Important candidate genes for abdominal fat content identified by linkage disequilibrium and fixation index information

Wei Na,[*,†,‡] Jia-Qiang Yu,[*,†,‡] Zi-Chun Xu,[*,†,‡] Xin-Yang Zhang,[*,†,‡] Li-Li Yang,[*,†,‡] Zhi-Ping Cao,[*,†,‡] Hui Li,[*,†,‡] and Hui Zhang[*,†,‡,1]

[*]*Key Laboratory of Chicken Genetics and Breeding, Ministry of Agriculture and Rural Affairs;* [†]*Key Laboratory of Animal Genetics, Breeding and Reproduction, Education Department of Heilongjiang Province; and* [‡]*College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, P. R. China*

ABSTRACT Selection for rapid growth in chickens has always been accompanied by increased fat deposition and excessive fat deposition, especially abdominal fat, cannot only decrease feed efficiency but also cause many diseases. Finding the candidate genes associated with abdominal fat deposition is essential for breeding. To identify these candidate genes, we applied linkage disequilibrium and selection signature analysis using chicken 60 k single nucleotide polymorphism (SNP) chips in two broiler lines divergently selected for abdominal fat content for 11 generations. After quality control, 46,033 SNPs were left for analysis. Using these SNPs, we found that r^2 was 0.06 to 0.14 in the lean line and 0.07 to 0.13 in the fat line for all 28 chromosomes (except GGA16). Pairwise SNP distances <25 kb showed a mean $r^2 = 0.33$ in the lean line and $r^2 = 0.32$ in the fat line. The fixation index (F_{ST}) analysis was carried out and 46 SNPs with the top 0.1% of the F_{ST} value was detected as the loci with selection signatures. Besides F_{ST}, hapFLK was also used to detect selection signatures for abdominal fat content. A total of 11 genes, including transient receptor potential cation channel subfamily C member 4, estrogen related receptor gamma, fibroblast growth factor 13, G-protein-signaling modulator 2, RAR related orphan receptor A, phospholipase A2 group X, mitochondrial ribosomal protein L28, metadherin, calcitonin receptor like receptor, serine/threonine kinase 39, and nuclear factor I A, were detected as the important candidate genes for abdominal fat deposition based on their basic functions. The results of the present study may benefit the understanding of genetic mechanism of abdominal fat deposition in chicken.

Key words: chicken, abdominal fat, linkage disequilibrium, F_{ST}, hapFLK

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INTRODUCTION

The chicken (*Gallus gallus*) is an important model organism that bridges the evolutionary gap between mammals and other vertebrates (International Chicken Genome Sequencing Consortium, 2004). Selection for rapid growth in chickens has always been accompanied by an increased fat deposition (Havenstein et al., 1994; Nones et al., 2006). Excessive fat deposition can decrease feed efficiency and cause consumer rejection of the meat (Kessler et al., 2000), and increase difficulties in meat processing (Chambers, 1990). Knowledge of the genetic factors associated with fatness will facilitate genetic selection using genetic markers. Linkage disequilibrium (**LD**) is the nonrandom association of alleles at two or more loci (Qanbari et al., 2010). Generally speaking, selection could affect the extent of disequilibrium (Ardlie et al., 2002). The allele frequency that related with a favored variant had important effects on the traits of interest can rapidly increase or even fix after several generations of selection (Parsch et al., 2001; Verrelli and Eanes, 2001; Wang et al., 2002). There are several methods to detect this kind of selection signatures in domestic animals, including fixation index (**F_{ST}**), which was originally proposed by Wright (1922). F_{ST} approach was most frequently used in comparing between two or more breeds to detect selection signatures (Flori et al., 2009; Kijas et al., 2009; MacEachern et al., 2009; Pintus et al., 2014). F_{ST} core could also be used as an external source of information in genomics selection analysis (Chang et al., 2018). In the present study, the Northeast Agricultural University broiler lines divergently selected for abdominal fat content (**NEAUHLF**) for 11 yr was used. Therefore, we proposed that the structure of the genome may affected by the selection for abdominal fat content and the regions containing single nucleotide polymorphism (**SNPs**) with a high F_{ST} value may harbor genes that important for abdominal fat deposition. The aims of

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[1]Corresponding author: huizhang@neau.edu.cn

the present study were to construct a high-resolution LD map of broilers and to detect the important genes for abdominal fat deposition in chicken based on the LD and F_{ST} results.

MATERIALS AND METHODS

Ethics Statement

All animal work was conducted according to the guidelines for the Care and Use of Experimental Animals established by the Ministry of Science and Technology of the People's Republic of China (approval number: 2006–398), and approved by the Laboratory Animal Management Committee of Northeast Agricultural University.

