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Technical Note

Genome-Wide Association Study of Abdominal Fat in Wenshang Barred Chicken Based on the Slaf-Seq Technology

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ABSTRACT

Chicken abdominal fat (AF) is an economically important trait, and many studies have been conducted on genetic selection for AF. However, previous studies have focused on detecting functional chromosome mutations or regions using gene chips. The present study used the specific-locus amplified fragment sequencing (SLAF-seq) technology to perform a genome-wide association study (GWAS) on purebred Wengshang Barred chicken. A total of 1,286,715 single-nucleotide polymorphisms (SNPs) were detected, and 175,211 SNPs were selected as candidate SNPs for genome-wide association analysis using TASSEL general linear models. Two SNPs markers reached genome-wide significance. Of these, rs7943847, rs127627362 were significantly associated with AF at 120 days. These SNPs are close to eight genes (SLC16A6, ARSG, WIPI1, PRKAR1A, FAM20A, ABCA8, ABCA9, CPQ,). These results would enrich the studies on AF and promote the use of Chinese chicken, especially the Wenshang Barred chicken.

INTRODUCTION

Abdominal fat (AF) is one of the by-products of chicken slaughter and is rarely used in the food industry. Chicken AF deposition is regulated by genetics, endocrine hormones, environmental factors multiple behavioral factors (Cahaner *et al.*, 1985; Fouad *et al.*, 2014; Resnyk *et al.*, 2015). The genes or molecules that regulate AF deposition or abdominal adipose tissue development can be identified by many different genomic approaches (Tatsuda *et al.*, 2001; Abasht *et al.*, 2007; Huang *et al.*, 2015; Ouyang *et al.*, 2016; Jin *et al.*, 2017; Zhang *et al.*, 2017).

For the past few years, the genome-wide association study (GWAS) technique has been developed for detecting single-nucleotide polymorphisms (SNPs) and functional genes that affect quantitative traits (Jin *et al.*, 2015; Li *et al.*, 2018). Fan *et al.* (2014) screened 25 SNPs affecting slaughter traits in Jinghai Yellow Chicken by genome-wide association analysis, of which 5 SNPs were associated with AF. Several polymorphic loci that were significantly associated with chicken AF traits were identified by GWAS during the pretest period, of which six loci were located in type III tyrosine kinase receptor (Wu *et al.*, 2012).

In this study, the specific-locus amplified fragment sequencing (SLAF-seq) technology was used to perform a GWAS of AF traits in Wenshang Barred chicken (a Chinese chicken breed) to identify the associated SNPs and predict functional genes. These results will enrich the study of the chicken AF trait and may be helpful for the use of local breeds.

MATERIAL AND METHODS

Experimental animals

The animals used in this study were 250 1-day-old male chickens of the same hatch from the same generation chosen randomly and obtained from the base population of the Institute of Poultry Sciences, Shandong Academy of Agricultural Sciences (SAAS). All animals were reared in stair-step cages and had genealogical records. All environmental and nutritional conditions were the same. Blood samples were collected from 250 male chickens at 120 days of age. After blood collection, the same birds were weighed and killed by cervical dislocation. The AF was removed and weighed.

Specific-locus amplified fragment sequencing

Genomic DNA was isolated from whole blood samples using the phenol–chloroform method. The quality and quantity of DNA was then inspected using gel electophoresis. The quantified DNA was diluted to 20 μg/μL and was stored at -20 °C before use.

The procedure of SLAF-seq in this experiment is shown in Figure 1. The Gallus gallus sequences were analyzed using the SLAF_Predict (Biomarker, Beijing, China), based on the GC content, repeat sequences, and gene characteristics. The marker selection, digestion conditions, gel cutting range, and total sequencing volume were determined to ensure consistency of marker coverage throughout the genome.

