Haplotype-based genome-wide association studies for carcass and growth traits in chicken

Hui Zhang  
Northeast Agricultural University

Lin-Yong Shen  
Northeast Agricultural University

Zi-Chun Xu  
Northeast Agricultural University

Luke M. Kramer  
Iowa State University, lmkramer@iastate.edu

Jia-Qiang Yu  
Northeast Agricultural University

See next page for additional authors

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Abstract
There have been several genome-wide association study (GWAS) reported for carcass, growth, and meat traits in chickens. Most of these studies have been based on single SNPs GWAS. In contrast, haplotype-based GWAS reports have been limited. In the present study, 2 Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF) and genotyped with the chicken 60K SNP chip were used to perform a haplotype-based GWAS. The lean and fat chicken lines were selected for abdominal fat content for 11 yr. Abdominal fat weight was significantly different between the 2 lines; however, there was no difference for body weight between the lean and fat lines. A total of 132 haplotype windows were significantly associated with abdominal fat weight. These significantly associated haplotype windows were primarily located on chromosomes 2, 4, 8, 10, and 26. Seven candidate genes, including SHH, LMBR1, FGF7, IL16, PLIN1, IGF1R, and SLC16A1, were located within these associated regions. These genes may play important roles in the control of abdominal fat content. Two regions on chromosomes 3 and 10 were significantly associated with testis weight. These 2 regions were previously detected by the single SNP GWAS using this same resource population. TCF21 on chromosome 3 was identified as a potentially important candidate gene for testis growth and development based on gene expression analysis and the reported function of this gene. TCF12, which was previously detected in our SNP by SNP interaction analysis, was located in a region on chromosome 10 that was significantly associated with testis weight. Six candidate genes, including TNFRSF1B, PLOD1, NPPC, MTHFR, EPHB2, and SLC35A3, on chromosome 21 may play important roles in bone development based on the known function of these genes. In addition, several regions were significantly associated with other carcass and growth traits, but no candidate genes were identified. The results of the present study may be helpful in understanding the genetic mechanisms of carcass and growth traits in chickens.

Keywords
haplotype-based genome-wide association study (GWAS), abdominal fat, testis, candidate gene

Disciplines
Agriculture | Animal Sciences | Genetics and Genomics | Poultry or Avian Science

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Authors
Hui Zhang, Lin-Yong Shen, Zi-Chun Xu, Luke M. Kramer, Jia-Qiang Yu, Xin-Yang Zhang, Wei Na, Li-Li Yang, Zhi-Ping Cao, Peng Luan, James M. Reecy, and Hui Li

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Haplotype-based genome-wide association studies for carcass and growth traits in chicken

Hui Zhang,* Lin-Yong Shen,* Zi-Chun Xu,* Luke M. Kramer,† Jia-Qiang Yu,* Xin-Yang Zhang,* Wei Na,* Li-Li Yang,* Zhi-Ping Cao,* Peng Luan,* James M. Reecy,†,1 and Hui Li*,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, College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, PR China; and †Department of Animal Science, Iowa State University, Ames, IA 50011, USA

ABSTRACT There have been several genome-wide association study (GWAS) reported for carcass, growth, and meat traits in chickens. Most of these studies have been based on single SNPs GWAS. In contrast, haplotype-based GWAS reports have been limited. In the present study, 2 Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHLF) and genotyped with the chicken 60K SNP chip were used to perform a haplotype-based GWAS. The lean and fat chicken lines were selected for abdominal fat content for 11 yr. Abdominal fat weight was significantly different between the 2 lines; however, there was no difference for body weight between the lean and fat lines. A total of 132 haplotype windows were significantly associated with abdominal fat weight. These significantly associated haplotype windows were primarily located on chromosomes 2, 4, 8, 10, and 26. Seven candidate genes, including SHH, LMBR1, FGF7, IL16, PLIN1, IGF1R, and SLC16A1, were located within these associated regions. These genes may play important roles in the control of abdominal fat content. Two regions on chromosomes 3 and 10 were significantly associated with testis weight. These 2 regions were previously detected by the single SNP GWAS using this same resource population. TCF21 on chromosome 3 was identified as a potentially important candidate gene for testis growth and development based on gene expression analysis and the reported function of this gene. TCF12, which was previously detected in our SNP by SNP interaction analysis, was located in a region on chromosome 10 that was significantly associated with testis weight. Six candidate genes, including TNFRSF1B, PLOD1, NPPC, MTHFR, EPHB2, and SLC35A3, on chromosome 21 may play important roles in bone development based on the known function of these genes. In addition, several regions were significantly associated with other carcass and growth traits, but no candidate genes were identified. The results of the present study may be helpful in understanding the genetic mechanisms of carcass and growth traits in chickens.

