

Analysis of Selection Signatures on the Z Chromosome of Bidirectional Selection Broiler Lines for the Assessment of Abdominal fat Content

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Abstract

Background: The discovery of selection signatures has enabled the identification of genomics regions under selective pressure, enhancing knowledge of evolutionary genotype-phenotypes. Sex chromosomes play an important role in species formation and evolution. Therefore, the exploration of selection signals on sex chromosomes has important biological significance.

Results: In this study, we used the Cross Population Extend Haplotype Homozygosity Test (XPEHH), F-statistics (Fst) and EigenGWAS to assess selection footprints on the Z chromosome in 474 broiler chickens via Illumina chicken 60K SNP chips. SNP genotype data were downloaded from publicly available resources. We identified 35 selection regions, amongst which 1, 9 and 27 were identified by XPEHH, Fst, and EigenGWAS, respectively. Each end of the Z chromosome appeared to undergo the highest levels of selection pressure. A total of 219 candidate genes were located in 35 selection regions, some of which mediated lipogenesis, fatty acid production, fat metabolism, and fat decomposition, including *FGF10*, *ELOVL7*, and *IL6ST*. Using abdominal adipose tissue expression data of the chickens, 198 candidate genes were expressed with 11 differentially expressed genes (DEGs) in fat vs. lean lines identified. Amongst the DEGs, *VCAN* was related to fat metabolism. GO pathway enrichment analysis and QTL annotations were performed to fully characterize the selection mechanism(s) of chicken abdominal fat content.

Conclusions: We have found some selection regions and candidate genes involving in fat metabolism on the Z chromosome. These findings enhance our understanding of sex chromosome selection signals.

Background

The domestication of chickens in Asia (*Gallus gallus*) occurred around 5,400 BC with Darwin suggesting their evolution from red jungle fowl [1, 2]. Chickens hold value from an evolutionary perspective as they provide information that bridges knowledge between mammals and other vertebrates [3]. Domestic chickens have genetically adapted to unique habitats through strong genetic and phenotypic alterations. To date, an array of specialized commercial populations and inbred chicken lines have subsequently been developed.

Selection has many effects on the genome. Allele frequencies and polymorphism underlying selection are expected to change. With the availability of high-quality draft sequences of the chicken genome, high-density single nucleotide polymorphism (SNP) genotyping chips, and whole-genome re-sequencing technologies, the detection of selection signatures on the chicken genome have been reported. Rubin et al. identified the *TSHR* gene (thyroid stimulating hormone receptor) as a prominent selection marker in all domestic chickens [4]. Guo et al. identified 413 candidate genes in Xishuangbanna fighting chickens that were related to aggressive behavior, including *BDNF*, *NTS* and *GNAO1* [5]. Boschiero et al. revealed ≥ 300 regions of selection with many important genes, including *AKAP6*, *IGFBP2* and *IGF1R*, associated with fat deposition and muscle development [6].

Sex chromosomes play an important role in species formation and evolution. Mcvicker et al. analyzed the selective forces that shape hominid evolution and found that under natural selection, the selection pressure of sex chromosomes (12% – 40%) exceeded those of the autosome (19% – 26%) [7]. The selection pressure of autosomes and sex chromosomes is different, and when considering sex-specific dosage compensation, genes on the sex chromosomes are more directly and efficiently selected than those on autosomes [8–10]. The size of the chicken Z chromosome is approximately 83 Mb, accounting for 7.9% of the chicken genome. The Z chromosome contains 1,345 genes, and some genes, including *FGF10* (fibroblast growth factor 10), *ELOVL7* (ELOVL fatty acid elongase 7) and *ACO1* (aconitase 1, soluble), regulated fat deposition and development. Previous studies have focused on the selection footprints of chicken autosomes, but the selection signals of the chicken Z chromosome less well studied. It is therefore necessary to identify selection sweeps on the Z chromosome in chickens.

In this study, we used the XPEHH, Fst and EigenGWAS methods to identify the selection signatures associated with abdominal fat in the Z chromosomes of broilers with divergent fat content from Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF). Through the integration of gene microarrays in the adipose tissue of NEAUHLF populations, we investigated the expression profiles of the candidate genes on selection regions at 7 weeks of age. Gene annotations and functional enrichment were implemented to elucidate the significance of the identified selection footprints to fat containing traits.

Results

Population structure

We performed principal component analysis (PCA) on 1937 SNPs on the Z chromosome to identify individual patterns. The first principal component (21.5% of the total variance) could separate the two lines (Fig. 1A). The second principal component (6.1% of the total variance) revealed genetic differences in the fat lines, whilst the third principal component (5.3% of the total variance) revealed differences in the lean lines (Fig. 1B).

Selection signatures on the Z chromosome

Chicken Z chromosome selection signatures were identified between fat and lean line populations. Table 1 summarizes the selection signals obtained using XPEHH, Fst and EigenGWAS. For the fat-lean line pair, 1, 9 and 27 selection regions were identified using XPEHH, Fst and EigenGWAS methods, respectively (Table 2 and Fig. 2). A total of 35 candidate regions were identified. The majority of the identified selection markers were present on both ends of the Z chromosome. Amongst them, XPEHH and EigenGWAS were identified with four overlapping and near candidate regions, whilst Fst and EigenGWAS identified five overlapping and near candidate regions. However and unexpectedly, those detected with Fst showed minimal overlap with XPEHH.

Table 1
 Selection signals in the two chicken lines.

Items	Fat - Lean		
	XPEHH	Fst	EigenGWAS
Number of significant SNPs	50	45	83
Number of regions	1	9	27
Average length (Mb)	2.24	0.70	0.76
Total length (Mb)	2.24	6.33	20.53

Table 2
Selection regions on the Z chromosome and candidate genes detected in the regions.