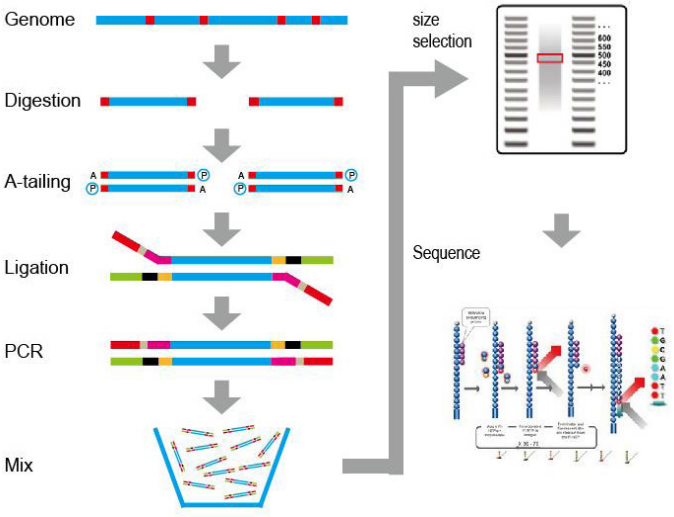

Figure 1 – SLAF-seq flowchart.

Genomic DNA was digested by HaeIII [New England Biolabs (NEB), Ipswich, MA, USA]. A single-nucleotide A overhang was added to the digested fragments with Klenow fragment (3′→5′ exonuclease and 5′→3′ polymerase) (NEB) and dATP at 37°C, and then duplex tag labeled sequencing adapters (Life Technologies, Carlsbad, CA, USA) were ligated to the A-tailed DNA with T4 DNA ligase. Polymerase chain reaction (PCR) was performed with diluted restriction-ligation DNA samples, dNTPs, Q5 High-Fidelity DNA Polymerase, and PAGE-purified PCR primers AATGATACGGCGACCACCGA and CAAGCAGAAGACGGCATACG (Life Technologies, Carlsbad, CA, USA). The PCR products were purified using the Agencourt AMPure XP beads (Beckman Coulter, High Wycombe, UK) and pooled. The pooled sample was separated via electrophoresis in a 2% agarose gel. Fragments with indexes and adaptors from 300 to 500 bp were excised and purified using a QIAquick Gel Extraction Kit (Qiagen, Germany). The gel-purified products were sequenced using the Illumina HiSeq 2500 system (Illumina, Inc., CA, USA). Sequencing produced paired-end reads that were evaluated and mapped using SOAP 2.20 software (Li *et al.*, 2009) to assemble newly referenced genomes (http://ftp.ensembl.org/pub/release-75/fasta/gallus gallus/DNA/). Paired-end reads that can be mapped to the reference genome were reserved for analysis.

Statistical analysis

The structure of the state of population was analyzed using ADMIXTURE software (Alexander *et al.*, 2009) based on SNP genotype data. In the cluster analysis, the subgroup number (Q value) of the 250 samples was 10, which was confirmed by its peak ΔQ value position. The subgroup with the minimum ΔQ peak value was considered to be the best.

The GWAS analysis was performed using a general linear model (GLM) of the TASSEL program (Zhang *et al.*, 2010), as follows:

$$Y = \mu + X\alpha + Q\beta + e$$

Where Y is the phenotypic value, X is the genotype, Q is the population structure matrix calculated by the ADMIXTURE program, β is the weight vector of each group, α is the weight vector of each marker, and e is the random error. The threshold P value for declaring genome-wide significance was 5.7E-07 (0.1/175211). The P value for communicating "suggestive" genome-wide matter, allowing for one false-positive effect in a genome-wide test, was 5.7E-06 (1/175211), based on a Bonferroni correction.

RESULTS

Phenotype

The Barred chicken breed (Gallus gallus domesticus), found initially mainly in Wenshang county of Shandong

province, China, is a commercial dual-purpose egg-meat-type chicken. The Barred chicken is popular because of its meat quality. Table 1 lists descriptive statistics of AF. Their distributions approximately fitted normal distributions.

Table 1 – Statistics of AF measurements.

AF	Minimum	Maximum	Mean	Standard error	Coefficient of variation
AFW	0	57.8	11.61	0.618	0.0831
AFR	0	5.58	1.267	0.065	0.0804

AFW abdominal fat weight, AFR abdominal fat rate.

