The Myostatin gene: an overview of mechanisms of action and its relevance to livestock animals

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Review: The *Myostatin* gene: an overview of mechanisms of action and its relevance to livestock animals

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Summary

Myostatin, also known as Growth Differentiation Factor 8, a member of the Transforming Growth Factor-beta (TGF-β) super-family is a negative regulator of muscle development. Myostatin acts at key points during pre- and post-natal life of amniotes which ultimately determine the overall muscle mass of an animal. Mutations have already demonstrated the impact of attenuating Myostatin activity on muscle development. A number of large animals including cattle, sheep, dogs and humans display the 'double muscled' phenotype due to mutations in the Myostatin gene. Here we firstly give an overview of the molecular pathways regulated by Myostatin that control muscle development. Then we describe the natural mutations and their associated phenotypes as well as the physiological influence of altering Myostatin expression in livestock animals (cattle, sheep, goat, horse, pig, rabbit and chicken). Knowledge of null alleles and polymorphisms in the *Myostatin* gene are of great interest in the animal breeding field and it could be utilized to improve meat production in livestock animals.

Keywords: double muscling, single nucleotide polymorphisms, muscle hypertrophy, muscle hyperplasia, meat production.

Introduction

Myostatin

Myostatin (MSTN), also known as Growth and Differentiation Factor 8 (GDF8), is one of the major regulators of skeletal muscle development (Beyer *et al.,* 2013). The MSTN gene (*MSTN*) is highly conserved among mammalian species and it acts in an

almost unique manner to reduce muscle size. MSTN-deficient animals display an increase in skeletal muscle mass known as double-muscling (DBM). Mutations in *MSTN* have been described in numerous species including dog (Mosher *et al.,* 2007), sheep (Kijas *et al.,* 2007), cattle (Grobet *et al.,* 1997), pig (Stinckens *et al*., 2008) as well as in one human (Schuelke *et al*., 2004).

Myostatin signalling pathway and its control of skeletal muscle development

MSTN is expressed in many tissues (including the mammary gland) but most prominently in skeletal muscle (Ji *et al*., 1998). The *MSTN* has been highly conserved throughout evolution and comprises 3 exons and 2 introns.

In all species reported in this review, *MSTN* exons code for a 375 amino acid latent protein which undergoes significant post-translational modification in order to become biologically active (Wolfman *et al*., 2003). Firstly, the polypeptide undergoes intracellular homodimerization through the formation of disulphide bonds. Thereafter it is cleaved to form the N-terminal propeptide region and the C-terminal mature region. The 12- KDa C-terminal mature fragment of MSTN initiates an intracellular signalling cascade through its ability to bind and activate the Activin type II receptor at the cell surface *(*ActRIIB and to a lesser extent ActRIIA). Subsequent autophosphorylation of the ActRIIB leads to the recruitment and activation of low affinity type I receptor for Activin ALK-4 or ALK-5. Activated type I receptor kinase phosphorylates the transcription factors Smad2 and Smad3, allowing them to interact with Smad4 (co-Smad) and translocate to the nucleus, to activate target gene transcription. Importantly the activation of the MSTN receptor also inhibits Akt (protein kinase B) activity, a major determinant in muscle protein synthesis and cell proliferation. Enlargement of muscle fibre size, a process called fibre hypertrophy (or

simply hypertrophy) is in large part controlled by Akt activity (Trendelenburg *et al.,* 2009). Myogenic differentiation is a highly orchestrated sequential program that ultimately generates mature skeletal muscle. Highly proliferative muscle precursors which arise during embryogenesis differentiate into myoblasts. The commitment of the myogenic lineage is regulated by Muscle Regulatory Factors (MRFs) a collective group of helix-loop-helix transcription factors; namely, MyoD, Myf5, Myogenin and MRF4 (Fig. 1). Additionally, exit from the cell cycle is a vital step during myoblast differentiation (Bryson-Richardson & Currie, 2008).

MSTN regulates muscle development at key points during the process of pre-natal muscle development: muscle precursor proliferation, myoblast proliferation and differentiation. Studies by Amthor *et al*. (2002) have shown that ectopic expression (in limb muscle) of *MSTN* down regulates *Pax3;* a key marker of proliferating muscle precursors (Amthor *et al*., 2002). Additionally, MSTN upregulates p21 expression, which ultimately inhibits proliferation of MyoD expressing myoblasts (Thomas *et al.*, 2000). Of relevance to this review is the relationship between MyoD activity and the expression of *MSTN*. MyoD is an important regulator of *MSTN* expression during myogenesis. This is demonstrated by a critical role of E-box motifs that were identified in the *MSTN* promoter region; these motifs are known to be the binding sites for basic helix-loop-helix transcription factors (MRFs) (Hu et al., 2013).

The interrelationship between MyoD and MSTN ensure that promiscuous differentiation mediated by an over-active MyoD induced cascade is checked by the up-regulation of *MSTN*. Therefore MSTN serves to limit the size of both the myoblast precursor ($Pax3^{+}/MyoD^{+}$) and myoblast ($Pax3^{-}/MyoD^{+}$) pools. Down-regulating the expression of *MSTN* would lead to an expansion of both populations (Amthor *et al*., 1999).

Examination of mouse development shows that muscle mass is determined by the ability of myoblasts to form fibres, a process that occurs in two phases; primary and secondary fibre formation. Matsakas *et al*. (2010) have shown an increase in the myoblast pool, just before the fibre formation process in *Myostatin* null mouse (*Mstn*$^{-/-}$) embryos, which supports the development of extranumerary primary and secondary myofibres. Any programme that promotes an increase in fibre formation is called fibre hyperplasia or simply, hyperplasia (Amthor *et al*., 2002). Therefore the *Mstn*$^{-/-}$ mouse displays hyperplasia as a consequence of developing an increased number of mono-nucleated muscle cells (Matsakas *et al*., 2010).

Shortly before birth, muscle in *Mstn*$^{-/-}$ mice not only contain extra muscle fibres, but also each fibre has undergone a small, albeit significant, increase in size (18%). However this is not enough to explain why the muscles in this species often weigh 2-3 times more than their normal counterpart (Omairi *et al*., 2016). The resolution to this issue comes by examining the size of each muscle fibre in adult mice. This reveals that in the mouse, the increased muscle mass has arisen due to a combination of a pre-natal increase in the number of fibres (hyperplasia) and a precocious post-natal increase (43%) in the size of each fibre (hypertrophy) (McPherron & Lee, 1997).

These studies are extremely insightful when attempting to determine the cellular mechanism underpinning double muscling in large mammalian species harbouring a *MSTN* mutation (Elashry *et al*., 2012). They predict that for an animal to develop fibre hyperplasia and a small degree of hypertrophy as a consequence of a *MSTN* mutation, the gene must normally be expressed and properly translated into a mature form during pre-natal development. However, in order to display significant fibre hypertrophy these conditions need to be satisfied during post-natal life. If the mouse

is taken as a guide, then changes in fibre number and small changes in fibre diameter (less than 20%) can be explained by pre-natal action of MSTN. In cattle, very low levels of MSTN are detected from day 15 to day 29 embryos, and increased expression is detected from day 31 onwards (Kambadur *et al.,* 1997). The increase of *MSTN* expression in the bovine embryos is thought to occur at a gestational stage when primary myoblasts are starting to fuse and differentiate into myofibres. Therefore the null mutation in the bovine *MSTN* lead to hyperplasia.