Method	Region (Mb)	Top SNP	Effect value	Candidate Genes
XPEHH	65.73–67.97	rs14776247	-2.7762	<i>AKAP2, SLC46A2, SNX30, INIP, KIAA1958, HSDL2, PTBP3, SUSL1, UGCG, C9orf84, GNG10, DNAJC25, KIAA0368, SMC2, PTGR1, TXN, SVEP1, MUSK, LPAR1, NPR1, MIR1452, MIR1459</i>
Fst	4.37–4.77	rs314369012	0.7226	-
	6.79–7.19	rs14689482	0.6864	<i>KIAA1328, TPGS2</i>
	19.79–20.19	rs14753903	0.7915	<i>HTR1A, RNF180, RGS7BP</i>
	20.01–22.11	rs16101791	0.8044	<i>MAST4, CD180, PIK3R1, SLC30A5, CENPH, MRPS36, CDK7, THBS4, MTX3</i>
	36.11–36.51	rs14694887	0.7650	-
	37.18–38.61	rs16111480	0.7721	<i>PCSK5, GCNT1, PRUNE2, FOXB2, VPS13A, GNAQ, CEP78, PSAT1, TLE4, TLE4Z1</i>
	51.05–51.71	rs316461367	0.7340	<i>CHD1Z, RGMB, RIOK2, LIX1, LNPEP</i>
	52.61–53.02	rs14769085	0.7009	<i>PCGF3, CHRNA7L, CHRNA8, TRABD2A</i>
EgienGWAS	56.25–57.68	rs14751538	0.7744	<i>PHAX, LMNB1, MEGF10, PRRC1, CTXN3, SLC12A2, FBN2, ERAP1, CAST, PCSK1, ELL2, GLRX, RHOBTB3, RFESD, ARSK, TTC37, FAM81B</i>
	1.91–2.31	rs312273884	2.24E-07	<i>LOXHD1, RNF165, CZH18ORF25, HAUS1, ATP5A1Z, PSTPIP2, SLC14A2</i>
	6.95–7.35	rs14689552	2.40E-05	<i>KIAA1328, TPGS2, AQP3, NOL6, UBE2R2</i>
	9.25–9.65	rs14784526	9.10E-07	<i>ARHGEF39, MRPL17, CCBE1, PDZD2, PMIP1</i>
	13.95–14.35	rs16103326	7.97E-06	<i>FGF10, MRPS30</i>
16.75–17.42	rs16760225	7.23E-06	<i>PPAP2A, SLC38A9, DDX4, IL6ST, ANKRD55, SKIV2L2, MAP3K1</i>	

Method	Region (Mb)	Top SNP	Effect value	Candidate Genes
	17.80–18.20	rs16099146	1.05E-06	<i>PLK2, RAB3C</i>
	18.71–19.11	rs736458094	1.18E-05	<i>DEPDC1B, ELOVL7, NDUFAF2, SMIM15, MIR1584</i>
	22.90–23.50	rs313728591	7.91E-08	<i>AP3B1, TBCA, OTP, WDR41, PDE8B, AGGF1, IQGAP2, CRHBP, S100Z, F2RL1, F2R, F2RL2</i>
	32.13–32.53	rs16108416	2.11E-05	<i>BNC2, MIR1779</i>
	33.03–33.63	rs314666735	1.66E-05	<i>SH3GL2, ADAMTSL1, HAUS6, PLIN2</i>
	39.07–39.87	rs16781643	3.03E-06	<i>TLE1, FRMD3</i>
	48.33–51.04	rs314662658	6.33E-06	<i>ST8SIA4, FAM174A, EFNA5, MIR6651</i>
	52.48–52.88	rs14768956	1.78E-06	<i>PCGF3, MFSD7, CHRNA8, CHRNA7L</i>
	53.37–53.77	rs14769714	2.52E-05	<i>PIGG, TMEM175, ATP5ME, PDE6B, RBP4, IDUA, SLC26A1, CHRNA6, CHRNB3, THAP1, RNF170, ATP5I, RBP4B</i>
	61.68–62.12	rs14774149	6.47E-07	-
	62.78–63.58	rs14773275	3.68E-07	<i>TMEM167A, ATP6AP1L, RPS23, ATG10, VCAN</i>
	63.88–64.28	rs16118182	2.81E-08	<i>CKMT2, DHFR</i>
	64.82–66.08	rs14774834	1.72E-07	<i>GRIN3A, ALDOB, MRPL50, RLPPR1, AKAP2, SLC46A2, SNX30, MIR7440, RNF20,</i>
	66.24–66.67	rs13788226	2.66E-07	<i>HSDL2, PTBP3, SUSD1, UGCG, GNG10, DNAJC25, KIAA0368, MIR1452, MIR1459, SMC2</i>
	66.84–67.88	rs16775262	1.59E-06	<i>SVEP1, MUSK, LPAR1, NPR1, TOPORS, ELAVL2</i>
	68.59–68.99	rs16776912	2.02E-08	<i>CAAP1, PLAA, IFT74, LRRC19, TEK, MOB3B, C9orf72, MIR7437</i>
	69.41–70.09	rs14780063	2.38E-05	-
	70.35–71.36	rs16125209	2.28E-06	<i>CORO2A, TBC1D2, IKBKAP, APTX, DNAJA1, SMU1, B4GALT1, SPINK4, HEMGN, XPA, NCBP1, TSTD2, TMOD1, TDRD7, ACO1</i>

