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ORIGINAL ARTICLE

Fine-mapping of quantitative trait loci for body weight and bone traits and positional cloning of the *RB1* gene in chicken

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Summary

Previously, a quantitative trait locus (QTL) that affects body weight (BW) at 4–12 weeks of age and carcass weight at 12 weeks of age had been mapped on chicken chromosome 1. After including more markers and individuals, the confidence interval was narrowed down to approximately 5.5 Mbps and located this QTL near a microsatellite marker (ADL328). This QTL is the same as the QTL for 12 bone traits, including metatarsus length and metatarsus circumference at 4, 6, 8, 10 and 12 weeks of age and keel length and metatarsus claw weight at 12 weeks of age, that was identified using the same population. In the current study, 1010 individuals from the Northeast Agricultural University F_2 resource population were used and 14 single-nucleotide polymorphism (SNPs) around ADL328 were developed to construct haplotypes, and an association analysis was performed to fine-map the QTL. The haplotypes were constructed on the basis of a sliding 'window', with three SNP markers included in each 'window'. The association analysis results indicated that the haplotypes in 'windows' 6–12 were significantly associated with BW and bone traits and suggested that the QTL for BW and bone traits was located between SNP8 and SNP14 or was in linkage disequilibrium with this region. The interval from SNP8 to SNP14 was approximately 400 kbps. This region contained five RefSeq genes (*RB1, P2RY5, FNDC3A, MLNR* and *CAB39L*) on the University of California Santa Cruz website. The *RB1* gene was selected as a candidate gene and five SNPs were identified in the gene. The association results indicated that the *RB1* gene was a major gene for BW and bone traits. The SNPs g.39692 G>A and g.77260 A>G in *RB1* gene might be two quantitative trait nucleotides for BW and bone traits.

Introduction

For more than 50 years, substantial advances have been made in the improvement of traits of interest in chicken by artificial selection. However, most selection has been made on the basis of observable phenotypes and with no knowledge of the genetic background of the selected characteristics (Dekkers & Hospital 2002). The development of methods to characterize genetic variation at the DNA level has enabled the mapping of genes that affect quantitative traits of interest in agricultural animals (e.g. Lander & Kruglyak 1995). Currently, mapping of quantitative trait loci (QTL) is the preferred approach

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to detect genetic variation of economically important traits at a molecular level. Using this strategy, a number of chromosomal locations predicted to harbour genes that influence traits of interest have been identified in chicken (e.g. Abasht *et al.* 2006). However, the confidence intervals of the QTL are often more than 20 cM (Zhou *et al.* 2007), which is too large for positional cloning of the underlying candidate genes. Therefore, fine-mapping of QTL and identification of the underlying genes remain a major challenge in the genetic analysis of traits of interest.

Three factors that limit the resolution of fine-mapping are marker density, sample size and crossover density. Although increasing marker density remains time-consuming, it is conceptually the simplest bottleneck to resolve (Nezer *et al.* 2003). In addition, simply increasing the sample size can make a substantial contribution to the fine-mapping of QTL owing to the inverse relationship between resolving power and sample size (Darvasi & Soller 1997). There are two options to increase the density of observable crossover: (i) breed recombinants de novo or (ii) exploit historical recombination events (Nezer *et al.* 2003). In the first approach, additional recombination events are created through selective breeding, and this has been used generally with model organisms that have a short generation interval (Darvasi 1998). The second approach takes advantage of historical recombinants to refine the location of QTL. For example, haplotype analysis utilizes historical recombinants to fine-map the QTL of interest (Lopes *et al.* 2009).

Recently, a QTL with major effect on body weight (BW) from 4 to 12 weeks of age and carcass weight (CW) at 12 weeks of age had been mapped on chicken chromosome 1 using a F_2 population for a broiler × layer cross (Liu *et al.* 2007). For BW at 12 weeks of age (BW12), the QTL confidence interval spans 50.8 cM in genetic distance or 24 Mbps of the chicken genome (http://www.genome.ucsc.edu/). To fine-map this QTL, nine additional microsatellite markers and eight F_1 half-sib families were used to narrow down the confidence interval to 5.5 Mbps of chicken genomic sequence (http://www.genome.ucsc.edu/) (Liu *et al.* 2008) and the QTL was located near the microsatellite marker ADL328. The QTL for BW from 4 to 12 weeks of age and CW at 12 weeks of age was the same as the QTL for bone traits, including metatarsus length (MeL) and metatarsus circumference (MeC) at 4, 6, 8, 10 and 12 weeks of age and keel length (KeL) and metatarsus claw weight (MeCW) at 12 weeks of age, that was reported by Zhang *et al.* (2010) using the same F_2 population. However, the confidence interval of this QTL was still large, and there were hundreds of genes in this region. The objective of the present study was to fine-map this QTL using a haplotype approach and to identify the major gene for BW and bone traits.

Materials and methods

Animals and phenotypic measurements

The Northeast Agricultural University Resource Population (NEAURP) was used in the current study (Liu *et al.* 2007, 2008; Zhang *et al.* 2010). The traits analysed in the current study included BW from 4 to 12 weeks of age and CW at 12 weeks of age and 12 bone traits (MeL and MeC at 4, 6, 8, 10 and 12 weeks of age and KeL and MeCW at 12 weeks of age) had been fine-mapped previously (Liu *et al.* 2008; Zhang *et al.* 2010). A total of 1010 F_2 individuals were used in this study and were derived from 12 half-sib families. All F_2 birds had free access to feed and water. Commercial corn/soybean-based diets that met all National Research Council (NRC, 1994) requirements were provided in the current study. From hatching to 3 weeks of age, birds received a starter feed (3000 kcal of ME/kg and 210 g/kg of CP), and from 3 to 12 weeks of age, birds were fed a grower diet (3100 kcal of ME/kg and 190 g/kg of CP) (Wang *et al.* 2006).