Double muscling phenotypes

The term hypertrophy has often been used to describe large mammalian species, which display at the gross anatomical level, the enlargement of muscle. Mechanistically this term has been used loosely, since in many cases enlargement of muscle is solely through pre-natal muscle hyperplasia without any post-natal fibre hypertrophy.

DBM in large animals has been reported in several species. Generally, muscle with a large superficial area tends to be the most enlarged, while deeper muscles tend to be reduced in size relative to normal muscle (Ouhayon & Beaumont, 1968). Large commercially important DBM animals, especially cattle, have an excellent conformation and an extremely high carcass yield, coinciding with a reduced internal organ mass (Fiems, 2012).

However, these animals are more susceptible to respiratory disease, urolithiasis, lameness, nutritional stress, heat and dystocia resulting in lower robustness (Holmes *et al.*, 1973). Also the reproductive performance can be influenced by hypertrophy: i.e. in the South Devon breed, the gestation period for DBM calves is longer, resulting in offspring with higher birth weights than the normal calves, also evidenced by the

139 higher instances of dystocia with high mortality rates if births are unassisted; the
140 findings highlighted therefore that the segregating alleles at the *MSTN* have
141 significant effects on calving ease in this breed (Wiener *et al*., 2002).
142 DBM cattle showed signs of fatiguing faster than normal cattle during forced
143 exercise; relating to metabolic acidosis, because of a reduced blood circulation
144 leading to a deficiency in the transport of oxygen and a reduction of aerobic
145 metabolic activity in the muscle (Holmes *et al*., 1973). DBM cattle have in fact an
146 increase in the proportion of fast twitch glycolytic fibres, resulting in a faster and more
147 glycolytic phenotype (Girgenrath *et al*., 2005).
148 Mutations in the *MSTN* are responsible for DBM in other large animals including one
149 case in humans. In the latter, Schuelke *et al*. (2004), observed that a G to A transition
150 at nucleotide gIVS1+5 caused extraordinary muscling in a young boy, especially in
151 the thighs and upper arms. No health problems were reported in the patient and the
152 testosterone and IGF-1 levels were normal. In dogs known as "bully" whippets, a 2-
153 bp deletion was discovered in the third exon of the *MSTN* is associated with the DBM
154 phenotype. This deletion removes nucleotides 939 and 940 within exon three and
155 leads to a premature stop codon at amino-acid 313 instead of the normal cysteine,
156 removing 63 amino acids from the predicted 375-aa protein (Mosher *et al*., 2007). A
157 gene targeting approach using the CRISPR/Cas9 system has been used to create
158 *MSTN* null Beagles; although mutant dogs displayed the DMB phenotype, very little
159 detail is available regarding their cellular phenotype (Zou *et al*., 2015). Due to the
160 effects of MSTN on muscle mass, growth and other traits, the variations in *MSTN*
161 expression levels in skeletal muscles are of great interest in the animal breeding
162 field. Knowledge of null alleles and polymorphisms in the *MSTN* has been utilized to
163 improve the selection of beef cattle and sheep (Georges, 2010). The aim of this

164 section of the review is to describe known double-muscling in livestock animals that
165 harbour *MSTN* mutations.
166
167 Mutations in the Myostatin gene in cattle
168 Monogenic determination of muscular hypertrophy in Belgian Blue cattle was first
169 described in the 1980's (Hanset & Michaux, 1985; Grobet *et al.*, 1997). Double
170 muscling was shown to be inherited as a single major autosomal locus which
171 nevertheless was affected by several modifier loci manifesting in incomplete
172 penetrance. The causal loss of function mutation in Belgian Blue *MSTN*, located on
173 chromosome 2, was first reported by Grobet (1997) followed shortly thereafter by the
174 study of McPherron and Lee who not only substantiated the finding of Grobet but
175 also reported a missense mutation in exon 3 in the Piedmontese breed *MSTN*
176 (McPherron & Lee, 1997). Approximately 20 different types of genetic variants
177 (deletions, insertions and nucleotide substitutions, also known as single nucleotide
178 polymorphisms - SNPs) have been identified in the bovine *MSTN*. Some of these
179 genetic variants give rise to muscular hypertrophy by inactivation of the gene (Grobet
180 *et al.*, 1997). Mutated alleles and inactive *MSTN* have a significant association with
181 growth speed and carcass favourite traits, so these polymorphisms could be used in
182 beef cattle in order to increase the quality and quantity of meat (Mirhoseini & Zare,
183 2012). In the view of quality meat production, this is an outstanding trait, since these
184 animals produce not just more, but leaner and more tender meat (Kobolák & Gócza,
185 2002). The carcass and meat quality traits are superior in these animals because of a
186 reduction in fat (decreased by 50%), muscle mass increase (by 20%) lower
187 proportions of bone and also less connective tissue, which contributes to tenderness
188 (McPherron & Lee, 1997; Vincenti *et al.*, 2007). However, dystocia-related problems

189 are often observed in DBM cattle because hyperplasia occurs before birth, resulting
190 in larger calves (Deveaux *et al*., 2001). Homozygous DBM animals manifest more
191 problems of dystocia than heterozygous. Therefore in order to generate homozygous
192 animals and at the same time keep costs down as well as reducing calve death
193 probability, it is worth considering mating heterozygous animals (Bellinge *et al*.,
194 2005).
195 A summary of the detected genetic variants in cattle is reported in Table 1.
196
197 Double muscled cattle breeds
198 Belgian Blue
199 The breed in which this muscular hypertrophy and its effects have been analysed
200 most extensively is the Belgian Blue breed, which has been systematically selected
201 for double muscling to the point of fixation in many herds. Research by Grobet *et al.*
202 (1997) revealed an 11-bp deletion (nucleotides 821-831) in the open reading frame of
203 the Belgian Blue *MSTN* allele which results in the loss of 3 amino acids (275, 276,
204 and 277) and a frameshift after amino acid 274. The frameshift leads to a stop codon
205 after amino acid 287. Work by Wegner *et al.* (2000) showed that *Semitendinosus*
206 from Belgian Blue was 1.6 times the weight of normal breeds solely due to an
207 increase in muscle fibre number. Indeed, muscle fibre size from the Belgian Blue was
208 actually smaller than other breeds (Wegner *et al*., 2000). Furthermore, these animals
209 have less collagen and connective tissue than the normal animals. The carcass fat
210 content in these animals is significantly lower than in normal cattle, especially
211 intramuscular fat (marbling) being influenced by the DBM phenotype with a strong
212 reduction of subcutaneous and internal fat tissues (Mirhoseini & Zare, 2012). The
213 results of many studies in fact have indicated that MSTN plays key roles in not only

myogenesis but also adipogenesis. *MSTN* deletion and inhibition in animals mainly lead to increased muscle mass and reduced fat mass (Deng *et al.*, 2017).

