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Molecular Characteristics of the Porcine *MUSTN1* Gene and its Significant Association with Economic Traits

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Abstract: The *MUSTN1* (musculoskeletal, embryonic nuclear protein 1) gene plays an important role in the development and regeneration of the mammalian musculoskeletal system and has a high expression in the muscle. In the present study, we cloned the porcine *MUSTN1* genome DNA sequence including the full-length cDNA. Analysis of the genomic sequence revealed that porcine *MUSTN1* consists of 3 exons separated by 2 introns. It contains a 237 bp open reading frame flanked by 251 bp 5'UTR and 216 bp 3'UTR. Radiation hybrid mapping data indicated that the porcine *MUSTN1* gene was located to chromosome 13q12-q22. The expression data revealed that the porcine *MUSTN1* gene is widely expressed in the examined tissues and has an especially high expression level in muscle tissues. One single nucleotide polymorphism c. 265C >T was identified in the 3'UTR of the porcine *MUSTN1* gene and genotyped by the NruI PCR-RFLP. Association analysis showed that different genotypes of the *MUSTN1* gene were significantly associated with ADG (average daily gain from birth to 100 kg), percentage of ham, proportion of lean and bone of the ham and meat color ($p<0.05$). Thus, the mutation can be applied to pig breeding by molecular Marker-Assisted Selection (MAS).

Key words: Pig, *MUSTN1*, SNP, association analysis, meat quality traits, expression

INTRODUCTION

The *MUSTN1* (musculoskeletal, embryonic nuclear protein 1) gene plays an important role in the development and regeneration of the mammalian musculoskeletal system which was previously identified in examining bone remodeling in mice after fracture. It was also up-regulated after 24 h to lengthening and shortening contractions in human muscle tissues (Kostek *et al*., 2007).

The *MUSTN1* gene was highly expressed during the early phases of bone regeneration and also had high level expression in terminally differentiated adult skeletal muscles (Lombardo *et al*., 2004). The other study indicated that *MUSTN1* have upregulated expression during hypertrophy in the sheep with the callipyge mutation (Vuocolo *et al*., 2007). Previous research also indicated that the expression of *MUSTN1* increases as C2C12 cells undergo differentiation into myocytes and this coincides with the up-regulation expression of skeletal muscle-specific transcription factors, MyoD and myogenin. Liu *et al*. (2010), recently study indicated that *MUSTN1* have a important role in myoblast differentiation which can inhibit the myoblast differentitaion and myofusion and which have the same expression pattern with the MyoD. There are also some multiple transcription factor binding sites in the *MUSTN1* gene promoter of mice, especially AP-1 sites. These sites represent regulator binding sites in many other musculoskeletal genes (MyoD and β-catenin) (Pedraza-Alva *et al*., 1994; Goldberg *et al*., 1996; Winchester *et al*., 2000; Ionescu *et al*., 2001; Andreucci *et al*., 2002; Li *et al*., 2004; Liu and Hadjiargyrou, 2006). Thus the *MUSTN1* gene may be linked to the MyoD-associated signaling pathway which participates in the process of myogenesis. Based on the previous finding, It speculated that the *MUSTN1* gene represents a kind of skeletal muscle marker gene with a probable function in cell differentiation and may play an important role in myogenic differentiation.

It is well known that there are multiple factors controlling growth speed and meat quality, so it is important that we further study muscle development and the differentiation mechanism. At present, there are already many genes which are known to influence meat muscle development and differentiation and some of them can improve meat quality and growth speed, such as RN

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(Rendement Napole) (Carr *et al*., 2006) and RYR1 (ryanodine receptor 1) (Fujii *et al*., 1991). However, there are still some potential candidate genes that to be discovered and studied. Previous studies have indicated that *MUSTN1* is involved in differentiating and maturing muscle tissues of humans and mice. The purpose of this study is to gain an insight into the function of *MUSTN1* by isolating the porcine *MUSTN1* gene, predicting its amino acid sequence, testing the expression profile in different tissues and associating the genotypes with economic traits. This is the first report showing the functions of the *MUSTN1* gene in pigs and as such, it aims at helping to understand the porcine development of economic traits. It also points out that, by molecular marker-assisted selection, the c. 265C>T mutation site can be applied to pig breeding as a molecule marker.

