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Associations between variants of bone morphogenetic protein 7 gene and growth traits in chickens

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Short title: BMP 7 gene variants and growth

Abstract:
1. Enhancing bone strength to solve leg disorders in poultry has become an important goal in broiler production.
2. Bone morphogenetic protein 7 (BMP7), a member of the BMP family, represents an attractive therapeutic target for bone regeneration in humans and plays critical roles in skeletal development.
3. The objective of this study was to investigate the relationship between BMP7 gene expression, single nucleotide polymorphisms (SNPs) and growth traits in chickens. Here, a SNP (c.1995T>C) in the chicken (Gallus gallus) BMP7 gene was identified,
that was associated with growth and carcass traits.

4. Genotyping revealed that the T allele occurred more frequently in breeds with high growth rates, whereas the C allele was predominant in those with low growth rates. The expression level of \textit{BMP7} in the thigh bone of birds with the TT genotype was significantly higher than in those with the CC genotype at 21, 42 and 91 days of age.

5. These findings suggest that selecting the birds with the TT genotype of SNP c.1995T>C could improve bone growth, could reduce leg disorders in fast-growing birds. The SNP c.1995T>C may serve as a selective marker for improving bone growth and increasing the consistency of body weights in poultry breeding.

\textbf{Keywords:} bone formation, single nucleotide polymorphism, gene expression, shank circumference, shank length

\textbf{Introduction}

In past decades, broiler chickens (\textit{Gallus gallus}) have experienced significant increases in the growth rates and meat yields. However, certain problems, such as obesity, ascites and leg disorders have accompanied the improvement of these production traits (Dunnington and Siegel, 1996). Birds with leg disorders show reduced feed intake, poor growth, loss of immunity and declining carcass quality, increasing the cost of rearing. In particularly, birds with leg problems are characterised by decreased uniformity and poor economic performance. Enhancing bone strength to maintain bone proportions as a method to prevent chicken leg problems has become an important goal in current broiler production. Some studies have already reported that leg problems have a genetic component (Rekaya \textit{et al.}, 2013; González-Cerón \textit{et al.}, 2015).
Bone morphogenetic proteins (BMPs) are growth factors and secreted signalling molecules that can induce ectopic bone growth. BMPs belong to the transforming growth factor β (TGFβ) superfamily. BMPs play critical roles in embryonic development and cellular functions, both in postnatal and adult animals, especially in postnatal bone formation (Chen et al., 2004). Bone morphogenetic protein 7 (BMP7) is an attractive therapeutic agent for bone regeneration in humans, because it can induce new bone formation when implanted within a suitable matrix (Sampath et al., 1990; Sengle et al., 2008). In fact, BMP7 plays important roles in multiple processes, such as murine hind brain development, tooth and eye development, nephrogenesis and skeletal patterning (Luo et al., 1995; Arkell and Beddington, 1997; Helder et al., 1998). In 1994, Houston et al. (1994) cloned and expressed chicken BMP7 in the epiphyseal growth plate. BMP7 has been previously identified as a likely candidate gene affecting hyperpigmentation of the visceral peritoneum (HVP) in chickens by using a genome wide association study (Luo et al., 2013). Body weights (BW) of Huiyang Beard chickens (HB; a Chinese chicken breed native to the Guangdong province with a low growth rate and high meat quality, tailored to Chinese tastes) with HVP was significantly lower than that of normal Huiyang Beard chickens (P < 0.05, unpublished data). Such results revealed that BMP7 plays a specific role in the growth plate, possibly in osteoblast activation or as a chemotactic agent for the metaphyseal vessels.

This study investigated the functional importance of BMP7 by analysing its sequence and identifying polymorphisms associated with growth and body composition in an F2 intercross between Huiyang Bearded (HB) breed and the fast-growing Lingnanhuang Line A (HQLA, which has undergone over 10 generations of selection for a high growth rate). Differences in gene expression were
validated among birds with different genotypes. The information concerning the polymorphisms could be integrated into breeding programs to address leg problems and increase body weights in broiler breeders.

Materials and methods

Sample collection and phenotypic measurements

Screening for polymorphisms in the chicken BMP7 gene was performed on 10 birds each from the HB and HQLA breeds. In the association analysis, the birds were from the HB × HQLA F2 resource population, which included 824 birds (436 males, 388 females) from six hatches. The F2 individuals were generated and fed as described in previous studies (Sheng et al., 2013; Luo et al., 2014). All birds had free access to feed and water, and were sacrificed at 91 days of age, at which point their vein blood was collected into centrifuge tubes with anticoagulant containing ethylenediaminetetraacetic acid disodium salt solution and stored at -80°C.

