Mutations in myostatin (GDF8) in Double-Muscled Belgian Blue and Piedmontese Cattle

Ravi Kambadur, Mridula Sharma, Timothy P.L. Smith and John J. Bass

Genome Res. 1997 7: 910-915
Access the most recent version at doi:10.1101/gr.7.9.910

RESEARCH

Mutations in *myostatin* (*GDF8*) in Double-Muscled Belgian Blue and Piedmontese Cattle

Ravi Kambadur,[1,2] Mridula Sharma,[1] Timothy P.L. Smith,[3] and John J. Bass

AgResearch, Ruakura, Hamilton, New Zealand; [3] U.S. Department of Agriculture (USDA)/Agricultural Research Service (ARS) Meat Animal Research Center, Clay Center, Nebraska 68933-0166

A visibly distinct muscular hypertrophy (mh), commonly known as double muscling, occurs with high frequency in the Belgian Blue and Piedmontese cattle breeds. The autosomal recessive *mh* locus causing double-muscling condition in these cattle maps to bovine chromosome 2 within the same interval as *myostatin,* a member of the TGF-β superfamily of genes. Because targeted disruption of *myostatin* in mice results in a muscular phenotype very similar to that seen in double-muscled cattle, we have evaluated this gene as a candidate gene for double-muscling condition by cloning the bovine myostatin cDNA and examining the expression pattern and sequence of the gene in normal and double-muscled cattle. The analysis demonstrates that the levels and timing of expression do not appear to differ between Belgian Blue and normal animals, as both classes show expression initiating during fetal development and being maintained in adult muscle. Moreover, sequence analysis reveals mutations in heavy-muscled cattle of both breeds. Belgian Blue cattle are homozygous for an 11-bp deletion in the coding region that is not detected in cDNA of any normal animals examined. This deletion results in a frame-shift mutation that removes the portion of the Myostatin protein that is most highly conserved among TGF-β family members and that is the portion targeted for disruption in the mouse study. Piedmontese animals tested have a G–A transition in the same region that changes a cysteine residue to a tyrosine. This mutation alters one of the residues that are hallmarks of the TGF-β family and are highly conserved during evolution and among members of the gene family. It therefore appears likely that the *mh* allele in these breeds involves mutation within the myostatin gene and that *myostatin* is a negative regulator of muscle growth in cattle as well as mice.

[The sequence data for bovine myostatin has been submitted to GenBank under accession no. AF019761.]

The muscular hypertrophy (mh), or double-muscle phenotype, is a heritable condition in cattle that primarily results from an increase in number of muscle fibers (hyperplasia) rather than the enlargement of individual muscle fibers (hypertrophy), relative to normal cattle (Hanset et al. 1982). The relative increase in fiber number is observed early in pregnancy (Swatland and Kieffer 1974) and results in a calf possessing nearly twice the number of muscle fibers at the time of birth. The occurrence of double muscling has been observed in several cattle breeds worldwide since it was first documented by Culley in 1807. The breed in which this muscular hypertrophy and its effects have been analyzed most extensively is the Belgian Blue breed, which has been systematically selected for double muscling to the point of fixation in many herds. Domestic animals other than cattle also show dramatic increases in muscle mass. Malignant hyperthermia of pigs with muscular hypertrophy (Brenig and Brem 1992) and muscle hypertrophy of cats associated with a dystrophin deficiency (Gaschen et al. 1992) have been analyzed at the molecular level.

Compared with normal cattle, Belgian Blue and Piedmontese animals have an increased proficiency to convert feed into lean muscle and produce a higher percentage of the most desirable cuts of meat (Casas et al. 1997). These animals have less bone, less fat, and 20% more muscle on average (Shahin and Berg 1985; Hanset 1986, 1991). However, problems associated with the trait, such as reduction in stress tolerance, fertility, and calf viability in Belgian

[1]These authors contributed equally to this work.
[2]Corresponding author.
E-MAIL KambadurR@agresearch.cri.nz; FAX 64 7 838 5536.

7:910–915 ©1997 by Cold Spring Harbor Laboratory Press ISSN 1054-9803/97 $5.00

Blue have hindered exploitation of the hypertrophy by classical genetic selection (Arthur 1995).