MATERIALS AND METHODS

Isolation full-length cDNA of porcine *MUSTN1*: The mRNA of the human *MUSTN1* gene (GenBank accession no. NC_000003.11) from NCBI and search for homologous pig EST in the porcine-EST databases using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast/) and shared at least 80% sequence homology with the human cDNA in order to assemble the potential porcine gene contig sequence by the seqman of DNAStar and the consensus sequences of porcine ESTs were used to design primers for the amplification of the porcine *MUSTN1* gene.

Total RNA was isolated from the freshly obtained tissue of mature Tongcheng (domestic breed) pig using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was treated with RQ1 RNase free DNase (Promega, Madison, WI, USA) and transcripted by using M-MLV reverse transcriptase (Promega, Madison, WI, USA). The primers (Table 1) were designed based on the porcine ESTs to amplify the porcine *MUSTN1* gene cDNA. Three pairs of primers is deoigned (Table 1) in order to amplify the complete mRNA sequence and genomic sequence of the porcine *MUSTN1*. The PCR (Polymerase Chain Reaction) profile was 5 min at 95°C, followed by 34 cycles of 40 sec at 94°C, 30 sec at annealing temperature (Table 1), 1 min at 72°C and a final extension of 5 min at 72°C.

SMART cDNA were synthesized from the total RNA of a mature Large White pig's skeletal muscle by using the SMART™ RACE cDNA amplification kit (Clontech) for Rapid Amplification of the cDNA ends (RACE)-Polymerase Chain Reaction (PCR). 5' RACE and 3' RACE was performed according to the instructions of the SMART™ RACE cDNA Amplification Kit protocol. The PCR product was purified with the 3S Spin DNA Agarose Gel Purification system (Shenergy Biocolor, Shanghai, China) and cloned into the pMD18-T vector (TakaRa, Dalian, China) then sequenced by commercial service. Touchdown PCR was carried out for 5 min at 95°C, followed by 5 cycles at (94°C 30 sec, 73°C 30 sec, 72°C 30 sec) and 30 cycles at (94°C 30 sec, 68°C 30 sec, 72°C 30 sec) and a final extension of 5 min at 72°C by using 5' RACE and 3' RACE universal primers and *MUSTN1* specificity primers (Table 1). The ORF (open reading frame) and the amino acid sequences were deduced with the program Seqman (DNAstar, Madison, WI, USA). The secondary structure was performed by online tools of the ExPASy website (http://cn.expasy.org/tools/).

Table 1: Primer pairs designed for the porcine *MUSTN1* gene

Primer name	Primer sequence (5'- 3')	Tm (°C)	Size (bp)
CDS-F	TCTACCGCACCCACCATGT	61	305
CDS-R	AAGGTGGCAGGTGAGAAGGT		
3-RACE	GTCCCAGGAAGCCCCCATCAAGAAGA	73	521
	CCTCCTCACCACCAGCAACA		
5-RACE	ACTGCTTTCACCAGGGCTG	60	433
	AAGTGGGTATCTGGGTTGTTCT		
RH-F	TTGGCTTGGTGGCATCTGA	60	160
RH-R	GGACTGGCTTTGAGGCTGAA		
EX-F	CCAAGAACCAGGAGATCAAGTC	60	165
EX-R	GCACTTCTCAGCCAAAGACACT		
18S-F	TTTCGCTCTGGTCCGTCTTG	60	101
18S-R	TTCGGAACTGAGGCCATGAT		
SNP-F	GGACTCTGCACCAGCACTCG	60	157
SNP-R	CATTCCTCTGAGACCCTTCCAA		

Amplification genomic sequence of porcine *MUSTN1*: The exons of porcine *MUSTN1* were deduced based on the genomic structure of the human *MUSTN1* gene. The introns were then amplified by PCR using the genomic DNA template and primers EX-F/ EX-R and CDS-F/CDS-R (Table 1). The PCR was carried out as described earlier on 10 ng genomics DNA as template in a total volume of 10 μL. The obtained genomic fragments with overlapping ends were purified, cloned and sequenced. The *MUSTN1* genomic sequence was obtained by assembling the overlapping genomic fragments. To verify the integrity of the assembled sequence, relevant porcine genomic sequences in the GenBank database were retrieved and aligned with the assembled sequence.

