Journal of Genetics, Vol. 96, No. 4, September 2017, pp. 535–544
DOI 10.1007/s12041-017-0804-9

RESEARCH ARTICLE

Effects of genetic variants of the bovine *WNT8A* gene on nine important growth traits in beef cattle

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Received 3 June 2016; revised 23 October 2016; accepted 10 November 2016; published online 8 August 2017

Abstract. *WNT-β*-catenin-TCF pathway is involved in carcinogenesis and foetal development. As a member of the *WNT* gene family, *Wnt8A* encodes secreted signalling proteins and responds to many biological processes. However, similar research on the effects of genetic variations of *Wnt8A* gene on growth traits is lacking. Therefore, in this study, polymorphisms of *Wnt8A* were detected in 396 animals from Chinese Qinchuan cattle using DNA pool sequencing and PCR-RFLP methods. Four novel single-nucleotide polymorphisms (SNPs) of *Wnt8A* gene were identified, including three mutations in introns (g.T-445C, g.G244C and g.G910A) and one in exon (g.T4922C). Additionally, we examined the associations of four SNPs with growth traits. The results revealed that SNP2 (g.G244C) was significantly associated with shoulder height, hip height, body length, hip width, and body weight ($P < 0.05$). SNP3 (g.G910A) also displayed notable effects on hip width ($P < 0.05$). Meanwhile, the haplotype combination CC-GC-GA-CC was strongly associated with heavier, taller and longer animals ($P < 0.05$). These results show that the *Wnt8A* gene may be a potential candidate gene, and the SNPs could be used as molecular markers in early marker-assisted selection in beef cattle breeding programmes.

Keywords. polymorphisms; *Wnt8A* gene; growth traits; Qinchuan cattle.

Introduction

Wnt/*β*-catenin signalling has been discovered in many animals, which plays an important role in carcinogenesis and foetal development (Moon *et al.* 1997; Peifer and Polakis 2000). Wnt proteins are a family of paracrine and autocrine factors that regulate many aspects of cell growth and differentiation (Cadigan and Nusse 1997). Several field investigations have clearly demonstrated that *Wnt* signalling is essential for osteogenic differentiation and bone formation. During embryonic development, *Wnt* signalling increase bone mass through numerous mechanisms, including renewal of stem cells (Reya and Clevers 2005), stimulation of preosteoblast replication and induction of osteoblastogenesis (Kato *et al.* 2002). Other studies suggested that the Wnt signalling pathway is involved in adipogenesis. The small interfering RNA (siRNA) treatment in rat hepatoma cells confirmed that *Wnt*/β-catenin signalling is involved in hepatic fat metabolism (Zhou *et al.* 2014) and disruption of *Wnt* signalling caused transdifferentiation of myoblasts into adipocytes *in vitro* (Ross *et al.* 2000). Additionally, the canonical *Wnt* signal regulated myogenic differentiation through activation of reserve myoblasts (Rochat *et al.* 2004).

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Wnt8A is an extremely important member of the *Wnt* gene family, located on chromosome 7, contains six exons and five introns, encodes 351 amino acids in human (Saitoh and Katoh 2001). *Wnt8A* takes part in a number of basic developmental processes. Several studies have demonstrated that *Wnt8A* signalling can prevent the dorsal organizer from expansion by regulating the expression of the transcriptional repressors, vent, vox and ved in the ventrolateral mesoderm (Ramel and Lekven 2004) and contribute to the development of the nervous system, especially in neuroectoderm patterning (Erter *et al.* 2001; Rhinn *et al.* 2005; Luz *et al.* 2014). Some studies indicated that *Wnt8A* is also involved in early inner ear development (Urness *et al.* 2010; Rogers *et al.* 2011; Vendrell *et al.* 2013) and plays key roles in embryonal tumours and embryonic stem cells through synergistic activation of the β-catenin-TCF signalling pathway (Saitoh *et al.* 2002). Other studies in zebrafish showed that *Wnt8A* was essential to fine tune the balance of the signalling outputs of the complex *Wnt8A* locus and regulated the mesodermal patterning and morphogenesis as well as patterning between brain subdivisions (Lekven *et al.* 2011; Wylie *et al.* 2014). The previous studies on *Wnt8A* function have indicated that *Wnt8A* had a great effect on body patterning during early embryogenesis. Loss of *Wnt8A* function has resulted in reduction or shrink of the posterior body and the expansion of dorsal axial tissues (Shimizu *et al.* 2005) and the loss of *Wnt8A* function has also been reported to prevent tail development (Agathon *et al.* 2003). Consequently, we propose the hypothesis that *Wnt8A* is a candidate gene for growth traits in cattle based on those emerging evidences.