SLAF-seq results

In total, 288,130,000 paired-end reads were generated. Finally, 294,133 SLAF markers spread throughout the genome were selected (Table 2). The average sequencing depth was 7.95*x*. The distribution of all SLAFs in the genomes of the 250 samples was determined by the number of SLAFs per 100 kb in the genomes (Fig 2). SLAFs were relatively evenly distributed throughout the genomes, indicating that the SLAF data were reliable. A total of 1,286,715 SNPs were detected, and 175,211 SNPs were selected as candidate SNPs for genome-wide association analysis using the integrity >0.5 and minor allele frequency >0.05.

Table 2 – SLAF marker numbers.

Chromosome ID	SLAF number	Polymorphic SLAF
chr1	55,577	53,042
chr2	39,305	37,371
chr3	30,065	28,698
chr4	23,736	22,694
chr5	16,142	15,399
chr6	10,806	10,410
chr7	10,528	10,082
chr8	8,400	8,004
chr9	7,578	7,322
chr10	6,406	6,168
chr11	5,300	4,991
chr12	6,340	6,112
chr13	6,095	5,839
chr14	5,286	5,112
chr15	4,525	4,321
chr16	140	132
chr17	4,416	4,266
chr18	4,349	4,172
chr19	3,988	3,841
chr20	5,275	5,070
chr21	2,778	2,679
chr22	1,045	937
chr23	2,482	2,405
chr24	2,791	2,696
chr25	826	798
chr26	2,185	2,116
chr27	2,085	1,962
chr28	1,913	1,850
chr30	1	1
chr31	5	4
chr32	19	19
chr33	562	537
chrW	210	21
chrZ	14,688	12,581
Scaffold	8,286	5,842
Total	294,133	175,211

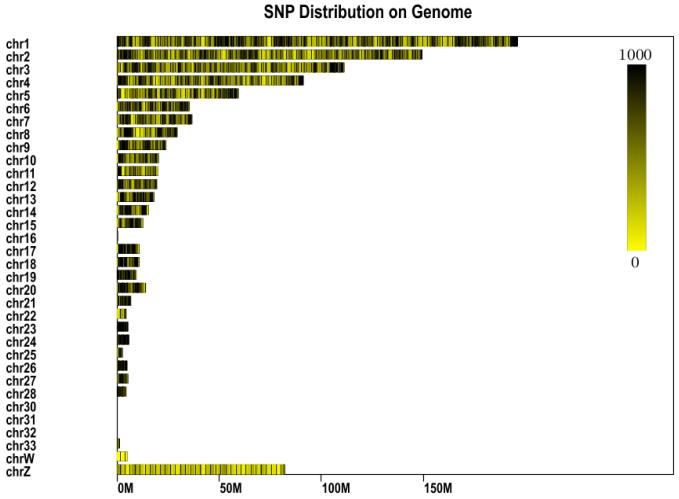

Figure 2 – Chromosomal SNP distribution.

Group structure and cluster analysis

The group structure of the samples indicated that the best dataset was produced using a K-value of 2, indicating that the models probably derived from two ancestors. A clustering strategy was applied to the models using ADMIXTURE software (Fig 3). The quantile-quantile plots of AFR and AFW did not find false positives due to population stratification (Fig 4 and Fig 5). Therefore, the result of the association analysis was reliable.

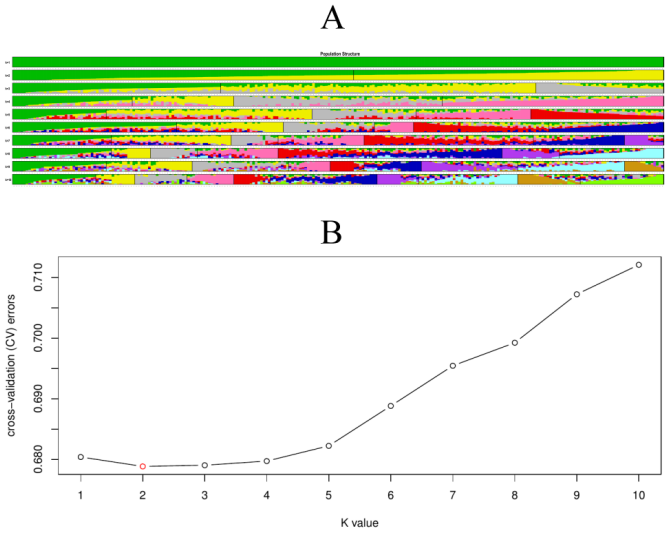

Figure 3 – Group structure and clustering results. (A) The colors represent separate groups, with lines giving the group value. (B) The coefficient of variation value for each K-value.