Genotyping

Genomic DNA was isolated from venous blood samples using a phenol–chloroform method (Wang *et al.* 2006). A total of 14 single-nucleotide polymorphism (SNP) markers were selected from the SNP database on the University of California Santa Cruz (UCSC) website (http://www.genome.ucsc.edu/) to carry out the haplotype analysis. Another five SNPs in the *RB1* gene, three (g.2174 G>A, g.39692 G>A, and g.77260 A>G) from the sequencing results and two (g.33349 A>G and g.72532 G>A) from the SNP database (http://www.genome.ucsc.edu/), were identified to perform the association analysis. All primers were designed on the basis of the chicken genome sequence in the GenBank database (http://www.ncbi.nlm.nih.gov) using the software Primer 5.0 (PREMIER Biosoft International, Palo Alto, CA, USA). Polymerase chain reactions (PCR) for all the SNP markers were carried out separately in a reaction volume of 10 μl, which included 1× PCR reaction buffer, 200 μM each dNTP, 0.25 μM each primer, 1 μl of genomic DNA (50 ng/μl)

and 0.5 U of *Taq* polymerase. The thermal profiles were 95°C for 7 min followed by 35 cycles of 95°C for 30 s, 45–65°C for 30 s and 72°C for 45 s. Typing of the SNPs was performed using the PCR-restriction fragment length polymorphism (RFLP) and length polymorphism (PCR-LP) method. For RFLP, the PCR products were digested using 0.5–2 U of the restriction enzyme at 37°C overnight. Restriction patterns were visualized by electrophoresing the digestion product in a 2–3% agarose gel stained with ethidium bromide. For PCR-LP, polymorphism patterns were visualized by electrophoresing 1 μl of the PCR product in a 14% non-denaturing polyacrylamide gel stained with silver nitrate. Information about all the SNPs and the restriction enzymes used is listed in Table 1.

Statistical analysis

Haplotypes were constructed based on a sliding 'window' that comprised three markers and slide one marker each time (e.g. Calus *et al.* 2009). The haplotypes of each 'window' were constructed using HAPLOTYPE procedure in SAS 9.1.3 software (SAS Institute Inc., Cary, NC 2004). The combination

Table 1 Information on SNP markers used in the current study

Markers[1]	Position[2] (bp)	Type	Forward primer (5′->3′) Reverse primer (5′->3′)	Restriction enzyme
SNP1	172747132	C>T	CACCTAAGCACCACAAAACC CTTTTTGTTCATCAGGACGC	*AfaI*
SNP2	172794063	AATATTGTATTTGGGTGGATCAT (deletion)	ACAGCGATAGTTGTAAGCAG CCTGGAGTATTAGGCTTTT	–
SNP3	172843508	A>T	GAAGTTTTTCACACAGAGGG CAGGACTTGAAGGATGTTGT	*DraI*
SNP4	172945958	ATTTCAAGT (deletion)	AATAAGGAGAAGTGTGAT GTGTAAGAGATGAAAAAC	–
SNP5	173046793	G>A	CCCTCCTGTAGTCTGTATT AAGTCAACAACTATCAGGG	*AfaI*
SNP6	173144757	C>T	CGAATGGCTAACCCTAC GAAGTAGTGGGCTGGAG	*HindIII*
SNP7	173173350	GCACTTCA (deletion)	TATTTCTCGCCTTCTTTC AATCACCAGGGTTAGTTT	–
SNP8	173196313	G>A	GTGTTTATCAGCACGAGCC CAGGAACTGTCAAGGTGGG	*MspI*
SNP9	173239127	A>G	AGAAAGCCACTATCAAGAAC TTGGGTGTCAACAAGGAT	*MboI*
SNP10	173306996	T>G	GAAGTGAGGGTGTTGGAGAC GAGCAGGTGAGTTTCAAATAGG	*XbaI*
SNP11	173492453	C>T	CTTCAAAGTGCTCCTATCCC GTGGTATTACTTGTGGGAGC	*Eco72I*
SNP12	173511382	A>G	ATTGACCGTGCCAGATTACC ACGACCCAAACTACCTGACC	*EcoRV*
SNP13	173524229	T>G	CCACAACAACTTCAGGGATG TGTGCTGAGGTAGCCAAGAG	*XbaI*
SNP14	173539900	T>C	CCTATGTGGAAGCGTGAG TCTTGGGAATGAGGAGTT	*XbaI*
g.2174 G>A	*RB1*	G>A	CAAATAGCGTTGCTGACCCG ACACCGAGAGGCTCCTGGAT	*Hae III*
g.33349 A>G	*RB1*	A>G	GGAAAAGTCTTCTCAATA AGTCTCCCACCTCTGT	*Ban II*
g.39692 G>A	*RB1*	G>A	TGTTTTCGTGACCATACCAT CAGAGTTCCACTATCCATTCC	*Asu I*
g.72532 G>A	*RB1*	G>A	AGACTTGAAGGGAGCATA CAGGGTGAGAAATAAACAT	*Hha I*
g.77260 A>G	*RB1*	A>G	TGAGTTGCTTCTTCAGTCGCTTT ATTCAGCGACCAATCCGTGTG	*Hha I*

SNP, single-nucleotide polymorphism.

[1]Names of SNP markers used in the current study.

[2]Physical positions (Mbps) of SNP markers in UCSC and the last five SNPs in the *RB1* gene.

