Effect of Myostatin (*MSTN*) g+6223G>A on Production and Carcass Traits in New Zealand Romney Sheep

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ABSTRACT : Myostatin, which is also known as growth and differentiation factor 8 (GDF8), has been reported to act as a negative regulator of skeletal muscle development. Variation in the myostatin gene (*MSTN*) has been associated with variation in muscularity in certain "meaty" sheep breeds. Polymerase Chain Reaction-Single Strand Conformational Polymorphism (PCR-SSCP) analysis was used to investigate allelic variation in the previously described g+6223G>A single-nucleotide polymorphism (SNP) in the 3' untranslated region (3'UTR) of *MSTN*. The sheep studied were 79 New Zealand (NZ) Romney lambs derived from a single sire heterozyous for g+6223G>A, which is in itself notable as this polymorphism has not been described previously in this breed. Allelic variation was observed to be associated with an abnormal gender ratio (p = 0.046) in the progeny. The presence of allele A was observed to have an effect (p<0.05) on birth weight, mean loin yield, proportion yield loin and total muscle yield. Allelic variation did not significantly affect mean shoulder yield, leg yield, proportion yield shoulder and proportion yield leg. This preliminary result suggests that while the A allele at *MSTN* g+6223 appears to improve some valuable traits in NZ Romney sheep, further research is required to understand if and how it may affect other traits. (**Key Words:** Myostatin, MSTN, GDF8, g+6223G>A, Sheep, Carcass Trait)

INTRODUCTION

Myostatin, which is also known as growth and differentiation factor 8 (GDF8), acts as a negative regulator of skeletal muscle growth (McPherron et al., 1997). It may also contribute both to the regulation of adipogenesis (Lee and McPherron, 2001) due to reduced production and secretion of leptin (McPherron and Lee, 2002), and to the regulation of tendon structure and function during prenatal and postnatal development (Mendias et al., 2008). Variation in the myostatin gene (*MSTN*) has been associated with a "double-muscling" phenotype in various mammalian species including mice (McPherron et al., 1997), cattle (Kambadur et al., 1997), humans (Schuelke et al., 2004), dogs (Mosher et al., 2007), pigs (Stinckens et al., 2008) and

sheep (Clop et al., 2006; Kijas et al., 2007; Johnson et al., 2009).

The association of muscular hypertrophy with ovine *MSTN* variation was first reported in Belgian Texel sheep (Clop et al., 2006), which have been described as a "meaty" sheep breed. Twenty single-nucleotide polymorphisms (SNPs) were identified in the Texel sheep across an extended region of the gene. Among these, a SNP in the 3' untranslated region (3'UTR) g+6223G>A (previously called g+6723G>A) was shown to create or destroy putative miRNA target sites and hence affect muscularity (Clop et al., 2006). Subsequently, this SNP was also found in other sheep including Australian Texel (Kijas et al., 2007), New Zealand (NZ) Texel (Johnson et al., 2009), Charollais (Hadjipavlou et al., 2008), and White Suffolk, Poll Dorset and Lincoln sheep in Australia (Kijas et al., 2007). Thus, *MSTN* g+6223G>A has been suggested as a useful gene-marker for breeding for improved carcass traits in sheep (Kijas et al., 2007; Hadjipavlou et al., 2008; Johnson et al., 2009). *MSTN* g+6223G>A is now used as a commercial gene-marker for sheep breeding selection in NZ and elsewhere under the name MyoMAX® (Pfizer Animal Health, Dunedin, NZ). It is claimed by this company that a

sheep with MyoMAX®, having one or two copies of the A allele of *MSTN* g+6223G>A, has at least 5% increased muscling in the leg and rump.

In this study, Polymerase Chain Reaction-Single Strand Conformational Polymorphism (PCR-SSCP) analysis was used to investigate the association of *MSTN* g+6223G>A with carcass production traits in a single sire-line of NZ's most common maternal breed, the NZ Romney, a breed developed over a period of about 140 years from English Romney Marsh sheep and which constitutes 41.1% of the NZ flock (Meat and Wool NZ, 2008).

MATERIALS AND METHODS

Sheep investigated and data collection

The presence of *MSTN* g+6223G>A allelic variation was investigated in 79 lambs, derived from a single NZ Romney sire, heterozygous for *MSTN* g+6223G>A. These lambs were produced from 60 ewes that were mated and lambed outdoors on pasture and that were all homozygous for *MSTN* g+6223G. The lambs were raised outside on mixed ryegrass-clover pasture without supplementation. They were slaughtered when their live-weight exceeded 38 kg. The lambs and ewes were part of a much larger trial involving 17 sire lines and 60 ewes each, but none of the other rams were positive for g+6223G. Birth rank (single or multiple birth), birth weight and gender were recorded within 12 h of birth.

