

Myostatin and its implications on animal breeding: a review

R. H. S. Bellinge^{*,†}, D. A. Liberles[‡], S. P. A. Iaschi[§], P. A. O'Brien^{*} and G. K. Tay^{*,§,¶}

^{*}School of Biological Sciences and Biotechnology, Murdoch University, Western Australia 6150, Australia. [†]Concept Fertility Centre, Subiaco, Western Australia 6008, Australia. [‡]Computational Biology Unit, Bergen Centre for Computational Science, University of Bergen, Bergen, 5020 Norway. [§]Department of Pathology, The University of Western Australia, Nedlands, Western Australia 6907, Australia.

[¶]Agri-BIOTECH Pty Ltd, Claremont, 6010 Western Australia, Australia

Summary

Myostatin, or growth and differentiation factor 8 (*GDF8*), has been identified as the factor causing a phenotype known as double muscling, in which a series of mutations render the gene inactive, and therefore, unable to regulate muscle fibre deposition. This phenotype occurs at a high frequency in some breeds of cattle such as Belgian Blue and Peidmontese. Phylogenetic analysis has shown that there has been positive selection pressure for non-synonymous mutations within the *myostatin* gene family, around the time of the divergence of cattle, sheep and goats, and these positive selective pressures on non-ancestral *myostatin* are relatively recent. To date, there have been reports of nine mutations in coding regions of *myostatin* that cause non-synonymous changes, of which three cause missense mutations, including two in exon 1 and one in exon 2. The remaining six mutations, located in exons 2 and 3, result in premature stop codons, which are the mutations responsible for the double-muscling phenotype. Unfortunately, breed management problems exist for double-muscling cattle, such as birthing difficulties, which can be overcome through genetically controlled breeding programmes, as shown in this review.

Keywords Belgian Blue, carcass traits, double muscling, growth and differentiation factor 8, *myostatin*.

Introduction

The first written account of bovine muscular hypertrophy by a British farmer in his stock almanac (Culley 1807), and later described in some depth by Kaiser (1888), marked the beginning of a novel perspective on animal breeding associated with carcass characteristics. The phenotype has since become increasingly widespread among European beef cattle. This phenotype involves an exaggerated muscle development, termed 'double muscling' (DM), as can be seen in Fig. 1. It has been referred to by various authors as culard, agroppa dopia, bottle-thighed, doppelender (or double loin), Yorkshire, greyhound belly, Teeswater and double rumped.

Different symbols have been used by authors to differentiate between the DM phenotype among animals and the normal phenotype. These include double muscled or nor-

mal, DM or N, D or n, DM or dm, C or N, c or n, A or a, and mh or +.

The DM phenotype is characterized by hypertrophy of muscles, most prominently in the regions of the proximal fore- and hind quarters, and prominent muscular protrusion, with intermuscular boundaries and grooves clearly visible beneath the skin (Menissier 1982a). Other physical characteristics include fineness of the limb bones, a higher incidence of underdeveloped external genitalia, and enlarged tongues in newborn calves (Kieffer & Cartwright 1980). Double-muscled animals also have less bone, less fat and more muscle, with a higher proportion of 'expensive' cuts of meat (Menissier 1982a; Shahin & Berg 1985). However, there are some drawbacks of the DM phenotype, including reduced fertility, low calf viability, increased stress susceptibility (Arthur *et al.* 1988) and dystocia (Arthur *et al.* 1989).

History of double-muscling in cattle

Having initially being detailed by Kaiser (1888), it was then proposed by Wriedt (1929) that the phenotype was monofactorial (i.e. dictated by a single gene). This was later challenged by Kronacher (1934), who suggested the

Address for correspondence

Dr Guan K. Tay, Department of Pathology, The University of Western Australia, Nedlands, Western Australia 6907, Australia.