Key words: haplotype-based genome-wide association study (GWAS), abdominal fat, testis, candidate gene

INTRODUCTION

Single nucleotide polymorphisms (SNP) are the most common type of variant within a genome. They have been extensively used to carry out genome-wide association studies (GWAS). SNP chips have made it possible and affordable to conduct GWAS for complex traits, especially for important economic traits in livestock (Goddard et al., 2016). Therefore, many studies about the successful applications of GWAS in animal breeding and genetics have been reported, and many genes or markers for economically important traits have been identified (Goddard et al., 2016). These results not only supply a number of molecular markers that can be used in prediction/genomic selection but they can also provide important information to help explain the genetic mechanisms that underlie these traits. However, most of these GWAS were based on single SNPs. Single SNP-based GWAS is unlikely to fully capture the variations in regions surrounding the genotyped markers. Instead, haplotype-based GWAS may help to improve this defect and could detect new discoveries of important traits (Howard et al., 2017). In addition, utilization of the haplotype-based approach delivered greater power with...
no inflation in type I error rate for association studies. The most important process to carry out the haplotype-based GWAS is to construct phasing of the genome, which means that the haplotypes are needed to be constructed. He et al. (2011) developed an efficient approach to accelerate the phasing process and reduce the potential bias generated by unrealistic assumptions in the phasing process. Recently, haplotyped-based GWASs have been conducted and have obtained some useful results (Wu et al., 2014; Sato et al., 2016; Chen et al., 2018). In chickens, GWAS identified genetic variation that has been associated with disease (Raeesi et al., 2017), carcass (Huang et al., 2018), growth (Guo et al., 2017; Pértille et al., 2017), and meat quantitative traits (Moreira et al., 2018). However, nearly all of these GWAS reports were based on single SNP, and no haplotype associations were reported.

The aim of the present study is to identify potentially important genes for carcass and growth traits using a haplotype-based GWAS approach in 2 Northeast Agricultural University broiler lines divergently selected for abdominal fat content (NEAUHFLF) for 11 yr. The

![Manhattan plots](image)

**Figure 1.** Results of haplotype-based genome-wide association studies using PLINK for abdominal fat weight (AFW). The results are presented as Manhattan plots based on haplotype 11-specified, 12-specified, 21-specified, and 22-specified, respectively. The solid line indicates the Bonferroni threshold for multiple test correction with a type I error of 5% ($P$-value $<1.04 \times 10^{-6}$).
results of this study may supply useful information for prediction/genomic selection in chicken breeding programs and may also provide important information to explain the genetic mechanisms that underlie carcass and growth traits in chicken.

MATERIALS AND METHODS

Ethics Statement

All animal work was conducted as per 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 was approved by the Laboratory Animal Management Committee of Northeast Agricultural University.

Experimental Populations

Two NEAUHLF were used to carry out the haplotype-based association study (Guo et al., 2011). The population used in the present study included 475 males (203 and 272 birds from the lean and fat lines, respectively) from the 11th generation of NEAUHLF (Li et al., 2013). The birds were weighed at 0, 1, 3, 5, and 7 wk of age (BW0, BW1, BW3, BW5, and BW7, respectively). At 7 wk of age, the metatarsus length (MeL), metatarsus circumference (MeC), keel length (KeL), and chest width (ChWi) were measured before slaughter as previously described (Zhang et al., 2010). Abdominal fat weight (AFW), testis weight (TeW), carcass weight (CW), heart weight (HW), liver weight (LW), spleen weight (SW), and muscular and glandular stomach weight (MGSW) were obtained after the birds were slaughtered.

SNP Genotyping

Genotyping was carried out using the chicken 60 K SNP chip (Illumina Inc., San Diego, CA), which contained 57,636 SNP. After quality control, 48,034 SNP in 475 individuals located on 28 autosomal and Z chromosomes were used in the haplotype-based GWAS. The quality control of the SNP genotypes was described previously by Zhang et al. (2012).

