Nucleotide Sequence Variation in the Insulin-Like Growth Factor 1 Gene Affects Growth and Carcass Traits in New Zealand Romney Sheep

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Insulin-like growth factor 1 (IGF1) is a mediator of the effects of growth hormone and polymorphism in the IGF1 gene (IGF1) is reported to affect fat deposition in some livestock species. In this study, nucleotide sequence variation in three regions of ovine IGF1 (part of the 5’ flanking region, the exon 3 region, and the exon 4 region) was investigated in 848 New Zealand Romney lambs using PCR-single strand conformation polymorphism (SSCP) analyses to ascertain if single nucleotide polymorphisms (SNPs) existed. Six SNPs were identified across these three regions. The effect of the sequence variation in the exon 3 and exon 4 regions on growth and carcass traits were investigated. One of the PCR-SSCP sequence variants in the exon 3 region was associated with variation in hot carcass weight, carcass fat depth at the 12th rib measured using video imaging and the percentage proportion of leg lean meat, whereas the other was associated with variation in growth rate to weaning. No associations were detected for the other gene regions analyzed. The results suggest that polymorphism in exon 3 of ovine IGF1 has potential for use as a gene-marker for some carcass and growth traits.

Keywords: insulin-like growth factor 1 gene, sheep, polymorphism, carcass, growth

Introduction

Insulin-like growth factor 1 (IGF1) is encoded by the IGF1 gene (IGF1) (Jansen et al., 1983; Hoppener et al., 1985). It has “non-suppressible insulin-like activity” (Salmon and Daughaday, 1957) and is a primary mediator of the effects of growth hormone. Growth hormone is synthesized in the anterior pituitary gland and released into the blood stream. It stimulates the liver to produce IGF1, which can then fuel body growth by having growth-promoting effects on almost every cell in the body, while also regulating cellular DNA synthesis (Yakar et al., 2002).

In mammals, IGF1 is composed of six exons separated by five introns, and it spans >80 kb (Rotwein, 2012). The nucleotide sequence and length of exons 1–4 are conserved across species, whereas exons 5 and 6 are more variable. Exons 1 and 2 determine the class of the protein and encode the signal peptide for cellular localization after translation, whereas exons 3 and 4 primarily encode the mature IGF1 peptide. This ultimately becomes the receptor-binding ligand (Rotwein, 2012).

Polymorphism of IGF1 has been reported to affect growth and production traits in a number of livestock species. It has been reported that a single nucleotide polymorphism (SNP) in the promoter of IGF1 affects fat deposition and carcass merit traits in hybrid Angus and Charolais beef cattle (Islam et al., 2009); and in dairy cattle, SNPs in IGF1 have been associated with growth-related traits, carcass fat, milk production, and milk fatty acid traits (Mullen et al., 2011; Li et al., 2016). In pigs polymorphism of IGF1 is associated with final body weight, average daily gain and back-fat thickness (Niu et al., 2013), whereas in goats an IGF1 SNP affects growth traits (Zhang et al., 2008).

There have been a number of studies investigating the effects of IGF1 polymorphism on growth and production traits in different sheep breeds, but the results do at times
conflict. Some researchers have reported polymorphism in the 5′-flanking region of IGF1 associated with growth traits in Baluchi (Tahmoorespur et al., 2009), Makui (Hajhosseinilo et al., 2013) and Makooei sheep (Negahdary et al., 2013), but no associations between IGF1 polymorphism and growth traits were detected in Indian Madras Red sheep (Ramasamy, 2018), Polish Pomeranina coarse-wool sheep (Proskura and Szewczuk, 2014), Zandi sheep (Nazari et al., 2016) and Baluchi sheep (Gholibeikifard et al., 2013). With Colored Polish Merino sheep, polymorphism in the 5′-flanking region of IGF1 is known about whether nucleotide sequence variation in the region affects growth and body size, but also affects carcass and meat quality traits (Grochowska et al., 2017). SNPs in IGF1 intron 1 were found to be associated with a number of carcass traits in Santa Ines sheep, including internal carcass length, rump girth, rib yield and neck weight (Meira et al., 2019).