Segregation analysis has indicated a monogenic autosomal segregation pattern for the double-muscling trait (Hanset and Michaux 1985a,b; Charlier et al. 1995). The locus has been termed ''partially recessive'' because there is some effect of a single copy of the allele, but generally the truly double muscled phenotype requires that the animal be homozygous. A mapping study utilizing a panel of microsatellite markers to scan the bovine genome (Charlier et al. 1995) localized the *mh* locus in Belgian Blue cattle to the centromeric end of the bovine Chromosome 2 (BTA2) linkage group. The map position of the *mh* locus has been refined and extended to the Piedmontese breed using additional genetic and physical markers to a 3- to 5-cM interval near the centromere of BTA2 (Casas et al. 1997) close to the position of the α collagen type III (*COL3AI*) locus (Sonstegard et al. 1997).

A recent study demonstrated that mice lacking a normal copy of the myostatin gene (*GDF8*) display a phenotype with significant similarities to the double muscling seen in cattle. *myostatin* is a member of the transforming growth factor β (TGF-β) gene superfamily specifically expressed in skeletal muscle of adult mice, as well as during early development. In the mouse study, the third exon of the gene was replaced with a *neo* cassette, removing the portion of the protein that is highly conserved among the TGF-β superfamily of genes. Animals homozygous for the disruption display an increase in skeletal muscle mass similar to that observed in homozygous *mh* cattle.

The bovine myostatin gene recently has been mapped to the same interval as the *mh* locus by genetic linkage (Smith et al. 1997), which strongly suggests that it may be the gene causing double muscling in cattle. Here we report the sequence of bovine *myostatin* and evaluate it as a candidate gene by sequence and expression analysis. Although the expression of myostatin mRNA does not appear disrupted in Belgian Blue cattle, mutation analysis reveals an 11-bp deletion mutation in the coding region of the myostatin gene in Belgian Blue cattle that would be predicted to abolish the activity of the protein, as the truncated portion encodes the peptide sequence thought to mediate essential functions (McPherron et al. 1997). In addition, a transition mutation found in animals of the Piedmontese breed affects a conserved cysteine in exon 3 of the myostatin that is also likely to affect function of Myostatin.

RESULTS

Cloning and Sequencing of bovine myostatin cDNA

The first step in these experiments was to obtain a cDNA clone of bovine myostatin (bmyostatin) from a normal animal to use as a probe and to provide sequence information for comparison to the double-muscle allele. Primers were designed based on the murine sequence (GenBank accession no. U84005) of the 5′ and 3′-untranslated regions and used for RT–PCR to amplify the entire coding region of the bovine homolog (bmyostatin) from total RNA isolated from skeletal muscles of normal Friesian cattle. Comparison of the predicted amino acid sequence of murine and bmyostatin indicates that it is highly conserved, with 93% homology between the two proteins (Fig. 1A). All of the hallmarks of the TGF-β superfamily, including signal sequence for secretion, a proteolytic processing site, and a conserved pattern of cysteine residues in the carboxy-terminal region, are conserved between mouse and cattle forms (Fig. 1A).

Developmental Expression of the bmyostatin Gene in Normal and Belgian Blue Cattle

The phenotype of the *myostatin* knockout mice suggests that *myostatin* is a negative regulator of muscle growth, because mice lacking normal gene function displayed enlarged muscles. Therefore, any mutation that decreases the amount or activity of Myostatin at the critical developmental period could lead to an increase in muscle mass. The decrease could result either from changes in the mRNA expression pattern due to mutations that affect transcription/transcript stability or from changes in the translated portion that affect the function of the protein. First, we addressed the possibility that the *mh* allele affects transcription of *bmyostatin* by comparing the pattern of expression of bmyostatin between normal and Belgian Blue animals.

