


## IDENTIFICATION OF TWO NOVEL SINGLE NUCLEOTIDE POLYMORPHISM SITES IN THE *MYOSTATIN* (*MSTN*) GENE AND THEIR ASSOCIATION WITH CARCASS TRAITS IN MEAT-TYPE RABBITS (*ORYCTOLAGUS CUNICULUS*)

YANG L.Q.\*, ZHANG K.\*, WU Q.Y.\*, LI J.†, LAI S.J.‡, SONG T.Z.¶, ZHANG M. 

\*College of Animal Science & Technology, Sichuan Agricultural University, Wenjiang, CHENGDU, 611130, China.

†Institute of Animal Nutrition, Sichuan Agricultural University, Wenjiang, CHENGDU, 611130, China.

‡Institute of Animal Breeding and Genetic, Sichuan Agricultural University, Wenjiang, CHENGDU, 611130, China.

¶Institute of Animal Science, Tibet Academy of Agricultural and Animal Husbandry Science, Lhasa, Tibet, 850009, China.

**Abstract:** Two unknown single nucleotide polymorphism (SNP) sites in exons 1 (c.194C>T) and 2 (c.445T>A) of meat-type rabbit *MSTN* gene were identified in the study. Our objective was to analyse the population genetics structure of the two novel SNP sites in 230 individuals from six breeds and their associations with carcass traits of rabbits. We found that live body weight (BW), cold carcass weight (CCW), reference carcass weight (RCW), CCW percentage ( $P_{CCW}$ ) and RCW percentage ( $P_{RCW}$ ) of the rabbits with the genotype CC at the c.194C>T of exon 1 or AA at the c.445T>A of exon 2 were significantly higher than those with other genotypes. Diplotypes significantly affected BW, RCW, CCW,  $P_{RCW}$  ( $P<0.01$ ) and  $P_{CCW}$  and  $P_{CM}$  ( $P<0.05$ ). CC/AA was the advantageous diplotypes for BW, RCW, CCW and  $P_{CM}$ , and TT/AA was the advantageous diplotypes for  $P_{CCW}$  and  $P_{RCW}$ . In contrast, TT/TT was the negative diplotypes for BW, CCW, RCW,  $P_{CCW}$  and  $P_{RCW}$ , and TT/AA was the negative diplotypes for  $P_{CM}$ . The results suggest that the two new mutations of *MSTN* gene significantly affected BW, CCW, RCW,  $P_{CCW}$  and  $P_{RCW}$  of rabbits, and *MSTN* may be an important candidate gene of carcass traits in meat-type rabbits.

**Key Words:** rabbit, *myostatin* gene, single nucleotide polymorphisms, carcass traits.

## INTRODUCTION

Myostatin (*MSTN*), or growth and differentiation factor 8 (GDF-8), is a member of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily that acts as a negative regulator of skeletal muscle development and growth in mammals (McPherron *et al.*, 1997, 2002). *MSTN* can inhibit myogenic and sarcogenic cell proliferation by down-regulating the expression of MyoD, Pax and Myf25 (Rios *et al.*, 2002; Joulia *et al.*, 2003; McCroskery *et al.*, 2003) and has been shown to regulate muscle development and growth by suppressing the transcription of MyoG family members (Joulia *et al.*, 2003). Consequently, *MSTN* has received much attention from the meat-producing animal breeding industry as a candidate gene that can control growth and carcass traits (Bellinge *et al.*, 2004). Some mutations disrupting *MSTN* function caused double-muscling phenotypes in cattle (Kambadur *et al.*, 1997; Grobet *et al.*, 1997, 1998; McPherron *et al.*, 1997, 2002; Marchitelli *et al.*, 2003) and dogs (Mosher *et al.*, 2007). In addition, *MSTN* knock-out goat (Guo *et al.*, 2016) and mouse (Mcpherron *et al.*, 1997; Lin *et al.*, 2002) performed a high growth rate in the early stage and muscular hypertrophy and hyperplasia, respectively. Associations between *MSTN* polymorphisms and carcass traits have been reported for numerous livestock species including pig (Li *et al.*, 2002; Jiang *et al.*, 2008), goat (Liu *et al.*, 2006), cattle (Grobet *et al.*, 1998; Guo *et al.*, 2007), chicken (Zhu *et al.*, 2007), duck (Lu *et al.*, 2008) and rabbit (Fontanesi *et al.*, 2011; Qiao *et al.*, 2014; Abdel-Kafy *et al.*, 2016; El-Sabrou and Aggag, 2017), but few reports have investigated the effects of different SNPs in rabbit *MSTN* gene on production traits.

**Correspondence:** M. Zang, [zhangming@sicau.edu.cn](mailto:zhangming@sicau.edu.cn). Received August 2018 - Accepted October 2019.  
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Rabbits exhibit several traits of economic importance as meat livestock and as a common experimental animal. More than 750 thousand tons of rabbit meat has been produced in China every year since 2010, and improving the yield of rabbit meat by genetic selection or gene modification is a highly significant aim. Therefore, the exploration of all genetic mutation sites which affect the growth of skeletal muscles has an important theoretical and practical significance.

