Molecular Genetics Underlying Ovine Growth and Carcass Traits in New Zealand Breeds

A thesis submitted in fulfilment of the

requirements for the Degree of Doctor of Philosophy

at

Lincoln University

By

Ugonna Jane Ekegbu

Lincoln University

2019

Abstract of a thesis submitted in fulfilment of the

requirements for the Degree of Doctor of Philosophy

Molecular Genetics Underlying Ovine Growth and Carcass Traits in New Zealand Breeds

by

Ugonna Jane Ekegbu

As a major source of protein in many nations, the global demand for meat is rising. The multi-billion dollar New Zealand (NZ) export sheep meat industry is always searching for new ways to increase profitability, improve productivity and increase efficiency. Sheep growth and carcass traits are therefore considered to be of economic importance; with greater emphasis being placed on the use of genetic tools in breeding for leaner, faster-growing animals that yield more meat with good taste characteristics.

This research examined four sheep genes: insulin-like growth factor 1 receptor (*IGF1R*), growth hormone (*GH)*, POU class 1 homeobox 1 (*POU1F1*), and PROP paired-like homeobox 1 (*PROP1*). The biological function of the protein products of these genes, and their direct or indirect effect on traits associated with meat production, suggested they were good candidates for further study. This research investigated whether nucleotide sequence variation was present in these genes, and whether associations exist between variation in the genes and variation in selected sheep growth and carcass traits.

To achieve this goal, firstly, ten different NZ sheep breeds were screened to ascertain whether nucleotide sequence variation was present in selected regions of the genes. This involved using a combination of polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis, coupled with nucleotide sequencing. Next, with NZ Romney sheep that had well-recorded pedigree and growth and carcass traits, general linear mixed-effects models (GLMMs) were then used to ascertain whether or not any nucleotide sequence variation detected was associated with the growth and carcass traits.

The IGF1 receptor mediates key signalling processes that are crucial to cell growth, protein synthesis, proliferation, survival and metabolism. This study examined three fragments of ovine *IGF1R*, one of which spanned from exon 9 to intron 10. Five variants (A_9 to E_9) defined by ten nucleotide substitutions, including a deletion (c.2201+114_118delCGCAG), were detected in the exon 9 to intron 10 region. Lambs possessing the A_9 variant grew faster by 10.8 g/day (P = 0.001), had 9% less fat thickness (P = 0.038), weighed 3.2% more at slaughter (P < 0.001), and had 1.2% increase in total lean meat yield (P = 0.002). In contrast, the E_9 variant was associated with slower-growing lambs (P = 0.001), increased fat depth (P = 0.022), and decreased muscularity (P = 0.009).

Growth hormone is a key mediator of growth and other metabolic processes. Variation in *GH* has been reported in several species where it showed association with production traits. In this study, a fragment of ovine *GH* spanning the exon 2, intron 2 and exon 3 regions was found to have 11 nucleotide substitutions that were arranged in seven variants (named A_3 to G_3). Four of the substitutions were found in the coding region of *GH*, namely c.51C>T (p.Leu17Leu), c.59C>T (p.Pro20Leu), c.79G>A (p.Ala27Thr), and c.103G>C (p.Gly35Arg). The B_3 variant was associated with an increase in average daily weight gain by 17.4 g/day (P < 0.001), lower fat depth at the 12th rib by 0.48 mm (P = 0.015), and increased total lean meat yield by 1.2% (P = 0.007). In contrast, the F_3 variant was associated with decreased average daily weight gain (P < 0.001), lower weight at slaughter (P < 0.001), and decreased total lean meat yield (P < 0.001). Lambs possessing the A_3B_3 genotype grew the fastest (274.59 ± 10.56 g/day; P < 0.001) and had the highest lean meat yield (53.47 ± 0.19%, P < 0.001).

POU1F1 positively regulates the functions of three key hormones: GH, thyroid-stimulating hormone, and prolactin. Three fragments were explored in this study, but only the exon 1 fragment revealed any sequence variation with two variants, *A* and *B*. Associations with growth and carcass traits were not tested.

PROP1 controls prenatal muscle development and differentiation. Two fragments spanning the exon 1 and exon 2 regions of ovine *PROP1* were examined. Three variant sequences were revealed in each region: exon 1 - A_1, B_1 and C_1, and exon 2 - A_2, B_2 and C_2. The more abundant A_1 and A_2 variants were associated with an increase in growth rate (P < 0.001) and higher total lean meat yield (P = 0.001). Variant B_1 was associated with a decrease in birth weight by 0.14 kg (P = 0.025) and an increase in fat thickness by 0.45 mm (P = 0.013), while variant C_2 was associated with lower growth rate of 22.3 g/day (P = < 0.001) and decreased total lean meat yield by 1% (P = 0.025).

The sequence variations found in this study could potentially be used in marker-assisted selection (MAS) for improving growth and carcass traits in sheep.

Keywords: Sheep, growth, carcass, MAS, breeding, selection, *GH*, *IGF1R*, *POU1F1*, *PROP1*

Publications and Conference presentations arising from this thesis

Papers

Ekegbu, U.J., Burrows, L., Amirpour-Najafabadi, H., Zhou, H. and Hickford, J.G. (2019) Gene polymorphisms in PROP1 associated with growth traits in sheep. *Gene* 683, pp. 41-46.

Ekegbu, U.J., Haruna, I.L., Mahmoud, G., Zhou, H. and Hickford, J.G. (2019) Genetic polymorphisms in New Zealand sheep breeds. *World Journal of Agriculture and Soil Science* 1 (2), pp. 1 – 4.

Conferences

Ekegbu, U.J. (2017). Gene and Juice: Effect of gene variations on NZ sheep meat quality. Lincoln University Postgraduate conference, 20 – 21, 24 September 2018 at Lincoln University, Canterbury, New Zealand.

Ekegbu, U.J. (2018). Polymorphisms in somatotropic genes underlying growth and carcass traits in NZ sheep. 408th International Conference on Agricultural and Biological Sciences (ICABS), 29 – 30 July 2018 at Lagos, Nigeria.

Ekegbu, U.J. (2017). Polymorphisms in *PROP1* gene and association with growth and carcass traits. Lincoln University Postgraduate conference, 30 – 31 August 2017 at Lincoln University, Canterbury, New Zealand.

Sequences Submitted to NCBI GenBank

Ovine *GH2*

Ovine growth hormone (GH) gene, *GH2* allele variant 1, *A* sequence: MK573367

Ovine growth hormone (GH) gene, *GH2* allele variant 2, *B* sequence: MK573368

Ovine growth hormone (GH) gene, *GH2* allele variant 3, *C* sequence: MK573369

Ovine growth hormone (GH) gene, *GH2* allele variant 4, *D* sequence: MK573370

Ovine growth hormone (GH) gene, *GH2* allele variant 5, *E* sequence: MK573371

Ovine growth hormone (GH) gene, *GH2* allele variant 6, *F* sequence: MK573372

Ovine growth hormone (GH) gene, *GH2* allele variant 7, *G* sequence: MK573373

Ovine *IGF1R*

Ovine insulin-like growth factor 1 receptor (IGF1R) gene, variant 1, *A* sequence: MK210245

Ovine insulin-like growth factor 1 receptor (IGF1R) gene, variant 2, *B* sequence: MK210246

Ovine insulin-like growth factor 1 receptor (IGF1R) gene, variant 3, *C* sequence: MK210247

Ovine insulin-like growth factor 1 receptor (IGF1R) gene, variant 4, *D* sequence: MK210248

Ovine insulin-like growth factor 1 receptor (IGF1R) gene, variant 5, *E* sequence: MK210249

Ovine *PROP1*

Ovine PROP-paired like homeobox 1 (PROP1) gene, exon 1, *A* sequence: MF785097

Ovine PROP-paired like homeobox 1 (PROP1) gene, exon 1, *B* sequence: MF785098

Ovine PROP-paired like homeobox 1 (PROP1) gene, exon 1, *C* sequence: MF785099

Ovine PROP-paired like homeobox 1 (PROP1) gene, exon 2, *A* sequence: MH469232

Ovine PROP-paired like homeobox 1 (PROP1) gene, exon 2, *B* sequence: MH469233

Ovine PROP-paired like homeobox 1 (PROP1) gene, exon 2, *C* sequence: MH469234

Acknowledgements

"You never know how strong you are, until being strong is your only option" – Mom / Bob Marley

The journey towards a PhD has been an awe-inspiring, yet emotional rollercoaster. Some days I soared effortlessly, and other days I crawled through paragraphs. In the end, I was able to surmount a plethora of challenges. By means of this experience, I have become more resourceful, resilient, and proactive; essentially a better scientist.

My deepest gratitude goes to Professor Jon Hickford, my awesome supervisor. Thank you, Jon for being patient and insightful, for responding to my 2 am emails of manuscripts and drafts, and for your generous 'open-door' policy. Knowledgeable and sagacious, he gave me constructive advice, and taught me to write a compelling scientific narrative. This PhD would not have been possible without Jon's encouragement, unwavering confidence in me, and invaluable guidance. He is truly proof that 'not all heroes wear capes'.

I am also thankful to my co-supervisor, Dr Huitong Zhou. I am grateful for his help with experiments and data analysis, straight-forward advice and attention to detail. I owe thanks to Andrea Hogan, for her help with data collection and analyses, and for ensuring adequate amenities were available in the lab. Her meticulous and detail-oriented work enhanced the quality of this thesis. Thank you to the Gene Marker Laboratory for providing the facilities. I thank all the farmers and breeders who helped with sample collection. I thank all those who were involved in providing me with financial support, especially the John W & Carrie McLean Trust Scholarship 2019 in the final months of my PhD.

I am also grateful for the great group of friends and colleagues I have come to know over the years including staff and students. These include Ishaku, John, Joshua, Aimi, Mimi, Robyn, Hamed, Yun-Hai, Ghassan, Lucy, Chris, Freeman and Hua. Thank you to my extraordinary husband, Samuel Nwasimuo, for being my rock and support during despondent times.

My greatest gratitude goes to God, and to my phenomenal parents. Thanks Dad, for making all this possible at the start. It saddens me that you could not see my success all the way through, but I am sure you would have been very proud if you were alive. Thank you Mom, for believing in me and for supporting my ambitions against all odds posed by society. Thank you for instilling in me drive, strength, passion and compassion.

Table of Contents

List of Tables

List of Figures

Abbreviations

$	New Zealand dollar	MAPK	mitogen-activated protein kinase
α	alpha	MAS	marker-assisted selection
β	beta	mM	millimolar
◦C	degree Celsius	MSTN	myostatin
χ^2	Chi-square	ng	nanogram
µg	microgram	nm	nanometre
µL	microlitre	nt	nucleotide
µM	micromolar	NZ	New Zealand
Akt/PKB	Protein kinase B	OAR	*Ovis aries* chromosome
ANOVA	Analysis of Variance	Oct-01	Octamer-binding factor 1
bHLH	basic helix-loop-helix	PCR	polymerase chain reaction
bp	base pair	PI3K	phosphatidylinositol 3-kinase
C-terminal	carboxy-terminal	pH	potential hydrogen, $-\log[H^+]$
cal	calories	POU1F1	POU class 1 homeobox 1
dATP	deoxyadenosine triphosphate	PROP1	PROP paired-like homeobox 1
dCTP	deoxycytidine triphosphate	QTL	quantitative trait loci
dGTP	deoxyguanosine triphosphate	RNA	ribonucleic acid
DNA	deoxyribonucleic acid	rpm	revolutions per minute
dNTP	deoxynucleotide triphosphate	SE	standard error
dsRNA	double-stranded RNA	SIL	Sheep Improvement Limited
dTTP	deoxythymidine triphosphate	SNP	single nucleotide polymorphism
EDTA	ethylenediaminetetraacetic acid	SP	signal peptide
ERK	extracellular signal-regulated kinases	SSCP	single-strand conformational polymorphism
FOXO	Forkhead box	*Taq*	*Thermus aquaticus*
g	gram	TATA	TATA box
GH	growth hormone	TGF-β	transforming growth factor-β
GLMM	general linear mixed-effect models	TE	Tris EDTA
h	hour	Tris	tris(hydroxymethyl) aminomethane
HCW	hot carcass weight	U	unit
HTH	helix-turn-helix	UTR	un-translated region
IGF1	insulin-like growth factor 1	UV	ultraviolet
IGF1R	insulin-like growth factor 1 receptor	V	volt
IUGR	intrauterine growth restriction	V-GR	Viascan fat depth
kb	kilobase	VIAscan®	video imaging analyses
kg	kilogram	wt	weight

Amino Acid Abbreviations

Amino Acid	3-letter code	1-letter code	R-group class
Alanine	Ala	A	Non-polar
Arginine	Arg	R	*Basic*
Asparagine	Asn	N	Polar
Aspartic acid	Asp	D	*Acidic*
Cysteine	Cys	C	Polar
Glutamic acid	Glu	E	*Acidic*
Glutamine	Gln	Q	Polar
Glycine	Gly	G	Non-polar
Histidine	His	H	*Basic*
Isoleucine	Ile	I	Non-polar
Leucine	Leu	L	Non-polar
Lysine	Lys	K	*Basic*
Methionine	Met	M	Non-polar
Phenylalanine	Phe	F	Non-polar
Proline	Pro	P	Non-polar
Serine	Ser	S	Polar
Threonine	Thr	T	Polar
Tryptophan	Trp	W	Non-polar
Tyrosine	Tyr	Y	Polar
Valine	Val	V	Non-polar
Unknown/Stop	-	X	

Chapter 1
General Introduction

1.1 Background of this study

Agriculture is the largest sector of the tradable economy in New Zealand (NZ), accounting for about five percent of the GDP, with red meat production and export being a significant contributor and principal driver of the NZ economy (NZ Treasury, 2010; Meat Industry Association, 2018). In the year ending December 2018, the NZ red meat industry generated over $8 billion in export revenue, with about 30 million sheep and 8 million cattle being slaughtered and processed, and over 1 million tonnes of red meat and its co-products exported to 120 countries (Beef + Lamb NZ, 2018a; Meat Industry Association, 2018). Lamb and mutton exports constituted about half of the revenue. Not only does the red meat sector generate export revenue, but it also creates employment for thousands of people in NZ.

Growth in the world human population has brought about an increased need for food, and red meat is a major component in many diets. In 2017 alone, over 330 million tonnes of meat were produced globally, and by 2050, demand is projected to double (Rojas-Downing et al., 2017; FAO, 2018). Sheep meat holds high global demand because it faces fewer cultural and religious restrictions to consumption compared to other meat sources.

Increasing productivity in livestock species farmed mainly for meat production often entails increasing muscling, growth rates and litter size. The growth rate of lambs is a chief determinant of farm profit (Golding et al., 2008). Faster-growing lambs, from the perspective of production efficiency, can attain target market weights earlier than their slower-growing counterparts. This means that they can be weaned and drafted for slaughter more rapidly, hence yielding higher premiums and reducing production costs associated with labour input, feeding and healthcare. The gross efficiency of energy conversion, expressed as the amount of lamb meat produced in kg per unit feed intake, is higher for faster-growing lambs when compared to their slower-growing counterparts of the same weight (Elmes, 2013). Slower growing animals have a higher proportional maintenance cost in feeding (primarily a function of their live-weight), versus the additional feed needed to enable growth.

In some markets, the industry is being shaped by increasing consumer demands for better quality products, with juicier yet leaner meat being favoured in higher value markets. Consumer perception and increased awareness linking dietary fat to obesity, cardiovascular disease and mortality, has brought about this shift in consumer preference to lean meat (Bray and Popkin, 1998), such that only a minimal amount of intramuscular fat is considered acceptable for reasons of tenderness and taste. The meat industry is constantly seeking to strike a balance by ensuring the smallest fat content, whilst retaining palatability.

Carcass quality is affected by the weight, muscularity and body composition (fat to bone and muscle ratio) of animals at slaughter (Stanford et al., 1998). In some markets, the weight of a carcass is of critical importance, while in others, the amount of saleable meat on a carcass governs its financial value. Accordingly, lambs that produce higher yields of lean meat can be of importance to farmers, processors and consumers. Previous research has suggested that about half of all NZ lamb carcasses do not meet industry standards (Gooch and Firth, 2007), and this along with declining sheep numbers, poses a huge threat to the NZ meat industry. There is therefore an on-going need to improve lamb growth and carcass traits.

Short-term or transient improvement of carcass value can be achieved by using different management systems, including improving pre-slaughter handling of sheep, hormone administration, altering nutrition and feed quality/quantity, changing slaughter time and post-slaughter processing, and changing the on-farm environment (Freer, 2002). However, these strategies face limitations, such as the decreased efficiency of meat output per unit of land, as lamb slaughtered early has not attained its full growth potential (Lewis et al., 1996). In addition, altering forage composition in extensive farming systems and the post-slaughter trimming of fat can be both expensive and labour intensive. Results from these strategies are also not guaranteed, as they may vary across farms and processing plants.

Long-term improvement of carcass quality would require the selection of superior animals with commercially desirable traits for breeding. Traditional livestock selection based on visible ancestral performance is, for some traits, being superseded by genetic selection approaches, largely because the physical measurement of meat quality traits using *ex-vivo* approaches can be expensive, unreliable and difficult to standardise (Dunner et al., 2013). As such, they are impractical for testing a large number of animals from across different breeds. While it is true that various factors contribute to growth and development in

animals, including genes, metabolism, nutrition and environment, the assessment of the genetic components of growth is a reliable method to predict improvement in these production traits. Vital production traits such as carcass weight, lean meat yield, and growth rate all exhibit moderate heritability (0.25 – 0.5) (Safari et al., 2005). Hence, genetic selection could potentially improve these traits; and in an NZ context, the large and diverse population of sheep makes it ideal for the exploration of genetic markers.

Much of the diversity observed in species are governed by genetic loci, known as quantitative trait loci (QTL), exerting measurable effects on the phenotypic traits of an animal (Collard et al., 2005). Genes located in the QTL of established traits are known as candidate genes. Genetic improvement by means of molecular techniques relies on identifying, mapping and analysing genetic variations at important genetic loci. Hence, there is an exigent need to identify novel variations in candidate genes that are associated with key production traits. While some research has been completed in various ovine breeds, there is much work to be done with the NZ sheep breeds and in unexplored genes. Studies of diverse populations are then required to affirm the robustness of associations with key production traits reported for gene variations.

The selective breeding of animals based on genotypic variation at targeted loci, as an approach to improve livestock performance, is gaining more popularity in the meat industry. Companies such as GeneSTAR® and Igenity® utilise genetic markers to commercially offer gene typing tests to livestock breeders (Igenity, 2012).

Quantitative trait loci associated with liveweight, and growth and carcass traits in sheep, are found on chromosomes 1, 2, 3, 4, 5, 6, 11, 14, 18, 20, and 21 (Walling et al., 2004). Of the many genes housed in those regions, the ones selected for this study were: insulin-like growth factor 1 receptor (*IGF1R*), growth hormone (*GH*), POU class 1 homeobox 1 (*POU1F1*), and PROP paired-like homeobox 1 (*PROP1*). These genes were chosen because the physiological functions of their products directly or indirectly link to growth and carcass traits in sheep. Hence, nucleotide variation found therein could serve as potential markers for selective breeding.

This research thus aims to evaluate sequence variation in selected candidate genes and their association with growth and carcass traits in NZ sheep breeds. Genetic selection strategies utilising polymorphisms uncovered in this study have the potential to provide long-lasting approaches to boosting the profitability and sustainability of the NZ sheep industry, as well as promoting sheep production at higher market value.

1.2 Chapters Outline

Chapter 1
General Introduction

Chapter 2
Literature Review

Practical Chapters

	Objectives
Chapter 3 The relationship between growth and carcass traits in NZ sheep breeds	To provide insight into the growth and carcass traits distribution within NZ sheep breeds.
Chapter 4 Investigation of variation in ovine *IGF1R* and its association with growth and carcass traits	To uncover variation within sparsely elucidated regions of the *IGF1R* namely, exon 9, intron 9, exon 10 and intron 10. Further investigation of the effect on growth and carcass traits will be undertaken for any found variation.
Chapter 5 Variation in ovine *GH* and its association with growth and carcass traits in NZ sheep	To identify variation in *GH* and explore its association with growth and meat quality traits in NZ sheep breeds. Three major regions will be examined: exon 2, intron 2, and exon 3.
Chapter 6 Analysis of *POU1F1* variation across different NZ sheep breeds	To investigate nucleotide variation within the catalytic domains of *POU1F1* in NZ sheep breeds.
Chapter 7 Characterisation of ovine *PROP1* variation and its association with growth and carcass traits	To detect nucleotide polymorphisms in the coding regions, exon 1 and exon 2, of *PROP1* in ten NZ sheep breeds. Polymorphisms will be investigated for association with ovine growth and carcass traits.

Chapter 8
General Summary and Future Direction

Chapter 2
Literature Review

2.1 Sheep Production

Sheep (*Ovis aries*) provide many products of value to humans in the form of meat, wool, milk, skin and manure. While most sheep are 'multi-purpose' with respect to the things they produce, they are, nevertheless, commonly classified and farmed based on the products of commercial importance they deliver. The relative economic significance attributed to a product is influenced by consumer demand, and hence varies between different countries and markets.

Wool is the most widely used animal fibre. It is typically harvested by shearing and used in a variety of ways such as in the manufacture of clothing, furniture, mattresses, and carpets. During the early 20th century, wool exports provided the majority of income from sheep farming in NZ, with an economic peak in 1950 termed the 'NZ wool boom' (Hawke, 1985). The end of World War II saw a rapid decline in wool demand production, partly because wool was no longer required for uniforms and blankets for soldiers, but also because the industry encountered increased competition from competing fibres, including human-made fibres, in subsequent decades (Evans et al., 1996). Changing consumer taste in favour of the cheaper synthetic fibres or synthetic/natural blends made wool a less important export earner, but despite not being in its economic prime, wool still appeals to high-end consumers, and creates value for sheep farming, textile manufacture, and the fashion industry (Wuliji et al., 2001). NZ is particularly renowned for merino wool – a highly sought after wool with extremely fine fibres used to produce soft, elastic and breathable textiles.

Globally, Australia is the largest producer and exporter of wool, distributing over half of the world's export by volume. New Zealand comes in second, supplying about one-fifth of the world's wool which were mainly of coarse or strong variety (Teara, 2008). This type of wool is mainly used in the manufacture of carpets, bedding, upholstery and yarn. The top export markets for wool include China, United Kingdom, Italy, India and Belgium.

Sheep milk production is growing in popularity. Sheep milk has a relatively higher content of micronutrients such as calcium, phosphorous, and potassium, as well as vitamins A, B, D, and E, compared to cow milk (Campbell and Marshall, 1975). It also contains a higher

amount of conjugated linolenic acid (CLA) than milk from cattle, goats and humans, which has been reported to have cancer-fighting properties (Park et al., 2007). Milk obtained from sheep is commonly used in the production of cultured dairy products like cheese. The economic value of cheese is enhanced by its high solids content, greater than that of goats or cows (Raynal-Ljutovac et al., 2008). A higher solid content means that a gallon of sheep milk would produce more cheese than a similar gallon of goat or cow milk, as well as contributing to its rich creamy texture. Popular cheese derived from sheep milk include feta (Greece), ricotta (Italy) and Roquefort (France). Another factor that makes sheep milk aesthetically pleasing is the presence of smaller fat globules compared to cattle, which makes it easier to digest (Giorgio et al., 2018). This is also a limitation, as the larger fat globules in cow's milk aid the separation of cream, and subsequently the production of other products like butter.

Although history asserts that the commodity first associated with sheep was wool, a paradigm shift in global demand has increasingly placed greater economic importance to meat relative to wool. Compared to its cattle or pig counterparts, sheep meat is in high demand globally, because it encounters fewer religious and dietary constraints to its consumption, especially in the Middle-Eastern and the Asian Sub-Continent States, where Islamic, Hindu and Jewish beliefs dictate the type of meat that can be eaten. In 2017 alone, over 14 million tonnes of sheep were produced worldwide (FAO, 2018). World sheep meat production and export over three years is summed up in Table 2.1.

Table 2.1 Total sheep meat produced and exported in the world and selected countries

	2015	**2016**	**2017**
Total sheep meat production (x1000 tonnes)			
World	14607	14747	14839
China	4410	4617	4691
EU	908	877	893
India	732	740	741
Australia	755	715	1.8
Total sheep meat export (x1000 tonnes)			
World	960	910	980
Australia	442	428	454
New Zealand	400	370	396
EU	18	16	30
India	22	21	23

Sources: FAO (Food and Agriculture Organization of the United Nations) (FAO, 2018)

Per capita, Oceania boasts the largest production and consumption of sheep meat, with its major countries, Australia and New Zealand, accounting for about 90% of the global sheep meat export (FAO, 2018). The sheep meat enterprise is highly profitable since sheep meat can be obtained from all types of sheep, including those bred primarily for wool or milk.

2.1.1 The New Zealand sheep meat industry

Sheep meat exports constitute a major proportion of NZ red meat exports. Since the early 21st century, NZ has become one of the larger exporters of sheep meat in the world, exporting over 92% of the sheep meat it produces (FAO, 2018; Meat Industry Association, 2018). Over $8 billion was generated from red meat exports in 2018, a remarkable 21% increase compared to the previous year (Beef + Lamb NZ, 2018a; Meat Industry Association, 2018), with lamb exports surpassing beef and comprising nearly half (Figure 2.1).

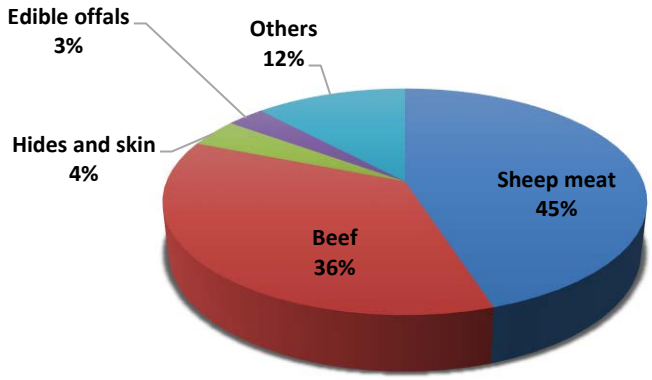


Figure 2.1 New Zealand red meat export value for the 2017/2018 period

(Meat Industry Association, 2018)

Over one million tonnes of red meat and co-products produced in NZ are shipped to more than 120 countries annually (Meat Industry Association, 2016). The EU constitutes NZ's largest market, especially for high-value products, with over 80,000 tonnes of lamb exported in 2016 (Beef + Lamb NZ, 2017). Another estimated 80,000 tonnes of lamb meat was exported to North Asia in 2017, as growing opportunities for new markets in that region arise (Beef + Lamb NZ, 2017). Increase in global sheep meat consumption has facilitated exports to other regions such as North America and the Middle East. The red

meat industry has enjoyed remarkable success with about 27 million sheep, with over 24 million processed annually (Beef + Lamb NZ, 2018b; Stats NZ, 2018). In NZ, while some sheep are bred solely for meat or wool production, a good number of sheep are dual-purpose. A brief history and detail of the ten sheep breeds investigated in this study is given below.

Romney

New Zealand Romney sheep originated from the wet marshes Kent in South England, and was brought to NZ by Messrs Bennett and Young, reaching Wellington in 1843 (Stringleman and Peden, 2008). Romney was then established in the early 1900s. The Romney was initially bred for its long heavy fleece due to the necessity of wool in the 17th century for making carpets and furnishing, but a shift in the industry to meat production meant NZ Romney was bred for both meat and wool (Meadows, 1997). The emphasis on meat production has led to an increase in the weight, size and fertility of Romney breeds. These traits, along with their adaptability to rough terrains and areas of heavy rainfall makes the Romney a highly sought-after breed.

About 50% of all the sheep in NZ are Romney breeds, making them a principal contributor to the economy (Dalton and Ackerley, 1974; Beef + Lamb NZ, 2018b). The Romney breed is also a favourite for use in crosses aimed at creating better sheep breeds.

Perendale

The Romney breeds experienced many difficulties in the steep landscapes of the North Island. The Perendale was formed from a cross between Romney and the Cheviot breeds to allow for adaptability on steep hill country (Stringleman and Peden, 2008). The Cheviot added its hardy constitution, easy shepherding and high lambing attributes to the mix. In addition to having a higher fertility rate than Romney, the Perendale has better foraging ability and hardiness (Dalton and Ackerley, 1974).

Coopworth

The Coopworth breed is the result of a Romney-Border Leicester cross in the 1960s. The Romney was added to the mix for its high-quality meat, while Border Leicester was selected primarily for its fertility, and also excellent maternal instinct. Coopworth ewes have a higher lambing percentage (30% triplets), and are best suited to low-land environments (Stringleman and Peden, 2008).

Poll Dorset

The Poll Dorset is a critical breed in the NZ meat industry popular for its succulent and lean meat, good muscling and faster growth rates. Its high growth rate, leanness, ease of lambing and out-of-season lambing makes the Poll Dorset a popular choice as a terminal sire breed for crosses (NZ Sheep Breeders Association, 2019).

Suffolk

The Suffolk sheep are large-size sheep known for their characteristic dark faces and dark long legs with a lack of horns. Suffolks were created by a cross between Southdown and Norfolk Horned breeds (NZ Sheep Breeders Association, 2019). In the past, the Suffolk was the most common terminal meat sire, but is largely being supplanted by the Poll Dorset breed. Both breeds share similar traits such as early maturation, high muscularity and lean-carcass.

White Dorper

The Dorper, a popular breed in Africa and the Middle East, is a cross between a Dorset Horn and Blackhead Persian, and hence Dorpers can be either all white-head or black-head (NZ Sheep Breeders Association, 2019). The Dorper is bred purely for meat, with little or no wool, thus producing high quality carcass. They are used as sires in crosses favouring larger carcass sizes.

Merino

The NZ economy is renowned for its wool production and export. The Merino is a medium-sized sheep breed originating in Spain, and was introduced into NZ in the early 1800s, making it one of the oldest NZ sheep breeds (NZ Sheep Breeders Association, 2019). On average, the NZ Merino sheep produces fine wool with a fibre diameter ranging from 13-25 microns (about one-third of the human hair diameter), weight of 3-6 kg, and a staple length of 65-100 mm (Wuliji et al., 2001). The Merino is bred for its highly prized ultra-fine and soft wool which is used in luxury garments like suits, insulation and upholstery.

The resilience and high adaptability of Merinos to climates of different temperatures due to the nature of its wool, enables farming in NZ's South Island where temperatures can climb to 35 ºC in summer, and drop to -10 ºC in winter. Merino breeds could hence stay cool in

summer and warm in winter. However, the stress brought upon the animal by harsh climates or neglect can be evident as weakened wool fibres (Wuliji et al., 2001). The The Merino breed was not ideal for meat production, as the carcass was very lean and produced tough meat.

Some challenges associated with Merino farming includes their slower growth and maturation as compared to meat breeds, susceptibility to the footrot and flystrike diseases which requires costly management by drenching (Dalton and Ackerley, 1974). Footrot is a contagious infection of tissue inside the hoof, while flystrike is a parasitic infection caused by flies attracted to the sheep's wool when it is contaminated with dirt, faeces and fluids.

Corriedale

In an attempt to improve wool production and inhibit footrot disease, a cross between long-wool Lincoln and Merino took place in the Otago areas of NZ during the late 1800s to early 1900s (NZ Sheep Breeders Association, 2019). Initially known as the inbred half-bred, the new breed was soon established as Corriedale, a dual-purpose breed reared for its wool and meat. Adapted for grazing on drier hilled areas, the Corriedale is farmed in both the North and South Island of NZ. Compared to the Merino breed, the Corriedale has higher fecundity and early maturation. The Corriedale lambs produce carcass with better muscling making them more profitable to rear (Stringleman and Peden, 2008). The Corriedale produces medium-fine wool (28-33 microns) that is versatile for use in knitting yarns, blankets, and carpets.

Texel

Originating from the cold and harsh environment of Texel in Holland, the Texel breed has adapted to difficult conditions by developing useful traits such as hardiness, low maintenance, high feed-to-energy conversion ratio and good mothering ability (NZ Sheep Breeders Association, 2019).

Dorset Down

The Dorset Down breeds are known for their early maturing and very lean carcass. Initially, the breed did not adapt well to the NZ climate following import from Europe. The Dorset Down is now considered a valuable sire breed, bringing excellent growth rate, and low-fat carcass into the genetic mix (NZ Sheep Breeders Association, 2019).

2.1.2 Role of growth and carcass traits in sheep production

Meat and meat products are an indisputable part of human diets which contain vital proteins, fatty acids, vitamins and minerals. A complex interplay of various factors including socio-economic status, price, religion, tradition, culture and geographical location influence dietary consumption. Consumers constitute the final step in the production chain, and meeting their expectations is of vital importance to the meat industry. The choice of the consumer is influenced by many factors including visual, sensory and marketing characteristics (Jopson et al., 2009). The consumer evaluation process can be broadly divided into two stages: before purchase and eating, and after purchase and eating. Prior to purchase, a key index of quality is the appearance of the meat, with colour being a major indicator of freshness (Pannier et al., 2018). Following consumption, the perception of taste in terms of tenderness, juiciness, and flavour of meat play important roles in the complete sensory experience.

From the perspective of the efficiency of meat production, slower-growing animals have higher proportional maintenance in feeding (primarily a function of their live-weight), versus the additional feed needed to enable growth. As a function of its size, all animals exert a maintenance cost, in the form of energy required for normal body function and homeostasis, the left-over of which is used for growth (Dickerson, 1978; Dawson and Steen, 1998). Faster-growing animals are better in this regard as they require less maintenance cost.

A carcass is composed of muscle, bone and fat, in varying proportions. Ideally, the animal carcass should have muscle in the highest proportion, sufficient fat, and very little bone. Although excessive fat is undesirable, a reasonable proportion enhances flavour (Pearce, 2016). Visceral fat, subcutaneous fat, and intramuscular fat are the three major kinds of carcass fat. The fats serve as energy reserves and in visceral insulation, while bones provide a rigid support. The muscle of the carcass comprises mainly of water (over 70%), along with some proteins, minerals and lipids (Pannier et al., 2014).

Animal growth is simply the deposition of protein, fat and bone in different quantities. As the animal approaches its mature body size plateau, protein deposition significantly decreases whilst fat deposition might continue (Irshad et al., 2013). Mature body size is thought to be the juncture at which muscle mass reaches its maximum. Typically, the protein mass increases proportionally to body weight. Consequently, increasing the mature body size through the use of genetic or nutritional techniques, such as the administration of

hormones or hormone modifiers, as a means to increase the protein:fat ratio of the carcass is commonplace (Owens et al., 1995). Alternatively, a reduction in carcass fat deposition can be achieved by limiting the energy intake or by the use of high concentrate diets.

Figure 2.2 Primal meat cuts on lamb carcass

Image sourced from Southdown Sheep Society NZ (2019).

At the processing plant, the carcass is weighed after being eviscerated, and the head pelt and gut are removed (Figure 2.2). Measurement of carcass weight as opposed to liveweight eradicates the effect of other factors such as gut fill (Allen et al., 1979).

The quantity of saleable meat of a carcass determines its economic value, thus lambs producing higher yields of lean meat are of prime importance to farmers, producers and consumers. In many regions, penalties are imposed for excess carcass fat as it is undesirable to consumers (Pearce, 2016). Lambs with higher lean meat yield also have greater efficiency with regards to feed conversion, and less labour and time required for trimming off excess fat. The visual component of meat, which is largely attributed to growth and carcass traits, undoubtedly plays a principal role in the consumer's perception and decision. Increasing literacy and growing awareness have skewed consumer demand for better quality meat (Bray and Popkin, 1998). While some consumers express concern over the cost of quality meat, high-end customers are willing to pay more for high-grade quality such as increased leanness. Preference varies across markets and ethnicities and is often influenced by culture and meat availability, with fatty meat preferred in the Middle

East and parts of Asia, while lean meat is considered ideal in parts of Europe and North America. A consensus points towards lean meat, with consumers insisting extra fat is trimmed off from meat to avoid being charged for a meat part that is considered inedible (Pearce, 2016).

Classification and grading of meat in NZ abattoir and processing plants are determined by defined characteristics that are crucial to consumers and the meat industry, such as carcass weight, muscle composition and fat depth over rib eye (Gooch and Firth, 2007). This, in turn, determines the selling price, and how much farmers are paid. Being regions of prime cuts, the leg, loin and shoulder regions are carefully inspected (Figure 2.2).

2.2 The Physiology of Animal Growth

Growth is one of the primary characteristics of any living organism. Understanding the processes that govern animal growth and development is essential to achieving human's dietary and other needs (Lawrence et al., 2012). The importance of growth and development in animal production cannot be overstated as they have a substantial influence on the commercial value of the animal. Essential food products such as meat and milk are the end products of animal growth. Growth is said to be bi-faceted: the first involving an increase in tissue mass, while the second entails a change in body composition due to varying degrees of specialised growth in different components of the body. Various methods have been employed to quantify growth, including a physical measurement of the animal's length or height (Hopkins et al., 2005). The measurement of the change in the liveweight of an animal remains the most popular technique, while production economics gives preference to the evaluation of carcass weight and composition.

Growth is a dynamic process that involves complex, yet coordinated interactions between various factors such as diet, genetics, immunity, metabolism and environment. Growth may result from an increase in size and diameter of already existing muscle fibres (hypertrophy), or an increase in the number of cells (hyperplasia), or both (Campbell, 1997). Since animals grow to a maximum size, hypertrophy is often favoured in the later stages of growth. Being a reflection of how adaptable and viable an animal is, growth and its associating traits are commonly considered as selection criteria in breeding programs and evaluation.

2.2.1 Skeletal muscle growth and development

Dissecting the molecular processes governing skeletal muscle growth and development is of great importance to the livestock production industry, since muscle is the desired component of meat. Growth is simply an accumulation of protein, fat and bone. Although protein accretion declines to a near halt as the animal approaches its mature body size, fat accumulation can continue through the end of the animal's lifespan (Dickerson, 1978).

Growth in animals entails an increase in tissue mass, and can be broadly classified into prenatal and postnatal growth. The major distinction between these two is the pattern of growth; with prenatal or embryonic growth characterised by hyperplasia, whilst hypertrophy is prevalent in postnatal growth (Rehfeldt et al., 2000).

Skeletal muscle development is a multi-step process. Early embryonic skeletal muscle development can be broadly classified into three stages: (1) determination, (2) differentiation, and (3) maturation (Musumeci et al., 2015). In vertebrates, skeletal muscle primarily originates from somites. Somites are transient ball-like segments that bud off from the paraxial mesoderm on either side of the neural tube and notochord in the developing vertebral embryo (Buckingham et al., 2003).

The immature somites are multipotent which means that any of its component cells could divide to yield more somites. During maturation, the somites form different compartments namely: (i) the sclerotome, which generates the cartilage of vertebrae and ribs, and (ii) the dermamyotome which forms the muscles and dermis (Goulding et al., 1994). In response to inductive cues from the neural tube and notochord, myogenesis commences in the somites. Determination begins with the assembly of multipotent mesodermal precursor cells present in the somites (Musumeci et al., 2015). These precursor cells proliferate to form myogenic progenitor cells, which further divide to yield proliferating myoblasts committed to the muscle-lineage (Figure 2.3). In the development process, myoblasts are thought to be the first group of cells identified as muscle cells (Chen and Goldhamer, 2003). The ensuing differentiation of the myoblasts is a hallmark of skeletal muscle development. A major characteristic of myoblast differentiation is their withdrawal from the cell cycle, and the fine-tuning of their transcriptional circuitry towards the expression of muscle-specific genes allowing them to synthesise proteins that characterise a mature muscle cell (Walsh and Perlman, 1997). Differentiated myoblasts subsequently align and fuse to form elongated, multi-nucleated myotubes. While most undergo this process, a small population of myoblasts do not fuse; rather they dwell on the exterior of the

myotubes as a group of mesenchymal cells called satellite cells. Muscle injury or trauma actuates the division and proliferation of these cells to form new muscle fibres (Goulding et al., 1994; Chen and Goldhamer, 2003).

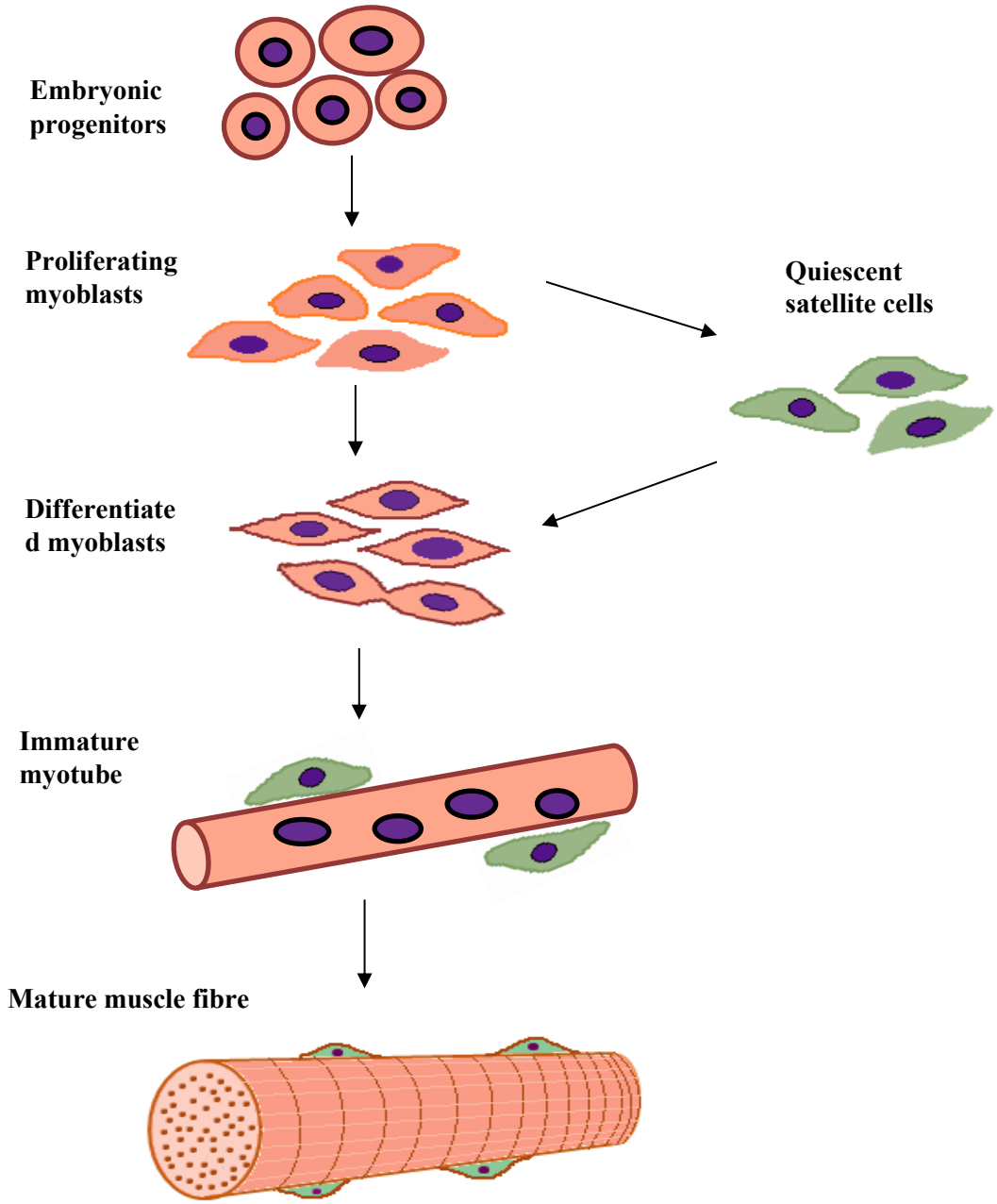


Figure 2.3 Skeletal muscle growth and development

In response to inductive cues, embryonic progenitors proliferate to form myoblasts, some of which transform into quiescent satellite cells, while others divide to form differentiating myoblasts. This group of myoblasts withdraw from the cell cycle and commit to the myogenic pathway. Differentiating myoblasts fuse to form a multinucleated myotube surrounded by satellite cells. These myotubes are bundled together to yield the mature muscle fibre.

Postnatal skeletal muscle growth and development occurs via hypertrophy. Although mature muscle fibres lose the ability to divide and proliferate, the presence of quiescent satellite cells allows a muscle fibre to retain a measure of its proliferative capacity (Figure 2.3). Satellite cells have the ability to generate both muscle-progenitor cells that fuse with and repair muscle fibres, and stem cells that allow for the self-renewal of the satellite cell population (Goulding et al., 1994). Unlike skeletal muscle, adipose tissue retains the ability to undergo hyperplasia throughout the lifespan of the animal.

Skeletal muscle mass is a delicate balance between two dynamic processes: muscle protein synthesis and muscle protein breakdown. While hypertrophy is the result of increased protein synthesis, atrophy is caused by protein degradation. The regulation of skeletal muscle size can occur via three major mechanisms, each yielding a different phenotypic outcome (Glass, 2005).

Firstly, the activation of satellite cells into myoblasts, followed by differentiation and fusion to pre-existing muscle fibres (Figure 2.3), causing an expansion in the mass as new myonuclei are added (Frontera and Ochala, 2015). The extracellular milieu of a muscle fibre plays a crucial role in its development. The second way is increasing the protein content by inducing protein synthesis signalling pathways such as IGF1/IGF1R signalling (Glass, 2005). Finally, the inhibition of muscle atrophy pathways to retain current muscle mass. All three signalling branches intersect at several downstream messenger factors. A harmonised interplay between the three mechanisms allow muscles to adapt readily in response to environmental cues including mechanical load, nutrition, stress, injury and illness (Clemmons, 2009; Otto and Patel, 2010). Skeletal muscle bears some remarkable plasticity.

2.2.2 Energy utilisation, partitioning and metabolism

Feed cost and nutrition account for over half of the total production costs in many livestock production systems (Freer, 2002). It is, therefore, imperative that nutrients from the feed are utilised to the fullest.

Feed efficiency is a measure of the weight gain of an animal over the dry matter of feed consumed. The feed utilisation of an animal is based upon a variety of factors such as age, length of time spent foraging, rumen constitution and type of feed (Rojas-Downing et al., 2017). Partitioning of the energy obtained from feed occurs shortly after consumption. The total energy intake obtained from the feed is called gross energy (GE), some of which is

absorbed by the rumen and used for metabolic processes (digestible energy), while the rest of the indigestible energy is excreted in the form of faecal waste (Dickerson, 1978). A portion of the digestible energy (DE) is lost during rumen activity as urine and methane. The energy left is known as metabolisable energy (ME), and represents the energy available for an animal's growth, maintenance, reproduction and metabolism (Freer, 2002).

Growth takes place when the energy and protein available surpasses that required for maintenance. Energy and metabolite partitioning vary for fast-growing and slow-growing animals (Webster, 1980). Since feed efficiency is proportional to growth rate, fast-growing animals are more efficient in utilising nutrients obtained from feed. Slower-growing lambs take longer to attain target market weights compared to their faster-growing counterparts. As a result, lambs with lower growth rates accrue higher costs with regards to maintenance, feed use, health care and land use, thus reducing production efficiency (Freer, 2002).

The gross efficiency of conversion, represented by the meat yield in kg per unit feed intake, has been found to be higher for faster-growing lambs compared to their slower-growing counterparts of the same weight (Elmes, 2013). The production efficiency and growth rates of livestock differ both within breeds and across breeds.

2.3 Breeding Selection Technologies

The performance of an animal is a combined product of the genetics inherited from their parents and their environments - such as nutrition, management, and rearing. The primary objective of animal breeding is to produce a successive generation of livestock that would yield desired traits with increased efficacy. Prior to the development of a breeding programme, the breeding objectives would have to be defined (Greenwood et al., 2006). This entails the calculation of the economic importance of production traits with a view to profitability. Economic value is measured as the economic impact on profit per unit independent change in each trait, while other traits are kept constant (Hazel, 1943; Byrne et al., 2010). Some of the common breeding objectives include: improving growth and carcass traits, enhancing reproduction and survival rates, increasing feed conversion and improving disease resistance (Kosgey et al., 2004).

Genetic evaluation systems examine how genes regulate production and how they can subsequently be passed down to an animal's offspring. Since the late 1900s, several

genetic evaluations schemes have been made available to NZ sheep breeders including the National Flock Recording Scheme in 1967, Sheeplan in 1976 and Animalplan in 1988 (Newman et al., 2000). The existence of multiple competing software and reporting systems led to inconsistencies that obfuscated the analysis, presentation, and interpretation of industry data and information. Striving for uniformity, a national genetic evaluation scheme was established in 1995 called the Sheep Improvement Limited (SIL) (Newman et al., 2000). SIL was designed to serve as a transparent industry genetic database utilising a singular yet comprehensive evaluation system that takes into account multi-trait and repeated-measure analyses. The major traits of interest on the SIL database include growth, meat production, disease resilience and resistance, fertility, and fecundity (Newman et al., 2002; Jopson et al., 2009). By providing insight into the genetic connection between flocks, the SIL database has enabled breeders to improve the genetic gain of their sheep and increase profitability.

Animal breeding has undergone tremendous transformations in recent years. Early breeding strategies employed the estimation of breeding values to bring about genetic improvement. The estimated breeding value (EBV) assesses the level of performance of an animal for a given trait when compared to flock/population average, or base figures set by SIL (Jopson et al., 2009). The EBV entails the measurement of phenotypic traits on either the animal itself or its genetic relation, such as parents, progeny or siblings. The unit for an EBV is based on the trait being measured, for instance, grams/day for growth rate. When EBVs for multiple traits are considered, and weighting is applied to each trait, this is known as a breeding index (Hopkins et al., 2005). Expressed as a proportion, the weighting value signifies which traits are of greater interest to the breeder.

A breeding index is beneficial for improving genetic gains on different traits. Depending on the production system, the SIL database has enabled the reliable determination of breeding indices for different traits which is reflective of an animal's breeding worth (BW) when economic influence is accounted for (Dodds et al., 2013). The economic index of a trait is essentially the "dollar value per unit change in a trait, given that all other traits are constant", and is an efficacious method of estimating a trait's economic importance (Harris, 1970). The heritability of a trait can influence the EBV and BW (Van der Werf et al., 2010).

Heritability refers to the proportion of total phenotypic variation in a trait that is explained by genetic variation (Smith and Simpson, 1986). A heritability value of 1 suggests that

genes alone control an animal's phenotypic trait, while other factors such as nutrition, management and environment are inconsequential (Safari et al., 2005). For traits with high heritability, trait measurement of the animal itself would suffice. However selection accuracy is reduced with low heritable traits, thus necessitating a more accurate assessment – a progeny test. A progeny test evaluates traits of interest in the offspring of the animals (Van der Werf et al., 2010). Animals, especially males, found to possess higher genetic merit by virtue of their EBVs are selected to form a 'superior breeding stock'. This superior stock is then applied intensively using reproductive technology to inseminate various females to yield improved offspring.