Expression profile analysis of porcine *MUSTN1*: The porcine *MUSTN1* gene mRNA expression in different tissues was detected by semi-quantitative RT-PCR (Reverse-Transcription Polymerase Chain Reaction) with 18S ribosome RNA (rRNA) as an internal control which showed very consistent expression among different samples. The total RNA was isolated from the freshly harvested tissues (heart, liver, spleen, lung, kidney, skeletal muscle, fat and small intestine) of three mature Tongcheng pigs°C Chinese breed°C. Primers among exon 2 and exon 3 were designed to amplify various tissue cDNA according to the mRNA sequence (Table 1). PCR amplification was done in a total volume of 20 μL

containing 50 ng cDNA, 10×PCR buffer, 0.3 μM of each primer, 75 μM of each dNTP and 1 U Taq DNA polymerase (Takara, Dalian, China). PCR conditions were 4 min at 94°C followed by 30 cycles of 30 sec at 94°C, 30 sec at 60°C, 20 sec at 72°C and a final extension of 5 min at 72°C. Finally, 10 μL of each PCR product was electrophoresed on a 2.0% agarose gel stained with GelRed.

Chromosomal localization of porcine *MUSTN1*: The INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel (Yerle *et al*., 1998) was employed to refine the mapping resolution. The porcine-specific primers (Table 1) located in the intron 1 were amplified using the porcine radiation hybrid (IMpRH) panel DNA samples. The PCR was performed in 10 μL volume containing 25 ng panel DNA, 10×PCR buffer, 0.3 μM of each primer, 35 μM of each dNTP and 0.5 U Taq DNA polymerase (Takara, Dalian, China). The PCR conditions were 5 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 60°C, 20 sec at 72°C and a final extension of 5 min at 72°C. The PCR results were recorded and analyzed on http://imprh.toulouse.inra.fr/ to get the mapping information (Milan *et al*., 2000).

SNP identification, genotyping and association analysis of porcine *MUSTN1*: PCR products from Landrace and Tongcheng pigs were amplified by using primers (EX-F and EX-R) and sequenced in order to detect potential mutations. The PCR for genotyping was performed in a volume of 10 μL consisting of 25 ng of genomic DNA, 10×PCR buffer, 0.3 μM of each primer, 35 μM of each dNTP and 0.5 U Taq DNA Polymerase (Takara, Dalian, China). PCR protocols were 4 min at 94°C followed by 35 cycles of 30 sec at 94°C, 30 sec at 59°C, 25 sec at 72°C and a final extension of 5 min at 72°C. About 5 μL of each PCR product was digested overnight with 3 U NruI (Jingmei Biotech, China) at 37°C for 8 h and then size-separated on GelRed stained 3.0% agarose gel. The animals used in the association analysis included 182 pigs: Tongcheng (34), Large White (30), Landrace (31) and crossbreds of Large White (Landrace x Tongcheng) (40) and Landrace x (Large White x Tongcheng) (47). Nineteen traits were recorded as well as the detail information about the population reference (Xu *et al*., 2008). An analysis method using the PROC GLM procedure in the SAS software package (SAS Inst. Inc., Cary, NC, USA) was employed to analyze the association between genotypes and traits. The linear model with the fixed effects is:

$$Y_{ijklm} = \mu + G_i + B_j + S_k + C_l + F_m(C) + \varepsilon_{ijklm}$$

Where:

Y_{ijklm} = The ijkl the traits' observation value
μ = The mean
G_i = The effect of the ith genotypes
B_j = The effect of jth batch
S_k = The effect of jth sex
C_l = The effect of lth population
$F_m(C)$ = Family effects within breed
ε_{ijklm} = The random residual corresponding to the traits observation value with var (ε) = $I\sigma_e^2$

RESULTS

Molecular characterization of the porcine *MUSTN1* gene: The genome sequence of porcine *MUSTN1* was deposited in the GenBank (accession No. GU345805). The sequence was obtained in combination with the four PCR products (Table 1: primer name: CDS-F/R, 3-RACE, 5-RACE and EX-F/R). Sequence analysis showed that the porcine *MUSTN1* gene consists of 3 exons and 2 introns and includes a 237bp ORF (open reading frame) flanked by a 251bp 5'UTR and a 216 bp 3'UTR. Analysis showed that *MUSTN1* gene encoded 78 amino acids with a calculated molecular mass of 8.479 kDa and an idoelectric point of 9.87. DNAMAN analysis showed that the predicted porcine amino acid sequence shared 92% and 89% identity with the corresponding human (NP_995325.2) and mouse (NP_852055.1) sequences, respectively. This suggested that the *MUSTN1* gene is evolutionarily conserved in large mammals. A multiple sequence alignment of the *MUSTN1* proteins of several species, including *xenopus laevis*, cattle, *canis lupus familiaris*, *Gallus gallus*, humans, mouse, rats and pigs is shown in Fig. 1.