Body weight was measured at hatching and every other week from hatch until the birds reached 84 days of age. Shank length (SL) and circumference (SC) were measured every 14 days during 28–84 days of age. Feed intake (FI) was measured every seven days from 77–84 days of age, and feed conversion ratio (FCR) was calculated during 77–84 days of age. At 91 days of age, the BW, SL and SC were measured and the birds were slaughtered. Anatomical and carcass traits, such as carcass weight (CW), thigh and shankbone weight (LBW), metatarsal and phalanx weight (MPW; these two bones being tested together), leg muscle weight (LMW) and breast muscle weight (BMW) were recorded.

A total of 1029 DNA samples from eight breeds were genotyped to determine the allele frequencies. These included the Red Jungle Fowl, Recessive Rock White,
HQLA, HB, Silkies, Beijing-You, Xiayan and Xinghua Chicken, were tested. All experimental birds were from the Guangdong Wiz Agricultural Science & Technology Co. (Guangzhou, China). Genomic DNA was isolated from the blood samples of 1853 birds using a standard phenol and phenol/chloroform purification-based protocol.

**Polymorphism identification**

Seven primer pairs were used to amplify a fragment of the *BMP7* gene to identify polymorphic sites in the exons of *BMP7* from the HB breed and HQLA chickens (Table 1). PCR amplification was performed in a 25-μl reaction volume on a GeneAmp PCR system 9600 PCR Cycler (Perkin Elmer, Foster City, CA, USA). The PCR reaction mixture contained 100 ng of chicken genomic DNA as a template, primers at 10 μM each, 1×PCR reaction mix and 1 U of Taq DNA polymerase (Dongsheng, Guangzhou, China). The PCR amplification was performed with the following cycling parameters; initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation for 45 s at 94°C, then annealing for 45 s at the optimal temperature and elongation for 90 s at 72°C (Table 1). A final extension was performed at 72°C for 10 min. All amplified products were separated on agarose gels, purified and then sequenced in both directions. The DNAstar software (DNASTar Inc., Madison, WI, USA) was used to analyse and align the potential polymorphic sites.

**Table 1. Primers used to study *BMP7* gene and bone traits in broiler chickens**

<table>
<thead>
<tr>
<th>Gene names</th>
<th>Primer names</th>
<th>Primer sequences (5’-3’)</th>
<th>Binding regions</th>
<th>Product sizes (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>BMP7</em></td>
<td>BMP7-exo n1-F</td>
<td>GCGTCCTCGCGACCTTC</td>
<td>Exon 1</td>
<td>411</td>
<td>58</td>
</tr>
</tbody>
</table>

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</thead>
<tbody>
<tr>
<td><strong>BMP7-exo n1</strong>-R</td>
<td>CTCGCCACCCCATCCAC</td>
<td>CT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n2</strong>-F</td>
<td>ACAGACAACAGGACCA</td>
<td>GGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n2</strong>-R</td>
<td>AGATTTCAACCATAACCAC</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n3</strong>-F</td>
<td>GTTTTGAGTCGGTTGTG</td>
<td>TG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n3</strong>-R</td>
<td>TTCTGTTGTGCTCTGTTG</td>
<td>C</td>
<td></td>
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<tr>
<td><strong>BMP7-exo n4</strong>-F</td>
<td>GGACTGATCGTGATGAT</td>
<td>GT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n4</strong>-R</td>
<td>TCGCTGTGTTTGTGTG</td>
<td>TG</td>
<td></td>
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<td></td>
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<tr>
<td><strong>BMP7-exo n5</strong>-F</td>
<td>TGACTACAGCAACAGAG</td>
<td>ACT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n5</strong>-R</td>
<td>GTGAAATACACAGCAGA</td>
<td>ATA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n6</strong>-F</td>
<td>TCCACAATCTAATGTCTT</td>
<td>CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n6</strong>-R</td>
<td>ATCCTGCTCTTCCCTCCC</td>
<td>TG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n7</strong>-F</td>
<td>CTGTGTTGTGTTATGGG</td>
<td>GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-exo n7</strong>-R</td>
<td>TGTGGTGTTTGTGTTG</td>
<td>GTT</td>
<td></td>
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</tbody>
</table>

