Journal of Animal and Veterinary Advances 8 (8): 1609-1615, 2009
ISSN: 1680-5593

Detection of DNA Polymorphisms in the MSTN Gene and the Effect of F94L on Meat Quantity and Quality Traits in Japanese Black x Limousin F2 Population

[1]Tsuyoshi Abe, [1]Hiroyuki Hasebe, [1]Toshiaki Okumura, [2]Keigo Kuchida,
[1]Tadashi Kawamura and [1]Eiji Kobayashi
[1]National Livestock Breeding Center, Fukushima, Japan
[2]Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan

Abstract: In this study, we searched for the mutations in Myostatin (MSTN) gene among the parental generation of our F2 population, consisted of Japanese Black and Limousin breeds, as the positional candidate gene of the QTL for several beef quality and quantity traits detected at the centromeric end of BTA2. Twenty single nucleotide polymorphisms were detected in MSTN gene and the one of them was nonsynonymous mutation, which cause amino acid substitution of phenylalanine to leucine at position 94 (F94L). The significant genotypic effects of F94L mutation for 5 carcass grading traits (carcass grade, *M. longissimus* area, BMS, luster and firmness), 3 meat quality traits (crude fat, crude protein and moisture of the *M. longissimus*) and 8 computer image analysis traits (*M. longissimus* area, *M. longissimus* lean area, Ratio of Fat Area (RFA) to *M. longissimus* area, RFA to *M. semispinalis* capitis area, *M. semispinalis* lean area, RFA to *M. semispinalis* area, *M. trapezius* lean area and RFA to *M. trapezius* area) were determined. Seventy five Japanese Black and 147 Holstein bull samples were then genotyped for assessing the frequency of F94L in the commercial population in Japan, but there observed no variation and fixed to phenylalanine homozygote in both 2 breeds. It was strongly suggested that F94L mutation of MSTN gene have an effect for beef quality and quantity traits, however, it seems almost impossible to apply the F94L information to the current breeding system of the Japanese Black breed.

Key words: Myostatin, MSTN, F94L, beef, Japanese Black, QTL

INTRODUCTION

The Quantitative Trait Loci (QTL) effects on 18 meat quality and quantity traits have been detected in the same centromeric region of BTA2 in our F2 population from a cross between Japanese Black sires and Limousin dams (Abe *et al*., 2008). Among the 18 traits, 6 were related to muscle mass and 8 were related to fat quality or fat accumulation. F2 animals that inherited the Limousin allele had greater muscle area and conversely, animals that inherited the Japanese Black allele gained higher values in fat-related traits. The highest F-value was observed in the lean area of *M. longissimus* derived from Computer Image Analysis (CIA) and a QTL effect for this lean area trait was also detected in other muscle parts (*M. semispinalis* and *M. trapezius*). Hence, this QTL seemed to play a significant role in muscle mass.

There are several reports on the detection of beef quality or quantity traits in the centromeric region of BTA2 (Casas *et al*., 1998, 2001; Alexander *et al*., 2007; Sellick *et al*., 2007). This region is also known as the muscular hypertrophy locus, where the Myostatin (MSTN) gene was isolated as the causative gene of the double-muscling phenotype in cattle (Grobet *et al*., 1997; Kambadur *et al*., 1997; McPherron and Lee, 1997b). MSTN is a Transforming Growth Factor-β (TGF-β) family member that plays an essential role in regulating muscle growth (McPherron *et al*., 1997a). Several distinct mutations have been identified that explain the increased muscling in cattle (Grobet *et al*., 1998; Dunner *et al*., 2003). This study fosuc on the detection of DNA polymorphisms in the MSTN gene of the F2 parental generation. A detected mutation was then genotyped in the F2 population and its relationship to the observed traits was assessed. Sire bulls of Japanese Black and Holstein breeds were also genotyped to estimate its genotypic frequency in the commercial population in Japan.