Fig. 1: The deduced amino acid sequence of the porcine *MUSTN1* gene was aligned to xenopus laevis, cattle, canis lupus familiaris, *Gallus gallus*; humans, mouse, rats and pigs sequence

Expression profile analysis of the porcine *MUSTN1* gene: Semi-quantitative RT-PCR was used to detect the tissue distribution of the porcine *MUSTN1* gene, excluding genomic DNA contamination of the RNA preparations and thus, confirming the RT-PCR results of the expression profile analysis of the porcine *MUSTN1* gene. Positive control PCR (18S rRNA) in each tissue displayed a basically identical signal. The gene was widely expressed in the examined tissues, including heart, liver, spleen, lung, kidney, muscle, intestine, fat and stomach. Muscle tissues showed an especially high level of expression (Fig. 2).

Chromosomal localization mapping of porcine *MUSTN1* gene: The chromosomal localization of the porcine *MUSTN1* gene was determined using a porcine radiation hybrid (IMpRH) panel. The porcine *MUSTN1* gene was located to chromosome 13q12-q22 with the most significantly linked marker being SW1400 (Distance = 60 cR, LOD = 5.85 and Retention frequency = 22%). In humans, the *MUSTN1* has been mapped to HSA3p21. This information confirmed the established conservation of synteny between pigs and humans through chromosome comparison which demonstrates synteny between SSC13 and HSA3 (https://www-lgc.toulouse.inra.fr/pig/compare/SSCHTML/SSC13S.HTM) (Goureau *et al.*, 1996). One mutation c. 265C>T was identified by comparative sequencing. The substitution occurred in the 3'UTR. The polymorphism c. 265C>T in the experimental population was analyzed by NruI PCR-RFLP. A mutation primer was designed to amplify the 157 bp genome fragment and was genotyped by using the NruI restriction enzyme (157 bp for allele T, 20 bp and 137 bp for allele C) (Fig. 3).

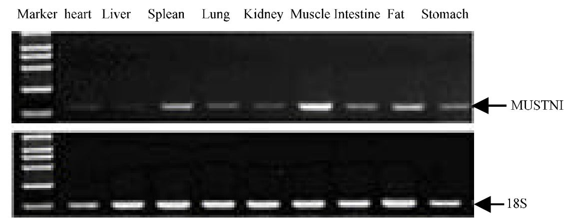

Fig. 2: The expression profile analysis of the porcine *MUSTN1* gene

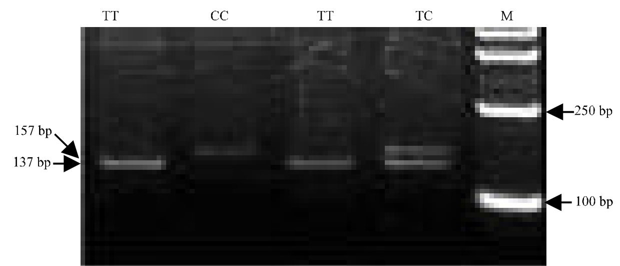

Fig. 3: One c. 265C>T Single Nucleotide Polymorphism (SNP) was detected in 3'UTR. PCR products were digested with NruI restriction enzyme to distinguish different alleles (157 bp for allele T, 20 bp and 137 bp for allele C)

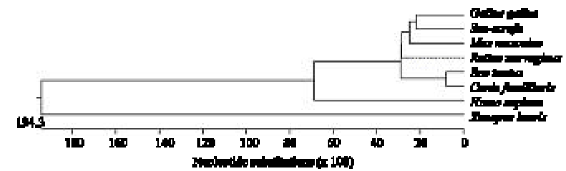

Fig. 4: The *MUSTN1* phylogenetic tree with amino acid sequences. The sequences used for analysis are derived from GenBank. The horizontal branch lengths are proportional to the estimated divergence of the sequence from the branch point

DISCUSSION

By using the PSOTR II, the multiple sequences indicated that different proteins had high similarity in both the N-terminal and C-terminal and there is also a classic nuclear import signal (PIKKKRPPA) in the N-terminal algorithm (http://psort.nibb.ac.jp). The previous studies indicated that rat *MUSTN1* is a nuclear protein in transient transfestion of preosteoblastic MC3T3 cells by using assays and Mustang-GFP fusion construct (Lombardo *et al.*, 2004) which consistent with the prediction. There are some processes which occur in the nucleus, including DNA replication, transcription and post-transcriptional processing. In order to determine the evolutionary relationship between different species, researchers analyzed the unrooted phylogenetic tree using DNAStar's LaserGene software (DNAStar, Madison, USA) (Fig. 4). The results revealed that the porcine *MUSTN1* had a closer genetic relationship with the *Gallus gallus*, mouse and rat.