To date, only a few *Wnt8A* polymorphisms have been reported. Two polymorphisms of the *Wnt8A* gene (rs78301778 and rs6596422) were associated with the risk and the development of Hirschsprung's disease (Gao *et al.* 2013). In humans, variation in *WNT* genes is associated with nonsyndromic cleft lip with or without cleft palate (Chiquet *et al.* 2008). However, the polymorphisms and function of bovine Wnt8A have not been elucidated. The purpose of this study was to identify SNPs, to carry out haplotype construction as well as association analysis, so as to investigate whether genetic variations in the *Wnt8A* gene are associated with growth traits in Chinese Qinchuan cattle, which will possibly offer some useful information about animal breeding and genetics.

Materials and methods

Cattle populations, genomic DNA isolation and data collection

In this study, blood samples were collected from 396 individuals of Qingchuan cattle, which were healthy breeding females without pregnancy and reared in the breeding farm (Fufeng, Shaanxi, China). They were raised on a corn–corn silage diet after weaning at an average of six months. Genomic DNA was extracted from these blood samples according to the standard methods (Müllenbach *et al.* 1989), and then were diluted to 50 ng/μL. The data of 10 important growth traits (shoulder height, hip height, body length, heart girth, chest width, chest depth, rump length, hucklebone width, hip width and body weight) in 2-years old Qinchuan cattle ($n = 140$) were collected for statistical analysis and were measured with reference to Gilbert's method (Gilbert *et al.* 1993).

SNP detection, DNA pooling and polymerase chain reaction (PCR) amplification

Based on the nucleotide sequence of the *Bos taurus Wnt8A* gene (GenBank accession number AC_000164), eight pairs of primers were designed to amplify the *Wnt8A* gene from cattle genomic DNA with Primer premier 5.0 software. Partial primers' information used for detecting all novel mutations are shown in table 1. DNA pool sequencing was utilized to detect mutations in the *Wnt8A* gene. One-hundred randomly selected DNA samples were mixed to form a DNA pool. Then the PCR amplifications were executed with pooled DNA as template. Each PCR amplification was performed in 25 μL of reaction volume containing 50 ng genomic DNA, 10 μM of each primer, 1× buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs and 0.6 U of *Taq* DNA polymerase (MBI, Vilnius, Lithuania). The PCR fragments were sequenced by Sangon Biotech, Shanghai, China, to search for possible mutations. Then the sequencing results were perused using the Chromas Pro 2.33 software (http://technelysium.com.au/wp/chromaspro/), and locations with two peaks at the same locus were considered as the candidate SNPs. The sequences were imported into the BioXM software ver. 2.6, to compare with the reference sequence (GenBank accession number AC_000164) and to locate the SNPs.

Genotyping and PCR-RFLP

Based on the sequencing results, four pairs of restriction enzyme digestion primers were projected to identify the genotypes of the possible mutations using PCR-RFLP method. The detailed information about the primers are provided in table 2. The SNP1 is genotyped by PCR-RFLP method; SNP2, SNP3 and SNP4 were genotyped by a forced PCR-RFLP method, which required mismatches changed in primers for creating restriction sites. Four μL PCR products were mixed with 6 μL enzyme digestion solution (4.8 μL water, 0.2 μL enzyme and 1 μL 10× buffer), and then the 10 μL reaction was conducted according to the supplier's instructions. Finally, the digested products were detected by electrophoresis in 2.5% agarose gel, which were run at a constant voltage (120 V) for about 0.5 to 1.0 h to identify the genotypes.

Table 1. Partial primers with mutations detected.

Primer name	Loci[a]	Primer sequences (5′–3′)	T_m (°C)	Length (bp)	Composition of fragments
P1	SNP1	F: AGGGACTGTTGCAGAGGTGT	62	718	5′ Flanking regulatory region and partial exon 1
		R: CAAGCAGAGGCACTGAAGGT			
P2	SNP2	F: GGGACAAATGAGAAGAGC	60	852	Exons 1, 2, 3 / partial introns 1, 4 and intron 2, 3
		R: GGAAATAACCCACAACAAG			
P3	SNP3	F: TCCTTGTTGTGGGTTAT	49	920	Partial intron 4
		R: CTCCCTCTATGGGTTTA			
P4	SNP4	F: AATCACGCAGAAGAGC	60	1051	Exon 6 and 3′ flanking regulatory region
		R: TAGGCAGGAAGACCAG			

[a]SNP loci: SNP1, g.T-445C; SNP2, g.G244C; SNP3, g.G910A; SNP4, g.T4922C.