E-mail: guan@cyllene.uwa.edu.au

Accepted for publication 1 December 2004



Figure 1 An example of a bull homozygous for the mutant *mh* allele, the current terminal sire, urbane, from the Belazure Belgian Blue Stud, Perth, Western Australia, at 2 years of age.

phenotype was trifactorial, in which two genes partly determine the variability of the trait, and one controlled the expression or suppression. Sometime later it was proposed by Quesada & Cachafeiro (1971) that DM was controlled by a difactorial model, with nine different genotypes expressed as four different phenotypic classes.

The Belgian Blue (Blanc Bleu Belge, Belgian White and Blue, or BBB) has long been the subject of intense scrutiny as an archetypal depiction of the DM condition. Originally resulting from crosses between English Shorthorns and Dutch Friesians with the native cattle herds of Belgium around 1850 (Friend 1978), it was selected as a dual purpose animal, for both carcass and milk characteristics. In the 1940s, the breed was segregated into two, one for carcass and the other for milk. But then the breed has become highly specialized for superior carcass characteristics.

Physiological evaluation of double muscling

Despite the naming, the DM phenotype has no association with a duplication of muscles, but rather an increase in the number of muscle fibres (hyperplasia), and fibre enlargement (hypertrophy) (Swatland & Kieffer 1974), with DM cattle possessing nearly twice the number of muscle fibres at birth than normal cattle (Gerrard *et al.* 1991). The DM cattle have a higher percentage of white muscle fibres (West 1974), as well as lower collagen content (Uytterhaegen *et al.* 1994). It has also been reported that the muscle of DM cattle contains less connective tissue, which would contribute to its increased tenderness (Hanset 1991). In addition, differing levels of circulating hormones have been found in DM compared with normal cattle. It was originally held that higher growth hormone (GH) levels were found in DM compared with normal cattle (Michaux *et al.* 1982), although a recent study by Hocquette *et al.* (1999) showed that the blood plasma levels of GH in DM cattle were consistently lower than those of normal cattle. A similar result was found for insulin, with normal

animals higher in insulin than DM cattle (Arthur *et al.* 1990), as well as serum concentrations of creatine and creatinine (Hanset & Michaux 1982), and muscle IGF2 mRNA concentration (Gerrard *et al.* 1991).

During forced exercise, DM cattle showed signs of fatigue faster than normal cattle (Holmes *et al.* 1973), relating to metabolic acidosis because of a reduced blood circulation (lower blood volume and lower haematocrit count), leading to a deficiency in the transport of oxygen on the one hand and a reduction of aerobic metabolic activity in the muscle of DM cattle on the other (Menissier 1982a). The large muscle mass of DM cattle results in higher heat production during heat stress, which is compounded by a lower capacity for heat dissipation (a reduction in respiratory capacity). However, it should be noted that expression of the DM phenotype is influenced not only by breed, but by nutrition and sex (Menissier 1982b).

As previously mentioned, the amount of fat in the carcass of DM cattle is significantly less than normal cattle, and in particular, intramuscular fat, known as marbling, are reported to be significantly affected by the DM phenotype (Hanset 1991). The minimal marbling contributes to a reduced flavour rating, and the cause for this reduced marbling is reported to be a reduced subcutaneous and internal fatty tissue adipocyte size, although the adipocyte size within the intramuscular fat appear to be the same between DM and normal cattle (Hocquette *et al.* 1999). Studies by Webb *et al.* (1998) demonstrated that variation also exists in the composition of fat depots in the Belgian Blue breed when compared with normal breeds. Such variations were encountered when assessing the total lipid content and percentage polyunsaturated fat levels of the DM intramuscular fat content, with a lower value, and much higher percentage value, respectively, for DM cattle in comparison with normal cattle breeds (11% fatty acids compared with 2.7–6.1% reported for other breeds). Also, a significantly higher proportion of polar lipid fatty acids and linoleic acids were found in the intramuscular fat of the Belgian Blue breed (Smet *et al.* 2000). The effect on producers and consumers is that DM animals develop a significantly higher proportion of lean meat than normal cattle types. The meat of DM animals is also significantly lower in fat, and those fats remaining tend to be polyunsaturated varieties (Smet *et al.* 2000). Both of these characteristics conform strongly to current health standards.