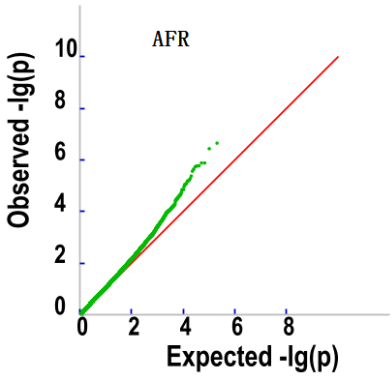

Figure 4 – Quantile–quantile (QQ) plots of abdominal fat rate.

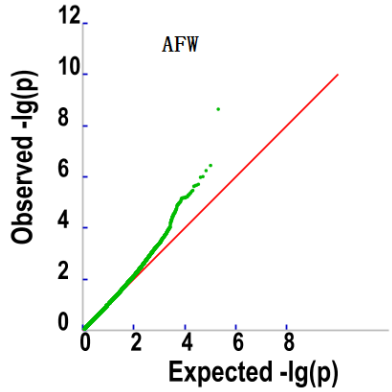

Figure 5 – Quantile–quantile (QQ) plots of abdominal fat weight.

Genome-wide association analysis

According to the quality control criteria, 250 chickens and 175,211 SNPs (Table 2) were eligible for GWAS analysis. Based on the TASSEL GLM, and a Bonferroni correction, two SNPs exhibited GWAS with AF in Wenshang Barred chickens (Table 3). Of these, rs13508784 at 7,943,847 bp of GGA18 and rs736609926 at 127,627,362 bp of GGA2 were significantly associated with AF. These SNPs are close to eight genes, including *SLC16A6*, *ARSG*, *WIPI1*, *PRKARIA*, *FAM20A*, *ABCA8*, *ABCA9*, and *CPQ*.

DISCUSSION

Excessive AF deposition often reduces feed utilization efficiency and causes waste. Therefore, the control of fat deposition in broilers has become an essential goal of the broiler industry. Some results showed that the percentage of AF in broilers was highly hereditary, and the weight of AF was controlled by multiple genes. Some genes affecting AF deposition and body weight gain have been found, and they differ in conformation between breeds, resulting in differences in their mechanism of action. The selection of chickens for rapid growth has been accompanied by an increase of fat deposition, and excessive fat deposition, especially AF, can not only decrease feed efficiency, but also cause many diseases (Na *et al.*, 2019). Finding the candidate genes associated with AF deposition is therefore essential for breeding.

GWAS is a powerful tool for the genetic analysis of important production traits in farm animals. Genomic heritability was estimated using relationships inferred from high-density SNP panel genotypes instead of pedigree-based relationships. The use of close relatives and a higher density of SNPs may lead to a better genomic prediction with less bias than can be achieved

Table 3 – Significant SNPs for Abdominal Fat.

Trait	SNP ID	Chr	Position	*p*-value	Nearest genes	Distance
AFR	rs13508784	18	7943847	2.59E-07	*SLC16A6*	64.61kb-65.29kb Upstream
					ARSG	67.89kb Upstream
					WIPI1	25.27kb Upstream
					PRKARIA	0.354kb-0.358kb Upstream
					FAM20A	1.703kbdownstream
					ABCA8	32.78kb downstream
					ABCA9	55.9kb downstream
	rs736609926	2	127627362	4.27E-07	*CPQ*	Within
AFW		18			*SLC16A6*	64.2kb-65.29kb downstream
					ARSG	67.89kb downstream
					WIPI1	25.27kb downstream
	rs13508784		7943847	2.63E-09	*PRKARIA*	0.354kb-0.358kb downstream
					FAM20A	1.305kb-2.308kb Upstream
					ABCA8	32.78kb Upstream

using pedigree-based connections (De *et al.*, 2015; Tsai *et al.*, 2016).