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genotypes of the two polymorphisms in *RB1* gene were constructed. For the term combination genotype, it is that if genotypes of a given individual at two loci are GG and AA, respectively, the combination genotype of the individual at the two loci is GGAA. The association analysis was carried out using the GLM procedure of JMP 4.0. The model used for association analysis was fitted with single SNP genotypes (s), haplotypes (h), combination genotypes (c), sex (S), hatch (H, 2 hatches), s*S, s*H, h*S, h*H, c*S, and c*H as fixed effects (*means interaction), and family (F) and dam nested within the family [D (F)] as random effects. BW at 0 week (BW0) was the linear covariate for BW from 4 to 12 weeks of age, CW at 12 weeks of age, and MeL and MeC at 4, 6, 8, 10 and 12 weeks of age. BW at 12 weeks of age was the linear covariate for KeL and MeCW at 12 weeks of age

$$Y = \mu + \mathrm{s} + \mathrm{S} + \mathrm{H} + \mathrm{F} + \mathrm{D}\ (\mathrm{F}) + \mathrm{s}^*\mathrm{S} + \mathrm{s}^*\mathrm{H} + \mathrm{BW0}\ (\text{or BW12}) + e \quad (1)$$

$$Y = \mu + \mathrm{h} + \mathrm{S} + \mathrm{H} + \mathrm{F} + \mathrm{D}\ (\mathrm{F}) + \mathrm{h}^*\mathrm{S} + \mathrm{h}^*\mathrm{H} + \mathrm{BW0}\ (\text{or BW12}) + e \quad (2)$$

$$Y = \mu + \mathrm{c} + \mathrm{S} + \mathrm{H} + \mathrm{F} + \mathrm{D}\ (\mathrm{F}) + \mathrm{c}^*\mathrm{S} + \mathrm{c}^*\mathrm{H} + \mathrm{BW0}\ (\text{or BW12}) + e \quad (3)$$

The models Equations (1–3) were used for single SNP, haplotype and combination genotype associations with both BW and bone traits, respectively, where Y is the dependent variable, μ is the population mean and e is the random error. Significant differences between least square means of different genotypes were calculated using a contrast test. Significant associations were adjusted by false discovery rate (FDR), which was calculated using the formula FDR = mp(i)/i, where m was the total number of tests (12 haplotype windows × 22 traits) and p(i) was the p-value at rank i when the p-values were ranked from least to highest (Benjamini & Hochberg 1995; Weller *et al.* 1998).

Results

The haplotypes were constructed on the basis of a sliding 'window' of three markers on chicken chromosome 1. Using 14 SNPs, a total of 12 'windows'

Table 2 Association analysis between different haplotypes in every 'window' and body weight and bone traits (p-value)

Traits[1]	1 (8)[2]	2 (8)	3 (7)	4 (7)	5 (7)	6 (7)	7 (8)	8 (6)	9 (6)	10 (7)	11 (7)	12 (6)
BW4 (g)	0.0204*	0.1432	0.0900	0.1146	0.0090*	<0.0001**	<0.0001**	<0.0001**	0.0014**	<0.0001**	<0.0001**	<0.0001**
BW5 (g)	0.0063**	0.0258*	0.0042**	0.0688	0.0006**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW6 (g)	0.0288*	0.0765	0.0252*	0.1774	0.0003**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW7 (g)	0.0030**	0.0372*	0.0141*	0.3597	0.0018**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW8 (g)	0.0028**	0.0258*	0.0111*	0.1882	0.0006**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW9 (g)	0.0130*	0.0792	0.0362*	0.2205	0.0015**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW10 (g)	0.0043**	0.0522	0.0227*	0.2386	0.0014**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW11 (g)	0.0053**	0.0823	0.0340*	0.3429	0.0009**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
BW12 (g)	0.0120*	0.1827	0.0429	0.4686	0.0011**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
CW (g)	0.0133*	0.1736	0.0323*	0.5126	0.0007**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
MeL4 (cm)	0.2707	0.1585	0.3142	0.6837	0.2934	0.0015**	0.0007**	<0.0001**	0.0007**	<0.0001**	<0.0001**	<0.0001**
MeC4 (cm)	0.1013	0.2054	0.0931	0.1725	0.0738	<0.0001**	0.0002**	<0.0001**	0.0031**	<0.0001**	<0.0001**	<0.0001**
MeL6 (cm)	0.0295*	0.1144	0.0290*	0.0679	0.0089*	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
MeC6 (cm)	0.5215	0.3951	0.1085	0.4939	0.0256*	<0.0001**	<0.0001**	<0.0001**	0.0008**	<0.0001**	<0.0001**	<0.0001**
MeL8 (cm)	0.2198	0.1657	0.2525	0.3955	0.0289*	<0.0001**	0.0001**	<0.0001**	0.0001**	<0.0001**	<0.0001**	<0.0001**
MeC8 (cm)	0.0003**	0.0014**	0.0003**	0.0834	0.0005**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
MeL10 (cm)	0.3072	0.3420	0.0738	0.3697	0.0107*	<0.0001**	<0.0001**	<0.0001**	0.0009**	<0.0001**	<0.0001**	<0.0001**
MeC10 (cm)	0.0001**	0.0061**	0.0009**	0.0523	0.0004**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
MeL12 (cm)	0.6509	0.6621	0.3433	0.5431	0.0389*	<0.0001**	0.0001**	<0.0001**	0.0013**	<0.0001**	<0.0001**	<0.0001**
MeC12 (cm)	0.0007**	0.0367*	0.0044**	0.3753	0.0131*	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**
KeL (cm)	0.0469	0.8529	0.0154*	0.6147	0.0151*	<0.0001**	<0.0001**	<0.0001**	0.0015**	0.0022**	<0.0001**	0.0002**
MeCW (g)	0.6731	0.6651	0.2498	0.7134	0.4657	0.0027**	0.0016**	0.0034**	0.0035**	<0.0001**	0.0003**	<0.0001**

BW, body weight; CW, carcass weight; FDR, false discovery rate; KeL, keel length; MeCW, metatarsus claw weight; MeL, metatarsus length.
[1]Numbers after BW, MeL and metatarsus circumference indicate age in weeks.
[2]Numbers in parentheses after the sliding window number indicate the number of haplotypes.
*Significant at FDR<5% level. **Significant at FDR<1% level.

were constructed. The number of haplotypes in each 'window' (Table 2) was eight in theory, but some haplotypes did not exist in the current population.