Double-muscled cattle also show a reduced fertility relative to normal cattle, and it has been observed that DM cattle were less capable of carrying to term (Strath *et al.* 1981). It has been suggested that DM embryos have a higher mortality rate (Rollins *et al.* 1972).

Genetic evaluation of *myostatin* and its role in muscle regulation

The mutation for muscle hypertrophy (*mh*) is located in the *myostatin* (*MSTN*) or growth and differentiation factor 8

(*GDF8*) gene, which is highly conserved across species and is expressed in developing and mature skeletal muscle (McPherron *et al.* 1997). The *myostatin* gene has been mapped to the distal end of chromosome 2 in cattle (Grobet *et al.* 1997), and consists of three exons and two intronic regions in all species studied including the pig (AY208121), buffalo (AH013313), zebra fish (AY323521), gilthead sea bream (AF258447), chicken (AF346599) and house mouse (AY204900) (Grobet *et al.* 1997; McPherron *et al.* 1997).

Myostatin is a member of the transforming growth factor (TGF)- β superfamily and is dissimilar enough to resist classification into the existing TGF- β subfamilies, such as the bone morphogenic proteins or the inhibins. This deviation from the typical TGF- β family is especially evident in the C-terminal region (McPherron *et al.* 1997).

Myostatin actively represses skeletal muscle growth. The presence of the mutant *mh* allele in loss-of-function homozygotes thus leads to muscular hypertrophy. However, the mutation does not have to be present in the homozygous condition in order for an effect to be detected. A study by Casas *et al.* (1998) found that animals inheriting a single copy of the mutant *mh* allele from a crossbred Belgian Blue, or crossbred Piedmontese sire, had increased longissimus muscle area and retail yield, and reduced external and intramuscular fat deposition compared with animals receiving no copies of the mutant *mh* allele.

There are at present six identified mutations within the *MSTN* sequence that give rise to muscular hypertrophy by inactivating this gene (Grobet *et al.* 1997). In the Belgian Blue, *mh* is due to an 11-bp deletion of nucleotides 821–831 inclusive, and is referred to as *nt821(del11)*. This results in a frame shift and subsequent premature termination in the bioactive carboxy-terminal domain of the gene (Grobet *et al.* 1997), which is highly conserved in the TGF- β superfamily. The same mutation is responsible for DM in the Asturiana breed (Georges *et al.* 1998). On the contrary, DM Piedmontese and Gasconne cattle have a G to A transition that changes a cysteine residue to a tyrosine in the same highly conserved region of the gene, known as *C313Y*, and occurring as nucleotide position 938, corresponding to exon 3, which disrupts a disulphide bridge that is essential for the proper conformation of the protein (Kambadur *et al.* 1997; McPherron & Lee 1997). Other reported mutations include an indel in which 10 unrelated bases are inserted in the place of seven bases that have been deleted at nucleotide 419 in exon 2, known as *nt419(del7-ins10)*, a C to T transition at nucleotide 610 in exon 2, known as *Q204X*, a G to T transversion at nucleotide 676 in exon 2, known as *E226X*, and a G to T transversion at nucleotide 874 in exon 3, known as *E291X* (Kambadur *et al.* 1997; Grobet *et al.* 1998; Cappucio *et al.* 1998). These mutations result in an inactive protein that is unable to regulate muscle fibre deposition.