In beef cattle production, crossing with Belgian Blue cattle shows that although the gene is recessive and monofactorial, its effect is apparent even in heterozygous animals due to its partial dominance (Kobolák & Gócza, 2002).

The same mutation was also found in the Asturiana de los Valles (AV), a Spanish beef cattle breed. *MSTN* polymorphisms in the AV breed have been described and its diffusion into the breed has been continuous due to economic reasons (Grobet *et al.*, 1997).

Piedmontese

In Piedmontese cattle the double-muscled phenotype is an inherited condition associated with a G to A mutation on nucleotide 938 (in exon 3) which translates to C313Y in a highly conserved cysteine-knot structural motif region of the protein. This is in the pre-helix loop, a region known to be important for ALK4/5 receptor interaction (Cash *et al.*, 2012). The mutation alters the function of MSTN, which disrupts a disulphide bridge that is essential for the correct conformation of the protein (Kambadur *et al.*, 1997). This breed has been systematically selected for double muscling to the point of fixation in many herds (> 96% homozygous in the Piedmonte region in Italy), but variability in muscle mass is still present (Miretti *et al.*, 2013). Several studies support the notion that the double muscling phenotype, a partially recessive trait, causes the relatively large effects on carcass conformation, without a negative effect on calving, compared with animals with no copies of the mutated allele (Casas *et al.*, 1998).

Marchigiana

The Marchigiana is one of the most important Italian beef cattle breeds and it is renowned for its large body size, high weight daily gains and superior carcass dressing percent. Marchigiana breed have a G to T transversion mutation at nucleotide 874 in exon 3 (g.874G>T), translating to E291X in the MSTN. This point mutation has a remarkable effect on the MSTN as it changes a codon for glutamic acid into a stop codon (Marchitelli *et al.*, 2003). In Marchigiana, as in the other double muscling breeds, the *MSTN* genotypes yield three different and distinct phenotypes. The homozygous G/G displays the normal phenotype whereas the T/T genotype manifests as a double muscled body shape while maintaining its small frame, and is frequently associated with skeletal defects and serious survival problems due to macroglossia and hypoplasia of the heart, lungs and other vital organs. The heterozygous genotype (G/T) produces a well-muscled and large body structure and excellent conformation without any of the above mentioned defects. Therefore, the heterozygous animals are frequently selected as sires (Cappuccio *et al.*, 1998). Moreover heterozygous animals show a better meat quality than animals with a normal genotype (Vincenti *et al.*, 2007). Therefore they could be useful for breeders to plan the matings to obtain a higher number of heterozygous animals. Obviously this is possible only if the genotype at the *MSTN* locus of each animal is available. Additionally two different SNPs have been found in the promoter region: g.-371T>A and g.-805G>C, although Sarti *et al.* (2014) reported that these substitutions may not be useful to be considered in the selection criteria, because there is no correlation with productive traits or due to their homozygous genotype.

Other cattle breeds

An 11 bp deletion (nt821(del11)) resulting in a truncation of the bioactive C-terminal domain of the protein has been found in Blonde d'Aquitaine, Limousine, and Parthenaise and Rubia Gallega breeds (Kambadur *et al*., 1997; Dunner et al., 2003). A recent study (Bouyer *et al*., 2014) identified an unexpected mutation in the *MSTN* in Blonde D'Aquitaine cattle. The mutant allele is highly expressed leading to an abnormal transcript consisting of a 41-bp inclusion between the exons 2 and 3, with a premature termination codon predicted to translate into a protein lacking the entire bioactive region.

An additional transversion mutation (g.433C>A) in Limousine breed has been described that was shown to be functionally associated with the increased muscle mass and carcass yield without any associated reproductive disadvantages (Sellick *et al*., 2007; Esmailizadeh *et al*., 2008; Vankan *et al*., 2010).

As in Piedmontese cattle, a G to A transition at nucleotide position 938 has been reported in Gasconne (Kambadur *et al*., 1997; Dunner *et al*., 2003). An insertion/deletion at position 419 replacing 7 bp with an unrelated stretch of 10 bp was reported in Maine-Anjou cattle, resulting in a premature stop codon in the N-terminal latency-associated peptide at amino-acid position 140 (nt419 (del7- ins10)) (McPherron & Lee*,* 1997). Additionally, a transversion (G to T) at nucleotide position 676, also causing a premature stop codon in the same N-terminal latency-associated peptide at amino-acid position 226 (E226X) was identified in the same breed (Grobet *et al*., 1997). Charolaise and Limousine have a C to T transition at nucleotide position 610 yielding a premature stop codon in the N-terminal latency associated peptide at amino-acid positions 204 (Q204X) (Cappuccio *et al.,* 1998). In addition to the genetic variants found in *Bos taurus*, 14 polymorphisms (three in exon one, seven in exon two, and four in exon three) have been reported in the coding part of the *MSTN* in

289 Nellore cattle (*Bos indicus*) genome. However, whether these polymorphisms are
290 functional mutations still remains to be elucidated (Grisolia *et al*., 2009).
291
292 Double muscling in sheep
293 The *MSTN* is located at the end of the long arm (2q32.2 locus) on chromosome 2 in
294 the sheep (*Ovis aries*) (Bellinge *et al*., 2005). During the past decade a total of 77
295 *MSTN* SNPs have been reported in various sheep breeds such as Texel, Norwegian
296 Spælsau, commercial New Zealand sheep breeds and Latvian Darkhead (Kijas *et al*.,
297 2007, Sjakste *et al*., 2011; Han *et al.,* 2013), and the majority of these SNPs are
298 located in the non-coding regions of the gene. The exception is a 1-bp deletion
299 identified in nucleotide position 960 in the *MSTN* of Norwegian White Sheep and
300 c.101G/A in New Zealand Romney, c.120insA (Boman *et al*., 2009). Lastly in 2018,
301 Trukhachev *et al*., described for the first time eight variations in non-coding regions of
302 *MSTN* in the Stavropol Merino, a breed used for meat production in Russia. A
303 summary of the detected genetic variants in sheep is reported in Table 2.
304
305 Texel
306 Belgian Texel sheep muscle fibres show enlargement and therefore can be
307 considered to have fibre hypertrophy. Texels are utilized extensively as a terminal
308 crossbreed because of their exceptional conformation and potential to produce
309 higher-yielding carcasses with increased lean and decreased fat content (Leymaster
310 & Jenkins, 1993). Analysing the *MSTN* revealed no nucleotide differences in the
311 coding regions between DBM and normally muscled breeds (Kijas *et al*., 2007). This
312 suggests that genetic variation located outside the coding regions plays a more
313 important role in the regulation of muscle development in contrast to cattle, where

MSTN loss of function variants have been found within the three coding exons (Grobet *et al.*, 1997). Quantitative trait locus (QTL) analysis in Texel sheep characterized a mutation (g.6723G>A) in the 3' UTR (Untranslated Region) of the *MSTN* on chromosome 2 which has an effect on muscle mass. This creates a target site for *miR1* and *miR206*; microRNAs (miRNAs) that are highly expressed in skeletal muscle (Kijas *et al.*, 2007). Other genetic variants have also been found including c.*1232A, g+391G>T and another 18 SNPs: g.2449C>G; g.2379C>T; g.1405A>T; g.1402G>A; g.1214C>T; g.1129C>T; g.41A>C; g.39T>C; g+474C>T; G+613T>C; g+616G>A; g+619T>C; g+622T>C; g+632G>T; g+696C>T; g+3135C>T; g+4036A>C; g+4044C>T (Kijas *et al.*, 2007).