The associations of the haplotypes in each 'window' with both BW and bone traits were analysed and the results are listed in Table 2. The haplotypes in 'windows' 1–5 were only significantly associated with some of the traits in the current study. The haplotypes in 'windows' 6–12 were associated significantly with all the traits including BW from 4 to 12 weeks of age, CW and the 12 bone traits at an FDR <1%. The haplotypes in 'window' 6 were associated significantly with all the traits of interest at an FDR <1% but haplotypes in 'window' 5 were only associated significantly with some of the traits in the current study at an FDR <1%. 'Window' 5 contained SNP5, SNP6 and SNP7, and 'window' 6 contained SNP6, SNP7 and SNP8. Therefore, two SNPs (SNP6 and SNP7) overlapped in these two windows, which suggested that SNP8, but not SNP6 and SNP7, might be associated significantly with the QTL for BW and bone traits. The associations of single SNPs with both BW and bone traits were also analysed, and the results indicated that SNP8 was associated significantly with BW and bone traits but SNP6 and SNP7 were not (data not shown). This suggested that the QTL for BW and bone traits might be located in the interval from SNP8 to SNP14.

The estimated size of the SNP8–SNP14 interval spanned approximately 400 kbps of the chicken genome (http://www.genome.ucsc.edu/), and there were five RefSeq genes (*RB1, P2RY5, FNDC3A, MLNR* and *CAB39L*) on the UCSC website (http://genome.ucsc.edu/) in this region. *RB1* gene was selected as a candidate gene for BW and bone traits and five SNPs were identified in the gene. The associations of the five SNPs with both BW and bone traits were analysed. The results indicated that g.39692 G>A and g.77260 A>G were significantly (at FDR <5% or FDR <1%) or suggestively significantly ($p < 0.05$) associated with BW and bone traits (Table 3). Birds with genotypes GG (g.39692 G>A) and AA (g.77260 A>G) had a significantly higher BW and MeCW and longer MeL, MeC and KeL than birds with genotypes AA (g.39692 G>A) and GG (g.77260 A>G) (Table 4). The haplotypes of g.39692 G>A and g.77260 A>G were constructed, and the results of the association analysis indicated that the haplotypes were associated significantly with BW and bone traits (Table 5). The combination genotypes of the two SNPs

Table 3 Association analysis of five single-nucleotide polymorphisms in the *RB1* gene with both body weight and bone traits (p-value)

Traits[1]	g.2174 G>A	g.33349 A>G	g.39692 G>A	g.72532 G>A	g.77260 A>G
BW4 (g)	0.1989	0.1787	0.0134*	0.3033	0.0268†
BW5 (g)	0.1081	0.6817	0.0154*	0.1267	<0.0001**
BW6 (g)	0.2392	0.9321	0.0024**	0.1008	<0.0001**
BW7 (g)	0.3212	0.9164	0.0175*	0.1310	<0.0001**
BW8 (g)	0.3995	0.7905	0.0085*	0.1367	<0.0001**
BW9 (g)	0.2321	0.5485	0.0096*	0.2113	<0.0001**
BW10 (g)	0.3837	0.6921	0.0028*	0.3236	<0.0001**
BW11 (g)	0.4628	0.9076	0.0005**	0.2117	<0.0001**
BW12 (g)	0.5485	0.9947	0.0038*	0.0648	<0.0001**
CW (g)	0.6269	0.8798	0.0060*	0.0726	<0.0001**
MeL4 (cm)	0.6217	0.8898	0.0010**	0.8695	0.0116*
MeC4 (cm)	0.4150	0.5375	0.0476†	0.5600	0.0070*
MeL6 (cm)	0.6537	0.8966	0.0002*	0.7687	0.0006**
MeC6 (cm)	0.5386	0.1318	0.0467†	0.0352†	0.0308†
MeL8 (cm)	0.5874	0.4408	0.0349†	0.5925	0.0006**
MeC8 (cm)	0.2349	0.8463	<0.0001*	0.0182†	<0.0001**
MeL10 (cm)	0.2847	0.6643	0.0017**	0.1123	0.0005**
MeC10 (cm)	0.1492	0.9736	0.0013**	0.0552	<0.0001**
MeL12 (cm)	0.3847	0.5987	<0.0001**	0.3518	0.0015**
MeC12 (cm)	0.2621	0.9516	<0.0001**	0.0652	<0.0001**
KeL (cm)	0.8525	0.2237	0.0023**	0.0904	0.0007**
MeCW (g)	0.3286	0.5128	0.0094*	0.0262†	0.0045*

BW, body weight; CW, carcass weight; FDR, false discovery rate; KeL, keel length; MeCW, metatarsus claw weight; MeL, metatarsus length.

[1]Numbers after BW, MeL and metatarsus circumference indicate age in weeks.

†Suggestive of significance ($p < 0.05$).

*Significant at the FDR<5% level. **Significant at the FDR<1% level.

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Table 4 Effects of different genotypes (g.39692 G>A and g.77260 A>G) on body weight and bone traits (least squares means)[1]