Despite the interest in ovine IGF1, research has tended to focus on SNPs in the 5′ region and introns. Little is known about whether nucleotide sequence variation in the other regions of IGF1 has an effect on growth and carcass traits, and whether the effect exists in common breeds in major sheep-farming countries.

In this study, we used PCR-single strand conformation polymorphism (PCR-SSCP) analyses to search for SNPs in the IGF1 5′ flanking region, and in the exon 3 and 4 region that encode the IGF1 mature peptide in New Zealand (NZ) Romney sheep, the most popular sheep breed in NZ. The effect of the PCR-SSCP haplotypes on growth and carcass traits was subsequently investigated.

Materials and Methods

All research involving animals was carried out in accordance with the Animal Welfare Act 1999 (NZ Government) and the collection of sheep blood drops by nicking sheep ears is covered by Section 7.5 Animal Identification of the Animal Welfare (Sheep and Beef Cattle) Code of Welfare 2010, which is a code of welfare issued under the Animal Welfare Act 1999 (NZ Government).

Sheep investigated and data collection

Eight hundred forty-eight NZ Romney lambs, the progeny of 19 unrelated industry-sourced rams that were part of a progeny test on a commercial farm, were investigated. The gender, birth weight, birth rank (i.e., whether they were a single, twin, or triplet), and rearing rank were recorded for each lamb. All the lambs were weaned at ~90 days of age, weighed, and separated based on gender into two mobs. The preweaning growth rate of the lambs was calculated as the average daily weight gain (grams/day) from birth to weaning.

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. Lambs weighing >37 kg were first drafted for slaughter at around 16 weeks of age, with a second draft at ~20 weeks of age. All remaining male lambs were slaughtered at ~24 weeks of age. Draft weight and draft age were recorded for each lamb.

Hot carcass weights (HCWs) were measured directly on the processing chain (Alliance Food Limited, Smithfield, Timaru, NZ), which is the weight in kilograms of the carcass minus the head, gut, and pelt. 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 shoulder (shoulder yield), loin (loin yield) and leg (leg yield), and total yield (the sum of the shoulder, loin and leg yields for any given carcass), and V-GR (a VIAScan assessment of subcutaneous fat depth near the 12th rib). To describe the distribution of lean meat across the carcass, the proportion of total yield of shoulder, loin, or leg was also recorded, this being the yield of the specific part of the carcass divided by the total yield and expressed as a percentage.

At tailing, 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 IGF1

Three pairs of PCR primers were designed manually to amplify a portion of the 5′-flanking region, the entirety of the exon 3 region (including parts of its flanking introns) and the exon 4 region (including parts of its flanking introns) of IGF1 (Table 1). The PCR primers were chosen based on analysis of the ovine genome sequence Oar_v4.0 NC_019475.2, and checked for suitability as primers using DNAMAN (version 5.2.10; Lynnon BioSoft, Vaudreuil, Canada). The primers were synthesized by Integrated DNA Technologies (Coralville, IA).

The PCR amplifications were carried out using S1000 thermal cyclers (Bio-Rad, Hercules, CA), and were

| Region amplified | Primer sequence (5′-3′) | Predicted amplicon size | PCR annealing temperature | SSCP condition |
|------------------|-------------------------|--------------------------|---------------------------|----------------|
| 5′ flanking      | CAGTGGGCTTTACAGCTCAG    | 340 bp                   | 60°C                      | 25°C, 270 V, 15 h |
|                  | CAYGCAATAATACCTTACC     |                          |                           |                |
| Exon 3           | CTGCTCAGAGGCTACCTAC    | 452 bp                   | 62°C                      | 31°C, 250 V, 19 h |
|                  | GCTGAAACACTAGGGCTGCG    |                          |                           |                |
| Exon 4           | GACTGCTGGAGATATACCTGG  | 389 bp                   | 62°C                      | 28°C, 250 V, 15 h |
|                  | CTTGATGCGCTTTACCCTTCTG |                          |                           |                |