Corresponding Author: Tsuyoshi Abe, Department of Technology, National Livestock Breeding Center, 1-Odakurahara, Nishigo-Mura, Fukushima, 961-8511, Japan

MATERIALS AND METHODS

Animals and phenotypes: The F2 population used in this study is described by Abe *et al*. (2008). Five carcass grade traits (carcass grade, *M. longissimus* area, BMS, luster and firmness), 5 meat quality traits by physiochemical analysis (crude fat, crude protein, moisture and C16:1 and C18:2 contents in intramuscular fat of *M. longissimus*) and 8 Computer Image Analysis (CIA) traits (*M. longissimus* area, *M. longissimus* lean area, Ratio of Fat Area (RFA) to *M. longissimus* area, RFA to *M. semispinalis* capitis area, *M. semispinalis* lean area, RFA to *M. semispinalis* area, *M. trapezius* lean area and RFA to *M. trapezius* area) of 186 F2 individuals

Table 1: Oligonucleotide primers used in this study

Ologionucleotide	Sequence (5'-3')	Annealing temperature	Nucleotide position
Mseq 01	AGAAAAATGGGCACCCTTCA	60	Promoter
	TTCCCTGAATAAATTCTTAACACA	60	Promoter
Mseq 02	TTATTTAAAAGTAATTCCATGAGCAA	60	Promoter
	GCTGTATGTGACATGCGAAAA	60	Promoter
Mseq 03	TTTCTGAGGGAAAAGCATATCAA	60	Promoter
	ACAACTTGCCACACCAGTGA	60	5' Untranslated
Mseq 04	TGGAATATAAAAAGCCACTTGGA	60	Promoter
	AGCCCCTCTTTTTCCACATT	60	Exon1
Mseq 05	GATTCACTGGTGTGGCAAGTT	60	5' Untranslated
	CAGCAGTCAGCAGAGTCGTT	60	Intron1
Mseq 06	CTCCTCCACTCCTGGAACTG	60	Exon1
	GCTCTTTCCCCTCCTCCTTA	60	Intron1
Mseq 07	GCTCCTTGGAAGACGATGAC	60	Intron1
	GTTCAGCAAACGCCCAAATA	60	Intron1
Mseq 08	CCAATGAGACTGAAAGCTGCTA	60	Intron1
	TGCTGTGTTTACTTCCTTATTGC	60	Intron1
Mseq 09	TTTGGTTGCCTGAAATATGC	60	Intron1
	CCTGTCTCCCTTTCCTTGAG	60	Intron1
Mseq 10	AAGTTCCACTTATACCCTGACCA	60	Intron1
	GGAGGCACATGCTTAATAACCT	60	Intron1
Mseq 11	AAAGCAAGATCGCAGACACA	60	Intron1
	ATGTTAGGACGCTGGGACAG	60	Intron1
Mseq 12	AGCCTGGCCCTAAAGACAAT	60	Intron1
	TTTTTAATTGGAATAATGTGTGCAT	60	Intron1
Mseq 13	GGCTGCTCATAACAGCTGAA	60	Intron1
	TCTGGGAAGGTTACAGCAAGA	60	Exon2
Mseq 14	AACTTGACATGAACCCAGGC	60	Exon2
	TCACTCTTCTGGGGGAAAAA	60	Intron2
Mseq 15	GGGAATTCTTTGGAATTGAGAA	60	Intron2
	GCCAGAAGAGTGAGTAGCTCTAAA	60	Intron2
Mseq 16	TTTTCTGAACTGTCTTACTGTTCTTT	60	Intron2
	CCTCTGTCACGTCACCAACTT	60	Intron2
Mseq 17	TTCCAGGTGATCAATTTTTCTTT	60	Intron2
	CAATTTGCTAATCCTCCTCCTG	60	Intron2
Mseq 18	TGTTATTGACTCCCAAAATGATG	60	Intron2
	TCATCCTCCATTCACTTGGTC	60	Intron2
Mseq 19	GGGAATATCATTGTATCTTCTTCTGA	60	Intron2
	TGCCTGACACATAATAGATGCTG	60	Intron2
Mseq 20	TGGCAACTATTGTGTTTCGG	60	Intron2
	CTTTTTGGTGTGTCTGTTACCTTG	60	Exon3
Mseq 21	AAGAAATGTGACATAAGCAAAATGA	60	Intron2
	TCAAAATTGTTGAGGGGAAGA	60	Exon3
Mseq 22	TATACGGGAAGATTCCAGCC	60	3' UTR[a]
	CGTTGTGCCATCCCTATTTT	60	3' UTR
Mseq 23	ATTTGAAACATCCTTAAACACTTGAA	60	3' UTR
	ATCCATTGCCCATAGGGAG	60	3' UTR
Mseq 24	TAGGCTGAATGGCTGATGTT	60	3' UTR
	ATATTGATAGAATCAGTCATTTCCATT	60	3' UTR
Mseq 25	TGCAATACTGCAGCTTTTAGG	60	3' UTR
	TCATGAATCCATAAGTGAATGCT	60	3' UTR
Mseq 26	TCCATATGCTAATGGTTAGATGG	60	3' UTR
	AATAGAGTTAAATCATTTTGGTATGCT	60	3' UTR
Mseq 27	TGGCTGTATAATGTGAATGTGAAA	60	3' UTR
	AAGCAACTTGACCAGAACCAA	60	3' UTR
F94L_TaqI	GCATGTTTGTGGAGGGAAAA	60	Exon1
	TGCACCAGCAGGACTACTCA	60	Intron1