	g.39692 G>A				g.77260 A>G			
Traits	AA (14)[2]	AG (272)	GG (717)	Phenotypic variance[3] (%)	AA (14)	AG (238)	GG (754)	Phenotypic variance[3] (%)
BW4 (g)	407.4 ± 17.0^{B}	443.6 ± 5.2^{B}	451.6 ± 4.3^{A}	0.70	478.1 ± 17.5^{A}	456.7 ± 5.6^{A}	445.6 ± 4.5^{B}	0.65
BW5 (g)	575.8 ± 23.3^{B}	616.9 ± 7.0^{A}	629.9 ± 5.6^{A}	0.77	678.9 ± 26.7^{A}	644.8 ± 7.7^{A}	618.0 ± 6.1^{B}	1.90
BW6 (g)	725.3 ± 36.3^{C}	809.5 ± 9.9^{B}	829.6 ± 8.3^{A}	1.29	880.0 ± 33.9^{A}	850.0 ± 11.0^{A}	811.9 ± 9.1^{B}	1.86
BW7 (g)	949.5 ± 41.9^{B}	1029.0 ± 13.3^{A}	1050.2 ± 11.0^{A}	0.68	1119.3 ± 45.0^{A}	1080.2 ± 14.7^{A}	1028.4 ± 12.2^{B}	1.82
BW8 (g)	1141.8 ± 53.6^{B}	1232.8 ± 15.7^{A}	1264.9 ± 13.0^{A}	0.83	1344.6 ± 53.9^{A}	1306.0 ± 17.8^{A}	1233.9 ± 14.9^{B}	2.30
BW9 (g)	1367.9 ± 64.2^{B}	1461.8 ± 18.5^{A}	1502.4 ± 15.2^{A}	0.77	1603.6 ± 64.5^{A}	1546.8 ± 21.0^{A}	1466.5 ± 17.5^{B}	1.94
BW10 (g)	1574.6 ± 69.7^{B}	1641.3 ± 20.1^{A}	1700.3 ± 16.1^{A}	0.92	1846.1 ± 73.5^{A}	1747.7 ± 23.2^{A}	1655.3 ± 18.9^{B}	2.09
BW11 (g)	1755.7 ± 76.1^{B}	1824.1 ± 21.4^{A}	1898.4 ± 16.8^{A}	1.05	2008.7 ± 88.3^{A}	1952.5 ± 24.7^{A}	1844.8 ± 20.0^{B}	1.91
BW12 (g)	1942.2 ± 83.4^{B}	2011.6 ± 23.4^{A}	2080.7 ± 18.3^{A}	0.73	2169.8 ± 89.7^{A}	2146.3 ± 26.2^{A}	2027.9 ± 20.8^{B}	1.74
CW (g)	1718.9 ± 75.2^{B}	1782.5 ± 20.9^{A}	1841.9 ± 16.3^{A}	0.67	1945.4 ± 80.6^{A}	1903.1 ± 23.1^{A}	1794.2 ± 18.0^{B}	1.85
MeL4 (cm)	5.39 ± 0.09^{B}	5.75 ± 0.03^{B}	5.76 ± 0.03^{A}	1.24	5.89 ± 0.11^{A}	5.80 ± 0.03^{A}	5.73 ± 0.03^{B}	0.77
MeC4 (cm)	2.83 ± 0.06^{B}	2.89 ± 0.02^{A}	2.92 ± 0.01^{A}	0.45	3.03 ± 0.05^{A}	2.94 ± 0.02^{A}	2.90 ± 0.01^{B}	0.81
MeL6 (cm)	6.61 ± 0.14^{C}	7.06 ± 0.04^{B}	7.12 ± 0.03^{A}	1.68	7.32 ± 0.13^{A}	7.18 ± 0.04^{A}	7.07 ± 0.03^{B}	1.18
MeC6 (cm)	3.54 ± 0.07^{B}	3.61 ± 0.02^{A}	3.65 ± 0.01^{A}	0.40	3.69 ± 0.06^{A}	3.68 ± 0.02^{A}	3.63 ± 0.02^{B}	0.43
MeL8 (cm)	8.03 ± 0.14^{B}	8.31 ± 0.04^{B}	8.36 ± 0.04^{A}	0.50	8.63 ± 0.14^{A}	8.43 ± 0.05^{A}	8.31 ± 0.04^{B}	1.00
MeC8 (cm)	3.84 ± 0.08^{B}	3.91 ± 0.02^{A}	3.99 ± 0.02^{A}	1.36	4.08 ± 0.08^{A}	4.06 ± 0.02^{A}	3.93 ± 0.02^{B}	2.96
MeL10 (cm)	9.07 ± 0.14^{B}	9.25 ± 0.05^{A}	9.37 ± 0.04^{A}	0.71	9.54 ± 0.15^{A}	9.44 ± 0.05^{A}	9.29 ± 0.04^{B}	0.87
MeC10 (cm)	4.04 ± 0.07^{B}	4.12 ± 0.02^{B}	4.20 ± 0.02^{A}	1.01	4.30 ± 0.09^{A}	4.27 ± 0.03^{A}	4.14 ± 0.02^{B}	2.18
MeL12 (cm)	9.33 ± 0.16^{C}	9.72 ± 0.05^{B}	9.86 ± 0.04^{A}	0.79	10.01 ± 0.15^{A}	9.92 ± 0.05^{A}	9.77 ± 0.04^{B}	0.51
MeC12 (cm)	4.20 ± 0.09^{B}	4.30 ± 0.03^{A}	4.40 ± 0.02^{A}	1.28	4.56 ± 0.09^{A}	4.47 ± 0.03^{A}	4.34 ± 0.02^{B}	1.90
KeL (cm)	13.30 ± 0.13^{C}	13.54 ± 0.05^{B}	13.63 ± 0.05^{A}	0.34	13.94 ± 0.14^{A}	13.67 ± 0.05^{A}	13.57 ± 0.05^{B}	0.40
MeCW (g)	69.8 ± 2.2^{B}	73.5 ± 0.8^{A}	74.9 ± 0.7^{A}	0.15	76.4 ± 2.1^{A}	75.9 ± 0.8^{A}	73.9 ± 0.6^{B}	0.17

BW, body weight; CW, carcass weight; KeL, keel length; MeCW, metatarsus claw weight; MeL, metatarsus length.

[1]Least square means ± standard error.

[2]Different letters in the same row indicate significant differences between groups ($p < 0.05$); the same letter in the same row indicates the absence of significant differences between groups ($p > 0.05$).

[3]Percentage of phenotypic variance in the F_2 population.

(g.39692 G>A and g.77260 A>G) were also constructed, and the association results indicated that these combination genotypes were associated significantly with BW and bone traits (Table 6). The haplotype G-A and combination genotype GGAA had the same effects as the genotypes AA (g.39692 G>A) and GG (g.77260 A>G) (Table 6). The per cent of phenotypic variance that the single SNPs, haplotypes and combination genotypes can explain was 0.15–2.96%, 0.11–1.27% and 0.38–4.06%, respectively.