After a series of analyses, including GWAS, linkage disequilibrium analysis, and association analysis between SNP marker genotypes and AF, two SNPs involved in eight valuable genes (*SLC16A6*, *ARSG*, *WIPI1*, *PRKARIA*, *FAM20A*, *ABCA8*, *ABCA9*, and *CPQ*) were detected as significant markers in the present study.

A previous study reported that the *SLC16A6* transporter may be a determinant of adult height (Santhosh *et al.*, 2019). In adult *SLC16A6* animals, research revealed a molecular mechanism for this selective diversion of carbon atoms to fatty acyl chains (and into triacylglycerol) but not into cholesterol (Karanth *et al.*, 2013; Santhosh *et al.*, 2019). This evidence suggests that *SLC16A6* is crucial in increasing AF by influencing lipid metabolism. *ABCA1* is a protein that plays a significant role in HDL biosynthesis and cellular cholesterol homeostasis (Oram., 2000). The tissue distribution of *ABCA1* is ubiquitous, but its activity in hepatocytes and enterocytes is primarily responsible for plasma HDL production (Timmins *et al.*, 2005; Brunham *et al.*, 2006). Several genes, including *ABCA1* and *ABCA8,* have been identified in patients with reduced HDL-c (Trigueros-Motos *et al.*, 2017). *ABCA1* dysfunction in chickens results in increased intracellular cholesterol ester accumulation in the liver and intestine, suggesting that these issues are a significant source of HDL lipids (Mulligan *et al.*, 2003). Therefore, these genes play regulatory roles in fat metabolism.

ARSG is a recently identified lysosomal sulfatase that was responsible for the degradation of 3-O-sulfated N-sulfoglucosamine residues of heparan sulfate glycosaminoglycans. Deficiency of *ARSG* leads to a new type of mucopolysaccharidosis, as described in a mouse model. *WIPI1* was first discovered for its role in nascent autophagosome formation and subsequently implicated in canonical and noncanonical autophagy pathways (Proikas *et al.*, 2015). There have been research reports that point to a role for *PRKAR1A* in pituitary tumorigenesis in CNC, and suggest the possibility of *PRKAR1A*'s involvement in endocrine-mesenchymal pituicyte interactions in this process (Bossis *et al.*, 2004). *FAM20A* is believed to be a pseudokinase and does not have kinase activity itself. Still, it can form a complex with *FAM20C* and enhance *FAM20C*'s kinase activity to phosphorylate secreted proteins within the secretory pathway (Cui *et al.*, 2015). Research demonstrates that the molecular structure of *ABCA9* is highly similar to those of *ABCA8* and *ABCA6*, respectively, and provides evidence for sterol-dependent regulation of *ABCA9* in human macrophages (Piehler *et al.*, 2002). A previous study could identify particular alleles of the carboxypeptidase Q (CPQ) gene that would specifically confer resistance to ascites in a gender-biased manner (Dey *et al.*, 2018). Although ARSG, WIPI1, PRKAR1A, FAM20A, and ABCA9 have not been reported to influence the AF traits of chicken, this evidence suggests it is vital to chicken growth.

CONCLUSIONS

The present study discovered two SNPs in eight genes using genome-wide association based on the SLAF-seq technology. All these genes have essential biological functions that might regulate the AF of Wenshang Barred chickens. Further investigation is necessary to determine how these genes influence AF. The findings of the present study provide new insight into the molecular mechanisms underlying AF traits, which will improve the use of Wenshang Barred chickens and other Chinese chicken breeds.

AUTHOR CONTRIBUTIONS

W L, HM and FW conceived the study project, analyzed the microarray data, and prepared the manuscript. QX, DG, DP, and HX contributed to the data analysis and preparation of the manuscript. JL, YZ, WJ, and JB provided help in sample collection. WL and FW discussed the manuscript and contributed to data interpretation. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

All experiments were approved by the Animal Care Committee of the Academy of Agricultural Sciences, Shandong Province, Ji'nan, China. The care and use of experimental animals were carried out in accordance with the Directory Proposals on the Ethical Treatment of the Experimental Animals, established by the Ministry of Science and Technology (Beijing, China).

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