In addition to these loss-of-function mutations within the coding regions of *myostatin*, a study by Grobet *et al.* (1998)

of 35 double-musced cattle from 10 European breeds determined a number of mutations in the intronic as well as the coding regions of the *myostatin* gene, most notably a C to A transition at nucleotide 282 in exon 1, known as *F94L*, resulting in a conservative phenylalanine to leucine substitution at amino acid position 94. Recently, two additional non-synonymous missense mutations have been characterized by Miranda *et al.* (2000), referred to as *S105C* and *D182N*, occurring in exons 1 and 2, respectively.

It was also determined by Grobet *et al.* (1998) that the Limousin and Blonde d'Aquitaine breeds had unidentifiable loss-of-function mutations within the *MSTN* gene. Most Limousin animals were homozygous for the *F94L* mutation, although this mutation is unlikely to cause the loss of the regulatory function of *MSTN*. The Blonde d'Aquitaine animals were typically homozygous wild type. This indicates that there are as yet unidentified factors leading to muscular hypertrophy, perhaps located outside the *MSTN* coding region.

Haplotypes in cattle breeds

Haplotype diversity across breeds has been assessed by Dunner *et al.* (2003) who have shown that, of the six breeds to display the *nt821(del11)* mutation, only the Belgian Blue breed seems to be genetically homogeneous. It was determined that the European muscular hypertrophy, defined as that caused by the *nt821(del11)* mutation, came about from different waves from northern European breeds whose purpose was mainly for milk. It was also surmised by Dunner *et al.* (2003) that some of the other mutations arose in single breeds, and were thus highly selected, and as such have not evolved in other populations to any notable extent.

Calving difficulties

Hanset (1991) determined that the gestation period for DM calves is longer, resulting in offspring with a higher birth weight than normal calves. This leads to higher instances of dystocia (calving difficulties) with high mortality rates if births are unassisted (Wiener *et al.* 2002). Further exacerbating this problem is high levels of muscling in the cow pelvis, preventing pelvic distension, or 'springing', thus preventing calving without assistance (Wiener *et al.* 2002). Animals that are heterozygous for the DM mutation are larger than their wild type counterparts, and do not suffer the same birthing difficulties occurring in homozygous mutant animals. Whereas a dam that is homozygous for DM has little to no chance of calving without assistance, a heterozygous dam will have a far greater capacity for unassisted birthing. This problem renders the DM phenotype cost-ineffective because of the absolute requirement for calving assistance without the assessment of the dam for homo/heterozygosity.

Because of the birthing difficulties encountered by mothers that are homozygous for the mutant *mh* allele, a simple typing method for the animals would prove to be a benefit for the breeder, as mothers heterozygous for the mutant *mh* allele encounter a low frequency of birthing difficulties, and are capable of delivering calves homozygous for the mutant *mh* allele. The reduction in birthing difficulties not only reduces cost but also reduces the risk of loss of the animal, either mother or calf.

Identification of double muscling

Identification of DM is typically based on the degree of hypertrophy, assessed by presence of intermuscular grooves and other external characteristics associated with the phenotype, such as pelvic inclination, and the apparent higher attachment point of the tail. Identification in the past was dictated purely by the above-mentioned phenotypic traits, but since the characterization of the *myostatin* gene by McPherron *et al.* (1997), and soon after the identification of mutant *mh* in cattle (Grobet *et al.* 1997), the identification is almost totally achieved via genetic marker testing.

As previously mentioned, the *mh* locus is characterized by an 11-bp deletion within exon 3 in Belgian Blues. As this is located within a highly conserved region, the bioactive carboxy-terminal domain, this deletion can be used as a marker for DM. Multiple molecular techniques have been used to identify carriers of the mutant *mh* locus. Fahrenkrug *et al.* (1999) first described a test using fluorescent polymerase chain reaction (PCR) to test for the presence of the *nt821(del11)* and *C313Y* mutations in Belgian Blue and Piedmontese breeds. A year later Karim *et al.* (2000) described a procedure that allowed for the simultaneous genotyping of six loss-of-function mutations in the bovine *myostatin* gene. These six mutations were *nt419(del7-ins10)*, *Q204X*, *E226X*, *nt821(del11)*, *C313Y* and *E291X*. These assays present a distinct advantage to breeders in managing their breeding programmes. These tests use simple sequence specific primers (SSP) to detect the presence or absence of the mutations and are cost-effective, bypassing the need to sequence products, a costly and time-consuming exercise.

