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Growth and carcass trait association with variation in the somatostatin receptor 1 (SSTR1) gene in New Zealand Romney sheep

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ABSTRACT

Somatostatin receptors (SSTRs) are thought to regulate the growth inhibitory effect of somatostatin and play a role in regulating growth hormone secretion. In this study, polymerase chain reaction-single-stranded conformational polymorphism (PCR-SSCP) analysis was used to screen for variation in the 3′-untranslated region of the SSTR1 gene (SSTR1) in 941 New Zealand Romney sheep. Phenotypic data were available for birth weight, weaning weight, pre-weaning growth rate, hot carcass weight (HCW), subcutaneous fat depth [measured as VIAscan-GR (V-GR)], and leg, loin, shoulder and total lean meat yield. Weaning weight was correlated (r = 0.854; P < .001) with pre-weaning growth rate; and leg, loin and shoulder lean meat yield were correlated with total lean meat yield (r = 0.878, 0.835 and 0.739, respectively, all P < .001). Three PCR-SSCP banding patterns were detected and DNA sequencing revealed three different nucleotide sequences (A–C). The presence of A was found to be associated with a decrease in HCW, while the presence of C was found to be associated with an increase in V-GR and lower birth weights.

Introduction

Somatostatin (SST or somatotropin release-inhibiting factor) is known to have inhibitory effects on both endocrine and exocrine secretions, and it is therefore important in metabolism, tissue differentiation and development (Sheridan et al. 2000; Weckbecker et al. 2003). SST binds receptors and there are five known SST receptor subtypes (SSTRs, named SSTR1–5), which belong to the G-protein-coupled receptor superfamily (Rajput et al. 2011). The receptors are distributed throughout many organs and tissues including the central nervous system, gut, pituitary, kidneys, thyroid, lungs, immune cells and...
various cancer cells (Weckbecker et al. 2003; Cakir et al. 2010). Each SSTR has a subtype-selective, tissue-specific and species-specific distribution pattern, and they are involved in different activation mechanisms for intracellular signalling. The SSTRs are thought to be able to regulate the growth inhibitory effect of SST (Kreienkamp et al. 1999; Zatelli et al. 2003).

In humans, SSTR1 has been revealed to be associated with neurodegeneration, endocrine gastroentero-pancreatic tumours and breast tumours, and its selective agonists have been used as cancer therapies (Rajput et al. 2011). Research suggests that SSTR1 plays an important role in regulating growth hormone secretion, and that it may influence body weight and cause growth retardation in mice (Wang et al. 2006). In goats, SSTR1 has been reported to affect body size, including body length, body height and chest circumference (Jin et al. 2011).

Regulatory regions within the 3'-untranslated region (3'-UTR) can influence polyadenylation, translation efficiency, localisation and stability of the mRNA (Barrett, et al. 2012). Together with Jin et al. (2011) describing both 3'-UTR and intronic variation in goats and Iida et al. (2004) describing variation in the 3'-UTR of human SSTR1, then this region seems a logical place to look for variation that may affect the expression of the SSTR1 gene in sheep.

The tissue distribution of ovine SSTR1 expression has been analysed and its coding sequence has been identified. According to ovine genome sequence v4.0 (NC_019475.2), SSTR1 is located on chromosome 18 and contains one large coding exon of 1173 bp (nt 47172694-47173866). The gene is conserved structurally and functionally across mammalian species (Debus, et al. 2001). The sheep and goat SSTR1 nucleotide sequences reported in GenBank are highly similar, and most of the sequence differences are found in the 3'-UTR.

In this study, we report variation in the 3'-UTR of ovine SSTR1 detected using polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) analysis, and reveal associations between this genetic variation and variation in some growth and carcass traits in New Zealand (NZ) Romney sheep.

**Materials and methods**

**Sheep investigated and data collection**

Nine hundred and forty-one NZ Romney lambs, the progeny of 19 un-related rams that were part of a progeny test on a commercial farm, were investigated: (1) to screen for variation in SSTR1 and (2) to assess whether the variation was associated with variation in growth and carcass traits.