Haplotype-Based GWAS

Haplotypes were constructed by LinkPHASE3 using pedigree information (Druet and Georges, 2015). Missing haplotypes were inferred by DAGPHASE and Beagle, which use an efficient approach based on hidden Markov models (Druet and Georges, 2010). Haplotypes were extracted using every 2 neighboring SNP. Thus, 4 kinds of haplotype (11, 12, 21, and 22) were detected. For the haplotype-based GWAS, we compared each haplotype vs. all others, which means that when haplotype 11 was specified, the individuals with 2 copies of the specified haplotype 11 had the diplotype of AA, the individuals with only one copy of the specified haplotype 11 had the diplotype of AB, and the individuals with no copy of the specified haplotype 11 had the diplotype of BB. In turn, when haplotype 12 was specified, the individuals with 2 copies of the specified haplotype 12 had the diplotype of AA, the individuals with only one copy of the specified haplotype 12 had the diplotype of AB, and the individuals with no copy of the specified haplotype 12 had the diplotype of BB, and so on. The genotype file of all individuals was generated with only 3 diplotypes, AA, AB, and BB. The haplotype-based GWAS was then conducted by Plink v1.07 using a linear regression method (Purcell et al., 2007). Family and Line were used as a 2 fix effects for all the traits to adjust the population structure’s effect. BW0 was used as a covariate for BW1, BW3, BW5, and

| Traits | No. of significant windows |
|--------|---------------------------|
|        | 11-Specified | 12-Specified | 21-Specified | 22-Specified | Total |
| AFW    | 41           | 40           | 50           | 41           | 132   |
| BW1    | 3            | 2            | 3            | 1            | 9     |
| BW3    | 4            | 8            | 6            | 3            | 18    |
| BW5    | 4            | 4            | 7            | 6            | 21    |
| BW7    | 1            | 2            | 2            | 1            | 6     |
| ChWi   | 5            | 3            | 4            | 3            | 14    |
| CW     | 0            | 1            | 1            | 0            | 2     |
| HW     | 1            | 1            | 1            | 0            | 3     |
| KeL    | 11           | 6            | 9            | 6            | 32    |
| LW     | 0            | 1            | 2            | 0            | 3     |
| MeC    | 24           | 33           | 35           | 30           | 110   |
| MeL    | 1            | 2            | 1            | 2            | 6     |
| MGSW   | 2            | 0            | 0            | 2            | 4     |
| SW     | 0            | 0            | 0            | 0            | 0     |
| TeW    | 34           | 33           | 35           | 31           | 123   |

Abbreviations: AFW, abdominal fat weight; ChWi, chest width; CW, carcass weight; HW, heart weight; KeL, keel length; LW, liver weight; MeC, metatarsus circumference; MeL, metatarsus length; MGSW, muscular and glandular stomach weight; SW, spleen weight; TeW, testis weight.
Table 2. Important chromosome regions for carcass and growth traits.

| Chromosome | Start_SNP     | Rs#         | Start_position | End_SNP     | Rs#         | End_position | Length   | Traits | Genes in the region |
|------------|---------------|-------------|----------------|-------------|-------------|--------------|----------|--------|---------------------|
| 2          | GGaluGA132691 | rs313439121 | 7969784        | Gga_rs15068089 | rs15068089 | 8567871      | 671,087  | AFW    | SHH, LMBR1, MNX1, UBE3C |
| 2          | Gga_rs14219117 | 14219117    | 9318534        | Gga_rs14219515 | 14219515    | 9373230      | 546,087  | AFW    |                       |
| 2          | GGaluGA158673 | rs120677977 | 90017288       | GGaluGA159074 | rs17455927 | 98822750     | 2,806,462 | AFW    |                       |
| 2          | GGaluGA159507 | rs315053861 | 100237421      | Gga_rs14224613 | 14224613    | 10038735     | 59,614   | AFW    | RTTN, MIR1681, TMX3, CDH19, CDH7, MC2R |
| 2          | Gga_rs13803296 | 13803296    | 102079036      | GGaluGA160440 | rs314547993 | 10396655     | 1,917,319 | AFW    | LAMA1, ZBTB14, AKAIN1, TGF1, MYL12A |
| 2          | Gga_rs16142136 | 16142136    | 139745278      | Gga_rs16141958 | 16141958    | 14009369     | 344,091  | AFW    |                       |
| 4          | Gga_rs1436487 | rs14436487  | 21729261       | Gga_rs14436961 | 14436961    | 22258381     | 529,120  | AFW    | CTOS    |
| 8          | Gga_rs15006323 | 15006323    | 8094782        | Gga_rs14985809 | 14985809    | 9028904      | 934,122  | AFW    |                       |
| 8          | Gga_rs14642420 | 14642420    | 14253680       | Gga_rs14642444 | 14642444    | 14296548     | 42,868   | AFW    |                       |
| 10         | GGaluGA069041 | rs317193761 | 12108078       | Gga_rs14088746 | rs14088746 | 14892303     | 2784,225 | AFW    |                       |