[a]UTR: Untranslated Region

were measured to assess their relationship with the detected mutation in the MSTN gene. All these traits had a detected QTL in the centromeric region of BTA2. The details of the measurement methodologies and values are also described by Abe *et al.* (2008).

DNA extraction: To detect mutations in the MSTN gene, the genomic DNA of the parental generation (2 Japanese Black sires (JB-A and JB-B) and 2 Limousin dams (L-A and L-B)) of the F2 family were used. In addition, 1 Japanese Black and 1 Holstein bull samples (JB-50 and Hol-1) were used to detect mutations. This Japanese Black bull (JB-50) did not have a strong blood relationship with JB-A or JB-B. Genomic DNA was extracted from frozen semen from the 2 Japanese Black sires and from the whole blood of the 2 Limousin dams. Genomic DNA of JB-50 and Hol-1 was also extracted from frozen semen.

The DNAs of F2 individuals were extracted from whole blood samples. Seventy five Japanese Black and 147 Holstein bull samples were genotyped for the F94L polymorphism in the MSTN gene, for assessing the genotypic frequency. DNA was extracted from all the frozen semen stored in our stations.

Detection of DNA polymorphisms in the MSTN gene: To design primers for the detection of DNA polymorphisms in the MSTN gene, 3 nucleotide sequences deposited in the GenBank database (AJ438578 for the promoter region of MSTN, AB076403 for the MSTN gene and the partial sequence of AAFC03052712.1 (nt20353-nt20681) for the 3' untranslated region of MSTN) were used. The nucleotide position numbers in this report are relative to the nucleotide sequence of AB076403. The primers designed are shwon in Table 1. Initially, Polymerase Chain Reaction (PCR) amplification with genomic DNAs of 4 parents and 2 bulls (Japanese Black and Holstein) was performed using the primers listed in Table 1 (Mseq 01-27), in a 15 μL reaction mixture containing 20 ng of cDNA, 1.67 mM $MgCl_2$, 6.25 pmol of each primer, 0.2 mM deoxynucleotides (dNTPs) and 0.375 U Taq DNA polymerase (ABgene, Epsom, UK). Amplifications were carried out under the following conditions: 5 min at 94°C; 30 cycles of 30 sec at 94°C, 30 sec at the annealing temperature and 30 sec at 72°C and a final extension of 7 min at 72°C. The annealing temperature was 60°C for all primers. After amplification, DNA cycle sequencing was performed with approximately 20 ng amplified product by using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster city, CA) in an ABI PRISM® 3130× L genetic analyzer, using both forward and reverse primers.

Genotyping of F94L mutation by PCR-restriction fragment length polymorphism: In order to perform PCR-Restriction Fragment Length Polymorphism (RFLP) detection, a primer pair to amplify the fragment containing the MSTN mutations g. 415 C>A was designed (Table 1, F94L_TaqI). The PCR conditions were the same as those described earlier. The amplified 274 bp fragment was subsequently digested with TaqI to detect the g 415 C>A genotype. The restriction enzyme digestions were performed using 5 μL of PCR product mixed with 5 units of the appropriate restriction enzyme, followed by incubation at 65°C for 4 h. All the animals used in this study were genotyped using this method.