**Quantitative real-time PCR analyses**

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</tr>
</thead>
<tbody>
<tr>
<td><strong>BMP7-Rt-F</strong></td>
<td>GAGAACAGCAGCAGCAGCG</td>
<td>GAGAACAGCAGCAGCAGCG</td>
<td>Exon 4-5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMP7-Rt-R</strong></td>
<td>ACC</td>
<td>ACC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ACTB-β-actin-F</strong></td>
<td>CAAAATAGAGCAGCTGAG</td>
<td>CAAAATAGAGCAGCTGAG</td>
<td>Exon 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ACTB-β-actin-R</strong></td>
<td>ATGGC</td>
<td>ATGGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GAPDH-F</strong></td>
<td>CCCCCAAGCCACAGAG</td>
<td>CCCCCAAGCCACAGAG</td>
<td>Exon 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GAPDH-R</strong></td>
<td>AGA</td>
<td>AGA</td>
<td></td>
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</tr>
</tbody>
</table>

**SNP analyses**

SNP analysis was performed for a polymorphic site located in exon 7 using the PCR-restriction fragment length polymorphism method. A 1352 bp fragment was
amplified using specific primer pairs (BMP7-exon7-F/R; Table 1) and digested with EcoRI (Thermo scientific, Shanghai, China) for 6 h at 37°C. Then, the digested products were analysed by agarose gel electrophoresis on 2% agarose gels.

**Association of polymorphisms with growth and carcass traits**

All 824 birds from the F₂ resource population were used for the association analysis. The GLM program from JMP software (SAS Institute Inc., Cary, NC USA) evaluated the associations between genotypes and phenotypes. The model used to analyse the data was assumed to be:

\[
Y_{ijklm} = \mu + S_i + H_j + S_k + D_{kl} + G_m + e_{ijklm}
\]

where \(Y_{ijklm}\) was the phenotypic value of the trait, \(\mu\) was the overall mean, \(S_i\) was the effect of the \(i\)th sex, \(H_j\) was the effect of the \(j\)th hatch, \(S_k\) was the effect of the \(k\)th sire, \(D_{kl}\) was the effect of the \(l\)th dam within the \(k\)th sire, \(G_m\) was the effect of the \(m\)th genotype and \(e_{ijklm}\) was the residual effect. The adjusted phenotypic value was calculated as \(\mu + e_{ijklm}\).

**Quantitative real-time PCR (qPCR)**

The TRIzol reagent (Life Technologies, Rockville, MD USA) was used to isolate total RNA from the thigh bones of the F₈ birds that were obtained from six generations in the HB x HQLA F₂ resource population at 21, 42 and 91 days of age. Bone tissue samples were weighed (approximately 0.2 g), ground into a powder in liquid nitrogen, and then homogenised in 2 ml of TRIzol using a handheld electric homogenate instrument (Germany, Staufen, IKA). The homogenised samples were left for 5 min at room temperature and then centrifuged at 12,000 rpm at 4°C for 10 min. Subsequent operations were performed according the manufacturer’s instructions. There were eight individuals with the TT, TC and CC genotypes of SNP c.1995T>C, respectively; 50% of the birds in each group were male and female.

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First-strand cDNA synthesis and reverse transcriptase PCR were performed as described in the instructions for the PrimeScript™ II reagent Kit with gDNA Eraser (TaKara, Dalian, China). Primer pairs \( \text{BMP7-Rt-F/ BMP7-Rt-R} \) (Table 1) were used to determine the relative expression values of the \( \text{BMP7} \) gene by qPCR. The PCR amplifications were performed in 20 \( \mu l \) reaction volumes comprising 0.5 \( \mu l \) of chicken cDNA, 0.5 \( \mu l \) of each primer and 10 \( \mu l \) of SYBR Green Real-time PCR Master Mix (TOYOBO, Tokyo, Japan) in a LightCycler 480 Real-Time PCR System (Roche Applied Science, Indianapolis, IN, USA). The PCR conditions were: 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 58°C for 15 s, and 72°C for 20 s. The level of fluorescence was used to calculate the threshold cycle (Ct) value for each sample. The relative expression levels of gene were analysed using the comparative Ct method, in which housekeeping genes \( \beta\text{-actin} \) and \( \text{GAPDH} \) (Table 1) were used as internal controls and the geometric averaging of those two internal control genes was calculated according a previously published method (Vandesompele \textit{et al.}, 2002). The quantitation procedures recommended by Roche Applied Science were used to correct for differences in mRNA quantities. All 72 birds (24 from each age) from the F\(_8\) population were used for gene expression analysis. Each time point was analysed separately.