At the slaughter of the male progeny (n = 32), data were recorded for hot carcass weight, loin yield, shoulder yield, leg yield, total yield, proportion yield loin, proportion yield shoulder and proportion yield leg, which were estimated with video imaging analysis (VIAscan® Sastek, Hamilton, QLD Australia) at the Alliance Food Processing Smithfield Plant, Timaru, NZ. Loin yield, shoulder yield and leg yield are the percentages of lean tissues as a proportion of the hot carcass weight. Total yield is the sum of the leg, loin and shoulder yield. The proportion yield of leg, loin or shoulder is the yield of the specific cut, divided by the total yield.

Genotyping of *MSTN* g+6223G>A using PCR-SSCP

Blood samples from all of the lambs and the sire were collected on FTA cards (Whatman, Middlesex, UK) and DNA was purified using a two-step procedure described by Zhou et al. (2006). A 273 bp fragment of the 3'UTR region of *MSTN* was amplified using the PCR primers 5'-AATTA GTTGATTAAATAGTGGT-3' and 5'-ACAATTTGTAAGA TACCATCAG-3' designed to span the g+6223G>A SNP. Amplicons were subject to SSCP analysis in 14% polyacrylamide gels at 390 V and 4°C for 19 h in 0.5×TBE buffer. Amplicons of previously identified g+6223G and g+6223A alleles were also included in each gel and their banding patterns were used as standards for determining the alleles present in individual sheep.

Statistical analysis

GenStat (Version 11, VSN International Ltd, Hempstead, UK) was used to analyze the data. Cross classification models were used to explore associations between genotype, gender and birth rank and REML mixed model analysis used to study the effect of these factors on meat traits.

A half-sib (segregation) analysis was performed to ascertain if the presence (or absence) of *MSTN* g+6223A in a lamb's genotype was associated with variation in birth weight and various carcass traits. Birth weight data were available for all progeny, whereas carcass data were only available for male lambs. In all cases *MSTN* g+6223A was a paternally inherited allele, as all the dams were *MSTN* g+6223G homozygous.

For each trait (birth weight, loin yield, shoulder yield, leg yield, total yield, proportion yield loin, proportion yield shoulder and proportion yield leg), a factorial REML was performed to assess the effect of sire-inherited allele, gender and birth rank (single or multiple), as well as the effect of those factors on the carcass traits. All non-significant interactions were removed from the model and only main effects are reported.

RESULTS

Allele and genotype frequencies

Two unique PCR-SSCP banding patterns were observed, corresponding to the *MSTN* g+6223A and *MSTN* g+6223G alleles (Figure 1). The genotypes observed were GG (n =
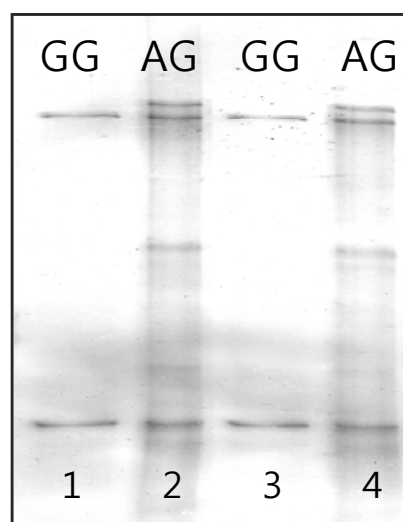
Figure 1. PCR-Single-Strand Conformational Polymorphism (PCR-SSCP) analysis of the ovine *MSTN* g+6223G>A. Two banding patterns, representing two alleles A and G were detected with homozygous genotype GG (lanes 1 and 3) and heterozygous genotype AG (lanes 2 and 4) in NZ Romney sheep.

Table 1. The effect of the ovine *MSTN* g+6223G>A on gender

	Number of progeny		p value
	GG (n = 43)	AG (n = 36)	
Female	19	24	0.046
Male	24	12	

43) and AG (n = 36) with allelic frequencies of 77.2% and 22.8% for g+6223G and g+6223A, respectively.