10        | Gga_rs15587351 | rs15587351  | 17309049       | Gga_rs14011820 | rs14011820 | 18758907     | 1,449,858 | AFW    |                       |

26        | GGaluGA196948 | rs318060966 | 3156806        | Gga_rs16203115 | rs16203115 | 3520068      | 363,262  | AFW    |                       |

1          | Gga_rs13895241 | rs13895241  | 88063956       | GGaluGA029830 | rs312695192 | 88670466     | 606,510  | MeC    |                       |

1          | GGaluGA0311230 | rs132759218 | 92236963       | Gga_rs14857266 | 14857266    | 93018416     | 781,453  | MeC    |                       |

2          | Gga_rs13669384 | rs13669384  | 57647772       | Gga_rs14165766 | 14165766    | 37653833     | 60,016   | MeC    |                       |

2          | Gga_rs1606608 | rs318119261 | 104397754      | Gga_rs13794375 | 13794375    | 10427095     | 230,051  | MeC    |                       |

2          | GGaluGA162581 | rs341838007 | 110512137      | Gga_rs14232072 | 14232072    | 11138788     | 875,751  | MeC    |                       |

2          | Gga_rs16149569 | rs16149569  | 148367066      | Gga_rs1730555 | rs135266923 | 150658423    | 2,290,757 | MeC    |                       |

4          | Gga_rs16404447 | rs16404447  | 4102957        | Gga_rs14727013 | 14727013    | 49961124     | 858,167  | MeC    |                       |

6          | Gga_rs14593228 | rs14593228  | 32615659       | GGaluGA305949 | rs312809174 | 33657322     | 751,063  | MeC    |                       |

6          | GGaluGA311411 | rs315499140 | 18350464       | Gga_rs31411444 | 31411444    | 18364173     | 13,900   | MeC    |                       |

7          | Gga_rs15862567 | rs15862567  | 24373174       | GGaluGA316074 | rs316254621 | 24467515     | 273,797  | MeC    |                       |

8          | Gga_rs13663515 | rs13663515  | 7859868        | GGaluGA325359 | rs316864055 | 7906123     | 46,255   | MeC    |                       |

8          | Gga_rs15910167 | rs15910167  | 10374244       | Gga_rs146146384 | rs146146384 | 12029311     | 2,835,507 | MeC    |                       |

21         | GGaluGA184599 | rs316833978 | 4781321        | Gga_rs15185019 | rs15185019  | 6179695      | 1,395,644 | MeC    |                       |

Z          | Gga_rs14689552 | rs14689552  | 6673737        | Gga_rs14783328 | rs14783328 | 6729085      | 617,348  | MeC    |                       |

Z          | Gga_rs16129856 | rs16129856  | 9391651        | Gga_rs14785793 | rs14785793 | 10575355     | 1,183,704 | MeC    |                       |
|   | SNP ID | Chromosome | Position | Ref SNP (rsID) | SNP ID | Chromosome | Position | Position | p-value |
|---|--------|------------|----------|---------------|--------|------------|----------|----------|---------|
| 1 | GGaluGA043278 | 1 | 315995993 | rs315995993 | Gga_rs13936329 | 1 | 131588419 | rs31936329 | 131711376 | 122957 |
| 2 | Gga_rs13534898 | 1 | 13534898 | rs13534898 | GGaluGA131254 | 1 | 314054036 | rs314054036 | 4866215 | 348502 |
| 2 | Gga_rs14240062 | 1 | 14240062 | rs14240062 | Gga_rs14241677 | 1 | 121959284 | rs121959284 | 123380687 | 1426803 |
| 2 | Gga_rs14245700 | 1 | 14245700 | rs14245700 | Gga_rs13730959 | 1 | 13730959 | rs13730959 | 127499632 | 56029 |
| 3 | GGaluGA222074 | 2 | 317102159 | rs317102159 | Gga_rs10729720 | 2 | 67517313 | rs67517313 | 67517313 | 14240369 |
| 10 | Gga_rs14002765 | 10 | 14002765 | rs14002765 | Gga_rs14003104 | 10 | 14003104 | rs14003104 | 6635581 | 672614 |
| 10 | Gga_rs1495763 | 10 | 1495763 | rs1495763 | Gga_rs14722408 | 10 | 14722408 | rs14722408 | 13180860 | 4720525 |
| 11 | GGaluGA074107 | 11 | 312924990 | rs312924990 | Gga_rs14958653 | 11 | 14958653 | rs14958653 | 1864531 | 829048 |