RT–PCR analysis was performed on total RNA isolated from either whole embryo or M. semitendinosus muscle from various gestation stages of normal and Belgian Blue animals. Oligonucleotide primers were designed such that 513-bp coding region was amplified in a combined RT–PCR reaction (see Methods for primer sequences). As shown in Figure 2A, the expression of bmyostatin gene was found at all developmental stages in normal cattle. Low levels of message were detected up to day 29 embryos in normal animals, and increased expression of bmyostatin was detected from day 31 on-

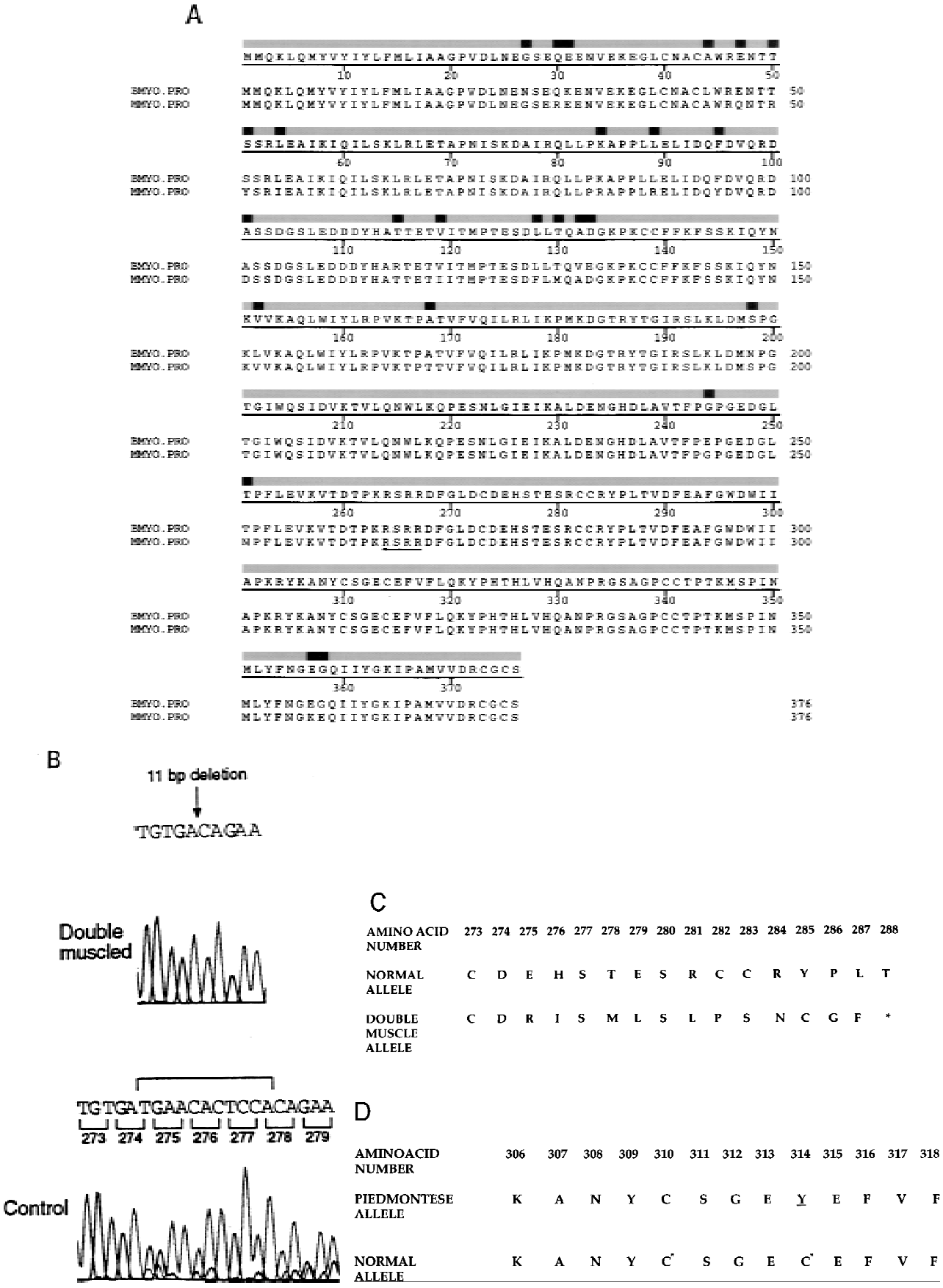

Figure 1 (*See facing page for legend.*)