Method	Region (Mb)	Top SNP	Effect value	Candidate Genes
	72.16–73.63	rs14781762	7.41E-09	<i>SEMA6A, COMMD10, LVRN, AP3S1, ATG12, CDO1, MARVELD2, GTF2H2, SMN, BDP1, MCCC2, KCNN2, TMED7</i>
	78.84–79.27	rs15992604	5.72E-09	<i>CDKN2A, CDKN2B, TRIM36, PGGT1B, CCDC112, FEM1C, ALDH7A1, GRAMD3, MIR31, MTAP</i>
	79.67–80.45	rs14685714	1.75E-07	<i>ZNF608</i>
	80.01–82.42	rs16683478	9.91E-09	<i>SRFBP1, LOX, SNCAIP, SNX2, SNX24, PPIC, PRDM6, CEP120, TRMT10B, DCAF10, POLR1E, ZBTB5, GRHPR, PAX5, MELK, MIR7483, SNCAIP</i>

Candidate gene annotations for functional analysis

According to the chicken gene annotation data (*Gallus gallus 6.0*) in the ENSEMBL database, we detected 219 candidate genes within 35 selection regions. Table 2 summarizes the genes in each selection region on the Z chromosome. A number of genes were found to regulate lipogenesis, fatty acid production, fat metabolism, or fat decomposition, including *FGF10*, *ELOVL7* and *IL6ST*.

To reveal the biological functions of the genes within the identified regions, gene Ontology (GO) pathway enrichment analyses were performed using DAVID (v6.7) [11]. Significant GO functional terms ($P < 0.05$) are listed in Table 3, but these terms were not significant upon Benjamini correction.

Table 3
Functional enrichment analysis of the selected genes.

Category	GO ID	Term	P value
Biological Progresses	GO:0043171	peptide catabolic process	0.0195
Cellular Components	GO:0005892	acetylcholine-gated channel complex	0.0231
	GO:0045211	postsynaptic membrane	0.0204
Molecular Function	GO:0070006	Metalloaminopeptidase activity	0.0154
	GO:0042166	acetylcholine binding	0.0230
	GO:0004889	acetylcholine-activated cation-selective channel activity	0.0230

Using the online quantitative trait loci (QTL) database of chickens, the selection signatures overlapped on 111 QTLs of health, physiology, exterior and production categories. Interestingly, 39 of the candidate

genes that overlapped with the QTL region were related to abdominal fat weight, 22 were related to liver weight, 7 were related to food intake, 11 to residual food intake, and 10 to food conversion ratios.

Expression profiles of the candidate genes in the selection regions

We extracted probe sets of the Gene Chip Chicken Genome Array to represent all candidate genes located on the selection regions. A total of 414 probes representing 198 candidate genes were identified. As shown in Figs. 3, 5 and 11 genes ($p < 0.05$) were only absent in lean and fat chicken lines at 7 weeks, respectively, whilst 11 DEGs ($P < 0.05$, fold change > 2) between fat and lean lines were identified. A total of 6 genes (*ADAMTSL1*, *FRMD3*, *MOB3B*, *ARHGEF39*, *VCAN* and *MELK*) were up-regulated and 5 (*HAUS6*, *MAST4*, *VPS13A*, *SPINK4* and *EFNA5*) were down-regulated in fat vs. lean lines. *MAST4* and *SPINK4* regulate serine metabolism; *MAST4* encodes a microtubule-associated serine/threonine kinase; *SPINK4* is a serine peptidase inhibitor; and *ARHGEF39* encodes a rho guanine nucleotide exchange factor key to Rho mediated signal transduction.

Discussion

High-density SNPs chips permit the identification of genome-wide selection signatures using site frequent spectrums, population differentiation, and linkage disequilibrium, with known strengths and weaknesses. In this study, we used three complementary statistical approaches (Fst, XPEHH, EigenGWAS) to explore the selection footprints on the Z chromosome to minimize bias and false positives in the broiler chickens. The Fst method is best suited for the detection of distant events [12]. The XPEHH test compares extended haplotype homozygosity between populations to detect selection footprints, which are segregated in populations and represent points of ongoing selection. XPEHH is therefore useful for the detection of entirely or approximately fixed loci [13]. The EigenGWAS algorithm combines the statistical framework of GWAS with eigenvector decomposition to identify selection footprints in the genomes of the underlying population. The EigenGWAS method uses multi-point information to identify core SNPs and grid windows, and can identify potential loci during selection, and a larger number of selection regions than Fst and XPEHH [14]. Due to the similarities and differences principles between Fst, XPEHH and EigenGWAS, there are differently selection regions can be obtained using the different statistical approaches.

Selection footprints determined by multiple methods are deemed more credible [15–17]. Zhang et al. has reported 55.43-56.16Mb regions of chromosome Z (including *PC1/PCSK1*, *FBN2*, *ELL2* genes) was identified as selection signature related on abdominal fat weight and percentage based on long-range heterozygosity changes or allele frequency differences methods using the same populations [18]. In this study, we also detected this region using the Fst method based on population differentiation (lean vs fat

lines). Furthermore, we identified another nine selection regions using two simultaneous algorithms. These novel selection regions will provide specific gene targets for the control of chicken fatness traits or other traits significantly genetically correlated with abdominal fat weight. For example, we identified 65–68 Mb regions detected by XPEHH and EigenGWAS, and 24 genes that overlapped with the region, including *DNAJC25*, *GNG10* and *AKAP2*. Interestingly, *DNAJC25* is a member of DNAJ gene family identified by Liu et al. as expressed in chicken liver tissue using transcriptome sequencing analysis [19]. The *DNAJB6* gene, located on gga2, is a member of the DNAJ gene family and has a similar sequence to the *DNAJC25* gene. Jin et al. identified *DNAJB6* expressed in the liver and adipose tissue of G14 NEAUHLF populations, with differential expression patterns between lines that negatively correlate with both abdomen fat weight and abdomen fat percentage [20].