IGF1, insulin-like growth factor 1; SSCP, single strand conformation polymorphism.
performed in a 15-µL reaction containing the purified genomic DNA on a 1.2-mm punch of the TFN paper, 0.5 U Taq DNA polymerase (Qiagen, Hilden, Germany), 0.25 mM of each primer, 2.5 mM Mg²⁺, 150 µM of each dNTP (Bioline, London, UK) and 1× the reaction buffer supplied with the enzyme. The thermal profile for amplification consisted of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at the annealing temperatures shown in Table 1, and 30 s at 72°C; with a final extension of 5 min at 72°C.

Screening for sequence variation and sequencing of PCR-SSCP haplotypes

The PCR amplicons were screened for nucleotide 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 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 Protein II xi cells (Bio-Rad) in 0.5×TBE buffer, and the electrophoretic conditions shown in Table 1. Gels were silver stained according to the method of Byun et al. (2009).

The PCR amplicons identified as homozygous by SSCP analysis were directly sequenced at the Lincoln University Sequencing Facility, NZ. Sequence alignments, translations, and comparisons were carried out using DNA MAN. The SNPs that were revealed were named using the nomenclature described online and aligned to GenBank NC_019475.2 (Ovis aries breed Texel chromosome 18), Oar_v4.0.

Statistical analyses

There were some missing data, and sheep with incomplete records were removed from some analyses. Sample numbers, therefore, vary in different analyses. Statistical analyses were performed using Minitab version 17 (Minitab, Inc., State College, PA).

Two types of General Linear Mixed-Effects Models (GLMMs) were used to ascertain the effect of IGF1 genotype on the measured traits. The first models ascertained the effect of the presence/absence (recorded as 1 and 0) of the PCR-SSCP variant sequences on the measured traits. The second models were pairwise comparisons between genotypes using a Tukey test with Bonferroni corrections. The core model for these analyses was Yijklm=μ + Si + Gj + Bk + Di + Vm + eijklm, where Yijklm is the trait measured on each animal (birth weight, etc.), μ is the mean for the trait, Si is the random effect of sire, Gj is the effect of gender, Bk is the effect of birth weight, birth rank, or rearing rank, Di is draft age, Vm is the fixed effect of genotype or the presence/absence of each variant, and eijklm is the random residual error. For the birth weight GLMM, gender and birth rank were fitted into the models as fixed factors, but with the growth to weaning GLMMs, gender and rearing rank were fitted into the models. For carcass and yield traits, gender, birth weight, and draft age were fitted into the models as covariates. Only main effects were tested, and associations were considered significant at the 5% level.

Results

Nucleotide sequence variation in ovine IGF1

Two unique PCR-SSCP banding patterns were detected in each region of ovine IGF1, with either one or a combination of two banding patterns observed for each sheep (Fig. 1). DNA sequencing revealed that these PCR-SSCP patterns represented six unique sequences of IGF1. The six sequences have been deposited into GenBank with accession numbers MH144564–MH144569. In total, six SNPs were identified (Fig. 1). There was only one SNP in the exon 3 coding region, which was a synonymous SNP c.153T>C. The frequencies of these sequences in the NZ Romney sheep investigated are illustrated in Figure 1.

Effect of sequence variation in IGF1 on carcass and growth traits

In the 5′ flanking region one sequence (B1; c.-648C and c.-646A) was predominant, whereas the other sequence (A1; c.-648G and c.-646G) occurred at a frequency of <5%, hence the association analyses were only undertaken for the exon 3 and exon 4 regions.