The rams used were all ranked in the top 20% of the Sheep Improvement Limited (SIL – a Division of Meat and Wool NZ, Wellington, NZ) Dual Purpose index and were deliberately selected from different NZ Romney studs from across NZ prior to being brought to the North Canterbury farm for mating. Each ram was single-sire mated to a group of randomly selected NZ Romney ewes (n ≈ 40–60) that were non-first-parity and ranged in age from 4 to 7 years. Each ewe was identified to sire group by a numbered plastic ear-tag. Single-bearing and multiple-bearing ewes were kept in separately and were differentially fed. Just prior to lambing, ewes were set-stocked at approximately 12 stock units per hectare.
All lambs were ear-tagged with a unique identification number within 12 hours of birth and the gender, birth rank (i.e. whether they were a single, twin, triplet or quad), rearing rank and birth weight were recorded for each lamb. All the lambs were weaned at approximately 90 days of age, weighed and separated based on their gender. As most of the female lambs were kept as ewe replacements for the larger commercial base flock, the draft weight and carcass data were only available from male lambs, and a small number of cull ewe lambs.

The pre-weaning growth rate of the lambs was calculated as the average daily weight gain (g/d) from birth to weaning. Hot carcass weights (HCW) were measured directly on the processing chain. HCW is the weight in kilograms of the carcass minus the pelt, head and gut. Video image analysis (VIAscan; Sastek, Australia), developed by Meat and Livestock Australia and described by Hopkins et al. (2004), was used to estimate the following carcass traits: lean meat yield (expressed as a percentage of HCW) in the leg (leg yield), loin (loin yield) and shoulder (shoulder yield), and total yield (the sum of the leg, loin and shoulder yields for any given carcass), and V-GR (a VIAscan assessment of subcutaneous fat depth near the 12th rib).

Blood samples from all these sheep were collected onto TFN paper (Munktell Filter AB, Sweden) by nicking the lamb’s ears and genomic DNA was then purified for PCR analysis, using a two-step procedure described by Zhou et al. (2006).

**PCR primers and amplification of ovine SSTR1**

Two PCR primers, 5'-GCACGTCCAGGATCACGAC-3' and 5'-AGTTACCACCTGCACCCCTG-CACCTG-3' (Ovine genome sequence NC_019475.2, 47173841-47173859 and 47174191-47174173, respectively), were designed to amplify a 351-bp fragment of the 3'-UTR of ovine SSTR1. This fragment covered a region from 26 bp upstream of the 3'-UTR to 325 bp downstream of the coding sequence (c*325). The primers were synthesised by Integrated DNA Technologies (Coralville, IA, USA).

PCR amplifications were performed in a 15-µL reaction containing the purified genomic DNA on a 1.2-mm punch of the TFN paper, 0.25 µM of primer, 150 µM dNTPs (Bioline, London, UK), 2.5 mM Mg²⁺, 0.5 U Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× the reaction buffer supplied with the enzyme. The thermal profile for amplification consisted of an initial hold 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C; with a final extension of 5 min at 72°C. Amplification was carried out using S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA).

Amplicons 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 Na₂EDTA) containing 200 ng/mL of ethidium bromide.

**Screening for variation in ovine SSTR1**

The PCR amplicons were screened for sequence variation using SSCP analysis. A 0.7-µL aliquot of each amplicon was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue and 0.025% xylene-cyanol). After denaturation at 95°C for 5 min, the samples were rapidly cooled on wet ice and then loaded on 16 cm × 18 cm, 14% acrylamide:bisacrylamide (37:5:1) (Bio-Rad) gels. Electrophoresis was performed
using Protean II xi cells (Bio-Rad) in 0.5× TBE buffer, under the electrophoretic conditions of 32°C, 180 V for 16 h. Gels were silver-stained according to the method of Byun et al. (2009).

**Sequencing of ovine SSTR1 variants and sequence analyses**

PCR amplicons identified as homozygous by SSCP analysis were directly sequenced at the Lincoln University Sequencing Facility, NZ. Weak bands are observed at times with PCR-SSCP, resulting in more complex patterns. When present within apparently heterozygous genotypes, bands of similar mobility to the homozygous variants were excised and sequenced to confirm the sequence using an approach described by Gong et al. (2011). Briefly, the single bands of interest were recovered directly from the SSCP gels as a gel slice. This was macerated and the DNA was eluted into 50 μL TE buffer by incubating at 70°C for 20 min. One micro-litre of the eluted solution was used as a template for the second round of PCR amplification with the original primers, to produce a simple SSCP gel pattern which could be directly compared to, or found in, the pattern derived from the original heterozygous amplicon. When the banding patterns could be matched and identified, then the second PCR amplicons were directly sequenced at the Lincoln University DNA Sequencing Facility.

Sequence alignments, translations and phylogenetic analysis were carried out using DNAMAN (version 5.2.10, Lynnon BioSoft, Vaudreuil, Canada).