To-date, majority of selection signatures or candidate genes has been identified in chickens. In this study, there are 219 candidate genes overlapped or near 35 selection regions on chromosome Z. Amongst the candidate genes, *IL6ST*, *ELOVL7*, *CKMT2* and *FGF10* genes were also identified by Gholami et al. in three commercial layer breeds and 14 non-commercial breeds [21]. The *VCAN*, *ST8SIA4*, *FBN2*, *ERAP1* and *CAST* were also identified by Fu et al., showing 10 regions of high confidence for selection on the Z chromosome, detected in male Cornish lines (a meat type breed), and female lines from White Rock (a dual-purpose breed) [22]. We also found that the majority of candidate genes expressed in the adipose tissue of G8 NEAUHLF fat and lean lines, and 11 genes (including *VCAN*, *EFNA5*, *ARHGEF39*, etc) in the adipose tissue significantly differed between fat vs. lean birds using microarray gene expression data.

According to known gene functions, some candidate genes were associated with the fat content of chickens, such as the *FGF10* gene. *FGF10* is a mesenchymal factor affecting epithelial cells. Matsubara et al. reported that *FGF10* when secreted in chicken adipose tissue contributes to adipose genesis, and is downregulated during the early stages of chicken adipocyte differentiation [23]. Konishi et al. showed that *FGF10* stimulates proliferation in the white adipose tissue of mice [24]. In addition, Yamasaki et al. highlighted *FGF10* as an important intercellular signaling molecule during lipogenesis that is abundantly expressed in the adipose tissue of adult rats [25].

Due to high species conservation, the identified genes related to human or mice obesity traits may hold importance for adipose deposition in chickens, such as *ELOVL7*, *IL6ST*, *IQGAP2*, *PAX5* and *CKMT2* (Table 4). *ELOVL7* shows altered affinity for the elongation of precursor fatty acids and mediates the extension of saturated fatty acids of up to 24 carbon atoms [26]. *IL6ST* is an IL-6 transducer and a potent modulator of fat metabolism in humans, known to increase fat oxidation and fatty acid re-esterification [27]. *IQGAP2* deficiency influences hepatic free fatty acid uptake, fatty acid synthesis, and lipogenesis suggesting its importance in obesity [28]. *PAX5* is a paired box 5 gene for which Melka et al. performed GWAS in adolescents from the French-Canadian founder population, revealing the association of its locus with total fat mass (TFM) and body mass index (BMI) in 6.4% and 3.7% of TFM and BMI heritability estimates, respectively [29]. These results imply that *PAX5* plays a key role in obesity regulation. *CKMT2* (creatine kinase, mitochondrial 2) is a creatine kinase isoenzyme. Müller et al. showed that *CKMT2* is an

effective modulator of ATP synthase coupled respiration and is exclusively expressed in human brown adipose tissue [30]. *CKMT2* also regulates energy metabolism.

Table 4
Candidate genes in the selection regions and their functions.

Gene symbol	Location(Mb)	Full name	Function of association
<i>FGF10</i>	13.97–14.03	Fibroblast growth factor 10	Promotes fat formation (Matsubara et al., 2013)
<i>IL6ST</i>	17.02–17.06	interleukin 6 signal transducer	Increases fat oxidation and fatty acid re-esterification (Van et al., 2003).
<i>ELOVL7</i>	18.87–18.91	ELOVL fatty acid elongase 7	Extension of saturated fatty acids (Guillou et al., 2010)
<i>IQGAP2</i>	23.46–23.58	IQ motif containing GTPase activating protein 2	Influences the liver uptake of free fatty acids, fatty acid synthesis and adipogenesis (Chiariello et al., 2012)
<i>CKMT2</i>	63.95–63.98	Creatine kinase, mitochondrial 2	Regulation of energy metabolism, expression in brown adipose tissue (Müller et al.,2016)
<i>PAX5</i>	82.01–82.12	paired box 5	Regulates obesity (Melka et al., 2012)

Conclusion

In this study, 35 selection regions were screened through the analysis of selection signals in the chicken Z chromosome, including 219 candidate genes, some of which are involved in lipogenesis, fatty acid production, fat metabolism and fat decomposition, such as *FGF10*, *ELOVL7*, *IL6ST* and so on. Moreover, in the candidate region, using abdominal fat expression data from chickens, 198 candidate genes were identified as expressed in the fat and lean lines, with 11 genes identified as differentially expressed. GO pathways enrichment and QTL annotations provided additional information on the selection mechanism(s) of chicken abdominal fat content. The culmination of these data enhances our understanding of sex chromosome selection signals and their role in fat deposition in chickens.

Methods

Genotype data and population

SNP genotype data were downloaded from GEO Datasets on the NCBI website (GEO accession: GSE58551) [31]. Based on Illumina chicken 60K SNP chips, 48,035 SNPs from 28 autosomes and Z chromosomes in 475 male broilers of the 11th generation (G11) (203 lean lines and 272 fat lines) were identified from NEAUHLF [18]. We mapped the SNP loci on the Z chromosome of all birds to the chicken reference genome (*Gallus gallus* 6.0), resulting in 1,973 SNP loci. We applied QC measurements on the

SNP loci on the Z chromosome of all birds using PLINK (v1.90) software: (1) SNP loci call rates of 0.95; (2) Sample call rates of 0.95; and (3) Minor allele frequencies (MAF) of 0.01 were discounted. In total, 1,937 SNPs and 474 birds were investigated to detect selective sweeps in the chicken sex chromosomes. Specific details of broiler breeding strategy have been described by Zhang et al. [31].

Principal component analysis

We performed PCA to distinguish population structures using EigenGWAS software [14] based on 1,937 SNPs on the Z chromosome. The first ten eigenvalues and their corresponding eigenvectors were then calculated.

