46,665 SNPs. The method was used to calculate the R^2 value of SNPs within a 200-kb window on the same chromosome. If $R^2 > 0.8$, the 2 SNPs were considered to be linked.

The general linear regression model (GLM, I) in PLINK was used in this study. The following model equation was used:

$$Y = G\alpha + X\beta + e$$

where *Y* is the vector of observations; *G* is genetic effect vector, *X* is a matrix containing all other fixed effects, including population structure (PCA1 and PCA2), α and β are incidence matrix, and *e* is the vector of random residual. Furthermore, the association analysis between haplotypes and meat quality traits was also performed using the GLM. The Bonferroni P value was calculated based on the independent SNPs of autosomes, which were defined using the indep-pairwise option with $r^2 = 0.4$ (Johnson et al., 2010). Ultimately, 27,824 SNPs were identified. The threshold P value for Bonferroni potential significance was 3.59E-5 (1/27,824), and the P value for Bonferroni genome-wide significance was 1.80E-6 (0.05/27,824). QQ plots and Manhattan plots were created using the R (2.15.1) software.

RESULTS

Population structure

The result showed that the distribution of 200 chickens from the 19 half-sibling families showed some degree of stratification (Figure 1). The population structure was corrected using principal component analysis (PCA). PCA1 and PCA2 were used in models to reduce the population stratification effect.

Genome-wide association analysis

Two SNPs showing genome-wide significance (P < 1.8E-6) with FLM were identified using the GLM model. Two SNPs, rs312796105 and rs15469825, were located on GGA1 and shared the same proximal gene of *LOC101747478* (Table 3). Seven SNPs showing "suggestive" genome-wide significance (3.59E-5) were identified to be associated with FLM, fat content in breast muscle and protein content in leg muscle using the GLM model. Four

SNPs, including rs313563594, rs313986006, rs14743029, and rs 16136932, were located on GGA2. The rs1z59929, rs15544591, and rs14300880 were located on GGA1, 4, and 26, respectively (Table 4). No significant SNP was found to be associated with protein content in breast muscle. The Manhattan plots for FLM with significant SNPs were drawn using the R (2.15.1) software (Figure 2). A total of 5650 haplotypes were identified based on the 46,665 SNPs. Only 1 haplotype showing "suggestive" genome-wide significance was identified to be associated with FLM. Names of genes in 1-Mb segments surrounding each significant SNP were downloaded from Ensembl and NCBI. Nine SNPs were located in or nearby 7 candidate genes, including *LOC101747478*, *CBLN2*, and *HPGDS*, among others.

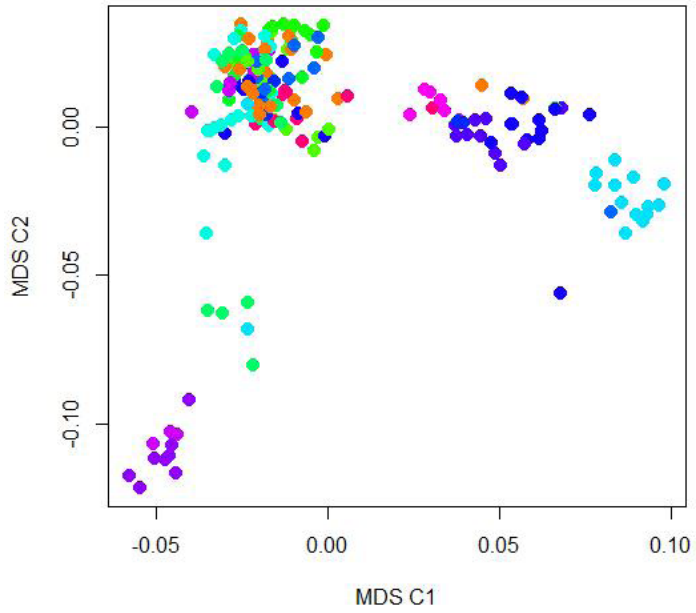

Figure 1. Population structure identified by Multidimensional Scaling analysis.

Table 3. Significant small nucleotide polymorphisms for meat quality traits.

Trait	SNP ID	Chromosome	Position (bp)	P value	Nearest gene	Distance
FLM	rs312796105	1	159933083	1.18E-06	*LOC101747478*	15 kb downstream
	rs15469825	1	159851745	1.60E-06	*LOC101747478*	56 kb downstream

Table 4. "Suggestive" significant small nucleotide polymorphismsfor meat quality traits.

	SNP ID	Chromosome	Position (bp)	P value	Nearest gene	Distance
FLM	rs313563594	2	92854994	4.85E-06	*CBLN2*	224.659 kb upstream
	rs1z59929	1	165000000	3.02E-05	*LOC101747478*	20 kb downstream
	rs15544591	4	36643430	3.10E-06	*HPGDS*	7.362 kb upstream
FBM	rs313986006	2	3876584	2.86E-05	*SETD2*	Within
	rs14743029	2	128320097	9.72E-06	*ANKRD46*	7.910 kb downstream
	rs16136932	2	130238033	2.04E-05	*ZFPM2*	Within
PLM	rs14300880	26	4378338	1.50E-05	*GRM4*	1.814 kb downstream

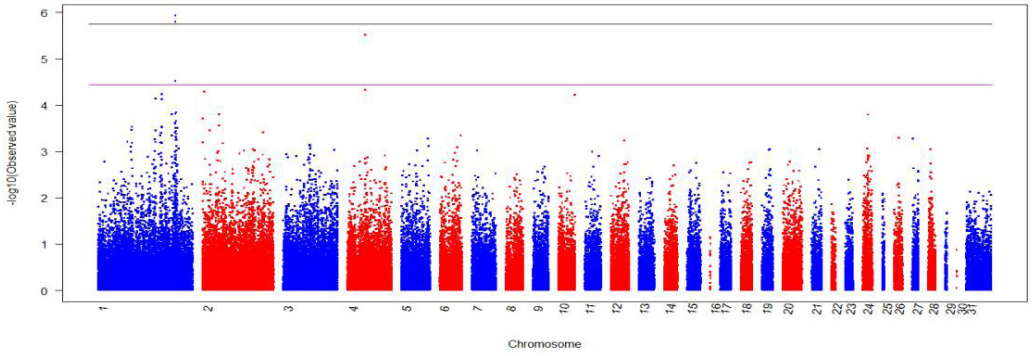

Figure 2. Manhattan plot for genome-wide association study on FLM.