By means of genetic selection techniques, a remarkable increase in the annual carcass gain in NZ lambs has been reported over the last decade (Beef + Lamb NZ, 2012; Brito et al., 2017). Genome-wide or genomic selection (GS) is a type of marker-assisted selection that allows the estimation of marker effect across the genome of a reference population consisting of thousands of sheep (Desta and Ortiz, 2014) Genomic selection is capable of capturing genetic variation at any loci, irrespective of the size of the effect they may have on a phenotypic trait. This genetic tool allows for a more accurate prediction of the breeding value of a trait and genetic merit of an animal by assessing genomic relationship within the reference breeding population.

The introduction of whole-genome sequencing was a break-through for genomic selection. It paved the way for the detection of thousands of DNA markers in the form of nucleotide variations that are present across the whole genome (Smith and Simpson, 1986). These markers are relatively stable, with low mutation rates. They are also easy to amplify with PCR, and have been used to develop chip-based DNA typing technologies that can be employed to detect genetic markers. Decisions regarding breeding and selection, especially for harder to measure traits, will be made easier with the use of these markers (Schaeffer, 2006). The approach could potentially address a major challenge in breeding, by reducing reliance on visual assessment of the sheep prior to selecting for genetic merit, thus decreasing the time interval from testing to selection. Genomic selection has been reported to decreased the generation interval, and increase the rate of genetic gain in dairy cows and sheep by 60-120% (Schaeffer, 2006; Hayes et al., 2013). Such gain is beneficial to farmers and breeders. Intensive breeding for a given trait can, however, negatively impact animal welfare and other production traits (Rauw et al., 1998).

2.3.1 Quantitative trait loci (QTL)

Understanding the genetic architecture governing complex traits is crucial to livestock farming. A quantitative trait locus (QTL) is a region of the DNA containing genes associated with a phenotypic trait. Over the past decades, advances in genomic research and QTL identification has invigorated its application in animal breeding (Collard et al., 2005). Identification of genetic markers associated with phenotypic traits was a major scientific breakthrough that has led to the detection of QTL. Genetic markers are essentially 'tags' or 'signs' that are adjacent (that is tightly linked) to genes of interest (Vignal et al., 2002). The three major types of markers are: morphological, biochemical and DNA markers.

Morphological traits represent visible phenotypic characteristics such as height, body length, chest size and tail length. Visual appraisal provided the basis upon which these traits were recorded, and hence was unsuitable for traits with low heritabilities, or ones that were costly or hard to measure (disease resistance) (Jopson et al., 2009). Many of the morphological traits can only be measured when the animal is of adult age (body weight and composition), or upon slaughter (carcass traits), which impedes utility. Additionally, visible features are influenced by environmental factors, diet and the developmental stage of the animal (Dickerson, 1978). On the other hand, biochemical markers employ isozymes which are different forms of an enzyme. Whilst biochemical markers have great use for the diagnosis of conditions, they are limited by the small number of different marker loci available.

Molecular markers (DNA or genetic markers) are the ultimate and most widely used markers, as they provide a vast array of utility and application. The fundamental construction of genome-wide molecular maps is made possible by means of molecular markers (Gupta et al., 2005). A key distinction between DNA markers and biochemical or morphological markers is that DNA markers are abundant in number and are not affected by environmental and developmental factors.

A linkage map is a map of the relative positions and distances of genes (as indicated by their genetic markers) along a chromosome (Montaldo and Meza-Herrera, 1998). These distances do not refer to physical distances, rather are determined linkage analysis as the recombination frequency of the two gene loci. Chromosomal recombination or crossing over of genes and markers during meiosis is the basis for constructing a linkage map (Ardlie et al., 2002).

Genes located on different chromosomes, or situated far apart assort independently, and thus are unlinked. In contrast, genes (or alleles) located in close proximity on the same chromosome are often inherited as a single unit, and hence are said to be linked. Therefore, the closer the genes, the more tightly linked they are, and the shorter their linkage distance (Ardlie et al., 2002). Linkage distance is measured in centiMorgans (cM). Major drawbacks hampering this traditional form of linkage analysis, also known as family linkage analysis, include the need for pedigree information, and limitations for use in larger population size (Liu, 1997). Although linkage mapping is the classical QTL mapping technique, advancements in mapping technology popularised the association mapping method.

Linkage disequilibrium (LD) mapping, also known as association mapping, is a high-resolution technique used in exploring the genetic undertone of production traits (McRae et al., 2002). Linkage disequilibrium is the non-random association of alleles at different loci within the population. By utilising information on the history of recombination and marker-trait associations within the population, linkage disequilibrium improves the power and accuracy of QTL mapping, compared to the conventional linkage analysis (Kruglyak, 1999; Meuwissen and Goddard, 2004). The LD approach successfully integrates the effects of many past generational cross-overs between the gene locus and markers. Association analyses depend on the principle that common genetic variants underpin traits of interest such as susceptibility to diseases.

Association studies have been applied extensively in human genetics in the mapping of alleles that influence common diseases. These include the association of Factor V Leiden with clotting disorders (Weiss and Clark, 2002), LDL receptors with heart disease (Reich and Lander, 2001), and ApoE-e4 with Alzheimer's (Corder et al., 1993). Genetic linkage mapping is an essential component in the study of plants and animals. Molecular markers facilitate the identification of QTL and candidate genes influencing variations in traits of interest (Collard et al., 2005). DNA markers are useful in constructing linkage maps.

Marker-assisted selection (MAS) has received increased attention in the past few years, which has translated into intensified efforts towards QTL and marker identification. Remarkable success has been attained with the use of MAS in livestock evidenced by an increase in the annual genetic gain by up to 30% (Ge et al., 2001). Growth and body composition, meat yield, milk production, disease resistance, fertility and fecundity are some of the commonly investigated traits for QTL analyses.

In NZ, genetic technologies are largely employed within the dairy industry in addition to other livestock improvement techniques such as artificial insemination, multiple ovulation and embryo transfer (Lopez-Villalobos et al., 2000; Hayes et al., 2009). In comparison, the sheep industry is slower with adopting genetics-oriented techniques. This is largely because the NZ sheep industry operates as a 2-3 tier breeding system, where the top tier breeders control genetic advancement within the industry, who supply rams to small-scale breeders, who in turn supply them to commercial farms (Gooch and Firth, 2007). Top-tier breeders can enhance flock productivity by the selection of traits of economic importance using modern genetic schemes such as MAS, alongside accurate performance recording.

Traditional breeding methods are being supplemented by genetic technologies such as MAS, a powerful tool that employs the use of DNA markers to select for traits of economic importance. MAS aims to produce, with greater accuracy, sheep of superior genetic merit. The efficacy and accuracy of QTL mapping and MAS increases with the number of genetic markers used, hence the need for the type of research being carried out in this thesis.

2.4 Implications of Genes in Growth and Carcass Traits

Research into livestock production begs the age-old question: do genes influence performance traits? If so, how far-reaching are these effects? Indisputably, nothing develops in a genetic void or is free of environmental influence. Growth entails the change in the size, composition and shape of an animal, often expressed as the ability to get larger with time (Dickerson, 1978). Genes play a pivotal role in the growth and development of animals. Various production traits are complex, in that they are multi-factorial, with both genes and the environment having a substantial effect.

Continuous variation is observed with most traits of economic value, thus indicating a complex underlying genetic architecture. Growth and carcass traits are high-interest traits in meat production with moderate heritabilities (Safari et al., 2005). Lamb growth rate and carcass qualities are crucial determining factors of profitability. Breeding programmes favour faster-growing animals because they achieve target market weight earlier, and hence are more efficient.

The growth rate of an animal is determined by the protein turnover within the skeletal muscle. When protein synthesis exceeds protein degradation, a faster growth rate is

witnessed. Muscle protein turnover or accretion is regulated by several genes including growth hormone (*GH*) and insulin-like growth factor receptor (*IGF1R*). Equally impacting growth, are genes involved in early prenatal development, such as POU class 1 homeobox 1 (*POU1F1*) and PROP paired-like homeobox 1 (*PROP1*). The QTL associated with liveweight, and growth and carcass traits, are found on sheep chromosomes OAR 1, 2, 3, 4, 5, 6, 11, 14, 18, 20, and 21 (Walling et al., 2004). The aforementioned genes which will be examined in this thesis, are found within some of these QTL – *IGF1R* (OAR 18), *GH* (OAR 11), *POU1F1* (OAR 1), and *PROP1* (OAR 5).

Dissecting the genetic makeup underpinning economically important traits is of great interest to researchers and farmers alike. Fundamental to this goal is the detection of marker-trait associations by means of QTL analyses (Hopkins et al., 2011). Nucleotide sequence variation found within the chosen genes that occur within QTL associated with animal productivity could serve as potential markers for selective breeding. They could also ensure that breeders and farmers have a better understanding, and can make more accurate decisions when selecting breeding stock.

2.4.1 Insulin-like growth factor 1 receptor (IGF1R)

Insulin-like growth factor 1 receptor (IGF1R) plays a vital role in cell growth, metabolism and survival in vertebrates. IGF1R is a tyrosine kinase receptor with an $(\alpha\beta)_2$ structure, and comprises of an extracellular ligand-binding domain, a membrane-spanning region and an intracellular tyrosine kinase (TK) region (Adams et al., 2000). IGF1R also promotes the stable retention of muscle fibres and architecture by regulating protein accretion and breakdown, neuronal survival and plasticity.

IGF1R signalling occurs upon binding to any of its ligands, IGF1, IGF2 or insulin. The ensuing dynamic structural transformation and signal transduction brings about the auto-phosphorylation of the tyrosine kinase region, followed by a cascade of molecular events culminating in the activation of mitogenic genes and inhibition of apoptosis (Chitnis et al., 2008; Girnita et al., 2014). Signalling by IGF1R inherently upregulates pathways leading to muscle hypertrophy, while simultaneously inhibiting pathways favouring muscle atrophy.

The *IGF1R* disruption in mice is associated with a significant decrease in the size and number of muscle fibres, with null mice weighing only 40% of the normal, and *in utero* growth retardation in humans (Liu et al., 1993; Baker et al., 1996). Additionally, *IGF1R*

over-expression has been found to accelerate cell proliferation and differentiation, and IGF signalling can facilitate the attenuation of atrophy in skeletal muscles. Abnormal expression of *IGF1R* in humans has been reported to cause growth retardation, cancers, and impaired mental development (Kawashima et al., 2005; Yang et al., 2018).

Nucleotide sequence variations in *IGF1R* have been reported to have an effect on the growth and performance traits of livestock including cattle (De la Rosa Reyna et al., 2010), buffalos (El-Magd et al., 2013), pigs (Wang et al., 2006) and chickens (Lei et al., 2008).

Notwithstanding its predominant role in mammalian function, much remains to be learned about the role of *IGF1R* in livestock breeds. Given the ubiquitous presence of IGF1R in nearly all body cells, as well as its crucial function in growth and metabolic processes, sequence variation within its gene could likely have a multi-faceted effect on the resulting protein, and impact the phenotypic attributes of an animal. Therefore, further research examining the effect of sequence variation in *IGF1R* on sheep growth and carcass traits is required to provide more enlightenment.

2.4.2 Growth hormone (GH)

Growth is a principal characteristic of all living organisms. This biological process involves the coordinated networking of various genes, hormones and environmental factors.

Growth hormone (GH) is a major regulator of growth and metabolism through direct signalling on target cells and indirect signalling via insulin-like growth factor 1 (IGF1) (Curi et al., 2006). GH binding to its receptor, GHR, brings about receptor dimerisation and activation. Cascading events due to this signal transduction lead to the activation of downstream effectors and secondary messengers, ultimately resulting in the GH-dependent regulation of gene expression, metabolism and somatic growth (Campbell, 1997; Brooks et al., 2014). The effects of GH signalling include protein synthesis, lipolysis and glycolysis, which translates to the longitudinal increase in muscle and bone mass. Therefore, *GH* is a suitable candidate gene for investigating growth and carcass traits in livestock.

In sheep, *GH* is mapped to chromosome 11, a region harbouring the QTL for liveweight and growth and carcass traits (Carter-Su et al., 1996). The 1.8 kb long gene consists of five exons and four introns. Excessive GH secretion has been linked with acromegaly and gigantism in humans, while GH deficiency has been associated with dwarfism, congenital malformation and diabetes (Cogan et al., 1993; Giustina et al., 2008). Polymorphisms in

GH have been reported in various livestock such as cattle (Jia et al., 2014), goat (Malveiro et al., 2001), and sheep (Jia et al., 2014), and have been associated with key productive traits such as milk yield, and growth and carcass traits.

Additional research is required to confirm already reported variations and associations in ovine *GH*, especially in unexplored NZ sheep breeds. This could also involve searching for new sequence variation that could impact sheep growth and carcass traits.

2.4.3 POU class 1 homeobox 1 (POU1F1)

The POU class 1 homeobox 1 (POU1F1) protein, also called pituitary-specific transcription factor 1 (PIT-1), plays a major role in the embryonic development of cells and tissues of the pituitary gland. It positively regulates the secretion of three major hormones: growth hormone (GH), the beta subunit of thyroid-stimulating hormone (TSH-β) and prolactin (PRL) (Mangalam et al., 1989; Bastos et al., 2006b), by controlling the differentiation of the hormone-producing cells somatotropes, thyrotropes and lactotropes respectively. These hormones all have crucial roles in regulating growth, metabolism and homeostasis in mammals (Pierpaoli and Besedovsky, 1975; Wallis, 1988; Labad et al., 2016).

Being a part of the POU-domain family of transcription factors, POU1F1 has a common structure consisting of three domains: an N-terminal transactivation domain, which undertakes the transactivation of target genes such as GH, TSH and PRL, and a C-terminal POU-specific domain and the POU-homeodomain, both of which engage in high affinity DNA binding (Bastos et al., 2006a).

Ovine *POU1F1* is located on chromosome 1, is 17 kb long and comprises of six exons and five introns (Woollard et al., 2000). There exists close homology between ovine *POU1F1* and other species such as bovine (98.2%), human (91.2%) and murine (86.2%), thus indicating a high degree of evolutionary conservation. Nevertheless, variations have been reported for this gene in many breeds. Four sequence variations have been reported in Portuguese sheep breeds, including Gly89Asp and Ala105Thr in the exon 3 of ovine *POU1F1*, which were reported to affect sheep production traits (Bastos et al., 2006b). Other sequence variations in Sarda goat breed were found to impact milk yield and milk fatty acid content (Daga et al., 2013).

Despite all the research done on ovine *POU1F1*, very little is known about its effect on growth and carcass composition traits, and nothing at all about NZ breeds. Due to its role

in prenatal growth and development, variation within this gene would likely have a multi-faceted effect on the resulting protein and phenotypic attributes.

2.4.4 PROP paired-like homeobox 1 (PROP1)

Often referred to as the 'master gland', the pituitary gland serves a vital function in mammals. The PROP paired-like homeobox 1 (PROP1) gene encodes a paired-class homeodomain transcription factor essential in the early development of the pituitary. In response to intrinsic and extrinsic signals, the mammalian pituitary gland develops from a common primordium. The release of transcription factors and signalling molecules, such as PROP1 and HESX, in a spatiotemporal manner induces the differentiation and commitment of precursor cells into distinct hormone-producing cell populations (Deladoëy et al., 1999; Scully and Rosenfeld, 2002). The mature anterior lobe of the pituitary is inhabited by five distinct cell types: corticotropes (produce pro-opiomelanocortin which undergoes cleavage to adrenocorticotropin, ACTH), gonadotropes (produce luteinizing hormone, LH and follicle stimulating hormone, FSH), lactotropes (produce prolactin, PRL), somatotropes (produce GH) and thyrotropes (produce thyroid stimulating hormone, TSH).

Ovine *PROP1*, which encodes 226 amino acids, is a 3.5 kb long gene and is organised into three exons interspersed by two introns. Contained within PROP1 are two major conserved domains: a central paired-like DNA-binding homeodomain which interacts with AT-rich promoter region on target DNA, and a transcription activation located at the carboxyl terminus (Sornson et al., 1996; Guy et al., 2004). The less conserved amino terminus of PROP1 possesses repressor activities.

Prophet of POU1F1, another name for *(PROP1)*, is derived from its upstream expression with respect to *POU1F1*, as it is necessary for *POU1F1* expression. By virtue of its central function and the extensive reach of the hormone targets it governs, mutations in *PROP1* that result in dysfunction or hyperactivity of the anterior pituitary hormones would have sweeping effects on multiple organ systems. A total loss or impaired production of anterior pituitary hormones such as GH, PRL and TSH, commonly accompanies *PROP1* mutations, which sometimes translates phenotypically as dwarfism, as seen in the Ames dwarf mouse (df/df) (Sornson et al., 1996; Flück et al., 1998). Combined pituitary hormone disorders (CPHD) is the term given to conditions displaying low circulating levels of anterior pituitary hormones such as GH, PRL, TSH, LH and FSH (Parks et al., 1999).

Over fifteen *PROP1* mutations have been associated with CPHD in humans (Kelberman et al., 2009a). About twelve *PROP1* mutations have been uncovered in sheep, most notably of which is a *C* to *T* transversion at position 330 in intron 1 linked with increased litter size, and a Thr181Ala substitution brought about by a g.2647 A>G substitution in exon 3 associated with increased wool fibre diameter in Chinese sheep. (Zeng et al., 2011; Liu et al., 2015). Undoubtedly, more remains to be learned about *PROP1*, particularly in NZ sheep breeds.

2.5 Challenges Faced by the Sheep Industry

Rivalling the sheep industry, the dairy industry is NZ's top export earner within agriculture. The unparalleled success of the dairy industry has been detrimental to the sheep industry as more lands are being converted for dairy use. The dairy expansion has also forced the relocation of sheep farming to less productive areas. This has led to a significant decline in sheep numbers, especially for the ewe breeding flock (Meat Industry Association, 2016). Over the last decade, from 1994 to 2017, while dairy cattle numbers have increased by 70%, sheep numbers have suffered a 44% decline from 49.5 million to 27.5 million (Stats NZ, 2019).

Sheep farming in NZ is pastoral, and as such is influenced by climate and weather conditions which can vary significantly. Rising temperatures due to global warming could have an adverse impact on livestock production. Reduced nutrient availability in forages, decreased intake of feed and conversion efficiency, lower livestock fertility rates, and higher mortality rates would be some of the challenges presented by rising temperature (Rojas-Downing et al., 2017). Feed quantity and quality would also be affected by the surge in carbon dioxide levels and greenhouse gases. Phenotypic variability is also observable with carcass muscle and fat traits, indicating a polygenic inheritance or strong environmental effect.

Current consumer preference leans towards high meat to fat ratio. The ideal high quality carcass would have high lean meat yield, low levels of intermuscular and subcutaneous fat, and just enough intramuscular fat to improve palatability, tenderness and taste. Considering that lean meat only constitutes about 50-60% of the entire carcass, more effort is being put into breeding for carcasses with higher lean meat yield (Lewis et al., 1996).

Despite having enjoyed global success, conventional breeding methods are met with several limitations. Selection accuracy of estimated breeding values (EBV) is dependent on the abundance of data and its storage, which can be expensive and require certain infrastructure (Van der Werf et al., 2010). Data could include pedigree, sire and dam data. Repetition of measurements is often required as this authenticates results and minimises the influence of random environmental conditions.

Another quandary faced by traditional breeding strategies is the generation interval, which represents the average age of parents at which offspring are born. A shorter generation period allows for a quicker expression of genetic potential in the progeny, and subsequent comparison with parents to establish a trend (Hayes et al., 2013). Short generation intervals can be achieved with younger breeding animals. However, younger breeding animals usually have less information available such as fewer repeated records, absence of progeny test, and limited performance data, all of which decrease selection accuracy. Older breeding animals with higher selection accuracy, by virtue of copious data on their performance (repeated expression of traits) and possibly some progeny data, have higher generation intervals and are thus only available for fewer selection rounds during their lifetime, compared to their younger counterparts.

The breeding sheep for increased lean meat yield can have an antagonistic effect on other traits such as decreased eating quality of meat and palatability (Brito et al., 2017). This "trade-off" phenomenon is seen across different breeding programmes where the intensive selection of one trait weakens the performance of other production traits. Inbreeding is also a possible consequence of the excessive usage of superior breeding stock (Vostry et al., 2018). The resulting lack of genetic variation in the gene pool could put the animals at risk of being wiped out by a disease. Additionally, reproductive rates, which is the number of offspring born to an animal, and gestation periods are major determinants of the success of reproductive technology (Smith and Simpson, 1986). Animals with higher rates of reproduction are preferred since that reduces the number of breeding animals required. However, this is not always the case with available livestock.

Intensive selection for particular traits might prove counterproductive as other valuable traits diminish. For instance, variation in the myostatin gene gives rise to double-muscled cattle that produce more meat with leaner quality (McPherron and Lee, 1997). These cattle, however, have been reported to suffer from poor fertility, and respiratory and skeletal problems (McPherron and Lee, 1997). In other cases, selection for particular traits has led

to the loss of disease resistance genes in animals (Smith and Simpson, 1986). Cattle and sheep also have long gestation periods which might render reproductive technology inefficient.

A sustainable solution to this problem would be the simultaneous improvement of meat quality traits along with other production traits such as fertility and disease resistance.

2.6 Objectives of this Study

In an effort to improve sheep production and ensure sustainability and profitability of the meat industry, more focus is being placed on selecting sheep with faster growth rate, higher lean meat yield, and increased fecundity. This strategy effectively decreases labour inputs, cost of healthcare, and frees up land for subsequent use.

Intensive research has established the impact of genetics on the performance traits of livestock animals. Marker-assisted selection which targets genes associated with desired traits is coming into the limelight in the meat industry. Genetic variations in candidate genes located within QTL associated with economically important traits have been well documented in cattle. Small ruminants such as sheep, however, are yet to be comprehensively studied. While some research has been completed in various ovine breeds, there is much work to be done with the unexplored NZ sheep breeds.

Skeletal muscle yield and function, somatic growth, metabolism and protein accretion are regulated by the genes chosen for this study, namely: growth hormone (*GH*), insulin-like growth factor 1 receptor (*IGF1R*), POU-homeobox 1 (*POU1F1*), and PROP paired-like homeobox 1 (*PROP1*). This research aims to identify novel variations in these four genes of interest within NZ sheep breeds, and evaluate their association with growth and carcass traits. The sequence variations uncovered could then be applied in MAS and the breeding of animals with desirable traits, with a view to considerable economic gain.

This project will provide major contributions to the NZ meat and pastoral industry, including a possible multi-gene typing system. Furnished with the knowledge provided by this research, farmers could make more informed and effective breeding decisions that maximises desirable traits such as improved meat yield, flavour and nutritional value, while minimising undesired traits.

Chapter 3
The relationship between growth and carcass traits in NZ sheep breeds

3.1 Introduction

Growth and carcass traits are some of the most crucial traits of interest to the meat industry, as they often dictate how much consumers are willing to pay for a product, and the financial reward obtainable by sheep farmers and breeders. Increasing focus on personal fitness and health awareness has led to a paradigm shift in consumer taste, with high-fat meat considered less desirable, while lean cuts are seen as premium quality.

Birth weight, weaning weight, and growth rate-to-weaning (also called average daily gain) are common growth indices crucial to the sheep industry. In a like manner, hot carcass weight, fat depth around the 12^{th} rib, and lean meat yield in the leg, loin and shoulder of a lamb carcass are useful in appraising lamb meat yield (Sañudo et al., 1998). The economic value of lambs are determined by these phenotypic traits, and hence they will be examined in this study. Lamb production traits have moderate heritabilities, indicating that they can be influenced by both genetics and the environment (Hopkins et al., 2011).

Nutrition (both from maternal milk and later feed), type of feed, prevalence of intestinal parasites, and on-site farm management systems are environmental factors that can impact production traits. The genetic component is more complex, but its impact is extensive. Genotypes of both the foetus and mother have been found to influence *in utero* growth (Oldham et al., 2011). Genome-wide association studies have linked candidate genes on sheep chromosomes OAR 1, 2 and 3 to production traits such as pre- and post-weaning gains, weaning weight, chest girth and shin circumference (Zhang et al., 2013).

Birth weight is one of the most important determinants of lamb survival during the first few days of life (Oldham et al., 2011). Several factors can affect how much a lamb foetus grows and its birth weight, such as maternal and foetal genotype, lamb gender, maternal body condition, litter size, and maternal nutrition. Feed availability, energy intake and the maternal body condition for a pregnant ewe can have an immense impact on lamb birthweight. It is vital that nutrition is controlled during lamb gestation as underfeeding could result in low energy intake, depletion in energy reserves, and reduced mammary growth and development, while overfeeding may lead to birth issues and lambing

difficulties. Proper nutrition is especially crucial during the late stages of pregnancy, as studies have shown that a 10 kg gain in ewe liveweight from day 100 can increase lamb birth weight by about 0.45 kg (Oldham et al., 2011).

As the lactation phase commences, good nutritional provision is required to ensure the maintenance of the mammary gland and ensuring steady milk production. Postnatal nutrition, as determined by the quality of quantity of the ewe's milk, is crucial to the development of new lambs (Pearce, 2016). A restricted milk allowance is experienced by twin or triplet lambs who have to compete for suckling time, and is often reflected in weaning weights and growth gains. Administering high-energy diets consisting of concentrates to lambs following weaning has been associated with higher growth rates, but also fatter carcasses (Sañudo et al., 1998)

Determining the genetic relationship by means of correlation analyses between these traits of economic importance is imperative to the effective planning of a breeding program and design of an efficient genetic evaluation scheme.

3.2 Materials and Methods

3.2.1 Details of sheep to be investigated

A total of 800 NZ Romney lambs were investigated in this study. The lambs examined in this study were obtained from 40 unrelated sire-lines belonging to the top 20% of the SIL dual-purpose index ranking.

3.2.2 Sample collection and processing

All research, in this thesis, involving animals was performed in accordance with the Animal Welfare Act 1999 (NZ Government), and the collection of sheep blood drops by the nicking of their ears was covered by Section 7.5 Animal Identification, in: *Code of Welfare: Sheep and Beef Cattle (2016); a code of welfare issued under that Act*. This process is considered to be a regular practice in farm management system, and causes little or no harm to the animal, hence this study was considered exempt from formal ethics review.

Within 12 hours of birth, lambs were tagged with plastic ear tags containing a unique identification number. The date of birth, birth rank (single, twin or triplet assigned ranks 1,

2 and 3 respectively), birth weight (in kg), gender and sire details were documented concomitantly.

To enable future analyses, blood samples were taken from a small nick the end of the animal's ear using electric cutters (Appendix A), with several drops of whole blood collected on labelled FTA cards (Whatman, Middlesex, UK). The FTA card was then air-dried and stored at room temperature in a dark room until required for further analyses.

All the lambs were fed on pasture and placed together for three weeks until tailing was carried out using a rubber ring. The tailing date and weights were measured. Weaning occurred three months later, at which time weaning weights were recorded. The difference between the weaning weight and the birth weight divided by the age in days was used to calculate growth rate to weaning, expressed as grams/day. This parameter was calculated because it gives a useful indication of the lamb's growth pattern during its lifetime. Both male and female lamb data where used in growth trait analyses. Separation of the lambs took place shortly after weaning, with only male lambs applied for further use in carcass yield and meat quality investigations, while female lambs were retained for use as ewe replacements for the flock.

The target liveweight for male lambs in this study was 36 kg. Male lambs weighing 36 kg and over were immediately drafted for slaughter and transported to the meat processing plant. Underweight male lambs were kept of pasture for another four weeks, at which time a second draft took place for lambs hitting the target age. In the subsequent four-week period, a third and final draft occurred where all male lambs, regardless of liveweight, were taken to slaughter. The lamb weight and age at drafting were recorded.

Hot carcass weight (HCW) in kg, which refers to the weight of carcass components without the head, pelt or gut, was measured at slaughter. Other post-slaughter carcass traits were evaluated using the VIASCAN® Video Image Analysis system (VIASCAN, Sastek, Hamilton, Queensland, Australia) developed by Meat and Livestock Australia and described by Hopkins et al. (2004). The leg yield, loin yield, shoulder yield, were calculated as a percentage of the lean meat tissue in proportion to HCW. The total yield is the sum of the leg, loin and shoulder yields. The carcass fat depth over the 12th rib (V-GR) was also recorded in millimetres.

3.2.3 Statistical analysis

SPSS version 25 (SPSS Science Inc., Chicago, IL, USA) was used to calculate descriptive statistics, analysis of variance (ANOVA) and frequency data at a two-tailed significance level of $\alpha = 0.05$. Birth rank was fixed as factor with three levels (1, 2, and 3) in independent analysis against the response variables: birth weight, weaning weight, growth rate, HCW, VGR and total yield. Tukey post-hoc analyses were also carried out to determine where the differences lay between mean groups. Pearson correlation coefficient and scatter-plot analyses were used to assess the relationship between growth and carcass traits. Results were considered significant at $P < 0.05$ and trends noted at $P < 0.20$, unless indicated otherwise.

3.3 Results

3.3.1 Mean values of growth and carcass traits in NZ sheep breeds

Using one-way ANOVA, the means of the six major growth and carcass traits including birth weight, weaning weight, growth rate to weaning, hot carcass weight, fat depth at the 12th rib and total lean meat yield, were calculated with birth rank fitted as a factor (Table 3.1).

Table 3.1 Mean values of growth and carcass traits relative to birth rank

Birth Rank	Birth Weight (kg)	Weaning Weight (kg)	Growth Rate to weaning (g/day)	HCW[1] (kg)	V-GR[2] (mm)	Total Yield (%)
1	6.44 ± 0.94^{a}	34.0 ± 5.2^{a}	312.3 ± 50.8^{a}	17.9 ± 1.9^{a}	3.67 ± 3.08	52.1 ± 2.6
2	5.75 ± 0.87^{a}	31.3 ± 4.4^{ab}	289.0 ± 45.1^{ab}	16.9 ± 1.5^{b}	3.86 ± 2.56	52.6 ± 2.8
3	5.36 ± 0.80^{ab}	30.7 ± 4.4^{ab}	286.2 ± 43.8^{ab}	16.4 ± 1.4^{b}	3.44 ± 1.94	53.2 ± 2.9
P-value	**P = <0.001**	**P = <0.001**	**P = 0.001**	**P = <0.001**	P = 0.711	P = 0.213
Total Mean	5.79 ± 0.90	31.5 ± 4.6	291.0 ± 46.1	16.9 ± 1.6	3.81 ± 2.58	52.6 ± 2.8

[1] HCW = Hot carcass weight, [2] V-GR = Fat depth at the 12th rib

[a, b] Mean values within the same column with different superscripts differ at $P < 0.05$

A decrease in the mean birth weight was noticed with increasing birth rank, with triple-birth lambs weighing about 1 kg less than single-birth lambs. A similar pattern was observed with weaning weights as lambs with ranking of 1 weighed nearly 4 kg more than their counterparts with a ranking of 3. Lambs born single were also faster growing by an average of 23 to 26 grams per day, when compared to lambs of double and triple births respectively.

On the carcass side, single-born lambs had higher hot carcass weights in comparison to lambs born in a group (Table 3.1). These results may, however, be influenced by birth weight, as lambs with higher liveweight at birth and at weaning would very likely weigh more at slaughter too. A contrasting finding was observed for total yield, where lambs of single-birth produced lower lean meat than their multiple-birth counterparts. However, this finding along with those of fat depth were not statistically significant.

3.3.2 Relationship between growth and carcass traits

Table 3.2 Pearson correlation coefficient between different growth and carcass traits

		Tailing Wt[1] (kg)	Weaning Wt (kg)	Growth rate (g/day)	HCW[2] (kg)	V-GR[3] (mm)	Leg Yield[4] (%)	Loin Yield[4] (%)	Shoulder Yield[4] (%)	Total Yield[5] (%)
Birth Weight (kg)	r	0.314**	0.343**	0.264**	0.130**	0.056	0.133**	0.146**	0.156**	0.165**
	Sig.	0.000	0.000	0.000	0.001	0.151	0.001	0.000	0.000	0.000
Tailing Weight (kg)	r		0.531**	0.392**	0.437**	0.072	-0.137**	0.062	0.111**	-0.014
	Sig.		0.000	0.000	0.000	0.061	0.000	0.108	0.004	0.726
Weaning Weight (kg)	r			0.919**	0.574**	0.442**	0.195**	0.411**	0.362**	0.348**
	Sig.			0.000	0.000	0.000	0.000	0.000	0.000	0.000
Growth rate to weaning (g/day)	r				0.526**	0.424**	0.218**	0.426**	0.349**	0.359**
	Sig.				0.000	0.000	0.000	0.000	0.000	0.000
Hot Carcass Weight (kg)	r					0.410**	-0.106**	0.301**	.269**	0.130**
	Sig.					0.000	0.006	0.000	0.000	0.001
V-GR Fat Depth (mm)	r						-0.017	0.192**	0.055	0.070
	Sig.						0.661	0.000	0.156	0.068
Leg Yield (%)	r							0.726**	0.548**	0.916**
	Sig.							0.000	0.000	0.000
Loin Yield (%)	r								0.590**	0.878**
	Sig.								0.000	0.000
Shoulder Yield (%)	r									0.790**
	Sig.									0.000

** Correlation is significant at the 0.01 level (2-tailed), r = Pearson correlation coefficient

[1] Wt = Weight; [2] HCW – Hot carcass weight; [3] V-GR – Viascan fat depth around the 12th rib

[4] Lean meat yield expressed as a percentage of hot carcass weight; [5] Total yield is the sum of the leg, shoulder and loin yields

As illustrated in Table 3.2, most of the growth and carcass traits were linked as demonstrated by significant Pearson correlation coefficients. The strongest correlations were between weaning weight and growth rate-to-weaning (r = 0.919), and between total yield and leg yield (r = 0.916). This was expected because the growth rate calculation was derived from the weaning weight measurements, and loin yield represents a proportion of the total lean meat yield. Interestingly, unlike the loin and shoulder yields, leg yield had weak negative linear relationships with tailing weight (r = -0.137) and hot carcass weight (r = -0.106).

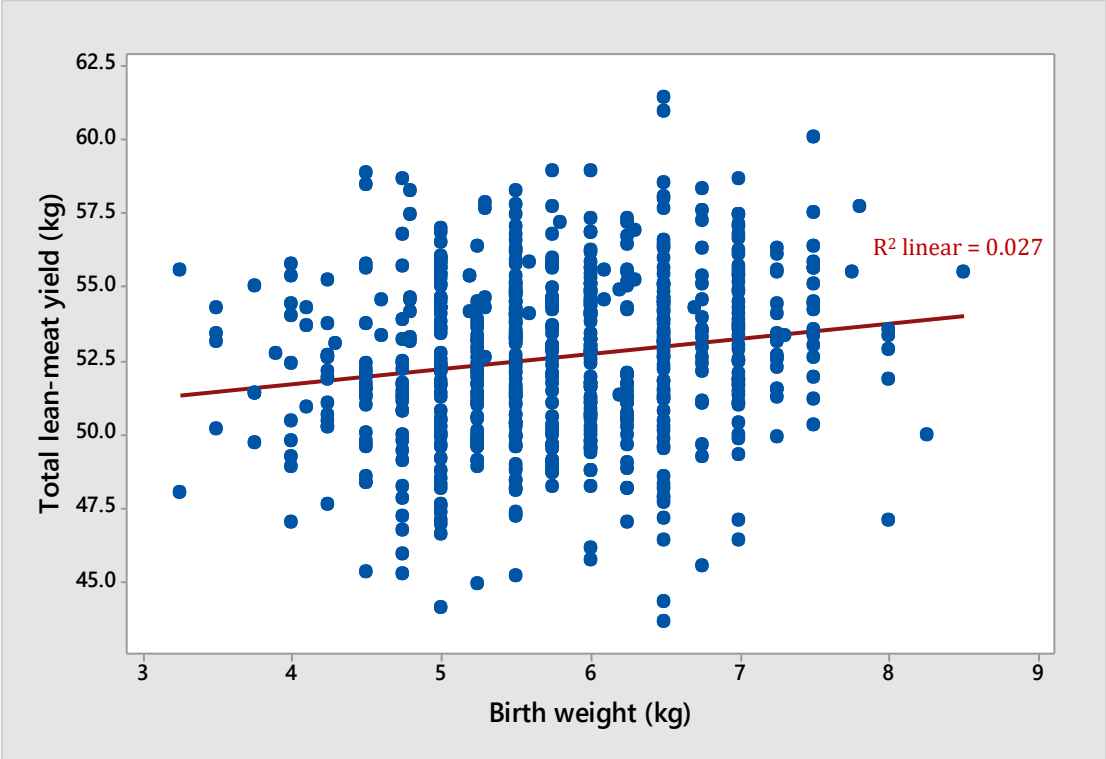


Figure 3.1 Scatterplot of total lean meat yield vs birth weight

A weak positive linear relationship between birth weight and total yield was observed (r = 0.165, P < 0.001). However, regression analysis showed that weight at birth only accounts for about 3% (R^2 = 0.027) of total lean meat yield (Figure 3.1). The results therefore, suggest that although higher birth weight animals tend to produce more lean meat, characteristics such as genetics may be better predictors of lean meat yield in NZ sheep breeds.

3.4 Discussion

In this thesis, the growth traits examined include birth weight, tailing weight, weaning weight, and growth rate-to-weaning, while the carcass traits examined include hot carcass weight, fat depth around the 12th rib, and lean meat yields in the leg, loin, and shoulder, and total lean meat yield.

The average birth weight recorded for lambs in this study was 5.79 kg which falls within the ideal range of 3.5 kg to 6 kg (Knight et al., 1988). Previous studies have demonstrated that the relationship between birth weight and lamb survival follows a bell-shaped curve with optimum survival at birth weight of 4.6 ± 0.03 kg, and minimal survival at either extremes of very high or very low birth weights, notwithstanding the source of variation (Knight et al., 1988; Dwyer, 2008). Lambs at the lower extreme of the birth weight scale are more likely to die due to starvation, injury, or susceptibility to exposure, while high birth weight lambs are more likely to die due to dystocia. This reinforces the influence of birth weight in lamb survival and growth.

Linear regression analysis showed that an increase in birth weight was weakly correlated with an increase in lean meat yields in the present study. This observation might be explained by the finding that heavier born lambs are at an advantage with respect to increased lamb vigour and independence (Dwyer and Bünger, 2012). Due to their agility, vigour, and better suckling ability, heavier born lambs are likely to receive more nutrition, whether in the form of milk or pasture, when compared to their lighter counterparts, which might translate to increased postnatal growth rate and subsequent meat yield. Birth weight is a unique production trait requiring a delicate balance when being selected for, as low birth weight has been linked with neonatal mortality, whereas high birth weight has been linked with dystocia (labour complications) and maternal mortality (Gardner et al., 2007).

Regarding birth rank, single-birth lambs tend to weigh more than twin lambs, who in turn weight more than triplet lambs, as demonstrated in this study. This is likely because the available nutrients provided by the ewe *in utero* is shared across lambs in multiple-lamb pregnancies, as compared to a sole recipient in a single-lamb pregnancy. This pattern was also consistent for weaning weight with single-birth lambs being heavier at weaning than lambs with birth ranks of 2 or 3. Competition for milk by sibling lambs in multiple births impact their performance and energy intake, hence can lead to decreased weight. Some studies have also reported that ewes giving birth to multiple lambs often do not suckle all their lambs due to a farm management decision in favour of hand-rearing, or as a result of

premature lamb death (Notter and Brown, 2015). Hand-rearing is however, not a common practice in NZ Romney farming.

An increase in growth rate-to-weaning was strongly correlated with an increase in both growth and carcass traits in the present study. The correlations with birth weight and weaning weight were expected as those parameters are directly applied in the calculation of growth rate. The findings are consistent with previous studies reporting a correlation between birth weight and weaning weight, as well as birth weight and average daily gain (Rashidi et al., 2008; Zhang et al., 2013). Worthy of note, however, was the positive correlation observed between growth rate and lean meat yields in the leg, loin and shoulders. This finding further confirms the notion that faster-growing lambs are more efficient in converting feed to usable energy that can be directed towards building up skeletal muscle, ultimately manifesting as higher lean meat yield. Therefore, a breeding program selecting for faster-growing lambs could produce a bi-faceted benefit by bringing about a simultaneous selection for higher lean meat yield in these lambs.

Based on these preliminary findings, birth rank, gender and sire were corrected for in subsequent models analysing growth traits; while draft age, sire and birth weight were adjusted for in models examining carcass traits. By convention, ram lambs were sent to the slaughter rather than ewe lambs. While this is largely because ewes are retained for reproduction and as flock replacements, other reasons come into play.

Gender has a huge influence on prenatal and postnatal lamb growth rate and performance. Previous studies have shown that male lambs tend to weigh more at birth and grow faster prior to weaning, when compared to than females lambs of the same age (Sañudo et al., 1998; Lind et al., 2011). Ram carcasses are also heavier with a greater proportion of lean muscle, when compared to ewe carcasses slaughtered at the same age. From an early age, ewe lambs have a greater tendency to amass fat, which ultimately results in higher fat content in meat from ewe lambs when compared to ram lambs (Sañudo et al., 1998; Lind et al., 2011). In spite of the differences in carcass fatty acid composition, gender has not been shown to influence sensorial characteristics, taste and palatability of meat produced by both male and female lambs (Tejeda et al., 2008). This indicates that the energy partitioning of a male lamb which yield the desirable lean-carcass is more suitable for meat production purposes. In this study, only male lambs were considered for the analyses of carcass traits.

3.5 Conclusion

This study demonstrated interactions between growth traits and carcass traits in NZ Romney sheep. Birth rank was found to influence the weights at birth and weaning, as well as growth rate and hot carcass weight. Single birth lambs exhibited better performance traits than their double- and triple-births counterparts. Insight into the interactions between performance traits may be useful in sheep improvement approaches.

Chapter 4
Investigation of variation in ovine *IGF1R* and its association with growth and carcass traits

4.1 Introduction

Insulin-like growth factors (IGFs), previously known as somatomedins, are a family of peptide hormones bearing structural similarity to insulin, and which play a central role in mammalian cell growth, differentiation and survival.

Within the IGF system there are three major ligands, IGF1, IGF2, and insulin. These are capable of interacting with two homologous cell surface receptors, the IGF1 receptor (IGF1R), and the insulin receptor (IR), and to a lesser degree the IGF-2 receptor (IGF2R) or mannose 6-phosphate receptor (Florini et al., 1996; Adams et al., 2000). The IGF1 receptor primarily mediates the physiological effects of IGF1 and IGF2, which have greater affinity to the receptor than insulin does. IGF1R is ubiquitously expressed in many cell types across many species, and in both foetal and adult tissues. This confirms its importance in animal growth and development. The IGF1 ligand is the chief mediator of biological action through IGF1R signalling, and serum IGF1 is largely produced by the liver, while most cells locally produce and secrete IGF1 into the extracellular matrix.

A family of six IGF-binding proteins (IGFBP-1-6) bind to the IGFs with high specificity and affinity to form a complex; the most common of which is a complex with IGFBP3 (Hwa et al., 1999). Although IGF-1 exists in high concentrations within the circulation, most of it is protein-bound, with less than one percent constituting the free ligand concentration (Baxter, 2014). Therefore, IGFBPs, along with their proteases, regulate IGF activity by determining IGF availability and accessibility to membrane receptors in tissues; and this provides the basis of a controlled-release system. The IGF-IGFBP complex also protects IGF molecules from being degraded within the circulation, thereby acting as a ligand reservoir that releases the molecules as needed. Some studies have even shown IGFBP to exert a ligand-independent modulatory effect on metabolism (LeRoith et al., 1995).

4.1.1 IGF1R structure

The structure of human IGF1R has been extensively studied and documented, with the first characterisation of the receptor undertaken using human placental tissue in the late 1800s (Bhaumick et al., 1981). The receptor is a transmembrane tyrosine kinase, and is initially synthesised as a 1367 amino acid precursor protein, with a signal peptide 30 residues long. This pro-receptor is processed into a mature peptide by the proteolytic cleavage of the signal peptide during its translocation into the endoplasmic reticulum, and the N-linked glycosylation of the remaining polypeptide (Sepp-Lorenzino, 1998).

The mature and functional IGF1R is 1337 residues long and consists of an αβ-heterodimer held together by disulphide bonds in a β-α-α-β pattern. It is encoded by a 21-exon gene (Adams et al., 2000). A single disulphide bond links the α and β subunit, while multiple disulphide bonds between the α-chains of each αβ monomer hold the entire complex together. The α subunit contains about 710 residues (1 – 710), and they are strictly extracellular in locality; while the β subunits span the membrane and enter the cytoplasm (Figure 4.1) (Girnita et al., 2014). Unlike many receptors which dimerise upon ligand contact, the mature IGF1R receptor exists as a dimer, prior to activation.

Each ectodomain α-monomer of IGF1R consists of two homologous leucine-rich domains called L1 and L2, connected by a cysteine-rich (CR) region (Cys^{148} to Cys^{298}) (Lawrence et al., 2007). Findings from the development of peptide chimeras and the site-specific mutagenesis of the gene reveal a unique orientation that allows ligand-binding to occur at the centre of the IGF1R ectodomain, as well as contact with the surrounding L1, CR and L2 domains (Molina et al., 2000). The CR domain is the major participant in ligand contact. The L1, L2 and CR regions consist of approximately 150 residues each. Adjacent to the L2 domain is a linear arrangement of three fibronectin III domains (FnIII-1 to FnIII-3) (Figure 4.1).

FnIII-2 consists of an insert domain (ID) of about 120 residues, within which resides an α-β furin-cleavage site that generates the distinct α and β subunits of IGF1R (Ward et al., 2008). The α-α disulphide bonds in IGFR1 are located within two sites: one bond linking the C524 pair on each α chain FnIII-1 region, while the other three bonds are between residue pairs Cys682, Cys683 and Cys685 in the insert domain (Adams et al., 2000; Ward et al., 2008). The α-β disulphide bond occurs between C647 in the FnIII-2 domain and C860 in the FnIII-1 domain (Ward and Lawrence, 2009). This bond introduces some stability into the complex, and is crucial for signal transduction. The last 20 residues at the

C-terminal end of the α-subunit contribute to ligand binding to IGF1R, and this was demonstrated by site-specific mutagenesis, chemical cross-linking analysis, and the creation of chimeric receptor proteins (Lawrence et al., 2007; Ward and Lawrence, 2009).

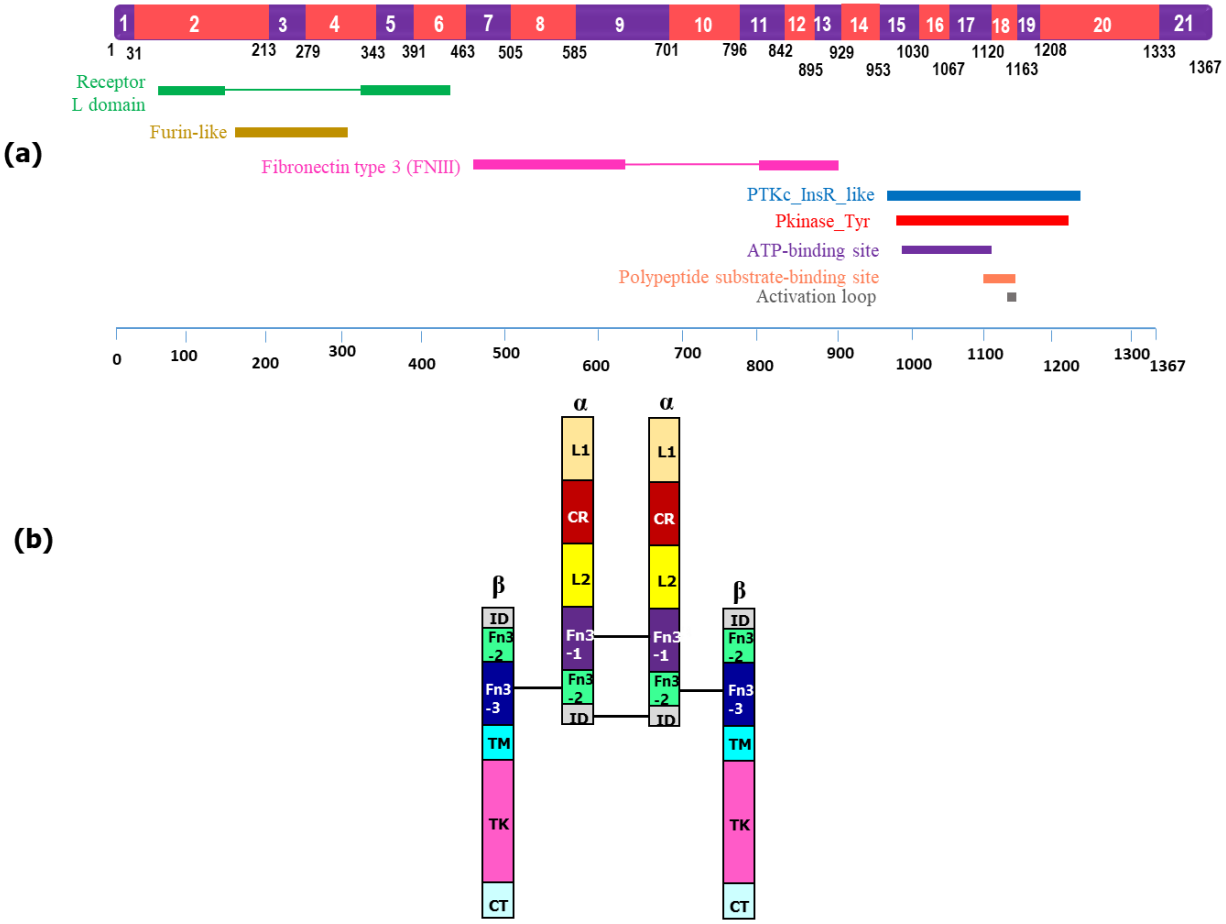


Figure 4.1 Human *IGF1R* structure and functional domains

Diagrammatic representation of *IGF1R* structure indicating (a) the 21 exons that encode the protein and the corresponding domains, and (b) the mature IGF1R αβ-heterodimer indicating the positions of the different domains. L1/L2 = leucine-rich domains 1 and 2, CR = cysteine-rich region, Fn3-(1-3) = fibronectin III domains (1-3), ID = insert domain, TM = transmembrane region, TK = tyrosine kinase domain, CT = C-terminal tail.

The β subunit consists of 627 amino acid residues (residues 711 – 1337), and is subdivided into three regions: an extracellular region consisting of five N-linked glycosylation sites through which it binds to the α subunit, a hydrophobic transmembrane region (906 – 929), and an intracellular region (Sepp-Lorenzino, 1998) (Figure 4.1). The intracellular portion of the β-subunit consists of two regulatory domains, a membrane-proximal region (residues 930 – 972) and a C-terminal tail (residues 1230 – 1337), interrupted by a catalytic tyrosine kinase (TK) domain (residues 973 – 1299) found in the middle (Ward et al., 2008).