Discussion

Growth reflects the development of various individual parts of a body, and the amount of growth achieved is the result of interaction among genetic, nutritional and environmental factors (Scanes *et al.* 1984). Growth is under complex genetic control, and uncovering the molecular mechanisms of growth will contribute to more efficient selection for growth in broiler chickens (Deeb & Lamont 2002). Although the weight of broilers has been increased significantly over recent decades, bone problems have persisted and bone structure might not be strong enough to support the weight of these animals (Bradshaw *et al.* 2002). In this study, the associations between BW and bone traits were very significant (data not shown). Therefore, investigation of the molecular basis of bone traits might be beneficial for the selection of rapid growth. Identification of QTL can lead to marker-assisted selection (MAS) (van der Beek & van Arendonk 1996), and the results might contribute to the improvement of rapid growth in broiler chickens.

A QTL with pleiotropic effects on BW and bone traits was mapped and fine-mapped near the marker ADL328 (Liu *et al.* 2007, 2008; Zhang *et al.* 2010). This QTL was also reported in several other studies (Sewalem *et al.* 2002; Nones *et al.* 2006; Zhou *et al.* 2006). In order to fine-map this QTL and find the causal gene for BW and bone traits, 14 SNPs around ADL328 were selected to determine the linkage phase of the F_2 birds in the current study. The 14 SNPs were distributed relatively regularly around

Table 5 Association analysis of different haplotypes for g.39692 G>A and g.77260 A>G with both body weight and bone traits[1]

Traits	p-value	A-A (5)[2]	A-G (295)	G-A (261)	G-G (1455)	Phenotypic Variance[3] (%)
BW4 (g)	0.0051**	451.7 ± 31.0^{ABC}	440.2 ± 5.3^{C}	458.3 ± 5.4^{A}	449.4 ± 4.3^{B}	0.43
BW5 (g)	<0.0001**	636.1 ± 42.5^{ABC}	612.1 ± 7.0^{C}	645.5 ± 7.2^{A}	624.8 ± 5.5^{B}	0.88
BW6 (g)	<0.0001**	860.3 ± 56.9^{ABC}	804.3 ± 9.9^{C}	848.8 ± 10.1^{A}	821.9 ± 8.1^{B}	0.85
BW7 (g)	<0.0001**	1090.2 ± 77.6^{AB}	1022.6 ± 13.2^{B}	1078.3 ± 13.5^{A}	1040.8 ± 10.8^{B}	0.74
BW8 (g)	<0.0001**	1298.3 ± 91.1^{ABC}	1227.2 ± 15.7^{C}	1301.7 ± 16.1^{A}	1250.9 ± 12.8^{B}	0.90
BW9 (g)	<0.0001**	1533.0 ± 109.2^{ABC}	1457.7 ± 18.6^{C}	1544.2 ± 19.0^{A}	1486.3 ± 15.0^{B}	0.79
BW10 (g)	<0.0001**	1705.8 ± 124.1^{ABC}	1640.4 ± 20.5^{C}	1746.7 ± 21.0^{A}	1679.0 ± 16.2^{B}	0.87
BW11 (g)	<0.0001**	1903.2 ± 143.2^{ABC}	1826.0 ± 21.7^{C}	1949.5 ± 22.3^{A}	1872.8 ± 16.7^{B}	0.87
BW12 (g)	<0.0001**	2080.3 ± 153.9^{ABC}	2014.5 ± 23.3^{C}	2137.3 ± 23.9^{A}	2056.9 ± 17.6^{B}	0.69
CW (g)	<0.0001**	1872.6 ± 138.6^{ABC}	1783.0 ± 20.5^{C}	1896.3 ± 21.1^{A}	1820.6 ± 15.2^{B}	0.73
MeL4 (cm)	0.0154*	5.88 ± 0.18^{AB}	5.71 ± 0.03^{B}	5.80 ± 0.03^{A}	5.75 ± 0.03^{B}	0.40
MeC4 (cm)	0.0043**	2.99 ± 0.09^{AB}	2.89 ± 0.02^{B}	2.94 ± 0.02^{A}	2.91 ± 0.01^{B}	0.32
MeL6 (cm)	0.0003**	7.04 ± 0.22^{ABC}	7.03 ± 0.04^{C}	7.18 ± 0.04^{A}	7.10 ± 0.03^{B}	0.64
MeC6 (cm)	0.0074**	3.61 ± 0.13^{ABC}	3.61 ± 0.02^{C}	3.68 ± 0.02^{A}	3.64 ± 0.01^{B}	0.30
MeL8 (cm)	0.0011**	8.58 ± 0.24^{AB}	8.29 ± 0.04^{B}	8.44 ± 0.04^{A}	8.34 ± 0.04^{B}	0.47
MeC8 (cm)	<0.0001**	3.95 ± 0.13^{ABC}	3.91 ± 0.02^{C}	4.05 ± 0.02^{A}	3.96 ± 0.02^{B}	1.27
MeL10 (cm)	0.0003**	9.39 ± 0.25^{ABC}	9.25 ± 0.05^{C}	9.43 ± 0.05^{A}	9.34 ± 0.04^{B}	0.41
MeC10 (cm)	<0.0001**	4.24 ± 0.15^{ABC}	4.13 ± 0.02^{C}	4.26 ± 0.02^{A}	4.17 ± 0.02^{B}	0.84
MeL12 (cm)	0.0002**	9.60 ± 0.29^{ABC}	9.71 ± 0.05^{C}	9.92 ± 0.05^{A}	9.82 ± 0.04^{B}	0.35
MeC12 (cm)	<0.0001**	4.37 ± 0.16^{ABC}	4.31 ± 0.03^{C}	4.46 ± 0.03^{A}	4.37 ± 0.02^{B}	0.84
KeL (cm)	<0.0001**	14.13 ± 0.23^{A}	13.52 ± 0.05^{D}	13.67 ± 0.05^{B}	13.60 ± 0.05^{C}	0.28
MeCW (g)	0.0014**	77.8 ± 4.1^{ABC}	73.2 ± 0.8^{C}	75.8 ± 0.8^{A}	74.4 ± 0.6^{B}	0.11

BW, body weight; CW, carcass weight; FDR, false discovery rate; KeL, keel length; MeCW, metatarsus claw weight; MeL, metatarsus length.
[1]Different letters in the same row indicate significant differences between groups ($p < 0.05$). *FDR<0.05. **FDR<0.01.
[2]Least square means±standard error.
[3]Percentage of phenotypic variance in the F_2 population.