The rabbit *MSTN* gene which consists of three exons and two introns is highly conserved and is expressed in developing and mature skeletal muscle (McPherron *et al.*, 1997; Bellinge *et al.*, 2004; Fontanesi *et al.*, 2008, 2011; Qiao *et al.*, 2014). *MSTN* knock-out rabbits exhibited increased birth weight and a significant increase in the weight ratios of the quadriceps and biceps muscles to the whole body (Guo *et al.*, 2016). The role of *MSTN* in growth and carcass-related traits suggests that it may be an important genetic marker in rabbits. Fontanesi *et al.* (2011) identified 4 SNPs in 14 rabbits representing breeding or lines, and Sternstein *et al.* (2014) identified two SNPs in crossbred population of Giant Grey and New Zealand white rabbit. The present research was carried out to detect the genetic variation within *MSTN* gene of the rabbits from six breeds and to estimate the association of *MSTN* SNPs, alone and in combination, with carcass traits in the rabbits.

## MATERIALS AND METHODS

### **Animal**

A batch of 230 rabbits from six breeds (30 Harbin white, 32 Belgians, 21 Hotot, 63 Zika, 36 California and 48 Tianfu black) was obtained from the Sichuan Agricultural University research farm. All rabbits were housed and fed under the same conditions. The nutritional levels and feeding management have been addressed by Zhang *et al.* (2011). All experimental procedures were approved by the Animal Ethics Monitoring Committee of Sichuan Agricultural University and The use of experimental rabbits followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (3rd Ed). The approval number is SCAUS20163636.

### **Growth and carcass traits measurement**

Live body weights (BW) of all rabbits were measured after 12h starvation at 135 d of age, while cold carcass weight (CCW) and reference carcass weight (RCW) were measured after slaughter at the age of 135 d according to the description from George *et al.* (2015). Then, the CCW and RCW percentages were calculated according to the formula:  $P_{CCW}=(CCW/BW)\times 100\%$  and  $P_{RCW}=(RCW/BW)\times 100\%$ , respectively. To measure the cooked meat percentage ( $P_{CM}$ ), 100 g right-leg semi-membranous muscle was collected 2 h post-slaughter, weighed ( $W_0$ ) and steamed at 100°C for 45 min, cooled at room temperature for 30 min and weighed again ( $W_1$ ). The cooked meat percentage (CMP) was calculated according to the formula:  $P_{CM}=(W_1/W_0)\times 100\%$  (Naveen *et al.*, 2016).

### **Sampling and DNA extraction**

Genomic DNA was isolated from 1.5 mL blood by standard phenol-chloroform extraction. The integrity and concentration of genomic DNA were assessed by 0.8% agarose gel electrophoresis and photometry using Gene Quant II (GE, USA), respectively. The purity of genomic DNA met the experimental requirements. Genomic DNA was diluted to a concentration of 20 ng/ $\mu$ L and stored at -80°C until polymerase chain reaction (PCR) amplification.

### **PCR amplification, PCR-Single Strand Conformation Polymorphism analysis (SSCP) and Sequencing**

All primers (Table 1) were designed according to their DNA sequence, containing two exons (AM931155; AM931156) of the *MSTN* gene in GenBank database of NCBI, using Oligo 6.0 software (<http://www.oligo.net/downloads.html>) and synthesised by InvitroGen Shanghai Company (Shanghai, China). PCR amplification was performed in 10  $\mu$ L reactions containing 5.0  $\mu$ L of 2 $\times$ PCR MasterMix from TaKaRa (Dalian, China), 0.4  $\mu$ L of 10 pmol/ $\mu$ L forward and reverse primer, respectively, 0.7  $\mu$ L 25 ng/ $\mu$ L template DNA and 3.5  $\mu$ L RNase free water. Samples were amplified in a PTC-100 thermal cycler (MJ Company, USA) by an initial denaturation at 96°C for 10 min, 35 cycles of denaturation (95°C, 30 s), annealing (see Table 1 for respective temperatures, 30 s) and extension (72°C, 15-30 s), and a final extension at 72°C for 5 min.

**Table 1:** Primer sequence and reaction conditions for the amplification of the three *myostatin* exons of meat-type rabbits.