Three tyrosine residues are found at the positions 1131, 1135 and 1136 of the TK domain, and are essential for the auto-phosphorylation of the receptor during signalling. Also within the TK domain at residues 976 – 981, is a catalytic section consisting of an ATP-binding motif (GXGXXG), and further downstream at position 1003 is a catalytic lysine residue that is crucial for Mg-ATP binding (Girnita et al., 2014). The membrane-proximal region plays a role in receptor internalisation, while the C-tail contains important regulatory elements of TK activity. In addition to the triple tyrosine cluster found in the TK domain, others residues necessary for receptor signal transduction include Y950, in the membrane-proximal region which serves as a docking site for Shc/IRS complex, and residues Y1250 and Y1251 in the C-terminal tail.

4.1.2 IGF1R signalling and function

Receptor signalling is a controlled process involving multiple factors including mitogen-activated protein kinase (MAPK), mammalian target of rapamycin (mTOR), phosphatidylinositol-3-kinase (PI3K), and serine/threonine kinase or protein kinase B (Akt or PKB). Upon proteolysis of the bound IGFBP, the ligand is free to bind to membrane receptors.

Ligand binding by IGF1, IGF2, or insulin to the extracellular domain of IGF1R brings about a conformational change, inducing the activation of its tyrosine kinase activity and the auto-phosphorylation of tyrosine residues 1131, 1135 and 1136 in the kinase domain (Chitnis et al., 2008). This action prompts the phosphorylation of juxtaposed tyrosine and serine residues outside the kinase domain, creating docking sites for downstream mediators such as insulin receptor substrates 1-4 (IRS-1-4), the Shc-adapter protein, and the Src kinase (Iams and Lovly, 2015). Amongst the four IRS proteins, IRS-1 and IRS-2 commonly mediate the physiological activities of IGFs in growth and development. Contained within the N-terminus of each IRS protein is a homologous phosphotyrosine binding (PTB) domain, through which they interact with membrane receptors (Girnita et

al., 2014). This cascade of events initiates downstream signal transduction via two major pathways: the PI3K/Akt pathway and the Ras/Raf/MAPK pathway. Figure 4.2 summarises the signalling process.

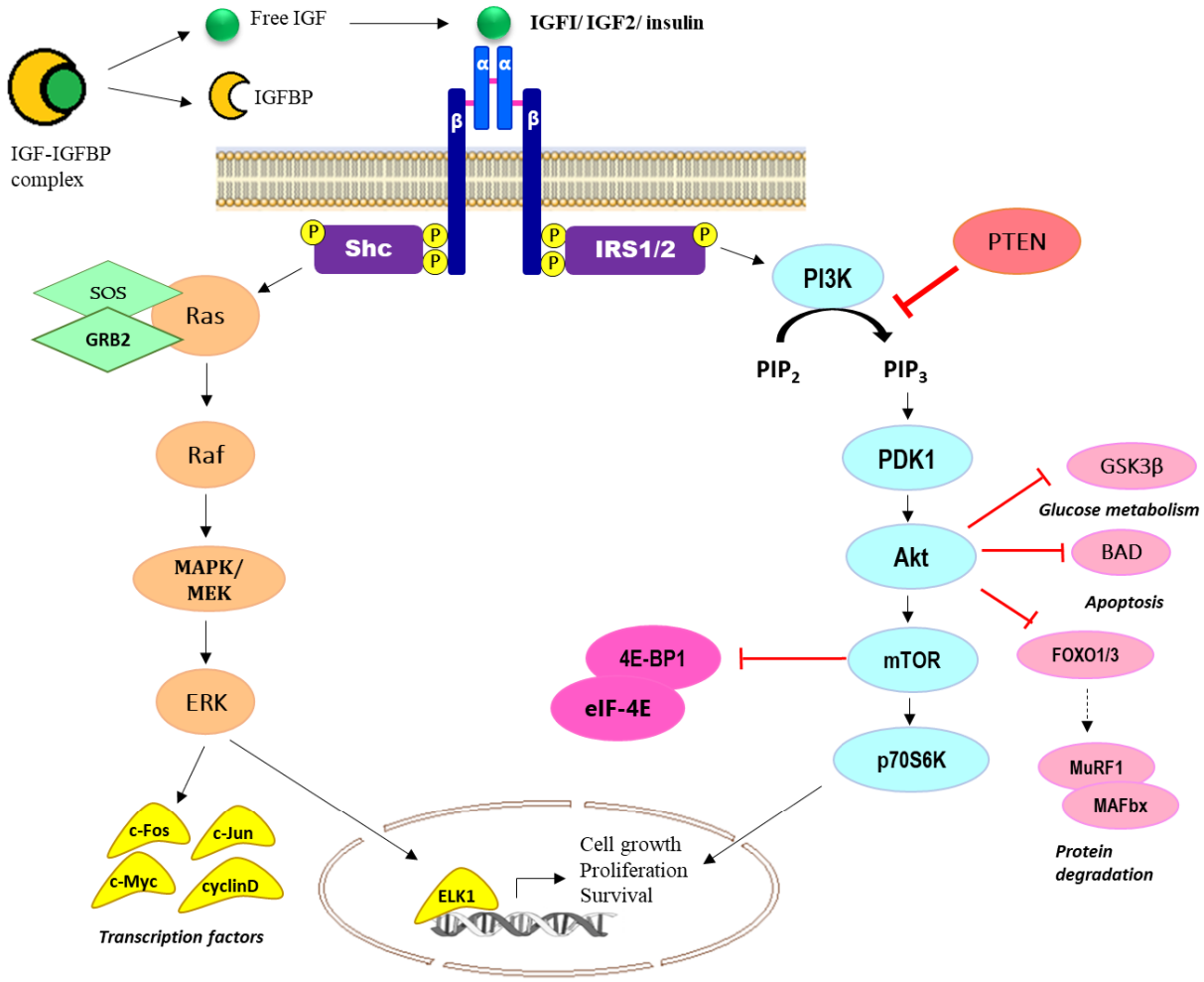


Figure 4.2 Molecular signalling pathway for IGF1R

Ligand binding to the α portion of IGF1R provokes the phosphorylation of the receptor tyrosine kinase domain, thus initiating a cascade of reactions including the recruitment of adapter proteins, including Shc and IRS protein complexes. Recurring phosphorylation leads to the activation of secondary messengers such as PI3K and Ras. PI3K triggers PDK, which in turn activates Akt. Akt is central to IGF1R function as it inhibits growth-inhibiting FOXO and BAD processes, while promoting cell growth and survival via mTOR and p70S6K activation. Ras, on the other hand, activates the MAPK/MEK/ERK pathways, which lead to the mobilisation of transcriptions factors such as c-Fos, cyclin-D, and ELK, which are capable of translocating to the nucleus, and ultimately bring about the expression of growth-promoting genes.

Following the phosphorylation of its tyrosines by IGF1R, IRS1/2 serves as the docking protein for p85, the regulatory subunit of P13K. The Src homology 2 (SH2) domain within p85 undergoes a high-affinity interaction with the phosphorylation-rich site in the C-terminal region of IRS1/2, triggering the conversion of inner membrane bound-phosphatidylinositol (4, 5)-bisphosphate (PIP_2) to phosphatidylinositol (3, 4, 5)-trisphosphate (PIP_3) by the catalytic domain of PI3K (Girnita et al., 2014). This conversion is antagonised by the phosphatase and tensin homolog (PTEN), which dephosphorylates PIP_3 to PIP_2, thereby serving as a negative regulation mechanism for this pathway (Hemmings and Restuccia, 2012) (Figure 4.2). The PIP_3 protein recruits other proteins possessing the pleckstrin homology (PH) domain, one of which is PI3K-dependent kinase 1 (PDK1). This kinase phosphorylates Akt at the conserved threonine-308 residue, priming it for phosphorylation at serine-473 by the mTORC2 complex to bring about complete Akt activation (Adams et al., 2000; Hemmings and Restuccia, 2012).

Phosphorylated Akt goes on to inhibit several atrophy-inducing factors including BAD (B-cell lymphoma 2, BCL-2-associated agonist of cell death), FOXO (forkhead box), and GSK3β (glycogen synthase kinase 3 beta) (Stitt et al., 2004). The factors, BAD and BAX (BCL-2-associated X protein), both belong to the pro-apoptotic class of BCL2 factors, and their inhibition by Akt promotes cell survival. The phosphorylation of the FOXO1/3 subfamily at multiple sites by Akt prevents nuclear entry. This consequently inhibits the expression of growth repressor genes encoding ubiquitin ligase proteins, including *MuRF1* (*muscle RING finger 1)*, and *muscle atrophy F box (MAFbx)* or *Atrogin-1* (Bodine et al., 2001; Hribal et al., 2003). These ubiquitin ligases, if upregulated by FOXO, conjugate ubiquitin to protein targets and marks them for degradation by proteasomes.

The fully active Akt subsequently stimulates the phosphorylation of cytosolic and nuclear substrates, including mTOR and p70 ribosomal S6 kinase (p70S6K). Phosphorylated mTOR downregulates the eukaryotic translation initiation factor 4E binding protein 1 (4EBP1), which acts as a translational repressor (Figure 4.2). On the other hand, phosphorylated p70S6K brings about protein synthesis by activating ribosomal protein subunit S6 and other factors involved in translation initiation and elongation. The function of p70S6K is so important to muscle function that its deletion resulted in a reduction in the size of myoblasts and muscle fibres (Ohanna et al., 2005; Clemmons, 2009). The Akt signalling mechanism induces the expression of muscle-specific genes, resulting in the differentiation of myoblasts. These signalling events collectively promote growth, protein synthesis, cell survival, migration and increased metabolism.

The second parallel pathway involves Ras/Raf/MAPK signal transduction (Figure 4.2). Signal transduction from ligand-binding leads to the docking and phosphorylation of Shc proteins at the phosphorylated C-terminal tail of IGF1R. The activated phosphotyrosine residues of Shc binds to the SH2 domain of the adaptor protein, GRB2 (growth factor receptor-bound protein 2), which subsequently forms a complex with SOS (Son of Sevenless), a guanine nucleotide exchange factor (Duan et al., 2010). From the cytosol, the GRB2-SOS complex migrates to the plasma membrane, where the activated SOS elicits the conversion of GDP (guanosine diphosphate) to GTP on Ras. The protein Ras is one of a family of small GTPase proteins. The active GTP-bound Ras recruits and activates Raf, a serine/threonine kinase, which in turn activates MEK (MAPK kinase), that goes on to activate MAPK (also called ERK) (Girnita et al., 2014). MAPK activates downstream transcription factors, such as ELK1, c-Fos, c-Jun, c-Myc and cyclinD (Coolican et al., 1997). This chain of events leads to growth and mitogenesis.

Ultimately, both pathways converge in a concerted effort to promote cell growth, survival and protein synthesis. The organised phosphorylation and dephosphorylation in IGF1R signalling thereby enables regulation through an ON/OFF type of system.

The IGF factors possess some uniqueness amongst other metabolic factors in that they can stimulate both proliferation and differentiation, two mutually exclusive processes within muscle fibres. Myoblast activation and proliferation occurs primarily via the MAPK pathway, while differentiation utilises the PI3K-Akt pathway (Coolican et al., 1997; Bodine et al., 2001; Rommel et al., 2001).

4.1.3 IGF1R effects on skeletal muscle growth

The size and strength of muscle fibres are often dependent on multiple factors including nutrient availability and gene expression. Despite sharing substantial similarity in their signalling pathways, insulin and IGFs diverge with respect to their physiological functions. While insulin primarily regulates fuel uptake, metabolism and homeostasis in cells, the IGFs stimulate cell survival, differentiation and growth.

Insulin-like growth factor 1 can function in an autocrine, paracrine or endocrine fashion, depending on its site of synthesis. It is commonly accepted that IGF1 produced by the liver dominates the circulation, while IGF1 synthesised by extrahepatic tissue is secreted into the extracellular space, where it acts in an autocrine and paracrine manner (Clemmons, 2016).

The signalling of IGF1 and IGF1R plays a vital role in skeletal myogenesis and maintenance, in both embryonic and postnatal stages. IGF1 expression has been found to induce the conservation of muscle architecture and satellite cell regeneration, while inhibiting atrophy. Studies undertaken with chicken embryos have shown IGF1 signalling to promote muscle hypertrophy, myoblast proliferation and differentiation, and an increase in the number of satellite cells, in a dose-dependent manner (Yu et al., 2015; Ma et al., 2017).

The IGF1 hormone is unique, in the sense that while most mitogenic growth factors promote myoblast proliferation and inhibit differentiation, IGF1-IGF1R signalling favours both myoblast proliferation and differentiation (Coolican et al., 1997; Hribal et al., 2003). Strong evidence demonstrating IGF1R participation in myogenesis include the observation that muscle hypoplasia and dwarfism result from the inactivation of *IGF1R* (Liu et al., 1993), and that myofibre hypertrophy is brought about *IGF1* transgene overexpression in the muscle (Coleman et al., 1995).

Postnatal skeletal muscle fibres are post-mitotic, hence further growth or repair is made possible by the mitotically-capable satellite cells that surround the muscle fibres. Aging can bring about depletion in the number and function of satellite cells. IGF1 signalling has a beneficial effect on muscle fibres and can prolong the life of adult muscle. The repair and regeneration of muscle following damage are influenced by IGF1R signalling.

4.1.4 Animal models of *IGF1R* inactivation and over-expression

Transgenic and knock-out mice models have shed light on the role of IGF1 in muscle growth and development, and during both the embryonic and postnatal stages of development (Woods et al., 1996). Using a targeted mutagenesis approach, Liu et al. (1993) revealed that homozygous *Igf1r(-/-)* null mutants at birth exhibit growth retardation achieving at most 45% of the normal size. They have organ and muscle hypoplasia and typically die at birth due to respiratory failure caused by having insufficient muscle mass needed to inflate their lungs. When compared to *Igf1r* knock-out mice, *Igf1*(-/-) and *Igf2*(-/-) null mutants experience less severe abnormalities, as some survive to adulthood and can grow up to 60% of the normal adult size (Liu et al., 1993; Baker et al., 1996; Woods et al., 1996).

Contrastingly, transgenic mice over-expressing a chimeric form of IGF1 manifested a 130% increase in weight, a reflection of increased somatic growth (Mathews et al., 1988;

Coleman et al., 1995). Increased muscle fibre numbers, improved regeneration and enhanced muscle strength, are characteristic of mice that have over-expression of IGF1 (Coleman et al., 1995; Barton-Davis et al., 1998; Barton et al., 2002).

In diseases such as muscular dystrophies, muscle degeneration exceeds regeneration, often resulting in the permanent breakdown and loss of myofibres. In a study comparing mice expressing transgenic IGF1 against non-transgenic control, the transgenic expression of IGF1 was found to result in a 40% increase in muscle mass with higher contractile force compared to their non-transgenic counterparts (Barton et al., 2002). IGF1 was also found to protect against muscle disintegration and necrosis (Musarò et al., 2001; Barton et al., 2002).

Varying results were obtained, possibly as a consequence of muscle type, with transgenic mice expressing muscle-specific IGF1. A general increase in muscle size was noted, with the increase varying from 75% in the glycolytic triceps muscles, to 23% in oxidative soleus muscles, to a two-fold increase in some biceps muscles (Musarò et al., 2001; Otto and Patel, 2010). In addition, mIgf1 was confirmed to antagonise atrophy and maintain mass within senescent muscle fibres (Musarò et al., 2001; Barton et al., 2002; Stitt et al., 2004).

The PI3K-Akt pathway has been demonstrated to be a critical driver of skeletal muscle hypertrophy activated by IGF1R signalling in transgenic mouse models. In these models, the conditional expression of Akt in transgenic mice possessing a constitutively active Akt mutant, brought about a rapid two-fold increase in the muscle fibre size (Lai et al., 2004; Stitt et al., 2004). Other studies, *in vivo* and *in vitro*, have demonstrated muscle hypertrophy following the expression of genes encoding active forms of the signalling mediators, PI3K or Akt (Bodine et al., 2001; Rommel et al., 2001; Lai et al., 2004).

In addition to the muscle, the IGF system has effects on the nervous system (Woods et al., 1996), bone (Zhao et al., 2000), and reproductive tissues (Baker et al., 1996). Increased brain growth, synaptogenesis, improved myelination and neurogenesis, were observed with the over-expression of a mouse IGF1 transgene in transgenic mice (D'Ercole et al., 2002; Duan et al., 2010), whereas IGF1-null mice exhibited significant neuronal loss, hypo-myelination, increased neuronal apoptosis, sensorineural deafness and a reduction in brain weight and size (Beck et al., 1995).

4.1.5 Human *IGF1R* mutations

Human *IGF1R* is located on the long arm of chromosome 15 (15q26.3) (Klammt et al., 2011; Janchevska et al., 2018). Pathological studies have implicated abnormalities in this region with syndromes such as 'short for gestational age' (SGA).

Abuzzahab et al. (2003) reported the first three *IGF1R* mutations documented in humans. These were located in the exon 2 region of the gene, and included a *C* to *T* transition resulting in a premature stop codon at position 59, a *C* to *A* transversion leading to the substitution of the 108th arginine for glutamine, and an *A* to *C* transversion resulting in lysine residue being substituted for asparagine at position 115. Fibroblast culture studies revealed that patients with the nonsense p.Arg59stop mutation exhibited a decline in the number of cell surface IGF1R peptides, whereas patients with the missense mutations, p.Arg108Gln and p.Lys115Asn, had reduced IGF1R function (Abuzzahab et al., 2003). Exon 2 encodes the start of the mature IGF1R protein, hence the p.Arg59stop mutation allele would result in a truncated and non-functional receptor. As a result of their location in the ligand binding domain of IGF1R, the two amino acid substitutions would bring about a diminished affinity of IGF1 for its receptor (Abuzzahab et al., 2003).

The cleavage site of IGF1R is conserved, and consists of the amino acids Arg-Lys-Arg-Arg (R-K-R-R). A missense substitution of arginine for glutamine (p.Arg709Gln) at residue 709 due to a *C* to *T* transition in exon 11 of human *IGF1R*, altered the cleavage site to R-K-Q-R (Kawashima et al., 2005). This mutation halted the post-translational processing of the IGF1R precursor protein into the mature α and β subunits, ultimately resulting in short stature and intrauterine growth retardation (Kawashima et al., 2005).

A c.3148G>A transition in the exon 16 of human *IGF1R* resulted in the substitution of glutamic acid for lysine at codon 1020 (Walenkamp et al., 2006). This p.Glu1020Lys substitution lies within the intracellular tyrosine kinase domain that is necessary for IGF1R signalling and function. Molecular and cell line studies revealed reduced auto-phosphorylation and downstream signal transduction of IGF1R, although the binding of IGF1 to its receptor was unperturbed (Walenkamp et al., 2006). Reduced receptor auto-phosphorylation and second messenger phosphorylation, as well as reduced cell proliferation were also reported for the following IGF1R mutations: an arginine substitution to leucine at position 431 (p.Arg431Leu) (Kawashima et al., 2012), an arginine 481 substitution to glutamine (p.Arg481Gln) (Inagaki et al., 2007), and a valine 599 substitution to glutamic acid (p.Val599Glu) (Wallborn et al., 2010).

Scanning microscopy and flow cytometric studies revealed that the p.Val599Glu substitution resulted in a misfolded IGF1R pro-receptor, which was withheld in the endoplasmic reticulum (ER), and for which cleavage to the mature form of the protein was abrogated (Wallborn et al., 2010). Intracellular retention in the ER, of the defective IGF1R pro-receptor was also postulated to occur with the p.Glu121Lys and p.Glu234Lys mutations described by Fang et al. (2012).

In the distal end of the β-subunit, a glycine to alanine substitution at codon 1125 (p.Gly1125Ala), caused by a *G* to *C* nucleotide transversion, disrupts a conserved region of the protein kinase activation loop motif in IGF1R (Kruis et al., 2010). Modelling analyses predict that the introduction of a methyl group by the arginine in this position would bring about a structural 'clash' with the other conserved motif residues, including the aspartate at position 1123, and the phenylalanine at position 1124, and this would culminate in abolished auto-phosphorylation (Kruis et al., 2010).

Duplication mutations are quite rare in the coding regions of crucial house-keeping genes, nonetheless a 19 bp duplication (c.3348_3366dup19) in exon 18 of human *IGF1R* results in a frameshift and formation of a premature stop codon at position 1106 of *IGF1R* (Fang et al., 2009; Klammt et al., 2011). The tyrosine kinase domain is partially encoded by exon 18, and thus this truncated IGF1R would be non-viable. The duplication resulted in mRNA degradation, indicated by the inability to detect mutant mRNA and subsequent IGF1R haplo-insufficiency (Fang et al., 2009).

Humans possessing one or more of these mutations exhibit, to varying degrees, the following clinical phenotypes: intrauterine growth retardation (IUGR), postnatal growth failure and short stature, delayed mental development, microcephaly, craniofacial abnormalities, hindered bone growth, and IGF1 resistance (Abuzzahab et al., 2003; Kawashima et al., 2005; Walenkamp et al., 2006; Inagaki et al., 2007; Fang et al., 2012; Leal et al., 2013; Yang et al., 2018).

Interestingly, one study revealed that humans possessing a p.Tyr387stop mutation, also had altered carbohydrate metabolism, including having reduced glucose tolerance and impaired insulin sensitivity (Mohn et al., 2011). In another rare condition, Cunningham et al. (2011) described five missense mutations (p.Pro190Ser, p.Arg406His, p.Met446Val, p.Arg595His, and p.Asn857Ser), that resulted in severe craniosynostosis and delayed neuronal development.

The ligand IGF1 has been identified as a risk factor in prostrate, lung, colon and breast cancers, with tumour cells expressing elevated levels of IGF1 (Duan et al., 2010). A homozygous partial deletion of IGF1 in humans has been associated with impaired foetal growth, mental and postnatal growth retardation, and sensorineural issues (Woods et al., 1996). Research into IGF1R could, therefore, provide clinical benefit and therapeutic relevance for treating pathologies and muscle-related diseases including cachexia, sepsis and dystrophy.

4.1.6 IGF1R variations in livestock

Ovine *IGF1R* is located on chromosome 18, and consists of 21 exons and 20 introns that encode a 1367-amino acid polypeptide. Extensive effort has gone into studying IGF1R in humans, largely due to its role in pathological conditions, such as dwarfism and intrauterine growth retardation. However, compared to the human counterpart, very little knowledge is available on the effect of *IGF1R* sequence variation on animal production traits.

In cattle, digestion of a 625 bp intronic fragment of bovine *IGF1R* by *TaqI* was found to yield two alleles, *A* and *B*, where the *AA* and *AB* genotypes were associated with increased milk production, while genotype *BB* was associated with a reduced interval for calving (Moody et al., 1996; Pereira et al., 2003; Curi et al., 2005). Another study in South Anatolian Red cattle reported a similar finding, with the *A* and *B* alleles presenting with frequencies of 82% and 18% respectively, although no association was observed with milk traits (Akis et al., 2010).

Digestion of bovine *IGF1R* by *MspI* and *TaqI* enzymes, was reported to bring about two variations, a synonymous variation in exon 12, and a non-coding variation in the 3' UTR, respectively. The *GG* genotype at the *IGF1R/MspI* locus showed association with increased weaning weight in Angus beef cattle, when compared to the *AG* genotype (Szewczuk et al., 2013).

In a recent study in Polish Holstein-Friesian cows, increased milk protein, milk fat and milk yield were observed with *IGF1R* genotype combinations, *TT/GG*, *TT/AG* and *CT/GG*, when compared to the *CC/AA* genotype. The variations were reported to reside within exon 2 of bovine *IGF1R*, which encodes the ligand binding domain that is crucial to IGF1R signalling (Szewczuk, 2017).

In sheep, the *TT* genotype of a *C* to *T* substitution in the intron 12 of ovine *IGF1R* showed significant association with higher body weight (at days 1, 30 and 90), and average daily weight gain in Pomeranian coarsewool ewes, when compared to the *CC* genotype (Proskura and Szewczuk, 2014). Three sequence variants (*A*, *B*, and *C*) were revealed in the intron 2-exon 3 region of ovine *IGF1R*, and a synonymous variation present in the *C* variant was associated with variation in the age and lifespan of NZ sheep breeds (Byun et al., 2008; Byun et al., 2012). When explored in Barki ewes, the same intron 2-exon 3 region of *IGF1R* was found to yield three genotypes, *AA*, *BB*, and *CC*, where ewes possessing the *AA* genotype showed increased litter size and longevity, when compared to their counterparts.

4.1.7 Rationale behind study

The ovine *IGF1R* was selected for this study due to the central function of its protein product in animal growth, protein turnover and energy metabolism, as demonstrated by its high evolutionary conservation and ubiquitous distribution. IGF1R is a receptor for three important ligands – IGF1, IGF2 and insulin, and its signalling promotes the proliferation and differentiation of muscle cells, while inhibiting cell death. In response to growth hormone stimulation, IGF1R can also bring about lipolysis in the adipose tissue. This suggests that IGF1R may be an important regulator of growth, muscling and leanness in sheep.

A region of ovine *IGF1R* spanning from exon 9 through to intron 10, which has not yet been explored in livestock, was selected for analysis. This region harbours the α-β cleavage site that results in the production of the two subunits of the mature protein. Also housed in this region is the fibronectin-III domain which enables the formation of disulphide bridges that maintain the structure and stability of the receptor.

Two additional gene regions were chosen for this investigation: one spanning known regulatory regions in the 5' UTR/promoter, and the other spanning exon 15 – a segment that encodes the transmembrane region of IGF1R.

Given its broad functionality, nucleotide sequence variation in ovine *IGF1R* could not only alter its own signalling and physiological activity, but potentially exert effects on growth, homeostasis and metabolism.

4.2 Materials and Methods

4.2.1 Sample collection and purification

Blood samples used in this investigation were collected from 1135 lambs from ten different NZ breeds, namely Romney (n = 743), Coopworth (n = 30), Corriedale (n = 31), Dorset Down (n = 32), Merino (n = 51), Perendale (n = 74), Poll Dorset (n = 30), Suffolk (n = 45), Texel (n = 31), and White Dorper (n = 68). See Chapter 3.2 for details on growth and carcass data recording for Romney lambs.

The search for genetic variation was performed on all sheep samples. Lambs lacking phenotypic data for growth and carcass traits were excluded from the statistical analyses, leaving 743 Romney lambs for which these data were available. The lambs examined in this study were obtained from 40 unrelated sire-lines belonging to the top 20% of Romney sheep in the Sheep Improvement Limited (SIL) dual-purpose index ranking.

The genomic DNA from the FTA blood samples was purified using a two-step procedure. This involved a cell and protein denaturation with 20 mM NaOH at 60 ºC heat for 30 min, and subsequent washing with 1 × TE buffer for 5 min, as outlined by Zhou et al. (2006).

4.2.2 Primer design and PCR analysis

Based on ovine *IGF1R* sequences with the accession numbers NC_030828.1 and NC_019475.2 obtained from the NCBI database, three primers were designed to amplify coding and non-coding regions of ovine *IGF1R*.

The ideal PCR and SSCP conditions for each primer set were established empirically following multiple optimisation experiments. For a start, PCR optimisations entailed low stringency PCR with standard temperatures and reagent concentrations. The annealing temperature was subsequently raised in stepwise increments of 1 ºC until a single band was observed on agarose gel electrophoresis. This was done to curb non-specific amplification. Similar increments in a stepwise fashion were employed to the reagents used until the best results were obtained. The *IGF1R* primer details and respective PCR-SSCP running conditions are stipulated in Table 4.1.

Table 4.1 *IGF1R* primers and PCR-SSCP conditions

Gene Region	Forward/reverse primers Oligonucleotide (5' to 3')	Amplicon size	Annealing temp (ºC)	SSCP conditions
Promoter/5' UTR	**F:** CCTCGGGGAGTGGTGTCGC **R:** CGACGGCGGCAACTCGGGT	319 bp	59 ºC	14%, 300 V, 30 ºC, 18 h
Exon 9, Intron 9, Exon 10, Intron 10	**F:** CTGAGCTACTACATCGTGCG **R:** CTCACTTCTGGGCTGTCAAC	548 bp	58 ºC	7%, 250 V, 30 ºC, 12 h
Exon 15	**F:** CTCAGCTCCGGCCTCTTCC **R:** GCCCTGCACCACCTCTGG	387 bp	59 ºC	12%, 270 V, 30 ºC, 19 h

The PCR amplifications were performed in 15 µl reactions containing the purified genomic DNA on a 1.2 mm diameter disc of FTA, 0.25 µM of each primer, 150 µM of each dNTP (Eppendorf, Hamburg, Germany), 3.0 mM Mg^{2+}, 0.5 U *Taq* DNA polymerase (Qiagen, Hilden, Germany), and 1 × the reaction buffer supplied with the enzyme. Amplifications were undertaken in Bio-Rad S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA).

The thermal profile entailed initial denaturation at 94 ºC for 2 mins, followed by 39 cycles of 94 ºC for 30 s, annealing at 58 ºC for 50 s, and extension at 72 ºC for 40 s, and a final extension step at 72 ºC for 5 min. The described profile is for the exon 9 – intron 10 fragment; the specific annealing temperatures for the other amplified fragments are given in Table 4.1.

Subsequent visualisation of the amplicons from the PCR reaction were carried out by means of agarose gel electrophoresis using 1% agarose (Quantum Scientific, Queensland, Australia) gels containing 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA), 200 ng/mL ethidium bromide. 2 µL aliquots of the PCR products were mixed with 2 µL of loading dye (0.2% bromophenol blue, 0.2% xylene cyanol, 40% (w/v) sucrose), and loaded into the gels that were run at a constant 5 V/cm for 2 hours. After electrophoresis, the gels were visualised using ultraviolet transillumination at 254 nm.

4.2.3 Single-stranded conformational polymorphism (SSCP) analyses

Optimisations for SSCP involved running amplicons at various conditions and using different gel compositions until consistently clear banding patterns were obtained.

Following PCR, 0.7 µL of each PCR product was mixed with 7µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). The samples were denatured at 105 ºC for 5 min, snap-chilled on ice, and 10 µL of each sample was loaded into 16 x 18 cm, 7% acrylamide:bisacrylamide (37.5:1, Bio-Rad) gels in Protean II xi cells (Bio-Rad). The samples were run at a voltage of 250 V at 30 ºC for 18 hours. The SSCP conditions stated above are for the exon 9 - intron 10 fragment, with the SSCP conditions for other amplified fragments are given in Table 4.1.

Silver-staining of the gels was performed according to the method described by Byun et al. (2009). Briefly, the gels were silver-stained with silver nitrate, washed with distilled water and subsequently developed using a solution containing sodium hydroxide and 40% formaldehyde.

In order to ensure the integrity of the experiments and to obtain reproducible banding patterns, freshly prepared PCR and SSCP products were always used. A negative control well containing no DNA was also run with each series of amplifications.

4.2.4 Nucleotide sequencing and genotyping

Following PCR-SSCP analysis, the banding patterns in the gels were observed. Amplicons deemed to be homozygous were sequenced in the forward and reverse directions using capillary sequencing at the Lincoln University DNA Sequencing Facility. For patterns that appeared to be heterozygous, single bands of non-homozygous samples were excised from the SSCP gel, washed repeatedly, and genomic DNA extracted. The resulting homozygous DNA was re-amplified, and then sequenced. Reactions were replicated to ensure accuracy and prevent PCR and sequencing errors.

Sequence alignments, translations and comparisons were carried out using DNAMAN version 5.2.10 (Lynnon BioSoft, Vaudreuil, Canada). The BLAST algorithm (http://blast.ncbi.nlm.nih.gov/) was used to search the NCBI GenBank and Ensembl databases for homologous sequences in various species.

4.2.5 Statistical analyses

IBM SPSS Statistics (Version 23, IBM, NY, USA) was used to perform all analyses. Trait data normality was tested using the Shapiro-Wilk test and Normal Q-Q plots. Variant and genotypic frequencies were calculated using the PopGene 3.2 software. The variant frequencies were then tested for deviation from Hardy-Weinberg equilibrium using a Chi-square test available on the Online Encyclopedia for Genetic Epidemiology Studies (OEGE) (Rodriguez et al., 2009).

The strength of relationships between the different traits was assessed using Pearson correlation coefficients. The growth traits investigated were birth weight, tailing weight, weaning weight, and growth rate-to-weaning. The carcass traits investigated were hot carcass weight (HCW), fat depth at the 12th rib (V-GR), hind-leg lean meat yield, loin lean meat yield, shoulder lean meat yield, and total carcass lean meat yield.

Two sets of general linear mixed-effect models (GLMMs) were carried out. The first model was used to investigate the effect of the presence and absence (coded as 1 and 0 respectively) of a specific variant on growth and carcass traits, while the second model assessed associations between the genotypes for each nucleotide substitution and growth and carcass traits. A Bonferroni correction was applied to the analyses.

The statistical model used was:

$$Y_{ijkl} = \mu + S_i + Gj + B_k + V_l + e_{ijkl}$$

where:

Y_{ijkl} is the trait measured on each animal (birth, tailing and weaning weights, growth-rate, HCW, V-GR, and leg, loin, shoulder, and total lean meat yields)

μ is the mean for the trait

S_i is the random effect of sire

Gj is the fixed effect of gender

B_j is the fixed effect of birth rank

V_l is the fixed effect of variant (presence or absence) or genotype

e_{ijkl} is the random residual error

Gender was fitted as a fixed factor, and sire as a random factor in all growth trait models. For the growth trait models assessing the relationship between nucleotide genotype (or the presence/absence of a sequence variant) and tailing and weaning weights, the age at tailing and weaning were fitted as covariates respectively, as variation in age at measurement could potentially influence these traits.

In order to account for the effect of prenatal growth and development, birth weight (which had a bigger effect than birth rank) was fitted as a covariate in models assessing the relationship between nucleotide variant genotype or the presence/absence of a sequence variant, and growth rate-to-weaning. Rearing rank, which is reflective of maternal influence was shown to have an effect, and hence was corrected for in growth rate models.

For all carcass trait models, genotype was fitted as a fixed factor, and sire as a random factor, while birth weight and slaughter age in days were fitted as covariates, so as to correct for the effect of prenatal muscle development and the variation in the age of the lambs at slaughter respectively. Gender was not corrected for in any of the carcass trait models, as only ram lambs were sent for slaughter.

The variant model was run in two phases. Firstly, a single variant model assessing the unilateral effect of variant presence/absence on individual traits was run. If a significant trend was observed ($P < 0.2$, the threshold for inclusion), then a subsequent multi-variant model investigating the effect of the presence/absence of a variant, within the context of the effect of the other variants, was performed.

Values from the GLMM pairwise comparison was presented as estimated marginal means ± standard error (mean ± SE). Unless otherwise specified, statistical significance was declared at $P < 0.05$, and trends were observed at $P < 0.2$.

4.3 Results

4.3.1 *IGF1R* gene variations

Three segments of ovine *IGF1R* were amplified and investigated for genetic variations in ten NZ sheep breeds (Figure 4.3). Following PCR-SSCP analysis, no variation was detected in the amplicons derived from the promoter and exon 15 regions.

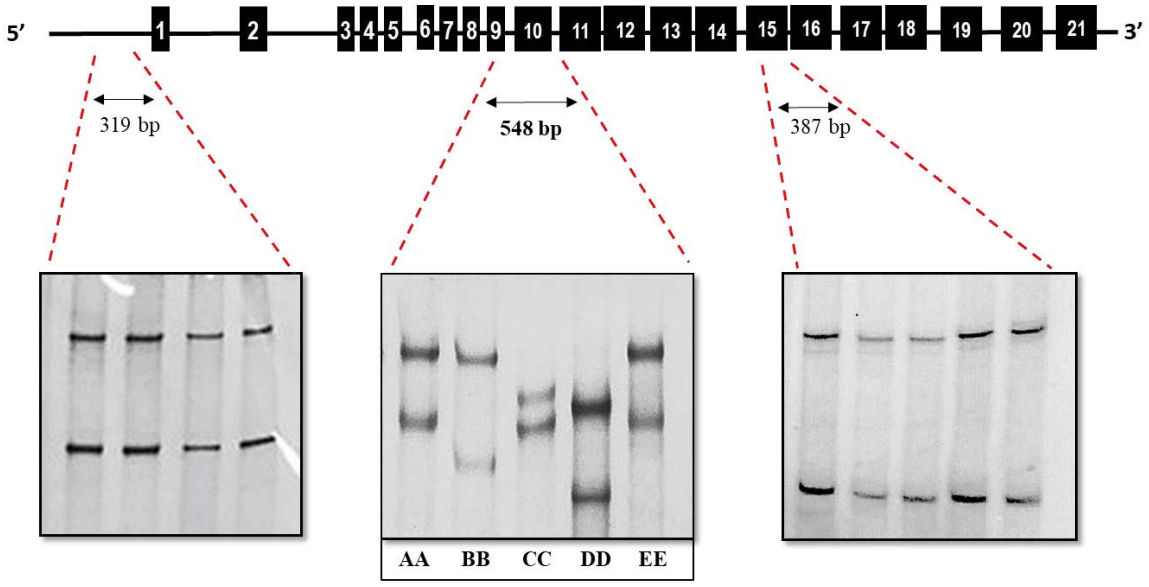


Figure 4.3 *IGF1R* regions amplified and the PCR-SSCP gel patterns

Diagrammatic representation of the ovine *IGF1R* showing all 21 exons, and the polymerase chain reaction-single-stranded conformational polymorphism (PCR-SSCP) gel results of the three amplified regions of ovine *IGF1R*, including the promoter (319 bp), exon 9 -intron 10 (548 bp), and exon 15 (387 bp) regions.

The 548 bp fragment spanning from exon 9 to intron 10 showed polymorphism, with five variants (*A_9, B_9, C_9, D_9,* and *E_9*) being detected (Figure 4.3). Upon sequencing, these revealed five unique DNA sequences that contained ten novel nucleotide sequence variations. The five variant sequences were submitted to the GenBank database and assigned the accession numbers: MK210245 - MK210249.

4.3.2 Multiple sequence alignment of *IGF1R* sequences

Alignments of the nucleotide sequences of the five *IGF1R* variants and other related species are presented in Figure 4.4. It can be seen from the alignment that the exon 9 - intron 10 region of *IGF1R* is conserved, with most species or breed-specific variations occurring in the non-coding regions.

```
Variant_A   CTGGCAGCGCCAGCCCCAGGATAGCTACCTGTACCGGCACAACTACTGCT     50
Variant_B   --------------------------------------------------     50
Variant_C   --------------------------------------------------     50
Variant_D   --------------------------------------------------     50
Variant_E   --------------------------------------------------     50
 Goat       --------------------C-----------------------------     50
 Cattle     --------------------C-----------------------------     50
 Yak        --------------------C-----------------------------     50

Variant_A   CCAAAGgtgaggggggcggccggg..cgcgcctgtgggcagtgagcggcag     98
Variant_B   ----------------------..-------------------------     98
Variant_C   ----------------------..-------------------------     98
Variant_D   ----------------------..-------------------------     98
Variant_E   ----------------------..-------------------------     98
 Goat       ----------------------..---a-------------g-------     98
 Cattle     ---------------a-------.cg---t-------g----g-------     99
 Yak        ---------------a-------gcg---t-------g----ga------    100

Variant_A   cggcggtcccgagcccgggggcgcgggctctcccctcccggcagcccgtg    148
Variant_B   --------------------------------------------------    148
Variant_C   ---------t----------------------------------------    148
Variant_D   --------------------t----------------------------    148
Variant_E   --------------------------------------------------    148
 Goat       gc------------------------------------------------    148
 Cattle     ------g--t-----------------------at---------------    149
 Yak        ------g--t-----------t-----------at---------------    150

Variant_A   taacctggctcgcaccgggccactctgttccgagcagACAAAATCCCCAT    198
Variant_B   --------------------------------------------------    198
Variant_C   --------------------------------------------------    198
Variant_D   --------------------------------------------------    198
Variant_E   --------------------------------------------------    198
 Goat       -------------g------------------------------------    198
 Cattle     ----t----------g----------c----------------------    199
 Yak        ----t----------g----------c----------------------    200

Variant_A   CAGGAAGTACGCCGACGGCACCATCGACGTCGAGGAGGTGACGGAGAACC    248
Variant_B   ---------T-----------------T----------------------    248
Variant_C   ----------------------------------------A-------    248
Variant_D   ---------T-----------------T----------------------    248
Variant_E   --------------------------------------------------    248
 Goat       ------------------------------------------------T-    248
 Cattle     --------------------------------------------------    249
 Yak        ---------------T----------------------------------    250

Variant_A   CCAAGACCGAGGTGTGCGGCGGGGAGAAAGGGCCGTGCTGCGCTTGCCCC    298
Variant_B   --------------------------------------------------    298
Variant_C   --------------------------------------------------    298
Variant_D   --------------------------------------------------    298
Variant_E   --------------------------------------------------    298
 Goat       --------------------------------------------------    298
 Cattle     ----------------------------------------------T---    299
 Yak        ----------------------------------------------T---    300
```

```
Variant_A   AAAACCGAAGCCGAGAAGCAGGCAGAGAAGGAGGAGGCCGAGTACCGCAA    348
Variant_B   ----------------------G-------------------------    348
Variant_C   ----------------------G-------------------------    348
Variant_D   ----------------------G-------------------------    348
Variant_E   ----------------------G-------------------------    348
 Goat       ----------------------G-------------------------    348
 Cattle     ----------T----------G-------------------------    349
 Yak        ----------T----------G-------------------------    350

Variant_A   GGTCTTCGAGAACTTCCTGCACAACGCCATCTTCGTGCCCAGgtactgct    398
Variant_B   ------------------------------------------------    398
Variant_C   ------------------------------------------------    398
Variant_D   ------------------------------------------------    398
Variant_E   ------------------------------------------------    398
 Goat       ------------------------------------------------    398
 Cattle     ----------------------------------------------c--c    399
 Yak        ----------------------------------------------c--c    400

Variant_A   atctttgtgcccaggtaccaccatcttggtgcccgggcaccaccatcttt    448
Variant_B   ----------------------t-------------------------    448
Variant_C   ------------------------------------------------    448
Variant_D   ------------------------------------------------    448
Variant_E   -------------------c----------------------------    448
 Goat       ------------------------------------------------    448
 Cattle     --t--c------g------g--------------------g--------    449
 Yak        --t--c------g------g--------------------g--------    450

Variant_A   gcacccaggccatatgtcctccgcggtgctggtgccccgaccccagccca    498
Variant_B   ----------------------t-------------------------    498
Variant_C   ------------------------------------------------    498
Variant_D   ----------------------t-------------------------    498
Variant_E   ----------------------t-------------------------    498
 Goat       ------------------------------------------------    498
 Cattle     -----------                                         460
 Yak        -----------cc-c---t----------c-ccc----c---a-------    500

Variant_A   ccatgcgcag                                         508
Variant_B   -----.....                                         503
Variant_C   ----------                                         508
Variant_D   -----.....                                         503
Variant_E   -----.....                                         503
 Goat       ----------                                         508
 Cattle                                                        460
 Yak        --t-------                                         510
```

Figure 4.4 Alignment of *IGF1R* sequences

The sequences *A* – *E* represent the five different variants (A_9 – E_9) detected in this study (excluding the primer region). The alignment was carried out using the DNAMAN software. Dashes (-) denote nucleotides identical to the reference variant A_9 sequence, while dots (.) denote missing nucleotides. The DNA sequences for the related species were extracted from GenBank with the following accession numbers: Goat, *Capra hircus* (NC_030828.1); Cattle, *Bos taurus* (JN204287); and Yak, *Bos mutus* (CP027089.1).

4.3.3 Variant diversities in ovine *IGF1R*

The ten *IGF1R* sequence variations detected and their defining haplotypes are summarised in Table 4.2.

Table 4.2 Variants and haplotypes detected in the intron 9, exon 10 and intron 10 regions of ovine *IGF1R*

Haplotype / Variant	Region	A_9 H1	B_9 H2	C_9 H3	D_9 H4	E_9 H5
c.1996+52C>T	Intron 9	c	c	t	c	c
c.1996+64C>T	Intron 9	c	c	c	t	c
c.2019C>T (p.Tyr673Tyr)	**Exon 10**	**C**	**T**	**C**	**T**	**C**
c.2037C>T (p.Asp679Asp)	**Exon 10**	**C**	**T**	**C**	**T**	**C**
c.2052G>A (p.Thr684Thr)	**Exon 10**	**G**	**G**	**A**	**G**	**G**
c.2133G>A (p.Ala711Ala)	**Exon 10**	**A**	**G**	**G**	**G**	**G**
c.2201+28A>C	Intron 10	a	a	a	a	c
c.2201+31A>T	Intron 10	a	t	a	a	a
c.2201+82T>C	Intron 10	c	t	c	t	t
c.2201+114_118delCGCAG	Intron 10	cgcag	del	cgcag	del	del

All of the variation found in exon 10 was synonymous, meaning that there would not be changes in the putative amino acid sequence at positions 673, 679, 684 and 711. Intron 10 had more variation than intron 9, with the most notable being a 5 bp deletion located 114 bases upstream of the coding nucleotide at position 2201. This c.2201+114_118delCGCAG deletion was present in three of the five variants – B_9, D_9 and E_9 (Table 4.2).

4.3.4 Variant and genotype frequencies of *IGF1R*

The genotypic frequencies of *IGF1R* variants across ten NZ sheep breeds are given in Table 4.3.

Table 4.3 Breed frequencies of ovine *IGF1R*

		***IGF1R* Variant Genotypes (%)**								
Breed	**n**	***A*9*A*9**	***A*9*B*9**	***A*9*C*9**	***A*9*D*9**	***A*9*E*9**	***B*9*B*9**	***B*9*C*9**	***B*9*E*9**	***E*9*E*9**
Romney	743	20.0	41.0	_	_	11.0	17.0	_	8.0	3.0
Coopworth	30	20.0	50.0	_	_	13.3	10.0	_	3.4	3.3
Corriedale	31	9.7	38.7	_	_	12.9	6.4	_	32.3	_
Dorset Down	32	37.5	18.7	15.6	_	_	15.6	6.3	6.3	_
Merino	51	13.7	43.1	_	_	11.8	5.9	_	25.5	_
Perendale	74	40.5	20.3	_	4.1	13.5	_	8.1	13.5	_
Poll Dorset	30	23.3	36.7	_	_	16.7	16.7	_	6.6	_
Suffolk	45	11.1	17.8	24.4	_	_	13.4	33.3	_	_
Texel	31	22.6	22.6	_	_	16.2	19.3	_	19.3	_
White Dorper	68	11.8	60.3	2.9	_	10.3	7.4	_	2.9	4.4
Total	1135									

Genotypes *A*9*A*9 and *A*9*B*9 were detected in all breeds, while *B*9*B*9 was present in all but the Perendale breed. The Perendale was the only sheep breed with the *A*9*D*9 genotype (4.1%). Both the Suffolk and Dorset Down breeds possessed the limited *A*9*C*9 and *B*9*C*9 genotypes, with the highest frequencies found in the former. The *B*9*D*9, *C*9*C*9, *C*9*D*9, *C*9*E*9, *D*9*D*9 and *D*9*E*9 genotypes were not observed in any of the lambs.

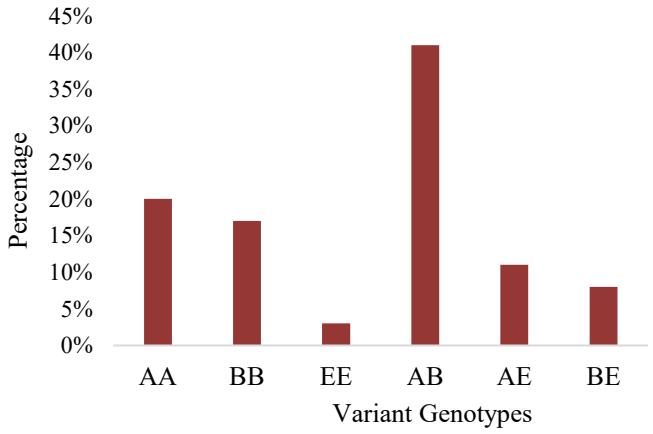


Figure 4.5 *IGF1R* variant genotype frequencies in NZ Romney breed

Only three variants (A_9, B_9 and E_9) were observed for NZ Romney breed (Table 4.3). Genotype A_9A_9 was the most common (20%), followed by B_9B_9 (17%). Only 3% of the Romney lambs possessed the least common E_9E_9 genotype (Figure 4.5), which was also present in the Coopworth and Dorper breeds. The A_9B_9 genotype (41%) occurred twice as commonly as A_9A_9, while the A_9E_9 and B_9E_9 genotypes had frequencies of 11% and 8% respectively (Figure 4.5).

Table 4.4 summarises the nucleotide and genotype frequencies of the detected variants, and their deviation from the Hardy-Weinberg Equilibrium.

Table 4.4 *IGF1R* genotype frequencies at variant loci in NZ Romney sheep

Variant	Nucleotide	Nucleotide frequency	n	Genotype	Genotype frequency	HWE[1]
c.2019C>T	C	0.58	253	CC	0.34	$\chi^2 = 0.06$
	T	0.42	364	CT	0.49	P = 0.970
			126	TT	0.17	
c.2037C>T	C	0.58	253	CC	0.34	$\chi^2 = 0.06$
	T	0.42	364	CT	0.49	P = 0.970
			126	TT	0.17	
c.2133G>A	A	0.46	149	AA	0.20	$\chi^2 = 1.48$
	G	0.54	387	AG	0.52	P = 0.477
			207	GG	0.28	
c.2201+28A>C	A	0.88	580	AA	0.78	$\chi^2 = 10.95$
	C	0.12	141	AC	0.19	**P = 0.004**
			22	CC	0.03	
c.2201+31A>T	A	0.58	253	AA	0.34	$\chi^2 = 0.06$
	T	0.42	364	AT	0.49	P = 0.970
			126	TT	0.17	
c.2201+82T>C	C	0.46	149	CC	0.20	$\chi^2 = 1.48$
	T	0.54	387	CT	0.52	P = 0.477
			207	TT	0.28	
c.2201+114_118 delCGCAG	CGCAG	0.46	149	CGCAG/CGCAG	0.20	$\chi^2 = 1.48$
	del	0.54	387	CGCAG/del	0.52	P = 0.477
			207	del/del	0.28	

[1] HWE = Hardy-Weinberg Equilibrium

Within the NZ Romney sheep, the nucleotide substitutions were all in agreement with the Hardy-Weinberg Equilibrium (P > 0.05), except for c.2201+28A>C (P = 0.004). This was likely a consequence of adenine being the major nucleotide (88%) with a predominant *AA* genotype (78%), while cytosine was the minor nucleotide (12%) with the *CC* genotype being rare (3%).