Detection selection signatures

Extended haplotype homozygosity (EHH) scores measure the probability that two randomly selected chromosomes carry a tested core haplotype that is homozygous at all SNPs [32]. XPEHH scores can detect selective sweeps in which a selected allele has achieved fixation in one population but remains polymorphic in another [16, 33]. Fst estimates heterozygosity between populations, detecting the degree of segregation according to breeding objectives and evolutionary history. The EigenGWAS algorithm [14] combines the statistical framework of genome-wide association studies with eigenvector decomposition to identify selection footprints on the underlying genome. The eigenvector generated from genome-wide SNPs acts as the phenotype, with the single-marker regression model implemented to detect significant signals defined as selection signatures. In this study, XPEHH, Fst, and EigenGWAS were used to detect selective footprints on the chicken Z chromosome.

We used SHAPEIT (v2.12) software to generate haplotype data based on the SNPs data. We used the LD package of R (v3.6.1) to compute the XPEHH values. The threshold of the XPEHH at a significance level of 0.05 was ± 2 . Using VCFtools [34], unbiased Fst estimations were calculated as described by Weir et al. [35]. Boxplots were constructed for the identification of the upper to lower thresholds and Fst outliers in the SNPs loci. For EigenGWAS, EMMAX software [36] was used for single-marker regression. Threshold P-values of $0.05/1937=2.58 \times 10^{-5}$ were used to confirm statistically significant differences.

To reveal the biological functions of the selection signals, additional analyses were performed. We identified candidate genes within the selection regions using chicken gene annotation data from the Ensembl database. We then used the online software DAVID (v6.7) [11] to perform GO analysis based on the candidate genes obtained. Thirdly, selection regions were mapped onto QTL obtained from the chicken QTL database (<https://www.animalgenome.org/cgi-bin/QTLdb/GG/index>).

Gene expression profiles

Gene expressions in the abdominal adipose tissue of seven-week-old NEAUHLF broilers were evaluated in chicken genome arrays. Raw data sets were normalized and downloaded from the GEO Datasets (GEO accession: GSE8010) [37]. Ten birds were selected based on the percentage of abdominal fat at 7-weeks for the 8th-generation of NEAUHLF broilers. Five birds from lean lines and others from fat lines were obtained. A one-way ANOVA was used to statistically compare the DEGs between fat and lean line chickens.

Abbreviations

XPEHH: Cross Population Extend Haplotype Homozygosity Test; Fst: F-statistics; DEG: differentially expressed genes; SNP: single nucleotide polymorphism; NEAUHLF: Northeast Agricultural University broiler lines divergently selected for abdominal fat content; PCA: principal component analysis; GO: gene Ontology; QTL: quantitative trait loci; TFM: total fat mass; BMI: body mass index; MAF: minor allele frequencies; EHH: extended haplotype homozygosity.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The SNP genotype data and the 7-week-old abdominal fat expression data of broilers used in this study were obtained from the Gene Expression Omnibus (GEO) database at the National Center for Biotechnology Information (NCBI) under accession numbers GSE58551 and GSE8010.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZW and TW conceived the project. TW, MZ, JG and YG performed the bioinformatics and data analysis. TW and ZW wrote the manuscript. KD and PW collected the samples and data. All authors read and approved the final manuscript.

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References

1. Xiang H, Gao J, Yu B, Zhou H, Cai D, Zhang Y, et al. Early Holocene chicken domestication in northern China. *Proc Natl Acad Sci U S A*. 2014; 111(49):17564-9. doi: 10.1073/pnas.1411882111.
2. Liu YP, Wu GS, Yao YG, Miao YW, Luikart G, Baig M, et al. Multiple maternal origins of chickens: out of the Asian jungles. *Mol Phylogenet Evol*. 2006; 38(1):12-9. doi: 10.1016/j.ympev.2005.09.014.
3. International Chicken Genome Sequencing Consortium. Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution. *Nature*. 2004; 432(7018):695-716. doi: 10.1038/nature03154.
4. Rubin CJ, Zody MC, Eriksson J, Meadows JR, Sherwood E, Webster MT, et al. Whole-genome resequencing reveals loci under selection during chicken domestication. *Nature*. 2010; 464(7288):587-91. doi: 10.1038/nature08832.
5. Guo X, Fang Q, Ma C, Zhou B, Wan Y, Jiang R. Whole-genome resequencing of Xishuangbanna fighting chicken to identify signatures of selection. *Genet Sel Evol*. 2016; 48(1):62. doi: 10.1186/s12711-016-0239-4.
6. Boschiero C, Moreira GCM, Gheyas AA, Godoy TF, Gasparin G, Mariani PDSC, et al. Genome-wide characterization of genetic variants and putative regions under selection in meat and egg-type chicken lines. *BMC Genomics*. 2018; 19(1):83. doi: 10.1186/s12864-018-4444-0.
7. McVicker G, Gordon D, Davis C, Green P. Widespread genomic signatures of natural selection in hominid evolution. *PLoS Genet*. 2009; 5(5):e1000471. doi: 10.1371/journal.pgen.1000471.
8. Rubin CJ, Megens HJ, Martinez Barrio A, Maqbool K, Sayyab S, Schwochow D, et al. Strong signatures of selection in the domestic pig genome. *Proc Natl Acad Sci U S A*. 2012; 109(48):19529-