Norwegian sheep

The DBM phenotype in Norwegian white sheep was described to have extraordinary over-development of the muscles, particularly on the hindquarters. Investigations showed that these animals have not only extremely low levels of subcutaneous fat, but also decreased internal fatty tissues. The DBM animals had lower bone mass compared with the wild type animal. Sequence analysis revealed a 1-bp deletion in the *MSTN* at nucleotide position 960 in DBM individuals. The deletion of a G residue (c.960delG) disrupted the reading frame from amino acid 320 onwards and produced a premature stop codon at amino acid position 359 (compared to position 375 in the wild type animals) (Boman & Vage, 2009).

The same *MSTN* 3'-UTR mutation (c.2360G>A) identified in Texel sheep was also found in the Norwegian breed but with a less profound effect (Boman & Vage, 2009). However a similar phenotype of increased muscle mass and fat was found in Norwegian Spælsau sheep. The sequencing of the *MSTN* coding region revealed a

1-bp insertion at nucleotide position 120 (c.120insA) in DBM animals. The insertion of an adenine residue disrupts the reading frame from amino acid position 40 onwards, and generates a premature stop codon at amino-acid position 49 (Boman & Vage, 2009).

New Zealand

A comprehensive investigation of polymorphisms in *MSTN* in a diverse range of sheep breeds (New Zealand Romney, Coopworth, Corriedale, Dorper, Perendale, Suffolk, Merino, Dorset Down, Poll Dorset, Texel and other NZ cross-bred sheep) was performed using polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis and DNA sequencing. A total of 28 nucleotide substitutions were identified from nucleotide c.-1199 (in the promoter region) to c.*1813 in the 3'UTR. Of these, three were located in the promoter region, three in the 5'UTR, 11 in intron 1, five in intron 2 and five in the 3' UTR. Ten new substitutions have been reported: c.-959C>T, c.-784A>G, c.373+563A>G, c.373+607A>G, c.374-654G>A, c.374-54T>C, c.748-54T>C, c.*83A>G, c.*455A>G and c.*709C>A (Han *et al.*, 2013).

The other 18 substitutions had been reported previously. These include c.101G>A which was already found in NZ Romney by Zhou *et al.* (2008) and also in Merino, Corriedale and NZ cross-bred sheep (Clop *et al.*, 2006; Kijas *et al.*, 2007). In NZ Romney a further two SNPs c.-2449G/C and c.-2379T/C were detected (Wang *et al.*, 2016). The SNP c.*123A observed in NZ cross-bred sheep was also reported in Texel (Kijas *et al.*, 2007), Charollais sheep from Britain (Hadjipavlou *et al.*, 2008), White Suffolk, Poll Dorset and Lincoln breeds from Australia and showed significant

363 association with DBM phenotype as well as the other substitution c.373+18 T>G,
364 reported in Texel sheep (Clop *et al.,* 2006).
365
366 Other sheep breeds
367 Zel sheep, a meat breed in northern Iran, has a polymorphism in intron 2 as does the
368 Iranian Baluchi sheep (Dehnavi *et al*., 2012). Three polymorphic sites in Indian sheep
369 have been identified in the 5'UTR, exon 1 and exon 2 regions. Both SNPs in the
370 exonic region were found to be non-synonymous. The genetic variants c.539T>G and
371 c.821T>A were in the exon 1 and exon 2, respectively (Pothuraju *et al*., 2015). All
372 these genetics variants are not significantly associated with DBM phenotype.
373
374 Myostatin polymorphisms in goat
375 Several studies investigated the allelic variation in the goat *MSTN*. A 5 bp indel (1256
376 TTTTA/-) was identified in 5'UTR region in Boer, Matou, Haimen and Nubi goat
377 breeds, and a substitution (1388 T/A) in exon 1 region was detected only in Boer
378 (Zhang *et al*., 2012). Two novel single nucleotide polymorphisms were also identified
379 in Boer and Anhui white goat: g.197G>A, a substitution located in the 5'-UTR, and
380 345A>T in the exon 1 (Zhang *et al*., 2013). A thorough investigation was conducted
381 in 22 different goat breeds (Inner Mongolia Cashmere, Liaoning Cashmere, Taihang
382 Mountain, Chengde Polled, Jining Grey, Tibetan, Chengdu Brown, Jianchang Black,
383 Guizhou White, Guizhou Black, Longlin, Duan goat, Leizhou, Matou, Yichang White,
384 Shannan White, Nanjiang Brown, Angora, Toggenburg, Nubian, Saanen and Boer
385 goat) and a total of eight SNPs were detected (A1980G, G1981C, A1982G, G1984T,
386 A2121G, T2124C, G2174A and A2246G) (Li *et al*., 2006). Recently Nguluma *et al.*
387 (2018) detected a polymorphic site T298C in the Boer goat population: the authors

388 concluded that the potential association of this polymorphism in *MSTN* with growth
389 performance could not be confirmed and that other genes for growth could be
390 responsible for the observed variation. A summary of the detected genetic variants in
391 goat is reported in Table 3.
392
393 Myostatin polymorphisms in horse
394 Hosoyama *et al*. (2002) isolated and sequenced *MSTN* cDNA from a Thoroughbred
395 horse which was mapped to chromosome 18. Mutations in the equine *MSTN* have
396 been identified and are associated with racing phenotypes influencing racing
397 performance and muscle fibre proportions (Petersen *et al.,* 2013). Dall'Olio *et al*.
398 (2010) sequenced in 16 horse breeds (Rapid Heavy Draft, Noric, Bardigiano,
399 Haflinger, Lipizzan, Murgese, Tolfetano, Uruguayan Creole, Italian Saddle,
400 Maremmano, Quarter Horse, Salernitano, Andalusian, Ventasso, Italian trotter,
401 Thoroughbred horse) revealing seven SNPs: two transitions were located in the
402 promoter region at -646 (GQ183900: g.26T>C) and -156 (GQ183900: g.156T>C) bp
403 upstream from the start codon and are associated with breeds of different
404 morphological types. The g.26T>C SNP was polymorphic in 6/16 breeds with higher
405 observed frequency of the g.26C allele. The g.156T>C polymorphism was detected
406 in 11/16 breeds and was identified in homozygous condition in a few Bardigiano,
407 Haflinger, Noric, Rapid Heavy Draft, and Uruguayan Creole horses (Dall'Olio *et al*.,
408 2010). The other five SNPs were in intronic regions: four were localized in intron 1
409 and one in intron 2. Three of the SNPs of intron 1 (g.1634T>G, g.2115A>G, and
410 g.2327A>C) were also identified in Thoroughbred breeds (Petersen *et al.,* 2013). One
411 polymorphism (g.2115A>G) has been associated with sprinting ability and racing
412 stamina in Thoroughbred horses. The association between *MSTN* and horse racing

performances was further evidenced by Binns *et al.* (2010) and Tozaki *et al.* (2010). Subsequently 15 Chinese breeds were studied to select the best Chinese domestic breed to evaluate the potential racing performances (Li *et al*., 2014). These studies found six different SNPs in *MSTN*: two SNPs (g.26T>C and g.156T>C) in the promoter region, two (g.587A>G and g.598C>T) in the 5'-UTR region, and two (g.1485C>T, g.2115A> G) in intron-1 of the equine *MSTN*, respectively. The SNPs g.587A>G and g.598C>T were novel whereas the others had been previously reported (Petersen *et al.,* 2013).