Animals

In total, 475 birds (203 from the lean line and 272 from the fat line) from the eleventh-generation population of NEAUHLF were used. Detailed information about NEAUHLF has been previously published (Zhang et al., 2012). After 11 generations of divergent selection for abdominal fatness, the abdominal fat percentage of the fat broiler line at 7 wk was 3.59 times higher than the lean line. All birds were kept in similar environmental conditions and had free access to feed and water.

Genotyping and Quality Control

Genomic DNA samples were extracted from blood using a standard phenol/chloroform method, and DNA sample quality was determined using spectrophotometry and agarose gel electrophoresis. Illumina chicken 60 k SNP chip containing 57,636 SNPs was used. Genotyping data were generated using BeadStudio (Version 3.2.2). We used only SNPs with assigned positions on autosomes (galGal 5). The SNPs were filtered with the call rate <95%, and individuals with pedigree error or 5% or more missing SNP genotypes were removed.

LD Estimation

Pairwise r^2 estimation was used to measure LD between pairs of SNPs using Haploview v4.1 (Barrett et al., 2005) for SNPs on autosomal chromosomes 1–28 that had passed the quality controls described above. The r^2, defined as squared correlation coefficient of alleles at two loci, was measured (Lu et al., 2012). To visualize LD patterns in the two lines, r^2 values were placed in ascending order based on physical distances between corresponding SNP pairs. A rolling average of LD was calculated as the arithmetic mean of all r^2 values for SNP pairs in 25-kb intervals and plotted against physical distance between SNPs. The average r^2 of the lean and fat lines was compared using t-test, and $P < 0.05$ means significant different.

Genome Scans for Selection Signatures Using FST and hapFLK

In the present study, the Genepop v4.2 was used to calculate F_{ST} using the 46,033 SNPs in the lean and fat lines. The top 0.1% and 1% of the SNPs according to the F_{ST} value were selected as harbor selection signatures at two threshold levels.

The hapFLK software, which could potentially account for population structure and reduce the number of false positives, was also used to detect selection signatures for abdominal fat deposition (Fariello et al., 2013; Gholami et al., 2015). The number K of haplotype clusters was set to 10 according to cross-validation procedure implemented in the fastPHASE software. Significant level was adjusted by false discovery rate (**FDR**), which was calculated using the formula $\mathrm{FDR} = m^* P(i) i$, where m was the total number of tests and $p(i)$ was the P-value at rank i when the P-values were ranked from least to highest (Benjamini and Hochberg 1995; Weller et al., 1998).

Annotated genes related to the SNPs identified with selection signatures were obtained from the UCSC Genome Browser Gateway (http://genome.ucsc.edu/) using the chicken genome galGal 5. Functional annotation of genes was performed using DAVID bioinformatics resources 6.8 (http://david.abcc.ncifcrf.gov/summary.jsp) for Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathway analysis. Statistical significance was determined using a P-value < 0.05.

RESULTS

Marker Statistics

The number of SNPs that remained after quality control that were used in subsequent analyses was 46,033 for the lean and fat lines (Table 1). These SNPs were distributed on 28 autosomes and covered about 950 Mb with an average SNP interval of 23 kb.

Distributions of minor allel frequency (**MAF**) for SNPs for the lean and fat lines are shown in Figure 1. More than 60% of SNPs for both lines had a MAF > 0.2.

Table 1. Characteristics of marker panels used in lean and fat lines.

Single nucleotide polymorphism (SNP) marker information	Lean line	Fat line
Number of SNPs used	46,033	46,033
Genome coverage (Mb)	950.43	950.95
Mean adjacent marker spacing (kb)	23.75	23.17
Mean MAF	0.25	0.26
Mean F_{ST}	0.12	

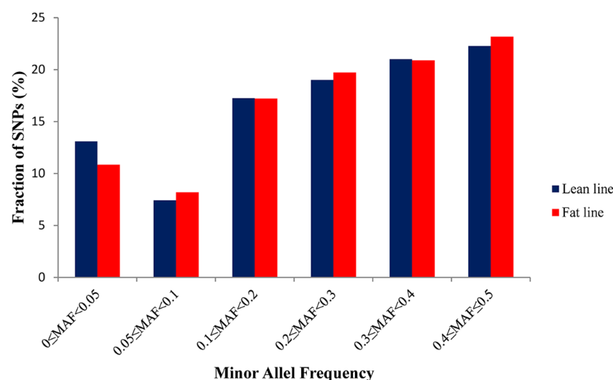

Figure 1. Distribution of MAF of single nucleotide polymorphism (SNPs) after quality control for the lean and fat lines.

Table 2. Average r^2 for each chromosome.