**References:**

- ACA1, MAPKKK3L, MYD88, MIR6610
- TRPA1, MIR1796, TERF1, RPL7, RDH10, STAU2, UBE2W, ELOC, TMEM76, PI15, CRISPLD1
- CASA
- GTF2H5, EZR, ADGR6, CITED2, TXLNB, ABRAC1, REFS1, MIR7462, PERP2, PERP1, IFNGR1, MIR6568, PEX7, MAP7, MYB, SGI1, TBP1L, TCF21, EYA4, RPS12, MIR1454, SLC18B1, VNN1, STX7, MOXD1, CTGF, MIR6582, MIR6697, MIR1660, ECHDC1, RSP03, CENPW, TRMT11, NCOA7, TPDS2L1, HDEC2, NKAIN2, FABP7, PKIB, SERINC1, HSF2, GJA1, MCM9, ASFA1, PLN, MIR199B, ROS1, VGLL2, SOT3A1L, RWDD1, FAM26E, HDAC2, MARCKS
- RORA, ANXA2, GTF2A2
- TCF12, PRTG, PYGO1, DYX1C1, CCPC1, PIGBOS1, RAB27A, RSL24D1, FAM31A, ARPP19, MYOSA, GNB5, BCL2L10, MAPK6, LYSMD2, LEO1, TMOD3, LYSMD2, SGC3, CYP19A1, MIR1744, SLC24A5, MYEF2, DUT, COP52, GALK2, FG7, MIR147-1, BLOC1S6, ITGB1BP3, MIR6596, GABPB1, TRPM7, GABPB1, HEC, GATM, SCARNA15, FAM103A1, FAM103A1, TM6SF1, BTBD1, SH3GL3, CTFCF, LOC15664, LOC15664, LOC769668, LOC10708643, LOC15662, AARS, MIR1616, FHOD1, ATP6V0D1, AGGP, SETD6, CNOT1, G0T2, CALB2, HYDIN, VAC14, COL4, ST3GAL2, GLG1
BW7. BW7 was used as a covariate for KeL, MeL, MeC, ChWi, AFW, CW, TeW, HW, LW, SW, and MGSW. A genome-wide 5% type I error after Bonferroni correction was used as the genome-wide significance level. The threshold $P$-value for declaring genome-wide significance was $0.05/48,005 = 1.04 \times 10^{-6}$. Manhattan plots of the $P$-values for all haplotypes associated with carcass and growth were plotted using SNPEVG1, version 2.1 (Wang et al., 2012). Gene locations and information were mined from Ensembl chicken genome galGal3 (https://www.genome.ucsc.edu).

Haplotypes were also extracted using the sliding windows of 3 SNP, 4 SNP, and 5 SNP. The haplotype frequencies were calculated, and the major haplotype was specified, which meant that the individuals with 2 copies of the major haplotype had the diplotype of AA, the individuals with only one copy of the major haplotype had the diplotype of AB, and the individuals with no copy of the major haplotype had the diplotype of BB. Therefore, we got the genotype file of all individuals with only 3 diplotypes, AA, AB, and BB. The haplotype-based GWAS was then conducted by the method described previously.

**RESULTS AND DISCUSSION**

**Haplotype-Based GWAS for Carcass Trait**

For more than 60 yr, broiler chicken breeders have focused on the selection of important economic traits and have made dramatic genetic improvements (Hill and Dansky, 1954; Bedford and Classen, 1992; Demeure et al., 2013). However, long-term intense selection for fast juvenile growth in broiler chickens has increased their abdominal fat deposition and resulted in metabolic changes (Pym, 1987; Emmerson, 1997; Scheele, 1997; Julian, 2005). Excessive deposition of abdominal fat has negative impacts on feed efficiency and carcass quality (Demeure et al., 2013; Ramiah et al., 2014). Therefore, the detection of important genes or markers for

**Figure 2.** The difference of abdominal fat weight (AFW) between the individuals with the major haplotype (Hap1) and the individuals with other haplotypes (Hap2) ($t$-test). Different alphabets means extremely significantly different ($P < 0.01$) and the error bar is the standard deviation (SD).

