GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Genome-wide association study of growth traits in the Jinghai Yellow chicken

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Genet. Mol. Res. 14 (4): 15331-15338 (2015)
Received June 27, 2015
Accepted October 8, 2015
Published November 30, 2015
DOI http://dx.doi.org/10.4238/2015.November.30.10

ABSTRACT. Growth is one of the most economically important traits in the poultry industry. In this study, we identified single-nucleotide polymorphisms (SNPs) and candidate genes associated with growth traits of the Jinghai Yellow chicken. Genome-wide association studies were conducted using the Illumina 60 K SNP Chicken array to genotype 400 Jinghai Yellow chickens. For each bird, the body weights at hatching and at 2, 4, 6, 8, 12, 14, and 16 weeks were recorded. The SNPs that were significantly associated with the growth traits were identified using the general linear regression model. The results revealed a total of 18 SNPs that reached Bonferroni genome-wide significance (P < 1.80E-6). Three proximal genes (*BTRC*, *NLK*, and *NF1*) were found to participate in the Wnt-signaling pathway and mitogen-activated protein kinase signaling pathway. Haplotype analysis identified 19 significant haplotypes and identified a region 152.4-156.3M on GGA1 affecting 3 growth traits (BW4, BW14, and BW16). These results may help identify the exact

locations of body weight quantitative trait loci on a genome level and indicate variants that can be used for subsequent investigations for Jinghai Yellow chicken.

Key words: Body weight; Genome-wide association study; PLINK; Jinghai Yellow chicken; Single-nucleotide polymorphism

INTRODUCTION

Growth is one of the most economically important traits in the poultry industry; therefore, studies exploring quantitative trait loci (QTLs) that affect the body weight of chickens are very important (Wahlberg et al., 2009; Gu et al., 2011; Xie et al., 2012). To date, more than 1500 QTLs, covering most of the genome, have been found to be associated with growth traits (Hu et al., 2013). Previous genomic studies have generally employed low-density microsatellites as markers. However, this approach may no longer provide novel information.

Currently, genome-wide association studies (GWAS) employ single-nucleotide polymorphisms (SNPs) as markers; these potential markers are distributed throughout the entire genome at a high density. Such studies have been used to comprehensively analyze complex, economically important traits using technical statistical tools (Fan et al., 2011; Liu et al., 2011; Onteru et al., 2012; Liu et al., 2013; Wolc et al., 2014). Thus, GWAS have been one of the most effective approaches for identifying related QTLs and functional genes (Xu et al., 2013). In chickens, Sun et al. (2013) identified 14 new genes related to meat quality traits in chicken using a GWAS. Liu et al. (2013) found that a consistent region on chicken *GGA4* was associated with carcass weight and eviscerated weight. Gu et al. (2011) used an F_2 resource population derived from Silky Fowl and White Plymouth Rock chickens to detect SNPs associated with body weight at 7-14 weeks of age in a region of *GGA4* (71.6-80.2 Mb). Xie et al. (2012) identified SNPs in a region of *GGA1* (173.5-175 Mb) that strongly affected the body weight of F_2 chickens based on an F_2 population derived from Xinghua and White Plymouth Rock chickens.

Chinese chicken breeds have gained increased attention for their high nutritional value and improved meat quality, although most breeds are slow-growing. Jinghai chicken is a national cultivated meat breed (minitype). In order to increase the growth rate of this chicken breed, the Illumina 60 K SNP Chicken array was used to identify key SNPs and functional genes affecting growth traits in the present study.

MATERIAL AND METHODS

Ethics statement

Blood samples of chickens were collected from the brachial vein using a standard venipuncture procedure (#XK622) approved by the Animal Welfare Committee of Yangzhou University.

Experimental animals

Jinghai Yellow chickens were obtained from the core group within the Jinghai Yellow

Chicken Breeding Station. Nineteen unrelated male chickens were selected to constitute 19 half-sibling families, and 400 female offspring were chosen randomly from the same batch. All chickens were raised in the pens and fed commercial diets meeting National Research Council's (NRC) requirements. Their body weights at hatching and at 2, 4, 6, 8, 12, 14, and 16 weeks were recorded. Before GWAS, the data were transformed in Minitab (v16.1.1) to approximate a standard normal distribution.