Chromosome	r^2 Lean line	r^2 Fat line
1	0.13	0.13
2	0.14	0.12
3	0.14	0.11
4	0.14	0.12
5	0.13	0.12
6	0.12	0.10
7	0.13	0.12
8	0.12	0.11
9	0.11	0.10
10	0.11	0.11
11	0.15	0.11
12	0.13	0.10
13	0.12	0.11
14	0.11	0.11
15	0.11	0.09
16	0.67	0.19
17	0.09	0.08
18	0.09	0.09
19	0.07	0.08
20	0.10	0.11
21	0.10	0.07
22	0.08	0.08
23	0.08	0.07
24	0.08	0.08
25	0.10	0.07
26	0.06	0.08
27	0.07	0.07
28	0.07	0.07

The lean and fat lines had similar patterns of MAF distributions.

Linkage Disequilibrium

The extent of LD in the lean and fat lines was examined using Haploview software. LD was calculated as r^2 for the two lines. In the lean line, r^2 ranged from 0.06 to 0.14 for every chromosome except GGA16 ($r^2 = 0.67$), which contained few SNPs. In the fat line, r^2 ranged from 0.07 to 0.13 for every chromosome except GGA16 ($r^2 = 0.19$), which also contained few SNPs (Table 2).

LD patterns were similar for the lean and fat lines. In both lines, LD declined as the distance between markers increased (Figure 2). The relationship between LD and physical distance of SNPs in the lines is in Table 3. In

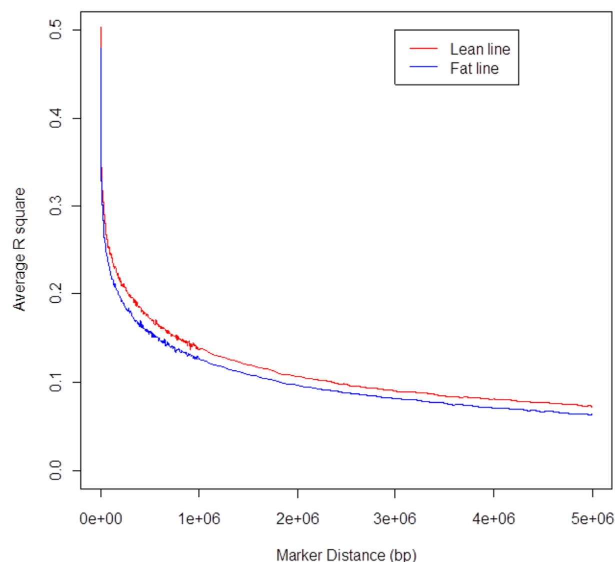

Figure 2. Decay of linkage disequilibrium (LD) with distance over the entire genome.

Table 3. Comparison of linkage disequilibrium (LD) strength vs. physical distance.

Distance (kb)	r^2 Lean line	r^2 Fat line
<25	0.33	0.32
25–50	0.29	0.27
50–75	0.26	0.24
75–100	0.25	0.23
100–200	0.23	0.21
200–500	0.19	0.17
500–1500	0.14	0.13
1500–3000	0.10	0.09
3000–5000	0.08	0.07

the lean line, mean $r^2 = 0.33$ was observed for pairwise distances <25 kb, dropping to $r^2 = 0.25$ for 75 to 100 kb. In the fat line, overall mean $r^2 = 0.32$ was observed for SNPs <25 kb apart, dropping to 0.23 at 75 to 100 kb (Table 3). Overall, the fat line had significant lower LD than the lean line (Table 3), indicating that selection led to different changes in genetic structure in the lean and fat lines.

Genome Scans for Selection Signatures Using FST and hapFLK

F_{ST} was estimated between the two lines using the common 46,033 SNPs after quality control (Figures 3 and 4). Overall, the average F_{ST} was 0.12, suggesting substantial genetic differentiation between the lean and fat lines. There were 460 SNPs with top 1% F_{ST} scores detected (Table S1, Supplementary Information). The 46 SNPs with the top 0.1% F_{ST} value were identified as the selection signatures (Table 4). The nearest genes of these 46 SNPs were detected and a total of 37 genes were identified (Table 4). Three Gene Ontology terms, including heterophilic cell–cell adhesion via plasma membrane cell adhesion molecules, intercalated

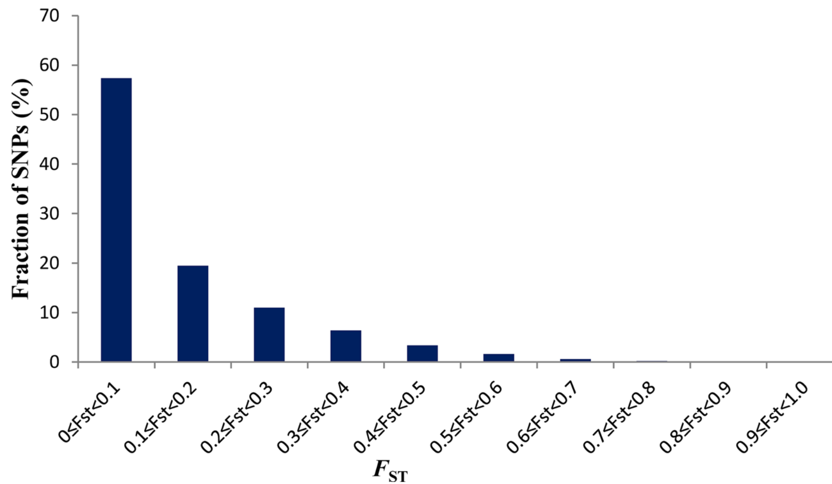

Figure 3. Distribution of F_{ST} scores in the lean and fat lines.