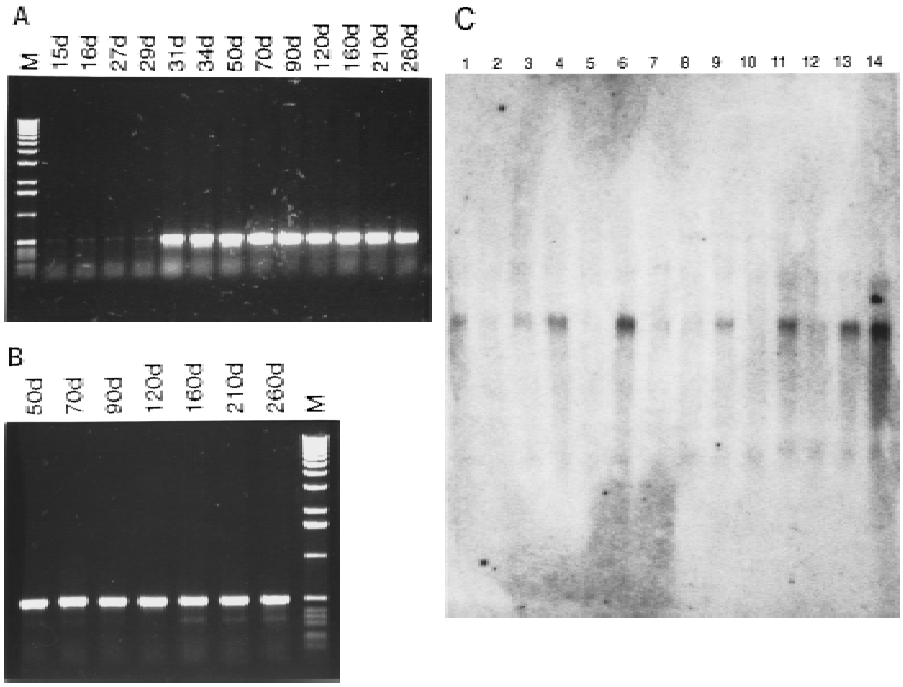

Figure 2 (*A,B*) Agarose gel electrophoresis of PCR products (513 bp) obtained from RT–PCR using total RNA from embryos or fetuses of different normal (*A*)- or double (*B*)-muscled Belgian Blue bovine developmental stages. (M) Markers (1-kb ladder from GIBCO BRL). Different embryonic or fetal ages are indicated in corresponding lanes. The locations of the primers used to amplify 513-bp partial cDNA are from amino acid 202 to 208 (sense primer) and from amino acid 365 to 371 (antisense primer). (See Methods for primer sequence.) This 513-bp partial cDNA contains the 11-bp deletion observed in double-muscled Belgian Blue cattle. (*C*) Expression of myostatin in different adult bovine muscles. Fifteen micrograms of total RNA was electrophoresed on formaldehyde–agarose gel, blotted onto nylon membrane, and probed with bovine myostatin cDNA. (*Lane 1*) M. gastrocnemius; (*lane 2*) M. psoas major; (*lane 3*) M. longissimus dorsi; (*lane 4*) M. biceps femoris; (*lane 5*) M. diaphragm; (*lane 6*) M. semimembranosus; (*lane 7*) M. flexor digitorum longus; (*lane 8*) M. vastus medialis; (*lane 9*) M. vastus lateralis; (*lane 10*) heart; (*lane 11*) M. cutaneus trunci; (*lane 12*) M. semitendinosus; (*lane 13*) M. semitendinosus (normal, 260 day); (*lane 14*) M. semitendinosus (double-muscle, 260 day).

wards to late in gestation (260-day-old fetuses). bmyostatin cDNA was also detected in Belgian Blue 50-day-old fetuses onwards and late in gestation (day 260) (Fig 2B). bmyostatin mRNA expression in fetuses prior to day 50 was not analyzed in Belgian Blue cattle. No changes were noted in the level of expression of bmyostatin between normal and Belgian Blue animals at various gestation stages examined (Fig. 2A,B), and bmyostatin continued to be expressed in the skeletal muscle of adult animals.