Primer Pair	Primer sequence(5'-3')	Product size (bp)	PCR conditions	Region
1	F: CAATTTTGCTTGCCATTACTG R:GAAGTGTGACATACACTAATAGGACT	493	96°C/10 min-(95°C/30 s-55.4°C/30 s-72°C/30 s) ×35-72°C/5 min	Exon1
2	F: TATTTGGCAGAGCATTGATGTG R:AACAAGGTTACTAGAGGGTCTTTTCAGT	184	96°C/10 min-(95°C/30 s-55.5°C/30 s-72°C/15 s) ×35-72°C/5 min	Exon2

To confirm the reliability of SNP detection, all PCR products from 230 individuals were sequenced in two directions by Sanger method after the amplification of products of *MSTN* gene was conducted in 12% non-denaturing polyacrylamide gel to determine single strand conformation polymorphism (SSCP) (Shi *et al.*, 2009). The sequences were compared by BLASTn searching in GenBank and analysed by DNAMAN Sequence Analysis Software (Lynnon Corporation, St-Louis, Pointe-Claire, Quebec, Canada). RNA structure fold was predicted by online tool (<http://rna.urmc.rochester.edu/RNAstructureWeb/Servers/>) to explain the potential effect of two SNPs on carcass traits.

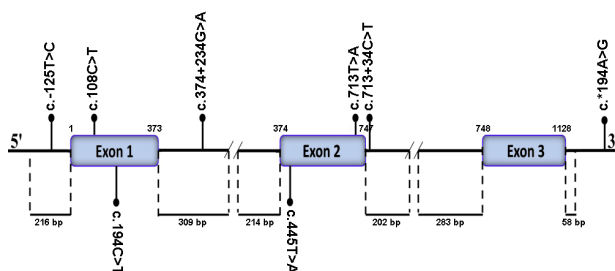
### Statistics

Data of  $P_{CCW}$ ,  $P_{RCW}$  and  $P_{CM}$  were not normally distributed by *Shapiro-Wilk* test (Meredith *et al.*, 2006) and the percentage data required arcsine transformation before further processing. The transformed data and BW, CCW and RCW were analysed by the general linear model (GLM) procedure of SAS (SAS Inst. Inc., Cary NC). The genetic effects were assessed by a mixed procedure according to the following GLM:  $Y = \mu + B + G + bX + e$ , where  $Y$  was the dependent variance;  $\mu$ , the population mean;  $B$ , the fixed effects of the breed;  $G$ , the fixed effects of the genetic (genotype or diplotype) background;  $X$ , the growth and carcass traits;  $b$ , the coefficient of regression; and  $e$ , random error. Multiple comparisons were performed by least squares means (LSM) analyses. Differences were considered significant ( $P < 0.05$ ), and all values are presented as the mean  $\pm$  standard deviation. Chi square test was performed in Hardy-Weinberg equilibrium of the frequency of alleles and genotype.

## RESULTS

### Single Nucleotide Polymorphisms (SNPs) in exons of the rabbit *MSTN* gene

The sequence data revealed two new SNPs which were localised in exon 1 and 2 (Figure 1). There was a C  $\rightarrow$  T mutation at position c.194 (relative to GenBank accession No: AM931155) in exon 1, and a T  $\rightarrow$  A mutation at position c.445 (relative to GenBank accession No: AM931156) in exon 2. Both SNPs were synonymous mutations that did not change the amino acid sequence of *MSTN*.



**Figure 1:** Structure of the rabbit *myostatin* gene and positions of mutations. We found the two novel SNPs are c.194C>T and c.445T>A, listed below the schematic gene structure map, and six SNPs (125T>C, 108C>T, 374+234G>A, 713T>A, 713+34C>T and 3'UTR 194A>G) which have been reported were marked on the schematic map of gene structure. The sequenced regions comprise the exons and 5' and 3' exon flanking regions.

### Association of the *MSTN* alleles and genotypes with rabbit carcass traits

The frequencies of the alleles and genotypes are summarised in Table 2. In exon 1, allele C was predominant over allele T in all six rabbit lines, and the homozygous genotype CC had the highest frequency in all populations. In exon 2, the heterozygous TA genotype had the highest frequency in all populations. The frequency of alleles and genotypes did not deviate from the Hardy-Weinberg equilibrium (HWE) in all six rabbit breeds by chi-square ( $\chi^2$ ) test, with the exception of exon 1 in *Zika* rabbits.

The associations between *MSTN* genotypes and carcass traits (BW, CCW, RCW,  $P_{CCW}$ ,  $P_{RCW}$ ,  $P_{CM}$ ) were assessed by GLM analysis (Table 3). In exon 1, genotype significantly affected BW, CCW, RCW,  $P_{RCW}$  and  $P_{CCW}$ . In particular, the BW, CCW, RCW,  $P_{RCW}$  and  $P_{CCW}$  of these rabbits with the CC genotype was significantly higher than those with the TT genotype ( $P < 0.05$ ). The BW, CCW and RCW of CC rabbits was significantly higher than those of CT rabbits ( $P < 0.05$ ). In exon 2, genotype strongly influenced the CCW,  $P_{RCW}$  and  $P_{CM}$ . Rabbits of the AA genotype had significantly higher CCW,  $P_{RCW}$  and  $P_{CM}$  ( $P < 0.05$ ) than those of the TT genotype. The results suggested that CC was the advantageous genotype for BW, RCW, CCW,  $P_{RCW}$  and  $P_{CCW}$  in exon 1, whereas TT was the negative genotype for BW, CCW, RCW,  $P_{CCW}$ ,  $P_{RCW}$  and  $P_{CM}$ . In exon 2, AA was the advantageous genotype for CCW,  $P_{RCW}$  and  $P_{CM}$  and TT was the negative genotype for CCW,  $P_{RCW}$  and  $P_{CM}$ .