Baron *et al.* (2012) described a genetic variant in exon 2 in some horse breeds. In fact, they identified a substitution g.2279A>C in Arabians horses and a substitution g.2478G>C in the Soraia breed horse.

Five polymorphisms (g.66495826T>C, g.66495696T>C, g.66493737T>C, g.66495254C>T and g.66490010T>C) were recently observed (Stefaniuk *et al*., 2016) in four Polish breeds (Arabians, Polish Konik, Hucul and Polish Heavy Draft). The polymorphism g.66495254C>T (also known as g.598C>T), has been described in Chinese horse breeds as well as in Polish Konik and Arabian horse breeds. The g.66493737C>T polymorphism known to predict optimum distance in Thoroughbred horses has been identified in four breeds in Egyptian bloodlines (Bower *et al*., 2012) which were introduced to Polish bloodstock through Egyptian stallions. The insertion g.66495326_66495327Ins227 has been described for the first time in *MSTN* in Thoroughbred horses. Recently, it has been found in the American Quarter Horse (Petersen *et al*., 2013), and in the Uruguayan Creole breeds (Dall'Olio *et al*., 2014). In the Quarter Horse breed, the Ins227 in *MSTN* is connected with changes to *Gluteus medius* muscle fibre proportions. The higher Myosin Heavy Chain 2B fibre type (fast contracting), is in line with pressure selection in Quarter Horse breed for

racing performance (Petersen *et al*., 2013). A summary of the detected genetic variants in horse is reported in Table 4.

Myostatin polymorphisms in pig

Jiang *et al*. (2002) reported three SNPs in porcine *MSTN* T>A, G>A and C>T, in the promoter, intron 1 and exon 3, respectively. Only one mutation (T to A) located in the region 383bp upstream of translation initiation site of porcine *MSTN* was associated with average daily gain in the growing period (from 60 to 100 kg of live weight) in Yorkshire pigs. Furthermore BW in pig with the heterozygous mutation (no AA was found) was increased (Jiang *et al*., 2002).

Stinckens *et al*. (2008) compared the *MSTN* sequence of Belgian Piétrain, which shows a heavily muscled phenotype with five other breeds (Piétrain, Landrace, Large White, Meishan and Wild Boar). Fifteen polymorphic loci were found, three of which were located in the promoter region (g.435G>A, g.447A>G, and g.879T>A), five in intron 1 and seven in intron 2. The SNP g.879T>A only appears in Chinese Meishan pigs whilst the polymorphism located at position 447 of the porcine *MSTN* promoter had a very high allele frequency in the Piétrain pig breed. A g.447A>G mutation which is associated with the expression of the porcine *MSTN* occurs at the putative myocyte enhancer factor 3 (MEF3) binding site on the negative DNA strand. This mutation disrupts a putative MEF3 binding site (Stinckens *et al*., 2008).

However, these results suggest that naturally occurring *MSTN* genetic variants identified thus far in pigs do not have significant association with muscle phenotypes. Nevertheless, a recent work, using an experimental approach has shown the role of MSTN in the development of muscle in pigs. Qian *et al.* (2015) generated *MSTN*-deficient Meishan pigs using zinc finger nucleases (ZFN) technology coupled with

463 somatic cell nucleus transfer. The resulting offspring show remarkable DBM
464 phenotype especially pronounced in the hindquarters. Muscle in the *MSTN* null pig
465 increased mass by 50-100%. Incredibly the muscle fibre size in the null pigs was
466 smaller than the wild type. All the increase in mass could be attributed to fibre
467 hyperplasia whereby some muscles from the null had twice the fibre number
468 compared to wild type. The animals displayed good overall health. As the technology
469 employed did not involve the introduction of any genetic material in to the genome
470 (e.g. selection markers), Qian *et al*. (2015) suggest that it is essentially the same as
471 double muscle cattle which are used for human consumption.
472 A summary of the detected genetic variants in pigs is reported in Table 5.
473
474 Myostatin polymorphisms in rabbit
475 Fontanesi *et al*. (2011) investigated the variability of the effects of *MSTN*
476 polymorphisms on rabbit production traits. Four single SNPs have been identified by
477 comparative sequencing of 14 rabbits representing breeds or lines having different
478 conformation and muscle mass: one rare synonymous SNP in exon 1 (c.108C>T),
479 one synonymous SNP in exon 2 (c.713T>A), one SNP in the 3'-untranslated region
480 (c.*194A>G) and another SNP in intron 2 (c.747+34C>T) in Belgian hare, Burgundy
481 fawn, Checkered giant and Giant grey.
482 In commercial hybrids, Qiao *et al*. (2014) detected a SNP (T to C) in the 5' regulatory
483 region, but no mutation sites were detected in the exons. The correlation analysis
484 showed that the mutation was associated with increased liver and carcass weight.
485 These results suggest that the mutations in the upstream regulatory region of the
486 *MSTN* are beneficial to the rabbit soma development, and the mutations can be used
487 as molecular markers for the selection of the meat quality in rabbits. Sternstein *et al.*

(2014) found polymorphisms in the *MSTN* in Giant Grey and NZ White breeds. Comparative sequencing of these breeds revealed two SNPs located in the regulatory region of the rabbit *MSTN* (c.-125T>C) and in intron 1 (c.373+234T>C). A summary of the detected genetic variants in rabbit is reported in Table 6.

Myostatin polymorphisms in poultry

In chickens *MSTN* maps to 7p11 (Sazanov *et al*., 1999), and like that of mammals is composed of three exons (373 bp, 374 bp and 1567 bp, respectively) and two introns. Gu *et al.* (2003) showed poultry *MSTN* not only regulates skeletal muscle development, but also participates in the fat metabolism and disposition. This research team identified seven SNPs: five were in the 5'-regulatory region (G167A, T177C, G304A, A322G, and C334T) and two were in the 3'-regulatory region of different chicken lines. These last two SNPs in the 3'-regulatory region of the *MSTN* are A to T (7263) and A to G (6935). Ye *et al*. (2007) studied the association of *MSTN* polymorphism with mortality rate, growth, feed conversion efficiency, ultrasound breast depth, breast percentage, eviscerated carcass weight, leg defects, blood oxygen level, and hen antibody titer to the infectious bursal disease virus in three commercial broiler chicken lines. The *MSTN* had pleiotropic effects on broiler performance. This conclusion was reached by the discovery of fourteen SNPs: seven genetic variants in exon 1 (G2100A, G2109A, G2244C, A2283G, C2346T, C2373T, A2416G), one in exon 2 (T4842G), three in exon 3 (C7434G, A7435G, C7436A), and three in intron 1 and 2 (A4405C, A4405T and A4954G).