**Table 3.** Candidate genes for AFW, TeW, and MeC identified from the haplotype-based GWAS results.

| Genes   | Haplotype window | Near or contained the haplotype window | Chromosome | Major haplotype | Trait |
|---------|-----------------|-----------------------------------------|------------|-----------------|-------|
| SHH     | WIN7856         | Near                                    | 2          | 21              | AFW   |
| LMBR1   | WIN7879         | Near                                    | 2          | 12              | AFW   |
| FGFL    | WIN3047         | Near                                    | 10         | 22              | AFW   |
| IL16    | WIN30558        | Near                                    | 10         | 11              | AFW   |
| PLIN1   | WIN30605        | Near                                    | 10         | 12              | AFW   |
| IGF1R   | WIN30893        | Contained                               | 10         | 22              | AFW   |
| SLC16A1 | WIN44687        | Near                                    | 26         | 12              | AFW   |
| TCF21   | WIN15421        | Near                                    | 3          | 11              | TeW   |
| TCF12   | WIN30233 and WIN30234 | Contained                   | 10         | 212             | TeW   |
| SLC35A3 | WIN27613        | Contained                               | 8          | 12              | MeC   |
| TNRFSF1B| WIN42161        | Near                                    | 21         | 22              | MeC   |
| PLOD1   | WIN42177        | Near                                    | 21         | 12              | MeC   |
| NPPC    | WIN27613        | Contained                               | 8          | 12              | MeC   |
| MTNR1   | WIN42231 and WIN42234 | Contained                  | 21         | 1,212           | MeC   |

Abbreviations: AFW, abdominal fat weight; GWAS, genome-wide association study; MeC, metatarsus circumference; TeW, testis weight.
Abdominal fat content will help to select lean chicken lines. In the present study, haplotype-based GWAS for AFW are carried out to identify genes for abdominal fat content (Figure 1). There were 156 haplotype windows that were significantly associated with AFW (Table 1 and Supplementary Table 1). A total of 132 haplotype windows that were significantly associated with AFW were obtained after combining overlapping windows. The SNP in these significant haplotype windows were concentrated on chromosomes 2, 4, 8, 10, and 26. The 12 regions on these chromosomes were obtained after combining windows that overlapped (Table 2). There were 70 RefGenes located in these 12 regions. Possible candidate genes for abdominal fat deposition include SHH, LMBR1, FGF7, IL16, PLIN1, IGF1R, and SLC16A1. These genes contained a haplotype window or located near a haplotype window with significant effects on AFW (Table 3). Individuals with the major haplotype (Hap1) had significantly lower or higher AFW than the individuals with the other haplotypes (Hap2, Figure 2). These results indicated that SHH (sonic hedgehog) is an obesity susceptibility gene in humans (Wu et al., 2017). This gene can reduce lipid accumulation in adipocytes and decrease the expression

Figure 3. Results of haplotype-based genome-wide association studies using PLINK for testis weight (TeW). The results are presented as Manhattan plots based on haplotype 11-specified, 12-specified, 21-specified, and 22-specified, respectively. The solid line indicates the Bonferroni threshold for multiple test correction with a type I error of 5% (P-value < 1.04 × 10⁻⁶).
of the adipocyte-specific gene (Fontaine et al., 2008). *LMBR1* is the limb development membrane protein 1, and the SNP in this gene was significantly associated with obesity in humans (Wu et al., 2017). *FGF7* is the fibroblast growth factor (FGF) 7, and the protein encoded by this gene is a member of the FGF family. Most FGF family members could promote the proliferation and differentiation of human preadipocytes by activating a family of receptor tyrosine kinases (Patel et al., 2005). *FGF7* was identified as a target of miR-143 in murine adipogenesis and it was plausible that the overexpression of miR-143 could promote adipogenesis by inhibiting its target *FGF7* (He et al., 2013). A functional SNP in *IL6* gene was strongly associated with waist circumference in a large Dutch study population, which indicated that IL6 may contribute to obesity in humans (van den Berg et al., 2009). Perilipin (*PLIN1*) is a lipid droplet coat protein that belongs to the lipid droplet–related protein family. Genetic variation in *PLIN1* has been significantly associated with adiposity in human (Ruiz et al., 2011), pig (Gandolfi et al., 2011), cattle (Fan et al., 2010), sheep (Gao et al., 2012), duck (Zhang et al., 2013), and chicken (Zhou et al., 2014; Zhang et al., 2015). In mice, knockout of insulin and/or IGF1 receptors (IR/IGF1R) was accompanied by a rapid loss of white and brown fat because of the increased lipolysis and adipocyte apoptosis (Sakaguchi et al., 2017). *SLC16A1* is the solute carrier family 16 member 1, which is also known as monocarboxylate transporter 1 (*MCT1*). *MCT1* is abundant in several tissues, including adipose, gut, brain, heart, muscle, liver, and kidney (Hajduch et al., 2000; Pierre and Pellerin, 2005; Iwanaga et al., 2006). It is also a carrier of short-chain fatty acids, ketone bodies, and lactate in several tissues, and *MCT1*+/− mice displayed resistance to development of diet-induced obesity when fed with high fat diet (HFD) (Lengacher et al., 2013).