36. doi: 10.1073/pnas.1217149109.
9. Nguyen DK, Disteché CM. Dosage compensation of the active X chromosome in mammals. *Nat Genet.* 2006; 38(1):47-53. doi: 10.1038/ng1705.
10. Graves JA. Sex chromosome specialization and degeneration in mammals. *Cell.* 2006; 124(5):901-14. doi: 10.1016/j.cell.2006.02.024.
11. Huang DW, Sherman BT, Tan Q, Kir J, Liu D, Bryant D, et al. DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res.* 2007; 35(Web Server issue):W169-75. doi: 10.1093/nar/gkm415.
12. Cadzow M, Boocock J, Nguyen HT, Wilcox P, Merriman TR, Black MA. A bioinformatics workflow for detecting signatures of selection in genomic data. *Front Genet.* 2014; 5:293. doi: 10.3389/fgene.2014.00293.
13. Sabeti PC, Varilly P, Fry B, Lohmueller J, Hostetter E, Cotsapas C, et al. Genome-wide detection and characterization of positive selection in human populations. *Nature.* 2007; 449(7164):913-8. doi: 10.1038/nature06250.
14. Chen GB, Lee SH, Zhu ZX, Benyamin B, Robinson MR. EigenGWAS: finding loci under selection through genome-wide association studies of eigenvectors in structured populations. *Heredity (Edinb).* 2016; 117(1):51-61. doi: 10.1038/hdy.2016.25.
15. Liu XX, Pan JF, Zhao QJ, He XH, Pu YB, Han JL, et al. Detecting selection signatures on the X chromosome of the Chinese Debao pony. *J Anim Breed Genet.* 2018; 135(1):84-92. doi: 10.1111/jbg.12314.
16. Ma Y, Zhang H, Zhang Q, Ding X. Identification of selection footprints on the X chromosome in pig. *PLoS One.* 2014; 9(4):e94911. doi: 10.1371/journal.pone.0094911.
17. Rothhammer S, Seichter D, Förster M, Medugorac I. A genome-wide scan for signatures of differential artificial selection in ten cattle breeds. *BMC Genomics.* 2013; 14:908. doi: 10.1186/1471-2164-14-908.
18. Zhang H, Hu X, Wang Z, Zhang Y, Wang S, Wang N, et al. Selection signature analysis implicates the PC1/PCSK1 region for chicken abdominal fat content. *PLoS One.* 2012; 7(7):e40736. doi: 10.1371/journal.pone.0040736.
19. Liu T, Jiang W, Han D, Yu L. DNAJC25 is downregulated in hepatocellular carcinoma and is a novel tumor suppressor gene. *Oncol Lett.* 2012; 4(6):1274-1280. doi: 10.3892/ol.2012.903.
20. Jin P, Wu X, Xu S, Zhang H, Li Y, Cao Z, et al. Differential expression of six genes and correlation with fatness traits in a unique broiler population. *Saudi J Biol Sci.* 2017; 24(4):945-949. doi: 10.1016/j.sjbs.2015.04.014.
21. Gholami M, Erbe M, Gärke C, Preisinger R, Weigend A, Weigend S, et al. Population genomic analyses based on 1 million SNPs in commercial egg layers. *PLoS One.* 2014; 9(4):e94509. doi: 10.1371/journal.pone.0094509.
22. Fu W, Lee WR, Abasht B. Detection of genomic signatures of recent selection in commercial broiler chickens. *BMC Genet.* 2016; 17(1):122. doi: 10.1186/s12863-016-0430-1.

23. Matsubara Y, Aoki M, Endo T, Sato K. Characterization of the expression profiles of adipogenesis-related factors, ZNF423, KLFs and FGF10, during preadipocyte differentiation and abdominal adipose tissue development in chickens. *Comp Biochem Physiol B Biochem Mol Biol*. 2013; 165(3):189-95. doi: 10.1016/j.cbpb.2013.04.002.
24. Konishi M, Asaki T, Koike N, Miwa H, Miyake A, Itoh N. Role of Fgf10 in cell proliferation in white adipose tissue. *Mol Cell Endocrinol*. 2006; 249(1-2):71-7. doi: 10.1016/j.mce.2006.01.010.
25. Yamasaki M, Emoto H, Konishi M, Mikami T, Ohuchi H, Nakao K, et al. FGF-10 is a growth factor for preadipocytes in white adipose tissue. *Biochem Biophys Res Commun*. 1999; 258(1):109-12. doi: 10.1006/bbrc.1999.0594.
26. Guillou H, Zadavec D, Martin PG, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog Lipid Res*. 2010; 49(2):186-99. doi: 10.1016/j.plipres.2009.12.002.
27. Van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, et al. Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J Clin Endocrinol Metab*. 2003; 88(7):3005-10. doi: 10.1210/jc.2002-021687.
28. Chiariello CS, LaComb JF, Bahou WF, Schmidt VA. Ablation of Iqgap2 protects from diet-induced hepatic steatosis due to impaired fatty acid uptake. *Regul Pept*. 2012; 173(1-3):36-46. doi: 10.1016/j.regpep.2011.09.003.
29. Melka MG, Bernard M, Mahboubi A, Abrahamowicz M, Paterson AD, Syme C, et al. Genome-wide scan for loci of adolescent obesity and their relationship with blood pressure. *J Clin Endocrinol Metab*. 2012; 97(1):E145-50. doi: 10.1210/jc.2011-1801.
30. Müller S, Balaz M, Stefanicka P, Varga L, Amri EZ, Ukropec J, et al. Proteomic Analysis of Human Brown Adipose Tissue Reveals Utilization of Coupled and Uncoupled Energy Expenditure Pathways. *Sci Rep*. 2016; 6:30030. doi: 10.1038/srep30030.
31. Zhang H, Du ZQ, Dong JQ, Wang HX, Shi HY, Wang N, et al. Detection of genome-wide copy number variations in two chicken lines divergently selected for abdominal fat content. *BMC Genomics*. 2014; 15:517. doi: 10.1186/1471-2164-15-517.
32. Voight BF, Kudaravalli S, Wen X, Pritchard JK. A map of recent positive selection in the human genome. *PLoS Biol*. 2006; 4(3):e72. doi: 10.1371/journal.pbio.0040072.
33. Sabeti PC, Reich DE, Higgins JM, Levine HZ, Richter DJ, Schaffner SF, et al. Detecting recent positive selection in the human genome from haplotype structure. *Nature*. 2002; 419(6909):832-7. doi: 10.1038/nature01140.
34. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. *Bioinformatics*. 2011; 27(15):2156-8. doi: 10.1093/bioinformatics/btr330.
35. Weir BS, Hill WG. Estimating F-statistics. *Annu Rev Genet*. 2002; 36:721-50. doi: 10.1146/annurev.genet.36.050802.093940.
36. Kang HM, Sul JH, Service SK, Zaitlen NA, Kong SY, Freimer NB, et al. Variance component model to account for sample structure in genome-wide association studies. *Nat Genet*. 2010; 42(4):348-54.

doi: 10.1038/ng.548.