To compare the expression of *bmyostatin* in different skeletal muscles, we performed Northern analysis on total RNA isolated from adult tissues from normal animals. As shown in Figure 2C, the bmyostatin probe detected a single-message of 2.9-kilobase mRNA expressed in both axial and paraxial musculature. High levels of expression were observed in the hindlimb muscles M. semimembranosus and M. biceps femoris, whereas low levels of expression were detected in other hindlimb muscles (Fig. 2C). *myostatin* expression could not be detected in the heart or in the diaphragm muscle.

Figure 1 Sequence analysis of normal- and double-muscled bovine myostatin. Sequencing was performed on three independent normal- and double-muscled alleles, and one representative sequence of both alleles is shown. (*A*) Amino acid sequence comparisons of mouse (MMYO.PRO) and bovine Myostatin (BMYO.PRO) proteins. Non-conserved amino acids are indicated by solid bars. The consensus amino acid sequence is shown at the *top.* The proteolytic processing site is underlined. (*B*) The deletion mutation is detected by fluorometric sequencing of myostatin cDNA from normal- and double-muscled cattle. The sequence of the double-muscled allele is shown above that of the normal allele (Control), and the position where 11 bp is deleted in the mutant allele is indicated by an arrow. The large bracket in the normal allele sequence denotes the region that is deleted in the double-muscled allele. (*C*) The amino acid sequence of Myostatin in normal cattle is shown above the predicted amino acid sequence of Myostatin in double-muscled cattle. The premature stop codon at amino acid position 288 in the double-muscle allele is indicated by an asterisk (*). (*D*) The predicted amino acid sequence of Myostatin in normal cattle is shown below that of the Piedmontese breed in the vicinity of the transition mutation. The altered residue in the Piedmontese allele is underlined. Asterisks (*) indicate two of the nine conserved cysteine residues in exon 3 of the normal bmyostatin allele.

Mutations in bmyostatin in Belgian Blue Double-Muscled Cattle

Because no differences in expression of the bmyostatin gene could be detected by RT–PCR, we examined the sequence of the cDNA in normal- and double-muscled animals to evaluate possible changes in the protein and correlated any changes with the observed phenotype. Myostatin cDNA from animals in three unrelated double-muscled pedigrees were sequenced and screened for mutations in the coding region. This analysis revealed an 11-bp deletion in the open reading frame of the Belgian Blue *bmyostatin* allele, which results in the loss of three amino acids (275, 276, and 277) and a frameshift after amino acid 274 (Fig. 1B,C). The frameshift leads to a stop codon after amino acid 287 that is predicted to truncate the protein such that most of the same portion that was deleted in the heavy muscled knockout mice would not be translated (Fig. 1C). Another 17 pedigrees of Belgian Blue cattle were subsequently tested for this deletion by simple sizing of PCR products that include this portion of the gene, including 16 pedigrees in New Zealand and 4 in the United States. All double-muscled, purebred animals tested were homozygous for this deletion, wherease none of 11 different normal-muscled dairy and traditional beef breed cattle showed evidence of deletion in this region. On the basis of the results from the Belgian Blue analysis, myostatin mRNA from a Piedmontese breed animal was then assessed for deletions or nonsense mutations that would abnormally truncate the protein. The resulting Piedmontese cDNA sequence predicted that a full-length Myostatin protein was coded for in this breed. This result demonstrates that in contrast to the Belgian Blue breed, mh in Piedmontese animals is not the result of an abnormally truncated Myostatin protein. However, examination of the cDNA sequence revealed a G–A transiton mutation at position 941 of the coding region. This mutation predicts the replacement of cysteine at amino acid 314 with tyrosine (Fig. 1D). This cysteine is the fifth in a series of nine whose appearance and spacing is extremely conserved throughout the TGF-β and inhibin-β gene families; thus, it is likely that the observed mutation would interfere with normal function of the protein (McPherron et al. 1997).