**Statistical analyses**

The Hardy–Weinberg equilibrium (HWE) for the SSTR1 genotypes was analysed using an online chi-square test (http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-3-alleles.html).

Statistical analyses were performed using Minitab version 17 (Minitab Inc., State College, PA, USA). Unless otherwise indicated, all P values were considered statistically significant when P < .05 and trends were noted when .05 ≤ P < .10. Measured traits were tested for normality using the Shapiro–Wilk test and Normal Q–Q plots.

Pearson’s correlation coefficients were calculated to test the strength of the relationship between the measured traits: birth weight, weaning weight, pre-weaning growth rate, HCW, V-GR, shoulder yield, leg yield, loin yield and total yield.

Generalised linear mixed models (GLMMs) were used to assess the effect of the presence or absence of the SSTR1 variants on these growth and carcass traits. Allele presence or absence (coded as 1 or 0, respectively) was fitted as a fixed factor, while sire was fitted as a random factor in each model. For the birth weight GLMMs, gender and birth rank were also fitted into the models as fixed factors, but with the weaning weight and growth to weaning GLMMs, gender and rearing rank were fitted into the models as a fixed factor. Weaning age was also included in the weaning weight model as a co-variate. For carcass and yield traits, birth weight and draft age were fitted into the models as covariates.

As only one variant in each of the first set of models was showing a trend or reached significance for association with the traits, additional models were not run where other variants were factored into the models to ascertain the effect of those variants in the genotypes.

In another set of models, any SSTR1 variant genotypes, present at a frequency of 5% or more (thereby ensuring adequate sample size), were tested to ascertain associations with
the traits. Multiple pairwise comparisons between genotypes were performed using a Tukey test with Bonferroni corrections. For the birth weight GLMMs, gender and birth rank were fitted into the models as fixed factors, but with the weaning weight and growth to weaning GLMMs, gender and rearing rank were fitted into the models. Weaning age was also included in the weaning weight model as a co-variate. For carcass and yield traits, birth weight and draft age were fitted into the models as covariates.

Results

Correlations between growth and carcass traits

Strong correlations ($|r| > 0.7$) were found between weaning weight and pre-weaning growth rate; and between total yield, and leg, loin and shoulder yield (Table 1). Moderate correlations ($0.3 < |r| \leq 0.7$) were found between birth weight and weaning weight; between pre-weaning growth rate and HCW; between weaning weight, and HCW, V-GR and shoulder yield; between pre-weaning growth rate, and HCW and V-GR; between HCW and V-GR; between V-GR, and leg yield and total yield; between leg yield, and loin yield and shoulder yield and between loin yield and shoulder yield. All these correlations were highly significant ($P < .001$). There were only weak or negligible correlations ($|r| \leq 0.3$) between the other traits.

Variation in ovine SSTR1

There were three PCR-SSCP banding patterns detected in the region of ovine SSTR1 that was amplified, with either one or a combination of two banding patterns observed for each sheep (Figure 1). DNA sequencing revealed that these PCR-SSCP patterns represented three distinct nucleotide sequences (named A, B and C). These sequences were deposited into GenBank with accession numbers MG591463–MG591465. Two single nucleotide polymorphisms (SNPs) were identified in the three sequences, and these were c.*17C/G and c.*167C/T. Sequence A was c.[*17C; *167C], sequence B was c.[*17G; *167C] and sequence C was c.[*17G; *167T]. Six genotypes were detected in the NZ Romney lambs, and they were as follows: AA, BB, CC, AB, AC and BC. The frequencies of the SSTR1 variants in the NZ Romney lambs were: A: 13.6%; B: 48.9% and C: 37.5%.

Table 1. Pearson correlation coefficients between various growth traits in 941 Romney lambs.