Sample preparation

The Dzup Genomic DNA Isolation Reagent Kit from Sangon Biotech Co., Ltd. (Shanghai, China) was used to extract genomic DNA, and a NanoDrop 2000 was used to assay the concentration and quality of genomic DNA to meet the requirements of the Infinium SNP genotyping platform. Subsequently, the DNA samples were genotyped using the Illumina 60 K SNP Chicken array (DNA LandMarks Inc., Quebec, Canada).

Data preparation

Quality control of the returned data was performed using PLINK (v1.07) (Purcell et al., 2007), and SNPs were removed for failing to meet one or more of the following criteria: low call rate (<90%), low call frequency (<95%), low Hardy-Weinberg equilibrium (<1E-6), and low minor allele frequency (<3%). As a result of this process, 396 samples and 46,665 SNPs were retained for further analysis.

Linkage disequilibrium based on SNP pruning of autosomes was applied using the indep-pairwise option with a window size of 25 SNPs, a step of 5 SNPs, and an r^2 threshold of 0.2, resulting in 12,877 independent SNPs. Principal component analysis (PCA) was performed in GCTA (v1.24), and PCA1 and PCA2 were used in models to reduce the population stratification effect (Wall et al., 2003). Moreover, haplotype analysis was performed with an r^2 threshold (>0.8) and window size (200 kb) in PLINK, and 5650 haplotypes were obtained for further study.

Statistical analysis

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

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

where *Y* is the vector of observations; *G* is genetic effect vector, *X* is a matrix containing all other fixed effects, including population structure (PCA1 and PCA2); α and β are incidence matrix, and *e* is the vector of random residual. Furthermore, association analysis between haplotypes and growth traits was also performed using the GLM. The Bonferroni P value was calculated based on the independent SNPs of autosomes, which were defined using the indep-pairwise option with $r^2 > 0.4$ (Liu et al., 2013). Ultimately, 27,824 SNPs were identified. The threshold P value for Bonferroni potential significance was 3.59E-5 (1/27,824), and the P value for Bonferroni genome-wide significance was 1.80E-6 (0.05/27,824). An empirical genome-wide P value was obtained using the maxT function in PLINK. Manhattan plots were

created using the R (2.15.1) software.

RESULTS

Descriptive statistics of the phenotypic measurements of growth traits in the 400 Jinghai Yellow chickens used for the present GWAS studies are shown in Table 1. All non-normal phenotypic data were normalized after Johnson transformation. Manhattan plots of the growth traits with significant SNPs are shown in Figure 1. A total of 18 SNPs with genome-wide significance (P < 1.80E-6) were shown (Table 2) to affect BW4 (2), BW6 (1), BW12 (3), BW14 (2), and BW16 (12). Among these SNPs, 6 were located on *GGA1* and 4 were located on *GGA19*. SNPs affecting body weight at 2, 8, and 10 weeks (BW2, BW8, and BW10) were not found.

Table 1. Basic growth-trait statistics.

Phenotype[1]	Sample size	Max	Min	Mean	Standard deviation
BW0(g)	344	46	25	33.9	3.7
BW2(g)	300	126	40	85.6	13.5
BW4(g)	348	324	106	205.3	29.9
BW6(g)	343	495	200	330.2	52.1
BW8(g)	293	695	310	513.5	72.7
BW10(g)	288	1290	405	706.4	106.7
BW12(g)	273	1170	605	870.7	100.5
BW14(g)	335	1480	680	1042.3	113.8
BW16(g)	398	1502	725	1123.5	124.8

[1]BW2, BW4, BW6, BW8, BW10, BW12, BW14, and BW16 indicate the body weights at the ages of 2, 4, 6, 8, 10, 12, 14, and 16 weeks, respectively.