For the nucleotide substitutions c.2019C>T, c.2037C>T and c.2201+31A>T, the major and minor nucleotides had frequencies of 58% and 42% respectively (Table 4.4). For the nucleotide substitutions c.2133G>A, c.2201+82T>C and c.2201+114_118delCGCAG, the major and minor nucleotides had frequencies of 54% and 46% respectively.

4.3.5 Association of *IGF1R* genetic variants with growth traits

The presence or absence of each *IGF1R* variant was investigated for association with growth traits in NZ Romney lambs, and the results are given in Table 4.5.

Table 4.5 Association between variants in ovine *IGF1R* and variation in growth traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
Birth weight (kg)	A_9		5.03 ± 0.21	207	5.13 ± 0.20	536	0.150
	B_9		5.13 ± 0.21	253	5.07 ± 0.20	490	0.368
	E_9		5.11 ± 0.20	580	5.00 ± 0.21	163	0.136
	A_9	E_9	5.01 ± 0.21	207	5.09 ± 0.21	536	0.268
	E_9	A_9	5.10 ± 0.20	580	5.00 ± 0.21	163	0.240
Tailing weight (kg)	A_9		11.75 ± 0.56	207	12.14 ± 0.56	536	**0.029**
	B_9		12.20 ± 0.56	253	11.87 ± 0.55	490	0.055
	E_9		12.03 ± 0.55	580	11.61 ± 0.58	163	**0.029**
	A_9	B_9, E_9	11.80 ± 0.56	207	11.91 ± 0.56	536	0.609
	B_9	A_9, E_9	12.10 ± 0.56	253	11.61 ± 0.56	490	**0.016**
	E_9	A_9, B_9	12.15 ± 0.55	580	11.56 ± 0.57	163	**0.008**
Weaning weight (kg)	A_9		28.62 ± 0.97	207	29.76 ± 0.96	536	**<0.001**
	B_9		29.93 ± 0.98	253	29.00 ± 0.96	490	**0.002**
	E_9		29.45 ± 0.95	580	28.27 ± 0.99	163	**0.001**
	A_9	B_9, E_9	28.79 ± 0.96	207	29.13 ± 0.96	536	0.327
	B_9	A_9, E_9	29.63 ± 0.96	253	28.29 ± 0.96	490	**<0.001**
	E_9	A_9, B_9	29.78 ± 0.95	580	28.14 ± 0.98	163	**<0.001**
Growth rate to weaning (g/day)	A_9		249.59 ± 10.57	207	260.35 ± 10.35	536	**0.001**
	B_9		264.91 ± 10.56	253	254.78 ± 10.34	490	**0.001**
	E_9		259.40 ± 10.36	580	249.61 ± 10.71	163	**0.006**
	A_9	B_9, E_9	254.20 ± 10.55	207	257.43 ± 10.30	536	0.374
	B_9	A_9, E_9	262.70 ± 10.54	253	248.93 ± 10.30	490	**<0.001**
	E_9	A_9, B_9	263.13 ± 10.38	580	248.50 ± 10.55	163	**<0.001**

The presence of A_9 was associated with a 3.3% increase in tailing weight and a 4.0% increase in weaning weight. A 0.42 kg decrease in tailing weight was associated with E_9 being present. Weaning weight was 1.18 kg lower when E_9 was present, and 0.93 kg lower when B_9 was present.

Animals possessing the A_9 variant grew 4.3% faster by 10.76 g/day when compared to their counterparts that did not possess A_9. In contrast, animals possessing the B_9 and E_9 variants grew more slowly by 10.13 g/day and 9.79 g/day respectively (Table 4.5). All the associations and trends observed for B_9 and E_9 remained when the other variants that may have had an effect were factored into the models.

Table 4.6 summarises results from association studies assessing the effect of individual variant loci on ovine growth traits.

Table 4.6 Association of genotypes at variant loci in ovine *IGF1R* with growth traits in NZ Romney sheep

Variant Loci	Genotypes	n	Birth weight (kg)	Tailing weight (kg)	Weaning weight (kg)	Growth rate-to-weaning (g/day)
c.2019C>T (p.Tyr673Tyr)	CC	253	5.14 ± 0.21	12.19 ± 0.57	29.90 ± 0.98[a]	265.01 ± 10.56[a]
	CT	364	5.09 ± 0.21	11.85 ± 0.56	28.92 ± 0.97[ab]	254.22 ± 10.37[a]
	TT	126	5.04 ± 0.21	11.91 ± 0.57	29.10 ± 0.98[b]	256.80 ± 10.81[b]
	P-value		0.557	0.153	**0.007**	**0.004**
c.2037C>T (p.Asp679Asp)	CC	253	5.14 ± 0.21	12.19 ± 0.57	29.90 ± 0.98[a]	265.01 ± 10.56[a]
	CT	364	5.09 ± 0.21	11.85 ± 0.56	28.92 ± 0.97[ab]	254.22 ± 10.37[a]
	TT	126	5.04 ± 0.21	11.91 ± 0.57	29.10 ± 0.98[b]	256.80 ± 10.81[b]
	P-value		0.557	0.153	**0.007**	**0.004**
c.2133G>A (p.Ala711Ala)	AA	149	5.24 ± 0.21[a]	12.51 ± 0.57[a]	31.01 ± 0.98	274.22 ± 10.65[a]
	AG	387	5.08 ± 0.21[b]	11.97 ± 0.56[b]	29.18 ± 0.95	256.51 ± 10.24[b]
	GG	207	5.02 ± 0.21[b]	11.72 ± 0.56[b]	28.52 ± 0.95	250.23 ± 10.42[b]
	P-value		**0.036**	**0.004**	**<0.001**	**<0.001**
c.2201+28A>C	AA	580	5.10 ± 0.20	12.03 ± 0.55	29.44 ± 0.95	259.35 ± 10.28[a]
	AC	141	5.02 ± 0.21	11.65 ± 0.58	28.62 ± 0.99	253.34 ± 10.68[a]
	CC	22	4.83 ± 0.27	11.33 ± 0.73	25.65 ± 1.25	222.57 ± 13.27[b]
	P-value		0.197	0.075	**<0.001**	**<0.001**
c.2201+31A>T	AA	253	5.14 ± 0.21	12.19 ± 0.57	29.90 ± 0.98[a]	265.01 ± 10.56[a]
	AT	364	5.09 ± 0.21	11.85 ± 0.56	28.92 ± 0.97[ab]	254.22 ± 10.37[a]
	TT	126	5.04 ± 0.21	11.91 ± 0.57	29.10 ± 0.98[b]	256.80 ± 10.81[b]
	P-value		0.557	0.153	**0.007**	**0.004**
c.2201+82T>C	CC	149	5.24 ± 0.21[a]	12.51 ± 0.57[a]	31.01 ± 0.98	274.22 ± 10.65[a]
	CT	387	5.08 ± 0.21[b]	11.97 ± 0.56[b]	29.18 ± 0.95	256.51 ± 10.24[b]
	TT	207	5.02 ± 0.21[b]	11.72 ± 0.56[b]	28.52 ± 0.95	250.23 ± 10.42[b]
	P-value		**0.036**	**0.004**	**<0.001**	**<0.001**
c.2201+114_118 delCGCAG	CGCAG/CGCAG	149	5.24 ± 0.21[a]	12.51 ± 0.57[a]	31.01 ± 0.98	274.22 ± 10.65[a]
	CGCAG/del	387	5.08 ± 0.21[b]	11.97 ± 0.56[b]	29.18 ± 0.95	256.51 ± 10.24[b]
	del/del	207	5.02 ± 0.21[b]	11.72 ± 0.56[b]	28.52 ± 0.95	250.23 ± 10.42[b]
	P-value		**0.036**	**0.004**	**<0.001**	**<0.001**

[a, b] Mean values within the same column group with different superscripts differ at $P < 0.05$

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

Nucleotide substitutions with similar genotype frequencies exhibited similar associations with growth traits, reflecting their linkage in the variants. The common homozygous genotypes *CC*, *CC*, and *AA* for c.2019C>T, c.2037C>T and c.2201+31A>T respectively, were associated with a 2.7% increase in weaning weight and a 3.1% increase in growth rate by 8.2 g/day, when compared to the less abundant *TT* genotypes (Table 4.6). No associations were found with birth weight and tailing weight, although a decreasing trend of association with *TT* was noted for the latter.

At loci c.2201+114_118delCGCAG, the more abundant *del/del* homozygous genotype was associated with a 6.3% decrease in tailing weight, an 8% decrease in weaning weight, and an 8.7% decrease in growth rate of 24.0 g/day, when compared to the less abundant *CGCAG/CGCAG* genotype that lacked the deletion. Individuals possessing the *del/del* genotype were also 4.2% lighter at birth. The same pattern was noted for major and minor genotypes of c.2133G>A and c.2201+82T>C (Table 4.6).

The *CC* genotype of c.2201+28A>C was associated with lower weight at weaning by 3.79 kg and lower growth rate by 36.8 g/day, when compared to its more predominant *AA* counterpart. Similar decreasing trends were seen with birth weights and tailing weight at this loci.

4.3.6 Association of *IGF1R* genetic variants with carcass traits

The presence and absence of each variant was investigated for association with carcass traits in NZ Romney lambs, and the results are presented in Table 4.7.

Table 4.7 Association of variants in ovine *IGF1R* with carcass traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
HCW[1] (kg)	A_9		16.74 ± 0.11	192	17.27 ± 0.08	491	**<0.001**
	B_9		17.39 ± 0.11	231	17.00 ± 0.08	452	**0.002**
	E_9		17.20 ± 0.08	533	16.85 ± 0.13	150	**0.009**
	A_9	B_9, E_9	16.88 ± 0.12	192	17.15 ± 0.09	491	0.056
	B_9	A_9, E_9	17.24 ± 0.12	231	16.78 ± 0.10	452	**0.001**
	E_9	A_9, B_9	17.25 ± 0.10	533	16.77 ± 0.13	150	**0.003**
V-GR[2] Fat Depth (mm)	A_9		4.44 ± 0.17	192	4.04 ± 0.12	491	**0.038**
	B_9		3.97 ± 0.16	231	4.24 ± 0.12	452	0.138
	E_9		4.05 ± 0.11	533	4.52 ± 0.19	150	**0.022**
	A_9	B_9, E_9	4.34 ± 0.19	192	4.22 ± 0.14	491	0.574
	B_9	A_9, E_9	4.07 ± 0.18	231	4.49 ± 0.15	452	**0.046**
	E_9	A_9, B_9	3.97 ± 0.15	533	4.59 ± 0.19	150	**0.010**
Leg Yield (kg)	A_9		21.35 ± 0.09	192	21.56 ± 0.06	491	**0.025**
	B_9		21.48 ± 0.08	231	21.51 ± 0.06	452	0.707
	E_9		21.55 ± 0.06	533	21.32 ± 0.10	150	**0.032**
	A_9	E_9	21.33 ± 0.09	192	21.50 ± 0.07	491	0.075
	E_9	A_9	21.50 ± 0.06	533	21.32 ± 0.10	150	0.100
Loin Yield (%)	A_9		14.45 ± 0.06	192	14.62 ± 0.04	491	**0.011**
	B_9		14.57 ± 0.06	231	14.58 ± 0.04	452	0.921
	E_9		14.60 ± 0.04	533	14.48 ± 0.07	150	0.097
	A_9	E_9	14.44 ± 0.06	192	14.59 ± 0.05	491	**0.028**
	E_9	A_9	14.56 ± 0.04	533	14.48 ± 0.07	150	0.282
Shoulder Yield (%)	A_9		16.66 ± 0.06	192	16.92 ± 0.04	491	**<0.001**
	B_9		16.85 ± 0.06	231	16.85 ± 0.04	452	0.981
	E_9		16.90 ± 0.04	533	16.69 ± 0.07	150	**0.005**
	A_9	E_9	16.64 ± 0.06	192	16.87 ± 0.05	491	**0.001**
	E_9	A_9	16.83 ± 0.05	533	16.68 ± 0.07	150	0.055
Total Yield (%)	A_9		52.46 ± 0.18	192	53.11 ± 0.12	491	**0.002**
	B_9		52.90 ± 0.17	231	52.94 ± 0.12	452	0.817
	E_9		53.05 ± 0.12	533	52.50 ± 0.20	150	**0.009**
	A_9	E_9	52.41 ± 0.18	192	52.96 ± 0.14	491	**0.005**
	E_9	A_9	52.89 ± 0.13	533	52.48 ± 0.197	150	0.061

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

The presence of the A_9 variant was associated with a 3.2% increase in hot carcass weight of 0.53 kg; whereas B_9 and E_9 were associated with decreases in hot carcass weight of 0.39 kg and 0.35 kg respectively. Variant A_9 was associated with an increase in lean meat yield in the leg (1%), loin (1.2%) and shoulder (1.6%) regions, and total yield (1.2%); while E_9 was associated with a decrease in leg (1.1%), loin (0.8%), shoulder (1.2%) and total (1%) yield.

A 9% (0.4 mm) decrease in fat depth around the 12th rib was associated with the presence of A_9, while E_9 was associated with an increase in fat depth by 0.47 mm (Table 4.7). Variant B_9 showed a trend of association with increased fat depth in the single-variant model, which became significant in the subsequent multi-variant model.

This is an interesting observation as it appears that the associations of the B_9 and E_9 variants were strengthened in multi-variant models where both variants were factored in (i.e. HCW and V-GR), as opposed to the multi-variant models where only E_9 was factored in (i.e. leg, loin, shoulder and total yields). In the multi-variant model, B_9 and E_9 were associated with a decrease in hot carcass weight of 0.46 kg (P = 0.001) and 0.48 kg (P = 0.003) respectively, compared to the single-variant model where the difference was 0.39 kg (P = 0.002) and 0.35 kg (P = 0.009) respectively (Table 4.7).

Table 4.8 summarises results from association studies assessing the effect of individual nucleotide substitutions on ovine growth traits in the Romney sheep breed.

Table 4.8 Association of genotypes at variant loci of *IGF1R* with carcass traits in NZ Romney sheep

Variant Loci	Genotypes	n	HCW[1] (kg)	V-GR[2] (mm)	Leg yield (%)	Loin yield (%)	Shoulder yield (%)	Total yield (%)
c.2019C>T (p.Tyr673Tyr)	CC	231	17.39 ± 0.12^{a}	3.97 ± 0.16	21.48 ± 0.08	14.57 ± 0.06	16.85 ± 0.06^{a}	52.90 ± 0.16^{a}
	CT	335	17.01 ± 0.09^{ab}	4.16 ± 0.13	21.57 ± 0.07	14.62 ± 0.05	16.92 ± 0.05^{ab}	53.11 ± 0.14^{b}
	TT	117	16.96 ± 0.14^{b}	4.47 ± 0.21	21.35 ± 0.11	14.46 ± 0.07	16.65 ± 0.08^{b}	52.46 ± 0.22^{b}
	P-value		**0.004**	0.145	0.178	0.154	**0.006**	**0.026**
c.2037C>T (p.Asp679Asp)	CC	231	17.39 ± 0.12^{a}	3.97 ± 0.16	21.48 ± 0.08	14.57 ± 0.06	16.85 ± 0.06^{a}	52.90 ± 0.16^{a}
	CT	335	17.01 ± 0.09^{ab}	4.16 ± 0.13	21.57 ± 0.07	14.62 ± 0.05	16.92 ± 0.05^{ab}	53.11 ± 0.14^{b}
	TT	117	16.96 ± 0.14^{b}	4.47 ± 0.21	21.35 ± 0.11	14.46 ± 0.07	16.65 ± 0.08^{b}	52.46 ± 0.22^{b}
	P-value		**0.004**	0.145	0.178	0.154	**0.006**	**0.026**
c.2133G>A (p.Ala711Ala)	AA	136	17.69 ± 0.14	3.18 ± 0.20^{a}	21.73 ± 0.10^{a}	14.72 ± 0.07^{a}	17.06 ± 0.07	53.51 ± 0.21
	AG	355	17.12 ± 0.08	4.34 ± 0.13^{b}	21.50 ± 0.06^{ab}	14.59 ± 0.04^{ab}	16.88 ± 0.05	52.97 ± 0.13
	GG	192	16.74 ± 0.11	4.44 ± 0.17^{b}	21.35 ± 0.09^{b}	14.45 ± 0.06^{b}	16.66 ± 0.06	52.46 ± 0.18
	P-value		**<0.001**	**<0.001**	**0.012**	**0.011**	**<0.001**	**<0.001**
c.2201+28A>C	AA	533	17.20 ± 0.07^{a}	4.05 ± 0.11^{a}	21.55 ± 0.06^{a}	14.60 ± 0.04^{a}	16.89 ± 0.04^{a}	53.05 ± 0.11^{a}
	AC	130	16.97 ± 0.13^{ab}	4.28 ± 0.20^{a}	21.44 ± 0.10^{a}	14.57 ± 0.07^{a}	16.77 ± 0.07^{ab}	52.78 ± 0.20^{a}
	CC	20	15.98 ± 0.33^{b}	6.32 ± 0.50^{b}	20.51 ± 0.25^{b}	13.85 ± 0.17^{b}	16.10 ± 0.18^{b}	50.45 ± 0.51^{b}
	P-value		**0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
c.2201+31A>T	AA	231	17.39 ± 0.12^{a}	3.97 ± 0.16	21.48 ± 0.08	14.57 ± 0.06	16.85 ± 0.06^{a}	52.90 ± 0.16^{a}
	AT	335	17.01 ± 0.09^{ab}	4.16 ± 0.13	21.57 ± 0.07	14.62 ± 0.05	16.92 ± 0.05^{ab}	53.11 ± 0.14^{b}
	TT	117	16.96 ± 0.14^{b}	4.47 ± 0.21	21.35 ± 0.11	14.46 ± 0.07	16.65 ± 0.08^{b}	52.46 ± 0.22^{b}
	P-value		**0.004**	0.145	0.178	0.154	**0.006**	**0.026**

c.2201+82T>C	CC	136	17.69 ± 0.14	3.18 ± 0.20[a]	21.73 ± 0.10[a]	14.72 ± 0.07[a]	17.06 ± 0.07	53.51 ± 0.21
	CT	355	17.12 ± 0.08	4.34 ± 0.13[b]	21.50 ± 0.06[ab]	14.59 ± 0.04[ab]	16.88 ± 0.05	52.97 ± 0.13
	TT	192	16.74 ± 0.11	4.44 ± 0.17[b]	21.35 ± 0.09[b]	14.45 ± 0.06[b]	16.66 ± 0.06	52.46 ± 0.18
	P-value		**<0.001**	**<0.001**	**0.012**	**0.011**	**<0.001**	**<0.001**
c.2201+114_118 delCGCAG	CGCAG/CGCAG	136	17.69 ± 0.14	3.18 ± 0.20[a]	21.73 ± 0.10[a]	14.72 ± 0.07[a]	17.06 ± 0.07	53.51 ± 0.21
	CGCAG/del	355	17.12 ± 0.08	4.34 ± 0.13[b]	21.50 ± 0.06[ab]	14.59 ± 0.04[ab]	16.88 ± 0.05	52.97 ± 0.13
	del/del	192	16.74 ± 0.11	4.44 ± 0.17[b]	21.35 ± 0.09[b]	14.45 ± 0.06[b]	16.66 ± 0.06	52.46 ± 0.18
	P-value		**<0.001**	**<0.001**	**0.012**	**0.011**	**<0.001**	**<0.001**

[a, b] Mean values within the same column group with different superscripts differ at $P < 0.05$

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

At the level of nucleotide substitutions, variants with similar genotype frequencies unsurprisingly exhibited similar associations with carcass traits, as the nucleotides were linked in the contiguous variant sequence. The common *CC*, *CC*, and *AA* homozygous genotypes of c.2019C>T, c.2037C>T, and c.2201+31A>T respectively, were associated with a 0.43 kg increase in hot carcass weight, as well as an increase in shoulder (1.1%) and total (0.8%) lean meat yields, when compared to the less abundant *TT* genotype (Table 4.8). No other significant associations were observed for the remaining carcass traits, although a trend of association with increased fat depth and decreased leg and loin yields were observed for the *TT* genotype.

At loci c.2201+114_118delCGCAG, the major *del/del* homozygous genotype was associated with a 0.95 kg decrease in hot carcass weight, a 1.26 mm decrease in fat depth, and decrease in lean meat yields in the leg (1.7%), loin (1.8%) and shoulder (2.3%) regions, and total yield (2%), when compared to minor *CGCAG/CGCAG* genotype. The same pattern was noted for major and minor genotypes of c.2133G>A and c.2201+82T>C (Table 4.8).

When compared to the *AA* genotype, the *CC* genotype of c.2201+28A>C, which is unique to the E_9 variant, was associated with decreased hot carcass weight (1.22 kg), leg (4.8%), loin (5.1%), shoulder (4.7%) and total (4.9%) lean meat yields, and an increase in fat depth by 2.27 mm, the largest difference observed between the nucleotide genotypes.

4.4 Discussion

Increasing consumer demand for quality products, prompts transformation in the meat industry. Interest is, therefore, being directed towards the identification of genetic markers that could improve animal breeding and meat production. IGF1R is a principal participant in crucial physiological processes, such as growth and metabolism in sheep.

This is the first report of associations between variation in sheep growth and carcass traits and sequence variation in ovine *IGF1R*, for a fragment spanning part of exon 9, all of intron 9, all of exon 10, and part of the intron 10 regions. Nucleotide sequencing revealed five variants and ten novel sequence variations in this region. The A_9B_9 genotype was the most frequently occurring across the NZ sheep breeds examined. Interestingly, the highest frequencies of the B_9E_9 genotype were found in the Corriedale and Merino breeds, thus suggesting a possible benefit to wool production. Observed only in the Perendale breed,

the A_9D_9 genotype could be advantageous toward other desired traits of the breed, such as better foraging abilities and adaptability for hilly landscapes.

The presence of the A_9 variant was associated with faster-growing lambs that weighed more at tailing, weaning and slaughter; while lambs possessing the B_9 and E_9 variants had lower tailing and weaning weights, and also grew slower than their counterparts that did not have these variants. This is perhaps unsurprising, given that regulating growth is the primary function of *IGF1R* (Clemmons, 2009).

The evolutionary conservation of mammalian *IGF1R* has been established in many studies. This notion is supported by findings from this study, as only four of the ten nucleotide variations detected were located within the coding region (exon 10 specifically), none of which resulted in amino acid changes. In addition, there were no nucleotide variations detected in the promoter and exon 15 regions of ovine *IGF1R*. This is consistent with the findings of Byun et al. (2012), who reported two nucleotide substitutions within an intron 2/exon 3 fragment of ovine *IGF1R*, both of which did not result in amino acid changes.

Through ligand binding (of IGF1, IGF2 or insulin), IGF1R signalling promotes cell proliferation and differentiation, inhibits apoptosis and ensures skeletal muscle maintenance (Florini et al., 1996; Girnita et al., 2014). In this context, *IGF1R* has been presumed to be a 'house-keeping' gene, and given the near-ubiquitous presence of the protein in cells and tissues, it would seem logical that the receptor would be subject of functional constraint. Changes in the primary amino acid sequence may lead to dysfunctionality, and hence any gene variation underpinning this would be selected against. Several reports in humans have demonstrated the grave clinical consequences of non-synonymous mutations within *IGF1R*, including growth retardation, poor brain and neuronal development, and metabolic disorders (Kawashima et al., 2005; Klammt et al., 2011).

The long-standing misconception that intronic and synonymous genetic variations that do not alter the amino acid sequence or reading frame of a protein are inconsequential or silent, has been debunked by various studies. The functional importance of introns is underscored by compelling evidence that genes containing introns are transcribed 10-100 fold more efficiently than their intronless counterparts (Brinster et al., 1988; Le Hir et al., 2003). Both synonymous and intronic variations can impact the rate and efficiency of transcription and translation, which in turn can affect protein folding, functionality and quantity (Barrett et al., 2012). The creation of rare codons by synonymous variations, for

which there are less available tRNAs, can also slow down translation (Sauna and Kimchi-Sarfaty, 2011). Synonymous and non-coding variations may also have an impact by being linked to other potent variations elsewhere within the coding sequence or regulatory regions of a gene.

Four synonymous substitutions, located in the exon 10 of ovine *IGF1R*, were reported in this study: c.2019C>T (p.Tyr673Tyr), c.2037C>T (p.Asp679Asp), c.2052G>A (p.Thr684Thr), and c.2133G>A (p.Ala711Ala), and they all showed significant association with growth and carcass traits in sheep. The *AA* genotype of c.2052G>A (p.Thr684Thr) was associated with lower fat thickness, and increased carcass weight and lean meat yield. The *CC* genotypes of c.2019C>T (p.Tyr673Tyr) and c.2037C>T (p.Asp679Asp) showed association with higher weaning weight and average daily weight gain. This is consistent with findings by (Szewczuk et al., 2013), where the *GG* genotype of a synonymous variation in the exon 12 of bovine *IGF1R* was associated with higher weaning weight in Angus beef cattle.

In order for translation to occur, the transcribed mRNA must fold into a stable secondary structure – a process that can be hindered by non-coding nucleotide sequence variations. Irregular pre-mRNA splicing and relocation of the splice site may lead to the formation of a dysfunctional protein (Cartegni et al., 2002). Non-coding variations can also impair the cytosolic export and degradation of mRNA (Sauna and Kimchi-Sarfaty, 2011). Overall, it appears that because introns play such a crucial role in gene regulation, then the more complex an organism is, the higher the presence of non-coding DNA regions that can produce RNAs, as opposed to protein-coding regions (Barrett et al., 2012).

The functional importance of introns was suggested by results of this study, as all four intron sequence variants were found to show association with growth and carcass traits in sheep. Most notably were the c.2201+28A>C and c.2201+114_118delCGCAG variants found in the intron 10 of *IGF1R*. The c.2201+114_118delCGCAG variant may account for the observed decrease in weaning and slaughter weights and growth rate with the B_9 and E_9 variants, as both variants have the deletion. A_9, on the other hand, possessed the *CGCAG* sequence. The presence of the homozygous '*deletion'* genotype was found to be associated with an 8.7% decrease in the average daily gain of lambs. The presence of the *del/del* genotype in 28% of Romney lambs suggests that although this genotype is not ideal for lamb growth, it might be useful for other commercially desirable traits such as increased fertility, cold tolerance or immunity.

The *C* nucleotide of c.2201+28A>C was unique to variant E_9, and thus it might account for the decrease in the leg, loin and total lean meat yields. Lambs possessing the *CC* genotype (in the form of E_9E_9), had the lowest total lean meat yield, and an increase in intermuscular fat when compared to other genotypes. This might explain the rarity of the E_9E_9 genotype, with it only being observed in three sheep breeds, and in no more than 5% of the population. In contrast, lambs possessing the A_9 variant had leaner high-yielding carcasses, with less intermuscular fat. They also had a higher average daily gain. The finding that the A_9 variant was the most common, suggests that it confers some advantage to the lambs.

These findings are consistent with those of Proskura and Szewczuk (2014), where a g.195C>T transition in the intron 12 of *IGF1R* was found to be associated with body weight variation and average daily gain in Pomeranian coarse wool sheep. Similar studies confirming the effect of *IGF1R* variations on growth and performance traits of livestock have also been reported in cattle (Pereira et al., 2003; Szewczuk et al., 2013), buffalos (El-Magd et al., 2013), pigs (Wang et al., 2006) and chickens (Lei et al., 2008).

The conserved cleavage site that yields the α and β subunits of the mature *IGF1R* protein is encoded by exons 9 and 10 (Adams et al., 2000), where the nucleotide sequence variations reported in this study were located. Also encoded by exons 9 and 10 of IGF1R is the third repeat of the fibronectin type III (FnIII) domain – the largest and most common fibronectin subdomain. In IGF1R, the FnIII domain plays a crucial role in dimerisation, as cysteine residues within the domain form the disulphide bonds that connect the α and β subunits (Molina et al., 2000; Girnita et al., 2014). In addition to dimer cross-linking, the FnIII domain facilitates protein-protein interactions and ligand binding, and act as spacers that ensure the correct positioning and orientation of functionally important regions of IGF1R (Campbell and Spitzfaden, 1994; Ward et al., 2001). Therefore, alterations to this domain might not only impact the FnIII structure and function, but also engender flow-on effects to other regions of IGF1R. Potential changes to the α-β cleavage site and the linking disulphide bridges by the variations reported in this study, could result in either enhanced or impaired IGF-IGF1R signalling, thus ultimately promoting or inhibiting growth. This might explain why some of the detected nucleotide variants improved growth and muscle development, while others were inhibitory.

The myostatin gene (*MSTN*), a negative regulator of muscle growth, is a notable example of how non-coding gene sequence variations can have significant effects on livestock production traits. In sheep, a c.*1232 G>A substitution in the 3'untranslated region of

MSTN was found to create a new microRNA target sites, which in turn inhibited *MSTN* translation and ultimately led to hypertrophy and increased muscularity in sheep (Clop et al., 2006).

In key mammalian genes, changes in the amino acid sequence might, however, be selected against as they could destroy or majorly impact protein structure and functionality, and thus phenotype. This reduces the likelihood of passing the underlying coding sequence variation on to offspring. In comparison, synonymous and intronic variations that do not alter the amino acid sequence, such as those reported in this study, may be better tolerated in otherwise conserved genes, as their effects are subtler.

Although these non-coding sequence variations are likely to only have a minor effect on protein functionality, they could still lead to phenotypic differences that can be passed on to future generations, since they are subject to less selection pressure. This is crucial if the variations are intended for use as markers in the genetic selection of sheep, since these minor effects could accumulate over time, leading to genetic gain.

The average pay-out scheme for farmers and producers in NZ is $6 per kg of lamb carcass. The A_9 variant of ovine *IGF1R* was associated with a 0.53 kg increase in hot carcass weight, which would putatively generate an extra $3 per carcass. The total lamb exports for NZ over the 2018 – 2019 season is estimated at about 27 million carcasses, generating an estimated $3.23 billion of revenue (Ministry of Primary Industries, 2019). This means that if the A_9 variant were incorporated into all flock, assuming all lambs responded in a similar fashion to this study, the small genetic gain could translate to an $81 million increase in sheep meat revenue.

4.5 Conclusion

IGF1R is a key regulator of growth and skeletal muscle function in mammals, however the encoding gene has not been studied in great detail in sheep. IGF1R is capable of eliciting diverse effects, which is supported by this study where sequence variation in the gene was shown to be associated with several sheep production traits. The finding of associations between ovine *IGF1R* variation and growth and carcass traits, may be useful for the genetic improvement of sheep.

Chapter 5
Variation in ovine *GH* and its association with growth and carcass traits in NZ sheep

5.1 Introduction

Animal growth is a complex physiological process involving a wide array of hormones and the intricate interplay of signalling pathways. Of the hormones, growth hormone (GH) or somatotropin, is a 191-amino acid long peptide hormone secreted by the anterior pituitary gland, which plays a key role in the control of animal growth and metabolism (Kannian and Ryan, 2019). It stimulates the growth and development of essentially all body tissues, as well as regulates metabolism and body composition. Growth hormone exerts its effects directly on target cells by promoting transcription and protein synthesis, intracellular amino acid transport, and lipolysis, while also inhibiting glucose mobilisation in to cells (Waters and Brooks, 2015).

The GH effect can be categorised into two groups, the direct and indirect effects. The direct effect of GH is mediated through GH receptors (GHRs), which are present on the membrane of target cells (Argetsinger et al., 1993). The indirect effect utilises an intermediary called insulin-like growth factor 1 (IGF1), to effect GH action. The complex molecular mechanisms underlying GH action have challenged scientists for decades, but the development of cDNA cloning methods and advances in biotechnology have provided better insight into these complex processes.

5.1.1 Growth hormone function

The discovery of GH and investigation of its function started with research into the role of the pituitary gland in the early 20th century. For example, rats injected with bovine pituitary extracts exhibited accelerated body growth (Dott and Fraser, 1923; Evans and Long, 1924; Putnam et al., 1929). Subsequently, experiments aimed at purifying the pituitary factor responsible for this increased somatic growth earned it the *apropos* name of somatotropin or growth hormone (Lindahl et al., 1987). The function of GH in growth and development was further established when the restoration of somatic and bone growth was observed in rats and dogs with surgically removed pituitary glands, but that had been treated with GH (Dott and Fraser, 1923; Putnam et al., 1929; Isaksson et al., 1985).

In spite of the progress being made, research into GH was challenged by the disparity observed between its *in vitro* and *in vivo* effects. The accelerated somatic growth, bone formation and myogenesis observed with the *in vivo* administration of GH to *GH*-null mice, were not replicated *in vitro* in muscle and cartilage cell cultures, where the effects seemed to be more subtle and inconsistent (Isaksson et al., 1985; Waters, 2016). This finding led to the discovery of other circulating GH-dependent peptides in the mid-1900s; these being termed the somatomedins, or insulin-like growth factors (IGFs). They are known to mediate the biological effects of GH *in vivo*. Subsequent experiments, in which a combination of GH and somatomedins were administered *in vitro*, led to an increase in growth-associated parameters as might be expected *in vivo*, indicating that the somatomedins were crucial to GH function (Fryklund et al., 1974; Holder et al., 1981). It is now accepted that the two major plasma factors secreted by the liver upon GH stimulation are IGF1 and IGF2.

As seen in Figure 5.1, GH is pleiotropic in nature, exerting multiple effects while being regulated by two other hormones: growth hormone releasing hormone (GHRH), the positive regulator, and somatostatin (SST), the negative regulator.

Growth hormone is unique in that it is one of the few hormones capable of inducing somatic growth in a dose-dependent manner, as well as promoting both insulin-like and insulin-antagonistic actions, two diametrically opposed phenomena (Barta et al., 1981; Isaksson et al., 1985) (Figure 5.1). Various scientific experiments have established the stimulatory effects of GH on cells and tissues, including connective, lymphocyte, adipose and muscle tissues (Lesniak et al., 1974; Isaksson et al., 1985). Growth hormone secretion from the anterior pituitary gland is also pulsatile, and dependent upon biological maturity, exercise, nutrition, health status, gender, stress, and the stage of growth of the organism.

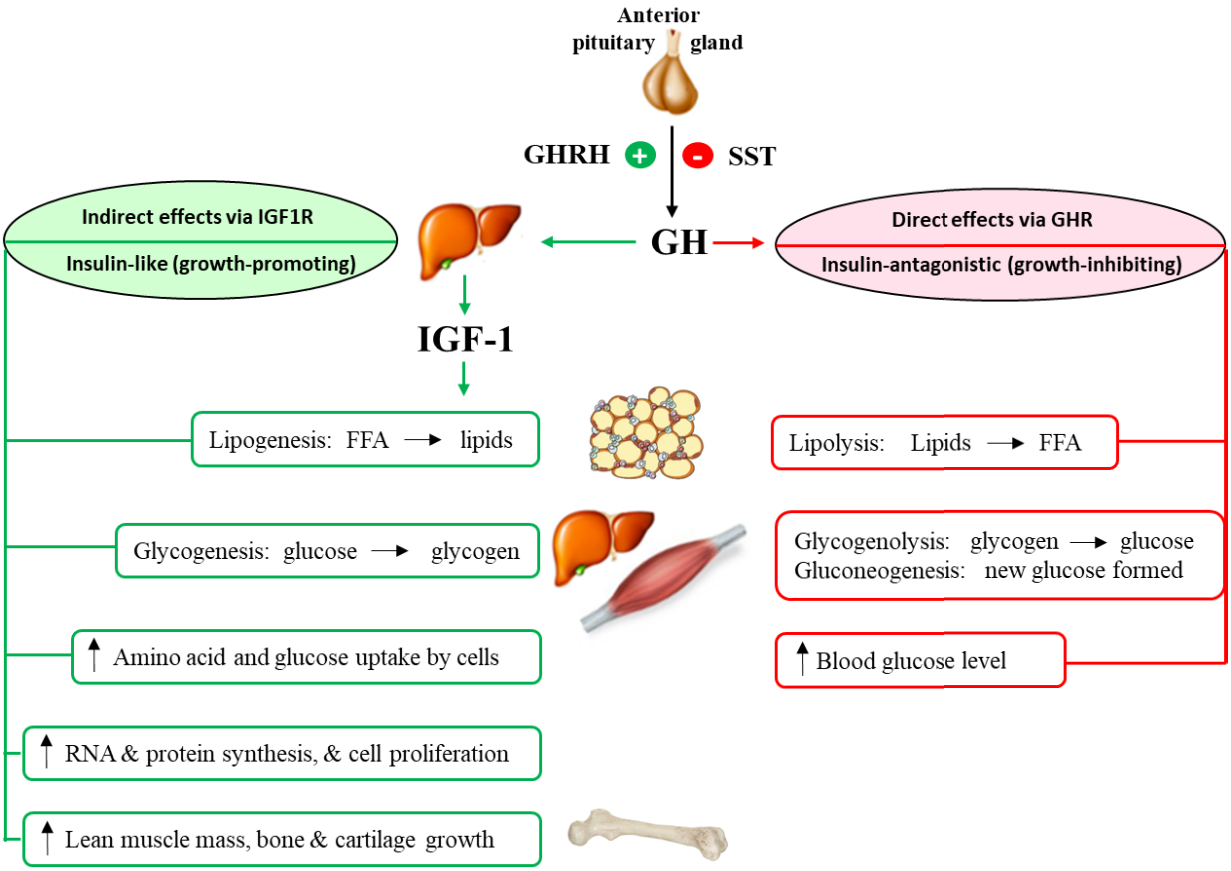


Figure 5.1 Diagrammatic outline of GH mechanism of action

Growth hormone can exert its effects directly or indirectly. The direct effects on adipose tissue include the increased breakdown of stored fat and subsequent release of free fatty acids (FFAs). GH triggers the liver to release IGF1, which in turn has indirect effects by stimulating lipid build-up from FFAs, and protein synthesis and growth in bone, cartilage, muscle and most other body organs. It also stimulates cellular proliferation and the uptake of amino acids in other tissues. GH secretion from the anterior pituitary gland is stimulated by growth hormone-releasing hormone (GHRH), and inhibited by somatostatin (SST).

Increased amino acid and glucose uptake by cells, lipogenesis (free fatty acid storage by conversion to lipids), glycogenesis (conversion of glucose to glycogen), a higher rate of RNA and protein synthesis, decreased protein degradation and enhanced muscle and cartilage growth, are some of the growth-enhancing effects of GH (Waters, 2016; Bergan-Roller and Sheridan, 2018). In contrast, the insulin-antagonistic effect involves lipolysis (lipid breakdown to produce free fatty acid), gluconeogenesis (production of new glucose molecules), glycogenolysis (the breakdown of glycogen to glucose), and hyper-

insulinaemia (Kopchick and Andry, 2000) (Figure 5.1). This insulin-counteracting effect of GH is thought to increase blood glucose levels in the event of hypoglycaemia, and occurs after the insulin-like effects are elicited.

The GH-IGF signalling pathway or axis, plays a crucial part in the regulation of growth and the differentiation of the skeletal muscle. It reportedly mediates 50-70% of the effects of GH, as compared to the 30-50% brought about by direct stimulation via GHR (Rosenfeld, 2003; Rosenfeld, 2006). IGF1 stimulates the proliferation and differentiation of myoblasts and chondrocytes. It also promotes cell survival and inhibits apoptosis.

In addition to somatic growth, GH regulates the metabolism of lipids, nitrogen, minerals, carbohydrates and proteins, liver detoxification via cytochrome P450 enzymes, cognitive and neuronal development, and synaptogenesis (Nyberg and Hallberg, 2013; Waters, 2016). Cell-line studies have also demonstrated that GH can stimulate the differentiation of pre-adipose 3T3 cells into mature adipocytes, and chondrocyte proliferation without the help of an intermediator (Morikawa et al., 1982; Kopchick and Andry, 2000). Growth hormone is also critical for prenatal and postnatal immune system development, modulation and maintenance, including the development of the thymus and T-cell selection (Chen et al., 1998). The B-cells, T-cells and other lymphoid cells, have been shown to express a high-affinity GHR.

Within cells of the immune system, GH is also reported to regulate the expression of cytokines and proto-oncogenes that are crucial for the proliferation and expansion of cells, such as c-fos and c-jun (Piechaczyk and Blanchard, 1994; Chen et al., 1998). This is possibly explained by the marked similarity between GHR, a type 1 cytokine receptor, and other cytokine receptors in the lymphopoietic system. Stress responses, neuropeptide activity, behaviour and emotion are some of the neuroendocrine actions modulated by GH effects in the amygdaloid and hypothalamic regions of the brain (Yoshizato et al., 1998; Kopchick and Andry, 2000).

Given its importance in so many biological systems, it is unsurprising that multiple mechanisms exist to modulate GH activity. The synthesis and secretion of GH by pituitary somatotropes is positively regulated by the hypothalamic hormone, GH releasing hormone (GHRH), and the enteric hormone, ghrelin; while GH release is inhibited by somatostatin. GHRH binding to its receptor on the surface of somatotropes causes a surge in cAMP (cyclic adenosine monophosphate) levels, triggering the transcription factor, POU1F1, which in turn stimulates GH expression and release (Kopchick and Andry, 2000).

As with IGF1 and the IGFBPs, the bioavailability of GH and binding to its receptor is modulated by GH binding proteins (GHBPs). More than half of the plasma GH has been revealed to be bound to GHBP (Baumann, 1991). Considering the pulsatile nature of GH secretion, GHBPs are effective at storing GH during secretion spikes, and releasing them into the circulation when the levels are low, thus ensuring constant availability of circulating GH. The GHBPs form a complex with circulating GH preventing degradation by plasma proteases and prolonging the half-life of GH. The inhibitory actions of GHBPs can involve the sequestration of GH, preventing GH binding to its receptor, and the competitive binding of GHBP to GHR (Baumann, 1991; Kopchick and Andry, 2000). GHBPs are found in many tissues, including adipose, hepatic and muscle tissues.

5.1.2 Growth hormone signalling

The GHR is a transmembrane cytokine receptor induced by ligand-dependent dimerisation. Upon binding to two molecules of GHR, GH brings about dimerisation and the subsequent activation of the tyrosine kinase called Janus Kinase 2 (JAK2) (Argetsinger et al., 1993; Carter-Su et al., 1996) (Figure 5.2). The activated JAK2 in turn phosphorylates tyrosine residues within itself, and those present in the cytoplasmic domain of the associated GHR (Carter-Su et al., 2000). These phosphorylated tyrosine residues form high-affinity docking sites for a variety of signalling molecules, such as signal transducers and activators of transcription (STAT), and the adapter protein, SH2-containing collagen-related proteins (SHC), and the insulin substrates (IRS) 1 and 2 (Campbell, 1997).

The STAT family of transcription factors (STAT 1, 3, 5a and 5b) are translocated to the nucleus where they stimulate the expression of the GH-dependent genes. Of particular significance is the stimulation of STAT-5b, which binds to specific recognition sequences to bring about the transcription of IGF1 (Chia, 2014). While IGF1 expression and secretion occur in various tissues, the liver is the major site of IGF1 synthesis.

The SHC proteins activate the Ras-Raf-MEK-MAPK and ERK pathways, while IRS initiates the phosphatidyl inositol 3-kinase (PI3K)-Akt pathways (Argetsinger and Carter-Su, 1996). These events initiate a downstream signalling cascade that leads to the release of various secondary messengers, and the ultimate regulation of cellular functions such as gene transcription, enzymatic action and metabolite transport (Campbell, 1997) (Figure 5.2). These effectively mediate the function of GH in the regulation of body growth and cell metabolism. This type of signalling is observed in adipocytes, where direct binding of

GH to its receptor stimulates the breakdown of fat cells, while inhibiting the uptake of free fatty acids.

Figure 5.2 Diagrammatic representation of growth hormone (GH) direct signalling [Adapted from Rosenfeld (2006)]

The binding of GH to two GHRs, brings about their subsequent dimerisation, which leads to the activation of GHR-associated JAK2 (Brooks et al., 2014). The activated JAK2 brings about the phosphorylation of tyrosine residues in both itself and GHR, leading to the recruitment and activation of various signalling intermediates such as STAT, SHC and IRS, and subsequently secondary messengers such as MAPK, PI3K, diacylglycerol kinase, and protein kinase C (Carter-Su et al., 1996). The IGF1 factor is a key transcriptional target of STAT signalling. The signalling cascade eventually brings about GH-induced growth and metabolism regulation in a number of ways, including changes in enzymatic activity, cellular transport and gene expression (Campbell, 1997).

Conventional views have long held that the GH-GHR signalling, which mediates the direct effects of GH, is confined solely to the plasma membrane. However, emerging evidence suggests that the GH-GHR complex is capable of nuclear translocation, where it can exert other biological functions. For example, Hainan et al. (2018) demonstrated the nuclear translocation of GH-GHR complexes in porcine liver cells. After GH ligand-binding and receptor activation is completed, the GH-GHR complex is internalised via either of two major pathways: clathrin-mediated endocytosis or caveolin-mediated endocytosis (Lobie et al., 1994a; van Kerkhof et al., 2001). The conventional view is that the internalised complex is marked for one of two fates: proteolytic degradation within the lysosomes, or targeted recycling back to the cell membrane to await subsequent signalling cycles. Recent studies have however led to an understanding that the internalised GH-GHR complex can still transmit signals from within whatever organelle that localisation has occurred, including the mitochondria, Golgi apparatus, endoplasmic reticulum, and even the cytoplasm (Lobie et al., 1994b; van Kerkhof et al., 2001; Hai-nan et al., 2019).

The localisation of GH-GHR complexes in the nucleus has been described. Endosomal vesicles carrying the ligand-receptor complex are believed to dock on and fuse with the nuclear membrane, where they empty their contents into the nucleoplasm. *In vivo* and *in vitro* studies with rat liver models, have demonstrated that the localisation of GHR within the nucleus, can result in accelerated cell proliferation via the STAT and P13K/Akt pathways, and that this may ultimately progress to cancer formation (Conway-Campbell et al., 2007; Hainan et al., 2018). The intracellular nuclear signalling of the GH-GHR complex is also reported to result in growth promoting effects, including increased gene expression, protein synthesis and carbohydrate metabolism (Lobie et al., 1994b; Hainan et al., 2018)

The ubiquitous distribution of GHR in many peripheral tissues including muscle, lymphopoietic tissues, liver, adipose tissue and bone, is testament to the diverse functions of growth hormone (Waters, 2016).

5.1.3 Growth hormone structure

Growth hormone is made up of 191 amino acids, encoded by a gene with five exons and four introns (Marques et al., 2006). The resulting GH polypeptide is arranged into four helices aligned in an up-up-down-down pattern that make up approximately half of the GH secondary structure. These helices are connected by non-helical loop regions (Kopchick and Andry, 2000). Hydrophobic residues within the GH core are responsible for

maintaining the stability of the four helices and for maintaining the secondary structure (Kopchick and Andry, 2000).

Located around the 5' UTR or promoter regions of the gene, there are regulatory sequences including TATAAA, at the -30 position, CATAAAT at -84, and a 41 bp sequence at -142, which is conserved in mammals (Byrne et al., 1987). The polyadenylation signal AATAAA is located at nucleotide 2734, along with a second polyadenylation site present at nucleotide 2758 (Byrne et al., 1987). The regions after the 5' UTR encoding the GH N-terminal (residues 1-44) have been associated with the modulation of carbohydrate metabolism.

The anti-insulin-like or diabetogenic function of GH appears to be encoded by exon 3. Experiments with human GH variants, have demonstrated that variants where part of exon 3 (amino acids 32-46) are deleted, do not exert insulin antagonistic actions, but are growth-enhancing; while variants possessing exon 3 amino acids (32-70) are anti-insulin-like in action, and do not promote growth (Lewis et al., 1980; Barta et al., 1981). Disulphide bridges between Cys53 and Cys164 in exon 3 and exon 5 respectively, and Cys181 and Cys189 (both in exon 5) of *GH* are crucial to the structure of the mature peptide and its growth-enhancing function (Lewis et al., 1980; Barta et al., 1981; Kopchick and Andry, 2000). These four cysteine residues, although in slightly different locations, are conserved across many species (Kopchick and Andry, 2000).

Human GH exists in multiple protein isoforms, the two most common of which are 22 kDa and 20 kDa in size that are brought about by the alternative splicing of a common mRNA. The 22 kDa isoform is more abundant in circulation and is 191 residues in length. Removal of amino acids 32-46 yields the 20 kDa isoform of length 176 residues (Baumann, 1991; Hai-nan et al., 2019). Potency-wise, both isoforms are mostly equivalent in how they exert biological functions such as growth-promoting effects, and both bind with similar affinities to GHR (Wada et al., 1998). However, studies in rats revealed that they differ in their effects on water retention and diabetogenic properties, with the 22 kDa having greater diabetogenic effects (Satozawa et al., 2000; Takahashi et al., 2001). This comes as no surprise, since the 20 kDa isoform lacks a significant portion of the exon 3 region that encodes amino acids creating the anti-insulin-like properties.

5.1.4 Growth hormone gene duplication

Growth hormone is considered to be part of a family of proteins (Kannian and Ryan, 2019). The family consists of three related proteins, namely GH, prolactin (PRL), and a placental hormone called placental lactogen (PL) (Barta et al., 1981; De Vos et al., 1992). All three are endocrine hormones, and they share several common features including having analogous protein structures and sizes (190-199 amino acids), being produced by genes that contain four introns, with exon-intron boundaries that occur in similar locations. The hormones can affect growth and lactogenesis, and each has an important tryptophan residue between amino acids 85-95 (Barta et al., 1981; Miller and Eberhardt, 1983). This similarity has led to the belief that the hormones in the GH family share a common ancestral precursor gene, which may have evolved to produce the observed genetic diversity by means of gene duplication, potentially as a consequence of unequal crossing-over events (Barta et al., 1981; Miller and Eberhardt, 1983).

The discovery of a repetitive DNA element within the nucleotide sequence of *GH* supports this theory. This pattern of genetic evolution is by no means uncommon for mammalian genes, and it has been suggested as the origin of human β-globin and human insulin gene families (Bell et al., 1980; Efstratiadis et al., 1980).

Duplication of entire genes and even genomes are not unheard of, and can arise by molecular interactions such as unequal crossing over, retroposition, and recombination (Ohta, 1987; Ohno, 1999; Gu et al., 2004). As long as a single gene locus controls a particular function in an organism and natural selection limits the accumulation of nucleotide variation (or mutation), functionality is maintained. The duplication of a gene could therefore, produce a ‘redundant’ gene locus with more freedom to accumulate sequence variation and possibly bring about phenotypic novelty. The accumulation of sequence variation could either be a deleterious mutation resulting in the failure to produce a protein, or bring about the emergence of a new and different function, or a different regulatory mechanism (Wapinski et al., 2007).