Manhattan plots of haplotype-based GWAS for TeW are shown in Figure 3. A total of 133 haplotype windows significantly associated with TeW were identified (Table 1 and Supplementary Table 1). These significant windows for TeW were mainly distributed on chromosomes 3 and 10. The haplotype windows with a significant effect on TeW on chromosome 3 were concentrated on a 14 Mb region from 53.28 Mb to 67.52 Mb. The significant haplotype windows for TeW on chromosome 10 were concentrated on the 4.72 Mb region from 8.46 Mb to 13.18 Mb. These 2 regions on chromosome 3 and 10 are same as previously detected by the single SNP GWAS (Zhang et al., 2017a). In these 2 regions, 2 transcription factors, including *TCF21* and *TCF12*, were detected as important genes for testis growth and development based on our previous studies (Zhang et al., 2017a, b). *TCF21* gene was located near a haplotype window (WIN15421) that was significantly associated with TeW and TeP (Zhang et al., 2017a). In humans and mice, *TCF21* plays important roles in hypertension, gastric cancer, and coronary heart disease (Miller et al., 2014; Fujimaki et al., 2015; Yang et al., 2015). In mice, *TCF21* is the first direct downstream target gene of the male sex-
determining factor (SRY) (Bhandari et al., 2011, 2012). The knockout of TCF21 in mice resulted in male-to-female sex reversal (Cui et al., 2004). SRY could bind to the TCF21 promoter and activate gene expression (Bhandari et al., 2012). In rats, TCF21 and SRY have similar effects on Sertoli cell differentiation and embryonic testis development (Bhandari et al., 2012). Taken together, these results indicated that TCF21 may play an important role in sex differentiation and testis development.

TCF12 was located within 2 consecutive haplotype windows (WIN30233 and WIN30234) that were significantly associated with TeW (Table 3). The 3 SNP that constituted these 2 haplotypes were used to construct 3 SNP haplotypes. Individuals with the major haplotype 212 (Hap1) had significantly higher TeW than the individuals with others haplotypes (Hap2) (Figure 4). TCF12 was in the same family as TCF21, which was also identified in the region for TeW on chromosome 10. In our previous study, TCF12 was detected as the important gene for testis growth and development from the SNP by SNP interaction analysis (Zhang et al., 2017b).

For TeW, a single SNP-based GWAS was carried out, previously (Zhang et al., 2017a). The haplotype-based GWAS results were compared with the single SNP-based GWAS, and we found that haplotype-based
GWAS identified all significant regions detected by single SNP-based GWAS for TeW. Furthermore, haplotype-based GWAS detected more significant regions for TeW than single SNP-based GWAS. Such significant regions on chromosomes 1 and 11 for TeW in the present study (Table 2) were not detected by single SNP-based GWAS as previously reported (Zhang et al., 2017a). Therefore, from these results we could conclude that the haplotype-based GWAS is a good supplement for single SNP-based GWAS.

For CW, HW, LW, SW, and MGSW, only a couple of haplotypes were significantly associated. Unfortunately, no interesting candidate genes were detected for these carcass traits (Table 1, Supplementary Table 1, and Supplementary Figure 1).