To verify this result and assess its generality, three unrelated double-muscled pedigrees of the Piedmontese breed were then examined for the transition mutation. A primer was developed from the sequence of the second intron of the bovine gene and used in combination with a primer designed from the downstream untranslated region (see Methods). These primers amplify a 493-bp fragment containing the entire coding portion of the third exon of *bmyostatin* from amino acid residue 251 to the carboxyl terminus at 376. The amplified fragment encompasses all of the coding region downstream of the putative proteolytic processing site (McPherron et al. 1997), including the observed mutation. This allowed us to use archival DNA from Piedmontese sires to assess the generality of the mutation in herds in the United States. The predicted amino acid sequence for all three pedigrees contained the replacement of cysteine with tyrosine at residue 314. Multiple independent PCR reactions from each animal were used to generate sequence, and the mutation was consistently observed; thus it appears that Piedmontese animals are homozygous for this mutation.

DISCUSSION

We report the cloning of bovine *myostatin* and an evaluation of this TGF-β family member as a candidate gene for *mh*. Recently the bovine myostatin gene has been mapped to the same interval as the *mh* locus that causes the double-muscle phenotype (Smith et al. 1997). Comparison of the bovine and murine proteins demonstrates that *myostatin* has been very highly conserved during mammalian evolution, suggesting an important role for this gene. This role has been demonstrated by the production of knockout mice, which develop greatly enlarged muscles (McPherron et al. 1997). The double-muscled trait in Belgian Blue and Piedmontese cattle has significant similarity to the phenotype of these mice, as both involve an increase in the muscle mass (Arthur 1995; McPherron et al. 1997). The increase in body weight found in mutant myostatin mice can be explained by an increase in muscle mass resulting from muscle fiber hyperplasia and hypertrophy. The increase in body weight of double-muscled cattle cannot be totally accounted for by an increase in muscle mass alone, as the weight of the skin, adipose and bone content, alimentary tract, and most other internal organs is reduced in double-muscled cattle. Also the increased musculature of double-muscled cattle results only from hyperplasia of muscle fibers and not muscle fiber hyperplasia and hypertrophy as found in the mutant mouse (Hanset et al. 1977). These differences in bovine and mouse phenotypes could be attributable to additional genes selected for during inbreeding of double-muscled cattle breeds.

How does *myostatin* negatively regulate myogenesis? Because myostatin expression is detected in the myotome early in myogenesis through to adult skeletal muscle, it may control fiber number and size during embryonic, fetal, and postnatal myogenesis. Based on the amino acid sequence, Myostatin is a secreted protein that is specifically synthesized by muscle tissue and therefore may be involved with autocrine or paracrine cell–cell communication that regulates proliferation and/or differentiation of myoblasts.

The phenotype of the knockout mice suggests that any mutation that affects myostatin production or activity could lead to muscle hyperplasia and hypertrophy. In both mice and cattle the heterozygotes display very mild abnormalities. The mild phenotypes displayed by *mh*/+ are probably caused by haploinsufficiency. Examination of the steady-state levels of myostatin mRNA through a range of developmental stages and in different adult muscles failed to reveal any significant difference in the levels of expression in double-muscled cattle, suggesting that differences in *myostatin* transcription do not underlie the *mh* phenotype. However, sequence analysis of *mh*/*mh* animals revealed the presence of significant mutations in the third exon of the gene. Presumably the loss of a critical cysteine residue has a negative effect on the activity of Myostatin in Piedmontese cattle, although the extent to which the activity is diminished is uncertain. Certainly the truncation observed in Belgian Blue cattle is likely to severely impair Myostatin function, as it removes the majority of the part of the protein that was disrupted in the knockout mice. We conclude that *myostatin* is probably the *mh* locus and that it appears that a nonfunctional Myostatin protein is responsible for loss of control of muscle growth in double-muscled Belgian Blue and Piedmontese cattle as well as knockout mice.