### Construction of haplotypes and their associations with rabbit carcass traits

Nine diplotypes (Table 4) were obtained based on the six haplotypes in Table 2. Among them, the frequency of the diplotype CC/AA was the highest (31.74%) and the frequency of the diplotype TT/AA was the lowest (0.87%). Mixed model analysis showed that the *MSTN* diplotype strongly correlated with carcass traits and significantly affected the BW, RCW, CCW and  $P_{RCW}$  ( $P < 0.01$ ) and the  $P_{CCW}$  and  $P_{CM}$  ( $P < 0.05$ ). The CC/AA diplotype was advantageous for BW, RCW, CCW and  $P_{CM}$  and the TT/AA diplotype was advantageous for  $P_{RCW}$  and  $P_{CCW}$ , whereas the TT/TT diplotype was negative for BW, RCW, CCW,  $P_{RCW}$  and  $P_{CCW}$ , and the TT/AA diplotype was negative for  $P_{CM}$ .

### Discussion

The identification of genetic variations in *MSTN* is of great interest to animal breeding, as a better understanding of the effects of *MSTN* on growth and carcass traits offers a potential genetic marker to improve meat production (Joullia-Ekaza *et al.*, 2007). In the present study, two SNP loci were first identified. At position c 194, there was a C to T transversion in exon 1 and a T to A transversion at position c 445 in exon 2. The two mutations were synonymous. Previous studies had identified a T to A transversion in exon 2 of rabbit *MSTN* gene (Fontanesi *et al.*, 2011; Abdel-Kafy *et al.*, 2016), and Li *et al.* (2002) identified a G to T transversion in exon 2. Moreover, an A to G

Table 2: Frequencies of *myostatin* alleles and genotypes in six rabbit lines.

Locus	Line	n	Genotypes and allele frequency					Pr
			CC	CT	TT	C	T	
Exon 1	Harbin	30	0.63	0.27	0.10	0.767	0.233	0.969
	Hotot	21	0.62	0.38	0.00	0.810	0.191	0.973
	Tianfu	48	0.60	0.31	0.08	0.761	0.240	0.991
	Belgian	32	0.66	0.28	0.06	0.797	0.203	0.992
	Zika	63	0.81	0.14	0.05	0.881	0.119	0.943*
	California	36	0.47	0.39	0.14	0.667	0.333	0.992
Exon 2	Line	n	TT	TA	AA	T	A	
	Harbin	30	0.37	0.50	0.13	0.617	0.383	0.998
	Hotot	21	0.29	0.62	0.10	0.595	0.405	0.963
	Tianfu	48	0.27	0.40	0.33	0.469	0.531	0.981
	Belgian	32	0.22	0.53	0.25	0.484	0.516	0.998
	Zika	63	0.17	0.54	0.29	0.444	0.556	0.995
	California	36	0.22	0.58	0.19	0.514	0.486	0.986

n: number of rabbits per breed; Pr is the probability of  $\chi^2$ -test for the Hardy-Weinberg equilibrium. \*Mean the frequency of alleles and genotypes deviate from the H-W equilibrium (HWE).

**Table 3:** GLM analysis of associations between rabbit carcass traits and *myostatin* gene SNPs.

Traits	Exon 1			Exon 2		
	CC (149)	CT (63)	TT (18)	TT (57)	TA (117)	AA (56)
BW (kg)	2.480±0.024 <sup>BP</sup>	2.378±0.037 <sup>a</sup>	2.316±0.070 <sup>aN</sup>	2.424±0.040	2.419±0.028	2.498±0.040
CCW (kg)	1.171±0.015 <sup>BP</sup>	1.096±0.023 <sup>a</sup>	1.049±0.043 <sup>aN</sup>	1.114±0.024 <sup>aN</sup>	1.130±0.017 <sup>a</sup>	1.191±0.025 <sup>BP</sup>
RCW(kg)	1.128±0.038 <sup>BP</sup>	1.051±0.029 <sup>a</sup>	0.992±0.026 <sup>aN</sup>	1.065±0.036	1.085±0.032	1.149±0.035
P <sub>RCW</sub> (%)	47.5±0.4 <sup>BP</sup>	46.4±0.5 <sup>ab</sup>	45.2±1.0 <sup>aN</sup>	46.1±0.6 <sup>aN</sup>	46.9±0.4 <sup>ab</sup>	48.0±0.6 <sup>BP</sup>
P <sub>CCW</sub> (%)	51.7±0.4 <sup>BP</sup>	50.3±0.6 <sup>ab</sup>	49.1±1.1 <sup>aN</sup>	50.1±0.6	51.2±0.5	51.8±0.7
P <sub>CM</sub> (%)	62.4±0.007	63.0±1.0	59.5±2.0	59.5±1.1 <sup>aN</sup>	62.9±0.7 <sup>b</sup>	63.4±1.1 <sup>BP</sup>