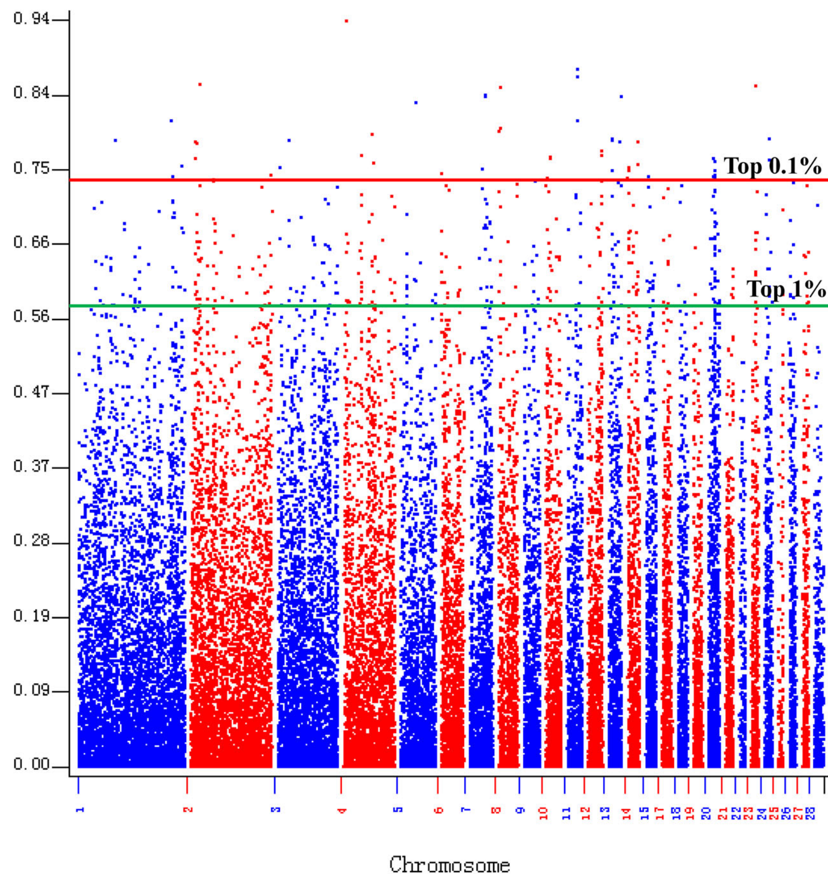

Figure 4. Manhattan plot of F_{ST} value between the lean and fat lines. The red and green lines means F_{ST} scores at top 0.1% and top 1% levels, respectively.

disc, and calcium ion binding, reached the significant level ($P < 0.05$). The Kyoto Encyclopedia of Genes and Genomes pathway, regulation of actin cytoskeleton, reached the significant level ($P < 0.05$). Seven genes, including transient receptor potential cation channel subfamily C member 4 (***TRPC4***), estrogen related receptor gamma (***ESRRG***), fibroblast growth factor 13 (***FGF13***), G-protein-signaling modulator 2 (***GPSM2***), RAR related orphan receptor A (***RORA***), phospholipase A2 group X (***PLA2G10***), and mitochondrial ribosomal protein L28 (***MRPL28***), were detected as the important candidate genes for abdominal fat deposition in chicken based on their basic functions.

The hapFLK was also used to detect selection signatures and a total of 23 SNPs reached the FDR < 0.05 level (Figure 5); however, these SNPs were not identified by F_{ST} analysis. The nearest genes of these 23 SNPs were detected and a total of 20 genes were

Table 4. A total of 46 SNPs with the top 0.1% F_{ST} value and the nearest genes.