Statistical analysis

Gene frequencies were determined for each breed by direct counting. Hardy–Weinberg equilibrium (HWE) was tested based on chi-square test for different loci (Yeh 1997). The three indices, expected heterozygosity (H_e), observed heterozygosity (H_o) and effective allele numbers (N_e, reciprocal of heterozygosity were used to measure genetic variation of a population, which was calculated according to Nei (1973). All the calculations and tests were performed using PopGene software ver. 1.32. Genotypic and allele frequencies, linkage disequilibrium (LD), and haplotype frequencies were analysed by SHEsis online platform (Li *et al.* 2009). Polymorphism information content (PIC) was calculated by PIC_Calc ver. 0.6 according to Botstein's methods (Botstein *et al.* 1980). Statistical analysis was performed on the basis of records of growth traits in Nanyang and Jiaxian, respectively. Then, a Bonferroni correction, the multiple trait derivative-free restricted maximum likelihood (MTDEREML) was used to analyse each trait with animal models (Boldman *et al.* 1993). The relationships between the variations of the *Wnt8A* gene and growth traits of Qinchuan cattle were analysed by analysis of variance (ANOVA). The general linear model (GLM) is as follows:

$$Y_{ijk} = \mu + G_j + E_{ijk},$$

where Y_{ijk} is the observation for a trait in the *ijk*th individual, μ is the overall population mean, G_j is the fixed effect of the *j*th genotype, and E_{ijk} is the random error.

Results

SNP detection and genotype identification

The bovine *Wnt8A* gene maps to chromosome 7 consists of six exons and five introns. Four novel SNPs (g.T-445C, g.G244C, g.G910A and g.T4922C) were detected in the *Wnt8A* gene in Qinchuan cattle (figure 1). Among these, mutations SNP2 (g.G244C) and SNP3 (g.G910A) were located in introns 2 and 3, respectively. The SNP4 (g.T4922C) was located in exon 6, which resulted in a missense mutation (Ser>Thr). The SNP1 was located in the 5′-flanking regulatory region. The four SNPs of *Wnt8A* gene were genotyped using the PCR-RFLP and PCR-forced RFLP methods. As a result, three genotypes were discovered in SNP1, SNP2 and SNP3, and two genotypes were found in SNP4 (figure 2). For SNP1, the digestion of the 718-bp PCR products with *Taq*I resulted in fragment lengths of 512 bp and 206 bp for genotype CC; 718 bp, 512 bp and 206 bp for genotype TC; and 718 bp for genotype TT (figure 2a). For SNP2, the digestion of the 334-bp PCR products with *Ava*I resulted in fragment lengths of 316 bp and 18 bp for genotype GG; 334 bp, 316 bp and 18 bp for genotype GC; and 334 bp for genotype CC (figure 2b). For SNP3, the digestion of the 169-bp PCR products with *Taq*I resulted in fragment lengths of 146 bp and 23 bp for genotype GG; 169 bp, 146 bp and 23 bp for genotype GA; and 169 bp for genotype AA (figure 2c). For SNP4, the digestion of the 227-bp PCR products with *Ava*I resulted in fragment lengths of 227 bp, 206 bp and 21 bp for genotype TC; and 227 bp for genotype TT (figure 2d). The 18 bp fragment in figure 2b, the 23 bp fragment in figure 2c and the 21 bp fragment in figure 2d were too short to be identified clearly. In this study, the PCR-RFLP method was successfully carried out to accurately detect the polymorphism of the bovine *Wnt8A* gene.

Genetic diversity analysis

Genotype distribution, genotype frequency, allele frequencies, and genetic indices (N_e, H_e, H_o and PIC) of the four SNPs were directly calculated and displayed in table 3. The frequencies of allele T in SNP1 and SNP4 were 0.34 and 0.27, respectively. The frequencies of allele C in SNP1, SNP2 and SNP4 were 0.66, 0.68 and 0.73, respectively, while the frequencies of allele G in SNP2 and SNP3 were

Table 2. Genetic variants identified in the bovine *Wnt8A* gene.

SNP	Location	Mutation type	Primer sequence (5′–3′)	T_m (°C)	Endonuclease	PCR-RFLP pattern
SNP1	5′-Flanking regulatory region	Noncoding	F: AGGGACTGTTGCAGAGGTGT R: CAAGCAGAGGCACTGAAGGT	62	*Taq*I	718/512+206 bp
SNP2	Intron 2	Noncoding	F: TTTACCCATTCATGTTCCCGA R: ACCACTTCTTAGCCTGTT	54	*Ava*I	347/330+17 bp
SNP3	Intron 3	Noncoding	F: CCAGGAGTGTCGTAAGGGTTG R: GTTGAAATTCTCTCAGGGGCTT	56	*Taq*I	169/147+22 bp
SNP4	Exon 6	Missense	F: CAGAACAGCCGCAACACATCCAGA R: CAGTCACTGCCCTTGCCCCCGG	63	*Ava*I	227/209+18 bp

SNPs, SNP1, g.T-445C; SNP2, g.G244C; SNP3, g.G910A; SNP4, g.T4922C. The underlined base show mismatches changed for creating restriction site.