ADL328 with an average interval of approximately 61.5 kb. The haplotypes were constructed using a 'sliding window' method. The results for two, three and four markers per 'window' were very similar; therefore, a 'window' size of three markers was used in the current study. A total of 12 'windows', which each contained three SNPs, were constructed across the 14 SNPs. The results of the association analysis between the haplotypes in every window and traits of interest suggested that the QTL for BW and bone traits was located in the region from SNP8 to SNP14. The estimated size of the SNP8–SNP14 interval spanned approximately 400 kbps. In this region, five RefSeq genes (*RB1, P2RY5, FNDC3A, MLNR* and *CAB39L*) were identified on the UCSC genomic biology database (http://genome.ucsc.edu/). These genes might have an important effect on BW and bone traits.

The *RB1* gene has an important role in the regulation of growth during mouse development (e.g. Zacksenhaus *et al.* 1996; Nikitin *et al.* 2001) and in osteogenic differentiation (e.g. Thomas *et al.* 2004; Berman *et al.* 2008). Therefore, we selected *RB1* as a candidate gene for BW and bone traits in the current study. Five SNPs were identified in the *RB1* gene, and the associations of the single SNPs with both BW and bone traits were analysed. The results showed that g.39692 G>A and g.77260 A>G were significantly or suggestively significantly associated with BW and bone traits. The haplotypes and combination genotypes of the two SNPs were constructed and shown to be associated significantly with BW and bone traits, which was in agreement with the single SNP analysis. Birds with the genotypes GG (g.39692 G>A) and AA (g.77260 A>G), the haplotype G-A and the combination genotype GGAA had a significantly higher BW and MeCW and a longer MeL, MeC and KeL than birds with the genotypes AA (g.39692 G>A) or GG (g.77260 A>G), the haplotype A-G and the combination genotype AAGG. Although less individuals with genotype AA (14 birds) were observed in the population, the results of associations of the single SNP, haplotype and combination genotype with traits of interest were consistent, which indicated that the *RB1* gene could be the major gene for BW and bone traits.

Chromosome 1 is the largest in the chicken genome. QTL that affect growth have been identified on

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Table 6 Association and effects of different combination genotypes for g.39692 G>A and g.77260 A>G with both body weight and bone traits[1]