The expression pattern study indicated the porcine *MUSTN1* gene is high expression levels in muscle tissues, previous studies revealed that the *MUSTN1* increases as C2C12 cells undergo differentiation into myocytes in humans (Liu and Hadjiargyrou, 2006), so it indicated that the porcine *MUSTN1* gene plays a role in muscle development and regeneration.

According to the information from the pig QTLs database (http://www.animalgenome.org/QTLdb/pig.html) (Hu *et al.*, 2007), there have been several papers which have reported that the Quantitative Trait Loci (QTL) on porcine chromosome 13q12-q22 includes responsible for meat color-B (De Koning *et al.*, 2001), pH 24 h, ham muscle weight, ham meat weight (Van Wijk *et al.*, 2006)

Table 2: The association results between the genotypes at c. 265C>T in 3'UTR polymorphism of the *MUSTN1* and economic traits

Genotype	Number	ADG	Percentage of ham	Proportion of lean and bone of the ham	Meat color
TT	88	491.73±4.19	30.63±0.19	70.50±0.45	2.99±0.04
CT	67	485.13±4.33	30.35±0.20	69.79±0.46	3.11±0.03
CC	27	476.49±6.61	29.80±0.30	68.41±0.71	3.15±0.06
p-value					
TT-CT		0.29	0.348	0.29	0.049*
TT-CC		0.048*	0.029*	0.014*	0.025*
CT-CC		0.27	0.142	0.098	0.47

*Indicates significant difference at $p<0.05$ level

and ADG (average daily gain) (De Koning *et al.*, 2001). The center location of this QTL is near the map position of *MUSTN1*. The effect of the c. 265C>T polymorphism on carcass and meat quality traits was also investigated in the experimental population described above. Association analyses revealed that the substitution of c. 265C>T had a significant effect on average daily gain from birth to 100 kg (ADG), meat color, percentage of ham, proportion of lean and bone of the ham ($p<0.05$) (Table 2). The ADG, percentage of ham and proportion of lean and bone of the ham in pigs with TT genotype was higher than in pigs with CC genotype ($p = 0.029$, $p = 0.014$ and $p = 0.048$, respectively) which means that the allele C was a predominant allele.

However, significant differences with opposite directions were found in meat color. The meat color of pigs with TT genotype was significantly lower than that of pigs with CC and CT genotypes ($p = 0.025$ and $p = 0.049$, respectively), in other words, allele T was a predominant allele in meat color.

Previous studies have shown that the porcine *MUSTN1* gene is exclusively and highly expressed during bone regeneration (Kostek *et al.*, 2007) and up-regulation expression of skeletal muscle-specific transcription factors (MyoD and myogenin) (Liu and Hadjiargyrou, 2006). And Zheng *et al.* (2009) also found that *MUSTN1* affect the skeletal muscles hypertrophy (Zheng *et al.*, 2009). Thus, the porcine *MUSTN1* gene may play an important role in bone and muscle development.

CONCLUSION

In summary, we have presented the complete genomic DNA sequence and organization of the porcine *MUSTN1* gene. The analysis of the generated genomic sequence indicated that the porcine *MUSTN1* gene consists of 3 exons and 2 introns. These results further support the claim that the porcine *MUSTN1* gene plays an important role in skeletal muscle differentiation and indicate a probability that c. 265C>T can be applied as a useful molecular marker in pig breeding.

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REFERENCES

Andreucci, J.J., D. Grant, D.M. Cox, L.K. Tomc and R. Prywes *et al.*, 2002. Composition and function of AP-1 transcription complexes during muscle cell differentiation. J. Biol. Chem., 277: 16426-16432.

Carr, C.C., J.B. Morgan, E.P. Berg, S.D. Carter and F.K. Ray, 2006. Growth performance, carcass composition, quality and enhancement treatment of fresh pork identified through deoxyribonucleic acid marker-assisted selection for the Rendement Napole gene. J. Anim. Sci., 84: 910-917.

De Koning, D.J., B. Harlizius, A.P. Rattink, M.A. Groenen, E.W. Brascamp and J.A. van Arendonk, 2001. Detection and characterization of quantitative trait loci for meat quality traits in pigs. J. Anim. Sci., 79: 2812-2819.