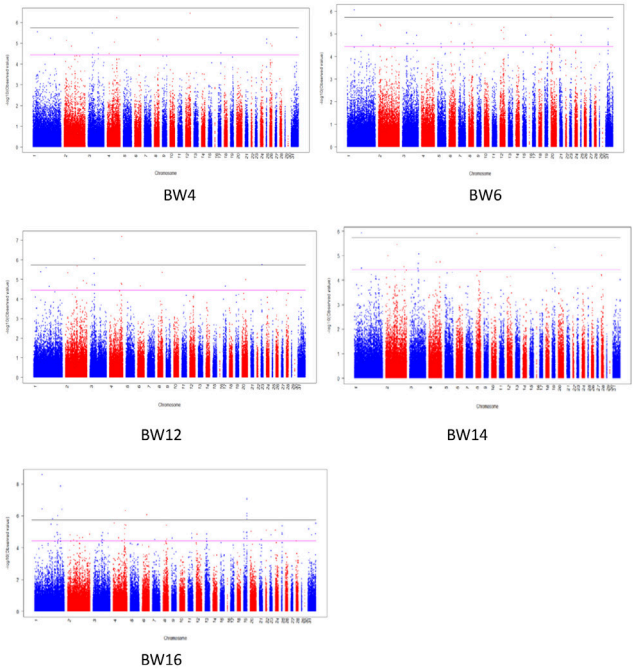

Figure 1. Manhattan plots for 5 growth traits with genome-wide significant SNPs. 1-28 on the x-axis indicate chromosomes 1-28, and 29, 30, and 31 indicate LGE22, LGE64, and chromosome Z respectively. The magenta horizontal line shows the potential significance threshold:-log10 (3.59E-5), while the black shows the potential significance genome-wise significance threshold:-log10 (1.80E-06).

Table 2. SNPs that reached genome-wide significance and their proximal genes.

Traits	SNP ID	Chr[1]	Pos (bp)	Alleles[2]	MAF	Pr[3]	P-adj	Proximal genes[4]
BW4	rs14047064	12	17762137	AG	0.03283	3.66E-07	0.010178	LOC771741
	GGaluGA263709	4	68188204	GA	0.4633	6.05E-07	0.016839	FRYL
BW6	rs13652021	1	50604748	CT	0.08418	8.71E-07	0.024207	22 U LOC101752136
BW12	GGaluGA267974	4	85148698	CT	0.4508	6.57E-08	0.001829	HTT
	rs14332284	3	29814153	CT	0.2184	8.7E-07	0.024193	94 U MOCS1
	rs16188810	23	3101987	CT	0.3099	1.75E-06	0.04872	PTPRU
BW14	rs13652021	1	50604748	CT	0.08418	1.19E-06	0.032971	22 U LOC101752136
	rs14637110	8	6077034	AG	0.3671	1.27E-06	0.035336	CACNA1E
BW16	rs13652021	1	50604748	CT	0.08418	2.62E-09	7.28E-05	22 U LOC101752136
	rs13973774	1	175116535	CT	0.2273	1.35E-08	0.000375	62 U COG6
	rs14123975	19	9331455	CT	0.351	8.49E-08	0.002361	SGSM2
	GGaluGA016599	1	50616709	AC	0.06962	3.64E-07	0.010128	10 U LOC101752136
	rs13983925	1	184074501	AG	0.1835	3.84E-07	0.010682	20 U LOC101748238
	GGaluGA267023	4	82146031	AG	0.3987	4.68E-07	0.013008	EVC
	rs15855551	19	9232211	GA	0.3797	7.01E-07	0.019496	NLK
	rs14583610	6	24330708	TC	0.1324	8.58E-07	0.023862	BTRC
	rs13957061	1	154795787	CT	0.3023	1.00E-06	0.027824	303 D SLITRK6
	rs13576057	19	9018028	TG	0.4356	1.03E-06	0.028547	NF1
	rs15397087	1	120766034	TC	0.4337	1.55E-06	0.043099	6 U LOC101749456
	rs15050713	19	9249624	AC	0.4293	1.57E-06	0.043656	NLK

[1]Position based on WADHUC2. [2]First allele is minor allele. [3]Pr indicates P value calculated by GLM, and P-adj indicated P value corrected by Bonferoni. [4]U = upstream, D = downstream. The unit of distance is kb.