Statistical analysis: The effects of the F94L genotype on traits observed in F2 were assessed for significance using a one way analysis of variance with the FASN genotype as a treatment following the SAS GLM procedure (SAS Inst., Inc. Cary, NC). To test the pairwise differences between the effects of genotype, Tukey's studentized range (HSD) test was also carried out.

RESULTS AND DISCUSSION

Twenty Single Nucleotide Polymorphisms (SNPs) were identified in the 6 investigated individuals (Table 2). Among these mutations, 2 SNPs were located in exon 1 (g 400 A>G and g 415 C>A). The SNP g 415 C>A was reflected in the codon at position 94 of the MSTN protein, TTC and TTA, which code for phenylalanine and leucine, respectively. This nonsynonymous mutation clearly differed between the 2 parental breeds of F2 and was C/C in Japanese Black and A/A in Limousin. The genotypes of F94L in JB-50 and Hol-1 were identical to JB-A and JB-B.

Next, F2 individuals were genotyped for the polymorphism of F94L in the MSTN gene by PCR-RFLP (Fig. 1) and the genotypic effects on 5 carcass grade, 5 meat quality and 8 computer image analysis traits were examined (Table 3-5). Significant effects were determined in all assessed traits, except for the C16:1 and C18:2 percentage of intramuscular fat, involved in 5 meat quality grade traits (Table 4). Overall, individuals that inherited the A allele at F94L showed higher values in muscle-related traits (crude protein (%), lean area of each of *M. longissimus*, *M. semispinalis* and *M. trapezius* (cm^2)). Especially, the mean value of the *M. longissimus* lean area differed by almost 10 cm^2 between the two kinds of individuals homozygous in F94L. This trait marked the highest F-value in QTL analysis and the alleles that originated from the Limousin parents had an enormous effect on muscle mass (Abe *et al.*, 2008). The observed effect of the A allele at F94L was the same as the QTL

Table 2: Detection of MSTN mutations in the 6 investigated individuals

Nucleotide position[a]	Region	P-generation of F2				Bull		Codon	Amino acid
		JB-A	JB-B	L-A	L-B	JB-50	Hol-1		
-338	Promoter	A/A	A/A	A/G	A/A	A/A	A/A		
400	Exon1	A/G	A/A	A/A	A/A	A/A	A/A	GA**A** to GA**G**	synonymous
415	Exon1	C/C	C/C	A/A	A/A	C/C	C/C	TT**C** to TT**A**	Phe to Leu
899	Intron1	A/T	A/A	A/A	A/A	A/A	A/A		
977	Intron1	T/T	C/T	C/C	C/C	C/C	C/C		
983	Intron1	G/G	G/G	A/A	A/A	G/G	A/A		
1044	Intron1	T/T	G/T	G/G	G/G	G/G	G/G		
1154	Intron1	A/A	G/A	G/G	G/G	G/G	G/G		
1309	Intron1	T/T	T/G	T/T	T/T	G/G	T/G		
1401	Intron1	G/G	G/G	G/G	G/G	G/G	G/C		
2290	Intron1	T/C	T/T	T/T	T/T	T/T	T/C		
4556	Intron2	C/T	C/C	C/C	C/C	C/C	C/C		
5283	3' UTR[b]	C/C	A/A	A/A	A/A	A/A	A/A		
5291	3' UTR	T/A	T/T	T/T	T/T	T/T	T/T		
5321	3' UTR	C/A	A/A	A/A	A/A	A/A	A/A		
5333	3' UTR	C/A	C/C	C/C	C/C	C/C	C/C		
5395	3' UTR	T/C	C/C	C/C	C/C	C/C	C/C		
6173	3' UTR	C/A	C/C	C/C	C/C	C/C	C/C		
6174	3' UTR	T/T	T/T	T/T	T/T	T/T	T/C		
6227	3' UTR	C/C	C/C	C/C	C/C	C/A	C/C		