As the main function of MSTN is the regulation of skeletal muscle growth, Ye *et al*. (2007) deemed that the non-synonymous SNP T4842G is associated with an amino acid change in the *MSTN* and it could be responsible for variability in body weight.

513 The Bian chicken breed raised for dual purposes, is an important Chinese breed and
514 has a 234G>A in exon 1 of the *MSTN* (Zhang *et al*., 2012). Other Chinese chicken
515 breeds (Jinghai, Youxi, and Arbor Acre) have shown four new mutations (A326G,
516 C334G, C1346T, G1375A) that were located in the 5'-regulatory region (Zhang *et al*.,
517 2012). Further studies on the growth traits show that the SNPs in chicken *MSTN* may
518 affect the abdominal fat weight and percentage, breast muscle weigh and
519 percentage, birth weight, and adult weight (Zhang *et al*. 2012). Zhiliang *et al.* (2004)
520 identified three SNPs in the 5' regulatory region and two SNPs in the 3' regulatory
521 region, and these differed in allele frequencies between breeds. They found that in
522 an F2 generation from a cross of broiler and silky chickens, homozygous genotypes
523 *AA* and *BB* at a locus in the 5' regulatory region have a higher abdominal fat weight
524 and abdominal fat percentage than *AB* genotype (Zhiliang *et al*., 2004). The
525 upstream promoter region of *MSTN* was analysed in Wenshang Luhua chicken DNA.
526 Thirteen E-boxes were identified upstream of *MSTN* and the polymorphisms of E-
527 boxes were explored for the first time (Hu *et al*., 2013).
528 Other interesting studies were carried out on ducks to investigate the association of
529 polymorphisms in *MSTN* with slaughter traits, breast muscle weight, breast muscle
530 percentage, leg muscle weight and leg muscle percentage. Analysis of the 5′
531 regulatory region of the *MSTN* showed that polymorphisms (753G>A, 658G>T and
532 235G>C) were associated with the breast muscle percentage and abdominal fat rate
533 (Lu *et al*., 2011). Furthermore Xu *et al*. (2013) studied polymorphisms in Pekin duck,
534 and identified three significant variations. The first is a transition T to C in the ORF
535 (position 129) and revealed an association with breast muscle thickness. The second
536 SNP was located at 708 bp for the T/C mutation in the ORF and last 952T<C had a
537 significant association with the "Fossilia Ossis Mastodi, or dragon bone" length. In

538 Gaoyou ducks, a transition G>A at 2701bp in exon 3 of the *MSTN* is correlated with
539 the abdominal fat rate (Liu *et al.*, 2012). In Sansui duck, six SNPs were identified in
540 the first and the third exons (g.106G>A, g.120A>G, g.159G>A, g.5368G>A,
541 g.5389A>C and g.5410G>A) with four loci seemingly associated to leg muscle
542 weight, leg muscle percentage and dressing percentage (Zhao *et al.*, 2016).
543 A summary of the detected genetic variants in poultry is reported in Table 7.
544
545 Myostatin and future implications
546 According to some investigators, *MSTN* mutations are the main cause of
547 hypertrophy, with a lesser roles played by other gene mutations (Kobolák & Gócza,
548 2002). Inactivation of *MSTN* has therefore been proposed to be a strategy for
549 improving muscle growth of food animals and treating human diseases associated
550 with muscle weakness and dystrophy (Chen & Lee, 2016).
551 Research, especially on mice, has highlighted the potential of manipulating MSTN
552 signalling in order to promote muscle growth. In null mutants of this species, some
553 muscles are approximately three times their normal weight. Impressive as they are,
554 muscle enlargement in large mammals carrying a null mutation in the same gene, to
555 our knowledge, do not approach this level of muscle growth. Therefore it is important
556 to ascertain the molecular basis underpinning these different responses with a view
557 of translating these findings into increased meat production.
558 One picture that emerges through this review is that mutations that compromise
559 MSTN function have a consequence during development and give rise to
560 supernumerary muscle fibres (hyperplasia). However, one of the clear differences
561 between mice and large animals (cattle and pigs) is the post-natal phenotype. Mice
562 show considerable fibre hypertrophy whereas in both cattle and pigs display no

563 increase in fibre size. These findings need to be used as a benchmark for future work
564 on doubling muscle in large animals. First and foremost is the need to understand the
565 basis of muscle growth in large mammals. Here it is very important to use the correct
566 terms to describe the phenotype of animals, as often this can lead to
567 misinterpretations regarding mechanism. Often DBM animals are referred to as being
568 'Hypertrophic'. However this could infer fibre enlargement. As we have discussed,
569 especially in the case of cattle and pig, there is no fibre enlargement. We suggest
570 that accurate mechanistic descriptors are used when they have been precisely
571 established and without this proof a more generic term needs to be applied. We
572 suggest the use of the four following terms: 1) Muscle enlargement through
573 hyperplasia; 2) Muscle enlargement through hypertrophy; 3) Muscle enlargement
574 through hyperplasia and hypertrophy; 4) Muscle enlargement through unknown
575 cellular mechanisms.
576 Research is required to understand the mechanisms that underpin the role of MSTN
577 in post-natal muscle development in mammals, to answer the question as to why in
578 the absence of MSTN, fibres from mice undergo enlargement, whereas those from
579 large mammals do not. For a number of years the naturally occurring mutants in
580 cattle were our only reference model for large animals lacking MSTN. The lack of
581 fibre hypertrophy was usually explained by the presence of a secondary (to date
582 unidentified) modifying mutation that interfered with the post-natal effect but sparred
583 the pre-natal phenotype. However the work by Qian *et al.* (2015) in the pig which
584 targets only the *MSTN* undermines the modifying gene idea. Therefore loss of
585 function mutation in both small and large animals leads to hyperplasia. However it is
586 only in mice that the mutation has an effect on muscle fibre size where it presents as
587 hypertrophy.

588 Clues to resolving this issue come from recent work in monkeys which shows that
589 MSTN and Activin act synergistically to inhibit fibre hypertrophy during adult life
590 (Latres *et al.*, 2017). Based on these findings we suggest that muscle fibres of both
591 cows and pigs are sensitive to Myostatin/Activin signalling, in a similar manner to
592 monkeys. But the issue that still needs to be resolved is why do fibres in adult cows
593 and pigs fail to enlarge in the absence of MSTN. The most parsimonious explanation
594 is that there is a partial redundancy relationship between MSTN and Activin; in the
595 absence of MSTN, the expression levels of Activin become elevated to such a
596 degree that in cows and pigs the latter can completely cover the loss of the former.
597 Examples of gene expression compensation by related molecules, similar to our
598 proposal are abound in mammalian biology (Barbaric *et al.*, 2007). One of the best
599 examples comes through the investigations of MRFs where genetic inactivation of
600 MyoD results in an up-regulation of the related gene-Myf5 (Rudnicki *et al.*, 1992).
601 The hypothesis outlined above has a number of important implications. Our assertion
602 of why the relationship between MSTN and Activin in cows and pigs is only partial
603 and not complete, come from the fact that loss of MSTN has some phenotypic
604 consequence (hyperplasia). Therefore compensation through an up-regulation of
605 Activin expression cannot have occurred during pre-natal life. The second implication
606 is that if there is a redundancy mechanism in mice, which must be very muted since
607 these animals develop a profound phenotype both during pre-natal and adult life. Our
608 suggestions can be validated by quantifying the levels of MSTN and Activin at
609 different developmental stages in both large and small animals, an avenue now
610 possible following the development of specific ELISA for MSTN and Activin (Latres *et*
611 *al.*, 2017).