For the exon 3 region, an effect of the presence/absence of PCR-SSCP variant was observed for growth rate to weaning (Table 2), with the presence of B2 being associated (p=0.048) with a lower growth rate (present: 384.9±3.77 grams/day; absent: 396.0±5.44 grams/day). An effect of the presence/absence of PCR-SSCP variant was also observed for HCW, V-GR, and proportion leg yield (Table 2), with the presence of B2 being associated with increased HCW (p=0.015) and increased V-GR (p=0.003), but decreased proportion leg yield (p=0.012). For this exon 3 region, an effect of genotype was observed for HCW, V-GR, and shoulder yield (Table 3). Sheep with genotype B2B2 (c.64-82CC and c.153CC) had lower HCW (p=0.010), lower V-GR (p=0.005) and less shoulder yield (p=0.021) than those sheep of genotype A2A2 (c.64-82AA and c.153TT) or A2B2 (c.64-82AC and c.153TC).

No associations were detected for the exon 4 region (results not shown).

Discussion

This is the first report describing associations between sequence variation in ovine IGF1 and growth and carcass traits in NZ Romney lambs. There was only a single synonymous SNP detected in the coding region of IGF1, which is in agreement with the observation that IGF1 is conserved among mammals and that the IGF1 protein, along with IGF2 and insulin, comprise a conserved protein family found in most mammalian species and in many other vertebrates (Rotwein, 2017). Highly conserved sequences are typically associated with proteins that underpin conserved or essential metabolic activities (Zhao et al., 2018), and mice that are IGF1-null (created by homologous recombination), exhibit postnatal lethality, growth retardation, infertility, and profound defects in the development of their major organ systems, with this confirming the essential nature of the protein’s activity (Liu et al., 2000).

The effect of SNPs in the 5′ flanking region cannot be reliably assessed in this study due to the minor sequence A (c.-648G and c.-646G) occurring at a low frequency (4.4%)
Association of IGF1 Exon 3 Sequences with Growth and Carcass Traits in New Zealand Romney Sheep

| Trait                        | Variant | Absent | Present | Absent | Present | Mean ± SE | p    |
|------------------------------|---------|--------|---------|--------|---------|----------|------|
| Birth weight (kg)            | A2      | 209    | 506     | 5.68 ± 0.07 | 5.77 ± 0.05 | 0.255    |      |
|                              | B2      | 144    | 571     | 5.78 ± 0.08 | 5.74 ± 0.05 | 0.659    |      |
| Growth rate to weaning (grams/day) | A2      | 209    | 506     | 390.1 ± 5.13 | 387.0 ± 3.68 | 0.532    |      |
|                              | B2      | 144    | 571     | 387.0 ± 3.68 | 384.9 ± 3.77 | 0.048    |      |
| HCW (kg)                     | A2      | 127    | 316     | 16.74 ± 0.23 | 17.21 ± 0.20 | 0.015    |      |
|                              | B2      | 93     | 350     | 17.22 ± 0.25 | 17.02 ± 0.20 | 0.350    |      |
| V-GR (mm)                    | A2      | 127    | 316     | 7.04 ± 0.32 | 7.84 ± 0.27 | 0.003    |      |
|                              | B2      | 93     | 350     | 7.82 ± 0.34 | 7.52 ± 0.27 | 0.316    |      |
| Shoulder yield (%)           | A2      | 127    | 316     | 17.01 ± 0.10 | 17.18 ± 0.09 | 0.052    |      |
|                              | B2      | 93     | 350     | 17.13 ± 0.11 | 17.12 ± 0.09 | 0.974    |      |
| Loin yield (%)               | A2      | 127    | 316     | 14.83 ± 0.10 | 14.92 ± 0.09 | 0.316    |      |
|                              | B2      | 93     | 350     | 14.90 ± 0.11 | 14.90 ± 0.09 | 0.869    |      |
| Leg yield (%)                | A2      | 127    | 316     | 22.19 ± 0.14 | 22.13 ± 0.12 | 0.591    |      |
|                              | B2      | 93     | 350     | 22.15 ± 0.15 | 22.15 ± 0.12 | 0.857    |      |
| Total yield (%)              | A2      | 127    | 316     | 54.03 ± 0.29 | 54.22 ± 0.24 | 0.415    |      |
|                              | B2      | 93     | 350     | 54.20 ± 0.31 | 54.15 ± 0.24 | 0.874    |      |
| Proportion shoulder yield (%)| A2      | 127    | 316     | 31.49 ± 0.13 | 31.68 ± 0.11 | 0.080    |      |
|                              | B2      | 93     | 350     | 31.61 ± 0.14 | 31.63 ± 0.11 | 0.843    |      |
| Proportion loin yield (%)     | A2      | 127    | 316     | 27.44 ± 0.11 | 27.50 ± 0.09 | 0.505    |      |
|                              | B2      | 93     | 350     | 27.49 ± 0.11 | 27.48 ± 0.09 | 0.918    |      |
| Proportion leg yield (%)      | A2      | 127    | 316     | 41.06 ± 0.12 | 40.81 ± 0.10 | 0.012    |      |
|                              | B2      | 93     | 350     | 40.90 ± 0.13 | 40.89 ± 0.10 | 0.900    |      |

