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Myostatin (*MSTN*) gene haplotypes and their association with growth and carcass traits in New Zealand Romney lambs

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ABSTRACT

Myostatin, which is also known as growth and differentiation factor 8 (GDF8), acts as a negative regulator of skeletal muscle growth. Genetic variation in the myostatin gene (*MSTN*) has been associated with variation in muscularity in many animals including sheep. Polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) analysis was used to investigate the haplotypic diversity of *MSTN* and the association between the *MSTN* haplotype and variation in lamb growth and carcass traits. A total of 1379 Romney male lambs from 19 sire-lines were tested in this study. Five *MSTN* haplotypes including *H1*, *H2*, *H3*, *H5* and *H7* were identified. Using a restricted maximum likelihood (REML) mixed-model approach in both half-sib and pooled-data analyses, *H1* was associated with an increase in birth weight, tailing weight and draft weight in lambs and had a significant effect on loin yield, leg yield, total yield and proportion loin yield from lamb carcasses. Haplotype *H2* in lambs was associated with an increase in draft weight and an earlier drafting age, together with an increase in loin, shoulder and total yield of lean meat. *H3* was associated with an increase in growth rate to weaning, leg yield and total yield of lean meat. The presence of haplotype *H5* in lambs was associated with a decrease in loin yield, shoulder yield and total yield of lean meat yield. The association of haplotype *H7* with a decrease in leg yield was identified for the first time in this study. These results suggest that the variation in ovine *MSTN* in NZ Romney is associated with differences in lamb growth and carcass traits and thus meat production. It also suggests that using *MSTN* as a marker-assisted selection tool for improved carcass traits in NZ Romney is a future possibility.

1. 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 to the regulation of adipogenesis (Lee and McPherron, 2001) and the regulation of tendon structure and function during both prenatal and postnatal development (Mendias et al., 2008). Variation in the myostatin gene (*MSTN*) has been associated with muscling in a variety of mammalian species including mice (McPherron et al., 1997), cattle (Dunner et al., 2003; Grobet et al., 1997), humans (Schuelke et al., 2004), dogs (Mosher et al., 2007), pigs (Stinckens et al., 2008) and sheep (Boman and Våge, 2009; Han et al., 2010, 2013; Hickford et al., 2010; Johnson et al., 2009; Kijas et al., 2007).

In sheep, variation in *MSTN* is already being used as a gene marker in selecting for improved muscle yield commercially (Zoetis, Florham Park, NJ, USA; Lincoln University Gene-Marker Laboratory, Christchurch, NZ). This is based

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on the detection of c.*1232G>A in the 3′UTR of *MSTN*, where it has been shown that SNP c.*1232A is associated with improved carcass muscle traits (Clop et al., 2006; Hadjipavlou et al., 2008; Johnson et al., 2009).

The high frequency of *MSTN* c.*1232A in Texel sheep may, however, limit its value in contributing to any further genetic gain. This leads to the question of whether other variation in *MSTN* affects carcass traits and whether such variation might therefore be of further value to improving carcass characteristics. In this respect, Kijas et al. (2007) demonstrated that four haplotypes not carrying *MSTN* c.*1232A had significant associations with variation in muscling and fatness. In addition, a recent trial with NZ Romney sheep revealed three haplotypes (*A*, *B* and *C*) of *MSTN* defined by variation in the exon 1–intron 1 region, with the presence of haplotypes *A* and *B*, being associated with variation in lean meat production (Hickford et al., 2010). Given the eight extended haplotypes of ovine *MSTN* reported by Han et al. (2013), it would therefore be reasonable to expect that this variation in sheep might also affect carcass characteristics.

In this study, the polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP) approach used by Han et al. (2013) to define the haplotypes will be used to investigate haplotypic diversity in a large number of NZ Romney lambs that have been slaughtered and for which records are available describing a variety of growth and carcass traits. Upon haplotyping, various statistical analyses will be employed to determine the relationship between the *MSTN* haplotypes and variation in the traits.

2. Materials and methods

2.1. Animals

The NZ Romney sheep selected for this study were part of the Ancare-Merial Romney NZ Saleable Meat Yield Trial. This trial has been run over 9 consecutive years from 2006 to 2014 at "Gleneyre", Oxford, North Canterbury, NZ, and "Osborne farm", Ashhurst, NZ.

For this study, the lambs were selected from both the North Island and South Island farms over four consecutive years from 2006 to 2009. Nineteen Romney sires and their progeny were studied (see Table S1) and all were ranked in the top 20% of Romney rams using the Sheep Improvement Limited (SIL—a Division of Beef+Lamb NZ, Wellington, NZ) Dual Purpose Overall (DPO) index.

Each ram was single-sire mated to a group of randomly selected commercial (non-stud) NZ Romney ewes ($n \approx 40$–60). Ewes varied in age from 2 to 7 years, with each ewe identified to the sire group by a numbered ear tag. These ewes were kept in one mob until pregnancy scanning and then the single-bearing ewes were drafted off from the multiple-bearing ewes to enable a differential feeding regime to be used. The ewes were not pre-lamb shorn. Prior to lambing, ewes were set-stocked at approximately 12 per hectare.