37. Wang HB, Li H, Wang QG, Zhang XY, Wang SZ, et al. Profiling of chicken adipose tissue gene expression by genome array. BMC Genomics. 2007; 8:193. doi: 10.1186/1471-2164-8-193.

Figures

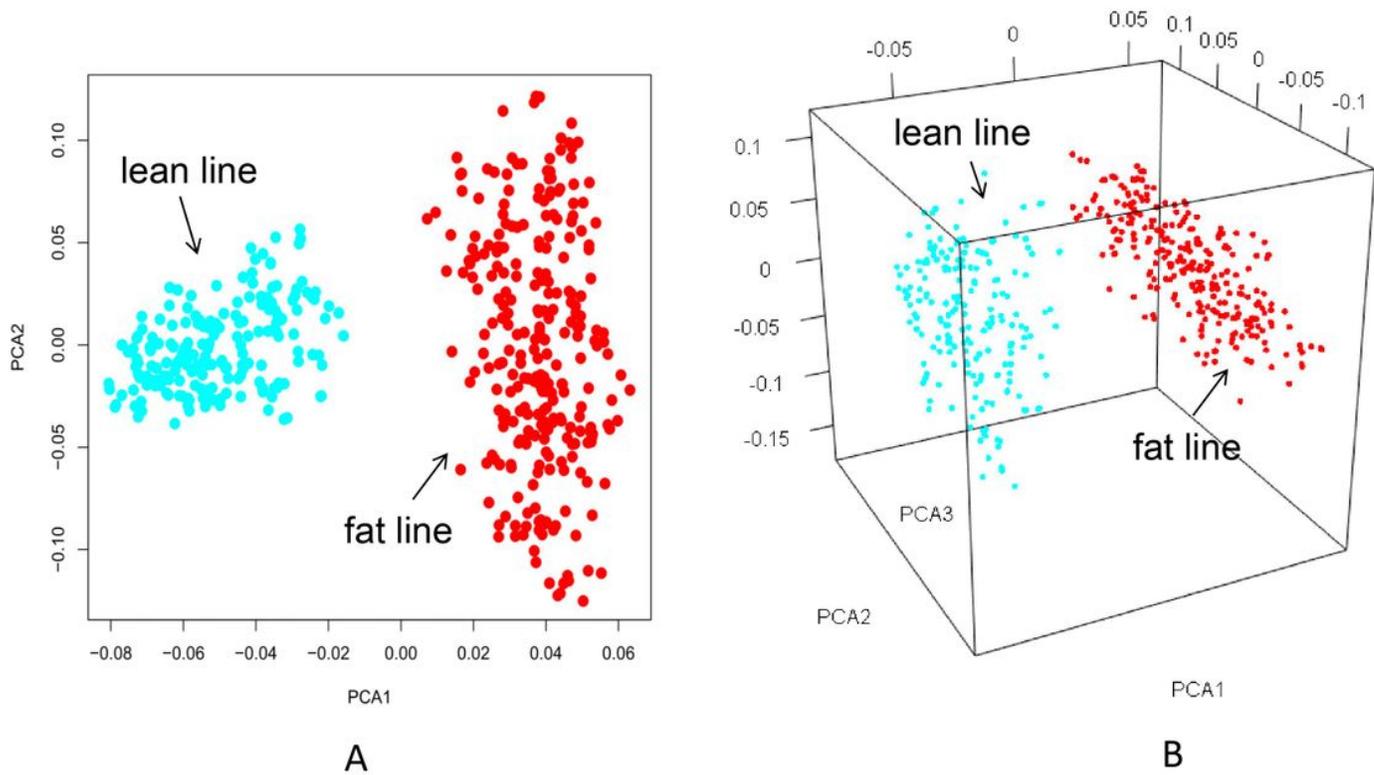


Figure 1

Population structure based on Z chromosome SNPs using principal component analysis. The subgraph (A) and (B) represent two-dimensional and three-dimensional PCA images.