5.1.5 Ovine growth hormone gene

Ovine *GH*, which maps to the long arm of chromosome 11 (11q25), encodes a 191-amino acid polypeptide and has five exons and four introns (Orian et al., 1988) (Figure 5.3). The ovine *GH* locus differs from the cattle and human structures, but is similar to the goat in having a more complex genetic architecture (Byrne et al., 1987; Ofir and Gootwine, 1997). Cattle and human possess a single copy of the gene, whereas two copies of *GH* are observed in some goat and sheep haplotypes (Woychik et al., 1982; De Vos et al., 1992; Vacca et al., 2013). This phenomenon was described by Valinsky et al. (1990) in multiple sheep and goat breeds, as the presence of 'two alleles at the *GH* locus'. In sheep, *GH1* is a single copy gene, but what is known as *GH2* contain two genes, *GH2-N* and *GH2-Z*, separated by a 3.5 kb region (Wallis et al., 1998) (Figure 5.3).

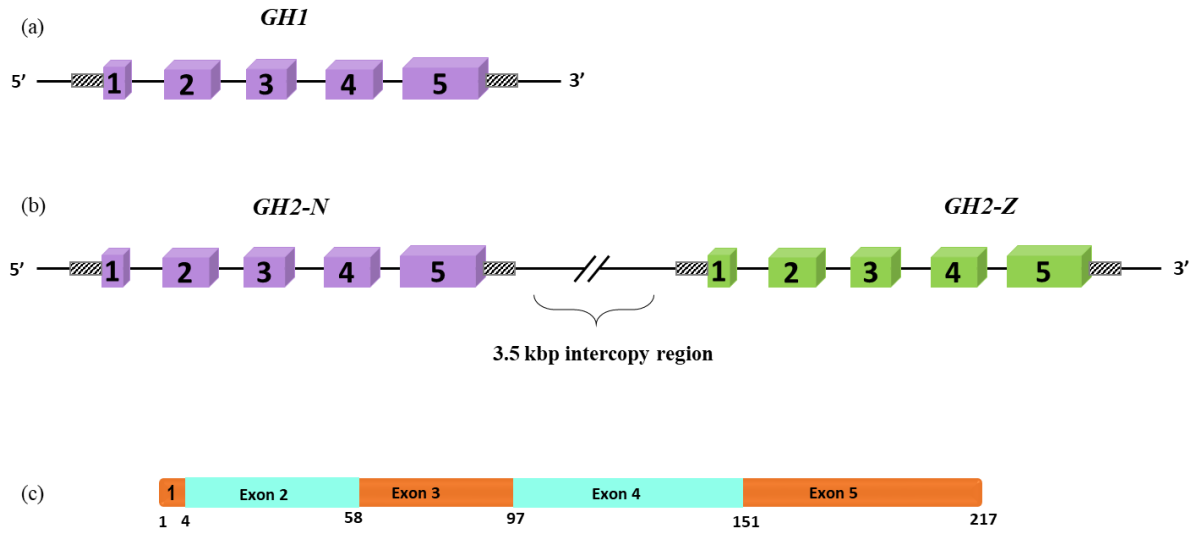


Figure 5.3 Schematic illustration of the ovine *GH* structure

The ovine GH genes are found at two locations, (a) *GH1* and (b) *GH2,* which contains two copies, *GH2-N* and *GH2-Z*, separated by a 3.5 kb region. The numbered boxes represent the exons, straight lines represent the introns, and shaded boxes represents the untranslated regions. (c) The polypeptide sequence of ovine GH showing amino acid number matched to the exons.

Despite their similarities, *GH2-N* and *GH2-Z* have been reported to exhibit certain differences, and are predominantly expressed in different organs; the pituitary gland for *GH2-N* and the placenta for the *GH2-Z* (Gootwine et al., 1996; Lacroix et al., 1996). The different ovine *GH* have sequence differences with some variations present in one and absent in the others, as well as having different receptor-binding affinities for their polypeptide products. It has been suggested that sequence divergence in duplicated genes impacts regulatory control, rather than biological function (Wapinski et al., 2007). The occurrence of these *GH* structures in sheep and goats, but not cattle, suggests their creation must have occurred in the *Caprinae* family after separation from the bovine species, but prior to the divergence of sheep and goats (Ofir and Gootwine, 1997).

5.1.6 Growth hormone gene variation

Growth hormone deficiency (GHD) is a broad term referring to conditions characterised by a low level of circulating GH. It can be an isolated event called isolated GH deficiency (IGHD), or part of a series of growth-related syndromes called combined pituitary hormone deficiencies (CPHD) (Cogan et al., 1993).

In humans, nucleotide sequence variations resulting in GH deficiency have been associated with dwarfism, congenital malformation and diabetes; while sequence variations causing excessive GH production have been linked to acromegaly and gigantism (Giustina et al., 2008; Renehan and Brennan, 2008). For example, in one study, Egyptians that presented with short stature and isolated growth hormone deficiency were found to have an *A* to *T* nucleotide substitution in intron 4 of *GH* (Esmaiel et al., 2019).

The functional consequences of *GH* sequence variation have been documented in livestock (Ge et al., 2003). Marques et al. (2006) detected 24 single nucleotide variations in *GH1/GH2-N*, and 13 single nucleotide variations (12 coding and one non-coding) in *GH2-Z*, in the Serra da Estrela sheep breed. The variations observed in *GH2-Z* were unique to that gene, whereas similar nucleotide sequence variations were reported to be present in when comparing the *GH1* and *GH2-N* genes (Marques et al., 2006). This suggest that *GH1* and *GH2-N* likely constitute a paired loci.

Vacca et al. (2013) reported five nucleotide sequence variations located in the 5' UTR and promoter regions of *GH2-Z*, and a nucleotide insertion in the 3' UTR region. These variations brought about putative changes in binding site conformation and the sequence of

enhancers and transcription factor-binding elements, which may influence the regulation of gene expression (Vacca et al., 2013).

Five variations found to be associated with body length, height and weight were reported in Chinese Tibetan sheep, including a single *T* nucleotide insertion in the 3' UTR (Jia et al., 2014). Similar associations with growth traits such as birth weight and body weight at six months and nine months of age, were reported with two ovine *GH* genotypes, *G1* and *G2*, in Iranian Baluchi sheep (Tahmoorespur et al., 2011).

The GH genes have also been found to have nucleotide sequence variability in other livestock breeds, such as goats and cattle. Gupta et al. (2007) found several variations in exon 4 of *GH* in Black Bengal goats, including an arginine to histidine substitution, an aspartic acid to valine substitution, an arginine to tryptophan substitution, and an arginine to proline substitution.

In the exon 3 of caprine *GH*, amino acid substitutions of glycine to serine, and arginine to tryptophan, caused by *C* to *T* nucleotide transition; and in exon 5, a proline to histidine substitution caused by a *C* to *A* nucleotide transversion, were thought to affect the structure and function of caprine *GH*, and revealed to be associated with growth traits in Chinese goat breeds (An et al., 2010). Variants *FF* and *AA* in exon 4 and exon 5 of caprine *GH*, were found to be associated with increased milk yield, and milk protein and fat content in goats (Malveiro et al., 2001).

In cattle, an *A* to *C* transversion in the exon 5 of bovine *GH*, and a *T* to *C* transition in intron 3 in Holstein-Friesian bulls were found to be associated with milk production traits (Yao et al., 1996). Variations in the promoter region of bovine *GH* were found to be associated with carcass weight, rib thickness and milk fat content in Japanese Black cattle (Sugita et al., 2014), and associations with milk production have also been reported in Simmental cattle, where the *BB* genotype of bovine *GH* was linked with higher milk yield and milk protein when compared to the *AA* and *AB* genotypes (Falaki et al., 1997).

5.1.7 Rationale behind study

Growth hormone was chosen for this study due to its well-founded effect on animal growth and muscling traits. It exerts interesting pleiotropic effects by regulating opposing biological phenomena such as glycogenesis/glycogenolysis, lipogenesis/lipolysis, and increased protein breakdown/decreased protein breakdown. Nucleotide variation in *GH* has been associated with productive traits in livestock. Notwithstanding the considerable research into GH effects in livestock, much remains to be revealed about nucleotide variations in the ovine *GH*, especially in NZ sheep breeds.

The region of *GH* selected to be studied spanned part of exon 2, intron 2 and part of exon 3. This region of the gene encodes amino acids that form the core of the α-helical motif that is responsible for GH binding to its receptor. Site-specific alanine mutagenesis has revealed that mutations in this region can decrease GH binding affinity by up to four-fold (Cunningham and Wells, 1989). It is therefore thought that nucleotide variation in this region might have a functional effect on sheep growth and carcass traits, and hence worthy of investigation.

5.2 Materials and Methods

5.2.1 Collection and purification of blood samples

The blood samples used in this investigation were randomly collected from 1143 lambs from ten different NZ breeds, namely Romney (n = 766), Coopworth (n = 32), Corriedale (n = 30), Dorset Down (n = 30), Merino (n = 58), Perendale (n = 62), Poll Dorset (n = 31), Suffolk (n = 30), Texel (n = 32), and White Dorper (n = 72). (See Chapter 3.2 for details on lamb growth and carcass data recording)

Although the search for variation was performed on all sheep samples, lambs lacking phenotypic data for growth and carcass traits were subsequently excluded from the statistical analyses, leaving 766 Romney lambs for which these data were available. The lambs examined in this study were obtained from 40 unrelated sire-lines belonging to the top 20% of Romney sheep in the Sheep Improvement Limited (SIL) dual-purpose index ranking.

The genomic DNA from the FTA blood samples was purified using a two-step procedure. This involved a cell and protein denaturation with 20 mM NaOH at 60 ºC heat for 30 min, and subsequent washing with 1 × TE buffer for 5 min, as outlined by Zhou et al. (2006).

5.2.2 Primer design and PCR analysis

A 560 bp fragment (primer inclusive) of ovine *GH* spanning part of exon 2, all of intron 2 and part of exon 3, was amplified using a pair of primers: forward primer was 5'-GCCCTCCTGGTCTCTCCC-3' and the reverse primer was 5'-GTCCCCTGCTCCTCGGTC-3'. Due to the unique nature of ovine *GH*, the primers designed were based on ovine *GH* nucleotide sequences obtained from the NCBI database, with accession numbers: DQ450147.1 (*GH1*), KU341778.1 (*GH2*), AH005493.2 (*GH2-N*) and DQ461615.1 (*GH2-Z*). BLAST comparisons between these database sequences revealed high homology across the different *GH* loci. The primers used in this study were then designed using the DNAMAN software version 5.2.10 (Lynnon BioSoft, Vaudreuil, Canada).

The ideal PCR and SSCP conditions for each primer set were established empirically following multiple optimisation experiments. For a start, PCR optimisations entailed low stringency PCR with standard temperatures and reagent concentrations. The annealing temperature was subsequently raised in stepwise increments of 1 ºC until a single band was observed on agarose gel electrophoresis. This was done to curb non-specific amplification. Similar increments in a stepwise fashion were employed to the reagents used until the best results were obtained.

PCR amplifications were performed in 15-µL reactions containing the purified genomic DNA on a 1.2 mm diameter disc of FTA, 0.25 µM of each primer, 150 µM of each dNTP (Eppendorf, Hamburg, Germany), 1.5 mM Mg^{2+}, 0.5 U *Taq* DNA polymerase (Qiagen, Hilden, Germany), and 1 × the reaction buffer supplied with the enzyme. Amplifications were undertaken in Bio-Rad S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA). The thermal profile entailed an initial denaturation at 94 ºC for 2 mins, followed by 39 cycles of 94 ºC for 30 s, annealing at 62 ºC for 50 s, and extension at 72 ºC for 40 S, and a final extension step at 72 ºC for 5 min.

Subsequent visualisation of the amplicons from the PCR reaction were carried out by means of agarose gel electrophoresis using 1% agarose (Quantum Scientific, Queensland, Australia) gels containing 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM

Na_2EDTA), 200 ng/mL ethidium bromide. 2 µL aliquots of the PCR products were mixed with 2 µL of loading dye (0.2% bromophenol blue, 0.2% xylene cyanol, 40% (w/v) sucrose), and loaded into the gels that were run at a constant 5 V/cm for 2 hours. After electrophoresis, the gels were visualised using ultraviolet transillumination at 254 nm.

5.2.3 Single-stranded conformational polymorphism (SSCP) analyses

Optimisations for SSCP involved running amplicons at various conditions and using different gel compositions until consistently clear banding patterns were obtained.

Following PCR, 0.7 µL of each PCR product was mixed with 7 µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). The samples were denatured at 105 ºC for 5 min, snap-chilled on ice, and 8 µL of each sample was loaded into 16 x 18 cm, 10% acrylamide:bisacrylamide (37.5:1, Bio-Rad) gels in Protean II xi cells (Bio-Rad). The samples were run at a voltage of 320 V at 20 ºC for 18 hours. Silver-staining of the gels was performed according to the method described by Byun et al. (2009). Briefly, the gels were silver-stained with silver nitrate, washed with distilled water, and subsequently developed using a solution containing sodium hydroxide and 40% formaldehyde.

In order to ensure the integrity of the experiments and to obtain reproducible banding patterns, freshly prepared PCR and SSCP products were always used. A negative control well containing no DNA was also run with each series of amplifications.

5.2.4 Nucleotide sequencing and genotyping

Following PCR-SSCP analysis, the banding patterns in the gels were observed. Amplicons deemed to be homozygous were sequenced in the forward and reverse directions using capillary sequencing at the Lincoln University DNA Sequencing Facility. For patterns that appeared to be heterozygous, single bands of non-homozygous samples were excised from the SSCP gel, washed repeatedly, and genomic DNA extracted. The resulting homozygous DNA was re-amplified, and then sequenced. Reactions were replicated to ensure accuracy and prevent PCR and sequencing errors.

Sequence alignments, translations and comparisons were carried out using DNAMAN version 5.2.10 (Lynnon BioSoft, Vaudreuil, Canada). The BLAST algorithm (http://blast.ncbi.nlm.nih.gov/) was used to search the NCBI GenBank and Ensembl databases for homologous sequences in various species.

5.2.5 Statistical analyses

IBM SPSS Statistics (Version 23, IBM, NY, USA) was used to perform all analyses. Trait data normality was tested using the Shapiro-Wilk test and Normal Q-Q plots. Variant and genotypic frequencies were calculated using the PopGene 3.2 software. The variant frequencies were then tested for deviation from Hardy-Weinberg equilibrium using a Chi-square test available on the Online Encyclopedia for Genetic Epidemiology Studies (OEGE) (Rodriguez et al., 2009).

Prediction of the effect of nucleotide variations on protein stability and function was performed using a variety of online software to ensure reliability, including SIFT (Ng and Henikoff, 2003), PROVEAN (Choi et al., 2012), HOPE (Venselaar et al., 2010), MuPro (Cheng et al., 2006), and iMutant 2.0 (Capriotti et al., 2005). Changes in protein stability were predicted using delta delta G (ΔΔG) in Kcal/mol, which refers to a change in the Gibbs free energy between folded and unfolded states of the wild-type and variant protein sequences. The analyses were carried out on the assumptions that temperature was 25 °C and pH was 7.0.

The strength of relationships between the different traits was assessed using Pearson correlation coefficients. A Bonferroni correction was applied to the analyses. The growth traits investigated were birth weight, tailing weight, weaning weight, and growth rate-to-weaning. The carcass traits investigated were hot carcass weight (HCW), fat depth at the 12th rib (V-GR), hind-leg lean meat yield, loin lean meat yield, shoulder lean meat yield, and total carcass lean meat yield.

Two sets of general linear mixed-effect models (GLMMs) were carried out. The first model was used to investigate the effect of the presence and absence (coded as 1 and 0 respectively) of a specific variant on growth and carcass traits, while the second model assessed associations between the genotypes for each nucleotide substitution and growth and carcass traits.

The statistical model used was:

$$Y_{ijkl} = \mu + S_i + Gj + B_k + V_l + e_{ijkl}$$

where:

Y_{ijkl} is the trait measured on each animal (birth, tailing and weaning weights, growth-rate, HCW, V-GR, and leg, loin, shoulder, and total lean meat yields)

μ is the mean for the trait

S_i is the random effect of sire

Gj is the fixed effect of gender

B_j is the fixed effect of birth rank

V_l is the fixed effect of variant (presence or absence) or genotype

e_{ijkl} is the random residual error

Gender was fitted as a fixed factor, and sire as a random factor in all growth trait models. For the growth trait models assessing the relationship between nucleotide genotype (or the presence/absence of a sequence variant) and tailing and weaning weights, the age at tailing and weaning were fitted as covariates respectively, as variation in age at measurement could potentially influence these traits.

In order to account for the effect of prenatal growth and development, birth weight (which had a bigger effect than birth rank) was fitted as a covariate in models assessing the relationship between nucleotide variant genotype or the presence/absence of a sequence variant, and growth rate-to-weaning. Rearing rank, which is reflective of maternal influence was shown to have an effect, and hence was corrected for in growth rate models.

For all carcass trait models, genotype was fitted as a fixed factor, and sire as a random factor, while birth weight and slaughter age in days were fitted as covariates, so as to correct for the effect of prenatal muscle development and the variation in the age of the lambs at slaughter respectively. Gender was not corrected for in any of the carcass trait models, as only ram lambs were sent for slaughter.

The variant model was run in two phases. Firstly, a single variant model assessing the unilateral effect of variant presence/absence on individual traits was run. If a significant trend was observed ($P < 0.2$, the threshold for inclusion), then a subsequent multi-variant model investigating the effect of the presence/absence of a variant, within the context of the effect of the other variants, was performed.

Values from the GLMM pairwise comparison was presented as estimated marginal means ± standard error (mean ± SE). Unless otherwise specified, statistical significance was declared at $P < 0.05$, and trends were observed at $P < 0.2$.

5.3 Results

5.3.1 *GH* variation

The 560 bp fragment spanning the exon 2, intron 2 and exon 3 regions of ovine *GH* was amplified in ten NZ sheep breeds (Figure 5.4). PCR-SSCP analysis and subsequent nucleotide sequencing revealed seven variant sequences (or haplotypes) named A_3, B_3, C_3, D_3, E_3, F_3 and G_3. The seven nucleotide sequences were submitted to the GenBank database where they were assigned the accession numbers: MK573367-MK573373. The heterozygous SSCP gel patterns are illustrated in Figure 5.5.

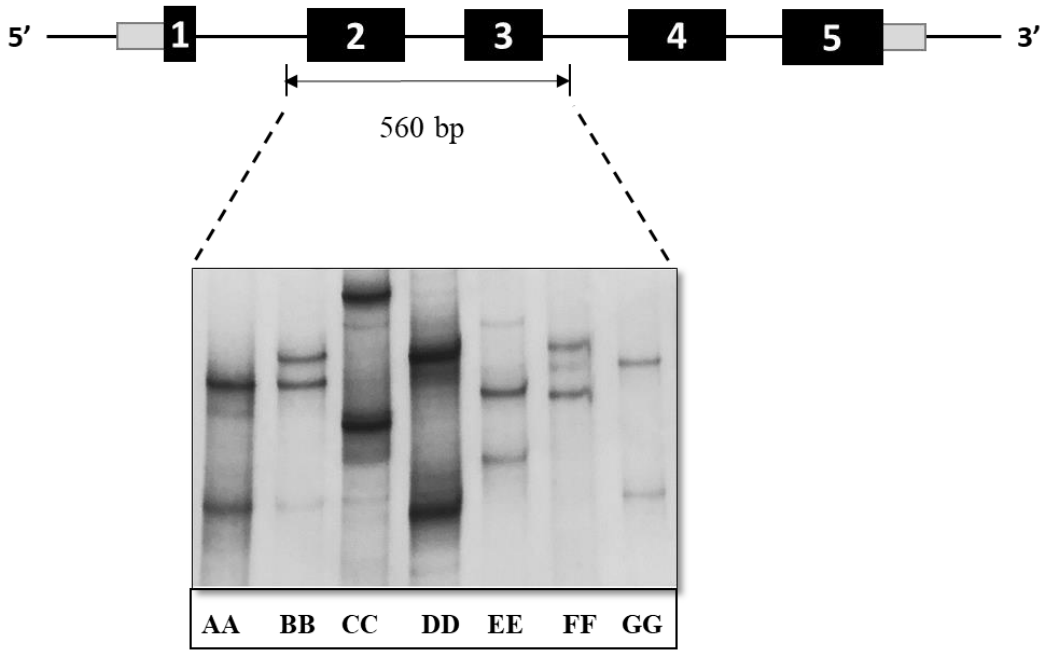


Figure 5.4 Ovine growth hormone gene (*GH)* region amplified and homozygous gel patterns from PCR-SSCP analysis

Stylistic map showing the exon and intron regions of ovine *GH*, and the 560 bp region amplified. The resulting gel patterns from polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) represented seven homozygous variants: A_3A_3, B_3B_3, C_3C_3, D_3D_3, E_3E_3, F_3F_3 and G_3G_3 (the subscript 3 denotes the region amplified, and differentiates variants from DNA nucleotides).

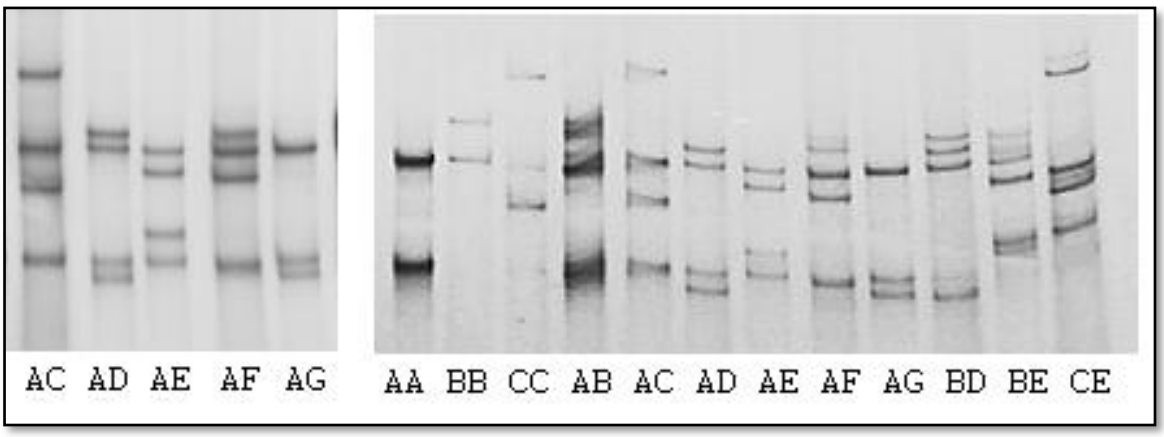


Figure 5.5 Homozygous and heterozygous PCR-SSCP patterns detected for ovine *GH*

The resulting gel patterns from polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) indicating homozygous and heterozygous genotypes of the sequence variants.

Results from BLAST analyses using sequences from the NCBI database (Accession numbers: DQ450147.1 (*GH1*), KU341778.1 (*GH2*), AH005493.2 (*GH2-N*) and DQ461615.1 (*GH2-Z*)), suggest that the *GH1* and *GH2-N* genes are homologues, and that the primers used in this study had the capacity to amplify congruous sequences within them. The PCR-SSCP analysis typically yielded simple gel banding patterns (at most four bands), which would appear to confirm that the gene regions were being amplified in any of the following genetic combinations: *GH1*/*GH1* or *GH1*/*GH2-N* or *GH2-N*/*GH2-N*.

Specific determination of whether *GH1* or *GH2-N* was being amplified could only be achieved by the amplification of a fragment spanning a much larger region of the gene (potentially > 7 kb – twice the distance between *GH2-N* and *GH2-Z*), which was not feasible in this study.

5.3.2 Sequence alignment of *GH* DNA and protein sequences

Sequence alignments of the nucleotide sequences of the seven *GH* variants are presented in Figure 5.6.

```
Template    tagGCCCCCGGACCTCCCTGCTCCTGGCTTTCACCCTGCTCTGCCTGCTC     50
Variant_A3  --------------------------------------------------     50
Variant_B3  ----------------------------------------------C-     50
Variant_C3  ----------------------------------------------C-     50
Variant_D3  ----------------------------------------------C-     50
Variant_E3  ----------------------------------------------C-     50
Variant_F3  ------------------------------------------T---------     50
```

```
Variant_G3  ------------------------------------------------C-     50

Template    TGGACTCAGGTGGTGGGCGCCTTCCCAGCCATGTCCTTGTCCCGCCTGTT    100
Variant_A3  -----------------------------------------------G-------  100
Variant_B3  -----------------------------------------------G-------  100
Variant_C3  -----------------------------------------------G-------  100
Variant_D3  -----------------------------------------------G-------  100
Variant_E3  ---------------------------------------------------------  100
Variant_F3  ----------------A-----------------------------------------  100
Variant_G3  -----------------------------------------------G-------  100

Template    TGCCAACGCTGTGCTCCGGGCTCAGCACCTGCATCAACTGGCTGCTGACA    150
Variant_A3  ---------------------------------------------------------  150
Variant_B3  ---------------------------------------------------------  150
Variant_C3  ---------------------------------------------------------  150
Variant_D3  ---------------------------------------------------------  150
Variant_E3  ---------------------------------------------------------  150
Variant_F3  ---------------------------------------------------------  150
Variant_G3  ---------------------------------------------------------  150

Template    CCTTCAAAGAGTTTgtaagctccccagagatgtgtcctagaggtggggag    200
Variant_A3  ---------------------------------------------------------  200
Variant_B3  ---------------------------------------------------------  200
Variant_C3  ---------------------------------------------------------  200
Variant_D3  ---------------------------------------------------------  200
Variant_E3  ---------------------------------------------------------  200
Variant_F3  ---------------------------------------------------------  200
Variant_G3  ---------------------------------------------------------  200

Template    gcaggaagggatgaatccgcaaccc.tccacacaatgggagggaactgag    249
Variant_A3  ----------g----------c---c------------------------  250
Variant_B3  ----------g----------c---c------------------------  250
Variant_C3  ----------g------t---c---c---------c--------------  250
Variant_D3  ----------g----------c---c------------------------  250
Variant_E3  ----------g----------c---c------------------------  250
Variant_F3  ----------g----------c---c------------------------  250
Variant_G3  ----------g----------c---c------------------------  250

Template    gacctcagtggtattttatccaagtaaggatgtggtcaggggagtagaaa    299
Variant_A3  ---------------------------------------------------------  300
Variant_B3  ---------------------------------------------------------  300
Variant_C3  ----------------------------a----------------------  300
Variant_D3  ---------------------------------------------------------  300
Variant_E3  ---------------------------------------------------------  300
Variant_F3  ---------------------------------------------g----  300
Variant_G3  ---------------------------------------------------------  300

Template    tgggggtgtgtggggtggggagggttccgaataaggcagtgaggggaacc    349
Variant_A3  ---------------------------------------------------------  350
Variant_B3  ---------------------------------------------------------  350
Variant_C3  ---------------------------------------------------------  350
Variant_D3  ---------------------------------------------------------  350
Variant_E3  ---------------------------------------------------------  350
Variant_F3  ---------------------------------------------------------  350
Variant_G3  ---------------------------------------------------------  350

Template    ccgcaccagctgagacctgggtgggtgtgttctccccccagGAGCGCACC    399
Variant_A3  ---------------------------------------------------------  400
Variant_B3  ---------------------------------------------------------  400
Variant_C3  a-------------------------------------------------------  400
Variant_D3  ---------------------------------------------A----  400
Variant_E3  ---------------------------------------------------------  400
Variant_F3  ---------------------------------------------------------  400
Variant_G3  ----------t----------------------------------------  400
```

```
Template    TACATCCCGGAGGGACAGAGATACTCCATCCAGAACACCCAGGTTGCCTT    449
Variant_A3  ---------------------------------------------------    450
Variant_B3  ---------------------------------------------------    450
Variant_C3  ---------------------------------------------------    450
Variant_D3  ---------------------------------------------------    450
Variant_E3  ---------------------------------------------------    450
Variant_F3  ---------------------------------------------------    450
Variant_G3  ---------------------------------------------------    450

Template    CTGCTTCTCTGAAACCATCCCAGCCCCCACGAGCAAGAATGAGGCCCAGC    499
Variant_A3  ---------C-----------G---------G------------------    500
Variant_B3  ---------C-----------G---------G------------------    500
Variant_C3  ---------C-----------G---------G------------------    500
Variant_D3  ---------C---------------------G------------------    500
Variant_E3  ---------C---------------------G------------------    500
Variant_F3  ---------C-----------G---------G------------------    500
Variant_G3  ---------C---------------------G------------------    500

Template    AGAAATCAGTGAgtggccacctag                             523
Variant_A3  ------------------------                             524
Variant_B3  ------------------------                             524
Variant_C3  ------------------------                             524
Variant_D3  ------------------------                             524
Variant_E3  ------------------------                             524
Variant_F3  ------------------------                             524
Variant_G3  ------------------------                             524
```

Figure 5.6 Sequence alignment of the *GH* variants

An alignment of the seven *GH* variants was undertaken using the DNAMAN software. Bars (-) denote nucleotides identical to the ovine *GH* sequence, while dots (.) denote missing nucleotides. The 524 bp region presented does not include the primer sequences. The ovine template sequence was obtained from GenBank (accession number DQ461615).

The nucleotide sequence alignment revealed coding and non-coding sequence variation in the *GH* variants (Figure 5.6). Subsequent alignment of the putative protein sequences of the *GH* variants, including a comparison with other mammalian species, revealed several non-synonymous amino acid changes (Figure 5.7).

```
A3       GPRTSLLLAFTLLCLLWTQVVGAFPAMSLSGLFANAVLRAQHLHQLAADTFKEFERT   5-61
B3       ---------------p-------------------------------------r--   5-61
C3       ---------------p-------------------------------------r--   5-61
D3       ---------------p-------------------------------------h--   5-61
E3       ---------------p-----------------r-------------------r--   5-61
F3       ---------------------t-----------r-------------------r--   5-61
G3       ---------------p-------------------------------------r--   5-61
SHEEP    GPRTSLLLAFTLLCLLWTQVVGAFPAMSLSRLFANAVLRAQHLHQLAADTFKEFERT   5-61
HUMAN    -s--------g----s-l-egs---tip-----d--m---rr-y---y--yq---ea   5-61
GOAT     ---------------p-------------------g-------------------r--   5-61
COW      ----------a----p-------------------g-------------------r--   5-61
PIG      -----v----a----p---e-------p--s-------------------y-----a   5-61
DOG      s--n-v----a----p-p-e-------p--s-------------------y-----a   5-61
RAT      dsq-pw--t-s------p-ea--l---p--s-------------------y-----a   5-61
MOUSE    ds---w--tvs------p-eas-----p--s--s-----------------y-----a   5-61

A3       YIPEGQRYS.IQNTQVAFCFSETIPAPTGKNEAQQKS              62-97
B3       ---------.---------------------------              62-97
C3       ---------.---------------------------              62-97
D3       ---------.---------------------------              62-97
E3       ---------.---------------------------              62-97
F3       ---------.---------------------------              62-97
G3       ---------.---------------------------              62-97
SHEEP    YIPEGQRYS.IQNTQVAFCFSETIPAPTSKNEAQQKS              62-97
HUMAN    --lke-k--fl--p-tsl----s--t-snrvkt----              62-98
GOAT     ---------.------------------g--------              62-97
COW      ---------.------------------g--------              62-97
PIG      ---------.---a-a------------g-d----r-              62-97
DOG      ---------.---a-a------------g-d----r-              62-97
RAT      ---------.---a-a------------g-e----rt              62-97
MOUSE    ---------.---a-a------------g-e----rt              62-97
```

Figure 5.7 Alignment of the putative protein sequences of ovine GH with other related species

Alignment was performed using the DNAMAN software across (a) the seven variants of ovine GH, starting from amino acid p.5 and ending at p.97, as well as (b) across different mammalian species. Bars (-) denote amino acids identical to the sheep GH protein sequence, while dots (.) denote missing amino acids. The amino acid sequences were derived from GenBank DNA sequences with the following accession numbers: Human, *Homo sapiens* (AY890602); Sheep, *Ovis aries* (DQ461615); Goat, *Capra hircus* (GU355687); Cow, *Bos taurus* (KP221576); Pig, *Sus scrofa* (AY536527); Dog, *Canis familiaris* (AF069071); Rat, *Rattus norvegicus* (U62779) and Mouse, *Mus musculus* (NM_008117.3). *A_3, B_3, C_3, D_3, E_3, F_3* and *G_3* depict amino acid sequences obtained from the putative translation of the variant DNA sequences reported in this study.

5.3.3 Diversity in ovine *GH*

The arrangement of the nucleotide substitutions across the seven haplotypes is summarised in Table 5.1.

Table 5.1 Nucleotide substitutions and haplotypes detected in a fragment spanning exon 2, intron 2 and intron 3 of ovine *GH*

	Haplotype / Variant	Region	A_3 H1	B_3 H2	C_3 H3	D_3 H4	E_3 H5	F_3 H6	G_3 H7
1	**c.51C>T (p.Leu17Leu)**	**Exon 2**	**C**	**C**	**C**	**C**	**C**	**T**	**C**
2	**c.59C>T (p.Pro20Leu)**	**Exon 2**	**T**	**C**	**C**	**C**	**C**	**T**	**C**
3	**c.79G>A (p.Ala27Thr)**	**Exon 2**	**G**	**G**	**G**	**G**	**G**	**A**	**G**
4	**c.103G>C (p.Gly35Arg)**	**Exon 2**	**G**	**G**	**G**	**G**	**C**	**C**	**G**
5	c.174+58A>C	Intron 2	c	c	t	c	c	c	c
6	c.174+72T>C	Intron 2	t	t	c	t	t	t	t
7	c.174+115G>A	Intron 2	g	g	a	g	g	g	g
8	c.174+132A>G	Intron 2	a	a	a	a	a	g	a
9	c.174+187C>A	Intron 2	c	c	a	c	c	c	c
10	c.174+198G>T	Intron 2	g	g	g	g	g	g	t
11	**c.179G>A (p.Arg60His)**	**Exon 3**	**G**	**G**	**G**	**A**	**G**	**G**	**G**

Four amino acid substitutions were predicted based on the nucleotide variations found in the exon 2 of ovine *GH*: c.51C>T (p.Leu17Leu), c.59C>T (p.Pro20Leu), c.79G>A (p.Ala27Thr), and c.103G>C (p.Gly35Arg). All of these were non-synonymous, except for c.51C>T (p.Leu17Leu). The variation c.179G>A (p.Arg60His) was the only amino acid variation revealed in exon 3 (Table 5.1). Predictions of the effects of putative amino acid substitutions on GH protein stability are summarized in Table 5.2.

Table 5.2 Protein stability prediction for missense variations in ovine *GH*

***GH* missense variant**	**Region**	**iMutant ΔΔG (Kcal/mol)**	**muPRO ΔΔG (Kcal/mol)**	**Effect on protein stability**
c.59C>T (p.Pro20Leu)	Exon 2	-1.19	-2.19	Decrease
c.79G>A (p.Ala27Thr)	Exon 2	-1.33	-0.89	Decrease
c.103G>C (p.Gly35Arg)	Exon 2	-0.84	-0.37	Decrease
c.179G>A (p.Arg60His)	Exon 3	-1.00	-1.11	Decrease

In all four detected non-synonymous *GH* variations, the ΔΔG value was negative. This means that the resulting putative amino acid change was predicted to bring about decreased GH protein stability (Table 5.2). These results were consistent across all other protein prediction software used.

The variant genotype frequencies for ovine *GH* across the ten NZ breeds examined are given in Table 5.3.

Table 5.3 Frequencies of ovine *GH* variant genotypes in different sheep breeds

		***GH* variant genotype frequencies**											
Breed	**n**	***A₃A₃***	***A₃B₃***	***A₃C₃***	***A₃D₃***	***A₃E₃***	***A₃F₃***	***A₃G₃***	***B₃B₃***	***B₃D₃***	***B₃E₃***	***C₃C₃***	***C₃E₃***
Romney	766	62.9	22.1	3.9	_	_	11.1	_	_	_	_	_	_
Coopworth	32	68.7	6.3	25.0	_	_	_	_	_	_	_	_	_
Corriedale	30	70.0	26.7	_	_	_	3.3	_	_	_	_	_	_
Dorset Down	30	43.3	13.4	43.3	_	_	_	_	_	_	_	_	_
Merino	58	15.5	5.2	19.0	_	_	55.2	_	1.7	1.7	_	1.7	_
Perendale	62	22.6	32.3	37.1	1.6	3.2	_	_	_	_	1.6	_	1.6
Poll Dorset	31	51.6	35.5	_	_	_	12.9	_	_	_	_	_	_
Suffolk	30	63.3	_	26.7	_	_	10.0	_	_	_	_	_	_
Texel	32	75.0	_	9.4	_	_	15.6	_	_	_	_	_	_
White Dorper	72	13.9	52.7	29.2	_	1.4	_	1.4	_	_	1.4	_	_
Total	1143												

Different *GH* genotype combinations were present in varying proportions across the ten examined NZ sheep breeds. Some rare genotypes were peculiar only to certain breeds: *A₃D₃* (Perendale), *A₃G₃* (White Dorper), *B₃B₃* (Merino), *B₃D₃* (Merino), *C₃C₃* (Merino) and *C₃E₃* (Perendale). The *A₃A₃* was the most predominant *GH* genotype across NZ sheep breeds, with the highest frequency (75%) observed for the Texel breed, and the lowest frequencies observed for the White Dorper (13.9%) and Merino (15.5%) breeds respectively (Table 5.3).

5.3.4 Variant and nucleotide substitution frequencies

The *A₃* variant was observed in every Romney lamb investigated, and hence it had a frequency of 100%, while *B₃*, *C₃* and *F₃* variants occurred at frequencies of 22%, 4% and 11% respectively. In the NZ Romney breed, *A₃* was the only variant observed in a

homozygous form (A_3A_3), while the other variants always existed in a heterozygous combination with A_3, namely A_3B_3, A_3C_3, and A_3F_3.

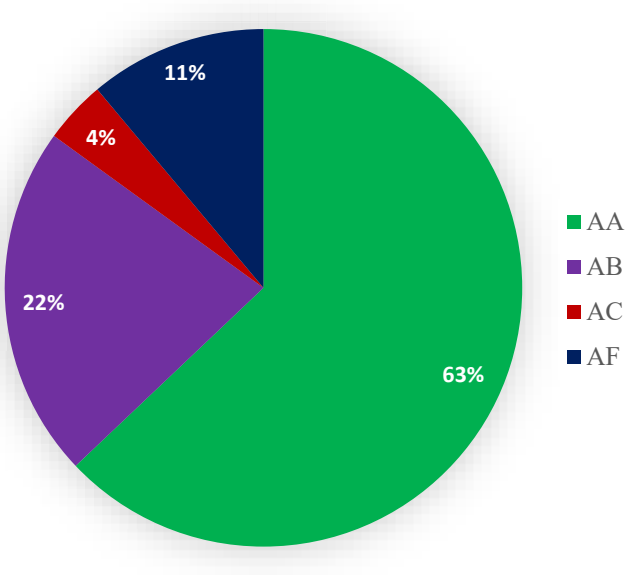


Figure 5.8 Pie chart of *GH* genotype frequencies in the NZ Romney sheep

The variant genotype A_3A_3 was the most common, being observed in more than half (63%) of NZ Romney lambs, followed by A_3B_3 (22%), and A_3F_3 (11%) (Figure 5.8). The A_3C_3 genotype had the lowest frequency (4%) in Romney lambs, and hence it was not included in further analyses as it was lower than the 5% statistical cut-off for analysis. Accordingly, the loci considered in nucleotide substitution genotype analyses where only those that differed between the A_3, B_3, and F_3 variants, namely c.51C>T, c.59C>T, c.79G>A, c.103G>C, and c.174+132A>G (Table 5.1).

As shown in Table 5.4, all the aforementioned variants except for c.59C>T, have a similar genotype frequency pattern where the major nucleotide was present in 94% of the population, while only 6% of population possessed the minor nucleotide. The major homozygous genotype was reported in 89% of all lambs, while the heterozygous form was found in 11%, and the minor homozygous nucleotide was not reported at all (0%).

The genotype and nucleotide frequencies for the *GH* substitutions are given in Table 5.4.

Table 5.4 Nucleotide and genotype frequencies for ovine *GH* variations

Variant	Nucleotide	Nucleotide frequency	n	Genotype	Genotype frequency	HWE[1]
c.51C>T	C	0.94	681	CC	0.89	χ^2 = 2.41
(p.Leu17Leu)	T	0.06	85	CT	0.11	P = 0.300
			0	TT	0.00	
c.59C>T	C	0.13	0	CC	0.00	χ^2 = 15.65
(p.Pro20Leu)	T	0.87	199	CT	0.26	**P = <0.001**
			567	TT	0.74	
c.79G>A	A	0.06	0	AA	0.00	χ^2 = 2.41
(p.Ala27Thr)	G	0.94	85	AG	0.11	P = 0.300
			681	GG	0.89	
c.103G>C	C	0.06	0	CC	0.00	χ^2 = 2.41
(p.Gly35Arg)	G	0.94	85	CG	0.11	P = 0.300
			681	GG	0.89	
c.174+132A>G	A	0.94	681	AA	0.89	χ^2 = 2.41
	G	0.06	85	AG	0.11	P = 0.300
			0	GG	0.00	

[1] HWE = Hardy-Weinberg Equilibrium

Notably, at all variant loci, the minor homozygous genotypes had frequencies of 0% as they were not detected in any lamb; as a result, these minor genotypes were excluded from further analyses. For the c.59C>T, *C* was the minor nucleotide occurring in about 13% of the lambs, while the more abundant *T* nucleotide occurred in 87% of lambs, and its homozygous form *TT* occurred in 74% of the lambs investigated (Table 5.4). The c.59C>T variation was the only one found to deviate from Hardy-Weinberg Equilibrium.

5.3.5 Association of *GH* genetic variants with growth traits

The presence and absence of each variant were investigated for association with growth traits in the NZ Romney lambs, and the results are summarised in Table 5.5. Due to the presence of the A_3 variant in all Romney lambs studied, it was excluded from the present-absent models.

Table 5.5 Association of variants in ovine *GH* with growth traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
Birth weight (kg)	B_3		5.05 ± 0.20	597	5.20 ± 0.21	169	**0.037**
	F_3		5.09 ± 0.20	681	4.97 ± 0.22	85	0.160
	B_3	F_3	5.02 ± 0.21	597	5.15 ± 0.21	169	0.063
	F_3	B_3	5.13± 0.20	681	5.04 ± 0.22	85	0.298
Tailing weight (kg)	B_3		11.86 ± 0.55	597	12.35 ± 0.57	169	**0.008**
	F_3		11.99 ± 0.55	681	11.37 ± 0.59	85	**0.012**
	B_3	F_3	11.64 ± 0.56	597	12.06 ± 0.58	169	**0.027**
	F_3	B_3	12.11 ± 0.55	681	11.59 ± 0.60	85	**0.038**
Weaning weight (kg)	B_3		28.91 ± 0.95	597	30.68 ± 0.98	169	**<0.001**
	F_3		29.42 ± 0.95	681	27.13 ± 1.03	85	**<0.001**
	B_3	F_3	28.13 ± 0.95	597	29.62 ± 1.00	169	**<0.001**
	F_3	B_3	29.84 ± 0.94	681	27.92 ± 1.03	85	**<0.001**
Growth rate to weaning (g/day)	B_3		253.59 ± 10.31	597	270.97 ± 10.63	169	**<0.001**
	F_3		264.06 ± 10.41	681	242.99 ± 10.76	85	**<0.001**
	B_3	F_3	250.91 ± 10.24	597	265.90 ± 10.61	169	**<0.001**
	F_3	B_3	267.16 ± 10.31	681	249.65 ± 10.74	85	**<0.001**

The presence of the B_3 variant of ovine *GH* was associated with increased weight at birth (0.15 kg), tailing (0.49 kg) and weaning (1.77 kg). Lambs possessing the B_3 variant also grew faster by 17.4 g/day (Table 5.5). In contrast, lambs possessing the F_3 variant had lower tailing (0.62 kg) and weaning (2.29 kg) weights, as well as slower growth rates (by 21.07 g/day). F_3 showed a decreasing trend of association with birth weight.

Table 5.6 summarises association studies assessing the effects of individual nucleotide variants on growth traits in the NZ Romney lambs.

Table 5.6 Association of nucleotide substitution genotypes in ovine *GH* with growth traits in the NZ Romney lambs

Variant Loci	Geno types	n	Birth weight (kg)	Tailing weight (kg)	Weaning weight (kg)	Growth rate-to-weaning (g/day)
c.51C>T (p.L17L)	CC	681	5.10 ± 0.20	11.99 ± 0.55	29.42 ± 0.95	264.06 ± 10.41
	CT	85	4.97 ± 0.22	11.37 ± 0.59	27.13 ± 1.03	242.99 ± 10.76
	P value		0.160	**0.012**	**<0.001**	**<0.001**
c.59C>T (p.P20L)	CT	199	5.24 ± 0.21	12.34 ± 0.56	30.66 ± 0.97	269.97 ± 10.56
	TT	567	5.04 ± 0.20	11.86 ± 0.55	28.87 ± 0.95	253.12 ± 10.31
	P value		**0.002**	**0.006**	**<0.001**	**<0.001**
c.79G>A (p.A27T)	GG	681	5.10 ± 0.20	11.99 ± 0.55	29.42 ± 0.95	264.06 ± 10.41
	AG	85	4.97 ± 0.22	11.37 ± 0.59	27.13 ± 1.03	242.99 ± 10.76
	P value		0.160	**0.012**	**<0.001**	**<0.001**
c.103G>C (p.G35R)	GG	681	5.10 ± 0.20	11.99 ± 0.55	29.42 ± 0.95	264.06 ± 10.41
	CG	85	4.97 ± 0.22	11.37 ± 0.59	27.13 ± 1.03	242.99 ± 10.76
	P value		0.160	**0.012**	**<0.001**	**<0.001**
c.174+132A>G	AA	681	5.10 ± 0.20	11.99 ± 0.55	29.42 ± 0.95	264.06 ± 10.41
	AG	85	4.97 ± 0.22	11.37 ± 0.59	27.13 ± 1.03	242.99 ± 10.76
	P value		0.160	**0.012**	**<0.001**	**<0.001**

Since the minor homozygous nucleotide genotypes were not observed for any lambs, these were excluded from further analyses. Hence for nucleotide substitution analyses, only the major homozygous and heterozygous variants were investigated. The four variants (c.51C>T, c.79G>A, c.103G>C and c.174+132A>G) exhibiting the same genotypic frequencies were unsurprisingly found to exert similar effects on growth traits (Table 5.6). Lambs possessing the major homozygous genotypes were found to have a 0.62 kg increase in tailing weight, a 2.29 kg increase in weaning weight, and grew faster by 21.1 g/day, when compared to their heterozygous counterparts. The variants showed no association with birth weight.

On the other hand, compared to the heterozygous *CC* genotype, the homozygous *TT* genotype of c.59C>T was associated with decreased birth (0.13 kg), tailing (0.48 kg) and weaning (16.85 kg) weights, and a diminished growth rate (16.8 g/day).

5.3.6 Association of genetic variants with carcass traits

The presence and absence of each variant were investigated for association with carcass traits in NZ Romney lambs, and the results are summarised in Table 5.7. The presence of the B_3 variant was associated with an increase in hot carcass weight (0.41 kg), and an increase in the leg (1.4%), loin (1.2%), shoulder (1.1%), and total lean meat yield (1.2%) (Table 5.7). Conversely, when present, the F_3 variant showed associations with decreased hot carcass weight (0.63 kg), leg yield (1.8%), loin yield (1.8%), shoulder yield (1.8%) and total yield (1.8%). Fat depth around the 12th rib was also lower by 0.48 mm with B_3, whilst being higher by 0.84 mm when F_3 was present.

Table 5.7 Association of variants in ovine *GH* with carcass traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
HCW[1] (kg)	B_3		17.03 ± 0.08	551	17.44 ± 0.13	155	**0.002**
	F_3		17.19 ± 0.07	628	16.56 ± 0.17	78	**<0.001**
	B_3	F_3	16.83 ± 0.10	551	17.17 ± 0.15	155	**0.011**
	F_3	B_3	17.28 ± 0.08	628	16.72 ± 0.18	78	**0.002**
V-GR[2] Fat	B_3		4.24 ± 0.11	551	3.76 ± 0.19	155	**0.015**
Depth (mm)	F_3		4.05 ± 0.11	628	4.89 ± 0.26	78	**0.001**
	B_3	F_3	4.52 ± 0.15	551	4.13 ± 0.23	155	**0.049**
	F_3	B_3	3.95 ± 0.12	628	4.70 ± 0.27	78	**0.004**
Leg	B_3		21.45 ± 0.06	551	21.75 ± 0.09	155	**0.003**
Yield (%)	F_3		21.56 ± 0.05	628	21.16 ± 0.13	78	**0.003**
	B_3	F_3	21.33 ± 0.07	551	21.58 ± 0.11	155	**0.011**
	F_3	B_3	21.62 ± 0.06	628	21.28 ± 0.14	78	**0.010**
Loin	B_3		14.54 ± 0.04	551	14.72 ± 0.06	155	**0.012**
Yield (%)	F_3		14.61 ± 0.04	628	14.35 ± 0.09	78	**0.005**
	B_3	F_3	14.46 ± 0.05	551	14.61 ± 0.08	155	**0.036**
	F_3	B_3	14.64 ± 0.04	628	14.41 ± 0.10	78	**0.015**
Shoulder	B_3		16.81 ± 0.04	551	17.00 ± 0.07	155	**0.010**
Yield (%)	F_3		16.88 ± 0.04	628	16.57 ± 0.09	78	**0.001**
	B_3	F_3	16.71 ± 0.05	551	16.86 ± 0.08	155	**0.037**
	F_3	B_3	16.92 ± 0.04	628	16.64 ± 0.10	78	**0.004**
Total	B_3		52.80 ± 0.12	551	53.47 ± 0.19	155	**0.001**
Yield (%)	F_3		53.05 ± 0.11	628	52.07 ± 0.26	78	**<0.001**
	B_3	F_3	52.49 ± 0.15	551	53.05 ± 0.23	155	**0.007**
	F_3	B_3	53.20 ± 0.12	628	52.34 ± 0.28	78	**0.002**

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

Association studies assessing the effect of individual nucleotide substitutions on variation in carcass traits were carried out for the NZ Romney lambs, and the results are presented in Table 5.8.