METHODS

Sample Collections

Muscle biopsies (0.5 gram) were obtained from the Musculus (Mo) biceps femoris muscle from 18 adult double-muscled Belgian Blue cattle. The cattle biopsied were derived from 17 different sire lines with limited inbreeding over the last three generations. The pedigrees sampled represent germ-plasm from 17 (NZ) and 4 (USA) sire lines imported from Belgium, with each animal carrying at least one allele from a unique sire. Muscle samples from normal Friesian cattle were obtained after slaughter at the AgResearch abattoir. Normal bovine embryos from day 15 to 34 were collected in triplicate by flushing the uterine tracks of cows; fetuses from day 50 to day 260 were collected after slaughter. Double-muscled Belgian Blue fetuses (in triplicate) were obtained from the recipient cows, which were implanted with purebred Belgian Blue embryos. DNA was obtained from blood sample of Piedmontese bulls by salt extraction (Miller et al. 1988). Adult Piedmontese muscle was obtained from the M. biceps femoris immediately after slaughter in the MARC abbatoir.

RNA Extraction and RT–PCR

RNA from muscle or embryo tissue was extracted using Trizol (GIBCO BRL), according to the manufacturer's protocol. First-strand cDNA was synthesized in a 20-µl reverse transcriptase (RT) reaction from 5 µg of total RNA using a Superscript pre-amplification kit (GIBCO-BRL), according to the manufacturers protocol.

Semiquantitative PCR was performed with 2 µl of the RT reaction at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min for 35 cycles. To clone the bmyostatin entire coding sequence we used the following primers: 5′-ATGATGCAAAAACTGCAA-3′ and 5′-TCATGAGCACCCACA-3′ (1127 bp) and 5′-TCGGA-CGGACATGCACTAA-3′ and 5′-GTCTACTACCATGGCTG-GAAT-3′ (1202 bp). The primers used to amplify the 513-bp cDNA shown in Figure 2, A and B, were 5′-GGTATTTG-GCAGAGTATTGAT-3′ and 5′-GTCTACTACCATGGCTG-GAAT-3′. To clone full-length cDNA from Piedmontese cattle we used alternate primers: 5′-TCACTTGGCATTACT-CAAAAGC-3′ and 5′-TCGAAATTGAGGGGAAGACC-3′. The 493-bp product containing exon 3 of myostatin gene from Piedmontese cattle was amplified from genomic DNA using the reverse primer noted above and an intron-specific primer: 5′-TGAGGTAGGAGAGTGTTTTGGG-3′.

Sequencing and Sequence Analysis

PCR-amplified bovine *myostatin* cDNA fragments were run on a low melting point agarose gel. DNA fragments were excised from the gel, and DNA was purified using the Wizard kit (Promega) and directly sequenced on an ABI automated sequencer (model no. 377). Sequence alignments were performed by using DNA Laser Gene software (DNA STAR).

Northern Analysis

Northern analysis was performed according to Sambrook et al. (1989). Fifteen micrograms of total RNA from various muscles was run on a 1.0% formaldehyde–agarose gel and transferred to Hybond N+ membrane (Amersham). The membrane was prehybridized in 5× SSC, 50% formamide, 5× Denhardt's solution, and 1% SDS, 0.25 mg/ml of Salmon sperm DNA for 2 hr, hybridized in the same solution with *bmyostatin* cDNA probe overnight, washed at 50°C for 15 min each with 2× SSC + 0.1% SDS, and then with 0.2× SSC + 0.1% SDS.

ACKNOWLEDGMENTS

We thank B. Worsnop and W. Hooper for providing material from their Belgian Blue pedigree herd and their enthusiasm and support for this research. We are indebted to the Foun-

dation of Research and Technology (New Zealand) for financial support. The support of all members of the Growth Physiology program who have provided the infrastructure enabling this research to progress so rapidly is acknowledged. We thank Stacey Farmer and Greg Baillie for timely and excellent sequencing of DNA. We thank Kevin Tennill for expert technical assistance, Dr. Roger Stone for helpful discussion and advice, and Dr. Aravinda Chakravarti for editing assistance.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

REFERENCES

Arthur, P.F. 1995. Double muscling in cattle: A review. *Aust. J. Agric. Res.* 46: 1493–1515.

Brenig, B. and G. Brem. 1992. Genomic organization and analysis of the 5′ end of the porcine ryanodine receptor gene (ryrl). *FEBS Lett.* 298: 277–279.