**Haplotype-Based GWAS for Growth Trait**

Manhattan plots of haplotype-based GWAS for MeC are shown in Figure 5. There were 122 haplotype windows that were significantly associated with MeC (Table 1 and Supplementary Table 1). A total of 110 haplotype windows were obtained after deleting the overlapped windows. Most of these significant haplotypes were distributed on chromosomes 1, 2, 8, 21, and Z. There were 66 RefGenes located in these regions, and possible candidate genes for bone traits include **TNFRSF1B**, **PLOD1**, **NPPC**, **MTHFR**, **EPHB2**, and **SLC35A3**. These genes were contained within or near a haplotype window that was significantly associated with MeC (Table 3). For each gene, individuals with the major haplotype (Hap1) had significantly lower (or higher) MeC than the individuals with the other haplotypes (Hap2, Figure 6). **EPHB2** spanned 2 haplotype windows (WIN42231 and WIN42234). These 4 SNP that constituted these 2 windows were used to construct 4 SNP haplotypes. Individuals with the major haplotype 1212 (Hap1) had significantly lower MeC than the individuals with the other haplotypes (Hap2) (Figure 6C). These results indicated that **TNFRSF1B**, **PLOD1**, **NPPC**, **MTHFR**, **EPHB2**, and **SLC35A3** are important for bone development. **TNFRSF1B** is a TNF receptor superfamily member, which could regulate the effects of TNF on osteoclastogenesis (Abu-Amer et al., 2000). The SNP in **TNFRSF1B** could contribute to the genetic regulation of bone mass (Albagha et al., 2002). **PLOD1** is procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1. Variants within this gene have been associated with bone mineral density (BMD) in humans (Spotila et al., 2003; Huang et al., 2009). **NPPC** is C-type natriuretic peptide 3, which is also known as **CNP**. Mice that overexpress **CNP** have longer bones (Chusho et al., 2001). **CNP** could stimulate chondrocyte proliferation and increase the size of individual hypertrophic chondrocytes (Yasoda et al., 1998; Mericq et al., 2000). **CNP** has been implicated in the regulation of bone growth.
skeletal growth in transgenic and knockout mice (Bartels et al., 2004). *MTHFR* is methylenetetrahydrofolate reductase, which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine. Variants within this gene have been associated with BMD (Li et al., 2016). *EPHB2* is EPH receptor B2. The GWAS meta-analysis of humar spine volumetric BMD measured by quantitative computed tomography was carried out and several loci were identified, including rs12742784 within *EPHB2*, which was associated with higher volumetric BMD and decreased risk of clinical vertebral fracture (Nielsen et al., 2016). This noncoding SNP has been associated with increased *EPHB2* mRNA expression levels in human bone biopsies (Nielsen et al., 2016). The basic function of *SLC35A3* is as a UDP-GlcNAc transporter. It has been shown to be expressed in all human tissues examined, including mesodermal derived tissues, skeletal muscle, and bone marrow (Ishida et al., 1999). A missense mutation in *SLC35A3* gene has been associated with complex vertebral malformations in bovine and revealed a new mechanism for malformation of the vertebral column caused by abnormal nucleotide sugar transport into the Golgi apparatus (Thomsen et al., 2006). Some other studies have also identified *SLC35A3* as having an important role in vertebral malformations (Ghebranious et al., 2006; Ruščič and Kamiński, 2007; Chu et al., 2008; Ghanem et al., 2008, 2009; Wang et al., 2011).

For BW1, BW3, BW5, ChWi, KeL, and MeL, only a couple of haplotype windows were significantly associated with these traits. No potential candidate genes were detected for these growth traits (Table 1, Supplementary Table 1 and Supplementary Figure 1).

**Haplotyping Using Sliding Window of 3 SNP, 4 SNP, and 5 SNP**

The GWAS results for carcass and growth traits aforementioned were all based on haplotypes extracted from sliding windows of 2 neighbor SNP. We also constructed haplotypes using 3 SNP in a sliding window, 4 SNP in a sliding window, and 5 SNP in a sliding window. Accordingly, haplotyping-based GWAS were carried out using 3-SNP, 4-SNP and 5-SNP sliding windows, respectively. Manhattan plots of 3-SNP, 4-SNP, and 5-SNP windows for carcass and growth traits are shown in Supplementary Figure 2. These results are similar as the results of 2-SNP window described previously.

In summary, the present study successfully used the haplotype-based GWAS method to detect important chromosome regions that harbor genes associated with carcass and growth traits in chicken. *SHH, LMBR1, FGF7, IL16, PLIN1, IGF1R*, and *SLC16A1* were identified as potential candidate genes for abdominal fat deposition. *TCF21* and *TCF12*, which were also previously detected by single SNP GWAS and epistatic effect analysis, were detected as important candidate genes for testis growth and development. *TNFRSF1B, PLOD1, NPPC, MTHFR, EPHB2*, and *SLC35A3* were potentially important genes for bone development. Only a couple of regions were detected as significantly associated with other carcass and growth traits. The results of this study may be helpful for exploring the metabolic mechanisms of fat deposition and testis growth in chicken.

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**SUPPLEMENTARY DATA**

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.01.009

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