Important candidate genes

Genes that were closest to the SNPs reaching genome-wide significance were searched in the NCBI database, and 16 genes were identified. Subsequently, pathway analysis of the genes was performed in the KEGG database. Only molybdopterin synthase, beta-transducin repeat containing E3 ubiquitin protein ligase (BTRC), nemo-like kinase (NLK), and neurofibromin 1 were found to be involved in pathways of chicken. Furthermore, BTRC and NLK were found to participate in the Wnt pathway: KEGG (gga04310) and NLK and neurofibromin 1 were involved in the mitogen-activated protein kinase (MAPK) pathway: KEGG (gga04010).

Haplotype analysis

Association analysis was performed between 5650 haplotypes and growth traits, and 19 haplotypes (Table 3) with genome-wide significance were identified to affect BW4 (1), BW6 (2), BW12 (2), BW14 (2), and BW16 (12). Twelve of the 19 haplotypes were located on *GGA1* and 7 of the 12 haplotypes were located in the region 52.4-156.3 Mb and affected BW6, BW14, and BW16, respectively.

DISCUSSION

Gu et al. (2011) found that SNPs associated with body weight at 7-14 weeks were in a region of *GGA4* (71.6-80.2 Mb) and Xie et al. (2012) identified SNPs in a region of *GGA1* (173.5-175 Mb) that strongly affected chicken body weight. In the present study, 18 SNPs reaching genome-wide significance in this study were located on 8 different chromosomes. No

obvious central region was found. Among these SNPs, 6 were located on *GGA1*, indicating that *GGA1* was an important candidate gene affecting body weight traits in the Jinghai Yellow chicken. The most important SNP appeared to be rs13652021, which was located 22 kb upstream of LOC101752136 and reached genome-wide significance with the BW6, BW14, and BW16 traits.

Table 3. Statistics of significant haplotypes with growth traits.

Traits	Chr	Pos I (bp)[1]	SNP I	Pos II (bp)	SNP II	Haplotype	Frequency	P[2]	P-adj
BW4	4	68174492	rs16428332	68188204	GGaluGA263709	AA	0.529	2.74E-07	7.62E-03
BW6	1	106629958	rs14866838	106815298	rs14866901	GCGGG	0.0139	3.05E-07	8.49E-03
BW6	1	154795787	rs13957061	154802557	rs13957071	CG	0.199	4.99E-07	1.39E-02
BW12	3	29814153	rs14332284	29832007	rs14332342	CC	0.216	1.56E-06	4.34E-02
BW12	24	2659651	GGaluGA191711	2668989	GGaluGA191716	AT	0.845	1.48E-06	4.12E-02
BW14	1	152462032	rs15461150	152587110	rs13956096	GGGG	0.0973	5.89E-07	1.64E-02
BW14	1	173531349	rs14916724	173536168	GGaluGA054842	AG	0.17	1.63E-07	4.54E-03
BW16	1	50616709	GGaluGA016599	50632934	rs13863600	AG	0.0696	3.18E-07	8.85E-03
BW16	1	120766034	rs15397087	120783594	rs13685727	CT	0.566	1.47E-06	4.09E-02
BW16	1	152462032	rs15461150	152587110	rs13956096	GGGG	0.0973	1.13E-06	3.14E-02
BW16	1	152930210	rs15461616	153065631	rs15461695	TGTCC	0.243	1.70E-06	4.73E-02
BW16	1	154795787	rs13957061	154802557	rs13957071	CG	0.199	1.61E-07	4.48E-03
BW16	1	154795787	rs13957061	154802557	rs13957071	TG	0.698	2.60E-07	7.23E-03
BW16	1	156277771	rs15465132	156390993	GGaluGA050635	ATGCGG	0.072	5.21E-07	1.45E-02
BW16	1	173531349	rs14916724	173536168	GGaluGA054842	AG	0.17	4.31E-09	1.20E-04
BW16	25	2007976	GGaluGA194718	2016848	GGaluGA194725	ACT	0.257	4.82E-07	1.34E-02
BW16	25	2007976	GGaluGA194718	2016848	GGaluGA194725	ACC	0.574	7.61E-07	2.12E-02
BW16	Z	44593823	rs14717488	44724842	rs14015587	GGACC	0.0606	3.80E-07	1.06E-02
BW16	Z	44743299	rs14955891	44760082	rs14015602	AC	0.0601	8.95E-08	2.49E-03