0.32. Additionally, at SNP3, the frequency of allele A was 0.68. The H_o values ranged from 0.458 to 0.553, and N_e values were from 1.653 to 1.811. According to the PIC values of the four mutations loci which were ~0.3, all the SNPs had moderate genetic diversity (PIC value < 0.25, low genetic diversity; 0.25 < PIC value < 0.50, intermediate genetic diversity; PIC value > 0.50, high genetic diversity).

LD and haplotype analyses

The LD analysis was performed using SHEsis and the values of D' and r^2 are shown in table 4. The LD between the four SNPs in Qinchuan cattle population was estimated, which indicated that the D' values ranged from 0.000 to 1.000; the r^2 values were also from 0.000 to 1.000. The case of $r^2 = 0$ is known as perfect linkage equilibrium, $r^2 > 0.33$ indicates sufficiently strong LD, and $r^2 = 1$ suggests complete LD (Ardlie *et al.* 2002). The estimated D values among the four SNPs ranged from 0.728 to 0.898; the r^2 values ranged from 0.414 to 0.725. SNP1 locus had a moderate linkage with SNP2 locus, SNP2 and SNP3 loci showed strong linkage as well as SNP1 and SNP3 loci, while the rest of the locus–locus combinations demonstrated weak linkage (figure 3). Haplotypes with frequency >0.03 were used for subsequent analysis (haplotypes with frequency <0.03 were ignored). Haplotype structure analysis of the *Wnt8A* gene in the Qinchuan cattle population is shown in table 5. The results indicated that Hap1 (-CCAC-) was predominant in Qinchuan cattle (59.7%), while Hap2 (-TGGC-) had the lowest frequency (6.8%).

Association analysis of single SNP marker and halotype combinations

The aim of this study was to investigate the four novel SNPs that are associated with five important growth traits in Nanyang cattle population. The results are displayed in table 6. We noticed that genotype of SNP1 and SNP4 did not have significant effects on growth traits ($P > 0.05$). At the SNP2 locus, GC genotype individuals had greater shoulder height ($P < 0.01$), body length ($P < 0.01$), hip height ($P < 0.01$), hip width ($P < 0.05$) and body weight ($P < 0.01$) than those of CC and GG genotypes. Referring to SNP3 loci, the animals with GG genotype had significantly greater hip width than those with genotypes AA and GG ($P < 0.05$). Three combined haplotypes composed of the four novel SNPs were constituted to analyse the conjunction effects on growth traits, and the detailed information is provided in table 7. The rest possibly combined haplotypes with frequencies less than 0.05 were excluded (data not shown). The association analysis of combined haplotypes of four SNPs with growth traits of Qinchuan cattle revealed that the individuals