612 For the meat industry and for the human health sector who focus on muscle growth,
613 the hypothesis outlined here advocates a strategy of dual MSTN and Activin
614 antagonism to promote the growth of the tissue. This could be achieved through the
615 use of a combination of molecules that specifically antagonise the activity of MSTN
616 and Activin (antibodies or protein specific propeptides) or a single protein which acts
617 at a signalling convergence point (at the receptor level through the deployment of a
618 ligand trap or blocking antibody (Omairi *et al.*, 2016, Lach-Trifilieff *et al.*, 2014).
619 Moreover for beef production it will be very interesting to better understand the role of
620 MSTN in adipogenesis; Deng *et al*. (2017) in fact reported that muscle and adipose
621 tissue develop from the same mesenchymal stem cells, and researchers have found
622 that MSTN is expressed in fat tissues and plays a key role in adipogenesis.
623 Finally *MSTN* is a prime target for transgenic approaches aimed at enhancing meat
624 production in livestock (Georges, 2010). Possible strategies for this outcome include
625 the generation of *MSTN* knock-out animals. Also more elaborate transgenic
626 approaches, such as targeting post-natal or sex specific inhibition of *MSTN* need to
627 be considered. Wang *et al.* (2017), reported the successful application of the
628 CRISPR/Cas9 system to engineer the goat genome through micro-injection of Cas9
629 mRNA and sgRNAs targeting *MSTN* in goat embryos. They demonstrate the utility of
630 this approach by disrupting *MSTN*, resulting in enhanced body weight and larger
631 muscle fiber size in Cas9-mediated gene modified goats. MSTN activity can also be
632 modified using non-genetic approaches using for example blocking antibodies or
633 ligand traps.
634
635 **Conclusions**
636 One picture that emerges through this review is that mutations that compromise

MSTN function have a consequence during development and give rise to supernumerary muscle fibres (hyperplasia). However, one of the clear differences between mice and large animals (cattle and pigs) is the post-natal phenotype. First and foremost there is the need to understand the basis of muscle growth in large mammals.

This review landscapes the genetics of DBM in mammalian species and chicken and demonstrates the huge number of genetic variants present in animals of commercial interest. It also highlights areas where greater research is required in order for progress to be made concerning the role of MSTN in the regulation of muscle development in economically important animals. Knowledge of null alleles and polymorphisms in *MSTN* are of great interest in the animal breeding field and could be utilized to improve the selection for meat production in livestock animals.

Conflict of interest

The authors have no conflict of interest to declare.

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LEGENDS TO FIGURES

Figure 1 Myostatin action during myoblast proliferation and differentiation (modified from Langley *et al.*, 2002). Retinoblastoma protein (Rb), in a low phosphorylated state, inhibits cell division. Rb activity is attenuated due to hyper-phosphorylation by the kinase action of CKD2. However the activity of CDK2 is inhibited by p21 which is induced by the action of MSTN. MSTN also activates Smad2/3 signalling which inhibits the expression of *MyoD* which is needed for normal myoblast differentiation. In the absence of MSTN, the activity of CDK2 is not inhibited which allows it to inactivate Rb resulting in increased proliferation of myoblasts. At the same time the expression of *MyoD* is no longer inhibited by Smad2/3 signalling pathways allowing it to promote differentiation of the extranumerary myoblasts.

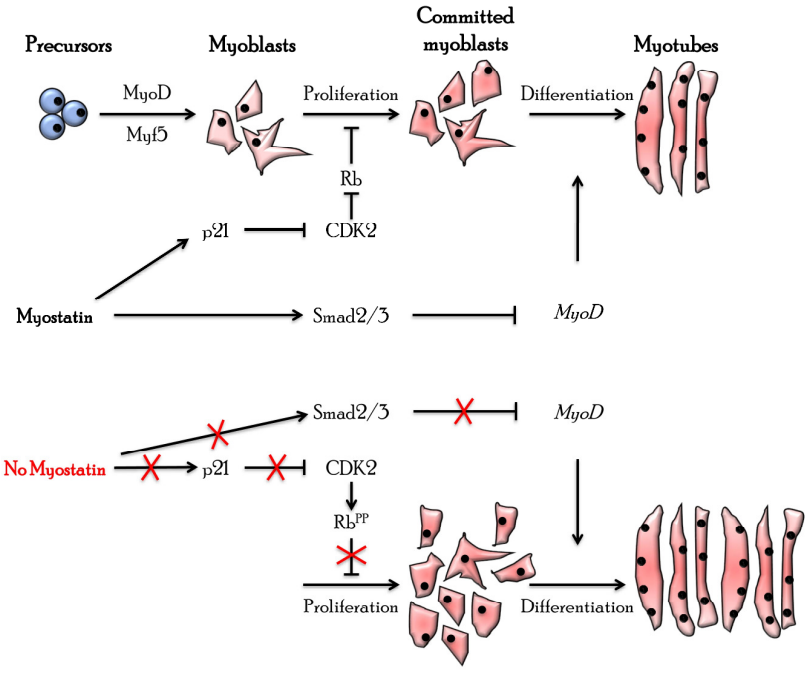

Table 1 Polymorphisms on *Myostatin* gene in cattle.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
Asturiana de los Valles	nt821	DEL11	Grobet *et al.*, 1997
Belgian Blue	nt821	DEL11	McPherron & Lee, 1997
Blonde d'Aquitaine	nt821 nt3811	DEL11 T>G	Kambadur *et al.*, 1997 Bouyer *et al.*, 2014
Charolaise	nt610	C>T	Kambadur *et al.*, 1998
Gasconne	nt938	G>A	Kambadur *et al.*, 1997 Dunner *et al.*, 2003
Limousine	nt821 nt610 g.433	DEL11 C>T C>A	Kambadur *et al.*, 1997 Cappuccio *et al.*, 1998 Sellick *et al.*, 2007
Maine-Anjou	nt419 nt676	del-7-ins10 G>T	McPherron & Lee, 1997 Grobet *et al.*, 1997
Marchigiana	g.874	G>T	Cappuccio *et al.*, 1998
Nellore	nt76 nt111 nt267 nt374 nt414 nt420 nt433 nt445 nt527 nt641 nt694 nt840 nt951 nt1083	A>T G>T A>G DEL16 C>T T>G A>T A>T T>A G>A G>A A>G T>G C>T	Grisolia *et al.*, 2009
Parthenoise	nt821	DEL11	Kambadur *et al.*, 1997
Piedmontese	nt938	G>A	Kambadur *et al.*, 1997
Rubia Gallega	nt821	DEL11	Kambadur *et al.*, 1997