Effect of genotype on lamb gender, birth weight and carcass traits

An association was revealed between *MSTN* g+6223 genotype and gender (p = 0.046) with the *MSTN* g+6223AG genotype being predominant in ewe lambs (Table 1).

An association (p = 0.038) was shown between *MSTN* g+6223A presence and birth weight. Those lambs with the g+6223GG genotype (n = 43) had a mean birth weight of 5.8±0.18 kg (SEM) while lambs with the g+6223AG genotype (n = 36) had a mean birth weight of 6.2±0.23 kg.

The presence (or absence) of *MSTN* g+6223A also had an effect (p<0.05) on the mean loin yield, total yield and proportion yield loin of the male lambs. For each of these traits, male lambs with the g+6223AG genotype had higher yields than the g+6223GG progeny (Table 2). No association was found between the g+6223 variation and mean shoulder yield, leg yield, proportion yield shoulder and proportion yield leg (Table 2).

DISCUSSION

This is the first study to report an association between *MSTN* g+6223 genotype and gender ratios in New Zealand's most common maternal sheep breed, the NZ Romney, a result that is unexpected. Myostatin is primarily expressed in skeletal muscle, but it is not restricted to this tissue. *MSTN* has also been detected in the heart muscle of the mouse and sheep (Sharma et al., 1999) and mammary glands of pigs (Ji et al., 1998). In chicken embryos, myostatin expression can be detected as early as the blastoderm stage (Kocamis et al., 1999). In addition,

myostatin is also involved in human maternal/foetal nutrient partitioning by acting as a paracrine regulator (Mitchell et al., 2006).

In light of these reports, we suggest that *MSTN* might also affect other physiological activities during embryonic development, which may explain the abnormal gender ratio observed here. The actual mechanism by which this could occur is however unclear. Furthermore, in transgenic mice with muscle-specific "over expression" of myostatin, muscle mass differences were only detected in male progeny and not females (Reisz-Porszasz et al., 2003). This suggests that in the female transgenic mice, there were gender-specific mechanisms that could override the effects of myostatin on muscle mass. The putative embryonic roles of myostatin may therefore be gender-specific.

The difference in gender *MSTN* g+6223 genotype ratio detected in this study could not be explained by genotyping those lambs that died at birth, and therefore is worthy of further investigation as g+6223A is currently being bred into sheep in an effort to increase meat yield. If the presence of g+6223A affects the viability of male embryos, then the potential exists for both a new threat and new opportunities in livestock reproduction.

The presence of *MSTN* g+6223A was found to have an effect on birth weight and mean loin yield, total yield and proportion yield loin in the male lambs, while no association was found between g+6223A and mean shoulder yield, leg yield, proportion yield shoulder and proportion yield leg. We tentatively conclude that the increased total yield is mainly due to the increased loin yield and not variation in shoulder and leg yield.

Birth weight effects attributable to *MSTN* have not been reported previously in sheep and are worthy of further investigation as there are important implications for lamb survival. In contrast, several studies have investigated the effect of *MSTN* g+6223A on carcass traits and the results of this study are consistent with a recent report of an increase in *M. longissimus* muscle in NZ Texel sheep with g+6223A (Johnson et al., 2009). The results of this study are, however, inconsistent with the previous observations that g+6223A is

Table 2. The effect of the ovine *MSTN* g+6223G>A on assessments of lamb carcass traits

Trait	Mean±Std		p value
	GG (n = 21)	AG (n = 11)	
Loin yield (% carcase)	14.4±0.23	15.3±0.28	0.002
Shoulder yield (% carcase)	16.8±0.26	17.2±0.32	0.190
Leg yield (% carcase)	21.2±0.35	21.8±0.43	0.140
Total yield (% carcase)	52.4±0.75	54.4±0.92	0.038
Proportion yield loin*	0.274±0.0018	0.281±0.0022	0.005
Proportion yield shoulder*	0.321±0.0028	0.317±0.0034	0.269
Proportion yield leg*	0.404±0.0021	0.400±0.0026	0.255

* As a proportion of Total yield

associated with heavier hindquarters in Texel sheep (Clop et al., 2006) and increased forequarters in Australian cross-bred half-sib progeny of Texel sheep (Kijas et al., 2007). Thus it is concluded that the benefits of using selection for g+6223A in sheep breeding may be breed and even gender-specific, although it is accepted that this is a small and preliminary study and that a trial involving many more sheep of the common NZ breeds, including the NZ Romney, is required to confirm these observations.

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