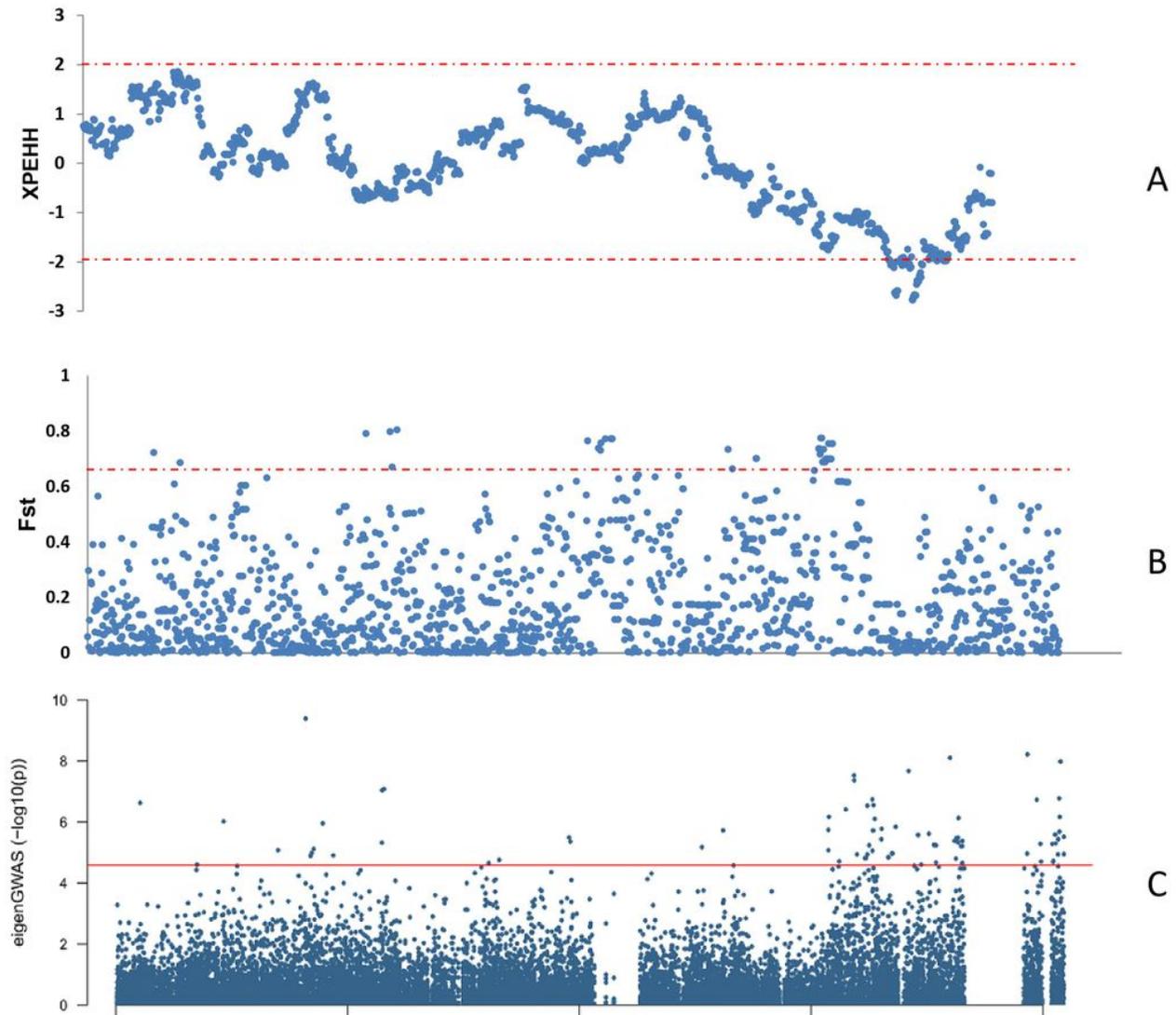


Figure 2

Selection signatures on the Z chromosome. The subgraphs (A), (B) and (C) are the selection signatures detected between the populations using the XPEHH, Fst and eigenGWAS methods, respectively.

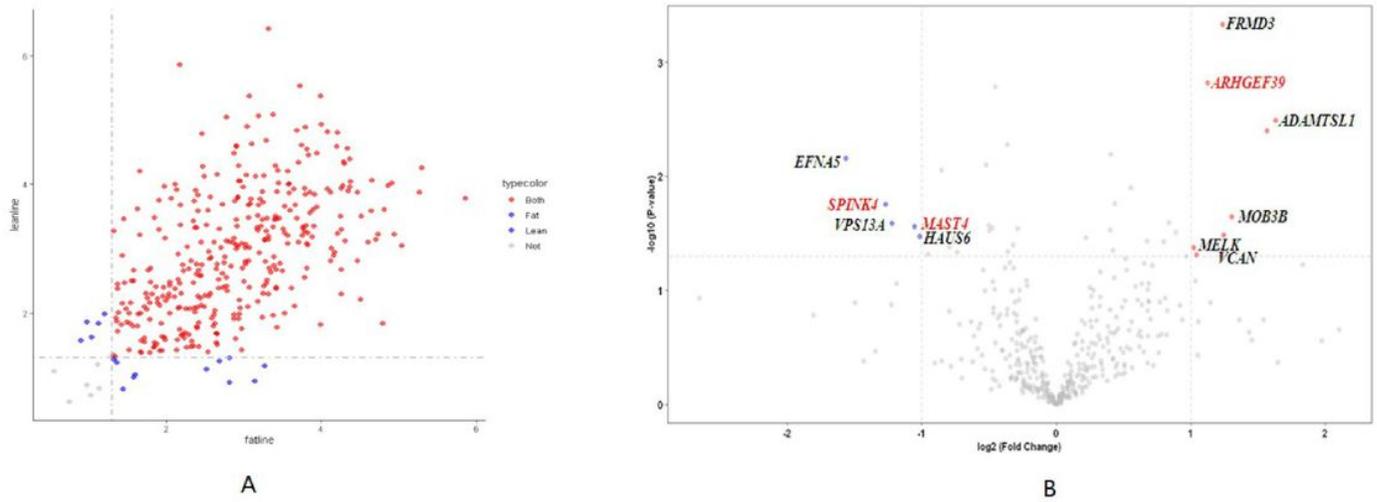


Figure 3

Candidate gene expression profiles in the adipose tissue of the NEAUHLF. The subgraphs (A) is the gene expression profile of two chicken strains. The X-axis and Y-axis is the $-\log_{10}(p\text{-value})$ of fat and lean meat lines, respectively. Figure (B) shows the differential expression of genes in adipose tissues in fat and lean lines at the 7th week of age.