The GLM program from JMP software (SAS Institute Inc., Cary, NC, USA) was used to evaluate the associations between genotypes and expressions. The model used to analyse the data was assumed to be:

\[
Y_{im} = \mu + S_i + G_m + e_{ijklm}
\]

where \( Y_{im} \) was the gene relative expression levels, \( \mu \) was the overall mean, \( S_i \) was the effect of the \( i^{th} \) sex, \( G_m \) was the effect of the \( m^{th} \) genotype and \( e_{ijklm} \) was the residual effect. Differences showing \( P<0.05 \) were considered significant.

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Ethics statement
The Animal Care Committee of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, People’s Republic of China) approved the study (approval number GAAS-IAS-2009-73).

RESULTS
Identification of SNPs in the BMP7 gene
Seven primer pairs were designed to detect single nucleotide polymorphisms (SNPs) in the 97.98% sequences of all exons of chicken BMP7. Comparative sequencing revealed only one potential polymorphic site, which created a restriction sites for the endonuclease EcoRI in the 3’ UTR of BMP7 (c.1995T>C, GenBank accession No. XM_417496.3). A 1352 bp DNA fragment containing the SNP, which was amplified using primers BMP7-exon7-F/R, was detected by digestion with EcoRI, allowing us to distinguish between the T allele (which produced a DNA fragment of 1352 bp) and the C allele (which produced two DNA fragments with lengths of 989 bp and 363 bp) (Figure 1).

FIG 1 HERE
Figure legends
Fig. 1. The electrophoresis patterns obtained after digestion with EcoRI endonuclease at the c.1995T>C locus. Agarose gel electrophoresis (2.0%) image showing the DNA restriction fragment patterns indicative of polymorphisms in BMP7 gene after amplification with the primer pair BMP7-exon7-F/R. The genotypes are shown at the top of the lanes, M, Marker DL2000 DNA Ladder (TaKara).
Allelic frequencies of the SNP c.1995T>C in different chicken breeds

The allelic frequencies of the SNP c.1995T>C in fast-growing and low-growing chicken breeds were notably different. As shown in Table 2, the C allele was predominant in those breeds with a low growth rate, such as Red Jungle Fowl, HB and Xinghua chicken. However, the T allele was predominant in high-growth-rate breeds, including Silkies, HQLA and White Recessive Rock chicken.

Table 2. Allelic frequencies for the SNP c.1995T>C in the *BMP7* gene in 8 chicken breeds

<table>
<thead>
<tr>
<th>Breeds</th>
<th>BW10 (μ±S.E.)</th>
<th>No. of birds</th>
<th>Genotypic number</th>
<th>Allelic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
<td>TT</td>
<td>TC</td>
</tr>
<tr>
<td>Silkies</td>
<td>1444±64.3</td>
<td>1340±50.3</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>Fast-growing Linhnanhuang Line A 2305±26.3 2017±25.5 70 61 8 1 0.929 0.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Recessive Rock Xiayan 1152±81.0 875±75.0 91 28 47 16 0.566 0.434</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beijing-You Xinghua 704±63.0 661±59.0 100 3 29 68 0.175 0.825</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huiyang Bearded 855±69.3 620.0±56.1 549 0 39 510 0.036 0.964</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red jungle fowl — 3 — 3 19 0 0 19 0.000 1.000</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

BW10: live body weight at 70 days of age, g.

1These data were from the Guangdong Wiz Agricultural Science & Technology Co. (Guangzhou, China).

2These data were from the “Animal Genetic Resources in China. Poultry” (Chen *et al.*, 2010).

3Data not collected

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Associations between the SNP c.1995T>C and growth traits

The SNP c.1995T>C in BMP7 was significantly associated with certain growth and carcass traits in the HB × HQLA F2 resource population. Furthermore, the growth traits, including BW, SC and SL at 56 and 91 days of age for the birds with the TT genotype were significantly higher than those for the birds with the CC genotype ($P<0.05$; Table 3). However, the birds with the TT genotype had the lowest FCR at 77–84 days of age in all birds tested ($P<0.05$; Table 3). For carcass traits, individuals with the TT genotype were heavier than those with the CC genotype in terms LBW and MPW ($P<0.05$, Table 3).