DISCUSSION

In the present study, 2 SNPs that showed genome-wide significance (P < 1 .8E-6) with FLM were identified using the GLM model. These 2 SNPs were located on GGA1 and shared the same proximal gene of *LOC101747478*. Seven SNPs showing "suggestive" genome-wide significance were identified (3.59E-5) using the GLM model. Nine SNPs were located in or nearby 7 different candidate genes.

Three SNPs associated with FLM were located in downstream of the *LOC101747478* gene, indicating that *LOC101747478* may influence FLM in chickens. *LOC101747478* is a novel gene that has not been previously reported. rs313563594 and rs15544591 were also found to be associated with FLM in chicken. They are located upstream of the *CBLN2* gene on GGA2 and the *HPGDS* gene on GGA4. It has been reported that *CBLN2* may play a role in synapse formation and in brain evolution (Reiner et al. 2011). *CBLN1*-null mice are severely ataxic, walk with an irregular gait, and do not maintain their balance on rotarod (Wei et al., 2009). Additionally, there is a high degree of sequence homology between *CBLN2* (91%, based on mouse sequences) and *CBLN4* (85%) with *CBLN1*, and they may play similar roles in synapse formation.

Hematopoietic prostaglandin D synthase (HPGDS) is mainly expressed in Th-2 cells, mast cells, and antigen-presenting cells. HPGDS can convert prostaglandin H2 into prostaglandin D2, which is a mediator thought to play a pivotal role in airway allergy and inflammatory processes as well as induce vasodilatation, bronchoconstriction, pulmonary eosinophil and lymphocyte infiltration, and cytokine release in asthmatics (Kanaoka and Urade, 2003). Inhibition of HPGDS may be therapeutically beneficial in the treatment of allergic disease and may be more effective than blocking either DP-1 or chemoattractant receptor-homologous molecules expressed on Th2 cells alone (Trivedi et al., 2006).

SET domain-containing 2 (*SETD2*) is a histone methyltransferase gene located on 3p21.31. *SETD2* is nonredundantly responsible for trimethylation of the histone mark *H3K36* and is a tumor suppressor gene involved in the development of clear cell renal cell carcinoma (Edmunds et al., 2008; Duns et al., 2010). Li et al. (2005) reported that SETD2 plays a role in chromatin structure modulation during transcriptional elongation via its interaction with hyperphosphorylated POLR2A. It also may act as a transcription activator that binds to promoters.

Ankyrin repeat domain 46 is a member of ankyrins. Ankyrins are adaptor proteins that attach membrane proteins to the cytoskeleton, and cytoskeletal proteins are important for the oncogenic process of migration and in disease states such as muscular dystrophy (Buscaglia and Li, 2011). Ankyrin repeat domain 46 was identified as a direct target of miR-21 (Cho, 2012). Yan et al. (2011) recently reported that in breast cancer cells, miR-21 is upregulated, corresponding with downregulation of ankyrin repeat domain 46, leading to increased proliferation and migration.

Zinc finger protein, multitype 2 (ZFPM2) is a target of miR-200 and can regulate the activity of phosphatidylinositol 3-kinase (the upstream activator of Akt) in insulin signaling (Park et al., 2013). Greenbaum et al. (2012) reported that the ZFPM2 SNP rs12678719 was associated with antipsychotic-induced parkinsonism. Tan et al. (2012) reported that variants of the ZFPM2/FOG2 gene may be a common cause of double outlet right ventricle.

Glutamate receptor, metabotropic 4 is a member of the group III metabotropic glutamate receptor genes. Presynaptic metabotropic glutamate receptors, including mGluR4, are known to modulate the release of glutamate and gamma-aminobutyric acid in the thalamocortical network, and perturbations of these pathways have been shown to play a role in several animal models of absence seizures. In particular, disruption of mGluR4 function in mice leads to increased susceptibility to absence seizures. Glutamate receptor, metabotropic 4 sequence variants may confer low-risk effects to the etiology of idiopathic generalized epilepsies. Jiang et al. (2014) reported that polymorphism in the metabotropic glutamate receptor 4 (*GRM4*) gene were associated with the susceptibility and prognosis of osteosarcoma in a Chinese Han population. In conclusion, all the 7 candidate genes discussed above are novel genes and have not been studied before in chicken before. However, most of those genes are important factors participating in many pathways. And the relationship between those candidate genes and meat quality traits of chicken will be verified in the further study.

CONCLUSIONS

In the present study, a genome-wide association study was carried out to identify candidate genes associated with meat quality traits in chicken. Nine significant SNPs were detected. Two SNPs, rs312796105, and rs15469825, associated with FLM reached a genome-wide significance level. Seven SNPs reached a "suggestive" genome-wide significance level associated with FLM, fat content in breast muscle, and protein content in leg muscle. Seven candidate genes in 1-Mb segments surrounding each significant SNP were obtained. This result indicated that these SNPs and genes are important candidate markers and genes and require further examination.

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