BW: live body weight at 135 d; CCW: cold carcass weight; RCW: reference carcass weight; P<sub>CCW</sub>=(CCW/BW)×100%; P<sub>RCW</sub>=(RCW/BW)×100%; P<sub>CM</sub>: cooked meat percentage. The numbers in the brackets indicate the number of rabbits corresponding to each genotype. <sup>a,b</sup>The least square means with different superscripts indicates significant differences ( $P<0.05$ ) among different genotypes within exon 1 or 2. P superscript represents the positive genotypes in exon 1 and 2, and N superscript represent the negative genotypes in exon 1 and 2.

mutation in exon 3 of porcine *MSTN* (Li *et al.*, 2002) and a conversion of G to A at position 84 in exon 1 of *MSTN* in German Merino sheep were examined by Zhang *et al.* (2007). These variations in *MSTN* gene from rabbit and other animals were all synonymous mutations. These studies suggest that *MSTN* has multiple SNP sites which often result in synonymous mutations. Synonymous single base mutation could not change the amino acid sequence of *MSTN*, but affected the modification or the folding of mRNA and thus resulted in altered biological functions (Simon *et al.*, 2002). The findings are further supported by our present study, which demonstrated that c.445T>A SNP resulted in a significant change in RNA secondary structure of exon 2. Moreover, the mutation caused the minimum free energy of RNA secondary structure to change from -75.2 kcal/mol to -72.8 kcal/mol, which affected the secondary structure stability of RNA and might affect the subsequent protein translation process. However, the c.194C>T SNP did not change the RNA secondary structure of exon 1, although the minimum free energy changed from -92.8 kcal/mol to -93.6 kcal/mol (Figure 2). Therefore, synonymous *MSTN* mutations may affect the biological phenotype, especially in terms of carcass traits.

Two SNPs of synonymous mutations in exon 1 of *MSTN* significantly impacted carcass weight, abdominal fat weight and breast muscle weight of chickens (Zhu *et al.*, 2007), and an exon 1 SNP in *MSTN* of Steppe red cattle and hybrid cattle (Limousin cattle×grassland red bull) had significant associations with the daily gain and dressing percentages (Guo *et al.*, 2007). An SNP (c.476T>A) in the 5' regulatory region of rabbit *MSTN* resulted in increased liver weight,

**Table 4:** Association between *myostatin* diplotype and growth and carcass traits.

Diplotype	Fr. (%)	Growth and carcass traits					
		BW (kg)**	RCW(kg)**	CCW (kg)**	P <sub>RCW</sub> (%)**	P <sub>CCW</sub> (%)*	P <sub>CM</sub> (%)*
CC/TT	15.65	2.463±0.050	1.099±0.028	1.126±0.030	46.0±0.7	50.0±0.8	59.7±1.4
CC/TA	17.39	2.472±0.035	1.093±0.026	1.172±0.021	47.7±0.5	52.2±0.6	62.9±1.0
CC/AA	31.74	2.511±0.047 <sup>P</sup>	1.142±0.012 <sup>P</sup>	1.208±0.029 <sup>P</sup>	48.4±0.7	52.2±0.8	63.6±1.3 <sup>P</sup>
CT/TT	6.09	2.413±0.079	1.072±0.034	1.141±0.048	47.5±1.1	51.4±1.3	59.1±2.2
CT/TA	15.22	2.327±0.050	1.003±0.013	1.061±0.031	45.8±0.7	49.8±0.8	63.6±1.4
CT/AA	6.09	2.470±0.079	1.081±0.040	1.140±0.048	46.6±1.1	50.4±1.3	63.9±2.2
TT/TT	3.04	2.246±0.112 <sup>N</sup>	0.892±0.026 <sup>N</sup>	0.994±0.068 <sup>N</sup>	43.8±1.6 <sup>N</sup>	48.1±1.8 <sup>N</sup>	59.2±3.1
TT/TA	3.91	2.344±0.099	1.031±0.027	1.056±0.060	45.2±1.4	48.9±1.6	60.7±2.7
TT/AA	0.87	2.438±0.210	1.068±0.010	1.208±0.128	49.7±3.0 <sup>P</sup>	53.4±3.4 <sup>P</sup>	55.1±5.8 <sup>N</sup>

BW: live body weight at 135 d; CCW: cold carcass weight; RCW: reference carcass weight; P<sub>CCW</sub>=(CCW/BW)×100%; P<sub>RCW</sub>=(RCW/BW)×100%; P<sub>CM</sub>: cooked meat percentage. P superscript represent the advantageous diplotypes, and N superscript represent the negative diplotypes. All values are presented as the least squares mean±standard deviation. \* $P<0.05$ , \*\* $P<0.01$ .