[a]Numbers of nucleotide position were based upon AB076403 of GenBank database. [b]UTR: Untranslated Region

Table 3: Carcass grade traits of MSTN genotype F94L mutation

F94L	n	Carcass grade (1-5)	*M. longissimus* area (cm^2)	BMS (1-12)	Luster (1-5)	Firmness (1-5)
C/C	36	2.68±0.10^c	48.66±0.82^c	3.44±0.15^c	3.07±0.06^b	2.78±0.10^c
C/A	78	2.43±0.06^b	51.46±0.54^d	3.09±0.10^c	2.94±0.04^b	2.51±0.06^b
A/A	36	2.09±0.04^a	57.43±1.00^e	2.32±0.08^a	2.57±0.07^a	2.15±0.05^a

Mean±SE: Mean values with different superscript letters in the same row differ significantly; $^{a,b,c,d,e}p<0.001$; $^{d,c}p<0.01$; $^{b,c}p<0.05$; $^{c,c}p<0.1$

analysis results. On the other hand, individuals that inherited the C allele at F94L showed higher values in fat-related traits (BMS, crude fat (%), RFA to *M. longissimus*, *M. semispinalis* capitis, *M semispinalis* and *M. trapezius* (%)) and its allelic effects were also the same as the QTL analysis results described in previous report (Abe *et al*., 2008).

Dunner *et al*. (2003) had already detected this mutation in several European cattle breeds, including the Limousin breed. All Limousin individuals in their study had the same non-disruptive MSTN haplotype, but their body conformational phenotypes, measured by their own criterion, were comparatively similar to those of individuals with the disruptive MSTN haplotype, which show the double-muscling phenotype. On the other hand, Sellick *et al*. (2007) reported the effect of MSTN F94L on carcass yield in their experimental back-cross population between Jersey and Limousin breeds. Like our study, they also detected QTL for similar traits such as meat percentage, eye muscle area and silverside (meat block composed of *M. gluteobiceps* and *M. semitendinosus*) percentage on the centromeric end of BTA2 by using their back-cross population. They focused on the MSTN gene as a candidate and observed a significant effect of F94L on carcass yield traits. Taken together, these data strongly suggest that the MSTN F94L mutation has an effect on carcass quality in beef cattle by regulating muscle mass.

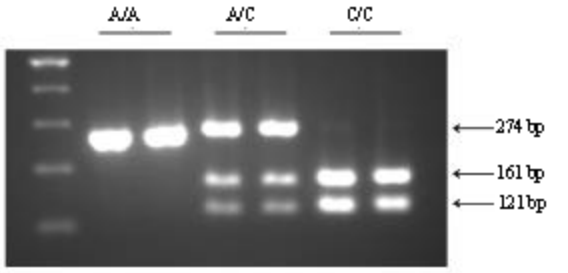

Fig. 1: Genotyping of F94L polymorphisms in MSTN gene. PCR products were digested with restriction enzyme TaqI. Arrows indicate the size of the DNA fragments. These fragments were size fractionated on 2% agarose gels

Significant effects on several fat-related traits were also detected. Because the amount of fat in the carcasses of double-muscled cattle is significantly less than that observed in the carcasses of conventional cattle (Hanset *et al*., 1982; Hocquette *et al*., 1999; Thiessen *et al*., 1982), these results could be explained simply as a reflection of the enormous effect of MSTN F94L on muscle mass.

However, there were several reports on the involvement of MSTN in adipogenesis, both *in vitro* (Rebbapragada *et al*., 2003; Artaza *et al*., 2005) and *in vivo*

Table 4: Meat quality traits of MSTN genotypes for F94L mutation

F94L	n	Crude fat	Crude protein	Moisture	C16:1	C18:2
C/C	36	20.28±0.86^{b}	18.45±0.20^{b}	60.74±0.66^{b}	4.95±0.14	2.40±0.11
C/A	78	18.84±0.57^{a}	18.62±0.13^{b}	61.87±0.43^{b}	4.85±0.09	2.56±0.09
A/A	36	13.08±0.56^{a}	20.19±0.13^{a}	66.03±0.45^{a}	4.63±0.10	2.63±0.10