aHCW—hot carcass weight; V-GR—VIAscan fat depth at the 12th rib.
bPredicted 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 < 0.05 are in bold, whereas 0.05 ≤ p < 0.10 are italicized.

GLMMs, General Linear Mixed-Effects Models.
Szewczuk (2014) and Gholibeikifard et al. (2016) found associations between the SNPs c.-5363C and c.-5188G in Haitihosseinlo. Tahmoorespur et al. (2016), Grochowska et al. (2019), suggest that c.-91A>T associated with growth traits in Baluchi sheep (IGF1 and growth traits is notable. The two SNPs in the 5' half-sibs at yearling shearing (Damak et al., 1996, 1999). The findings of this study in Pomeranian Coarse-wool sheep and Baluchi sheep, respectively, and did not find any association with growth traits. Ramasamy (2018), Nazari et al. (2016) and Grochowska et al. (2017) investigated polymorphism in the 5' flanking region of IGF1 and also did not detect any association with growth traits in different sheep breeds.

It is unknown whether the effect of IGF1 polymorphism on growth traits is breed dependent, but given some of the associations were typically detected with small numbers of sheep and/or there was the lack of genetic background for statistical correction, caution should be taken when interpreting these results, and further investigations may be required to confirm the findings.

The associations detected for the exon 3 PCR-SSCP variants and HCW, V-GR, and shoulder yield suggest that exon 3 nucleotide sequence variation affects selected carcass traits, although Trukhachev et al. (2016) revealed no associations between the synonymous substitution of c.81T>C in this exon and meat production parameters.

Given that HCW and V-GR have a moderate positive correlation (r=0.573; Supplementary Table S1), the associations detected for HCW and V-GR may be due to these traits being correlated. Polymorphism in the 5’ flanking region of IGF1 affected EUROP fat class, kidney fat class, and external fatness of carcass class in Colored Polish Merino sheep (Grochowska et al., 2017). Another study in Mehraban sheep describes how IGF1 polymorphism is associated with the triglyceride and cholesterol content of blood and the authors reported a tendency for association of the IGF1 polymorphism with dorsal fat thickness (Behzadi et al., 2015). In cattle, a SnaBI polymorphism in the regulatory region of the IGF1 associated with subcutaneous back fat (Curi et al., 2005), and a promoter SNP in IGF1 associated with ultrasound back fat thickness and carcass average back fat in the Angus beef (Islam et al., 2009). With transgenic mice, IGF1 has been shown to be involved in fat cell development (Rajkumar et al., 1999). The findings of this study and others suggest that IGF1 could be considered as a candidate gene for fat-related carcass traits.