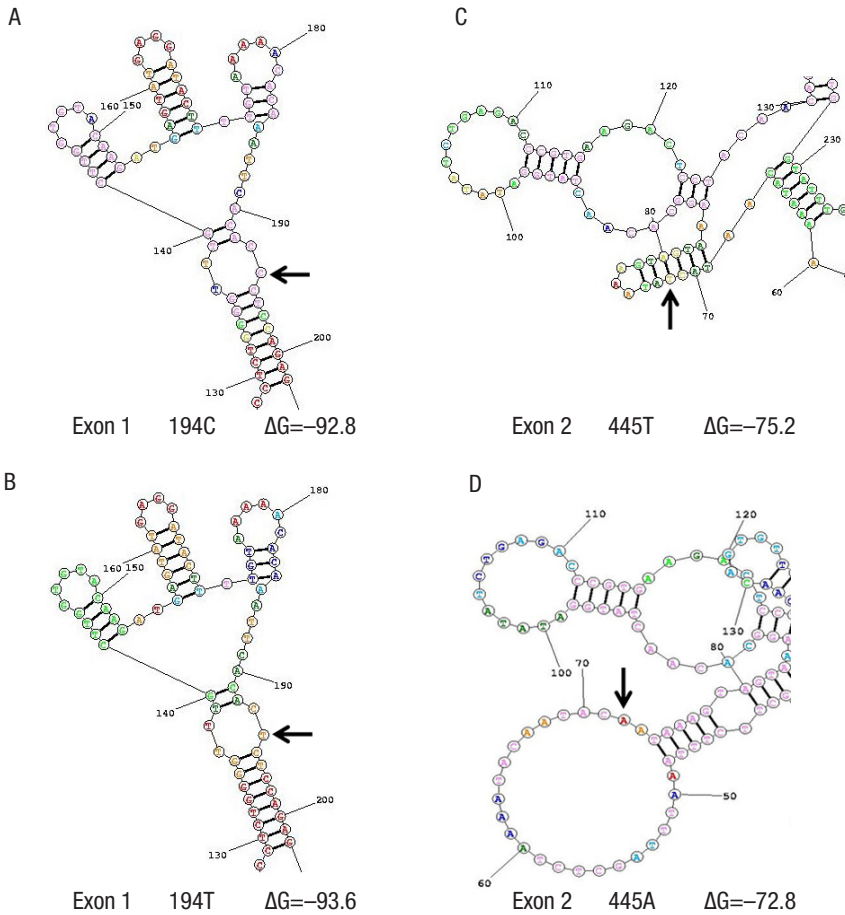


Figure 2: The effect of c.194C>T and c.445T>A mutations on RNA secondary structure of exon 1 and exon 2 of *myostatin* (*MSTN*). A, B: The secondary structure of the *MSTN* exon 1 of different alleles; C, D: The secondary structure of the *MSTN* exon 2 of different alleles. Arrows: Mutant site.

carcass weight, forelegs weight, back and waist weight, ham weight and tare weight (Qiao *et al.*, 2014), and Abdel-Kafy *et al.* (2016) also found that rabbit with the *MSTN* genotype (194GG) correlated with the highest values in BW and daily weight gain, and allele T at the c.747+34c>T SNP in intron 2 was significantly associated with increased BW, whereas no significant effects were found for c.-125T>C and c.747+34C>T in the coding region. In the present study, we also confirmed that the new SNPs in exon 1 and exon 2 of *MSTN* have significant associations with rabbit carcass traits.

The total effect of two variable sites on carcass traits was analysed by haplotype analysis. The results showed that individuals with the CC/AA or TT/AA diplotypes had higher BW, RCW and CCW or  $P_{RCW}$  and  $P_{CCW}$  than the other seven diplotypes. The frequency of the CC/AA diplotypes was highest among all diplotypes, and it was the advantageous diplotypes for BW, RCW and CCW, suggesting that BW, RCW and CCW may be preferential factors in the genetic selection of rabbits. Therefore, the SNPs in exon 1 or exon 2 of *MSTN* may be used as a potential site for BW, RCW and CCW selection. The absence of HWE deviation and lower than 50% frequency of advantageous diplotypes (CC/AA: 31.74%, TT/AA: 0.87%) suggest that genetic selection of *MSTN* still has great potential for improving the BW, RCW and CCW or  $P_{RCW}$  and  $P_{CCW}$ .



In conclusion, we found two novel variations in exon 1 (c.194C>T) and exon 2 (c.445T>A) of the *MSTN* gene and they significantly affect rabbit carcass traits. The advantageous diplotypes might be used in genetic selection for improving BW, RCW and CCW or  $P_{RCW}$  and  $P_{CCW}$ . Further population-wide studies are required to test the association of the two SNPs with carcass traits, and the stable verification of the SNPs and advantageous diplotypes in different generations may also be necessary.