Phylogenetics of *myostatin*

A recent systematic analysis for chordate protein-encoding genes under positive selective pressure implicated the *myostatin* gene family as having been under recent positive selective pressure in both Artiodactyls and in teleost fish, as detected by the ratio of non-synonymous to synonymous nucleotide substitution rates or Ka/Ks (Liberles *et al.* 2001). The Ka/Ks ratio detects periods when the averaged selective pressure for substitution in a gene is higher than the averaged selective pressure to retain the ancestral sequence

in the population (positive selection), and is a potential indicator of modified protein function.

Subsequent sequencing of *myostatin* from goat (Tay *et al.* 2004) and gaur (Tellgren *et al.*, 2004) and re-examination of the Artiodactyl gene tree using a more sensitive method based on the Ka/Ks ratio (Siltberg & Liberles 2002) resulted in the tree shown in Fig. 2. The branches stemming from the divergence of bovinæ and caprinae appear to be under positive selective pressure. Along the bovine lineage, it is likely that in addition to recent selective pressures on *myostatin* during the domestication process of cattle, *myostatin* has also been under selective pressure during a more ancient divergence event (Tellgren *et al.*, unpublished data).

Examination of the substitutions that have occurred between cow and sheep do not coincide with positions that are linked to the DM phenotype. This is not surprising, if one expects selective pressures for more subtle changes in activity to have occurred during the speciation process. An X-ray structure exists for a paralog, bone morphogenetic protein 2 (Scheufler *et al.* 1999), an α - β -fold possessing six conserved critical cysteine residues shared with *myostatin*. Examination of the cow–sheep substitution pattern in the context of the BMP2 structure indicates an Ala–Gly substitution at the dimer interface and two substitutions (Gly–Glu and Glu–Lys) near the expected receptor-binding site. The remaining substitutions lie adjacent to the α -helix. Understanding of the role of these substitutions on function awaits a better understanding of the sequence–structure–function relationship for *myostatin*.

It is unclear from current phylogenetic data exactly when the selective pressure for substitution in *myostatin* occurred. The divergence of bovinæ and caprinae occurred about 23 million years ago (Hassanin & Douzery 1999) and appears to correlate with a period of generally warm temperatures punctuated by brief cooler periods in northern latitudes (Zachos *et al.* 1997) where ancestral bovidæ lived

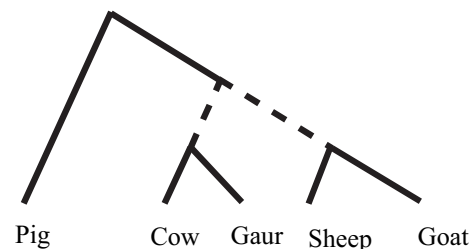


Figure 2 A phylogenetic tree shows the relationship between pig, cow, gaur, goat and sheep *myostatin* sequences as reported in The Adaptive Evolution Database (Liberles *et al.* 2001, see <http://www.sbc.su.se/~liberles/TAED2002/>), subsequently in Tellgren *et al.* (unpublished data), and corresponds with accepted species taxonomy. Ka/Ks calculation over this tree shows the dashed branches have Ka/Ks ratios >1. These branches are candidate periods for adaptive evolution in the *myostatin* gene family, which will be better resolved through ongoing sequencing efforts from additional species.

in Eurasia. It may be possible that differential adaptation to changing climates and/or migration south (in the bovine lineage) drove divergence in *myostatin* sequence, molecular functional interactions, and ultimately in body musculature and size.