A blood sample for DNA extraction was collected onto an FTA card (Whatman BioScience, Middlesex, UK) from the 19 rams at mating and the ewes at pregnancy scanning. A small notch was taken out of the end of an ear, and several drops of whole blood were collected onto a labelled FTA card (Whatman BioScience). The FTA card was allowed to air dry and was stored in darkness at room temperature until needed.

2.2. Data collection

During lambing, all lambs ($n = 1376$) were tagged within 12 h of birth using ear tags that carried a unique identification number. At tagging, birth date, birth rank (i.e. single or multiple birth), birth weight, gender and dam number were recorded. Individual lambing dates varied and extended over the period from 19 August to 23 September of each year.

All of the ewes and lambs were brought together at tailing (approximately 3 weeks after birth) and remained together until weaning. All lambs were tailed with a rubber ring, ear-marked and weighed. A blood sample for DNA extraction was collected onto an FTA card at tailing.

Weaning occurred at approximately 3 months after birth, although individual lamb ages varied. All the lambs were weighed and their weight recorded against their tag number. Pre-weaning growth rate was calculated as the difference between weaning weight and birth weight divided by lamb age in days (expressed in grams/day).

At weaning, the lambs were separated according to gender and only the male lambs were used in the subsequent carcass meat yield investigations. Of these male lambs, those that weighed 36 kg and more were drafted immediately for slaughter. Those under 36 kg were retained on pasture for another 4 weeks (until about 4 months of age) and drafted again for slaughter based on a target weight of 36 kg and above. A final draft occurred at approximately 5 months of age and all the remaining male lambs were sent for slaughter regardless of their weight (which ranged from 30.5 to 50.5 kg). Draft age and draft weight for each male lamb was recorded, so post-weaning growth rates could be calculated. All the female lambs were retained as flock ewe replacements.

Hot carcass weights (H-W) were measured on the male lambs directly at slaughter. H-W is the weight in kilograms of the carcass components minus the pelt, head and gut. Other carcass data including lean meat loin yield, shoulder yield, leg yield, total yield, proportion loin yield, proportion shoulder yield and proportion leg yield were subsequently evaluated using video imaging analyses (VIAScan® Sastek, Hamilton, Queensland, Australia). Loin yield, shoulder yield and leg yield is the percentage of lean tissue as a proportion of the H-W. Total yield is the sum of the leg, loin and shoulder yield for any given carcass. The proportion yield of leg, loin or shoulder is the yield of the specific area divided by the total yield, expressed as a percentage.

Of the 1376 lambs born, growth, blood sample and genotype data were collected for 1206 lambs. Of these, 582 were male lambs that were sent to the meat-processing plant. Of these male lambs, 79 were excluded from the dataset as only partial processing data were available as a result of either tags being mistakenly removed, or the presence of carcass faults that required trimming. Sample numbers therefore vary in the different analyses undertaken.

2.3. Genotyping of MSTN using PCR-SSCP

Blood samples from all the lambs, sires and ewes were collected on FTA cards and were purified using a two-step procedure (Zhou et al., 2006). Four fragments of *MSTN* spanning the 5′ untranslated region (UTR), Exon 1, Intron 1, Exon 3 and 3′UTR, were amplified and analysed using PCR-SSCP according to the method described by Han et al. (2013). These corresponded to amplicons 1, 5, 6 and 11 in Han et al. (2013).

Briefly, PCR amplifications were performed in 20 μL reactions containing the genomic DNA on a 1.2 mm diameter disc of FTA paper, 0.25 μM of each primer, 150 μM dNTPs (Eppendorf, Hamburg, Germany), 2.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× the reaction buffer supplied with the enzyme. Amplification was carried out in an iCycler (Bio-Rad Laboratories, Hercules, CA, USA).

The thermal profiles consisted of denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, annealing for 30 s at 58, 60, 55 and 59 °C (amplicons 1, 5, 6 and 11, respectively) and extension at 72 °C for 30 s, followed by a final extension step at 72 °C for 5 min.

The amplicons from the various PCR reactions were visualised by electrophoresis in 1% agarose (Quantum Scientific, Queensland, Australia) gels using 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2 EDTA), containing 200 ng/mL ethidium bromide. A 2 μL aliquot of PCR product was added to 2 μL of loading dye (0.2% bromophenol blue, 0.2% xylene cyanol, 40% (w/v) sucrose) and the gels were run at a constant 10 V/cm for 10 min, prior to visualisation by UV trans-illumination at 254 nm.

All SSCP gels were silver-stained according to the method of Sanguinetti et al. (1994) and the results were compared with the banding pattern standards described in Han et al. (2013). To asertain the haplotypes, sheep homozygous for one PCR-SSCP pattern (and sequence) in a region of the gene were then genotyped in another region of the MSTN, to ascertain whether they were homozygous or heterozygous in that second region. If heterozygous in the second region, then two haplotypes, spanning the first and second regions, could be defined.