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## REFERENCES

- Abdel-Kafy E.M., Darwish S.F., Elkhishin D. 2016. Correlating single nucleotide polymorphisms in the myostatin gene with performance traits in rabbit. *World Rabbit Sci.*, 24: 213-221. <https://doi.org/10.4995/wrs.2016.4026>
- Bellinge R.H.S., Liberles D.A., Iashi S.P.A., O'Brien P.A., Tay G.K. 2004. Myostatin and its implications on animal breeding: a review. *Anim. Gen.*, 36: 1-6. <https://doi.org/10.1111/j.1365-2052.2004.01229.x>
- El-Sabrouh K., Aggag S.A. 2017. Associations between single nucleotide polymorphisms in multiple candidate genes and body weight in rabbits. *Vet. World*, 10: 136-139. <https://doi.org/10.14202/vetworld.2017.136-139>
- Fontanesi L., Scotti E., Frabetti A., Fornasini D., Picconi A., Russo V. 2011. Identification of polymorphisms in the rabbit (*Oryctolagus cuniculus*) myostatin (*MSTN*) gene and association analysis with finishing weight in a commercial rabbit population. *Anim. Gen.*, 42: 339. <https://doi.org/10.1111/j.1365-2052.2010.02163.x>
- Fontanesi L., Tazzoli M., Scotti E., Russo V. 2008. Analysis of candidate genes for meat production traits in domestic rabbit breeds. *9th World Rabbit Congress, Verona, Italy, 10-13 June 2008*, 32: 73-87.
- George K.S., Michael G., Isosif B., George P., Olga P., Zafeiris A., Stellos G.D. 2015. Stella E.C. Effects of gestational maternal undernutrition on growth carcass composition and meat quality of rabbit offspring. *PLOS one*, 10: 1-11. <https://doi.org/10.1371/journal.pone.0118259>
- Grobet L., Poncelet D., Royo Martin L.J., Brouwers B., Pirottin D., Michaux C., Ménéssier F., Zanotti M., Dunner S., Georges M. 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double muscling in cattle. *Mamm. Genome*, 9: 210-213. <https://doi.org/10.1007/s003359900727>
- Grobet L., Royo Martin L.J., Poncelet D., Pirottin D., Brouwers B., Riquet J., Scheberlein A., Dunner S., Ménéssier F., Massabanda J., Fries R., Hanset R., Georges M. 1997. A deletion in the bovine myostatin gene causes the double-muscling phenotype in cattle. *Nat. Genet.*, 17: 71-74. <https://doi.org/10.1038/ng0997-71>
- Guo D., Cao Y., Zhang J.B., Han D., Zhang X.B., Jin H.G. 2007. Analysis of SNPs polymorphism in the exon 1 of *MSTN* gene in Grassland Red cattle. *Modern Journal of Animal Husbandry and Veterinary Medicine*, 11: 12-13 (In Chinese).
- Guo R., Wan Y., Xu D., Cui L., Deng M., Zhang G., Jia R., Zhou W., Wang Z., Deng K., Huang M., Wang F., Zhang Y. 2016. Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. *Sci. Rep-UK*, 6: 1-10. <https://doi.org/10.1038/srep29855>
- Jiang Y.L., Li N., Wu C.X., Li L.X. 2008. Analysis on nucleotide polymorphisms of porcine myostatin gene in different breeds. *J. Genet. Genomics*, 28: 840-845 (In Chinese).
- Jouliá-Ekaza D., Cabello G. 2007. The myostatin gene: physiology and pharmacological relevance. *Curr. Op. Pharmacol.*, 7: 310-315. <https://doi.org/10.1016/j.coph.2006.11.011>
- Jouliá D., Bernardi H., Garandel V., Rabenoelina F., Vernus B., Cabello G. 2003. Mechanisms involved in the inhibition of myoblast proliferation and differentiation by myostatin. *Exp. Cell Res*, 286: 263-275. [https://doi.org/10.1016/S0014-4827\(03\)00074-0](https://doi.org/10.1016/S0014-4827(03)00074-0)
- Jurečková J., Píček J. 2007. Shapiro-Wilk-type test of normality under nuisance regression and scale. *Computational Statistics & Data Analysis*, 51(10): 5184-5191. <https://doi.org/10.1016/j.csda.2006.08.026>
- Kambadur R., Sharma M., Smith T.P.L., Bass J.J. 1997. Mutation in myostatin (GDF8) in double-muscling Belgian blue and Piedmontese cattle. *Gen. Res.*, 7: 910-915. <https://doi.org/10.1101/gr.7.9.910>
- Lin J., Arnold H.B., Della-Fera M.A., Azain M.J., Hartzell D.L., Baile CA. 2002. Myostatin knock-out in mice increases myogenesis and decreases adipogenesis. *Biochem. Biophys. Res. Co.*, 292: 701-706. <https://doi.org/10.1006/bbrc.2002.6500>
- Li S.H., Xiong Y.Z., Zheng R., Li A.Y., Deng C.Y., Jiang S.W., Lei M.G., Weng Y.Q., Cao G.C. 2002. Polymorphism of porcine myostatin gene. *J. Genet. Genomics*, 29: 326-331 (In Chinese).
- Liu Z.Z., Li X.L., Gong Y.F., Jin X.M., Feng M.S. 2006. Relationship between polymorphism of goat *MSTN* gene intron 2 and body weight. *Acta Vet. Zootech. Sinica*, 37: 745-748.
- Lu J.Q., Huang W., Zhao N., Wang W.W., Hou S.S. 2008. Analysis on gene polymorphism of myostatin and carcass traits of Peking duck. *China Animal Husbandry & Veterinary Medicine* 35: 61-63 (In Chinese).
- Marchitelli C., Savarese M.C., Crisa A., Nardone A., Ajmone-Marsan P., Valentini A. 2003. Double muscling in marchigiana beef breed is caused by a stop codon in the third exon of myostatin gene. *Mamm. Genome*, 14: 392-395. <https://doi.org/10.1007/s00335-002-2176-5>
- McCroskery S., Thomas M., Maxwell L., Sharma M., Kambadur R. 2003. Myostatin negatively regulates satellite cell activation and self-renewal. *J. Cell Biol.*, 162: 1135-1147. <https://doi.org/10.1083/jcb.200207056>
- McPherron A.C., Lawler A.M., Lee S.J. 1997. Regulation of skeletal muscle mass in mice by a new TGF- $\beta$  superfamily member. *Nature*, 387: 83-90. <https://doi.org/10.1038/387083a0>