SNPs	Chr	Position	F_{st}	Nearest gene	Gene_position
Gga_rs13881527	1	67,479,454	0.79	*RASSF8*	chr1:67,608,332–67,626,872
GGaluGA055731	1	171,747,812	0.81	*TRPC4*	chr1:171,847,349–171,936,293
GGaluGA062629	1	190,848,717	0.75	*WASL*	chr1:190,601,144–190,603,566
Gga_rs14136449	2	9,139,060	0.78	*PTPRN2*	chr2:8,821,479–9,227,096
Gga_rs14136834	2	9,485,744	0.76	*NCAPG2*	chr2:9,476,880–9,502,645
GGaluGA133725	2	12,538,017	0.78	*FZD8*	chr2:12,831,550–12,834,103
Gga_rs14145514	2	17,766,808	0.86	*COMMD3*	chr2:17,759,601–17,762,528
GGaluGA173682	2	146,676,331	0.74	*PTP4A3*	chr2:146,364,086–146,370,748
Gga_rs14257243	2	146,790,299	0.74	*PTP4A3*	chr2:146,364,086–146,370,748
Gga_rs14309924	3	3,850,143	0.75	*CFAP61*	chr3:3,832,234–3,919,806
Gga_rs14323526	3	20,357,481	0.79	*ESRRG*	chr3:19,997,648–20,358,375
GGaluGA243716	4	4,737,670	0.94	*FGF13*	chr4:4,777,547–5,001,590
Gga_rs16382928	4	31,042,985	0.77	*HHIP*	chr4:31,101,428–31,170,692
GGaluGA258424	4	49,999,945	0.79	*SEPT11*	chr4:49,984,873–50,010,401
Gga_rs15579302	4	52,578,552	0.76	*FAT4*	chr4:52,850,935–52,987,324
Gga_rs14524001	5	24,477,980	0.83	*INO80*	chr5:24,449,297–24,509,836
GGaluGA293376	6	1,259,178	0.74	*CCSER2*	chr6:966,437–1,018,487
Gga_rs14613595	7	19,684,964	0.75	*SCN4A*	chr7:19,601,197–20,062,564
Gga_rs14618849	7	25,335,729	0.84	*MRAS*	chr7:25,370,032–25,403,821
Gga_rs14618860	7	25,340,862	0.84	*MRAS*	chr7:25,370,032–25,403,821
GGaluGA322085	8	1,322,382	0.80	*GPSM2*	chr8:1,316,627–1,333,182
GGaluGA322243	8	1,921,864	0.80	*MIR181A1*	chr8:2,005,169–2,005,272
Gga_rs15895615	8	1,987,987	0.85	*MIR181A1*	chr8:2,005,169–2,005,272
Gga_rs14943917	10	4,689,608	0.76	*RORA*	chr10:4,626,225–4,975,286
Gga_rs15569316	10	5,739,811	0.77	*MCEE*	chr10:5,733,315–5,743,137
Gga_rs14965369	11	13,417,788	0.87	*MIR6595*	chr11:13,466,650–13,466,759
Gga_rs13613944	11	13,426,811	0.87	*MIR6595*	chr11:13,466,650–13,466,759
Gga_rs15619912	11	13,508,154	0.87	*MIR6595*	chr11:13,466,650–13,466,759
Gga_rs14965441	11	13,571,160	0.81	*MIR6595*	chr11:13,466,650–13,466,759
GGaluGA089576	12	18,696,098	0.77	*EDEM1*	chr12:18,660,231–18,670,248
Gga_rs15672675	12	18,713,152	0.77	*EDEM1*	chr12:18,660,231–18,670,248
Gga_rs14054257	13	4,829,719	0.79	*TENM2*	chr13:4,820,043–5,306,069
Gga_rs14990229	13	5,194,596	0.79	*TENM2*	chr13:4,820,043–5,306,069
Gga_rs14990174	13	5,292,872	0.75	*TENM2*	chr13:4,820,043–5,306,069
Gga_rs15707857	13	16,155,589	0.78	*C13H5orf15*	chr13:16,135,944–16,141,737
Gga_rs14065195	13	17,196,636	0.84	*AFF4*	chr13:17,168,014–17,216,698
GGaluGA098657	14	843,034	0.75	*PLA2G10*	chr14:838,542–848,893
Gga_rs14069730	14	1,612,717	0.75	*FAM20C*	chr14:1,727,496–1,779,810
Gga_rs13815452	14	11,682,756	0.78	*GSG1L*	chr14:11,877,677–11,919,087
GGaluGA104264	14	12,185,673	0.76	*MRPL28*	chr14:12,187,984–12,191,135
Gga_rs16165606	20	5,598,435	0.76	*R3HDML*	chr20:5,594,252–5,596,876
Gga_rs14274693	20	7,122,530	0.75	*Fam217b*	chr20:7,064,875–7,065,282
Gga_rs16167541	20	7,252,909	0.76	*CDH4*	chr20:7,427,589–7,840,327
Gga_rs14290576	23	3,613,622	0.85	*EPHA10*	chr23:3,602,279–3,630,295
GGaluGA192499	24	3,854,003	0.76	*OAF*	chr24:3,833,146–3,843,905
Gga_rs15221004	24	3,893,861	0.79	*TRIM29*	chr24:3,869,163–4,216,162

identified (Table 5). The basic functions of these genes indicated that metadherin (***MTDH***), calcitonin receptor like receptor (***CALCRL***), serine/threonine kinase 39 (***STK39***), and nuclear factor I A (***NFIA***) may be important for abdominal fat deposition in chicken.