Mean±SE: Units are all percentage. Mean values with different superscript letters in the same row differ significantly: $^{a-b}p<0.001$; $^{c-d}p<0.1$

Table 5: Computer image analysis traits of MSTN genotype for F94L mutation

		M. longissimus			*M. semispinalis* capitis	*M. semispinalis*		*M. trapezius*	
F94L	n	Area (cm^2)	Lean area (cm^2)	RFA (%)	RFA (%)	Lean area (cm^2)	RFA (%)	Lean area (cm^2)	RFA (%)
C/C	36	43.72±0.75^{c}	33.03±0.65^{c}	24.35±0.91^{b}	24.71±0.67^{b}	20.90±0.38^{d}	29.67±0.87^{b}	24.50±0.49^{c}	25.58±1.12^{b}
C/A	78	46.89±0.57^{b}	35.99±0.51^{b}	23.23±0.60^{b}	23.93±0.46^{b}	22.60±0.29^{c}	29.62±0.54^{b}	26.36±0.34^{b}	23.89±0.63^{b}
A/A	36	51.34±1.07^{a}	42.62±0.93^{a}	16.94±0.66^{a}	20.49±0.80^{a}	24.37±0.47^{b}	25.16±0.78^{a}	29.89±0.61^{a}	19.24±0.92^{a}

Mean±SE; Mean values with different superscript letters in the same row differ significantly: $^{a-b, a-c, b-d}p<0.001$; $^{b-c, c-d}p<0.01$; RFA: Ratio of Fat Area

(Feldman *et al*., 2006) and another possibility could be discussed that the MSTN F94L mutation might also have an effect on fat-related traits.

The detected MSTN F94L mutation was located in the propeptide region and not in the functional mature region. Like other TGF-β super family members, MSTN is produced as a precursor protein (McPherron *et al*., 1997a; Derynck *et al*., 1985). Proteolytic processing cleaves between the propeptide and the C-terminal domain and the mature protein is released (McPherron *et al*., 1997a; Thies *et al*., 2001; Lee and McPherron, 2001). Both unprocessed and mature MSTN forms disulfide-linked dimers (McPherron *et al*., 1997a). *In vitro*, MSTN binds noncovalently to its propeptide after proteolytic processing, thereby producing a biologically inactive complex (Thies *et al*., 2001; Lee and McPherron, 2001).

Furthermore, overexpression of MSTN propeptide leads to an increase in muscle mass in transgenic animals (Lee and Mcpherron, 2001; Yang *et al*., 2001). Substitution at the 94th amino acid of MSTN may lead to some functional defects in the propeptide, which block inactivation of the mature peptide. Hence, it is very interesting and important to clarify whether F94L really has a functional effect.

Finally, to assess the genotypic frequency of F94L in the commercial population in Japan, 75 Japanese Black and 147 Holstein bull samples were genotyped. Surprisingly, no individuals with the A allele were found.

All animals of both breeds used in this study were C/C at F94L. In Europe, F94L mutation had limited distribution in only a few breeds such as Limousin, Pirenaica, Aubrac and Devon (Dunner *et al*., 2003). At the end of the 19th century, the Meiji government imported several European breeds into Japan. These European breeds included the Devon breed, which was crossed with native cattle in Shimane, Yamaguchi and Kagoshima prefectures (Namikawa, 1992). Thus, there remains a possibility of the existence of the F94L A allele in the Japanese Black breed in Japan. In the early modern breeding history of the Japanese Black, however, individuals that expressed the posterior muscle mass phenotype, the so-called pig rump phenotype, were eliminated from populations.

It may be that individuals with the A allele at F94L were involved in the pig rump population and exposed to the selection. In that case, it would be difficult to find such individuals in the Japanese Black population.

On the other hand, the allelic frequencies of the Japanese Black breed revealed more polymorphism than that in the Limousin breed (Table 2). It could be inferred that there exists a certain kinds of MSTN haplotypes in Japanese Black population and it is interesting to investigate the haplotypic effects of MSTN gene on beef quality and quantity traits in this breed, which was reconstructed with detected SNPs in this study.