Table 3. Association analyses in the “HB × HQLA” F2 resource population

<table>
<thead>
<tr>
<th>Traits</th>
<th>SNP c.1995T&gt;C Genotype ($\mu \pm$S.E.$)^2</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TT (n=100)</td>
<td>TC (n=416)</td>
</tr>
<tr>
<td>BW8</td>
<td>0.031</td>
<td>1271±16.1a</td>
</tr>
<tr>
<td>BW13</td>
<td>0.004</td>
<td>2180±29.1a</td>
</tr>
<tr>
<td>SC8</td>
<td>1.727E-4</td>
<td>3.81±0.020a</td>
</tr>
<tr>
<td>SC13</td>
<td>2.27E-3</td>
<td>4.25±0.023a</td>
</tr>
<tr>
<td>SL8</td>
<td>0.020</td>
<td>80.94±0.381a</td>
</tr>
<tr>
<td>SL13</td>
<td>7.04E-4</td>
<td>93.69±0.465a</td>
</tr>
<tr>
<td>FCR11–12</td>
<td>0.008</td>
<td>-0.27±0.110b</td>
</tr>
<tr>
<td>LBW</td>
<td>0.011</td>
<td>36.32±0.542a</td>
</tr>
<tr>
<td>MPW</td>
<td>0.032</td>
<td>34.09±0.539a</td>
</tr>
</tbody>
</table>

n: the number of birds studied.

BW8, 13: live body weight at 56 and 91 days of age, g.

SC8, 13: shank circumference at 56 and 91 days of age, cm.

SL8, 13: shank length at 56 and 91 days of age, cm.

FCR11–12: feed conversion ratio at 77–84 days of age.

LBW: thigh bone and shank bone weight, g.

MPW: metatarsal and phalanx weight, g.

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1 The adjusted phenotypic value was calculated twice as $\mu + e_{ijklm}$ by Johnson Su.

2 Means within a row lacking a common superscript are different ($P<0.05$).

**Gene expression in birds with different variants of BMP7**

The effects of the SNP c.1995T>C on the expressions of BMP7 gene were investigated in the thighbones of the HB × HQLA F8 birds at 21, 42 and 91 days of age. The expression level of the TT genotype of BMP7 was significantly higher than the CC genotype in thighbones both at 21, 42 and 91 days of age ($P<0.05$; Figure 2).

**FIG 2 HERE**

Fig. 2. Different expressions of BMP7 in different genotypes of SNP c.1995T>C. Relative expression analyses in chicken thighbones of BMP7 gene were normalized to the geometric average of ACTB ($\beta$-actin) and GAPDH genes for alleles at 21, 42 and 91 days of age that varied at SNP c.1995T>C. Error bars represent the SE, and the $P$-values for the expression of BMP7 at each time point were 0.019, 0.001, 0.038, respectively (from left to right in the order of the corresponding graph). a, b indicates significant differences between different genotypes on the same day ($P<0.05$).

**DISCUSSION**

Recombinant BMPs have been applied successfully to induce healing of segmental bone defects in number of species, including humans, rats, sheep and dogs (Yasko *et al.*, 1992; Gerhart *et al.*, 1993; Cook *et al.*, 1994; Kirker-Head *et al.*, 1998; Geesink *et al.*, 1999). BMPs are well-established agents for inducing orthotopic and ectopic bone formation. For gene therapy for bone formation, cells received the transfer by adenovirus vectors containing the BMP7 cDNA, which produce biologically active...
BMP7, which may be a viable route for stimulating bone regeneration (Franceschi et al., 2000). Similar to other BMPs, BMP7 stimulates the expression of the osteoblast phenotype in vitro, and possibly causes osteoblast precursors to migrate into the metaphyseal edge of the growth plate where they developed their physiological functions (Vukicevic et al., 1989; Ahrens et al., 1993; Asahina et al., 1993; Houston et al., 1994). BMP7-deficient mice exhibit various developmental defects, such as holes in the basisphenoid bone and the xyphoid cartilage, polydactyly in hind limbs, and cleft palate, indicating a requirement for BMP7 for the coordination of digit formation and palatogenesis (Jena et al., 1997; Kouskoura et al., 2013). Studies have suggested that the expression of BMP7 in the interdigital and peridigital tissues is responsible for the outgrowth of the digit cartilages; BMP7 plays an important role in the formation of the digits in chickens (Montero and Hurlé, 2007; Lorda-Diez et al., 2009) and has an important role in the process of skeletogenesis.