DISCUSSION

We constructed a genome-wide LD map of lean and fat broiler lines divergently selected for abdominal fat content. The genotyping data were from chicken 60k SNP chips covering 28 autosomal chromosomes. LD was calculated using 28 of 38 chicken autosomal chromosomes represented on chips. The 10 autosomes not used for analysis were microchromosomes not included in the design of the 60k SNP chip, as they are not yet covered by the genome build *Gallus gallus* v2.1 (Groenen et al., 2011). SNPs on linkage groups, sex chromosomes, and unknown marker positions were excluded from analysis. SNPs with MAF < 0.05 were also excluded from LD analysis in accordance with other studies (Qanbari et al., 2010; Fu et al., 2015). The high density of markers and the large number of animals in this study gave a better estimate of genome-wide LD.

We determined that different chromosomes had different LD, ranging from 0.06 to 0.14 in the lean line and from 0.07 to 0.13 in the fat line (except GGA16). The chromosomal difference in LD supports observations from other studies (Axelsson et al., 2005; Andreescu et al., 2007; Qanbari et al., 2010; Fu et al., 2015; Khanyile et al., 2015). The difference in LD for different chromosomes may be because of evolutionary, natural, and artificial selection (Axelsson et al., 2005; Megens et al., 2009). Our results showed a significant LD decay with increased marker interval: $r^2 = 0.33$ in the lean line and $r^2 = 0.32$ in the fat line in pairwise distances < 25 kb dropped to $r^2 = 0.25$ for the lean line and $r^2 = 0.23$ for the fat line at 75 to 100 kb; this is

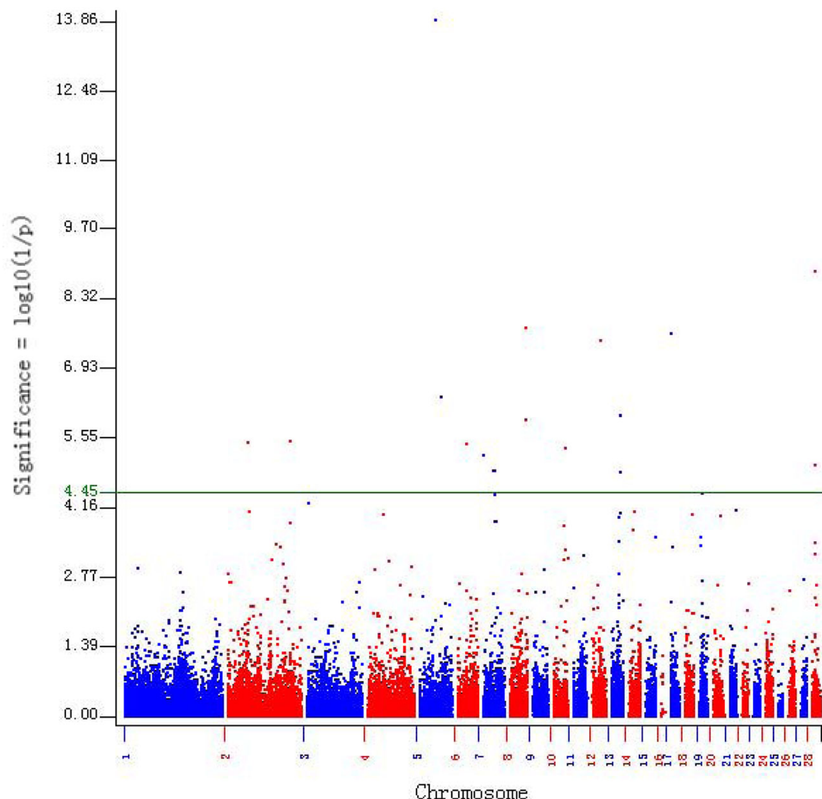

Figure 5. Manhattan plot of hapFLK result in the lean and fat lines. The green line mean false discovery rate (FDR) < 0.05 level.

Table 5. A total of 23 SNPs detected by hapFLK analysis and the nearest genes.