Table 3. Association of IGF1 Exon 3 Genotypes with Growth and Carcass Traits in New Zealand Romney Sheep

| Trait*                  | A2A2 (n=144) | A2B2 (n=362) | B2B2 (n=209) | p       |
|-------------------------|--------------|--------------|--------------|---------|
| Birth weight (kg)       | 5.78±0.08    | 5.76±0.05    | 5.68±0.07    | 0.515   |
| Growth rate to weaning (grams/day) | 395.07±5.08 | 385.96±3.54 | 388.76±4.55 | 0.256   |
| HCW (kg)                | 17.31±0.25a  | 17.17±0.20a  | 16.63±0.23b  | 0.010   |
| V-GR (mm)               | 7.91±0.34a   | 7.67±0.28a   | 6.88±0.31b   | 0.005   |
| Shoulder yield (%)      | 17.24±0.10a  | 17.24±0.08a  | 17.02±0.09b  | 0.021   |
| Loin yield (%)          | 14.91±0.11   | 14.92±0.09   | 14.83±0.11   | 0.604   |
| Leg yield (%)           | 22.16±0.15   | 22.12±0.13   | 22.19±0.14   | 0.824   |
| Total yield (%)         | 54.22±0.24   | 54.30±0.23   | 53.91±0.52   | 0.717   |
| Proportion shoulder yield (%) | 31.79±0.12  | 31.83±0.11   | 31.62±0.12   | 0.148   |
| Proportion loin yield (%) | 27.46±0.10  | 27.38±0.08   | 27.35±0.10   | 0.648   |
| Proportion leg yield (%) | 40.76±0.11   | 40.79±0.10   | 40.97±0.11   | 0.126   |

*HCW—hot carcass weight; V-GR—VIAScan fat depth at the 12th rib.
**Predicted means and standard error of those means derived from the GLMMs, with means that do not share a superscript letter (a or b) within rows being different at p<0.05 and shown in bold.

in the NZ Romney sheep investigated. However, the sequence frequencies in this region appear to be interesting. In Iranian Zandi sheep, a medium-sized dual-purpose breed used for meat and pelt production and found in the central region of Iran, those with the nucleotide sequence variation that was also revealed in A (c.-648G and c.-646G) constituted 47% of the population (Nazari et al., 2016). In Colored Polish Merino sheep, c.-648G and c.-646G are common, with a frequency of 91.6% reported (Grochowska et al., 2017) and it is detected at a frequency of 19.1% in Small Tail Han sheep (primarily a meat breed in China), and was very rare or absent in Texel and Dorset sheep (both meat breeds) (He et al., 2012). Whether this difference in sequence frequency is related to meat/wool/pelt production, or just reflects breed differences, awaits further investigation. However, the findings that the SNPs in this region affected wool production, with A (c.-648G and c.-646G) being associated with increased clean fleece weight in Egyptian Barki sheep (Darwish et al., 2017) and that IGF1 transgenic sheep produced more clean fleece than their nontransgenic half-sibs at yearling shearing (Damak et al., 1996), suggest that IGF1 may play a role in regulating wool growth and production.

The finding of associations between polymorphism in IGF1 and growth traits is notable. The two SNPs in the 5' flanking region described in Ramasamy (2018), Nazari et al. (2016), Grochowska et al. (2017), and in this study, were associated with growth traits in Baluchi sheep (n=102; Tahmoorespur et al., 2009), Makui sheep (n=100; Hajihosseinlo et al., 2013), and Makooei sheep (number unknown; Negahdary et al., 2013). In addition, Trukhachev et al. (2016) found associations between the SNPs c.-5363C>T, c.-5186G>C, c.-5186G>A and c.-4088G>A, and live weight, and reported that c.-91A>C had a correlation with live weight, withther weight, crump height, width and length, and other physical attributes in rams. Associations with the 5' flanking SNPs could not be tested in this study. However, Prosksura and Szewczuk (2014) and Gholibeikifard et al. (2013), investigated the effect of X69473.1:g271C>T (equivalent to c.153C>T in this study) in Pomeranian Coarse-wool sheep and Baluchi sheep, respectively, and did not find any association with growth traits. Ramasamy (2018), Nazari et al. (2016) and Grochowska et al. (2017) investigated polymorphism in the 5' flanking region of IGF1 and also did not detect any association with growth traits in different sheep breeds.