Table 5.8 Association of nucleotide genotypes at the different loci in ovine *GH* with carcass traits in the NZ Romney lambs

Variant Loci	Genotype	n	HCW[1] (kg)	V-GR[2] (mm)	Leg yield (%)	Loin yield (%)	Shoulder yield (%)	Total yield (%)
c.51C>T	CC	628	17.19 ± 0.07	4.05 ± 0.11	21.56 ± 0.05	14.61 ± 0.04	16.88 ± 0.04	53.05 ± 0.11
(p.L17L)	CT	78	16.56 ± 0.17	4.89 ± 0.26	21.16 ± 0.13	14.35 ± 0.09	16.57 ± 0.09	52.07 ± 0.26
	P value		**<0.001**	**0.001**	**0.003**	**0.005**	**0.001**	**<0.001**
c.59C>T	CT	183	17.45 ± 0.12	3.65 ± 0.17	21.78 ± 0.09	14.74 ± 0.06	17.00 ± 0.06	53.53 ± 0.18
(p.P20L)	TT	523	17.01 ± 0.08	4.31 ± 0.11	21.42 ± 0.06	14.52 ± 0.04	16.80 ± 0.04	52.75 ± 0.12
	P value		**0.001**	**<0.001**	**<0.001**	**0.001**	**0.004**	**<0.001**
c.79G>A	GG	628	17.19 ± 0.07	4.05 ± 0.11	21.56 ± 0.05	14.61 ± 0.04	16.88 ± 0.04	53.05 ± 0.11
(p.A27T)	AG	78	16.56 ± 0.17	4.89 ± 0.26	21.16 ± 0.13	14.35 ± 0.09	16.57 ± 0.09	52.07 ± 0.26
	P value		**<0.001**	**0.001**	**0.003**	**0.005**	**0.001**	**<0.001**
c.103G>C	GG	628	17.19 ± 0.07	4.05 ± 0.11	21.56 ± 0.05	14.61 ± 0.04	16.88 ± 0.04	53.05 ± 0.11
(p.G35R)	CG	78	16.56 ± 0.17	4.89 ± 0.26	21.16 ± 0.13	14.35 ± 0.09	16.57 ± 0.09	52.07 ± 0.26
	P value		**<0.001**	**0.001**	**0.003**	**0.005**	**0.001**	**<0.001**
c.174+132A>G	AA	628	17.19 ± 0.07	4.05 ± 0.11	21.56 ± 0.05	14.61 ± 0.04	16.88 ± 0.04	53.05 ± 0.11
	AG	78	16.56 ± 0.17	4.89 ± 0.26	21.16 ± 0.13	14.35 ± 0.09	16.57 ± 0.09	52.07 ± 0.26
	P value		**<0.001**	**0.001**	**0.003**	**0.005**	**0.001**	**<0.001**

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

The homozygous *CC, GG, GG* and *AA* genotypes of c.51C>T, c.79G>A, c.103G>C and c.174+132A>G respectively, showed association with an increase in hot carcass weight (0.63 kg), a 1.8% increase in leg, loin, shoulder and total lean meat yields; as well as a decrease in the fat depth (0.84 mm) (Table 5.8).

With the c.59C>T substitution, when two copies of the *T* nucleotide were present (*TT*), there was a decrease in hot carcass weight (0.44 kg), leg yield (1.6%), loin yield (1.5%), shoulder yield (1.2%) and total yield (1.4%).

5.3.7 Association of variant genotypes with carcass traits

The genotype combinations of the variants were assessed for their effects on growth and carcass traits in Romney lambs (Table 5.9). The variant genotypes assessed were A_3A_3, A_3B_3 and A_3F_3. A_3C_3 was not investigated as its frequency of 4% is lower than the 5% threshold required for analysis.

Table 5.9 Association of variant genotypes in ovine *GH* with growth and carcass traits in NZ Romney sheep

Growth traits	**A_3A_3 (n = 482)**	**A_3B_3 (n = 169)**	**A_3F_3 (n = 85)**	**P-value**
Birth weight (kg)	5.05 ± 0.20[a]	5.21 ± 0.21[ab]	4.98 ± 0.22[b]	**0.007**
Tailing weight (kg)	11.89 ± 0.55[a]	12.33 ± 0.56[a]	11.39 ± 0.59[b]	**0.008**
Weaning weight (kg)	29.05 ± 0.94[a]	30.61 ± 0.97[a]	27.21 ± 1.01[b]	**<0.001**
Growth rate to weaning (g/day)	259.13 ± 10.34[a]	274.59 ± 10.56[a]	242.10 ± 10.62[b]	**<0.001**

Carcass Traits	**A_3A_3 (n = 445)**	**A_3B_3 (n = 155)**	**A_3F_3 (n = 78)**	**P-value**
HCW[1] (kg)	17.09 ± 0.08[a]	17.45 ± 0.12[a]	16.55 ± 0.17[b]	**<0.001**
V-GR[1] Fat Depth (mm)	4.21 ± 0.12[a]	3.75 ± 0.18[ab]	4.91 ± 0.25[b]	**<0.001**
Leg Yield (%)	21.47 ± 0.06[a]	21.75 ± 0.09[ab]	21.15 ± 0.13[a]	**<0.001**
Loin Yield (%)	14.55 ± 0.04[a]	14.72 ± 0.06[ab]	14.34 ± 0.09[a]	**0.001**
Shoulder Yield (%)	16.84 ± 0.04[a]	17.00 ± 0.07[a]	16.56 ± 0.09[b]	**0.001**
Total Yield (%)	52.86 ± 0.12[a]	53.47 ± 0.19[ab]	52.05 ± 0.26[a]	**<0.001**

[a, b] Mean values in the same row with different superscripts differ at $P < 0.05$

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

Individuals possessing the A_3B_3 variant genotype had the highest mean growth rate, weights at birth, weaning and slaughter, and lean meat yield (Table 5.9). A_3B_3 lambs grew 5.6% faster than A_3A_3 lambs, and 11.8% faster than A_3F_3 lambs. At slaughter, A_3B_3 lambs weighed 0.36 kg more, and yielded 1.1% more total lean meat than their homozygous counterparts.

Compared to the A_3A_3 and A_3F_3 lambs, A_3B_3 lambs deposited 12.3% and 30.9% less intermuscular fat, respectively. Lambs possessing the A_3F_3 genotype weighed the least at birth and weaning, grew the slowest, and had the lowest lean meat yields.

5.4 Discussion

Growth hormone is a somatotropic hormone which regulates growth, nutrient partitioning and metabolism in nearly all body tissues and organs. GH promotes protein and DNA synthesis, muscle build-up, and fat breakdown and mobilisation.

In this study, eleven novel nucleotide variations defining seven sequence variants (A_3 to F_3) of ovine *GH*, were detected in ten NZ sheep breeds. The variations included four non-synonymous (exon 2 and 3), one synonymous (exon 2), and six intronic (intron 2) variants. Previous studies have reported *GH* variation in sheep (Jia et al., 2014; Bahrami et al., 2015; Gorlov et al., 2017), goats (Gupta et al., 2007; An et al., 2010), and cattle (Falaki et al., 1997; Sugita et al., 2014).

It has been reported that sheep *GH* consists of a single copy gene *(GH1)* at one locus, and two gene copies (*GH2-N* and *GH2-Z*) 3.5 kb apart, at a second locus (Valinsky et al., 1990; Gootwine et al., 1996; Gootwine et al., 1997). BLAST analyses revealed that the primers used to screen ovine *GH* in this study, could amplify both *GH1* and *GH2-N* genes. The BLAST analyses also revealed the lack of similarity between the reverse primer used and the *GH2-Z* gene. The PCR-SSCP confirms this notion, as there were at most four bands for each amplicon on the SSCP gels. This suggests that either *GH1*/*GH1*, or *GH1*/*GH2-N*, or *GH2-N*/*GH2-N*, but not *GH2-Z*, was being amplified. This finding also fits with the observation that *GH2-N* and *GH2-Z* have been reported to exhibit sequence differences, and are predominantly expressed in different organs; the pituitary gland for *GH2-N* and placenta for the *GH2-Z* (Gootwine et al., 1996; Gootwine et al., 1997).

In Serra de Estrela sheep, *GH1/GH2-N* and *GH2-Z* gene regions were found to be associated with milk yield traits, but animals with *GH2/GH2* genotype produced more milk when compared to *GH1/GH1* animals (Marques et al., 2006). The variants exhibited copy-specific variation despite being duplicates, and higher milk yield was also observed with a *GH2-N* and *GH2-Z* 'joined-effect' genotype (Marques et al., 2006).

The A_3A_3 genotype was the only one found in all NZ sheep breeds examined. Some *GH* genotypes were exclusive to certain breeds, which might indicate some possible breed-specific benefit besides growth and carcass traits. The B_3B_3, B_3D_3 and C_3C_3 genotypes observed solely in the Merino breeds might have an effect on wool traits and production. By the same token, the A_3D_3 and C_3E_3 genotypes unique to the Perendale breed, and the A_3G_3 genotype unique to the White Dorper breed, might influence hardiness and carcass quality traits which are sought after in these breeds, respectively.

It is interesting that although the A_3B_3 genotype is the second most abundant after A_3A_3, lambs exhibiting this genotype had the highest growth rates and lean meat yields, as well as the highest weights at tailing, weaning, and slaughter, when compared to lambs possessing other genotypes. Lambs possessing the A_3B_3 genotype also had the lowest intermuscular fat depth. The reverse was observed for the A_3F_3 genotype which exhibited the lowest growth traits and carcass traits, except for having the highest intermuscular fat depth. This agrees with previous findings in Russian Salsk sheep, where the *AB* genotype of *GH* had the highest carcass and slaughter weight, when compared to *AA* and *AC* genotypes (Gorlov et al., 2017). It is likely that the *AB* sequence reported in that study differs from that in this study; the similarities refer to the association of the *IGF1R* genotypes with ovine carcass traits in both studies.

The B_3 variant of *GH* was associated with higher weights at tailing and weaning, and faster growth rate. In contrast, the F_3 variant was associated with lower tailing and weaning weights, and slower growth rates. These findings are in agreement with other reports on ovine *GH*. A nucleotide transition from *T* to *C* in the exon 4 of ovine *GH*, was found to be associated with growth traits such as body length, weight, and height in Chinese Tibetan sheep (Jia et al., 2014).

The B_3 variant was associated with a decrease in fat thickness and higher lean meat yield, while the F_3 variant was associated with an increase in fat thickness and lower lean meat yield in the leg, loin and shoulder regions. This is consistent with the findings of Bahrami

et al. (2015), where nucleotide sequence variations in the exon 5 of ovine *GH* were associated with back fat thickness, body length, carcass weight and abdominal fat yield.

A c.59C>T transition in exon 2 of *GH* brings about a putative substitution of proline for leucine at position 20. Proline is a polar residue with an uncharged sidechain, whereas its substitute leucine, is a non-polar residue. Proline is unique, in that its side-chain connects directly to the residue's amino group to form a ring, hence giving it a compact and rigid structure (von Heijne, 1990). Proline, therefore, has the ability to 'kink' a protein, thus affecting the secondary and tertiary structures. The substitution to leucine, a larger amino acid, could result in bumps within the protein and disruption in the rigidity within the polypeptide backbone.

Signal peptides are short starter sequences, 15 to 30 amino acids long, at the N-terminal of a nascent polypeptide that is cleaved off by specialised signal peptidases to yield a mature protein. Given its crucial position within the signal peptide, the p.Pro20Leu variant could alter GH processing and function.

The substitution of alanine, a non-polar residue for threonine, a polar uncharged residue at position 27 is caused by a c.79G>A transition in the exon 2 of *GH*. This variation falls within the conserved boundary region of GH signal peptide. Around the signal peptide, a "-3, -1" rule applies, which states that in order for proteolytic cleavage to take place correctly, residues occupying the -3 and -1 amino acid positions, relative to the cleavage site, must be small and uncharged (Jain et al., 1994). These residues include alanine, glycine and serine. Alanine is conserved across various proteins and species as the most common residue found at the -3 and -1 positions relative the signal peptide cleavage sites (von Heijne, 1990; Nielsen et al., 1997). The substitution of any amino acid in the place of alanine could impair signal peptide cleavage and hence protein activity.

Although threonine satisfies the requirement of being small and uncharged, like serine and glycine, protein activity and stability prediction in this study revealed that the putative p.Ala27Thr variation is disruptive, and could decrease protein stability and alter normal functioning. This disruption could be explained by the loss of hydrophobicity in that region, as alanine is a hydrophobic non-polar residue, whereas threonine is polar and larger than alanine. Electrostatic interactions resulting from the threonine substitute could also be a contributing factor to the disruption. Several studies have established that the hydrophobicity of a signal peptide is crucial to its function (Fikes et al., 1990; von Heijne, 1990; Jain et al., 1994). The importance of Ala27 to GH function was illustrated by GH

protein alignment analyses across species, which revealed that the Ala27 residue is conserved across humans, cattle, goats, pigs, dogs, rats, mice, and other sheep breeds. Threonine 27 was unique only to the F_3 variant in this study, and not found in the other variants or mammalian species. This might explain why the F_3 variant was associated with less desirable traits such as slower-growing animals, decreased total lean meat yield and higher intermuscular fat deposition. The F_3 variant had the lowest frequency of all variants in NZ Romney sheep, thus suggesting that this variant was likely selected against.

The findings in this study are in agreement with an *in vivo* investigation by Fikes et al. (1990), which reported inefficient signal peptide processing and diminished protein function when threonine substitutes alanine at the -1 position of the maltose-binding protein. Due to this substitution, the normal cleavage site was obstructed, and processing was moved to a different site within the signal peptide (Fikes et al., 1990; Jain et al., 1994). Crystallographic analyses have demonstrated that alanine residues at the cleavage sites serve a crucial function in interacting with unique binding pockets within the active sites of signal peptidase enzymes, thus facilitating the fine-tuned positioning and correct cleavage of the signalling peptide (Paetzel et al., 1998; Chen et al., 1999).

The putative p.Pro20Leu and p.Ala27Thr missense variations both affect the GH signal peptide. Both variations may alter the function of the polypeptide product. While p.Pro20Leu occurs within the signal peptide sequence, p.Ala27Thr resides at the conserved cleavage site (von Heijne, 1990). Recognition of the signal peptide, and its subsequent cleavage by signal peptidases within the endoplasmic reticulum and Golgi apparatus is imperative to the processing of the polypeptide precursor into a mature GH protein. The threonine residue is less favourable and might bring about a blockage at the normal cleavage site, necessitating a redirection to an alternate site elsewhere within the signal peptide. This could potentially lead to the ineffective cleavage and processing of the signal peptide. By resulting in incorrect signal peptide processing, both p.Pro20Leu and p.Ala27Thr variants could bring about diminished or even loss of GH function and stability. Intracellular transport of the GH protein via endoplasmic reticulum to its final destination is also disrupted (Jain et al., 1994). This could explain findings from protein activity and stability analyses, which reveal both variations to be deleterious, and leading to decreased protein function and stability.

Within the alpha helices of GH, a glycine residue at position 35 of the polypeptide was exchanged for an arginine residue. This p.Gly35Arg substitution was the result of a

c.103G>C transversion in the exon 2 of *GH*. Being the smallest amino acid due to its lack of a carbon sidechain, glycine is able to reside in areas within the protein that are forbidden to other residues due to steric hindrance and rotation. Glycine also confers upon a protein some conformational flexibility due to its large torsional space that can accommodate unusual torsion angles, especially in areas of tight turns within the protein (Dong et al., 2012). Glycine is able to pack well against residues with both bulky and simple sidechains.

In helical proteins such as GH, glycine plays an essential role in the intimate packing of α-helices by permitting close proximity between the backbones (Javadpour et al., 1999). When performing their biological functions, some proteins undergo a certain degree of helix repacking and kinking, which is facilitated by the presence of glycine residues (Dong et al., 2012). By virtue of its larger torsional space, glycine, just like proline, can introduce kinks into tight spaces within a protein. Glycine also promotes helix-helix interactions, and can serve as a helix-breaker at the beginning or end of helices (O'Neil and DeGrado, 1990). Cumulative in effect, these characteristics of glycine promote stability in the crucial secondary and tertiary structures of a protein.

There are stark differences functionally and structurally between glycine and arginine residues. Arginine is both polar and charged. This means it is able to form salt bridges with negatively-charged residues, as well as intra-helical and inter-helical hydrogen bonds with other polar amino acids. These unsolicited interactions could disrupt the protein's secondary structure and natural conformation. In the glycophorin transmembrane receptor, an amino acid change from glycine to a larger amino acid in the α-helix region was reported to disrupt receptor dimerisation (Lemmon et al., 1994; Javadpour et al., 1999) Consistent with this study, there have been other reports of substitutions of glycine amino acids in the GH protein sequence. A glycine to serine substitution resulting from a c.265A>G transition (p.Gly89Ser), not observed in this study, was reported for the Serra de Estrela sheep breed (Marques et al., 2006).

Past the signal peptide region into the alpha helices, another substitution occurred at a conserved position. An arginine residue was putatively replaced with a histidine residue at amino acid position 60. This was brought about by a guanine to adenine transition at DNA position 179 (c.179G>A) of exon 3. Although this position is conserved across most mammalian species explored, multiple alignment analyses revealed a precedence for a substitution at this site, with arginine-60 being replaced with a glutamic acid residue (p.Arg60His) in humans. The sheep finding is also similar to the findings of Gupta et al.

(2007) in Black Bengal goats, where an amino acid substitution of arginine with histidine, encoded for by a substitution in exon 4 of caprine GH was reported.

The p.Arg60His variant, was only observed for the D_3 variant of ovine *GH*. Although arginine and histidine are both basic amino acids with positively charged sidechains, arginine is aliphatic whereas the sidechain of histidine is cyclic in nature. Protein structure prediction using online prediction software, postulated disruption in the ionic interactions between the wild-type arginine-60, and two other residues - aspartic acid at position 53, and glutamic acid at position 59.

Growth hormone is pleiotropic in its function, in that it is capable of stimulating both anabolic (growth-promoting) and catabolic (growth-antagonising) processes. Catabolically, GH promotes lipolysis, while also promoting anabolic growth (Bergan-Roller and Sheridan, 2018). Whether growth or lipolysis is induced, is dependent upon the signalling pathways activated upon GH binding to its receptor. GH has been reported to induce chondrogenesis in cartilage and longitudinal bone growth.

The only synonymous variant found in this study was c.51C>T (p.Leu17Leu) in exon 2, while intron 2 presented with six non-coding variations. These variations were found to show associations with growth and carcass traits in NZ Romney sheep. The variations could have impacted sheep phenotypic traits by influencing *GH* protein folding or stability, rate of transcription and translation, or by impacting mRNA stability and the rate and efficiency of transcription and translation (Cartegni et al., 2002; Le Hir et al., 2003).

From a commercial perspective, the findings of this study show potential profitability. Farmers and producers in NZ are paid on average $6 per kg of lamb carcass, and about 27 million carcasses were exported in the 2018 – 2019 period to yield an estimated revenue of $3.23 billion (Ministry of Primary Industries, 2019). The B_3 variant of ovine *GH* was associated with a 0.41 kg increase in hot carcass weight, which would putatively generate an extra $2.5 per carcass. This means that if the B_3 variant were incorporated into all flock, assuming all lambs responded in a similar fashion to this study, the small genetic gain could translate to a $67.5 million increase in sheep meat revenue.

5.5 Conclusion

This study detected novel variations in ovine *GH* which showed associations with growth and carcass traits in sheep. These findings are consistent with research in other sheep breeds and animal species, and hence could be valuable for use as potential markers in marker-assisted breeding programs with the goal of breeding faster-growing sheep with higher-yielding lean carcasses.

Chapter 6
Analysis of *POU1F1* variation across different NZ sheep breeds

6.1 Introduction

The POU class 1 homeobox 1 (POU1F1) protein, also called pituitary-specific transcription factor 1 (PIT-1), plays an essential role in the regulation of growth and development in vertebrates. The POU1F1 protein regulates the development of three pituitary cell types which secrete endocrine hormones, namely somatotropes (growth hormone, GH), thyrotropes (beta subunit of thyroid-stimulating hormone, TSH-β), and lactotropes (prolactin, PRL), respectively (Bodner et al., 1988; Mangalam et al., 1989).

Growth hormone regulates growth and development in mammals by promoting gene expression and protein synthesis. As a glycoprotein, TSH can interact with the thyroid gland via its G-protein coupled TSH receptor (TSHR) and prompt it to release triiodothyronine (T3) and thyroxine (T4) (Pierpaoli and Besedovsky, 1975). Both T3 and T4 act as potent regulators of growth, metabolism rate, heart rate, brain development and body temperature (Labad et al., 2016). Metabolic syndromes have been reported to result from TSH abnormality, including a single nucleotide substitution at the 29th amino acid of TSH-β (Hayashizaki et al., 1989).

PRL promotes milk production in lactating female mammals. Its biological function is not limited to lactation, however, as PRL also plays an important role in homeostasis and immune system regulation (Wallis, 1988).

6.1.1 POU1F1 structure and function

In sheep, *POU1F1* is a 17 kb gene located on the long arm of chromosome 1 (1q21-q22), consisting of six exons and five introns and encoding a 33 kDa protein (Woollard et al., 2000) (Figure 6.1). The coding region of ovine *POU1F1* is highly conserved as evidenced by its similarity with the bovine (98.2%), human (91.2%) and murine (86.2%) counterparts (Bastos et al., 2006b). POU1F1 is a positive regulator of GH, PRL and TSH-β in mammals, and is crucial to the differentiation and survival of three anterior pituitary cell types: thyrotropes, somatotropes and lactotropes (Cohen et al., 1996).

The POU1F1 protein belongs to the POU-domain family of transcription factors which share a common N-terminal transactivation domain (TAD), and a C-terminal POU-specific domain (POU_{SD}) and POU-homeodomain (POU_{HD}). The TAD domain is crucial for the transactivation of target genes – GH, TSH and PRL, while high-affinity DNA binding is ensured by the POU_{SD} and POU_{HD} regions (Bastos et al., 2006a).

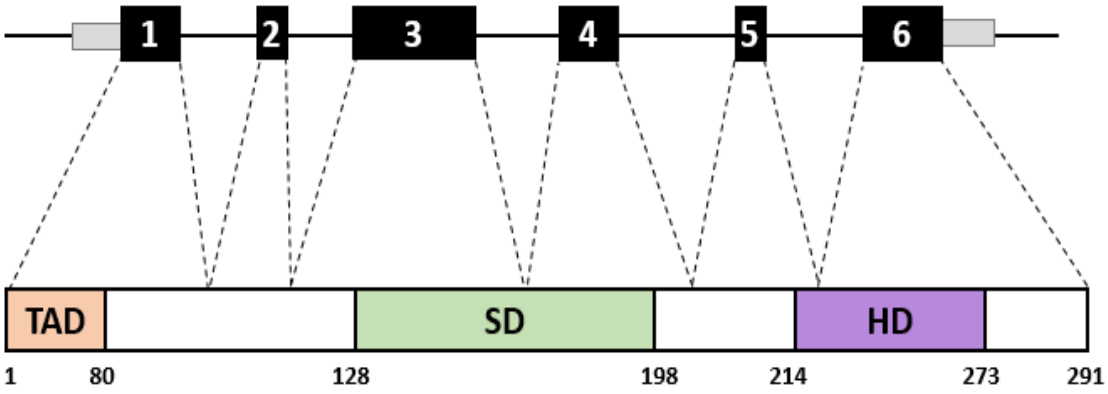


Figure 6.1 Diagrammatic alignment of POU1F1 gene and protein sequences showing peptide domains

The POU1F1 gene and the corresponding protein sequence with amino acid position numbers marking functional domain regions. TAD = Transactivation domain; SD = POU-specific domain; HD = POU-homeodomain. The TAD domain is located between amino acids 1 - 80, the specific domain between amino acids 128 - 198, and the homeodomain between amino acids 214 - 273.

The POU class of homeobox genes has been documented for its role in transcription and gene expression. The acronym POU (Pit-Oct-Unc) is derived from three mammalian genes whose products, the pituitary-specific factor 1 (Pit-1), octamer transcription factor (Oct-1 and Oct-2), and *Caenorhabditi elegans* factor (Unc-86), were found to share a common homologous region, later named the POU domain (Herr et al., 1988). The POU domain is a bipartite DNA-binding domain consisting of two subdomains, namely the N-terminal 75-amino acid POU-specific domain (POU_{SD}) and the 60-amino acid C-terminal POU-homeodomain (POU_{HD}), which are separated by a short linker region (Sturm and Herr, 1988) (Figure 6.1). Both subdomains are required for the high-affinity sequence-specific DNA binding of POU domain-containing transcription factors (Skowronska-Krawczyk et

al., 2016). Basic amino acid residues found in clusters in both POU_{SD} and POU_{HD} are essential to the function of the transcription factor.

Structural domains are an integral part of many proteins. A protein domain is an independent evolutionary unit of a given protein sequence, which can exist and function independently of the remaining sequence; and each domain can often be independently stable and folded into a compact 3D structure (Kissinger et al., 1990; Wegner et al., 1993). Some proteins have one protein domain, while others have multiple domains. The same domain may appear in a variety of proteins with distinct functions. Recombination of domains in different arrangements can produce functionally-different proteins and allow for molecular evolution (Banerjee-Basu and Baxevanis, 2001). By virtue of their independent stability, genetic engineering can be employed to swap domains between proteins to yield chimeric proteins.

The homeobox gene domain is a conserved DNA motif of about 180 bp in length, which was first described in Drosophila in the early 1980s (McGinnis and Krumlauf, 1992). The homeobox domain encodes the homeodomain protein, which has been reported to play a crucial role in the anatomical development of multicellular organisms. The homeodomain folds into a characteristic helix-turn-helix (HTH) structure, which can be a di-helical or tri-helical bundle consisting of alpha helices connected by a short loop turn that interact to create a DNA-protein interface (Sturm and Herr, 1988). For a tri-helical HTH motif, the third helix, which is the C-terminal recognition helix, aligns with and binds to the major groove of target DNA via a number of hydrogen bonds and hydrophobic interactions between specific amino acid sidechains and exposed bases in the DNA; whereas, the first two N-terminal helices stabilise the DNA-protein structure (Kissinger et al., 1990). The HTH motif in the homeobox shares some similarities in sequence and structure to various DNA-binding proteins, such as the cro repressor family of proteins.

During the later stages of pituitary development, around embryonic day 13, *POU1F1* is specifically expressed by the thyrotrope, somatotrope and lactotrope cell types of the anterior pituitary (Bodner et al., 1988; Kelberman et al., 2009a). POU1F1 promotes the proliferation and terminal differentiation of these cell populations. By means of its POU-homeodomain and POU-specific domain, POU1F1 binds with high affinity to regulatory regions on DNA sequences within these cells (Skowronska-Krawczyk et al., 2016). POU1F1 positively regulates the gene expression and synthesis of endocrine hormones, namely GH (somatotrope), TSH (thyrotrope) and PRL (lactotrope), within these anterior

pituitary cell populations (Pfäffle et al., 1992). Although, *POU1F1* expression climaxes around embryonic day 16, it is still maintained within these pituitary cell lineages, well into adulthood (Bodner et al., 1988).

6.1.2 *POU1F1* variations

Sequence variations in *POU1F1* were first described in dwarf mice. In this respect, the Snell dwarf and Jackson dwarf mice have been studied extensively. The Snell dwarf mouse is characterised by an amino acid substitution of tryptophan for cysteine at position 261 (p.Try261Cys) in the POU-homeodomain, resulting from a guanine to thymine mutation in murine *POU1F1* sequence (Li et al., 1990; Hendriks-Stegeman et al., 2001). This variation abolishes the ability of POU1F1 to bind to target genes, and ultimately results in a lack of somatotropes, lactotropes and thyrotropes.

On the other hand, the Jackson dwarf mouse is characterised by a complicated rearrangement of *POU1F1*, leading to a dysfunctional protein with a complete loss in its DNA-binding capacity (Li et al., 1990; Parks et al., 1999).

In humans, a substitution of arginine for tryptophan at position 271 (p.Arg271Trp), in the conserved POU-homeodomain acts as a dominant inhibitor of POU1F1 function, and presents with deficiencies in the endocrine hormones, GH, PRL and TSH (Kishimoto et al., 2003; Turton et al., 2005). Furthermore, an amino acid replacement of phenylalanine for cysteine at position 135 (p.Phe135Cys) in the POU-specific domain has been shown to decrease the transactivation of target genes by *POU1F1*, in cultured HeLa cell-lines (Vallette-Kasic et al., 2001b). Subsequent structural modelling revealed that the loss of function could have been due to the disruption of crucial intermolecular interactions between the wild-type phenylalanine residue and other proteins in the transcriptional machinery. Several polymorphisms have been described in the *POU1F1*, with some associated with production traits in mammals (Di Stasio et al., 2002; Huang et al., 2008).

Bastos et al. (2006b) described the first *POU1F1* polymorphisms in sheep. These included four sequence variations in the Churra da Terra Quente Portuguese sheep breed: a *G* to *A* transition in exon 2 that leads to a cysteine to tyrosine amino acid change at codon 58 (p.Cys58Tyr), two *G* to *A* transitions in exon 3 that resulted in the replacement of glycine with asparagine at position 89 (p.Gly89Asn), and the substitution of alanine for threonine at position 105 (p.Ala105Thr), and an *A* to *G* transversion in intron 4 (Bastos et al., 2006b).

These variations were confirmed by subsequent studies in ovine *POU1F1* (Sadeghi et al., 2014).

Seven nucleotide sequence variations were detected in Turkish sheep breeds, including a *T* to *C* transition in exon 6, and a *G* to *A* transition in the 3' UTR of ovine *POU1F1*; and they were found to be associated with milk fat, milk lactose and milk yield (Ozmen et al., 2014). Also showing associations with milk yield, fatty acid and protein content in Sarda goat breeds, were nine variations reported in the coding regions of caprine *POU1F1*. These variations included *G* to *C* and *G* to *A* substitutions in exon 1, a *C* to *T* transition in exon 3, and an *A* to *G* transition in exon 4 of caprine *POU1F1* (Daga et al., 2013).

Given its broad functionality, variation in *POU1F1* could not only alter its own physiological activity, but consequently affect its target genes and exert profound effects on growth, homeostasis and metabolism. This makes it a good candidate for investigating the association with growth and carcass traits in sheep. This research aims to search for sequence variation in ovine *POU1F1* in NZ sheep breeds. Exons 1, 3 and 6 of ovine *POU1F1* were chosen for exploration because their sequences encode the POU1F1 transactivation domain, POU-specific domain, and POU-homeodomain respectively.

6.2 Materials and Methods

6.2.1 Collection and purification of blood samples

Blood samples used in this investigation were randomly collected from 100 lambs from two different NZ breeds, namely Romney (n = 50) and Merino (n = 50). Exploring genetic diversity was the purpose of the samples used in this study; phenotypic data was not available for these samples.

The genomic DNA from the FTA blood samples was purified using a two-step procedure. This involved a cell and protein denaturation with 20 mM NaOH at 60 ºC heat for 30 min, and subsequent washing with 1 × TE buffer for 5 min, as outlined by Zhou et al. (2006).

6.2.2 Primer design and PCR analysis

Based on the accession number NC_019458.2 obtained from the NCBI database, three primers were designed to amplify coding and non-coding regions of ovine *POU1F1*. The

ideal PCR and SSCP conditions for each primer set were established empirically following multiple optimisation experiments.

For a start, PCR optimisations entailed low stringency PCR with standard temperatures and reagent concentrations. The annealing temperature was subsequently raised in stepwise increments of 1 ºC until a single band was observed on agarose gel electrophoresis. This was done to curb non-specific amplification. Similar increments in a stepwise fashion were employed to the reagents used until the best results were obtained. The *POU1F1* primer details and PCR-SSCP running conditions are stipulated in Table 6.1.

Table 6.1 *POU1F1* primers and PCR-SSCP conditions

Gene Region	Forward/reverse primers Oligonucleotide (5' to 3')	Amplicon size	Annealing temp (ºC)	SSCP conditions
Exon 1	**F:** TGAGATCTGAAACGGCCCTT **R:** ACAAGATTCAAAGCACCCATCC	301 bp	58 ºC	12%, 280 V, 20 ºC, 18 h
Exon 3	**F:** ACTGGCCTTCACAGAACAATC **R:** GACTTTGCAGATGGGGTTGT	365 bp	58 ºC	12%, 280 V, 20 ºC, 18h
Exon 6	**F:** CATCTCCCTTCTTCTTTCCTGC **R:** AGAAAAGCTCGCTGTTACACA	281 bp	58 ºC	12%, 280 V, 20 ºC, 18 h

PCR amplifications were performed in 15 µL reactions containing the purified genomic DNA on a 1.2 mm diameter disc of FTA, 0.25 µM of each primer, 150 µM of each dNTP (Eppendorf, Hamburg, Germany), 1.5 mM Mg^{2+}, 0.5 U *Taq* DNA polymerase (Qiagen, Hilden, Germany), and 1 × the reaction buffer supplied with the enzyme. Amplifications were undertaken in Bio-Rad S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA). The thermal profile entailed an initial denaturation at 94 ºC for 2 mins, followed by 34 cycles of 94 ºC for 30 s, annealing at 58 ºC for 30 s, and extension at 72 ºC for 30 s, and a final extension step at 72 ºC for 5 min.

Subsequent visualisation of the amplicons from the PCR reaction were carried out by means of agarose gel electrophoresis using 1% agarose (Quantum Scientific, Queensland, Australia) gels containing 1 × TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA), 200 ng/mL ethidium bromide. 2 µL aliquots of the PCR products were mixed with 2 µL of loading dye (0.2% bromophenol blue, 0.2% xylene cyanol, 40% (w/v)

sucrose), and loaded into the gels that were run at a constant 5 V/cm for 2 hours. After electrophoresis, the gels were visualised using ultraviolet transillumination at 254 nm.

6.2.3 Single-stranded conformational polymorphism (SSCP) analyses

Optimisations for SSCP involved running amplicons at various conditions and using different gel compositions until consistently clear banding patterns were obtained.

Following PCR, 0.7 µL of each PCR product was mixed with 7µL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). The samples were denatured at 105 ºC for 5 min, snap-chilled on ice, and 10 µL of each sample was loaded into 16 x 18 cm, 10% acrylamide:bisacrylamide (37.5:1, Bio-Rad) gels in Protean II xi cells (Bio-Rad). The samples were run at a voltage of 280 V at 12 ºC for 18 hours.

Silver-staining of the gels was performed according to the method described by Byun et al. (2009). Briefly, the gels were silver-stained with silver nitrate, washed with distilled water, and subsequently developed using a solution containing sodium hydroxide and 40% formaldehyde. Nucleotide sequencing was not performed due to insufficient variation detected.

In order to ensure the integrity of the experiments and to obtain reproducible banding patterns, freshly prepared PCR and SSCP products were always used. A negative control well containing no DNA was also run with each series of amplifications.

6.2.4 Statistical analyses

Variant and genotypic frequencies were calculated using the PopGene 3.2 software. The variant frequencies were then tested for deviation from Hardy-Weinberg equilibrium using a Chi-square test available on the Online Encyclopedia for Genetic Epidemiology Studies (OEGE) (Rodriguez et al., 2009).

Association studies were not performed due to insufficient variation, and phenotypic data was not available for the samples used.

6.3 Results

6.3.1 *POU1F1* gene variation

Three segments of ovine *POU1F1* were amplified and investigated for genetic variations in two NZ sheep breeds (Figure 6.2). No variations were detected in the amplicons of the exon 3 and exon 6 regions encoding the POU-specific domain (POU_{SD}) and POU-homeodomain (POU_{HD}) respectively.

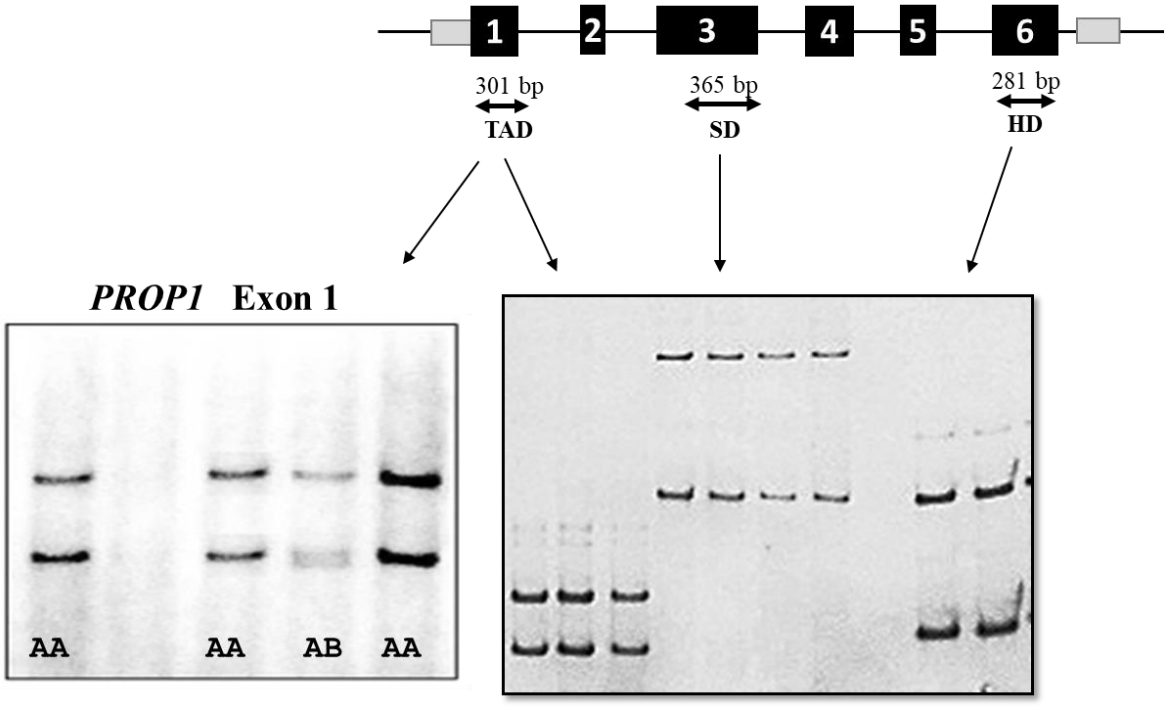


Figure 6.2 PCR-SSCP results of ovine *POU1F1* amplicons

Stylistic map of the ovine *POU1F1* gene indicating amplified regions with domains, and their resulting gel patterns from polymerase chain reaction-single-stranded conformational polymorphism. The three regions amplified were exon 1 (301 bp), exon 3 (365 bp) and exon 6 (281 bp). TAD = Transactivation domain; SD = POU-specific domain; HD = POU-homeodomain.

The 301 bp fragment amplifying the exon 1 of ovine *POU1F1* showed limited variation with two variants, *A* and *B* (Figure 6.2). The *B* variant could not be isolated, and hence nucleotide sequencing was not performed.

6.3.2 *POU1F1* genotype and variant frequencies

The genotypic frequencies of ovine *POU1F1* variants across Romney and Merino NZ sheep breeds are given in Table 6.2.

Table 6.2 Allelic and genotype frequencies of ovine *POU1F1* exon 1 in NZ Romney sheep

Gene (Breed)	Variant	Variant frequency	n	Genotype	Genotype frequency	HWE[1]
POU1F1	A	0.89	39	AA	0.78	$\chi^2 = 0.76$
(Romney)	B	0.11	11	AB	0.22	P = 0.383
			0	BB	0.00	
POU1F1	A	0.82	32	AA	0.64	$\chi^2 = 2.41$
(Merino)	B	0.18	18	AB	0.36	P = 0.120
			0	BB	0.00	

[1] HWE: Hardy-Weinberg Equilibrium

Fifty samples were investigated for the Romney breed, and another fifty for the Merino breed. For both sheep breeds, *A* was the major variant, with a frequency of 89% (Romney) and 82% (Merino), while the *B* variant had a smaller frequency of 11% (Romney) and 18% (Merino), respectively. The variant frequencies did not deviate from the Hardy-Weinberg equilibrium (P > 0.05). The *AA* homozygous genotype was predominant with a frequency of 78%, while the *BB* genotype (0%) was not observed.

6.4 Discussion

Genes that play essential roles in the early growth and development of an organism tend to be evolutionarily conserved, as sequence variations within them could have severe functional consequences, or even be lethal to the organism. The POU1F1 gene falls within this class of 'essential genes', as it is pivotal to mammalian embryogenesis and the prenatal development of organs.

In this study, the ovine *POU1F1* was screened for nucleotide sequence variation in the NZ Romney and Merino sheep breeds. Variation was not found in the exons 3 and 6 of ovine *POU1F1*, while exon 1 had limited variation with two variants, *A* and *B*, occurring at frequencies of 0.89 and 0.11 in Romney, and 0.82 and 0.18 in Merino, respectively. These

findings are unsurprising, as previous studies point to the strong conservation of ovine *POU1F1* evidenced by the similarity with its bovine (98.2%), human (91.2%) and murine (86.2%) counterparts (Bastos et al., 2006b).

The POU1F1 gene belongs to a family of homeobox genes involved in early embryonic development of pituitary cell types. The homeobox is a 180 bp DNA sequence that encodes a homeodomain that consists of 60 amino acid residues that are arranged into three alpha helices. By means of its helices, the homeodomain is able to bind to specific sequences in target DNA, altering its function or expression. Homeodomains, therefore, confer upon transcription factors their unique role in regulating gene expression.

By means of its POU-homeodomain, encoded in the exon 6 of the gene, POU1F1 is able to bind to regulatory and enhancer elements within the promoter and 3' UTR of target genes, including GH, PRL and TSH (Skowronska-Krawczyk et al., 2016). Genetic variations that alter the homeodomain can, therefore, be detrimental. It is therefore logical that there will be strong selection pressure against nucleotide sequence variations in the exon 6 of ovine *POU1F1*, which might explain the findings of this study.

In a similar fashion, the POU-specific domain assists the POU-homeodomain to bind to target DNA with high affinity and with the correct orientation. The POU-specific domain also interacts with the transcriptional machinery including co-activators and repressors, to ensure successful gene regulation; hence, this domain is crucial to POU1F1 function (Skowronska-Krawczyk et al., 2016). The POU-specific domain is encoded in the exon 3 of ovine *POU1F1*, within which no variation was observed in this study.

Given its function in the proliferation and differentiation of pituitary somatotropes, lactotropes and thyrotropes, sequence variations in *POU1F1* would not only affect its protein, but would exert a cascading downstream effect on the production of vital endocrine hormones including GH, PRL and TSH. Impaired pituitary function (Radovick et al., 1992; Salemi et al., 2003), inhibited cognitive function (Snabboon et al., 2008), and postnatal growth failure (Turton et al., 2005), have been reported as pathological phenotypes in humans with *POU1F1* mutations.

The findings of this study of no variation in exons 3 and 6 of ovine *POU1F1* are opposed to some reports in other sheep breeds. Bastos et al. (2006b) reported two *G* to *A* nucleotide variation in the exon 3 of *POU1F1* resulting in the amino acid changes, p.Gly89Asn and p.Ala105Thr, in Portuguese sheep breeds. Ozmen et al. (2014) reported a *T* to *C*

substitution in the exon 6 of ovine *POU1F1*, which showed association with milk traits in Turkish sheep breeds.

Variations do, however, occur within 'essential' genes, as they can be a source of functional divergence required for the evolution of genes and species. Nonetheless, these variations tend to occur in the non-coding regions of the DNA; or if they occur within the coding sequence, they are likely synonymous and do not lead to amino acid changes.

6.5 Conclusion

Although this study found limited variation in ovine *POU1F1*, the gene remains a good candidate gene to be explored for association with variation in sheep growth and carcass traits. Future studies could screen a larger number of sheep across multiple breeds, in the search for variation within *POU1F1*. Focus could be directed towards examining the non-coding regions of *POU1F1*, such as the introns, 3' UTR or 5' UTR regions, as they would likely be subject to less selection pressure than coding sequences that encode functional domains.

Chapter 7
Characterisation of ovine *PROP1* variation and its association with growth and carcass traits

7.1 Introduction

PROP paired-like homeobox 1 (PROP1) is a pituitary-specific paired-like homeodomain transcription factor that plays a crucial role in the development of the pituitary gland during embryonic development. The pituitary gland, also called the hypophysis, is a vital component of the neuroendocrine system, and serves as a mediator between the activities of the hypothalamus and peripheral target tissues (Zhu et al., 2007). It is often referred to as a "master gland" due to its role in the regulation of other endocrine glands, as well as modulating various physiological processes such as growth, metabolism, stress response, lactation and reproduction.

The mature pituitary gland consists of three lobes organised into two distinct entities: the anterior and intermediate lobes make up the adenohypophysis, while the posterior lobe makes up the neurohypophysis (Scully and Rosenfeld, 2002). The adenohypophysis arises from the oral ectoderm, and is mainly epithelial-containing endocrine cells that secrete a variety of hormones. The neurohypophysis is a collection of neural endings and supporting cells that do not secrete but instead store hormones from the infundibulum.

7.1.1 PROP1 structure and function

The PROP1 gene is 681 bp long, and it is comprised of three exons interspersed by two introns (Figure 7.1). The ovine *PROP1* is located on chromosome 5 and encodes a 226 amino acid protein. PROP1 has three major regions: the amino terminus (amino acids 1 - 68), the homeodomain (amino acids 69 - 128), and the carboxyl terminus (amino acids 129 - 226) (Osorio et al., 2000). The carboxyl-terminus possesses a transactivation domain that binds to and activates target genes, whereas the amino-terminus and homeodomain possess other DNA-binding and repressive capacities (Sornson et al., 1996; Showalter et al., 2002; Zhu et al., 2007).

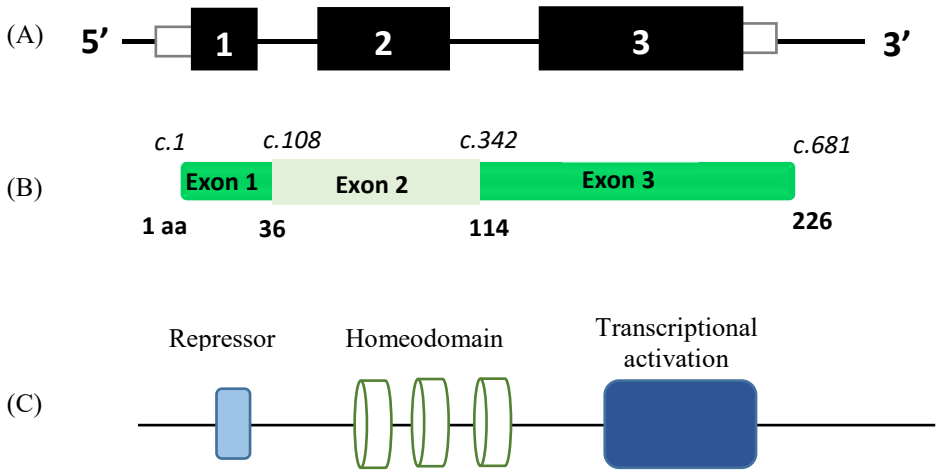


Figure 7.1 Schematic diagram of PROP1 gene and protein structure

(a) The three exons and two introns of *PROP1*. The black boxes represent the coding regions, the white boxes represent untranslated regions (UTR), and the single lines represent intronic regions. (b) The PROP1 amino acid structure showing the amino acid positions (bold case, bottom numbers) at exon boundaries, and the corresponding cDNA numbers (italics, top numbers). (c) The various domains of PROP1 including the amino-terminus repressor domain, the homeodomains, and the carboxyl-terminus transcriptional activation domain.

PROP1 has a unique function, in that it is both a transcriptional activator and a repressor. The negative and positive transcriptional activities of PROP1 depend on its pairing with other transcription factors and partner proteins (Showalter et al., 2002). Despite the evolutionarily divergent nature of the amino-terminus, it is, nevertheless, likely that amino acid residues vital to its repressive activity have been retained (Figure 7.1). This divergent nature could also serve another useful function by conferring upon PROP1 a species-specific functionality. In contrast, the highly conserved nature of most homeodomains suggests a strong selective pressure for the maintenance of their exact DNA-binding specificities (Berger et al., 2008).

PROP1 is a homeobox gene, and its product is required for the proliferation and differentiation of the hormone-producing cell types of the anterior pituitary. The homeobox genes are a family of regulatory genes that are expressed in a spatial and temporal manner during the anatomical development of vertebrates (Wolberger, 1996). The homeobox itself is a highly conserved 180-base-pair DNA sequence that encodes a compact 60-amino-acid homeodomain (Figure 7.2). These homeodomains regulate crucial biological processes by

binding to specific regulatory regions of the DNA, such as enhancers and promoters. The homeodomain comprises of a helix-turn-helix (HTH) structure, with which it binds to target DNA and brings about gene activation or repression (Bobola and Merabet, 2017). The HTH motif is formed by two alpha helices (helices 2 and 3) connected by a short amino acid loop. DNA binding by the second helix is mediated by hydrogen bonds and hydrophobic interactions between exposed bases on the target DNA and specific side-chains on the helix (Wolberger, 1996). Another helix, helix 1, stabilises the binding structure.

Homeodomains can often have dual functionality by binding DNA and also facilitating protein-protein interactions (Berger et al., 2008; Inukai et al., 2017). PROP1 possesses a paired-like homeodomain, and homeodomains within transcription factors regulate the activities of other genes by binding to DNA. To exert their gene expression function, the transcription factors (trans-elements) must interact with regulatory sequences such as promoters and enhancers (cis-elements). Some of the more common homeodomain classes include paired/Pax, POU, HOX and LIM, and these homeodomains have often been found to interact with one another (Bobola and Merabet, 2017).