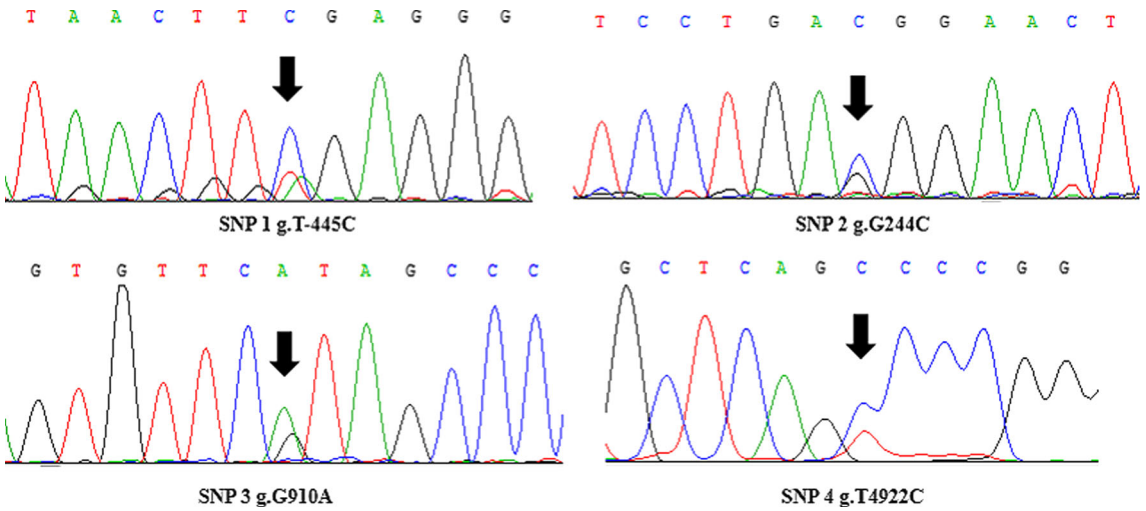

Figure 1. The sequencing maps of four novel SNPs in the bovine *Wnt8A* gene. The SNP positions are shown according to GenBank number AC_000164.

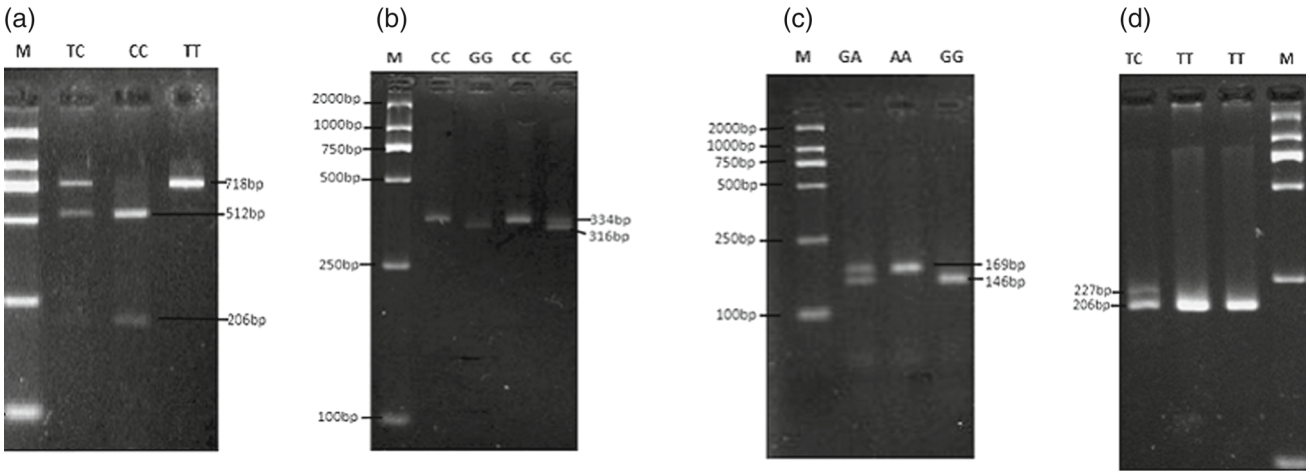

Figure 2. The electrophoresis patterns of PCR-RFLP analysis at four SNP loci in the bovine *Wnt8A* gene. (a) Digestion result by restriction enzyme *Taq*I at SNP1 locus, g.T-445C. (b) Digestion result by restriction enzyme *Ava*I at SNP2 locus, g.G244C. (c) Digestion result by restriction enzyme *Taq*I at SNP3 locus, g.G910A. (d) Digestion result by restriction enzyme *Ava*I at SNP4 locus, g.T4922C.

Table 3. Population genetic analyses of bovine *Wnt8A* gene in Qinchuan cattle.

SNP	Genotype frequency			Allele frequencies		PIC	χ^2 (HWE)[b]	N_e[c]	H_e	H_o
SNP1	TT/0.101	TC/0.477	CC/0.422	T/0.340	C/0.660	0.348	1.38*	1.811	0.475	0.525
SNP2	GG/0.091	GC/0.447	CC/0.462	G/0.314	C/0.686	0.338	0.50*	1.758	0.447	0.553
SNP3	GG/0.071	GA/0.490	AA/0.439	G/0.316	A/0.684	0.338	6.98**	1.758	0.490	0.510
SNP4	CC/0.460		TC/0.540	C/0.730	T/0.270	0.316	54.00**	1.758	0.542	0.458

PIC, polymorphism information content; χ^2 (HWE), Hardy–Weinberg equilibrium χ^2 value; N_e, effective allele numbers; H_e, expected heterozygosity of *Wnt8A* gene; H_o, observed heterozygosity of *Wnt8A* gene;
*$P > 0.05$ (HWE); **$P < 0.05$ (HW disequilibrium).

Table 4. Estimated values of LD for SNPs in bovine *Wnt8A* in Qinchuan cattle.