<table>
<thead>
<tr>
<th></th>
<th>Birth weight</th>
<th>Weaning weight</th>
<th>Pre-weaning growth rate</th>
<th>HCW</th>
<th>V-GR</th>
<th>Leg yield</th>
<th>Loin yield</th>
<th>Shoulder yield</th>
<th>Total yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning weight</td>
<td>0.460***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-weaning growth rate</td>
<td>0.562***</td>
<td><strong>0.854</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCW</td>
<td>0.362***</td>
<td>0.671***</td>
<td>0.581***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V-GR</td>
<td>0.223***</td>
<td>0.575***</td>
<td>0.524***</td>
<td>0.601***</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg yield</td>
<td>−0.125**</td>
<td>−0.206***</td>
<td>−0.236***</td>
<td>−0.194***</td>
<td>−0.568***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loin yield</td>
<td>0.005</td>
<td>0.068</td>
<td>0.013</td>
<td>0.238***</td>
<td>−0.159***</td>
<td>0.660***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoulder yield</td>
<td>0.132**</td>
<td>0.312**</td>
<td>0.230***</td>
<td>0.235***</td>
<td>0.070</td>
<td>0.424***</td>
<td>0.433***</td>
<td>0.739***</td>
<td></td>
</tr>
<tr>
<td>Total yield</td>
<td>−0.009</td>
<td>0.019</td>
<td>−0.022</td>
<td>0.079</td>
<td>−0.304***</td>
<td><strong>0.878</strong>*</td>
<td><strong>0.835</strong>*</td>
<td><strong>0.739</strong>*</td>
<td></td>
</tr>
</tbody>
</table>

Note: Correlations with $|r| > 0.7$ are in bold, and those with $0.3 < |r| \leq 0.7$ are underlined.

**$P < .01$.**

***$P < .001$.**
**Effect of variation in SSTR1 on growth and carcass traits**

The SSTR1 genotypes were found to be in HWE in the 941 NZ Romney lambs ($P = .1752$). The growth and carcass trait data exhibited a pattern consistent with being normally distributed.

The presence of C was found to be associated ($P = .029$) with decreased birth weight (Table 2). The presence of A was found to be associated ($P = .033$) with decreased HCW, whereas the presence of C was associated ($P = .018$) with an increase in V-GR (Table 2). A trend of association with shoulder yield was detected for variant B (Table 2).

Among the five genotypes with a frequency of over 5%, lambs with the C-containing genotypes (AB, BC and CC) had lower birth weight and higher V-GR than those with the genotypes (AB and BB) that did not contain C and lambs with the A-containing genotypes (AB and AC) had lower HCW than those with genotypes (BB, BC and CC) that did not contain A. However, after the correction for the multiple comparisons undertaken, these results lost their significance.

**Figure 1.** PCR-SSCP of the ovine SSTR1 gene. The bands corresponding to the two strands of the three variants of SSTR1 are annotated, and weak bands that are observed at times with PCR-SSCP, but that do not represent the variants are marked with a *. Selected bands within the heterozygous genotypes were excised and sequenced to confirm the variants present.
Discussion

This is the first report of both variation in ovine SSTR1 and associations between that variation and variation in growth and carcass traits.

The phenotypic correlations observed between the various growth and carcass traits are similar to those observed in other studies (Singh et al. 2006; Brito et al. 2015), but with some exceptions. The correlations between loin, shoulder, leg and total yield were moderate or strong and positive in this study, but were negligible in the study of Shrestha et al. (1986). The correlation between birth weight and pre-weaning growth rate was 0.562, but Singh et al. (2006) reported a value of 0.18. The correlation between V-GR, and loin yield and shoulder yield were −0.159 and 0.07, respectively, but Einarsson et al. (2015) reported the correlation between GR, and loin and shoulder yields were 0.37 and −0.43, respectively. This inconsistency may result from the different measurement methods used for V-GR and GR determination. McEwan et al. (1989) reported that mean fat depths measured ultrasonically were generally lower than physical measurements on carcasses. This was probably due to operator compression of the fat layers and operator inexperience. In this respect, some researchers suggest that ultrasound measurements of fat on live animals and the physical measurement of carcass fat traits should not be regarded

Table 2. Association between the presence/absence of ovine SSTR1 variants and growth traits (mean ± SE).a