(a)

```
SHEEP_PROP1 MDTEGRSEQAKQAKEQVCSSLWPEGYPAAETVTSSVDMNTQPYRNLSGVR     50
HUMAN_PROP1 -ea-r-rqae-pk-gr-g---l--rh--tg-p-tt--ssap-c-r-p-ag     50
MOUSE_PROP1 -eaqr--h-e--t-gha-grsl--prv-sg-li-t--rssea--r---te     50
  RAT_PROP1 -eaqr--q-e--t-gp--grsl--sqa-sg-li-t--rspetskr---tg     50
  PIG_PROP1 -ea---r--g-pr-gr-----------g-l-ar--is-r--------g     50
  DOG_PROP1 -ea-----rg--k-grl-g----ds----g-l--t--vsp--sk-----g     50
  COW_PROP1 -----s---g--t-------------------------------------     50
 GOAT_PROP1 -------------------------------------------------     50

SHEEP_PROP1 VGRPKLSLQGGQRGRPHSRRRHRTTFSPAQLEQLESAFGKNQYPDIWARE    100
HUMAN_PROP1 g--srf-p--------------------v----------r----------    100
MOUSE_PROP1 l-----cp-...--------------n------------r----------     97
  RAT_PROP1 l-----cp-...--------------n----g-------r-------v--     97
  PIG_PROP1 a---r--p-------------------------------r----------    100
  DOG_PROP1 -r--r--p-------l----------n-g------t---r----------    100
  COW_PROP1 ----------v---------------------------r----------    100
 GOAT_PROP1 a-------------------------------------------------    100

SHEEP_PROP1 SLAQDTGLSEARIQVWFQNRRAKQRKQERSLLQPLAHLSPATFSGFLPEP    150
HUMAN_PROP1 ---r-------------------------------------a--s----s    150
MOUSE_PROP1 g---------------------------------i----t---------s    147
  RAT_PROP1 g--------------------------------t-----------s-s    147
  PIG_PROP1 g--r-----------------------------------------------    150
  DOG_PROP1 g--r-------------------------------------------s    150
  COW_PROP1 -------------------------------------------------    150
 GOAT_PROP1 -------------------------h----------------------    150

SHEEP_PROP1 PSCPYPYPTPPPPMTCFPHPYNHALPSQPSTGSSFARPYQSEDWYPNLHP    200
```

```
HUMAN_PROP1 ta---s-aa----v-------s----------ga--lsh-------t---    200
MOUSE_PROP1 say--t-g-----ap------s-s-------aa-l-l-p-p-----t---    197
  RAT_PROP1 spy--t-t-----vp--------------c--a-ltl-a-p-----t---    197
  PIG_PROP1 -a---s--------------------------g----hp-------t---    200
  DOG_PROP1 -a---s--------------------------g-----h-------t---    200
  COW_PROP1 -----s-------------------------------------------    200
 GOAT_PROP1 -------------------------------------------------    200

SHEEP_PROP1 TPAGHLPCPPPPPMLPLSLEPPKSWN                          226
HUMAN_PROP1 a--------------------s----                          226
MOUSE_PROP1 a-t-----------f-----t-----                          223
  RAT_PROP1 -ht---a-------fs----t-----                          223
  PIG_PROP1 --t-------a--v------------                          226
  DOG_PROP1 p-t---------t-------------                          226
  COW_PROP1 ------a-------------------                          226
 GOAT_PROP1 -----------------g--------                          226
```

(b)

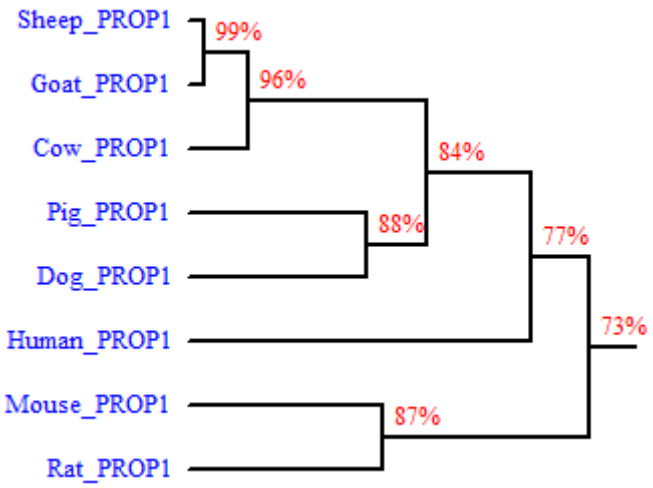


Figure 7.2 Multiple sequence alignment and homology tree of PROP1 protein sequences from different species

(a) Multiple sequence alignment of PROP1 protein sequences from different species using the MEGA Align software. Dashes (-) denote nucleotides identical to the Sheep_PROP1 sequence, while dots (.) denote missing nucleotides. The homeodomain is highlighted (amino acids 69 – 128).

(b) Homology tree of PROP1 protein sequences from various species. The dendogram depicts the relationships between mammalian PROP1 proteins, and was constructed using the multiple alignment algorithm in the DNAMAN package (DNAMAN 5.2.10, Lynnon Biosoft), and. Numbers represent the percentage of identities between the indicated pairs. The amino acid sequences were extracted from Genbank with the following accession numbers: Human, *Homo sapiens* (AF076215); Sheep, *Ovis aries* (AY533709); Goat, *Capra hircus* (XM_005682787); Cow, *Bos taurus* (XM_015472155); Pig, *Sus scrofa* (XM_013987527); Dog, *Canis familiaris* (NM_001020807); Rat, *Rattus norvegicus* (XM_008767664) and Mouse, *Mus musculus* (U77946).

PROP1 homeodomain binds DNA via its helix motifs (Figure 7.3). Being sequence-specific, PROP1 is also capable of recognising and binding to various palindromic TAAT motif sequences (two motifs separated by three nucleotides) with a varying range of binding affinities (Wilson et al., 1993; Kato et al., 2010). However, the PROP1 homeodomain preferentially and conventionally binds to its consensus binding sequence 5'-*TAATTGAATTA-3'*, termed PRDQ9, located in the promoter or enhancer regions of the DNA targets (Nakayama et al., 2009). PROP1, like most homeodomain proteins, bind DNA as a cooperative dimer formed with itself (homodimer) or other transcription factors (heterodimer). PROP1 mostly binds DNA as a dimer formed by intermolecular interactions between the Ile-96 in one PROP homeodomain and the Arg-71 in the other partner homeodomain, as well as hydrophobic interaction between the Ala-111 residues on both dimer proteins (Wilson et al., 1995; Nakayama et al., 2009) (Figure 7.3).

	Helix 1			Helix 2		Helix 1	
	p69	p78	p91	p96	p106	p110	p128
	1	10	23	28	38	42	60
Sheep	RRRHRTTFS	PAQLEQLESAFGK	NQYPDI	WARESLAQD	TGLS	EARIQVWFQNRRAKQRKQE	
Human	RRRHRTTFS	PVQLEQLESAFGR	NQYPDI	WARESLARD	TGLS	EARIQVWFQNRRAKQRKQE	
Mouse	RRRHRTTFN	PAQLEQLESAFGR	NQYPDI	WAREGLAQD	TGLS	EARIQVWFQNRRAKQRKQE	
Rat	RRRHRTTFN	PAQLGQLESAFGR	NQYPDI	WVREGLAQD	TGLS	EARIQVWFQNRRAKQRKQE	
Pig	RRRHRTTFS	PAQLEQLESAFGR	NQYPDI	WAREGLARD	TGLS	EARIQVWFQNRRAKQRKQE	
Dog	RRRHRTTFN	PGQLEQLETAFGR	NQYPDI	WAREGLARD	TGLS	EARIQVWFQNRRAKQRKQE	
Cow	RRRHRTTFS	PAQLEQLESAFGR	NQYPDI	WARESLAQD	TGLS	EARIQVWFQNRRAKQRKQE	
Goat	RRRHRTTFS	PAQLEQLESAFGK	NQYPDI	WARESLAQD	TGLS	EARIQVWFQNRRAKQRKHE	

Figure 7.3 Diagrammatic representation of helices in PROP1 homeodomain

PROP1 homeodomain is divided into segments of three helices, with numbering in reference to the homeodomain position (bottom row) or the PROP1 amino acid position (top row). Letters in red represent residues in the DNA binding sites of the major and minor groove.

Electrophoretic mobility assays have demonstrated that the PROP1 homodimer binds preferentially to an inverted TAAT motif possessing a T nucleotide immediately 3' to the motif (Nakayama et al., 2009; Kato et al., 2010). X-ray crystallography studies revealed that certain conserved residues within the homeodomain are required for binding to the major and minor grooves of target DNA (Table 7.1) (Wilson et al., 1995; Nakayama et al., 2009). The paired homeodomain typically lodges its third α-helix into the major groove of the DNA, while the adjoining minor groove binds to its N-terminal arm (Wolberger, 1996).

Table 7.1 PROP1 homeodomain DNA-binding residues

DNA major groove-binding residues		**DNA minor groove-binding residues**	
HD[1] location	**PROP1 location**	**HD location**	**PROP1 location**
Arg-44	**Arg-112**	Arg-2	Arg-70
Val-47	**Val-115**	Arg-3	Arg-71
Trp-48	**Trp-116**	Arg-5	Arg-73
Gln-50	**Gln-118**	Thr-6	Thr-74
Asn-51	**Asn-119**	Tyr-25	Tyr-93
Arg-57	**Arg-125**	Arg-31	Arg-99
		Glu-42	Glu-110
		Arg-53	Arg-121

[1] HD: Homeodomain

Since the eukaryotic binding consensus sequence is typically short (4-8 nucleotides), most transcription factors have the intrinsic ability to bind to a diverse range of DNA sequences (Table 7.1). To address this issue, homeodomain sequence recognition and function are dictated in a dynamic fashion by a variety of factors such as the shape of the DNA, chromatin accessibility and landscape, methylation status of the DNA, motif-flanking regions, intermolecular interactions with transcriptional partners (dimerisation), interactions with non-DNA-binding cofactors, and combinatorial binding (Wilson et al., 1995; Todeschini et al., 2014; Inukai et al., 2017). Unique features in DNA shape such as the width of the minor groove, and parameters of rotation and twist, can enable a transcription factor differentiate between motifs and decide whether or not to bind (Todeschini et al., 2014; Bobola and Merabet, 2017).

Chromatin accessibility further fine-tunes transcriptional regulation, as transcription factors would favour consensus sequences with open and accessible chromatin arrangement (free of hindering nucleosomes), over tight and compact ones (Todeschini et al., 2014; Bobola and Merabet, 2017). This limits the number of motifs available for binding, as unnecessary binding sites are buried within condensed chromatin arrangement. Homeodomain binding to a consensus sequence is, thus, 'condition-specific'.

Transient transfection assays showed that the dimeric binding of PROP1 to a TAAT motif could bring about transcriptional activation, whereas a PROP1 monomer cannot trigger activation despite recognising and binding to a single TAAT core sequence (Nakayama et al., 2009). Dimeric PROP1 binding is more successful than monomeric binding because

the binding of the first homeodomain has been found to bend the DNA and provide an interaction surface, which in turn facilitates the binding of the second homeodomain (Wilson et al., 1995; Berger et al., 2008). These conformational changes and distortion in the DNA are vital for transcriptional activation (Wilson et al., 1993; Berger et al., 2008). Following successful binding to target DNA consensus sequence, the PROP1-dimer then recruits transcriptional machinery that brings about gene expression.

Given the highly specific nature of DNA binding, the three-dimensional conformation of the PROP1 polypeptide plays a crucial role in successful activation. Nucleotide variations in *PROP1* can alter the three-dimensional shape of the protein, and hence impair or improve binding and activation. Differing hydrophobicity and length of side chains of substituted amino acids can alter the distance between residues, and hence modify the strength of homeodomain dimerisation and binding magnitude.

7.1.2 Organogenesis and development of the pituitary gland

The pituitary gland is an organ of dual origin, as the adenohypophysis and neurohypophysis arise from two different embryonic tissues (Sheng et al., 1997). The anterior and intermediate lobes derive from the oral ectoderm while the posterior lobe originates from the neural ectoderm. Pituitary organogenesis occurs in three successive events.

In mouse embryogenesis, at E8.5 – E9.0, the oral ectoderm thickens, with a simultaneous invagination or pouching of the oral epithelium to form Rathke's pouch (Dasen and Rosenfeld, 1999; Sloop et al., 1999). The creation of this rudimentary epithelial pouch (or placode) is considered to be the first step of organogenesis. During the second phase, between E10.5 and E12.5, the ventral region of the pouch epithelial cells continues to proliferate and extend upwards, separating off from the underlying oral ectoderm to form a definitive detached pouch (Sornson et al., 1996; Treier et al., 1998). Close physical contact is maintained between Rathke's pouch and the neural ectoderm of the ventral diencephalon (called infundibulum), prior to commitment to the pituitary lineage (Dasen and Rosenfeld, 1999; Davis et al., 2010). This direct interaction between Rathke's pouch and the infundibulum is essential to facilitate the pouch's further development and the terminal proliferation of the primordium (Takuma et al., 1998).

By E15, as Rathke's pouch continues to grow and divide, epithelial cells in the ventral portion of Rathke's pouch proliferate to yield primordial endocrine cell types that occupy

the anterior pituitary lobe (Treier et al., 1998). In the final phase by E17.5, progenitor cell types differentiate to produce a spectrum of hormone-secreting cell types arranged in a spatially and temporally specific manner (Sheng et al., 1996; Sloop et al., 1999). The infundibulum ultimately becomes the posterior lobe the pituitary gland.

7.1.3 PROP1 signalling pathways during pituitary organogenesis

A wide array of transcription factors function together in a delicate network to bring about pituitary ontogenesis. The spatial and temporal control of gene expression during pituitary organogenesis is a highly orchestrated process. Proper pituitary development requires both gene activation and gene repression at suitable and precise times. During the initial stage of pituitary development, several signalling factors are secreted by the neuroepithelium of the ventral diencephalon, including bone morphogenetic proteins (BMP) 2 and 4, fibroblast growth factor 8 (FGF8), sonic hedgehog (Shh) and Wnt5a which play a crucial role in the formation of Rathke's pouch (Voss and Rosenfeld, 1992; Sheng et al., 1996; Takuma et al., 1998). Other growth factors, mainly homeobox proteins, released in the oral ectoderm that derive from Rathke's pouch include Pitx2/RIEG, Hesx1 (or Rpx), Lhx4/Gsh4, Lhx3/P-Lim, and Pitx1/P-OTX (Watkins-Chow and Camper, 1998; Kioussi et al., 1999; Sloop et al., 1999).

PROP1 has a short period of expression, appearing at E10, peaking out at E12.5, and decreasing by E14.5 (Sornson et al., 1996). PROP1 is found solely in the developing pituitary, with no evidence of expression at or beyond birth. PROP1 expression is needed for the extinction of Hesx1 expression and the activation of POU1F1. PROP1 expression peaks one day before POU1F1, and is necessary for POU1F1expression. In turn, POU1F1 expression is needed for the silencing of PROP1 (Watkins-Chow and Camper, 1998). PROP1 is also a downstream target of Notch signalling (Zhu et al., 2007). Silencing of the Notch signalling is required for the terminal differentiation of POU1F1-dependent cell lineages (Zhu et al., 2006).

PROP1 plays a vital role in the intermediate stages of pituitary development in promoting the proliferation, specification and cellular differentiation of four hormone-secreting cell lineages of the anterior pituitary. Three of these cell types are POU1F1-dependent, namely somatotropes (secreting growth hormone, GH), lactotropes (secreting prolactin, PRL), and thyrotropes (producing thyroid stimulating hormone, TSH), while gonadotropes (producing luteinizing hormone, LH, and follicle stimulating hormone, FSH) do not require POU1F1 for their emergence.

In vitro studies also show that PROP1 forms a complex with β-catenin which can act as both a transcriptional activator or repressor, depending on the context (Olson et al., 2003). The PROP1/β-catenin complex enhances the expression of POU1F1, while down-regulating the expression of Hesx1 (or Rpx). The combined action of PROP1 and Wnt/β-catenin has been found to upregulate POU1F1 expression (Gage et al., 1996; Zhu et al., 2006).

Though the expression of PROP1 in the developing pituitary is transient, its function is by no means limited. The timely activation and repression of PROP1 is necessary, since premature expression can impair anterior pituitary development, and prolonged expression impedes terminal differentiation and can result in pituitary tumours (Bartke, 1965; Sornson et al., 1996).

7.1.4 *PROP1* knock-out mouse models

The effects of *PROP1* mutations were first described in spontaneous mouse mutants, the Ames dwarf mice (*df*) (Sornson et al., 1996). A similar phenomenon is described in the Snell dwarf mice (*dw and dwJ*), where defects are the result of mutation in *Pou1f1* (Bartke, 1965). In accordance with traditional nomenclature, lower case letters are used for the murine species (*Prop1*), while upper case letters are applied to the human species (*PROP1*)

Knock-out mice models comparing cell populations in the anterior pituitary, demonstrated that proliferating cells which typically migrate from the pouch to the developing anterior pituitary were present in *PROP1* wild-type mice, but severely impaired in their mutant counterparts (Ward et al., 2005). Additionally, $PROP1^{df/df}$ mice showed a reduction in pouch growth and size when compared to $PROP1^{wt/wt}$ mice (Sornson et al., 1996).

Sornson et al. (1996) were the first to describe a naturally occurring *Prop1* mutation – a *T* to *C* transition resulting in a p.Ser83Pro substitution in the Ames dwarf mouse. This serine to proline mutation occurs at the conserved residue 83, located in the first α-helix of the homeodomain of $PROP1^{df/df}$, thus leading to a failure to activate *Pou1f1*. This in turn significantly impairs the Pou1f1-dependent somatotropes, lactotropes and thyrotropes, with less than one percent of the regular number of cell types found in circulation (Sornson et al., 1996). The resulting phenomenon of low circulating levels of GH, PRL and TSH is thereby characteristic of Ames dwarf mice ($PROP1^{df/df}$) and the Snell dwarf mice ($Pou1f1^{dw/dw}$), although more pronounced in the former (Sornson et al., 1996). Supporting this notion, a 43% decrease in transcriptional activation by the S83P-mutant has been

demonstrated using transient transfection and luciferase assays (Osorio et al., 2000). The Ames dwarf mice also present with decreased levels of gonadotrope products, LH and FSH, which is exhibited phenotypically in the form of hypothyroidism, growth retardation and infertility.

7.1.5 *PROP1* mutations in humans

Mutations in *PROP1* are the major genetic cause of combined pituitary hormone deficiency (CPHD) in humans, accounting for about 50% of familial cases and 12% of sporadic cases (Cogan et al., 1998; Wu et al., 1998). The human PROP1 gene is situated on chromosome 5q35, comprising of three exons and two introns and encodes a 226-amino acid protein (Duquesnoy et al., 1998). Till date, about 22 recessive *PROP1* mutations have been identified in humans (Table 7.2). The classic phenotype of CPHD involves deficiencies in GH, PRL, TSH, LH and FSH.

Frameshift and nonsense mutations

Some of the earliest and most commonly reported *PROP1* mutations in humans include a p.R120C substitution, a p.F117I substitution, and a c.301-302delAG deletion (Cogan et al., 1998; Wu et al., 1998). The c.301-302delAG (also called c.296delGA) mutation accounts for about 50-72% of all familial CPHD-related *PROP1* mutations identified by several distinct pedigrees in various countries, thus making it the most commonly occurring *PROP1* mutation (Cogan et al., 1998; Wu et al., 1998). All of the deletion and nonsense mutations result in a premature stop codon that truncates the protein transcript.

The two most frequent mutations are two dinucleotide deletions: c.301-302delAG and c.149-150delGA. Both of these lead to a premature stop codon, and ultimately the truncation of the predicted protein product at serine 109 residue (p.S109X) (Cogan et al., 1998; Fofanova et al., 1998; Wu et al., 1998). The truncated PROP1 products were unable to bind to DNA target sites, and hence failed at the transactivation of genes (Cogan et al., 1998; Wu et al., 1998). This is expected as the deletions occur in the second α-helix of the homeodomain, thus eliminating completely the third α-helix and the carboxyl-terminus which are required for homeodomain DNA binding and transcriptional activation respectively. Patients with the deletion mutations failed to produce LH and FSH in response to gonadotropin releasing hormone (GnRH) stimulation (Wu et al., 1998). These mutations were also linked with CPHD in Russian children, as all patients possessing them exhibited deficiencies in GH, PRL and TSH (Fofanova et al., 1998). A noteworthy finding is the unique compound heterozygosity of the two distinct deletions (c.149-

150delGA/c.301-302delGA), which was identified in 36% of the cases (Fofanova et al., 1998). The Streisinger slippage-repair model postulates that the misalignment of DNA strands during reannealing can result in insertion and deletion mutations in repeat sequences, which is a leading cause of human diseases (Streisinger et al., 1966) Based on this theory, the AG repeat sequences in exon 2 (295-GAGAGAG-302) are potential mutational hotspots for deletions and insertions. Consistent with this notion, other deletion mutations have been found within the exon 2 vicinity of the AG tandem repeat.

Other frameshift mutations caused by deletions in the exon 2 of human PROP1 include: c.112-124del-13bp, c.150delA and c.157delA (Fofanova et al., 1998; Agarwal et al., 2000; Tatsumi et al., 2004). The 13 bp deletion spanning nucleotides 112-124 and located upstream of the homeodomain, is the largest ever reported for *PROP1* (Table 7.2). The truncated transcript is predicted to have only 37 out of the 226 amino acids in a normal PROP1 protein (Agarwal et al., 2000). The mutant protein would only possess the N-terminal region with a complete loss of the homeodomain and C-terminal regions. The 150delA and 157delA mutations lead to an untimely stop codon that disrupts the homeodomain, thus impairing its abilities to bind to target DNA and activate transcription (Riepe et al., 2001; Tatsumi et al., 2004).

A nonsense mutation, caused by a c.247C>T transition, resulted in a truncation of the PROP1 transcript at the glutamine-83 residue (Voutetakis et al., 2004). Due to its location in the highly conserved first α-helix, the p.Q83X mutation led to the loss of two functional domains, the homeodomain and the transactivation C-terminal domain. The nonsense mutation predicts a product with only 82 out of the 226 amino acids in a wild-type PROP1 protein. Truncated mRNA transcripts often undergo degradation by the cell via nonsense-mediated pathways, before they can even undergo translation. Individuals possessing the p.Q83X mutation showed a lack of growth acceleration in response to GH therapy. Neonates with the mutation also presented with prolonged jaundice, which is common with GH deficiency and hypothyroidism (Voutetakis et al., 2004).

Pituitary morphology differs across patients with PROP1 mutations. In most frameshift deletion cases, the pituitary is hypoplastic and reduced in size (Duquesnoy et al., 1998; Rosenbloom et al., 1999; Osorio et al., 2000; Vallette-Kasic et al., 2001a).

Table 7.2 *PROP1* mutations in humans

Location	Nucleotide change	Amino acid change	Type of mutation	References
Exon 1	c.2T>C	No translation	Nonsense	(Lemos et al., 2006)
Intron 1	c.109+1G>T	-	Splice site	(Böttner et al., 2004)
Exon 2	c.112-124del-13bp	S480X	Frameshift	(Duquesnoy et al., 1998; Agarwal et al., 2000)
Exon 2	c.149-150delGA	S109X	Frameshift	(Fofanova et al., 1998; Deladoëy et al., 1999; Kelberman et al., 2009b)
Exon 2	c.150delA	V164X	Frameshift	(Riepe et al., 2001; Kelberman et al., 2009b)
Exon 2	c.157delA	V164X	Frameshift	(Tatsumi et al., 2004)
Exon 2	c.211C>T	R71C	Missense	(Paracchini et al., 2003)
Exon 2	c.212G>A	R71H	Missense	(Paracchini et al., 2003)
Exon 2	c.217C>T	R73C	Missense	(Deladoëy et al., 1999; Paracchini et al., 2003; Voutetakis et al., 2004)
Exon 2	c.218G>A	R73H	Missense	(Vallette-Kasic et al., 2001a; Paracchini et al., 2003; Voutetakis et al., 2004)
Exon 2	c.247C>T	Q83X	Nonsense	(Voutetakis et al., 2004)
Exon 2	c.263T>C	F88S	Missense	(Osorio et al., 2000)
Exon 2	c.295C>T	R99X	Nonsense	(Vallette-Kasic et al., 2001a)
Exon 2	c.296G>A	R99Q	Missense	(Vieira et al., 2003)
Exon 2	c.301-302delAG (OR c.296delGA)	S109X	Frameshift	(Cogan et al., 1998; Fofanova et al., 1998; Wu et al., 1998)
Exon 2	c.310delC	R103X	Frameshift	(Kelberman et al., 2009b)
Intron 2	c.343-2A>T	-	Splice site	(Duquesnoy et al., 1998)
Intron 2	c.343-11C>G	-	Splice site	(Kelberman et al., 2009b)
Exon 3	c.349T>A	F117I	Missense	(Wu et al., 1998)
Exon 3	c.358C>T	R120C	Missense	(Flück et al., 1998; Wu et al., 1998; Deladoëy et al., 1999)
Exon 3	c.373C>T	R125W	Missense	(Kelberman et al., 2009b)
Exon 3	c.467insT	191X	Frameshift	(Nose et al., 2006)
Exon 3	c.582G>A	W194X	Nonsense	(Reynaud et al., 2005)
Exon 3	c.629delC		Frameshift	(Reynaud et al., 2006)

Missense mutations

An arginine to cysteine substitution at residue 120 (p.R120C) was brought about by a *C* to *T* transition; while a *T* to *A* transversion resulted in the replacement of phenylalanine by isoleucine (p.F117I) at amino acid position 117 (Wu et al., 1998) (Table 7.2). In addition to causing an impairment of transactivation activities in both cases, the p.R120C-mutant and p.F117I-mutant led to the decrease in DNA binding affinity by eight-fold and twenty-fold respectively, when compared to the wild-type PROP1 protein (Wu et al., 1998). Wu et al. (1998) reported that affected individuals homozygous for the p.R120C mutation exhibited lower circulating levels of the GH, PRL, TSH, FSH and LH hormones, while their adrenocorticotropic hormone (ACTH) levels were normal. They also failed to respond to external stimulations using LH-releasing hormone, GH-releasing hormone, and TSH-releasing hormone.

Of noteworthy attention, is the finding by Flück et al. (1998) in a different study where phenotypic variability in pituitary size and symptoms was reported amongst patients with the same p.R120C mutation (Flück et al., 1998). The patients presented with severe retardation in growth and an inability to thrive, as well as spontaneous commencement of puberty, attributable to TSH and/or GH deficiencies. The results indicated a deterioration in anterior pituitary function indicated by a gradual decline in peak levels of pituitary hormones (GH, TSH, PRL, LH and FSH) with age, the magnitude of which varied between patients (Flück et al., 1998; Böttner et al., 2004).

The impairment brought about by *PROP1* mutations in humans was comparatively more severe than that previously observed in the Ames dwarf mice ($PROP1^{df/df}$). Supporting this notion, is the complete loss of gonadotropin hormones (LH and FSH) in humans, which was not observed in their murine counterparts (Tang et al., 1993; Wu et al., 1998).

Two other mutations were reported in the same study: a c.310delC single nucleotide deletion resulting in a frameshift mutation that yielded a non-functional transcript, and a non-synonymous p.R125W substitution caused by a c.373C>T transition mutation in exon 3, which impaired the transcriptional activation of genetic targets, although the mutant protein retained its DNA-binding capacity (Kelberman et al., 2009b) (Table 7.2). Compared to wild-type PROP1, the p.R125W mutant had a 40% decrease in gene transactivation activity, while the c.310delC mutant showed no activity at all (Kelberman et al., 2009b). The p.R125W mutation was located within a highly conserved basic region of the homeodomain. Interestingly, Guy et al. (2004) previously described two highly

conserved basic regions, named B1 and B2, at the N-terminal (residues 69 - 73) and C-terminal (residues 120 - 126) ends of the homeodomain, found to contain nuclear localisation signals that are essential for the proper PROP1 protein transport into the nucleus. EMSA revealed faster migration by the p.R125W mutant-DNA complex compared to the wild-type, implying a change in protein conformation which possibly impacted the nuclear localisation signals, hence altering PROP1 nuclear transport and transactivation (Kelberman et al., 2009b).

A *T* to *C* transition at nucleotide 263 resulted in the substitution of phenylalanine for serine at residue 88 (Osorio et al., 2000). Phenylalanine is a non-polar residue which lies in a highly conserved region of the homeodomain consisting of the hydrophobic core of the first α-helix, which is required for stabilising the PROP1 dimer-DNA complex. Hence, the substitution by serine, which is a polar amino acid, was expected to modify the protein conformation and disrupt DNA binding. The p.F88S-mutant had a 34% decrease in transactivation activity and impaired DNA binding to the PRDQ9 consensus, compared to the wild-type PROP1 (Osorio et al., 2000). The human p.F88S mutation corresponds with the p.F85S mutation in mice, which is two codons away from the critical p.S83P mutation in Ames dwarf mice (Sornson et al., 1996). In a transfection analysis, Osorio et al. (2000) showed that transactivation activity was reduced by 34% in the p.F85S-mutant mouse, compared to a decrease in activity by nearly half in the p.S83P-mutant mouse.

Intronic mutations

While many reports of *PROP1* mutations in humans focus on the coding regions, interesting findings have been reported in the non-coding regions. Within affected individuals of three different pedigrees, Kelberman et al. (2009b) described a CPHD-associated c.343-11C>G mutation located in the intron 2 of human *PROP1*. When present in its homozygous form, this intronic mutation interferes with accurate splicing and ultimately results in a PROP1 transcript lacking an exon 3 region. Individuals carrying the c.343-11C>G intronic mutation exhibited a deficiency in ACTH.

7.1.6 *PROP1* mutations in livestock

Zeng et al. (2011) examined variations in exons 1 - 3 of ovine *PROP1* and their association with commercial wool traits such as fibre diameter, natural staple length, and stretch staple length in Chinese Merino Sheep. The study revealed one SNP in exon 1, four SNPs in exon 2, and one SNP in exon 3. For the non-coding regions, one SNP was described in intron 1 and four SNPs in intron 2.

A synonymous p.Glu15Glu substitution resulting from a g.45A>G SNP was described in exon 1 (P1 fragment); and for exon 2 a combination of synonymous and non-synonymous variations including g.1341G>C (Arg63Ser), g.1389G>A (Ala79Ala), g.1402C>T (Leu84Leu) and g.1424A>G (Asn91Ser) were found (Zeng et al., 2011). In intron 1, a g.1198A>G was also detected. However, these variations in intron 1 and exon 2 (P2 fragment) showed no significant association with wool traits.

None of the four SNPs found in intron 2 (P3 fragment) were associated with phenotypic traits, including g.1522C>T, g.1556A>T, g.1574T>C and g.2430C>G (Zeng et al., 2011). A final g.2647A>G variation resulting in a p.Thr181Ala substitution was found in exon 3 (P4 fragment). All three genotypes of this variant were found to be significantly associated with wool fibre diameter. Liu et al. (2015) reported a C330T variation in the intron 1 of *PROP1* in Chinese Small Tail Han sheep. The *TT* and *CT* genotypes were associated with an increase in litter size, compared to the *CC* genotype.

Showalter et al. (2002) investigated the molecular basis of PROP1 function by cloning the encoding gene in cattle and performing a comparative analysis on its protein activity and conservation. In that study, an amino acid change from histidine to arginine at position 173 in the exon 3 of bovine *PROP1* resulting from an *A* to *G* transition, was described. Although the location of this substitution was reasonably distant from the homeodomain (69 - 128 aa), it still had striking effects on PROP1 function. Two distinct protein products with varying DNA binding and activation capabilities were encoded by the two alleles (Arg-173 and His-173). *In vitro* analyses of transcription and translation using radiolabelled PROP1 protein isoforms demonstrated that a protein possessing the arginine isoform (*G* allele) in its carboxyl domain, had a 5-fold decrease in DNA binding and reduced gene activation capacity, compared to that possessing the histidine isoform (*A* allele) (Showalter et al., 2002).

The bovine *PROP1* differs markedly from that of other species due to the presence of a fourth exon and a corresponding third intron within its 3' UTR. The fourth exon is evidently non-coding, and intron 3 is thought to contain a SinA repetitive element (Showalter et al., 2002).

In the US Holstein cattle breed, the same p.His173Arg amino acid change, brought about by an *A* to *G* SNP in exon 3 of bovine *PROP1*, was found to be associated with production traits (Lan et al., 2013b). The arginine isoform (*G* allele) has a significant association with an increase in protein yield, total index and productive life, as well as a decrease in sire

conception rate. Another study in native Chinese cattle breeds corroborate the p.H173R (AC_000164:g.41208950A>G) findings in bovine *PROP1* (Pan et al., 2013). Association analysis revealed the p.H173R variation in exon 3 to have a significant effect on production traits. Cows possessing the *AA* genotype (histidine isoform) were found to have superior growth traits, such as increased body weight, average daily weight gain, body height, rump length and chest girth, compared to counterparts exhibiting the *GG* genotype (arginine isoform). Bovine *PROP1* maps to BTA7 spanning a QTL associated with ovulation rate and fertility (Showalter et al., 2002; Lan et al., 2013b).

In eight Chinese goat breeds, a g.1795C>T transition (Accession number: AF453512) brings about a missense p.Ala79Val mutation in caprine *PROP1.* The variation was, however, found to have no significant association with cashmere yield, wool thickness, fibre length and body weight (Lan et al., 2009).

7.1.7 Rationale for the study

PROP1 is crucial for the normal functioning of the pituitary gland – the centre for the regulation of growth and development in vertebrates. PROP1 also controls the expression and secretion of other growth-promoting proteins, such as POU1F1, GH and TSH. Despite being described in humans, there is limited knowledge on the effect of *PROP1* on sheep production traits.

Exons 1 and 2 of ovine *PROP1* were selected for this investigation. This is because exon 1 houses a DNA repressor domain, whereas exon 2 spans across the homeodomain which can bring about the activation or repression of target genes by binding directly to their DNA. These regions are crucial to PROP1 function, hence any variation found within them could have far-reaching effects on growth and carcass traits in sheep.

7.2 Materials and Methods

7.2.1 Sample collection and DNA isolation

Blood samples used in this investigation were randomly collected from 1018 lambs from ten different NZ breeds, namely Romney (n = 720), Coopworth (n = 30), Corriedale (n = 30), Dorset Down (n = 31), Merino (n = 32), Perendale (n = 30), Poll Dorset (n = 33), Suffolk (n = 31), Texel (n = 40) and White Dorper (n = 41). (See Chapter 3.2 for details on lamb growth and carcass data recording)

Although the search for variation was performed on all sheep samples, lambs lacking phenotypic data for growth and carcass traits were subsequently excluded from the statistical analyses, leaving 720 Romney lambs for which these data were available. The lambs examined in this study were obtained from 40 unrelated sire-lines belonging to the top 20% of Romney sheep in the Sheep Improvement Limited (SIL) dual-purpose index ranking.

The genomic DNA from the FTA blood samples was purified using a two-step procedure. This involved a cell and protein denaturation with 20 mM NaOH at 60 ºC heat for 30 min, and subsequent washing with 1 × TE buffer for 5 min, as outlined by Zhou et al. (2006).

7.2.2 Primer design and PCR-SSCP protocol

The primer pair 5'-AAGGGAGAAGTGAACAGGCT-3' forward, and 5'-AGTGAGGAGACAGATTTCAGCT -3' reverse, were used to amplify a 336 bp fragment of *PROP1* spanning the partial exon 1 and intron 1 regions. While a 385 bp fragment of *PROP1* spanning the exon 2 region, with flanking intron 1 and intron 2 regions, was amplified using the primer pair 5'-AGTCTGGGATGGATGGATGG-3' forward, and 5'-GCCCTGAGAATTTATGCCCC-3' reverse. The primers were designed based on the sheep *PROP1* sequence on the NCBI database (GenBank accession number AY533708), and synthesised by Integrated DNA Technologies (Coralville, IA, USA).

The ideal PCR and SSCP conditions for each primer set were established empirically following multiple optimisation experiments. For a start, PCR optimisations entailed low stringency PCR with standard temperatures and reagent concentrations. The annealing temperature was subsequently raised in stepwise increments of 1 ºC until a single band was observed on agarose gel electrophoresis. This was done to curb non-specific amplification. Similar increments in a stepwise fashion were employed to the reagents used until the best results were obtained. Table 7.3 outlines the primer details and PCR-SSCP running conditions for the investigated regions of ovine *PROP1*.

Table 7.3 *PROP1* primers and PCR-SSCP conditions

PROP1 **Region**	**Forward/reverse primers Oligonucleotide (5' to 3')**	**Amplicon size**	**Annealing temp (ºC)**	**SSCP conditions**
Exon 1 (partial) Intron 1 (partial)	**F:** AAGGGAGAAGTGAACAGGCT **R:** AGTGAGGAGACAGATTTCAGCT	336	61	14%, 300 V, 30 ºC, 13 h
Exon 2 (flanking intron 1 and 2)	**F:** AGTCTGGGATGGATGGATGG **R:** GCCCTGAGAATTTATGCCCC	385	60	14%, 300 V, 17 ºC, 19 h

The following protocol description is based on the conditions optimal for *PROP1* exon 1. The protocol was similar for exon 2, except for the different conditions listed in Table 7.3.

PCR amplifications were carried out in 15 µl reaction consisting of 1.2 mm punch of the sample FTA card, 0.25 µM of each primer, 3.0 mM Mg^{2+}, 150 µM of each deoxyribonucleoside triphosphate (dNTP) (Bioline, London, UK), 0.5 U of *Taq* DNA polymerase (Qiagen, Hilden, Germany), 1 × reaction buffer supplied with the enzyme, and double-distilled water (d.H_2O) to make up the volume. Amplifications took place in Bio-rad S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA). The thermal profile was as follows: initial denaturation at 94 ºC for 2 min, followed by 34 cycles of 94 ºC for 30 s (denaturation), 61 ºC for 30 s (annealing), 72 ºC for 30 s (elongation), with a final extension step at 72 ºC for 5 min. PCR products were visualised by agarose gel electrophoresis, where 2 µl of PCR products were run in 1% agarose (Quantum Scientific, Queensland, Australia) gels containing 200 ng/mL of ethidium bromide, using 1 × TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM Na_2EDTA) at a voltage of 5 V/cm for 2 hours, and visualised by ultraviolet transillumination.

7.2.3 Single-stranded conformational polymorphism (SSCP) analyses

Optimisations for SSCP involved running amplicons at various conditions and using different gel compositions until consistently clear banding patterns were obtained.

For SSCP analysis, a 0.7 µl aliquot of the amplicon was mixed with 7 µl of loading dye (98% formamide, 10mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). The samples were denatured at 95 ºC for 5 min and cooled rapidly on wet ice before being loaded on 16 x 18 cm, 14% acrylamide:bisacrylamide (37.5:1) (Bio-Rad) gels.

Electrophoresis was carried out at 300 V at 30 ºC for 13 h in 0.5 X TBE using Protean II xi cells (Bio-Rad). The gels were silver-stained using a method described by (Byun et al., 2009). Briefly, the gels were silver-stained with silver nitrate, washed with distilled water, and subsequently developed using a solution containing sodium hydroxide and 40% formaldehyde.

In order to ensure the integrity of the experiments and to obtain reproducible banding patterns, freshly prepared PCR and SSCP products were always used. A negative control well containing no DNA was also run with each series of amplifications.

7.2.4 Nucleotide sequencing and genotyping

Following PCR-SSCP analysis, the banding patterns in the gels were observed. Amplicons deemed to be homozygous were sequenced in the forward and reverse directions using capillary sequencing at the Lincoln University DNA Sequencing Facility. For patterns that appeared to be heterozygous, single bands of non-homozygous samples were excised from the SSCP gel, washed repeatedly, and genomic DNA extracted. The resulting homozygous DNA was re-amplified, and then sequenced. Reactions were replicated to ensure accuracy and prevent PCR and sequencing errors.

Sequence alignments, translations and comparisons were carried out using DNAMAN version 5.2.10 (Lynnon BioSoft, Vaudreuil, Canada). The BLAST algorithm (http://blast.ncbi.nlm.nih.gov/) was used to search the NCBI GenBank and Ensembl databases for homologous sequences in various species.

7.2.5 Statistical analysis

IBM SPSS Statistics (Version 23, IBM, NY, USA) was used to perform all analyses. Trait data normality was tested using the Shapiro-Wilk test and Normal Q-Q plots. Variant and genotypic frequencies were calculated using the PopGene 3.2 software. The variant frequencies were then tested for deviation from Hardy-Weinberg equilibrium using a Chi-square test available on the Online Encyclopedia for Genetic Epidemiology Studies (OEGE) (Rodriguez et al., 2009). The same software was used to test for measures of linkage disequilibrium.

Prediction of the effect of nucleotide variations on protein stability and function was performed using a variety of online software to ensure reliability, including SIFT (Ng and Henikoff, 2003), PROVEAN (Choi et al., 2012), HOPE (Venselaar et al., 2010), MuPro (Cheng et al., 2006), and iMutant 2.0 (Capriotti et al., 2005). Changes in protein stability

were predicted using delta delta G (ΔΔG) in Kcal/mol, which refers to a change in the Gibbs free energy between folded and unfolded states of the wild-type and variant protein sequences. The analyses were carried out on the assumptions that temperature was 25 °C and pH was 7.0.

The strength of relationships between the different traits was assessed using Pearson correlation coefficients. A Bonferroni correction was applied to the analyses. The growth traits investigated were birth weight, tailing weight, weaning weight, and growth rate-to-weaning. The carcass traits investigated were hot carcass weight (HCW), fat depth at the 12th rib (V-GR), hind-leg lean meat yield, loin lean meat yield, shoulder lean meat yield, and total carcass lean meat yield.

Two sets of general linear mixed-effect models (GLMMs) were carried out. The first model was used to investigate the effect of the presence and absence (coded as 1 and 0 respectively) of a specific variant on growth and carcass traits, while the second model assessed associations between the genotypes for each nucleotide substitution and growth and carcass traits.

The statistical model used was:

$$Y_{ijkl} = \mu + S_i + Gj + B_k + V_l + e_{ijkl}$$

where:

Y_{ijkl} is the trait measured on each animal (birth, tailing and weaning weights, growth-rate, HCW, V-GR, and leg, loin, shoulder, and total lean meat yields)

μ is the mean for the trait

S_i is the random effect of sire

Gj is the fixed effect of gender

B_j is the fixed effect of birth rank

V_l is the fixed effect of variant (presence or absence) or genotype

e_{ijkl} is the random residual error

Gender was fitted as a fixed factor, and sire as a random factor in all growth trait models. For the growth trait models assessing the relationship between nucleotide genotype (or the presence/absence of a sequence variant) and tailing and weaning weights, the age at tailing and weaning were fitted as covariates respectively, as variation in age at measurement could potentially influence these traits.

In order to account for the effect of prenatal growth and development, birth weight (which had a bigger effect than birth rank) was fitted as a covariate in models assessing the relationship between nucleotide variant genotype or the presence/absence of a sequence variant, and growth rate-to-weaning. Rearing rank, which is reflective of maternal influence was shown to have an effect, and hence was corrected for in growth rate models.

For all carcass trait models, genotype was fitted as a fixed factor, and sire as a random factor, while birth weight and slaughter age in days were fitted as covariates, so as to correct for the effect of prenatal muscle development and the variation in the age of the lambs at slaughter respectively. Gender was not corrected for in any of the carcass trait models, as only ram lambs were sent for slaughter.

The variant model was run in two phases. Firstly, a single variant model assessing the unilateral effect of variant presence/absence on individual traits was run. If a significant trend was observed ($P < 0.2$, the threshold for inclusion), then a subsequent multi-variant model investigating the effect of the presence/absence of a variant, within the context of the effect of the other variants, was performed.

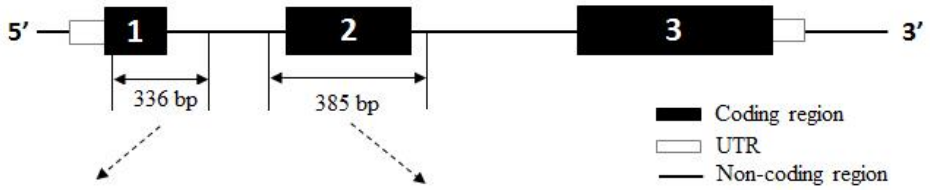
Values from the GLMM pairwise comparison was presented as estimated marginal means ± standard error (mean ± SE). Unless otherwise specified, statistical significance was declared at $P < 0.05$, and trends were observed at $P < 0.2$.

7.3 Results

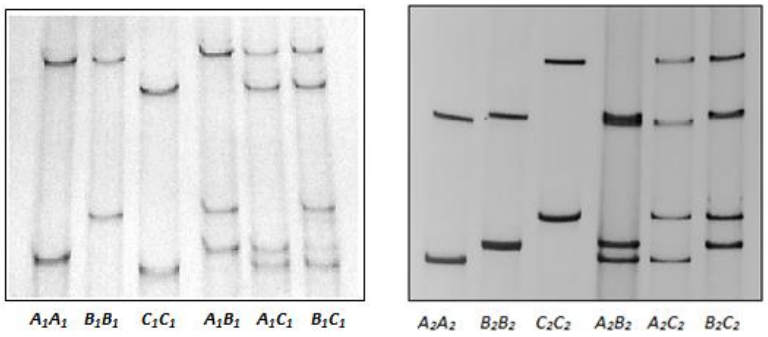
7.3.1 Variations in ovine *PROP1*

A 336 bp fragment spanning the partial exon 1 and intron 1 region of *PROP1* was amplified in ten NZ sheep breeds (Figure 7.4). The PCR-SSCP analysis and nucleotide sequencing revealed three SNPs organised into three variant patterns (A_1, B_1 and C_1). One of the SNPs, c.45A>G, was located in exon 1 and would putatively result in a synonymous p.Glu15Glu substitution. Located in intron 1, were the other two SNPs: c.109+40T>C and c.109+207C>T. The unique arrangement of the SNPs at loci c.45A>G, c.109+40 T>C and c.109+207C>T respectively, to form variants are as follows: A_1 (G-C-C), B_1 (A-T-C) and C_1 (G-C-T). The variant sequences were submitted to GenBank with the accession numbers: MF785097 - MF785099.

(a)



(b)



(c)

	A_1	B_1	C_1
c.45A>G	G	A	G
c.109+40T>C	C	T	C
c.109+207C>T	C	C	T

	A_2	B_2	C_2
c.189G>C	G	G	C
c.237G>A	A	G	G
c.342+62A>T	T	A	A

Figure 7.4 Amplified regions of *PROP1* and variants detected

(a) Stylistic map showing the exon and intron regions of *PROP1*, and the 336 bp and 385 bp regions amplified. (b) The resulting gel patterns from polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) indicating exon 1 (on the left) homozygous variants A_1 (well 1), B_1 (well 2) and C_1 (well 3), and exon 2 (on the right) homozygous variants A_2 (well 1), B_2 (well 2) and C_2 (well 3). The subscript '1' denotes the exon '1', while subscript '2' denotes the exon '2'; (c) Single nucleotide polymorphisms (SNPs) detected in the amplified regions of *PROP1* respectively.

On the other hand, three unique banding patterns (A_2, B_2 and C_2) corresponding to three unique DNA sequences were revealed in the 385 bp fragment of *PROP1* (Figure 7.4). Alignment of these sequences revealed three SNPs: c.189G>C and c.237G>A in exon 2, and c.342+62A>T in intron 2. These SNPs have been reported previously (Zeng et al., 2011). The variant sequences were deposited into GenBank with accession numbers MH469232 - MH469234. The c.237G>A SNP was synonymous (p.Ala79Ala).

The c.189G>C variation was found exclusively in the C_2 allele. If translated, this variation would lead to a non-synonymous amino acid substitution from arginine to serine at position 63 (p.Arg63Ser) in the PROP1 homeobox domain. Prediction analyses revealed the non-synonymous c.189G>C (p.Arg63Ser) to yield a ΔΔG value of -0.84 Kcal/mol, thus indicating that the putative amino acid change would result in a less stable PROP1 protein.

7.3.2 Variant, SNP and genotype frequencies of ovine *PROP1*

Table 7.4 summarises the frequencies for *PROP1* variant genotypes in exons 1 and 2 across ten NZ sheep breeds.

Table 7.4 Breed frequencies of ovine *PROP1*

Breed	n	***PROP1* Exon 1 Variant Genotypes (%)**					
		A_1A_1	A_1B_1	A_1C_1	B_1B_1	B_1C_1	C_1C_1
Romney	720	47.8	17.9	14.2	5.1	12.1	2.9
Coopworth	30	40.0	23.3	23.3	6.7	6.7	_
Corriedale	30	33.3	30.0	33.3	_	3.4	_
Dorset Down	31	29.0	12.9	51.7	3.2	_	3.2
Merino	32	50.0	18.7	12.5	6.3	12.5	_
Perendale	30	46.7	26.7	13.3	3.3	10.0	_
Poll Dorset	33	36.4	15.1	36.4	_	9.0	3.1
Suffolk	31	32.2	22.6	35.5	6.4	_	3.3
Texel	40	35.0	15.0	40.0	_	5.0	5.0
White Dorper	41	48.8	14.6	24.4	9.8	2.4	_
Total	**1018**						

Breed	n	***PROP1* Exon 2 Variant Genotypes (%)**					
		A_2A_2	A_2B_2	A_2C_2	B_2B_2	B_2C_2	C_2C_2
Romney	720	25.7	51.1	8.0	9.6	4.9	0.7
Coopworth	30	20.0	50.0	10.0	20.0	_	_
Corriedale	30	16.7	33.3	6.7	20.0	23.3	_
Dorset Down	31	29.0	51.6	3.3	16.1	_	_
Merino	32	18.7	25.0	12.6	18.7	25.0	_
Perendale	30	16.7	50.0	10.0	16.7	6.6	_
Poll Dorset	33	30.3	48.5	_	18.1	3.1	_
Suffolk	31	29.0	51.6	3.2	16.2	_	_
Texel	40	10.0	60.0	_	25.0	5.0	_
White Dorper	41	19.5	24.4	21.9	_	31.7	2.5
Total	**1018**						

The most abundant genotypes across most breeds were A_1A_1 for exon 1, and A_2B_2 for exon 2. The C_2C_2 genotype was the rarest, presenting only in the Romney and White Dorper breeds at 0.7% and 2.5% respectively, compared to C_1C_1 genotype which was present in five breeds (Table 7.4). The A_1C_1 was found in all sheep breeds examined, whereas the A_2C_2 genotype was not observed in the Poll Dorset and Texel breeds. The B_2B_2 genotype frequency was lowest in the Romney breed and highest in the Texel breed, and was observed in all but the White Dorper breed.

The genotypic and variant frequencies for exon 1 SNPs are presented in Table 7.5.

Table 7.5 Variant and genotype frequencies for exon 1 of ovine *PROP1* in NZ Romney sheep

SNP	AA[1] change	Variant	Variant frequency	n	Genotype	Genotype frequency	HWE[2]
c.45A>G	p.Glu15Glu	A	0.2	34	AA	0.07	$\chi^2 = 2.84$
(Exon 1)		G	0.8	201	AG	0.45	P = 0.092
				435	GG	0.48	
c.109+40T>C	-	C	0.8	435	CC	0.48	$\chi^2 = 2.84$
(Intron 1)		T	0.2	201	CT	0.45	P = 0.092
				34	TT	0.07	
c.109+207C>T	-	C	0.84	474	CC	0.89	$\chi^2 = 0.55$
(Intron 1)		T	0.16	176	CT	0.11	P = 0.474
				20	TT	0.00	

[1] AA – amino acid predicted, [2] HWE: Hardy-Weinberg Equilibrium

None of the variants deviated from Hardy-Weinberg Equilibrium ($P < 0.05$). The c.45A>G and c.109+40 T>C exhibited the same pattern of allelic and genotypic frequencies, with the *G* and *C* as major alleles with frequencies of 80%, while the *A* and *T* minor alleles had frequencies of 20% (Table 7.5).

On the genotypic end, the predominant homozygous genotypes (*GG* and *CC*) had frequencies of 48%, which was similar to the 45% frequencies observed for heterozygotes (*AG* and *CT*), while the *AA* and *TT* genotypes exhibited the lowest frequencies of 7%. More extreme values were observed for the c.109+207C>T with the major allele (*C*) frequency of 84%, and the minor allele (*T*) frequency of 16% (Table 7.5). Genotype analysis revealed the *CC* genotype to have the highest frequency (89%), followed by *CT* (11%), while *TT* was not observed (0%).

The genotypic and variant frequencies for exon 2 SNPs are presented in Table 7.6.