Conclusion

In cattle, the phenotype known as DM is attributed to mutations in the *myostatin* gene, which exerts a regulatory influence on muscle deposition. In double-muscled animals, the mutations render the gene inactive, and mutations in the gene can be utilized as markers to type for the double-muscled phenotype.

The identification and isolation of the *myostatin* gene brought a conclusion to the near two century debate on the origins of the DM phenotype. Rather than the physical observations previously used to identify *mh*, it is now possible to identify and differentiate via direct genotyping between those heterozygous individuals carrying one copy of the inactivated *myostatin* gene and those homozygous individuals carrying two inactivated *myostatin* gene copies (Fahrenkrug *et al.* 1999; Karim *et al.* 2000).

Although mutations in the *myostatin* gene have only been found in cattle breeds the genetic modification of other commercially important species, such as sheep, pig and goat may not be that far away, although there are many ethical hurdles. The prospect of artificially mutating the *myostatin* gene in other species is enticing from an economical standpoint. However, modification in animals is currently restricted to engineering animals either to render their organs immunologically compatible for human transplantation, or for improving the commercial production of recombinant proteins in the transgenic mammary gland (Thomson *et al.* 2003).

Acknowledgement

This work was funded by Agri-BIOTECH Pty Ltd, Claremont, Western Australia 6010.

References

- Arthur P.F., Makarechian M. & Price M.A. (1988) Incidence of dystocia and perinatal calf mortality resulting from reciprocal crossing of double-muscled and normal cattle. *Canadian Veterinary Journal* **29**, 163–7.
- Arthur P.F., Makarechian M., Price M.A. & Berg R.T. (1989) Heterosis, maternal and direct effects in double-muscled and normal cattle: I. Reproduction and growth traits. *Journal of Animal Science* **67**, 902–10.
- Arthur P.F., Makarechian M., Salmon R.K. & Price M.A. (1990) Plasma growth hormone and insulin concentrations in double-muscled and normal bull calves. *Journal of Animal Science* **68**, 1609–15.

- Cappuccio I., Marchitelli C., Serracchioli A., Nardone A., Filippini F., Ajmone-Marsan P. & Valentini A. (1998) A G-T Transversion Introduces a Stop Codon at the *mh* Locus in Hypertrophic Marchigiana Beef Subjects. Abstracts of the XXVth International Conference on Animal Genetics, 9–14 August 1998, Auckland, New Zealand.
- Casas E., Keele J.W., Shackelford S.D., Koohmaraie M., Sonstegard T.S., Smith T.P., Kappes S.M. & Stone R.T. (1998) Association of the muscle hypertrophy locus with carcass traits in beef cattle. *Journal of Animal Science* **76**, 468–73.
- Culley G. (1807) *Observations in Livestock*. G. Woodfall, London.
- Dunner S., Miranda M.E., Amigues Y., Canon J., Georges M., Hanset R., Williams J. & Menissier F. (2003) Haplotype diversity of the myostatin gene among beef cattle breeds. *Genetics Selection Evolution* **35**, 103–18.
- Fahrenkrug S.C., Casas E., Keele J.W. & Smith T.P. (1999) Technical note: direct genotyping of the double-muscling locus (*mh*) in Piedmontese and Belgian Blue cattle by fluorescent PCR. *Journal of Animal Science* **77**, 2028–30.
- Friend J. (1978) *Cattle of the World*. Blandford Press Ltd, Poole, UK.
- Georges M., Grobet L., Poncelet D., Royo L.J., Pirottin D. & Brouwers B. (1998) Positional candidate cloning of the bovine *mh* locus identifies an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Proceedings of the 6th World Congress on Genetics Applied to Livestock Production* **26**, 195–204.
- Gerrard D.E., Thrasher K.H., Grant A.L., Lemenager R.P. & Judge M.D. (1991) Serum-induced myoblast proliferation and gene expression during development of double muscled and normal cattle. *Journal of Animal Science* **69**, 317.
- Grobet L., Martin L.J., Poncelet D. *et al.* (1997) A deletion in the bovine myostatin gene causes the double-muscled phenotype in cattle. *Nature Genetics* **17**, 71–4.
- Grobet L., Poncelet D., Royo L.J., Brouwers B., Pirottin D., Michaux C., Menissier F., Zanotti M., Dunner S. & Georges M. (1998) Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mammalian Genome* **9**, 210–3.
- Hanset R. (1991) Breeding for disease resistance in farm animals. In: *CAB International* (Ed. by J. B. Owen & R. F. E. Axford), pp. 467–78. Axford, Wallingford, UK.
- Hanset R. & Michaux C. (1982) Creatine and creatinine levels in plasma red cells and muscles as characteristics of double-muscled cattle. In: *Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production* (Ed. by J.W.B. King & F. Menissier), pp. 237–56. Martinus Nijhoff Publishers, The Hague.
- Hassanin A. & Douzery E.J.P. (1999) The tribal radiation of the family Bovidae (Artiodactyla) and the evolution of the mitochondrial cytochrome b gene. *Molecular Phylogenetics and Evolution* **13**, 227–43.
- Hocquette J.F., Bas P., Bauchart D., Vermorel M. & Geay Y. (1999) Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. *Comparative Biochemistry and Physiology: Part A. Molecular and Integrative Physiology* **122**, 127–38.
- Holmes J.H., Ashmore C.R. & Robinson D.W. (1973) Effects of stress on cattle with hereditary muscular hypertrophy. *Journal of Animal Science* **36**, 684–94.