<table>
<thead>
<tr>
<th>Trait</th>
<th>Variant</th>
<th>n</th>
<th>Absent</th>
<th>Present</th>
<th>Absent</th>
<th>Present</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>A</td>
<td>696</td>
<td>245</td>
<td>5.74 ± 0.04</td>
<td>5.78 ± 0.08</td>
<td>0.537</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>241</td>
<td>700</td>
<td>5.69 ± 0.07</td>
<td>5.76 ± 0.05</td>
<td>0.344</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>369</td>
<td>572</td>
<td><strong>5.84 ± 0.06</strong></td>
<td><strong>5.69 ± 0.05</strong></td>
<td><strong>0.029</strong></td>
<td></td>
</tr>
<tr>
<td>Weaning weight (kg)</td>
<td>A</td>
<td>696</td>
<td>244</td>
<td>33.5 ± 0.33</td>
<td>33.5 ± 0.44</td>
<td>0.937</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>240</td>
<td>700</td>
<td>33.3 ± 0.40</td>
<td>33.6 ± 0.33</td>
<td>0.325</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>369</td>
<td>571</td>
<td>33.5 ± 0.39</td>
<td>33.5 ± 0.33</td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>Growth rate to weaning (g/d)</td>
<td>A</td>
<td>696</td>
<td>244</td>
<td>316.9 ± 3.4</td>
<td>316.4 ± 4.5</td>
<td>0.894</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>240</td>
<td>700</td>
<td>314.3 ± 4.1</td>
<td>316.9 ± 4.5</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>369</td>
<td>571</td>
<td>316.1 ± 4.1</td>
<td>317.1 ± 3.5</td>
<td>0.762</td>
<td></td>
</tr>
</tbody>
</table>

Table continued...

Note: HCW: hot carcass weight; V-GR: VIAscan fat depth at the 12th rib.

aPredicted means and standard error of those means derived from GLMMs, with various factors being included in the models for different traits as described in the Materials and Methods section. P < .05 are in bold, while .05 ≤ P < .10 are italicised.
as measurement of the same traits (Waldron et al. 1992), with phenotypic correlations in the range of 0.06–0.62 having been described between live animal measurements of weight at 5, 6, 8 and 14 months of age, and GR measured ultrasonically on those sheep at 14 months of age (McEwan et al. 1993). This suggests that age has an effect on the correlations of carcass traits with older sheep having higher weights and amounts of fat. In effect while Waldron et al. (1992) surmised that some differences in trait correlations may result from differences in sheep breeds or estimation errors, there may also be a maturity fat deposition effect.

In this study, birth weight showed a moderate correlation with weaning weight, pre-weaning growth rate and HCW, and a weak correlation with the other carcass traits. This is consistent with other studies that have reported that birth weight is moderately correlated with subsequent lamb growth (Bahreini-Behzadi et al. 2007), and that there is a low correlation between birth weight and carcass traits including scanned muscle depth and scanned fat depth (Ceyhana et al. 2015). Birth weight is considered to be an important trait for sheep production, as it has a direct effect on the survival of lambs (Oldham et al. 2011). Low birth weight lambs experience higher levels of postnatal mortality and higher birth weight lambs are more likely to experience lambing difficulties (Nowak and Poindron 2006).

Lambs with variant C had a lower birth weight, but a higher V-GR at slaughter. Birth weight and V-GR were weakly positively correlated (r = 0.223), suggesting that whatever effect C is having, it appears to be behaving differently in how it might affect birth weight, compared to how it might affect V-GR. While variant C had an effect on birth weight, it also had no measurable effect on weaning weight, growth to weaning or HCW. This supports the contention that the gene may be having an independent effect on birth weight, relative to its effect on other carcass traits. Taken together the evidence might also then suggest that C is of limited value to sheep production.

Variant A was associated with a small reduction in HCW. This could be reconciled with the effects of C, to suggest that variant B would be the most favourable as regards both increasing birth weight, increasing V-GR and not being associated with a reduction in HCW, but this would require further testing in more flocks of different breed, gender and age, to be confirmed.

Across mammalian species, SSTR1 contains a highly conserved amino acid sequence (Debus et al. 2001). Highly conserved sequences are typically associated with proteins that underpin conserved or essential metabolic activities. Wang et al. (2006) reported that SSTR1 knockout mice had changed patterns of insulin secretion and showed glucose intolerance, while also having a shortened lifespan. They presumed that SSTR1 not only regulates insulin secretion and glucose homeostasis but also plays a key role in regulating the overall growth of mice.

Although the SNPs identified in this study are not located in the coding regions of SSTR1, they may be linked to sequence variation in other regions of the gene that regulates gene expression. Equally, the 3′-UTR can in its own right play an important role in the post-transcriptional regulation of gene expression (Mignone et al. 2002), with, for example, a single base change in the 3′-UTR of the myostatin gene reducing the level of myostatin translation, and through this, having a major effect on muscularity in Texel sheep (Clop et al. 2006).
Conclusion

This study used PCR-SSCP to screen for variation in the 3’-UTR of ovine SSTR1 and identified two SNPs in NZ Romney sheep. The results suggest that variation in ovine SSTR1 may need to be considered when selecting for birth weight, HCW or V-GR, but this would require further testing in more flocks of different breed, gender and age.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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