Fujii, J., K. Otsu, F. Zorzato, S. de Leon and V.K. Khanna *et al.*, 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253: 448-451.

Goldberg, D., P. Polly, J.A. Eisman and N.A. Morrison, 1996. Identification of an osteocalcin gene promoter sequence that binds AP1. J. Cell. Biochem., 60: 447-457.

Goureau, A., M. Yerle, A. Schmitz, J. Riquet, D. Milan, P. Pinton, G. Frelat and J. Gellin, 1996. Human and porcine correspondence of chromosome segments using bidirectional chromosome painting. Genomics, 36: 252-262.

Hu, Z.L., E.R. Fritz and J.M. Reecy, 2007. AnimalQTLdb: A livestock QTL database tool set for positional QTL information mining and beyond. Nucleic. Acids. Res., 35: D604-D609.

Ionescu, A.M., E.M. Schwarz, C. Vinson, J.E. Puzas, R. Rosier, P.R. Reynolds and R.J. O'Keefe, 2001. PTHrP modulates chondrocyte differentiation through AP-1 and CREB signaling. J. Biol. Chem., 276: 11639-11647.

Kostek, M.C., Y.W. Chen, D.J. Cuthbertson, R. Shi, M.J. Fedele, K.A. Esser and M.J. Rennie, 2007. Gene expression responses over 24 h to lengthening and shortening contractions in human muscle: Major changes in CSRP3, *MUSTN1*, SIX1 and FBXO32. Physiol. Genomics, 31: 42-52.

Li, Q., W.M. Dashwood, X. Zhong, M. Al-Fageeh and R.H. Dashwood, 2004. Cloning of the rat beta-catenin gene (Ctnnb1) promoter and its functional analysis compared with the Catnb and CTNNB1 promoters. Genomics, 83: 231-242.

Liu, C. and M. Hadjiargyrou, 2006. Identification and characterization of the *Mustang promoter*: Regulation by AP-1 during myogenic differentiation. Bone, 39: 815-824.

Liu, C., R.P. Gersch, T.J. Hawke and M. Hadjiargyrou, 2010. Silencing of *MUSTN1* inhibits myogenic fusion and differentiation. Am. J. Physiol. Cell. Physiol., 298: C1100-C1108.

Lombardo, F., D. Komatsu and M. Hadjiargyrou, 2004. Molecular cloning and characterization of Mustang, a novel nuclear protein expressed during skeletal development and regeneration. FASEB. J., 18: 52-61.

Milan, D., R. Hawken, C. Cabau, S. Leroux and C. Genet *et al*., 2000. ImpRH server: An RH mapping server available on the Web. Bioinformatics, 16: 558-559.

Pedraza-Alva, G., J.M. Zingg and J.P. Jost, 1994. AP-1 binds to a putative cAMP response element of the MyoD1 promoter and negatively modulates MyoD1 expression in dividing myoblasts. J. Biol. Chem., 269: 6978-6985.

Van Wijk, H.J., B. Dibbits, E.E. Baron, A.D. Brings and B. Harlizius *et al*., 2006. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. J. Anim. Sci., 84: 789-799.

Vuocolo, T., K. Byrne, J. White, S. McWilliam, A. Reverter, N.E. Cockett and R.L. Tellam, 2007. Identification of a gene network contributing to hypertrophy in callipyge skeletal muscle. Physiol. Genomics, 28: 253-272.

Winchester, S.K., N. Selvamurugan, R.C. D'Alonzo and N.C. Partridge, 2000. Developmental regulation of collagenase-3 mRNA in normal, differentiating osteoblasts through the activator protein-1 and the runt domain binding sites. J. Biol. Chem., 275: 23310-23318.

Xu, X.L., K. Li, Z.Z. Peng, S.H. Zhao and M. Yu *et al*., 2008. Molecular characterization, expression and association analysis of the porcine CMYA4 gene with carcass traits. J. Anim. Breed. Genet., 125: 234-239.

Yerle, M., P. Pinton, A. Robic, A. Alfonso and Y. Palvadeau *et al*., 1998. Construction of a whole-genome radiation hybrid panel for high-resolution gene mapping in pigs. Cytogenet. Cell Genet., 82: 182-188.

Zheng, Q., Y. Zhang, Y. Chen, N. Yang, X.J. Wang and D. Zhu, 2009. Systematic identification of genes involved in divergent skeletal muscle growth rates of broiler and layer chickens. BMC Genomics, 10: 87-87.