SNPs	Chr	Position	*P*-value	Nearest_gene	Gene_position
Gga_rs15962679	2	41,566,664	3.59E-06	*METTL6*	chr2:41,549,975–41,556,164
GGaluGA168014	2	127,803,767	3.33E-06	*MTDH*	chr2:127,839,203–127,883,278
Gga_rs14527165	5	27,745,682	1.37E-14	*SMOC1*	chr5:27,724,356–27,795,694
Gga_rs14535900	5	39,248,439	4.49E-07	*TMED8*	chr5:39,245,129–39,250,750
Gga_rs16548338	6	17,197,430	3.76E-06	*PAX2*	chr6:17,099,350–17,178,107
Gga_rs15825209	7	1,175,317	6.19E-06	*CALCRL*	chr7:1,121,860–1,148,432
Gga_rs14611806	7	17,826,186	1.24E-05	*ITGA6*	chr7:17,799,938–17,838,370
Gga_rs15854475	7	17,967,155	1.24E-05	*HAT1*	chr7:17,979,544–17,997,798
Gga_rs14612642	7	18,754,459	1.24E-05	*FASTKD1*	chr7:18,741,873–18,753,403
GGaluGA314742	7	19,182,027	1.24E-05	*STK39*	chr7:19,181,467–19,265,132
GGaluGA314767	7	19,243,329	1.24E-05	*STK39*	chr7:19,181,467–19,265,132
GGaluGA332283	8	27,283,865	1.23E-06	*NFIA*	chr8:27,133,855–27,365,767
GGaluGA332288	8	27,297,098	1.83E-08	*NFIA*	chr8:27,133,855–27,365,767
Gga_rs15588563	10	16,591,461	4.6E-06	*MIR1813–2*	chr10:16,597,656–16,597,729
Gga_rs14042969	12	12,919,551	3.22E-08	*PTPRG*	chr12:12,750,103–13,124,192
Gga_rs14741569	13	13,618,657	1.05E-06	*RUFY1*	chr13:13,595,281–13,608,782
GGaluGA096397	13	13,657,155	1.05E-06	*ADAMTS2*	chr13:13,665,209–13,805,685
Gga_rs14998681	13	13,710,332	1.05E-06	*ADAMTS2*	chr13:13,665,209–13,805,685
GGaluGA096558	13	14,075,053	1.35E-05	*RASGEF1C*	chr13:14,051,308–14,115,158
Gga_rs14104760	17	1,265,213	2.39E-08	*EXD3*	chr17:1,149,946–1,308,298
Gga_rs15034738	17	1,533,422	2.39E-08	*LOC100216141.S*	chr17:1,534,629–1,539,805
Gga_rs14305753	28	2,069,358	9.52E-06	*DOT1L*	chr28:2,040,052–2,094,693
Gga_rs13545963	28	2,122,854	1.38E-09	*AP3D1*	chr28:2,096,479–2,123,705

generally a function of increased recombination events with increased genetic distance (Megens et al., 2009). The decay in LD showed a clear exponential trend with physical distance typically found in other data sets and in agreement with previous results (Heifetz et al., 2005; Aerts et al., 2007; Andreescu et al., 2007; Abasht et al., 2009; Megens et al., 2009; Qanbari et al., 2010; Fu et al., 2015; Khanyile et al., 2015). In the present study, LD was moderately high and remained well above 0.2 at marker distances of up to 200 kb when using genome-wide SNP data. This result indicated that LD of the broilers we used remained high over long distances. In contrast, LD decayed to relatively lower values of about 0.07 at marker distances of up to 5 Mb. The relatively high average LD that started at very short marker distances of 25 kb and persisted over long

distances could reflect artificial selection for abdominal fat content for more than 10 yrs. The decay of LD in a genome determines the resolution of quantitative trait locus (**QTL**) detection in QTL mapping studies, in particular fine-mapping studies. LD for chromosomes that extends over large genomic regions leads to a higher chance of finding associations between genes affecting a particular phenotype and a marker at a given distance. Our study showed an average $r^2 > 0.3$ for distances of <25 kb in both the lean and fat lines and a SNP density of one SNP per 23 kb. This result implied that the 60 k SNP chip used had a density that may be adequate for GWAS to identify genes important for traits of interest. However, for populations for which no selection is implemented, LD analysis should be carried out to determine if marker density is sufficient for GWAS. We found an average $r^2 > 0.3$ for distances < 25 kb in both the lean and fat lines. This result indicated that detected LD extent in the fat and lean lines was longer than other studies in both layer and broiler chickens. For example, using the same chicken 60 k SNP chip, Fu et al. found an average LD (measured by r^2) between SNPs markers of 0.24 in crossbred populations (2015). The higher LD in the present study indicated that selecting for abdominal fat content for more than 10 yr could affect the genome structure of chickens. This result indicated that the LD regions could harbor important genes or other elements for abdominal fat-related traits.