- McPherron A.C., Lee S.J. 1997. Double muscling in cattle due to mutations in myostatin gene. *In Proc.: National Academy of Sciences*, 94: 12457-12461. <https://doi.org/10.1073/pnas.94.23.12457>
- McPherron A.C., Lee S.J. 2002. Suppression of body fat accumulation in myostatin-deficient mice. *J. Clin. Invest.*, 109: 595-601. <https://doi.org/10.1172/JCI200213562>
- Mosher D.S., Quignon P., Bustamante C.D., Sutter N.B., Mellersh C.S., Parker H.G., Ostrander E.A. 2007. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genetics*, 3: e79. <https://doi.org/10.1371/journal.pgen.0030079>
- Naveen Z., Naik B.R., Subramanyam B.V., Reddy P.M. 2016. Studies on the quality of duck meat sausages during refrigeration. *Springer Plus*, 5: 2061. <https://doi.org/10.1186/s40064-016-3743-7>
- Qiao X.B., Xu K.Y., Li B., Luan X., Xia T., Fan X.Z. 2014. Rabbit *MSTN* gene polymorphisms and genetic effect analysis. *Genet. Mol. Res.*, 13: 2590-2597. <https://doi.org/10.4238/2014.April.8.1>
- Rios R., Carneiro I., Arce V.M., Devesa J. 2002. Myostatin is an inhibitor of myogenic differentiation. *Am. J. Physiol. Cell Physiol.*, 282: 993-999. <https://doi.org/10.1152/ajpcell.00372.2001>
- Shi B., Wen H.S., He F., Dong S.L., Ma S., Chen C.F., Wang L.S., Yao J., Mu X.J., Zhou Y.G. 2009. Association of reproductive performance with SNPs of *FOX L2* gene by SSCP in Japanese flounder (*Paralichthys olivaceus*). *Comp. Biochem. Physiol.*, 153: 1-7. <https://doi.org/10.1016/j.cbpb.2008.10.007>
- Simon D., Palatnik M., Roisenberg I. 2002. Analysis of the 1185A/G von Willebrand factor (VWF) gene polymorphism in two Brazilian ethnic groups and its effect on the plasma VWF levels. *Thromb. Res.*, 105: 519-522. [https://doi.org/10.1016/S0049-3848\(02\)00060-9](https://doi.org/10.1016/S0049-3848(02)00060-9)
- Sternstein I., Reissmann M., Maj D., Bieniek J., Brockmann G.A. 2014. A new single nucleotide polymorphism in the rabbit (*Oryctolagus cuniculus*) myostatin (*MSTN*) gene is associated with carcass composition traits. *Anim. Genet.*, 45: 596-599. <https://doi.org/10.1111/age.12165>
- Zhang G.W., Wang H.Z., Chen S.Y., Li Z.C., Zhang W.X., Lai S.J. 2011. A reduced incidence of digestive disorders in rabbits is associated with allelic diversity at the TLR4 locus. *Vet. Immunol. Immunop.*, 144: 482-486. <https://doi.org/10.1016/j.vetimm.2011.08.009>
- Zhang H.L., Shi H.C., Luo S.P., Niu Z.G., Tang S., Wu J., Liu M.J. 2007. Analysis on mononucleotide polymorphism of exon I of myostatin gene in sheep. *J. Xinjiang Agricultural University*, 30, 21-24 (In Chinese).
- Zhu Z., Wu D.J., Xu N.Y. 2007. SNPs of myostatin gene and its genetic effects on carcass traits in chicken. *Hereditas*, 29: 593-598 (In Chinese). <https://doi.org/10.1360/jc-007-0593>