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Given that HCW and V-GR have a moderate positive correlation (r=0.573; Supplementary Table S1), the associations detected for HCW and V-GR may be due to these traits being correlated. Polymorphism in the 5' flanking region of IGF1 affected EUROP fat class, kidney fat class, and external fatness of carcass class in Colored Polish Merino sheep (Grochowska et al., 2017). Another study in Mehraban sheep describes how IGF1 polymorphism is associated with the triglyceride and cholesterol content of blood and the authors reported a tendency for association of the IGF1 polymorphism with dorsal fat thickness (Behzadi et al., 2015). In cattle, a SnaBI polymorphism in the regulatory region of the IGF1 associated with subcutaneous back fat (Curi et al., 2005), and a promoter SNP in IGF1 associated with ultrasound back fat thickness and carcass average back fat in the Angus beef (Islam et al., 2009). With transgenic mice, IGF1 has been shown to be involved in fat cell development (Rajkumar et al., 1999). The findings of this study and others suggest that IGF1 could be considered as a candidate gene for fat-related carcass traits.
Shoulder yield only had a weak correlation with both HCW and V-GR, suggesting that whatever effect the IGF1 polymorphism was having, it may be different to how it might affect V-GR or HCW. The effect of IGF1 polymorphism on meat yield has been reported for both sheep and beef cattle, with Grochowska et al. (2017) describing how 5’ flanking region polymorphism affects fore shank weight, although they did not reveal an effect on shoulder yield in the Colored Polish Merino sheep. A promoter SNP associated with carcass lean meat yield in the Angus beef population, but not in Charolais cattle and hybrid Charolais×Angus cattle (Islam et al., 2009). This suggests that different SNPs in IGF1 may have different effects on meat yield and/or the effect may vary between breeds.

The IGF1 gene is located on ovine chromosome 3, which to date has had at least 60 quantitative trait loci (QTL) located on it (sheep QTL database, 2019), including markers for birth weight, body weight, muscle depth, and subcutaneous fat thickness.

The genotype associations detected for HCW, V-GR, and shoulder yield suggest that B2 is associated with a decrease in HCW, V-GR, and shoulder yield, whereas A2 (c.64-82A and c.153T) is associated with an increase in HCW, V-GR, and shoulder yield. As there was no difference in the marginal means for these traits between A2A2 (c.64-82AA and c.153TT) and A2B2 (c.64-82AC and c.153TC) sheep, this suggests that B2 (c.64-82C and c.153C) has a recessive effect, whereas A2 (c.64-82A and c.153T) has a dominant effect on these traits.

The effect of A2 (c.64-82A and c.153T) may come about directly as a result of the two SNPs. Although the SNP in the coding region (c.153T>C) was synonymous, and would not result in an amino acid substitution, it may affect the expression or structure of the protein. It has been reported that silent mutations may affect mRNA translation rates and thus potentially change the way that protein folds (Hurst, 2011). With the intronic SNP c.64-82A>C, introns are known to carry regulatory sequences, so although they may not have a direct involvement in the regulation of transcription of highly expressed genes (Mullen et al., 2011), they can affect alternative splicing mechanism and may be associated with mRNA transport or chromatin assembly (Jo and Choi, 2015).

Finally, it is quite possible that the effects observed in this research are due to the SNPs observed being linked to nucleotide sequence variation in other regions of the gene that regulate gene expression or function.

Conclusions

This study used PCR-SSCP to screen for nucleotide sequence variation in the 5′ flanking region, exon 3, and exon 4 regions of ovine IGF1. Six previously identified SNPs were identified in 848 NZ Romney sheep. In different models, sequence variation in exon 3 of IGF1 was associated with growth rate to weaning, HCW, V-GR, and shoulder lean meat yield and proportion leg yield. Verification of these findings will require further testing in more sheep from different flocks and breeds.

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Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Table S1

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