The selection for abdominal fat content of the population used in the present study may affect the LD structure and we supposed that the chromosome regions that harbor important genes for abdominal fat deposition may showed significant difference in allele or haplotype frequencies. Therefore, in the present study, F_{ST} and hapFLK were used to detect the important genes for abdominal fat content, and the results showed that a total of 11 genes, including *TRPC4*, *ESRRG*, *FGF13*, *GPSM2*, *RORA*, *PLA2G10*, *MRPL28*, *MTDH*, *CALCRL*, *STK39*, and *NFIA*, would be the candidate genes for abdominal fat deposition based on their basic functions. The *TRPC4* gene encodes a member of the canonical subfamily of transient receptor potential cation channels. *TRPC4* was differentially expressed in preadipocytes and adipocytes suggesting its significance in adipogenesis (Bishnoi et al., 2013). It was also differentially expressed in white and brown adipose tissues (Bishnoi et al., 2013). *ESRRG* belongs to an orphan nuclear receptor subfamily, which expressed mainly in brain, heart, kidney, skeletal muscle, and brown adipose tissue (Mangelsdorf et al., 1995; Heard et al., 2000). The previous results indicated that *ESRRG* could enhance the expression of uncoupling protein 1, and improve fatty acid oxidation in differentiating white pre-adipocytes and/or brown adipose tissue (Dixen et al., 2013). *FGF13* belongs to the fibroblast growth factor (FGF) family, which was a candidate obesity gene (Morton et al., 2011). The result of a genome wide association study identified that there was a locus, including three SNPs (rs193139, rs7523050, and rs1761621), near *GPSM2* and syntaxin-binding protein 3 were significantly associated with the lower body subcutaneous adipose tissue depots (Irvin et al., 2011), which mean that *GPSM2* may have an important effect on lipid metabolism. *RORA* can inhibit the differentiation of adipocyte (Duez et al., 2009) and also can regulate the expression level of gene that controls lipid metabolism in diet-induced obesity mice (Lau et al., 2008). *PLA2G10* significantly differently expressed between visceral adipose tissue and subcutaneous adipose tissue in human (Liesenfeld et al., 2015). In mice, there were 12 sex-dependent and also diet-dependent QTLs for dietary obesity on chromosome 7 and *MRPL28* was located in these QTL regions (Lin et al., 2013), which mean that *MRPL28* may be an obesity-related gene in mice. *MTDH*, also known as astrocyte elevated gene-1 (*AEG-1*), and *AEG-1* knock-out (AEG-1KO) mouse were significantly leaner with prominently less body fat compared with wild type (Robertson et al., 2015). The wild-type mice could rapidly gain weight when fed a high fat and cholesterol diet; however, AEG-1KO mice did not gain any weight, because the mice could decrease intestines fat absorption (Robertson et al., 2015). *CALCRL* was identified as a candidate gene in the etiology of obesity by using genome-wide expression analysis in obese prepubertal children visceral adipose tissue (Aguilera et al., 2015). *STK39* was suggested as one candidate gene for hypertension and obesity (Kim et al., 2012; Torre-Villalvazo et al., 2018). *NFIA* was identified as a transcriptional regulator of brown fat and exerted its effects by co-localizing with peroxisome proliferator-activated receptor gamma at cell-type-specific enhancers (Hiraike et al., 2017).

In our previous study, long-range allele frequency differences between the lean and fat lines were mainly used to detected selection signatures (Zhang et al., 2012). The long-range AFD and heterozygosity were measured using 0.5 Mb sliding windows of SNPs as the genome length, which was rough and only 10 genomics regions were detected as harboring important genes for abdominal fat content on the genome-wide level (Zhang et al., 2012). In the present study, we carried out all analysis based on every single SNP, which may obtain much more precise results and we aim to capture some other genes important for abdominal fat deposition compared with our previous study. F_{ST} statistic was most widely used approach to detect loci with outstanding genetic differentiation between different populations (Barreiro et al. 2008; Myles et al. 2008). And hapFLK statistic was a haplotype-based extension of the FLK statistic that accounts for both hierarchical structure and haplotype information (Fariello et al., 2013). The present study used both F_{ST} and hapFLK to detect selection signature and some genes were identified as important candidate genes for abdominal fat deposition in chicken. However, there was no overlap between the results of F_{ST} and hapFLK, which indicated that using different methods, single marker test for F_{ST} and haplotype

based for hapFLK, may capture different aparts of the whole selection signature map. Therefore, different methods were needed to get the details of selection signatures map for chicken abdominal fat content.

In summary, this was a comprehensive study of LD based on high-density SNP panels and two broilers lines divergently selected for abdominal fat content. An average $r^2 > 0.3$ for a distance of <25 kb was detected for both the lean and fat lines. The average marker density of the chicken 60k SNP chip was about 23 kb/SNP. Therefore, using a high-density SNP chip for association mapping or implementing genomic selection in lean and fat lines could achieve comparable resolution and accuracy. 11 genes, including *TRPC4*, *ESRRG*, *FGF13*, *GPSM2*, *RORA*, *PLA2G10*, *MRPL28*, *MTDH*, *CALCRL*, *STK39*, and *NFIA*, were detected as important genes for abdominal fat deposition.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

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