The chicken BMP7 gene is located between 12 Mb to 12.1 Mb on chromosome 20, and QTL analysis of bone traits in an F2 intercross, between domesticated white Leghorn and wild-type red jungle fowl chickens, identified a QTL that affects bone biomechanical strength (Rubin et al., 2000). This QTL is located on chicken chromosome 20 from 26 cM to 67 cM, which contains the BMP7 gene. Jacobsson et al. (2005) identified a QTL that located in a similar region on chicken chromosome 20 (from 26 cM to 62 cM) that affected BW in an F2 intercross from two growth-selected lines (http://www.animalgenome.org/cgi-bin/QTLdb/GG/). In this study, an SNP (c.1995T>C) was identified in the BMP7 gene that was significantly associated with partial growth and carcass traits, and genotyping results revealed that T alleles predominated in high-growth-rate chicken breeds, and C alleles predominated in breeds with a low growth rate (Table 3, Table 2). Significant
associations at 70 and 84 days of age showed that birds with the TT genotype had the highest growth rates at 28–56 and 56–84 days of age in all birds tested. However, for LMW and BMW, there was no statistically significant difference among the individuals with TT, TC and CC genotypes (data not shown). Therefore, the results suggest that SNP c.1995T>C can improve bone growth while maintaining high growth rates with no adverse effects on muscle growth and development. Phenotypic correlations of leg problems with growth rate were unfavourable, and this agreed with recent genetic analysis that indicated that the genetic relationship between growth traits and leg problems was extremely weak in chickens, ranging only from 0.01 to 0.08 (González-Cerón et al., 2015). Taken together, the BMP7 gene was identified as a likely candidate gene for bone and body growth traits in chickens.

To explore whether the SNP c.1995T>C could affect the expression of BMP7 in the bones of chicken, the expression of this gene in chicken thighbones with different alleles was examined. The expression level of the TT genotype was significantly higher than the CC genotype in the thighbone both at 21, 42 and 91 days of age (Figure 2). SNP c.1995T>C is in 3′ UTR of the chicken BMP7 gene, did not change the amino acid sequence of the protein, but it may be in linkage disequilibrium with a cis-regulatory mutation that affects the expression of BMP7 (Wang et al., 2014). Additionally, studies have indicated that the 3′ UTR plays an important role in the spatial and temporal regulation of mRNA translation (Dalby and Glover, 1993; Samson, 1998). In recent experiments, Kuree et al (2014) found a miRNA—miR-542-3p, which targets in the 3′UTR of BMP7, and over-expression of miR-542-3p which led to repression of BMP7 and inhibition of BMP7/PI3K-survivin signalling, which suppressed osteoblast cell proliferation and differentiation and inhibited bone formation. Unfortunately, the SNP c.1995T>C did not change the
miR-542-3p target sequences in the 3'UTR of *BMP7*, but this SNP may influence the structure of the 3'UTR of the *BMP7* gene, resulting in abatement of the binding efficiency of miR-542-3p, or change the target sequences, binding some miRNA that have not been found so far. However, this hypothesis requires further experimental validation. BMP can induce the phosphorylation of Smad1/5/8, which is essential for the activation of bone-specific genes (Chen et al., 2012). In *BMPR1b* and *BMP7* double mutant mice, the ulna and radius are severely affected, being either absent or shortened (Yi et al., 2000). In the chicken, *BMP7* is expressed throughout limb development, and has been implicated in early limb patterning, as well as in skeletogenesis (Francis et al., 1994). The *BMP7* as a member of the BMP family has an important role osteogenesis. The results suggested that SNP c.1995T>C in *BMP7* may play an important regulatory role in the process of skeletogenesis and improve growth in birds.

In this study, a SNP, c.1995T>C, was identified in the *BMP7* gene of chicken (*Gallus gallus*) genome and showed that selecting birds with the TT genotype of SNP c.1995T>C could improve their bone growth to reduce leg disorders during breeding for fast-growing broilers. Further confirmation in different populations is needed.

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**Disclosure statement**

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Figure 1
Figure 2

Expression of genes against the geometric average of GAPDH and ACTB internal control genes ($\times 10^{-5}$)

- TT genotype
- TC genotype
- CC genotype

21d: a, b
42d: a, a, b
91d: a, ab, b
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