Table 2 Polymorphisms on *Myostatin* gene in sheep.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
Texel	g.6723 g+391 g.2449 g.2379 g.1405 g.1402 g.1214 g.1129 g.41 g.39 g+474 G+613 g+616 g+619 g+622 g+632 g+696 g+3135 g+4036 g+4044	G>A G>T C>G C>T A>T G>A C>T C>T A>C T>C C>T T>C G>A T>C T>C G>T C>T C>T A>C C>T	Kijas *et al.*, 2007
Norwegian White Sheep	c.960 c.2360	DEL1 G>A	Wang *et al.*, 2016
New Zealand Romney	c.101 c.-959 c.-784 c.373+18 c.373+563 c.373+607 c.374-654 c.374-54 c.748-54 c.*83 c.*455 c.*709 c.*123A c.-2449 c.-2379	G>A C>T A>G A>G A>G G>A T>C T>C A>G A>G C>A INSA T>G G>C T>C	Wang *et al.*, 2016 Kijas *et al.*, 2007
Charollais	c.*123A		Kijas *et al.*, 2007
White Suffolk	c.*123A		Kijas *et al.*, 2007
Poll Dorset	c.*123A		Kijas *et al.*, 2007
Lincoln	c.*123A		Kijas *et al.*, 2007

Indian sheep	c.539 c.821	T>G T>A	Pothuraju *et al.*, 2015
Stavropol Merino	c.373+396 c.374-362 c.374-16 c.747+185 c.748-194 c.782_783 c.940 c.*310	T>C A>T DELT C>A C>A INST G>T G>T	Trukhachev *et al., 2018*

1003 **Table 3** Polymorphisms on *Myostatin* gene in goat.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
Anhui white	g.197 nt345	G>A A>T	Zhang *et al.*, 2013 Nguluma *et al.*, 2018
Boer	nt1256 g.197 nt1388 nt345 nt298	TTTA/- G>A T>A A>T T>C	
Haimen	nt1256	TTTA/-	
Motou	nt1256	TTTA/-	
Nubi	nt1256	TTTA/-	

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1006 **Table 4** Polymorphisms on *Myostatin* gene in horse.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
American Quarter Horse	g.66495326_66495327	INS227	Petersen *et al.*, 2013
Andalusian	g.26 g.156 g.1634 g.2024 g2115 g.2327 g.4230	T>C T>C T>G G>A A>G A>C T>A	Dall'Olio *et al.*, 2010
Arabians horses	g.2279 g.66495696 g.66495254	A>C T>C C>T	Baron *et al.*, 2012 Stefaniuk *et al.*, 2016
Bardigiano	g.156	T>C	Dall'Olio *et al.*, 2010
Haflinger	g.156	T>C	Dall'Olio *et al.*, 2010
Hucul	g.26 g.66495696 g.66493737 g.66490010	T>C T>C T>C T>C	Stefaniuk *et al.*, 2014 Stefaniuk *et al.*, 2016
Italian Saddle	g.26 g.156	T>C T>C	Dall'Olio *et al.*, 2010
Italian trotter	g.26	T>C	Dall'Olio *et al.*, 2010
Polish Konik	g.66495254 g.66495696 g.66493737 g.66495254 g.66490010	C>T T>C T>C C>T T>C	Stefaniuk *et al.*, 2014 Stefaniuk *et al.*, 2016
Lipizzan	g.26	T>C	Dall'Olio *et al.*, 2010
Maremmano	g.156	T>C	Dall'Olio *et al.*, 2010
Murgese	g.156	T>C	Dall'Olio *et al.*, 2010
Noric	g.26 g.156	T>C T>C	Dall'Olio *et al.*, 2010
Polish Heavy Draft	g.26 g.66495254 g.66495696 g.66493737 g.66490010	T>C C>T T>C T>C T>C	Stefaniuk *et al.*, 2014 Stefaniuk *et al.*, 2016
Rapid Heavy Draft	g.26 g.156	T>C T>C	Dall'Olio *et al.*, 2010
Salernitano	g.156	T>C	Dall'Olio *et al.*, 2010
Soraia	g.2478	G>C	Baron *et al.*, 2012
Thoroughbred horse	g.156 g.1634 g.2115 g.2327	T>C T>G A>G A>C	Dall'Olio *et al.*, 2010 Petersen *et al.*, 2013 Petersen *et al.*, 2013 Petersen *et al.*, 2013

Tolfetano	g.156	T>C	Dall'Olio *et al.*, 2010
Uruguayan Creole	g.156	T>C	Dall'Olio *et al.*, 2010
Ventasso	g.26	T>C	Dall'Olio *et al.*, 2010

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Table 5 Polymorphisms on *Myostatin* gene in pig.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
Belgian Pietrain	g.435 g.447 g.879	G>A A>G T>A	Stinckens *et al.*, 2008
Chinese Meishan	g.879	T>A	Qian *et al.*, 2015
Yorkshire pig	nt383 exon 3 (position no specified)	T>A G>A C>T	Jiang *et al.*, 2002

1011 **Table 6** Polymorphisms on *Myostatin* gene in rabbit.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
Belgian hare	c.108 c.713 c.*194 c.747+34	C>T T>A A>G C>T	Fontanesi *et al.*, 2013
Burgundy fawn	c.108 c.713 c.*194 c.747+34	C>T T>A A>G C>T	Fontanesi *et al.*, 2013
Checkered giant	c.108 c.713 c.*194 c.747+34	C>T T>A A>G C>T	Fontanesi *et al.*, 2013
Commercial breeds (not specified)	nt476	T>C	Qiao *et al.*, 2014
Giant grey	c.108 c.713 c.*194 c.747+34	C>T T>A A>G C>T	Fontanesi *et al.*, 2013
Giant Grey	c.-125 c.373+234	T>C T>C	Sternstein *et al.*, 2014
New Zealand White	c.-125 c.373+234	T>C T>C	Sternstein *et al.*, 2014

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1014 **Table 7** Polymorphisms on *Myostatin* gene in poultry.

Breed	Polymorphisms on *MSTN*		Reference
	position	mutation	
Arbor Acre	nt167 nt177 nt304 nt322 nt326 nt334 nt334 nt1346 nt1375 nt6935 nt7263	G>A T>C G>A A>G A>G C>T C>G C>T G>A A>G A>T	Gu *et al.*, 2003 Zhang *et al.*, 2012
Bian chicken	nt234	G>A	Zhang *et al.*, 2012
Gaoyou ducks	nt2701	G>A	Liu *et al.*, 2012
Jinghai	nt326 nt334 nt1346 nt1375	A>G C>G C>T G>A	Zhang *et al.*, 2012
Pekin duck	nt129 nt708 nt952	T>C T>C T>C	Xu *et al.*, 2013
Sansui duck	g.106 g.120 g.159 g.5368 g.5389 g.5410	G>A A>G G>A G>A A>C G>A	Zhao *et al.*, 2016
Youxi	nt326 nt334 nt1346 nt1375	A>G C>G C>T G>A	Zhang *et al.*, 2012

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