Casas, E., J.W. Keele, S.D. Shackelford, M. Koohmaraie, T.S. Sonstegard, T.P.L. Smith, S.M. Kapes, and R.T. Stone. 1997. Association of the muscle hypertrophy locus with carcass traits in beef cattle. *J. Anim. Sci.* (in press).

Charlier, C., W. Coppieters, F. Farnir, L. Grobet, P.L. Leroy, C. Michaux, M. Mni, A. Schwers, P. Vanmanshoven, R. Hanset, and M. Georges. 1995. The *mh* gene causing double-muscling in cattle maps to bovine chromosome 2. *Mamm. Genome* 6: 788–792.

Culley, G. 1807. *Observations on livestock,* 4th ed. G. Woodfall, London, UK.

Gaschen, F.P., E.P. Hoffman, J.R.M. Gorospe, E.W. Uhl, D.F. Senior, G.H. Cardinet III, and L.K. Pearce. 1992. Dystrophin deficiency causes lethal muscle hypertrophy in cats. *J. Neurol Sci.* 110: 149–159.

Hanset, R. 1967. Le probleme des bovins a croupe de poulain. *Ann. Med. Vet.* 105: 14–31.

———. 1986. Double-muscling in cattle. In *Exploiting new technologies in animal breeding: Genetic development* (ed. C. Smith, J.W. King, and J.C. McKay), pp. 71–80. Oxford University Press, Oxford, UK.

———. 1991. The major gene of muscular hypertrophy in the Belgian Blue cattle breed. In *Breeding for disease resistance in farm animals* (ed. A. Owen), pp. 467–478. Commonwealth Agricultural Bureaux International, London, UK.

Hanset, R. and C. Michaux. 1985a. On the genetic determinism of muscular hypertrophy in the Belgian White and Blue cattle breed. I. Experimental data. *Genet. Sel. Evol.* 17: 359–368.

———. 1985b. On the genetic determinism of muscular hypertrophy in the Belgian White and Blue cattle breed. II. Population data. *Genet. Sel. Evol.* 17: 369–386.

Hanset, R., M. Ansay, and M. Jandrain. 1977. Morphology of the double-muscled calf-discrimination between the double-muscled and the normal. In *Cross-breeding experiments and strategy of beef utilisation to increase beef production (ed. J.L. Mason and W. Pabst), pp. 399–411. Commission of the European Communities, Luxembourg.*

Hanset, R., C. Michaux, C. Dessy-Doize, and G. Burtonboy. 1982. Studies on the 7th rib cut in double muscled and conventional cattle. Anatomical, histological and biochemical aspects. In *Muscle hypertrophy of genetic origin and its use to improve beef production* (ed. J.W.B. King and F. Menissier), pp. 341–349. Martinus Nijhoff, The Hague, The Netherlands.

McPherron, A.C., A.M. Lawler, and S.-J. Lee. 1997. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. *Nature* 387: 83–90.

Miller, S.A., D.D. Dykes, and H.F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 16: 1215.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual,* 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Shahin, K.A. and R.T. Berg. 1985a. Growth patterns of muscle, fat, and bone, and carcass composition of double muscled and normal cattle. *Can. J. Anim. Sci.* 65: 279–293.

Smith, T.P.L., N.L. Lopez-Corrales, S.M. Kappes, and T.S. Sonstegard. 1997. Myostatin maps to the interval containing the bovine *mh* locus. *Mamm. Genome* (in press).

Sonstegaard, T.S., N.L. Lopez-Corrales, S.M. Kappes, C.W. Beattie, and T.P.L Smith. 1997. Comparative mapping of human chromosome 2 identifies segments of conserved synteny near bovine mh locus. *Mamm. Genome* (in press).

Swatland, H.J. and N.M. Kieffer. 1974. Fetal development of the double muscled condition in cattle. *J. Anim. Sci.* 38: 752–757.

Vissac, B., F. Menissier, and B. Perreau. 1973. Etude du caractere culard VII. Croissance et musculature des femelles, desequilibre morphologique an velage. *Ann. Genet. Sel. Anim.* 5: 23–38.

Received July 28, 1997; accepted in revised form August 18, 1997.