- Kaiser (1888) Über die sogenannten doppellendigen. *Rinderrassenerhebung Landwirtschaftliche Jahrbuch* **17**, 387–403.
- Kambadur R., Sharma M., Smith T.P. & Bass J.J. (1997) Mutations in myostatin (GDF8) in double-muscled Belgian Blue and Piedmontese cattle. *Genome Research* **7**, 910–6.
- Karim L., Coppieters W., Grobet L., Valentini A. & Georges M. (2000) Convenient genotyping of six myostatin mutations causing double-muscling in cattle using a multiplex oligonucleotide ligation assay. *Animal Genetics* **31**, 396–9.
- Kieffer K.M. & Cartwright T.C. (1980) *Double Muscling in Cattle*, Technical Report No. B-1325. The Texas A&M University System, College Station, TX.
- Kronacher C. (1934) Genetik und Tierzuchtung. In: *Handbuch der Vererbungswissenschaft* (Ed. by E. Baur & M. Hartmann), 3:139. Gerbruder Borntraeger, Berlin.
- Liberles D.A., Schreiber D.R., Govindarajan S., Chamberlin S.G. & Benner S.A. (2001) The adaptive evolution database (TAED). *Genome Biology* **2**, research0028.1-0028.6.
- McPherron A.C. & Lee S.J. (1997) Double muscling in cattle due to mutations in the myostatin gene. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12457–61.
- McPherron A.C., Lawler A.M. & Lee S.J. (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* **387**, 83–90.
- Menissier F. (1982a) General survey of the effect of double muscling on cattle performance. In: *Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production* (Ed. by J.W.B. King & F. Menissier), pp. 23–53. Martinus Nijhoff Publishers, The Hague.
- Menissier F. (1982b) Present state of knowledge about the genetic determination of muscular hypertrophy or the double muscled trait in cattle. In: *Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production* (Ed. by J.W.B. King & F. Menissier), pp. 387–428. Martinus Nijhoff Publishers, The Hague.
- Michaux C., van Sichem-Reynaert C.R. *et al.* (1982) Endocrinological studies on double muscled cattle: LH, GH, testosterone and insulin plasma levels during the first year of life. In: *Muscle Hypertrophy of Genetic Origin and its Use to Improve Beef Production* (Ed. by J.W.B. King & F. Menissier), pp. 350–67. Martinus Nijhoff Publishers, The Hague.
- Miranda M.E., Dunner S., Amigues Y. *et al.* (2000) *SNP Screening at the Myostatin Gene Level in European Cattle Breeds*. Proceedings of 27th International Conference on Animal Genetics Minneapolis, USA.
- Quesada S.A. & Cachafeiro B.M.E. (1971) Reproduction de la femelle cularde en race Asturienne. 22nd Reunion annuelle, Federation Europeenne de Zootechnie, Annales ge Genetique et de Selection Animale **4**, 132.
- Rollins W.C., Tanaka M., Nott C.F.G. & Thiessen R.B. (1972) On the mode of inheritance of double-muscled conformation in bovines. *Hilgardia* **41**, 433–55.