CONCLUSION

According to the results of this study, F94L mutation in MSTN gene has the strong effect on beef quality and quantity traits by regulating muscle mass, but it seems almost impossible to apply this information to the current breeding system of the Japanese Black breed because of its fixed genotype.

ACKNOWLEDGEMENT

We thank to the staff of Tokachi and Ohu station, National Livestock Breeding Center, for feeding and measuring various traits of F2 population.

REFERENCES

Abe, T., J. Saburi, H. Hasebe, T. Nakagawa, T. Kawamura, K. Saito, T. Nade, S. Misumi, T. Okumura, K. Kuchida, T. Hayashi, S. Nakane, T. Mitsuhasi, K. Nirasawa, Y. Sugimoto and E. Kobayashi, 2008. Bovine QTL analysis for growth, carcass and meat quality traits in an F2 population from a cross between Japanese Black and Limousin. J. Anim. Sci., 86: 2821-2832. DOI: 10.2527/jas.2007-0676. PMID: 18599673. http://jas.fass.org/cgi/content/full/86/11/2821.

Alexander, L.J., T.W. Geary, W.M. Snelling and M.D. Macneil, 2007. Quantitative trait loci with additive effects on growth and carcass traits in a Wagyu-Limousin F(2) population. Anim. Genet., 38: 413-416. DOI: 10.1111/j.1365-2052.2007.01616.x. PMID: 17596127. http://www3.interscience.wiley.com/journal/118504321/abstract.

Artaza, J.N., S. Bhasin, T.R. Magee, S. Reisz-Rorsasaz, R. Shen, N.P. Groome, M.M. Fareez and N.F. Gonzalez-Cadavid, 2005. Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. Endocrinology, 146: 3547-3557. DOI: 10.1210/en.2005-0362. PMID: 15878958. http://endo.endojournals.org/cgi/content/full/146/8/3547.

Casas, E., J.W. Keele, S.D. Shackelford, M. Koohmaraie, T.S. Sonstegard, T.P.L. Smith, S.M. Kappes and R.T. Stone, 1998. Association of the muscle hypertropht locus with carcass traits in beef cattle 1, 2. J. Anim. Sci., 76: 468-473. PMID: 9498354. http://jas.fass.org/cgi/reprint/76/2/468.

Casas, E., R.T. Stone, J.W. Keele, S.D. Shackelford, S.M. Kappes and M. Koohmaraie, 2001. A comprehensive search for quantitative trait loci affecting growth and carcass composition of cattle segregating alternative forms of the myostatin gene 1, 2. J. Anim. Sci., 79: 854-860. PMID: 11325189. http://jas.fass.org/cgi/reprint/79/4/854.

Derynck, R., J.A. Jarrett, E. Chen, D.H. Eaton, J.R. Bell, R.K. Sssoian, A.B. Roberts, M.B. Soprn and D.V. Goeddel, 1985. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells. Nature, 316: 701-705. PMID: 3861940.

Dunner, S., M.E. Miranda, Y. Amigues, J. Canon, M. Georges, R. Hanset, J. Williams and F. Menissier, 2003. Haplotype diversity of the myostatin gene among beef cattle breeds. Genet. Sel. Evol., 35: 103-118. DOI: 10.1051/gse:2002038. PMID: 12605853. http://www.gse-journal.org/index.php?option=article&access=doi&doi=10.1051/gse:2002038.

Feldman, B.J., R.S. Streeper, R.V. Farese Jr. and K.R. Yamamoto, 2006. Myostatin modulates adipogenesis to generagte adipocytes with favorable metabolic effect. Proc. Natl. Acad. Sci. USA, 103: 15675-15680. DOI: 10.1073/pnas.0607501103. PMID: 17030820. http://www.pnas.org/content/103/42/15675.long.

Grobet, L., D. Poncelet, L.J. Royo, B. Brouers, D. Pirottin, C. Michaux, F. Menissier, M. Zanotti, S. Dunner and M. Georges, 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. Mamm. Genome, 9: 210-213. DOI: 10.1007/s00335-9900727. PMID: 9501304. http://www.springerlink.com/content/31d3y7dkga9q4pe8.