SNP	SNP1 (g.T-445C)	SNP2 (g.G244C)	SNP3 (g.G910A)	SNP4 (g.T4922C)
SNP1 (g.T-445C)	–	$D' = 0.851$	$D' = 0.898$	$D' = 0.760$
SNP2 (g.G244C)	$r^2 = 0.646$	–	$D' = 0.854$	$D' = 0.728$
SNP3 (g.G910A)	$r^2 = 0.723$	$r^2 = 0.725$	–	$D' = 0.809$
SNP4 (g.T4922C)	$r^2 = 0.414$	$r^2 = 0.428$	$r^2 = 0.525$	–

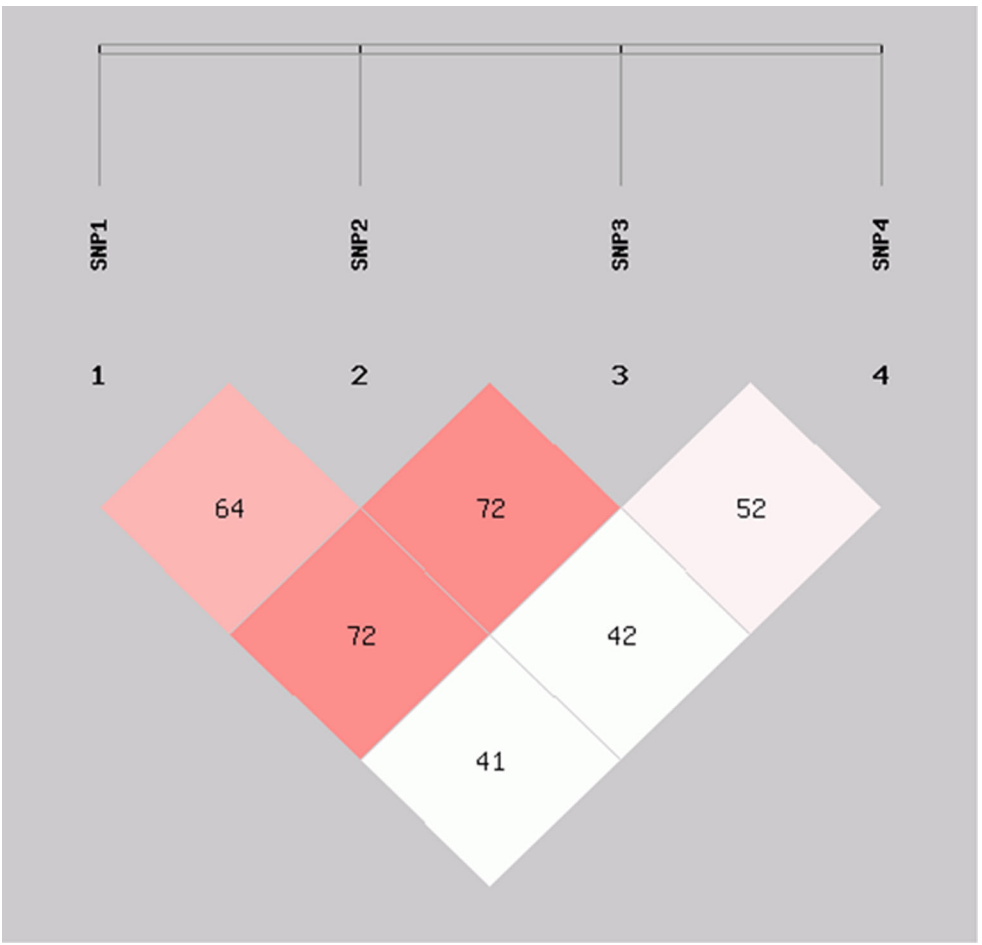

Figure 3. Pair-wise LD tests of four SNPs based on r^2, the deep colour denotes strong linkage between two loci. Numbers in each cell stand for pairwise r^2 values (%) and empty cells mean pairwise r^2 equals one between the corresponding SNPs.

Table 5. Haplotypes of *Wnt8A* gene and their frequencies in Qinchuan cattle.

Haplotype	SNP1 (g.T-445C)	SNP2 (g.G244C)	SNP3 (g.G910A)	SNP4 (g.T4922C)	Frequency
Hap 1 (CCAC)	C	C	A	C	0.597
Hap 2 (TGGT)	T	G	G	T	0.199
Hap 3 (TGGC)	T	G	G	C	0.068

Haplotypes with frequency <0.03 have been ignored.

Table 6. Association of different genotypes of SNPs in *Wnt8A* gene with growth traits in Qinchuan cattle (2 years old).