[1]Pos I and SNP I indicate the position and the SNP ID of the first allele of a haplotype, Pos II and SNP II indicate the position and the SNP ID of the last allele of a haplotype. [2]P indicates P value calculated by GLM, and P-adj indicated P value corrected by Bonferoni.

Sixteen candidate genes were detected in this study, with some genes related to tumors and growth. Most of these genes have not been previously reported in chickens. Among the SNPs in these genes, the largest effects were exerted by rs14047064 in LOC771741 on BW4; rs13652021 22 kb upstream of LOC101752136 on BW6, BW14, and BW16; GGaluGA267974 in huntingtin on BW12. The functions of LOC771741 and LOC101752136 remain unclear, although all may encode proteins. Huntingtin encodes a protein termed huntingtin. Wild-type huntingtin was reported to protect neurons and regulate cell apoptosis by binding with p21 protein (Cdc42/Rac)-activated kinase 2 (Leavitt et al., 2006; Luo and Rubinsztein, 2009).

The WNT pathway and the MAPK pathway were identified as being associated with growth traits involving BTRC, NLK, and neurofibromin 1. The Wnt pathway, which has been highly conserved throughout evolution, plays an important role in many biological processes, such as growth, development, and metabolism. Furthermore, this pathway is involved in regulating stem cell function in adult tissues, the development of muscle fibers, and the number of terminally myogenic cells (Anakwe et al., 2003; Borello et al., 2006). The MAPK pathway, which is also highly conserved between different species, can participate in many cell processes, such as cell growth, differentiation, migration, and apoptosis (Roberts et al., 2000). *BTRC* encodes a member of the F-box protein family that functions in phosphorylation-dependent ubiquitination. BTRC was found to be a tumor-inhibiting factor in previous studies (Wolter et al., 2003). *NLK* is involved in both the Wnt pathway and the MAPK pathway. Previous reports indicated that NLK inhibited Wnt/β-catenin and regulated cell growth and

apoptosis in *Drosophila* and mice (Kortenjann et al., 2001). Pathway analysis was limited because the functions of many of these genes are not definitively known (e.g., LOC101752136). However, based on our results, growth is clearly a highly complex biological process that is regulated by multiple pathways.

Nineteen haplotypes reaching genome-wide significance with growth traits were identified, and 7 of the 19 haplotypes contained SNPs with genome-wide significance. The results not only validated the results of our SNP association study, but also demonstrate that haplotype analysis can be used to identify QTLs that were lost by SNP analysis. Seven of the 19 haplotypes were located in the region 152.4-156.3 Mb of *GGA1* and were associated with affected BW6, BW14, and BW16 traits. This region should be further examined. Few studies have examined genes in this region, and only a few of functional genes were identified. These genes mainly found to belong to the SLITRK family (SLITRK1, SLITRK5, and SLITRK6) and are expressed mainly in the central nervous system and hematopoietic stem cells in human and mouse (Milde et al., 2007; Beaubien and Cloutier, 2009).

CONCLUSIONS

Eighteen SNPs with genome-wide significance were identified to affect growth traits in the Jinghai Yellow chicken. A few candidate genes that affected growth traits were also detected, and 2 possible pathways were found to regulate the growth of Jinghai Yellow chicken. Haplotype analysis identified 19 significant haplotypes and a region 151.3-156.7 Mb on *GGA1* that affected 3 growth traits. These results may be useful for identifying the exact locations of body weight QTL on a genome level and provide suggested variants for further studies in Jinghai Yellow chicken.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Broiler Industrial and Technology System (#nycytx-42-G1-05), the Priority Academic Program Development of Jiangsu Higher Education Institutions, and the New Century Talent Project of Yangzhou University.

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