Traits	p-value	AAGG (14)[2]	AGAA (5)	AGAG (46)	AGGG (221)	GGAA (9)	GGAG (190)	GGGG (516)	Phenotypic Variance[3] (%)
BW4(g)	0.0182*	407.6 ± 17.1^{C}	449.1 ± 31.8^{ABC}	446.4 ± 9.8^{AB}	442.7 ± 5.7^{B}	490.0 ± 24.6^{AB}	458.7 ± 5.9^{A}	447.9 ± 4.8^{AB}	1.26
BW5(g)	0.0002**	576.6 ± 23.4^{D}	630.9 ± 43.4^{ABCD}	628.1 ± 13.7^{BC}	613.5 ± 7.9^{CD}	700.7 ± 33.5^{A}	647.9 ± 8.3^{AB}	621.1 ± 6.7^{CD}	2.48
BW6(g)	<0.0001**	723.8 ± 36.4^{C}	851.9 ± 58.5^{ABC}	820.4 ± 18.5^{AB}	805.5 ± 11.5^{B}	882.1 ± 41.1^{AB}	855.8 ± 11.9^{A}	818.0 ± 10.0^{B}	2.75
BW7(g)	0.0002**	947.3 ± 42.3^{C}	1078.6 ± 79.4^{ABC}	1047.6 ± 25.1^{AB}	1023.4 ± 15.3^{BC}	1129.8 ± 54.4^{AB}	1085.4 ± 15.8^{A}	1034.4 ± 13.3^{B}	2.14
BW8(g)	<0.0001**	1139.2 ± 53.8^{D}	1275.5 ± 93.3^{ABCD}	1252.4 ± 29.7^{BC}	1226.7 ± 18.5^{CD}	1362.0 ± 65.5^{AB}	1316.7 ± 19.1^{A}	1241.7 ± 16.1^{BCD}	2.79
BW9(g)	<0.0001**	1366.2 ± 64.4^{C}	1499.4 ± 111.7^{ABC}	1480.4 ± 35.4^{BC}	1456.0 ± 21.7^{C}	1632.7 ± 78.3^{AB}	1560.0 ± 22.4^{A}	1476.2 ± 18.8^{C}	2.42
BW10(g)	<0.0001**	1573.8 ± 70.1^{B}	1670.2 ± 127.4^{AB}	1669.3 ± 40.3^{B}	1633.9 ± 23.8^{B}	1912.2 ± 89.2^{A}	1763.0 ± 24.7^{A}	1670.5 ± 20.3^{B}	2.68
BW11(g)	<0.0001**	1754.8 ± 76.4^{C}	1861.0 ± 145.3^{ABC}	1850.6 ± 43.2^{BC}	1816.9 ± 25.6^{C}	2073.9 ± 111.2^{AB}	1973.9 ± 26.4^{A}	1863.0 ± 21.6^{B}	2.65
BW12(g)	<0.0001**	1939.8 ± 83.3^{C}	2026.4 ± 155.5^{ABC}	2049.5 ± 46.9^{BC}	2002.7 ± 27.2^{BC}	2206.1 ± 108.2^{AB}	2166.8 ± 28.2^{A}	2041.6 ± 22.9^{BC}	2.23
CW(g)	<0.0001**	1717.1 ± 74.9^{C}	1830.1 ± 139.9^{ABC}	1815.6 ± 42.0^{BC}	1774.0 ± 24.1^{C}	1975.6 ± 97.4^{AB}	1922.0 ± 25.0^{A}	1805.0 ± 20.1^{BC}	2.30
MeL4(cm)	0.0014**	5.38 ± 0.09^{C}	5.85 ± 0.18^{AB}	5.77 ± 0.05^{AB}	5.73 ± 0.03^{B}	5.86 ± 0.12^{AB}	5.80 ± 0.03^{A}	5.73 ± 0.03^{B}	1.84
MeC4(cm)	0.0145*	2.87 ± 0.05^{BC}	2.98 ± 0.09^{AB}	2.89 ± 0.02^{BC}	2.88 ± 0.01^{B}	3.03 ± 0.06^{A}	2.94 ± 0.01^{AC}	2.90 ± 0.01^{B}	0.96
MeL6(cm)	<0.0001**	6.61 ± 0.13^{D}	6.99 ± 0.21^{ABCD}	7.09 ± 0.06^{BC}	7.04 ± 0.04^{C}	7.44 ± 0.15^{A}	7.20 ± 0.04^{AB}	7.08 ± 0.03^{C}	3.00
MeC6(cm)	0.0418*	3.48 ± 0.09^{C}	3.58 ± 0.12^{BC}	3.63 ± 0.03^{AC}	3.60 ± 0.02^{BC}	3.78 ± 0.09^{AB}	3.67 ± 0.02^{A}	3.63 ± 0.01^{AC}	0.91
MeL8(cm)	0.0024**	8.03 ± 0.14^{D}	8.59 ± 0.24^{ABC}	8.39 ± 0.07^{ABC}	8.28 ± 0.04^{CD}	8.65 ± 0.17^{AB}	8.43 ± 0.05^{A}	8.32 ± 0.04^{BC}	1.37
MeC8(cm)	<0.0001**	3.84 ± 0.07^{C}	3.92 ± 0.13^{ABC}	3.94 ± 0.04^{BC}	3.89 ± 0.02^{C}	4.11 ± 0.09^{AB}	4.08 ± 0.02^{A}	3.94 ± 0.02^{B}	4.06
MeL10(cm)	0.0002**	9.07 ± 0.14^{CD}	9.34 ± 0.26^{ABCD}	9.31 ± 0.08^{ABCD}	9.23 ± 0.05^{D}	9.61 ± 0.18^{AB}	9.46 ± 0.05^{A}	9.32 ± 0.04^{BC}	1.32
MeC10(cm)	<0.0001**	4.05 ± 0.08^{C}	4.20 ± 0.14^{AC}	4.15 ± 0.04^{BC}	4.11 ± 0.02^{C}	4.33 ± 0.10^{AB}	4.29 ± 0.02^{A}	4.15 ± 0.02^{BC}	2.79
MeL12(cm)	<0.0001**	9.34 ± 0.16^{D}	9.56 ± 0.29^{ABCD}	9.76 ± 0.09^{BC}	9.70 ± 0.05^{C}	10.18 ± 0.20^{AB}	9.95 ± 0.05^{A}	9.81 ± 0.04^{B}	1.22
MeC12(cm)	<0.0001**	4.20 ± 0.08^{B}	4.33 ± 0.16^{AB}	4.31 ± 0.04^{B}	4.30 ± 0.02^{B}	4.62 ± 0.11^{A}	4.50 ± 0.02^{A}	4.35 ± 0.02^{B}	3.02
KeL(cm)	0.0006**	13.31 ± 0.13^{E}	14.14 ± 0.23^{A}	13.60 ± 0.07^{BCD}	13.50 ± 0.05^{DE}	13.78 ± 0.16^{ABCD}	13.68 ± 0.05^{AB}	13.59 ± 0.04^{C}	0.73
MeCW(g)	0.0015**	68.7 ± 2.3^{C}	78.2 ± 4.2^{AB}	74.5 ± 1.3^{AB}	73.0 ± 0.8^{BC}	76.6 ± 3.1^{AB}	76.3 ± 0.8^{A}	74.3 ± 0.7^{B}	0.38

BW, body weight; CW, carcass weight; FDR, false discovery rate; KeL, keel length; MeCW, metatarsus claw weight; MeL, metatarsus length.

[1]Different letters in the same row indicate significant differences between groups ($p < 0.05$). *FDR<0.05. **FDR<0.01.

[2]Least square means ± standard error.

[3]Percentage of phenotypic variance in the F_2 population.

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chicken chromosome 1 in many studies (Abasht *et al.* 2006), including our study by Liu *et al.* (2007) and Zhang *et al.* (2010) using an F_2 resource population. More markers and individuals were used to refine the confidence interval of these QTL to a narrow region (Liu *et al.* 2008; Zhang *et al.* 2010). However, there were still many genes in this narrow region, and it was difficult to identify the major genes for growth by positional cloning. In the current study, the map position of the QTL for BW at 4–12 weeks of age and CW at 12 weeks of age, as well as bone traits, was refined to a subcentimorgan level by haplotype analysis and the narrow region was possible for the positional cloning of the underlying candidate genes. *RB1* gene was selected as a major gene for growth, and two quantitative trait nucleotides (QTNs) were identified. *IGF2* (Van Laere *et al.* 2003) and *GDF8* (Clop *et al.* 2006) were also identified as major candidate genes for muscle mass in pig and sheep, respectively, after fine-mapping of QTL using haplotype analysis. Taken together, these results indicate that haplotype analysis can be used to fine-map QTL to a narrow chromosome region that harbours only a few candidate genes.

The results of the current study indicated that the *RB1* gene was a major gene for BW and bone traits. Two SNPs (g.39692 G>A and g.77260 A>G) that were identified in the *RB1* gene might be the QTN for chicken growth. Functional confirmation of the involvement of the *RB1* gene by using electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (CHIP) and RNA interference (RNAi) is now needed.

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