			Growth trait (mean ± SE)								
SNP	Genotype	Sample size	Shoulder height (cm)	Hip height (cm)	Body length (cm)	Chest girth (cm)	Chest width (cm)	Chest depth (cm)	Hucklebone width (cm)	Hip width (cm)	Body weight (kg)
SNP1	TT	13	125.00 ± 1.31	122.43 ± 1.39	128.00 ± 3.86	171.08 ± 3.47	38.04 ± 1.51	60.69 ± 1.24	23.42 ± 1.10	41.00 ± 0.76	316.24 ± 19.58
	TC	59	128.44 ± 0.76	125.68 ± 0.79	135.79 ± 1.35	174.53 ± 1.30	36.77 ± 0.50	60.57 ± 0.50	22.30 ± 0.43	42.34 ± 0.38	348.17 ± 7.71
	CC	68	127.59 ± 0.71	125.02 ± 0.73	132.63 ± 1.81	173.78 ± 0.87	36.58 ± 0.49	60.64 ± 0.44	21.86 ± 0.42	41.44 ± 0.48	335.86 ± 8.16
P value			0.144	0.204	0.089	0.522	0.508	0.993	0.332	0.206	0.210
SNP2	CC	64	127.00 ± 0.69^{AB}	124.16 ± 0.76^{AB}	131.63 ± 1.67^{b}	172.12 ± 1.09	36.54 ± 0.48	60.54 ± 0.45	21.86 ± 0.42	41.18 ± 0.42^{B}	326.98 ± 6.79^{B}
	GC	63	129.35 ±0.76^{A}	126.79 ± 0.75 ^{A}	137.16 ± 1.41^{a}	175.79 ± 1.40	36.57 ±0.52	60.57 ±0.50	22.24 ± 0.44	42.82 ± 0.40^{A}	359.01 ± 7.49^{A}
	GG	13	123.85 ± 0.98^{B}	121.50 ± 1.09^{B}	127.54 ± 3.74^{b}	172.15 ± 3.21	39.27 ± 1.26	61.15 ± 1.24	23.92 ± 1.04	42.08 ± 0.83^{AB}	329.12 ± 17.92^{AB}
P value			0.003	0.003	0.009	0.107	0.074	0.867	0.148	0.019	0.006
SNP3	AA	57	127.12 ± 0.75	124.37 ± 0.79	131.91 ± 1.78	172.34 ± 1.19	36.65 ± 0.51	60.76 ± 0.45	21.80 ± 0.44	41.07 ± 0.46^{b}	329.34 ± 7.90
	GG	9	125.89 ± 1.73	123.00 ± 1.87	127.56 ± 3.95	170.89 ± 3.93	37.83 ± 1.95	59.67 ± 1.56	23.28 ± 1.48	40.78 ± 0.70^{b}	312.83 ±19.88
	GA	74	128.49 ± 0.70	125.91 ± 0.71	135.89 ± 1.40	175.23 ± 1.26	36.80 ± 0.48	60.61 ± 0.47	22.42 ± 0.40	42.55 ± 0.37^{a}	351.54 ± 7.71
P value			0.254	0.197	0.067	0.190	0.725	0.730	0.385	0.025	0.060
SNP4	CC	63	127.10 ± 0.70	124.25 ± 0.75	134.06 ± 1.52	172.77 ± 1.11	36.87 ± 0.50	60.92 ± 0.44	22.07 ± 0.47	41.68 ± 0.44	335.94 ± 7.01
	TC	77	128.31 ± 0.69	125.79 ± 0.69	133.47 ± 1.53	174.60 ± 1.28	36.75 ± 0.48	60.36 ± 0.47	22.34 ± 0.37	41.96 ± 0.37	343.35 ± 8.03
P value			0.22	0.14	0.79	0.30	0.86	0.39	0.65	0.63	0.49

SNPs, SNP1, g.T-445C; SNP2, g.G244C; SNP3, g.G910A; SNP4, g.T4922C; data are expressed as least square means ± SE: mean ± SE (cm or kg). Values with different superscripts within the same column differ significantly at $P < 0.05$ (a, b, ab) and $P < 0.01$ (A, B, AB); there is a significant difference among the mean values without common letters.

with the combined haplotype CC-GC-GA-CC had significantly greater body length, hip width and body weight ($P < 0.05$).