Grobet, L., L.J.R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset and M. Georges, 1997. A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. Nat. Genet., 17: 71-74. DOI: 10.1038/ng0997-71. PMID: 9288100. http://www.nature.com/ng/journal/v17/n1/abs/ng0997-71.html.

Hanset, R., J.B.W. King and J.D. McKay, 1982. Muscular hypertrophy as a racial characteristic: The case of the Belgian Blue breed. Curr. Topics Vet. Med. Anim. Sci., 16: 437-449.

Hocquette, J.F., P. Bas, D. Bauchart, M. Vermorel and Y. Geay, 1999. Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol., 122: 127-138. DOI:10.1016/S1095-6433(98)10172-1.PMID:10216937. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VNH-3VTSJ1G-D&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=558de29be21b5eceb10712bfff61ce93.

Kambadur, R., M. Sharma, T.P.L. Smith and J.J. Bass, 1997. Mutations in myostatin (GDF8) in double-muscled Belgian Blue? and Piedmontese? Cattle. Genome Res., 7: 910-915. DOI: 10.1101/gr.7.9.910. PMID: 9314496. http://genome.cshlp.org/content/ 7/9/910.long.

Lee, S.J. and A.C. McPherron, 2001. Regulation of myostatin activity and muscle growth. Proc. Natl. Acad. Sci. USA, 103: 9306-9311. DOI: 10.1073/pnas.151270098. PMID: 11459935. http://www.pnas.org/content/98/16/9306.long.

McPherron, A.C., A.M. Lawler and S.J. Lee, 1997a. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature, 387: 83-90. DOI: 10.1038/387083a0. PMID: 9139826. http://www.nature.com/nature/journal/v387/n6628/pdf/387083a0.pdf.

McPherron, A.C. and S.J. Lee, 1997b. Double muscling in cattle due to mutations in the myostatin? gene. Proc. Natl. Acad. Sci. USA, 94: 12457-12461. PMID: 9356471. http://www.pnas.org/content/94/23/12457.long.

Namikawa, K., 1992. Breeding History of Japanese Beef Cattle and Preservation of Genetic Resources as Economic Farm Animals: Wagyu. 2nd Edn. Wagyu Registry Association, Kyoto, Japan. http://web.kyoto-inet.or.jp/org/zwtkres.

Rebbapragada, A., H. Benchabane, J.L. Wrana, A.J. Celestle and L. Attisano, 2003. Myostatin signals through a transforming growth factor β-like signaling pathway to block adipogenesis. Mol. Cell. Biol., 23: 7230-7242. DOI: 10.1128/MCB.23.20.7230-7242.2003. PMID: 14517293. http://mcb.asm.org/cgi/content/full/23/20/7230?view=long&pmid=14517293.

Sellick, G.S., W.S. Pitchford, C.A. Morris, N.G. Cullen, A.M. Crawford, H.W. Raadsma and C.D. Bottema, 2007. Effect of myostatin F94L on carcass yield in cattle. Anim. Genet., 38: 440-446. DOI: 10.1111/j.1365-2052.2007.01623.x. PMID: 17617213. http://www3.interscience.wiley.com/journal/118504334/abstract?CRETRY=1&SRETRY=0.

Thies, R.S., T. Chen, M.V. Davies, K.N. Tomkinson, A.A. Pearson, Q.A. Shakey and N.M. Wolfman, 2001. GDF-8 propeptide binds to GDF-8 and antagonizes biological activity by inhibiting GDF-8 receptor binding. Growth Factors, 18: 251-259. PMID: 11519824.

Thiessen, R.B., W.C. Rollins, B.W. King and F. Mennissier, 1982. A comparison of mormal and heterozugous animals for double-muscling in British breeds of cattle. Curr. Topics Vet. Med. Anim. Sci., 16: 54-55.

Yang, J.Z., T. Ratovitski, J.P. Brady, M.B. Solomon, K.D. Wells and R.J. Wall, 2001. Expression of myostatin pro domain results in muscular transgenic mice. Mol. Reprod. Dev., 60: 351-361. DOI: 10.1002/mrd.1097.PMID:11599046.http://www3.interscience.wiley.com/journal/85513867/abstract.