- Scheufler C., Sebald W. & Hulsmeyer M. (1999) Crystal structure of human bone morphogenetic protein-2 at 2.7Å resolution. *Journal of Molecular Biology* **287**, 103–15.
- Shahin K.A. & Berg R.T. (1985) Growth patterns of muscle, fat and bone, and carcass composition of double muscled and normal cattle. *Canadian Journal of Animal Science* **65**, 279–93.
- Siltberg J. & Liberles D.A. (2002) A simple covarion-based approach to analyse nucleotide substitution rates. *Journal of Evolutionary Biology* **15**, 588–94.
- Smet S.D., Webb E.C., Claeys E., Uytterhaegen L. & Demeyer D.I. (2000) Effect of dietary energy and protein levels on fatty acid composition of intramuscular fat in double-muscled Belgian Blue bulls. *Meat Science* **56**, 73–9.
- Strath R.A., Thompson J.R. *et al.* (1981) *Reproduction in Cattle Displaying Muscular Hypertrophy*, 60th Annual Feeders' Day Report. Department of Animal Science, University of Alberta, Canada.
- Swatland H.J. & Kieffer N.M. (1974) Fetal development of the double muscled condition in cattle. *Journal of Animal Science* **38**, 752–7.
- Tay G.K., Iaschi S.P.A., Bellinge R.H.S., Chong F.N. & Hui J. (2004) The development of sequence-based-typing of myostatin (GDF-8) to identify the double muscling phenotype in the goat. *Small Ruminant Research* **52**, 1–12.
- Tellgren A., Berglund A.C., Savolainen P., Janis C.M. & Liberles D.A. (2004) Myostatin rapid sequence evolution in ruminants predates domestication. *Molecular Phylogenetics and Evolution* **33**, 782–90.
- Thomson A.J., Marques M.M. & McWhir J. (2003) Gene targeting in livestock. *Reproduction (Suppl)* **61**, 495–508.
- Uytterhaegen L., Claeys E. & Demeyer D. (1994) Effects of exogenous protease effectors on beef tenderness development and myofibrillar degradation and solubility. *Journal of Animal Science* **72**, 1209–23.
- Webb E.C., De Smet S., Van Nevel C., Martens B. & Demeyer D.I. (1998) Effect of anatomical location on the composition of fatty acids in double-muscled Belgian Blue cows. *Meat Science* **50**, 45–53.
- West R.L. (1974) Red to white fiber ratios as an index of double muscling in beef cattle. *Journal of Animal Science* **38**, 1165–75.
- Wiener P., Smith J.A., Lewis A.M., Woolliams J.A. & Williams J.L. (2002) Muscle-related traits in cattle: the role of the myostatin gene in the South Devon breed. *Genetics Selection Evolution* **34**, 221–32.
- Wriedt C. (1929) Die Vererbung des Doppellender – Kharacters die Rindern. *Zeitschrift fuer Induktive Abstammungs und Vererbungslehre* **51**, 482–6.
- Zachos J.C., Flower B.P. & Paul H. (1997) Orbitally paced climate oscillations across the oligocene/miocene boundary [Letter]. *Nature* **388**, 567–70.