Discussion

Wnt genes encode a large family of secreted proteins that play key roles as intercellular signalling molecules during development. In this research, four novel SNPs were found in the *Wnt8A* gene of Qinchuan cattle. Results suggested that the cattle with genotype SNP2-CC and SNP3-GG could be selected to obtain greater growth traits. The two SNP mutations (SNP2, intron 2; SNP3, intron 3) of bovine *Wnt8A* gene were noncoding mutations, which cannot result in the change of amino acid. Moreover, there were some reports concerning the effects of the silent mutations on the gene's function and phenotype (Komar 2007). A silent polymorphism in the *MDR1* gene resulted in substrate specificity change (Kimchi-Sarfaty *et al.* 2007). Three silent mutation in bovine *GLI3* gene were associated with body weight at birth and six months in Nanyang cattle population (Huang *et al.* 2013). The *IGF2* intron 3–G3072A mutation has major effects on muscle growth, size of the heart, and fat deposition (Van Laere *et al.* 2003). To date, numerous reports have illustrated that introns can influence gene expression from transcription to translation (Greenwood and Kelsoe 2003; Le Hir *et al.* 2003; Bicknell *et al.* 2012). Thus, SNP2 and SNP3 may impact the growth traits by regulating the gene expression.

As for SNP1 and SNP4 concerned, SNP1 located in the 5′-flanking regulatory region; SNP4, located in exon 6 have no significant effects on the growth traits of cattle. Interestingly, SNP1, SNP2 and SNP3 had three genotypes, but SNP4 had two genotypes. The main reason may be due to the effects of genetic drift, isolation or population substructure. The analysis of haplotype combination is superior to the analysis of a single SNP, which can play an important role in growth traits (Akey *et al.* 2001; Schaid 2004). The outcome of haplotype analysis indicated that genotype CC-GC-GA-CC was more highly associated with body length, hip width and body weight ($P < 0.05$) than genotypes CC-CC-AA-CC and TC-GC-GA-TC. Moreover, haplotypes can be population-specific, due to the population's ancestry and demography (Weiss and Clark 2002; Neale and Sham 2004). Thus, further study including increased sample size should be conducted to find more connections and mutual effects between the *Wnt8A* gene and growth traits in Chinese Qinchuan cattle.

In conclusion, in this study, we first reported the genetic variations of *Wnt8A* gene and found four novel SNPs as well as three combined haplotypes in Chinese Qinchuan cattle. The association analysis revealed that

Table 7. Association of combined haplotypes of four SNPs with growth traits in Qinchuan cattle (2 years old).

Combined haplotypes	Sample size	Growth trait (mean ± SE)							
		Shoulder height (cm)	Hip height (cm)	Body length (cm)	Chest girth (cm)	Chest width (cm)	Chest depth (cm)	Hucklebone width (cm)	Hip width (cm)	Body weight (kg)
CC-CC-AA-CC	45	127.44 ± 0.78	124.71 ± 0.84	132.51 ± 1.89^{b}	172.61 ± 1.29	36.87 ± 0.59	60.88 ± 0.52	21.61 ± 0.50	41.07 ± 0.54^{b}	331.49 ± 8.33^{b}
TC-GC-GA-CC	8	129.75 ± 1.88	127.12 ± 1.79	142.13 ± 3.05^{a}	174.75 ± 4.44	37.38 ± 1.80	61.69 ± 1.67	23.13 ± 1.72	44.00 ± 0.87^{a}	364.75 ± 23.43^{a}
TC-GC-GA-TC	45	129.76 ± 0.56	127.22 ± 0.82	137.22 ± 1.37^{a}	176.84 ± 1.55	36.92 ± 0.62	60.70 ± 0.62	22.10 ± 0.53	42.44 ± 0.47^{ab}	360.54 ± 8.87^{a}
P value		0.12	0.09	0.03	0.13	0.95	0.81	0.51	0.03	0.05

Data are expressed as least square means ± SE: mean ± SE (cm or kg). Values with different superscripts within the same column differ significantly at $P < 0.05$ (a, b, ab); there is a significant difference among the mean values without common letters.

SNP2 (g.G244C) and SNP3 (g.G910A) as well as combined genotypes (CC-GC-GA-CC) have a significant effect on growth traits, which suggest that they could be used as molecular markers in marker-assisted selection in the beef cattle industry. However, further research is essential to clarify the mechanisms by which these mutations affect growth traits in cattle.

Acknowledgements

This study was supported by National Natural Science Foundation of China (grant no. 31601926), Programme of National Beef Cattle Industrial Technology System (grant no. CARS-38), Postdoctoral Science Foundation of China (grant no. 2015M570857 and 2015M570856), Key Scientific Research Projects for Higher Education of Henan (17A230005), Bio-breeding capacity-building and industry specific projects from National Development and Reform Commission (grant no. 2014-2573), Specific Projects of Science and Technology in Henan Province (grant no. 141100110200), and Science and Technology Coordinator Innovative engineering projects of Shaanxi Province (grant no. 2015KTCL02-08).

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Corresponding editor: Indrajit Nanda