Table 7.6 Variant and genotype frequencies for exon 2/intron 2 of ovine *PROP1* in NZ Romney sheep

SNP	AA[1] change	Variant	Variant frequency	n	Genotype	Genotype frequency	HWE[2]
c.189G>C (Exon 2)	p.Arg63Ser	C	0.07	5	CC	0.01	$\chi^2 = 0.73$
		G	0.93	87	CG	0.13	P = 0.381
				578	GG	0.86	
c.237G>A (Exon 2)	p.Gly79Gly	A	0.55	172	AA	0.30	$\chi^2 = 25.51$
		G	0.45	396	AG	0.50	**P < 0.001**
				102	GG	0.20	
c.342+62A>T (Intron 2)	-	A	0.45	102	AA	0.20	$\chi^2 = 25.51$
		T	0.55	396	AT	0.50	**P < 0.001**
				172	TT	0.30	

[1] AA – amino acid predicted, [2] HWE: Hardy-Weinberg Equilibrium

For c.189G>C, the guanine frequency (93%) was much higher than that of cytosine (7%). The c.237G>A SNP had nucleotides of similar frequencies – adenine (55%) and guanine (45%), and for the c.342+62A>T SNP, adenine was found at a frequency of 45%. In both cases, upon HWE analysis, the P-value was less than 0.001, suggesting that the observed frequencies differed from the expected population frequency (Table 7.6).

The measures of LD (r^2 and D′) between pairs of SNPs in exon 2 of ovine *PROP1* were calculated using the OEGE software and the results are presented in Table 7.7.

Table 7.7 Linkage disequilibrium measures between SNP pairs detected in exon 2 of ovine *PROP1*

Locus 1	Locus 2	D'	r^2
c.189G>C	c.237G>A	1.0	0.096
c.189G>C	c.342+62A>T	1.0	0.096
c.237G>A	c.342+62A>T	-1.0	1.0

D′: Lewontin's coefficient; r^2: squared correlation coefficient

The D’ value for the three SNPs in exon 2 of ovine *PROP1* is 1, meaning that the SNPs are in high linkage disequilibrium, and hence are likely co-inherited.

7.3.3 Association of growth traits with *PROP1* exon 1 genetic variants

The results for the association of *PROP1* exon 1 variants with growth traits in NZ Romney lambs are given in Table 7.8.

Table 7.8 Association of *PROP1* exon 1 variants with growth traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
Birth weight (kg)	A_1		5.48 ± 0.22	145	5.54 ± 0.21	575	0.406
	B_1		5.56 ± 0.21	467	5.42 ± 0.22	253	**0.025**
	C_1		5.53 ± 0.21	510	5.57 ± 0.22	210	0.476
Tailing weight (kg)	A_1		11.09 ± 0.60	145	12.17 ± 0.58	575	**<0.001**
	B_1		12.17 ± 0.58	467	11.51 ± 0.60	253	**<0.001**
	C_1		12.10 ± 0.59	510	11.74 ± 0.60	210	0.053
	A_1	B_1, C_1	11.14 ± 0.61	145	12.11 ± 0.59	575	**0.001**
	B_1	A_1, C_1	11.74 ± 0.59	467	11.51 ± 0.59	253	0.275
	C_1	A_1, B_1	11.59 ± 0.59	510	11.66 ± 0.60	210	0.737
Weaning weight (kg)	A_1		28.00 ± 1.00	145	30.88 ± 0.95	575	**<0.001**
	B_1		30.90 ± 0.98	467	29.28 ± 1.00	253	**<0.001**
	C_1		30.75 ± 0.99	510	29.71 ± 1.02	210	**0.001**
	A_1	B_1, C_1	28.09 ± 1.01	145	30.78 ± 0.97	575	**<0.001**
	B_1	A_1, C_1	29.64 ± 0.97	467	29.23 ± 0.98	253	0.246
	C_1	A_1, B_1	29.37 ± 0.97	510	29.50 ± 0.98	210	0.719
Growth rate to weaning (g/day)	A_1		237.90 ± 10.38	145	270.11 ± 9.91	575	**<0.001**
	B_1		273.62 ± 10.38	467	257.67 ± 10.41	253	**<0.001**
	C_1		268.10 ± 10.34	510	254.36 ± 10.69	210	**<0.001**
	A_1	B_1, C_1	238.89 ± 10.58	145	269.65 ± 10.05	575	**<0.001**
	B_1	A_1, C_1	255.13 ± 10.31	467	253.41 ± 10.07	253	0.638
	C_1	A_1, B_1	254.59 ± 10.12	510	253.95 ± 10.26	210	0.860

Only the B_1 variant showed significant association with birth weight – a decrease from 5.56 kg to 5.42 kg. The A_1 variant exhibited strong association with an increase in tailing weight by 1.08 kg, weaning weight by 2.88 kg and growth rate-to-weaning by 32.21 g/day, with significance persisting in the multi-variant analysis (Table 7.8). Both the B_1 and C_1 variants, on the other hand, showed negative associations with tailing and weaning weights, and growth rate-to-weaning, which were no longer significant when other variants were accounted for. This suggests that only the A_1 effect is independent of other variants.

Association studies assessing the effect of individual SNPs in exon 1 with growth traits were carried out in NZ Romney breeds, and the results are summarised in Table 7.9.

Table 7.9 Association of *PROP1* exon 1 SNP genotypes with growth traits in NZ Romney sheep

SNP / Trait	c.45A>G (Glu15Glu)			
	AA (n = 37)	**AG (n = 216)**	**GG (n = 467)**	**P-value**
Birth weight (kg)	5.33 ± 0.25	5.43 ± 0.22	5.56 ± 0.21	0.063
Tailing weight (kg)	11.06 ± 0.69[a]	11.57 ± 0.60[a]	12.17 ± 0.58[b]	**<0.001**
Weaning weight (kg)	27.20 ± 1.15	29.59 ± 1.00	30.87 ± 0.97	**<0.001**
Growth rate to weaning (g/day)	234.60 ± 12.00	260.63 ± 10.34	272.90 ± 10.28	**<0.001**
	c.109+40T>C			
	TT (n = 37)	**CT (n = 216)**	**CC (n = 467)**	**P-value**
Birth weight (kg)	5.33 ± 0.25	5.43 ± 0.22	5.56 ± 0.21	0.063
Tailing weight (kg)	11.06 ± 0.69[a]	11.57 ± 0.60[a]	12.17 ± 0.58[b]	**<0.001**
Weaning weight (kg)	27.20 ± 1.15	29.59 ± 1.00	30.87 ± 0.97	**<0.001**
Growth rate to weaning (g/day)	234.60 ± 12.00	260.63 ± 10.34	272.90 ± 10.28	**<0.001**
	c.109+207C>T			
	CC (n = 510)	**CT (n = 189)**	**TT (n = 21)**	**P-value**
Birth weight (kg)	5.53 ± 0.21	5.55 ± 0.22	5.81 ± 0.27	0.281
Tailing weight (kg)	12.09 ± 0.59	11.80 ± 0.60	11.12 ± 0.76	0.060
Weaning weight (kg)	30.74 ± 0.99[a]	29.85 ± 1.02[b]	28.38 ± 1.28[b]	**0.001**
Growth rate to weaning (g/day)	268.41 ± 10.31[a]	256.45 ± 10.71[b]	237.85 ± 13.21[b]	**<0.001**

[a, b] Mean values in the same row with different superscripts differ at $P < 0.05$.

The *GG* and *CC* homozygotes at the c.45A>G and c.109+40T>C loci respectively, showed significant association with an increase in several growth traits when compared to the *AA/AG* and *TT/TC* genotypes (Table 7.9). An increase in tailing weight by 1.11 kg, weaning weight by 3.67 kg, and growth rate-to-weaning by 38.3 g/day was observed for both the *GG* and *CC* variant genotypes when compared to the *AA* and *TT* genotypes.

In contrast, individuals with the *TT* variant genotypes at the c.109+207C>T locus exhibited a trend ($P = 0.060$) toward a decrease in tailing weight by 0.97 kg. When compared to their *CC* counterparts, lambs possessing the *TT* genotype showed a decrease in weaning weight by 7.7% and growth rate by 11.4%. Although none of the genotypes demonstrated significant association with birth weight in NZ Romney sheep, a strong increasing trend was observed ($P = 0.063$) with the variant genotypes of c.45A>G and c.109+40T>C.

7.3.4 Association of growth traits with *PROP1* exon 2 genetic variants

The results for the association of *PROP1* exon 2 variants with growth traits in NZ Romney lambs are given in Table 7.10.

Table 7.10 Association of *PROP1* exon 2 variants with growth traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
Birth weight (kg)	A_2		5.51 ± 0.22	110	5.54 ± 0.21	610	0.787
	B_2		5.47 ± 0.22	248	5.56 ± 0.21	472	0.186
	C_2		5.55 ± 0.21	622	5.36 ± 0.23	98	**0.031**
	B_2	C_2	5.42 ± 0.22	248	5.47 ± 0.22	472	0.409
	C_2	B_2	5.53 ± 0.21	622	5.36 ± 0.23	98	0.058
Tailing weight (kg)	A_2		11.35 ± 0.62	110	12.10 ± 0.58	610	**0.001**
	B_2		11.92 ± 0.60	248	12.07 ± 0.59	472	0.390
	C_2		12.10 ± 0.58	622	11.23 ± 0.62	98	**<0.001**
	A_2	C_2	11.23 ± 0.62	110	11.78 ± 0.59	610	**0.022**
	C_2	A_2	11.86 ± 0.59	622	11.15 ± 0.62	98	**0.005**
Weaning weight (kg)	A_2		28.28 ± 1.03	110	30.85 ± 0.97	610	**<0.001**
	B_2		30.38 ± 1.02	248	30.65 ± 1.00	472	0.382
	C_2		30.74 ± 0.98	622	28.47 ± 1.05	98	**<0.001**
	A_2	C_2	27.98 ± 1.02	110	30.10 ± 0.98	610	**<0.001**
	C_2	A_2	29.86 ± 0.97	622	28.22 ± 1.03	98	**<0.001**
Growth rate to weaning (g/day)	A_2		247.91 ± 10.48	110	275.45 ± 10.23	610	**<0.001**
	B_2		265.04 ± 10.70	248	266.52 ± 10.49	472	0.630
	C_2		268.45 ± 10.27	622	246.12 ± 10.94	98	**<0.001**
	A_2	C_2	244.62 ± 10.43	248	267.93 ± 10.36	472	**<0.001**
	C_2	A_2	263.97 ± 10.09	622	248.58 ± 10.72	98	**<0.001**

Lambs possessing the A_2 variant grew faster by 27.5 g/day, and had higher weights at tailing (0.75 kg) and weaning (2.75 kg); while lambs possessing the C_2 variant had grew slower by 22.3 g/day, and had lower tailing (0.87 kg) and weaning (2.27 kg) weights (Table 7.10). The B_2 variant showed no association with ovine growth traits. The C_2 was the only variant to show a significant association with a decrease in birth weight by 0.19 kg.

Association studies assessing the effect of individual SNPs in exon 2 with growth traits were carried out in NZ Romney breeds, and the results are summarised in Table 7.11.

Table 7.11 Association of *PROP1* exon 2 SNP genotypes with growth traits in NZ Romney sheep

SNP / Trait	c.189G>C (Arg63Ser)			
	GG (n = 622)	**CG (n = 93)**	**CC (n = 5)**	**P-value**
Birth weight (kg)	5.54 ± 0.21[a]	5.40 ± 0.23[b]	4.34 ± 0.42[b]	**0.001**
Tailing weight (kg)	12.09 ± 0.58[a]	11.30 ± 0.62[a]	9.83 ± 1.14[b]	**0.007**
Weaning weight (kg)	30.69 ± 0.97	28.67 ± 1.04	24.08 ± 1.92	**<0.001**
Growth rate to weaning (g/day)	268.30 ± 10.23[a]	248.33 ± 10.93[a]	203.73 ± 20.00[b]	**<0.001**
	c.237G>A (Ala79Ala)			
	GG (n = 109)	**AG (n = 426)**	**AA (n = 185)**	**P-value**
Birth weight (kg)	5.52 ± 0.22	5.55 ± 0.21	5.51 ± 0.22	0.849
Tailing weight (kg)	11.29 ± 0.62[a]	12.15 ± 0.58[b]	12.00 ± 0.60[b]	**0.002**
Weaning weight (kg)	28.31 ± 1.03[a]	30.92 ± 0.97[b]	30.65 ± 1.00[b]	**<0.001**
Growth rate to weaning (g/day)	248.11 ± 10.48[a]	276.05 ± 10.28[b]	273.87 ± 10.49[b]	**<0.001**
	c.342+62A>T			
	AA (n = 109)	**AT (n = 426)**	**TT (n = 185)**	**P-value**
Birth weight (kg)	5.52 ± 0.22	5.55 ± 0.21	5.51 ± 0.22	0.849
Tailing weight (kg)	11.29 ± 0.62[a]	12.15 ± 0.58[b]	12.00 ± 0.60[b]	**0.002**
Weaning weight (kg)	28.31 ± 1.03[a]	30.92 ± 0.97[b]	30.65 ± 1.00[b]	**<0.001**
Growth rate to weaning (g/day)	248.11 ± 10.48[a]	276.05 ± 10.28[b]	273.87 ± 10.49[b]	**<0.001**

[a, b] Mean values in the same row with different superscripts differ at $P < 0.05$.

At the c.237G>A and c.342+62A>T variant loci, the heterozygous genotypes, *AG* and *AT* respectively, had the highest tailing weight, weaning weight and growth rate when compared to the homozygous genotypes (Table 7.11). When compared to lambs having the *GG* genotype, lambs possessing the *AG* genotype of c.237G>A weighed 7.6% and 9.2% more at tailing and weaning respectively, and grew 11.3% faster.

The rare *CC* variant of c.189G>C had the lowest weights at birth, tailing and weaning, and growth rate, compared to the *CG* and *GG* genotypes. Only five individuals expressed the *CC* genotype, hence the results presented with regards to this genotype might not be representative of the population.

7.3.5 Association of carcass traits with *PROP1* exon 1 genetic variants

The association of *PROP1* exon 1 variants with carcass traits in NZ Romney lambs are given in Table 7.12.

Table 7.12 Association of *PROP1* exon 1 variants with carcass traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
HCW[1] (kg)	A_1		16.62 ± 0.15	135	17.21 ± 0.09	535	**<0.001**
	B_1		17.19 ± 0.10	435	17.00 ± 0.11	235	0.132
	C_1		17.17 ± 0.09	474	16.93 ± 0.13	196	0.061
	A_1	B_1, C_1	16.57 ± 0.16	135	17.24 ± 0.11	535	**0.001**
	B_1	A_1, C_1	16.85 ± 0.13	435	16.96 ± 0.12	235	0.474
	C_1	A_1, B_1	16.90 ± 0.12	474	16.92 ± 0.13	196	0.905
V-GR[2] Fat Depth (mm)	A_1		4.73 ± 0.22	135	4.18 ± 0.13	535	**0.010**
	B_1		4.10 ± 0.14	435	4.55 ± 0.16	235	**0.013**
	C_1		4.22 ± 0.13	474	4.42 ± 0.19	196	0.274
	A_1	B_1	4.62 ± 0.23	135	4.24 ± 0.13	535	0.135
	B_1	A_1	4.29 ± 0.19	474	4.57 ± 0.17	196	0.184
Leg Yield (%)	A_1		21.21 ± 0.11	135	21.55 ± 0.06	535	**0.002**
	B_1		21.56 ± 0.07	435	21.39 ± 0.08	235	0.061
	C_1		21.55 ± 0.06	474	21.31 ± 0.09	196	**0.012**
	A_1	B_1, C_1	21.26 ± 0.12	135	21.48 ± 0.08	535	0.122
	B_1	A_1, C_1	21.40 ± 0.09	435	21.34 ± 0.08	235	0.584
	C_1	A_1, B_1	21.44 ± 0.08	474	21.30 ± 0.09	196	0.193
Loin Yield (%)	A_1		14.33 ± 0.08	135	14.59 ± 0.04	535	**0.001**
	B_1		14.60 ± 0.05	435	14.47 ± 0.06	235	**0.043**
	C_1		14.59 ± 0.04	474	14.40 ± 0.06	196	**0.005**
	A_1	B_1, C_1	14.37 ± 0.08	135	14.54 ± 0.06	535	0.115
	B_1	A_1, C_1	14.48 ± 0.07	435	14.43 ± 0.06	235	0.526
	C_1	A_1, B_1	14.51 ± 0.06	474	14.40 ± 0.07	196	0.120
Shoulder Yield (%)	A_1		16.54 ± 0.08	135	16.84 ± 0.05	535	**<0.001**
	B_1		16.88 ± 0.05	435	16.64 ± 0.06	235	**0.001**
	C_1		16.82 ± 0.05	474	16.70 ± 0.07	196	0.109
	A_1	B_1, C_1	16.60 ± 0.09	135	16.80 ± 0.06	535	0.077
	B_1	A_1, C_1	16.77 ± 0.07	435	16.62 ± 0.06	235	0.074
	C_1	A_1, B_1	16.71 ± 0.06	474	16.69 ± 0.07	196	0.767
Total Yield (%)	A_1		52.09 ± 0.22	135	52.98 ± 0.13	535	**<0.001**
	B_1		53.03 ± 0.14	435	52.50 ± 0.17	235	**0.004**
	C_1		52.95 ± 0.13	474	52.42 ± 0.19	196	**0.006**
	A_1	B_1, C_1	52.24 ± 0.25	135	52.82 ± 0.16	535	0.053
	B_1	A_1, C_1	52.66 ± 0.19	435	52.40 ± 0.17	235	0.254
	C_1	A_1, B_1	52.67 ± 0.17	474	52.39 ± 0.19	196	0.203

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

The A_1 variant was associated with an increase in carcass weight by 0.59 kg, and a decrease in fat depth by 0.55 mm, while the B_1 variant was associated with an increase in fat depth by 0.45 mm (Table 7.12).

The A_1 variant showed association with an increase in leg, loin, shoulder, and total lean meat yields by 1.6%, 1.8%, 1.8% and 1.7% respectively. The B_1 variant was associated with a decrease in loin, shoulder and total lean meat yields by 0.89%, 1.4% and 1% respectively. The C_1 variant was also associated with a decrease in leg, loin and total meat yields by 1.1%, 1.3% and 1% respectively.

Association studies assessing the effect of individual SNPs in exon 1 with carcass traits were carried out in NZ Romney breeds, and the results are summarised in Table 7.13.

Table 7.13 Association of *PROP1* exon 1 SNP genotypes with carcass traits in NZ Romney sheep

SNP / Trait	c.45A>G (Glu15Glu)			
	AA (n = 34)	**AG (n = 201)**	**GG (n = 435)**	**P-value**
HCW[1] (kg)	16.50 ± 0.27[a]	17.07 ± 0.12[b]	17.18 ± 0.10[b]	**0.038**
V-GR[2] Fat Depth (mm)	4.82 ± 0.39[a]	4.50 ± 0.17[ab]	4.10 ± 0.14[b]	**0.033**
Leg Yield (%)	21.09 ± 0.19[a]	21.43 ± 0.09[ab]	21.55 ± 0.07[a]	**0.039**
Loin Yield (%)	14.34 ± 0.13	14.49 ± 0.06	14.60 ± 0.05	0.076
Shoulder Yield (%)	16.34 ± 0.14	16.69 ± 0.06	16.88 ± 0.05	**<0.001**
Total Yield (%)	51.78 ± 0.40	52.61 ± 0.18	53.03 ± 0.14	**0.002**
	c.109+40T>C			
	TT (n = 34)	**CT (n = 201)**	**CC (n = 435)**	**P-value**
HCW[1] (kg)	16.50 ± 0.27[a]	17.07 ± 0.12[b]	17.18 ± 0.10[b]	**0.038**
V-GR[2] Fat Depth (mm)	4.82 ± 0.39[a]	4.50 ± 0.17[ab]	4.10 ± 0.14[b]	**0.033**
Leg Yield (%)	21.09 ± 0.19[a]	21.43 ± 0.09[b]	21.55 ± 0.07[a]	**0.039**
Loin Yield (%)	14.34 ± 0.13	14.49 ± 0.06	14.60 ± 0.05	0.076
Shoulder Yield (%)	16.34 ± 0.14	16.69 ± 0.06	16.88 ± 0.05	**<0.001**
Total Yield (%)	51.78 ± 0.40	52.61 ± 0.18	53.03 ± 0.14	**0.002**
	c.109+207C>T			
	CC (n = 474)	**CT (n = 176)**	**TT (n = 20)**	**P-value**
HCW[1] (kg)	17.17 ± 0.09	16.98 ± 0.13	16.44 ± 0.34	0.050
V-GR[2] Fat Depth (mm)	4.22 ± 0.13[a]	4.28 ± 0.19[a]	5.77 ± 0.49[b]	**0.006**
Leg Yield (%)	21.55 ± 0.06[a]	21.37 ± 0.10[a]	20.75 ± 0.24[b]	**0.002**
Loin Yield (%)	14.59 ± 0.04	14.44 ± 0.07	14.03 ± 0.17	**0.001**
Shoulder Yield (%)	16.82 ± 0.05[a]	16.74 ± 0.07[ab]	16.39 ± 0.18[a]	0.054
Total Yield (%)	52.96 ± 0.13[a]	52.55 ± 0.20[a]	51.18 ± 0.50[b]	**0.001**

[a, b] Mean values in the same row with different superscripts differ at $P < 0.05$

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

The predominant *GG* and *CC* genotypes of c.45A>G and c.109+40T>C respectively, had the highest carcass weight (17.18 ± 0.10), leg yield (21.55 ± 0.07), shoulder yield (16.88 ± 0.05), and total lean meat yield (53.03 ± 0.14), as well as the lowest fat depth (4.10 ± 0.14), compared to the heterozygous *AG/CT* and homozygous *AA/TT* (Table 7.13).

In contrast, the less abundant *TT* genotype of c.109+207C>T had the lowest leg yield (20.75 ± 0.24), loin yield (14.03 ± 0.17) and total yield (51.18 ± 0.5), but the highest fat depth (5.77 ± 0.49).

7.3.6 Association of carcass traits with *PROP1* exon 2 genetic variants

The results for the association of *PROP1* exon 2 variants with carcass traits in NZ Romney lambs are given in Table 7.14.

Table 7.14 Association of *PROP1* exon 2 variants with carcass traits in NZ Romney sheep

Trait (unit)	Variant assessed	Other variants	Mean ± SE				
			Variant absent (0)	n	Variant present (1)	n	P-value
HCW[1] (kg)	A_2		16.14 ± 0.16	103	17.30 ± 0.08	567	**<0.001**
	B_2		17.20 ± 0.11	231	17.07 ± 0.09	439	0.269
	C_2		17.17 ± 0.09	578	16.75 ± 0.17	92	**0.013**
	A_2	C_2	16.12 ± 0.16	103	17.25 ± 0.11	567	**<0.001**
	C_2	A_2	16.74 ± 0.10	578	16.64 ± 0.16	92	0.548
V-GR[2] Fat Depth (mm)	A_2		3.93 ± 0.23	103	4.33 ± 0.13	567	0.093
	B_2		4.47 ± 0.16	231	4.16 ± 0.14	439	0.075
	C_2		4.25 ± 0.13	578	4.35 ± 0.25	92	0.716
	A_2	B_2	4.04 ± 0.25	103	4.35 ± 0.13	567	0.208
	B_2	A_2	4.32 ± 0.20	231	4.07 ± 0.15	439	0.165
Leg Yield (%)	A_2		21.36 ± 0.12	103	21.52 ± 0.06	567	0.195
	B_2		21.47 ± 0.08	231	21.51 ± 0.07	439	0.647
	C_2		21.52 ± 0.06	578	21.31 ± 0.12	92	0.090
	A_2	C_2	21.34 ± 0.12	103	21.44 ± 0.08	567	0.384
	C_2	A_2	21.48 ± 0.08	578	21.30 ± 0.12	92	0.163
Loin Yield (%)	A_2		14.21 ± 0.08	103	14.61 ± 0.04	567	**<0.001**
	B_2		14.57 ± 0.06	231	14.53 ± 0.05	439	0.480
	C_2		14.57 ± 0.04	578	14.37 ± 0.09	92	**0.016**
	A_2	C_2	14.19 ± 0.08	103	14.57 ± 0.06	567	**<0.001**
	C_2	A_2	14.43 ± 0.05	578	14.33 ± 0.08	92	0.257
Shoulder Yield (%)	A_2		16.58 ± 0.09	103	16.83 ± 0.05	567	**0.005**
	B_2		16.76 ± 0.06	231	16.81 ± 0.05	439	0.511
	C_2		16.81 ± 0.05	578	16.66 ± 0.09	92	0.097
	A_2	C_2	16.56 ± 0.09	103	16.79 ± 0.06	567	**0.014**
	C_2	A_2	16.72 ± 0.06	578	16.63 ± 0.09	92	0.349
Total Yield (%)	A_2		52.15 ± 0.24	103	52.96 ± 0.13	567	**0.001**
	B_2		52.81 ± 0.17	231	52.85 ± 0.14	439	0.824
	C_2		52.90 ± 0.13	578	52.33 ± 0.25	92	**0.025**
	A_2	C_2	52.09 ± 0.24	103	52.81 ± 0.17	567	0.005
	C_2	A_2	52.63 ± 0.16	578	52.26 ± 0.25	92	0.159

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

For the variant model, GLMM revealed that the presence (and absence) of *PROP1* variants, A_2 and C_2, were associated with carcass traits. In the single-variant model, the presence of A_2 was associated with an increase in hot carcass weight by 1.16 kg, loin yield by 2.8%, shoulder yield by 1.5%, and total yield by 1.5% (Table 7.14).

In contrast, the C_2 variant was associated with a decrease in hot carcass weight by 0.42 kg, loin yield by 1.4%, and total yield by 1.1%. The associations observed for the A_2 variant persisted, while those observed for C_2 were lost in the multi-variant analyses. This means that the A_2 variant effect is not weakened by the presence of other variants.

In the multi-variant model, the effect of A_2 in increasing leg, loin, shoulder and total yields persisted. There was a trend for association (P = 0.075) between intermuscular fat depth trait and presence/absence of B_2. This trend was lost in the multi-variant model, suggesting the effect was probably driven by variant A_2 in the large number of A_2B_2 lambs.

Association studies assessing the effect of individual SNPs in exon 2 with carcass traits were carried out in NZ Romney breeds, and the results are summarised in Table 7.15.

Table 7.15 Association of *PROP1* exon 2 SNP genotypes with carcass traits in NZ Romney sheep

SNP / Trait	c.189G>C (Arg63Ser)			
	GG (n = 578)	**CG (n = 87)**	**CC (n = 5)**	**P-value**
HCW[1] (kg)	17.17 ± 0.09[a]	16.77 ± 0.17[b]	16.45 ± 0.67[b]	**0.042**
V-GR[2] Fat Depth (mm)	4.26 ± 0.12[a]	4.13 ± 0.25[b]	8.06 ± 0.96[b]	**<0.001**
Leg Yield (%)	21.52 ± 0.06	21.35 ± 0.13	20.58 ± 0.48	0.072
Loin Yield (%)	14.57 ± 0.04[a]	14.41 ± 0.09[ab]	13.58 ± 0.34[b]	**0.003**
Shoulder Yield (%)	16.81 ± 0.05[a]	16.72 ± 0.09[b]	15.49 ± 0.36[b]	**0.001**
Total Yield (%)	52.90 ± 0.13[a]	52.49 ± 0.26[ab]	49.65 ± 0.99[b]	**0.002**
	c.237G>A (Ala79Ala)			
	GG (n = 102)	**AG (n = 396)**	**AA (n = 172)**	**P-value**
HCW[1] (kg)	16.13 ± 0.16[a]	17.29 ± 0.09[b]	17.28 ± 0.12[b]	**<0.001**
V-GR[2] Fat Depth (mm)	3.93 ± 0.24	4.29 ± 0.14	4.41 ± 0.18	0.198
Leg Yield (%)	21.36 ± 0.12	21.52 ± 0.07	21.51 ± 0.09	0.430
Loin Yield (%)	14.20 ± 0.08[a]	14.60 ± 0.05[b]	14.63 ± 0.06[b]	**<0.001**
Shoulder Yield (%)	16.57 ± 0.09[a]	16.83 ± 0.05[ab]	16.82 ± 0.07[b]	**0.018**
Total Yield (%)	52.14 ± 0.24[a]	52.95 ± 0.14[ab]	52.96 ± 0.19[b]	**0.004**
	c.342+62A>T			
	AA (n = 102)	**AT (n = 396)**	**TT (n = 172)**	**P-value**
HCW[1] (kg)	16.13 ± 0.16[a]	17.29 ± 0.09[b]	17.29 ± 0.12[b]	**<0.001**
V-GR[2] Fat Depth (mm)	3.93 ± 0.24	4.29 ± 0.14	4.41 ± 0.18	0.198
Leg Yield (%)	21.36 ± 0.12	21.52 ± 0.07	21.51 ± 0.09	0.430
Loin Yield (%)	14.20 ± 0.08[a]	14.60 ± 0.05[b]	14.63 ± 0.06[b]	**<0.001**
Shoulder Yield (%)	16.57 ± 0.09[a]	16.83 ± 0.05[ab]	16.82 ± 0.07[b]	**0.018**
Total Yield (%)	52.14 ± 0.24[a]	52.95 ± 0.14[ab]	52.96 ± 0.19[b]	**0.004**

[a, b] Mean values in the same row with different superscripts differ at P < 0.05

[1] HCW = Hot carcass weight, [2] V-GR = Viascan fat depth around the 12th rib

The variant *CC* genotype at the c.189G>C locus was strongly associated with lower carcass yields when compared to its *GG* counterpart (Table 7.15). The trait values for the *CG* heterozygotes dwelled in a middle range between the *GG* and *CC* genotype values. The *CC* genotype exhibited a decrease in hot carcass weight by 4.2%, loin yield by 6.8%, shoulder yield by 7.8%, and total yield by 6.1%, when compared to *GG*.

While leg yield followed a similar trend to the other yields, its association was not significant (P = 0.072). Interestingly, the *CC* genotype was associated with a remarkable 89% increase in the fat depth around the 12th rib when compared to the *GG* genotype. It is worth noting that only a small number of animals (n = 5) carried the *CC* genotype, as compared to 578 *GG*-possessing animals. This enormous disparity in genotype distribution could justify some of the findings.

In contrast, the variant genotypes, *AA* and *TT*, at the c.237G>A and c.342+62A>T loci respectively, were both associated with a marked increase in hot carcass weight from 16.13 kg to 17.29 kg, loin yield from 14.20% to 14.63%, shoulder yield from 16.57% to 16.82%, and total yield from 52.14% to 52.96% (Table 7.15). Unlike the c.189 G>C SNP, the heterozygotes of c.237G>A and c.342+62A>T had very similar trait values to their variant homozygotes (*AA* and *TT* respectively), which were higher than those for the original homozygotes (*GG* and *AA* respectively).

For hot carcass weight, the heterozygotes (*AG/AT*) and variant homozygotes both weighed 17.29 kg; and heterozygotes and variant homozygotes only differed in total yield by 0.01%. It would appear that the variant allele, *A* and *T* for c.237G>A and c.342+62A>T respectively, has stronger trait influence that dominates over the original allele when both are present. No association was observed for leg yield and fat depth with genotypes at both loci.

7.3.7 Protein Alignment and Stability Assessment

Figure 7.5 compares the amino acid sequences of PROP1 in sheep to other species: human, goat, cow, pig, dog, mouse and rat. The region compared encompasses the fragment sequenced in this study, ranging from amino acid residues 38 to 114. The arginine amino acid at the 63rd position was found to be conserved across various species, differing only in the C_2 variant reported in this study, where it was putatively substituted for a serine. On the other hand, the alanine residue at position 79 was replaced by a valine in humans, and glycine in dogs. Online analysis performed in this study using a predictor software of

protein stability revealed that the substitution of arginine for serine at position 63 in exon 2 decreased the stability of the PROP1 protein.

```
Sheep_PROP1 MNTQPYRNLSGVRVGRPKLSLQGGQRGRPHSRRRHRTTFSPAQLEQLESA     38-87
Human_PROP1 ssap-c-r-p-agg--srf-p----------------------v--------     38-87
 Goat_PROP1 -------------a----------------------------------------     38-87
  Cow_PROP1 ----------------------v-----------------------------     38-87
  Pig_PROP1 is-r--------ga---r--p----------------------------------     38-87
  Dog_PROP1 vsp--sk-----g-r--r--p-------l----------n-g------t-     38-87
Mouse_PROP1 rssea--r---tel-----cp-...-------------n-----------     38-84
  Rat_PROP1 rspetskr---tgl-----cp-...-------------n---g-----     38-84
   A2 _PROP1 --------------------------------------------------     38-87
   B2 _PROP1 --------------------------------------------------     38-87
   C2 _PROP1 ------------------------s---------------------------     38-87

Sheep_PROP1 FGKNQYPDIWARESLAQDTGLSEARIQ                          88-114
Human_PROP1 --r-------------r----------                          88-114
 Goat_PROP1 ---------------------------                          88-114
  Cow_PROP1 --r------------------------                          88-114
  Pig_PROP1 --r----------g--r----------                          88-114
  Dog_PROP1 --r----------g--r----------                          88-114
Mouse_PROP1 --r----------g-------------                          85-111
  Rat_PROP1 --r-------v--g-------------                          85-111
   A2 _PROP1 ---------------------------                          88-114
   B2 _PROP1 ---------------------------                          88-114
   C2 _PROP1 ---------------------------                          88-114
```

Figure 7.5 Alignment of PROP1 exon 2 protein sequence across different species

Multiple sequence alignment of PROP1 exon 2 protein sequence across different species starting from amino acid p.38 to p.114, using the DNAMAN software. Bars (-) denote nucleotides identical to the 'Sheep_PROP1' sequence, while dots (.) denote missing nucleotides. The homeodomain is emboldened (residues 69 - 128), and two regions of nucleotide variation found in this study, p.Arg63Ser and p.Ala79Ala, are highlighted. The amino acid sequences were extracted from Genbank with the following accession numbers: Human, *Homo sapiens* (AF076215); Sheep, *Ovis aries* (AY533709); Goat, *Capra hircus* (XM_005682787); Cow, *Bos taurus* (XM_015472155); Pig, *Sus scrofa* (XM_013987527); Dog, *Canis familiaris* (NM_001020807); Rat, *Rattus norvegicus* (XM_008767664) and Mouse, *Mus musculus* (U77946). A_2, B_2, and C_2 are based on amino acid sequences predicted from the DNA sequences reported in this study for the respective variants.

7.4 Discussion

This is the first study demonstrating an association between variations in *PROP1* and growth and carcass traits in NZ Romney sheep. This study detected a total of six SNPs: one SNP in exon 1 (c.45A>G), two SNPs in intron 1 (c.109+40 T>C and c.109+207C>T), two SNPs in exon 2 (c.189G>C and c.237G>A), and one SNP in intron 2 (c.342+62A>T). The c.109+40 T>C and c.109+207C>T SNPs were novel, while the other four SNPs had been reported previously in Chinese Merino sheep (Zeng et al., 2011). Genotype and allele frequencies of *PROP1* similar to those found in this study were reported in the aforementioned investigation. However, two variations, g.1402C>T (p.Leu84Leu) and g.1424A>G (p.Asn91Ser), which were detected by Zeng et al. (2011) in the exon 2 of *PROP1*, were not observed in this study.

Two putative non-synonymous and synonymous amino acid substitutions, p.Arg63Ser (c.189G>C) and p.Ala79Ala (c.237G>A) respectively, were identified. For each of the 336 bp and 385 bp fragments, three different SNPs presenting with three genotypes at each locus were detected respectively, thus indicating that *PROP1* is quite variable. Supporting this notion, studies have reported *PROP1* variations in humans (Duquesnoy et al., 1998; Wu et al., 1998), cattle (Pan et al., 2007), goats (Lan et al., 2009), and mice (Sornson et al., 1996).

In the past, breeding for growth and carcass traits relied on the physical measurement of phenotypic traits, and the ancestral performance of individuals within the population (Lande and Thompson, 1990). This was both cumbersome and unreliable as some key production traits had low heritabilities and were often measured post-slaughter. Thus, achieving rapid genetic gain in some desirable traits was difficult. The emergence of QTL that linked phenotypic traits with particular genes, or regions in the chromosome, was a breakthrough for the meat industry. It enabled the identification of genes that underpinned key production traits, such as improved growth rate and increased musculature. In this respect, *PROP1* is located on chromosome 5, and in a region that harbours a QTL for growth and liveweight in sheep (Walling et al., 2004). The PROP1 protein also plays a key role in early growth and homeostasis, as it regulates the production of secretory cells of the anterior pituitary. *PROP1*, therefore, provides promising potential as a genetic marker for breeding sheep with improved growth and carcass performance.

Exon 1

Similar to the present study, Zeng et al. (2011) described an *A* to *G* variation in the exon 1 of ovine *PROP1*, potentially resulting in a synonymous substitution of the glutamic acid residue at position 15 of the polypeptide. The genotype frequencies noted for the Chinese Merino sheep examined were comparable to this study, with the major genotypes found in the population being *AG* and *GG*, whilst the *AA* genotype was not observed in the Chinese Merino sheep. This study did observe the occurrence of the *AA*, albeit at a low frequency, thus reflecting either breed differences and/or the larger sheep population examined. Zeng et al. (2011) found no association with wool traits, in contrast, results from this study demonstrated a strong association between the *GG* genotype of the c.45A>G variation and an increase in weaning weight, growth rate, hot carcass weight and total lean meat yield, as well as a decrease in intermuscular fat depth.

Emerging evidence suggests that synonymous and non-coding intronic variations, although not yielding any amino acid changes, can affect the phenotypic characteristics of the protein product in diverse ways, such as altering the mRNA stability, structure and codon usage during translation, changing the rate of translation, altering protein expression, stability and three-dimensional folding, and modifying the electrical charge of the resulting polypeptide (Le Hir et al., 2003; Sauna and Kimchi-Sarfaty, 2011; Hunt et al., 2014).

Within the non-coding region of ovine *PROP1*, Liu et al. (2015) examined small tail Han sheep and reported that sheep with the *TT* and *CT* genotypes of a C330T variation in intron 1 had a higher litter size compared to sheep with the *CC* genotype. However, this difference was not significant. A g.1198 A>G variation reported by Zeng et al. (2011) was also not found to have any significant association with wool traits, such as fibre diameter. Neither of those previously reported variations were observed in the present study. In contrast, this study described strong positive associations, with individuals possessing the variant *CC* genotype of c.109+40 T>C weighing more at tailing and slaughter, were faster-growing, and had higher leg and total lean meat yields. On the other hand, negative associations with sheep growth and carcass traits were observed with the *TT* homozygous genotypes of c.109+207C>T.

The first intron of *PROP1* plays an interesting role in the unique relationship between Notch and PROP1 signalling during embryonic pituitary development. Electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) analyses have revealed that Rbp-J, a DNA-binding protein that mediates Notch signalling, can regulate

the activity of PROP1 by binding to an evolutionarily conserved consensus sequence within the intron 1 of *PROP1* (Zhu et al., 2006). Although Notch signalling via Rbp-J does not initiate *PROP1* expression, it is required for the upregulation and sustained expression of PROP1 at embryonic day E12.5 of murine embryos (Sornson et al., 1996; Zhu et al., 2006). This suggests that the first intron of *PROP1* is a direct target of Rbp-J-mediated Notch activity, which plays a vital mechanistic role in upregulating PROP1 expression, and hence is crucial to the early lineage commitment of cells in the anterior pituitary.

Providing further credence to this view are studies into other genes that have demonstrated the regulatory effects of first introns in altering transcription efficiency and gene expression levels (Gaunitz et al., 2004; Chorev and Carmel, 2012). Gene modulation was achievable by means of regulatory elements, such as silencers and enhancers present in these 5'-proximal introns (Gaunitz et al., 2004; Chorev and Carmel, 2012). Supporting this notion and consistent with the findings of this study, variation in the intron 1 of the ovine myostatin gene showed association with meat production traits in NZ sheep breeds, including leg, loin, and total meat yields (Hickford et al., 2010).

The results observed for the genotypes were consistent in the variant model. The A_1 variant, which comprised of the *G*-*C*-*C* alleles at the three polymorphic loci in that order, was associated with an increase in weaning and hot carcass weights, growth rate-to-weaning, lean meat yields in the leg, loin and shoulder, and lower fat deposition. On the other hand, the B_1 variant, which consisted of *A*-*T*-*C* alleles, had negative effects on growth traits and carcass traits including lower birth weight, weaning weight, growth rate, loin yield, total yield, and increased intermuscular fat depth.

Exon 2

The A_2 variant was associated with higher weaning weight, growth rate, hot carcass weight, and loin, shoulder and total lean meat yields. Conversely, the C_2 variant showed association with decreased birth rate, weaning weight, growth rate, hot carcass weight, loin yield and total yield. The C_2 association with carcass traits was found to be diminished by the presence of other variants, unlike the persistent A_2 association.

The *G* allele of c.189G>C was more frequent than the *C* allele by thirteen-fold within the population. This stark difference could be due to the non-synonymous nature of the resulting putative p.Arg63Ser substitution, which was predicted by stability prediction software to yield a less stable PROP1 protein. Consistent with this notion is the finding that

the arginine residue at position 63 is highly conserved across various species. This evolutionary conservation, which is scarcely explored, points to the pronounced importance of Arg63 in the physiological function of PROP1. The two residues differ in chemical grouping, with arginine possessing a positively charged side chain, while serine contains an uncharged polar side chain (Wolfenden et al., 1981). The unique interactions between the side chains of specific amino acids which determine the three-dimensional shape of the polypeptide, could be distorted by substitution such as that detected in this study. Amino acid residues in core positions within a protein are particularly vital to its folding, stability and biological function (Bowie et al., 1990).

The p.Arg63Ser substitution lies in a central location in the vicinity of the homeobox domain of PROP1, and could potentially result in a significant change in the structure and conformation of the protein product, thus affecting its function. The homeobox domain carries out the primary function of PROP1 as a transcription factor, by interacting directly with target DNA through hydrogen bonding and hydrophobic interaction (Inukai et al., 2017). A change in protein conformation as a consequence of the presence of Ser63, could impair its ability to activate the transcription of downstream targets such as POU1F1-dependent somatotropes, lactotropes and thyrotropes. Subsequent reduction in the levels of GH, LH and FSH could then impede growth and muscle development, as has been documented for humans and mice possessing mutations in *PROP1* (Nakamura et al., 1999; Kelberman et al., 2009b). Guy et al. (2004) reported that variations within the basic regions of the *PROP1* homeodomain altered its DNA binding and transactivation activities in both humans and sheep.

This would ultimately bring about a cascade effect manifesting as decreased yield of production traits in livestock breeds. This is supported by findings in this study that individuals possessing the *CC* genotype of c.189G>C had lower hot carcass and weaning weights, growth rate and lean meat yield. On the other hand, the *GG* genotype was linked with a marked increase in weights at weaning and slaughter, higher growth rate, increased loin, shoulder and total lean meat yields, and decrease in intermuscular fat thickness. While intramuscular fat is generally indicative of the juicy texture of meat, excessive visceral or intermuscular fat however, is commercially undesirable as many consumers view it as unhealthy and unappetising. The breeder could, therefore, potentially breed in favour of the *GG* genotype, and against its *CC* counterpart.

It is notable that exon 2 of *PROP1*, within which the homeodomain lies, has been an area of growing attention since it is considered a 'hotspot' that accounts for over half of all reported mutations associated with CPHD in humans (Wu et al., 1998; Kelberman and Dattani, 2006; Lemos et al., 2006). Arginine residues frequently occur within and around the homeodomain, indicating their importance to PROP1 function. In humans, a p.Arg71Cys substitution has been revealed to underpin structural change in PROP1 associated with CPHD and the accompanying low circulating levels of GH, TSH and PRL hormones (Paracchini et al., 2003). This human substitution is sufficiently close to the ovine p.Arg63Ser described herein, to suggest a similar effect might occur in sheep.

A reported g.1389G>A SNP that would result in a putative synonymous p.Ala79Ala substitution was found to have no association with wool traits in Chinese Merino Sheep (Zeng et al., 2011). In contrast, this study revealed that despite its synonymous nature, the c.237G>A (p.Ala79Ala) variation was associated with various growth and carcass traits. The *AA* genotype, although producing the same alanine residue, was associated with increased weaning and carcass weights, and higher growth rate and lean meat yields, when compared to its *GG* counterpart. The *TT* genotype of the c.342+62A>T SNP present in the non-coding region was also associated with superior growth and carcass traits.

These associations could be due to the linkage of c.237G>A (Ala79Ala) and c.342+62A>T to other trait-influencing SNPs in its vicinity; or alternatively that the change in the nucleotide sequence affects some aspect of the transcription or translation of *PROP1*. The alanine residue at the 79th position is also fairly conserved across mammalian species, except for humans and dogs. Interestingly, a non-synonymous p.Ala79Val substitution (AF453512: g.1795C>T) at an analogous caprine *PROP1* region, has been described in Chinese goat breeds; however, it was found to have no significant associations with wool traits and milk yield (Lan et al., 2009).

The disparity in association and significance between the current study and the previous research could be attributed to the differences in the breeds, number of animals or traits investigated. While most studies so far examine ovine *PROP1* in relation to wool production, milk production or fertility (Zeng et al., 2011; Liu et al., 2015), this study explores a different direction of growth traits. Indeed, growth traits are considered to be some of the most important traits in animal production as they interest both breeders and consumers. However, some previous reports are consistent with the present study in describing the association between *PROP1* variations in other regions of the gene and

production traits in livestock breeds. This includes a g.2647A>G SNP in the exon 3 of *PROP1* found to be associated with wool traits in Chinese Merino Sheep (Zeng et al., 2011). Also in accordance with the present study, an *A* to *G* variation in the exon 3 of bovine *PROP1*, resulting in a p.His173Arg amino acid change, was found to be significantly associated with milk production traits, protein yield and fertility in Holstein Friesian cattle (Lan et al., 2013a; Lan et al., 2013b).

Selection for sheep possessing the A_1 and A_2 variants of ovine *PROP1*, or the *G*, *A* and *T* alleles of c.189G>C, c.237G>A, and c.342+62A>T respectively, may serve as a future strategy in selecting animals with superior meat qualities for breeding. Selection against the B_1 variant and the *C* allele of c.189G>C could be employed for healthier lean meat options with greater yield.

In a commercial context, the variations found in this study could be potentially profitable. The average pay-out scheme for farmers and producers in NZ is $6 per kg of lamb carcass. The A_1 and A_2 variants of ovine *PROP1* were associated with an increase in hot carcass weight by 0.59 kg and 1.16 kg, and hence putatively generating an extra $3.5 and $7 per carcass, respectively. The total lamb exports for NZ over the 2018 – 2019 period was estimated to be 27 million carcasses, which yielded an estimated $3.23 billion in revenue (Ministry of Primary Industries, 2019). This means that if the A_2 variant were incorporated into all flock, assuming all lambs responded in a similar fashion to this study, the small genetic gain could potentially translate to an increase in sheep meat revenue by $189 million; whereas the A_1 variant would generate half that amount. Further testing of allelic effects and validation would, however, be required to ensure the reliability of using these novel SNPs as markers.

7.5 Conclusion

In summary, this study reports novel associations between variants of *PROP1* and economically important growth and meat quality traits in sheep. These findings are consistent with research in other animal breeds, and hence could be valuable as potential markers for use in marker-assisted breeding programs. The findings of this study confirm the role of *PROP1* variations on growth traits in sheep. This improves understanding of the gene-trait interplay and could potentially contribute to advancing animal breeding techniques and MAS. Future research could look for variations in other regions of *PROP1* and across various sheep breeds, and assess their effects on commercially desirable traits.

Chapter 8
General Summary and Future Direction

In order to maintain viability, the multi-billion dollar NZ meat industry must aim for increased efficiency, by reducing production cost and increasing productivity. Using genetic markers, enhanced productivity in sheep production can be accomplished by selection and breeding for increased muscling and higher growth rates. The search for nucleotide sequence variations in specific loci influencing key production traits is a necessity for sheep genetic improvement approaches.

This thesis focused on identifying genetic variation within four genes of the somatotropic axis in sheep – *GH*, *IGF1R*, *POU1F1* and *PROP1*, and whether any variation found was associated with sheep production traits. These genes were selected because of their documented effects on growth and carcass traits in other mammals, including cattle, goats and sheep. The genes selected were involved in energy metabolism and protein turnover (*IGF1R*, Chapter 4), somatic growth and development (*GH*, Chapter 5), and regulation of the expression of other genes (*POU1F1* and *PROP1*, Chapters 6 and 7).

The IGF1 receptor, a potent effector of GH action, appeared to be more conserved through evolution. Of the three separate fragments amplified, only the fragment that spanned the exon 9 to intron 10 region had delectable nucleotide variation (Chapter 4). Being either synonymous or within introns, none of the nucleotide sequence variations were found to alter the amino acid sequence. Ten novel nucleotide variations were however revealed, and these defined five sequence variants (A_9 to E_9). Variant A_9 was associated with increased postnatal growth rate and higher lean meat yield. Given its central role in vertebrates, these 'subtle' *IGF1R* variations could be useful for developing genetic tools that select for faster-growing, higher-yielding lambs, with lower fat deposition, as they might persist long enough in future generations to reliably be passed onto offspring.

In Chapter 5, a fragment of ovine *GH* spanning exon 2 – exon 3 was revealed to be highly variable, with 11 nucleotide substitutions defining seven novel nucleotide sequences (A_3 to G_3). Four of the substitutions were non-synonymous, thereby putatively resulting in changes in the amino acid sequence. The B_3 variant was associated with an increase in average daily weight gain and higher lean meat yield. These findings suggest that

nucleotide sequence variation in *GH* could be applied in the development of markers for breeding faster-growing lambs that produce leaner meat.

The gene *POU1F1* also appeared to be evolutionarily conserved, with two (exons 3 and 6) of the three explored regions showing no variation (Chapter 6). This might be due to the crucial domains encoded by these gene regions. The exon 1 region showed limited variation with only two variants (*A* and *B*) being observed. Further investigation into the non-coding regions of *POU1F1* is required to determine if they have greater freedom to accumulate genetic variations.

The gene *PROP1* revealed greater variability (Chapter 7). Three nucleotide variants were identified for both the exon 1 (A_1, B_1 and C_1) and exon 2 (A_2, B_2 and C_2) regions; and three nucleotide substitutions were also described for each region. The A_1 variant was associated with increased muscling and lower intermuscular fat levels, while the A_2 variant was associated with higher lamb growth rates. These *PROP1* variants could therefore possibly be useful in breeding lambs that grow more quickly and produce more meat.

Strategies that use alterations in environmental factors such as farm management approaches, nutrition, and pre-slaughter handling, only provide short-lived or non-enduring improvements. Long-lasting solutions that can be passed down to future livestock generations would require breeding strategies utilising genetic technologies to bring about improved livestock performance.

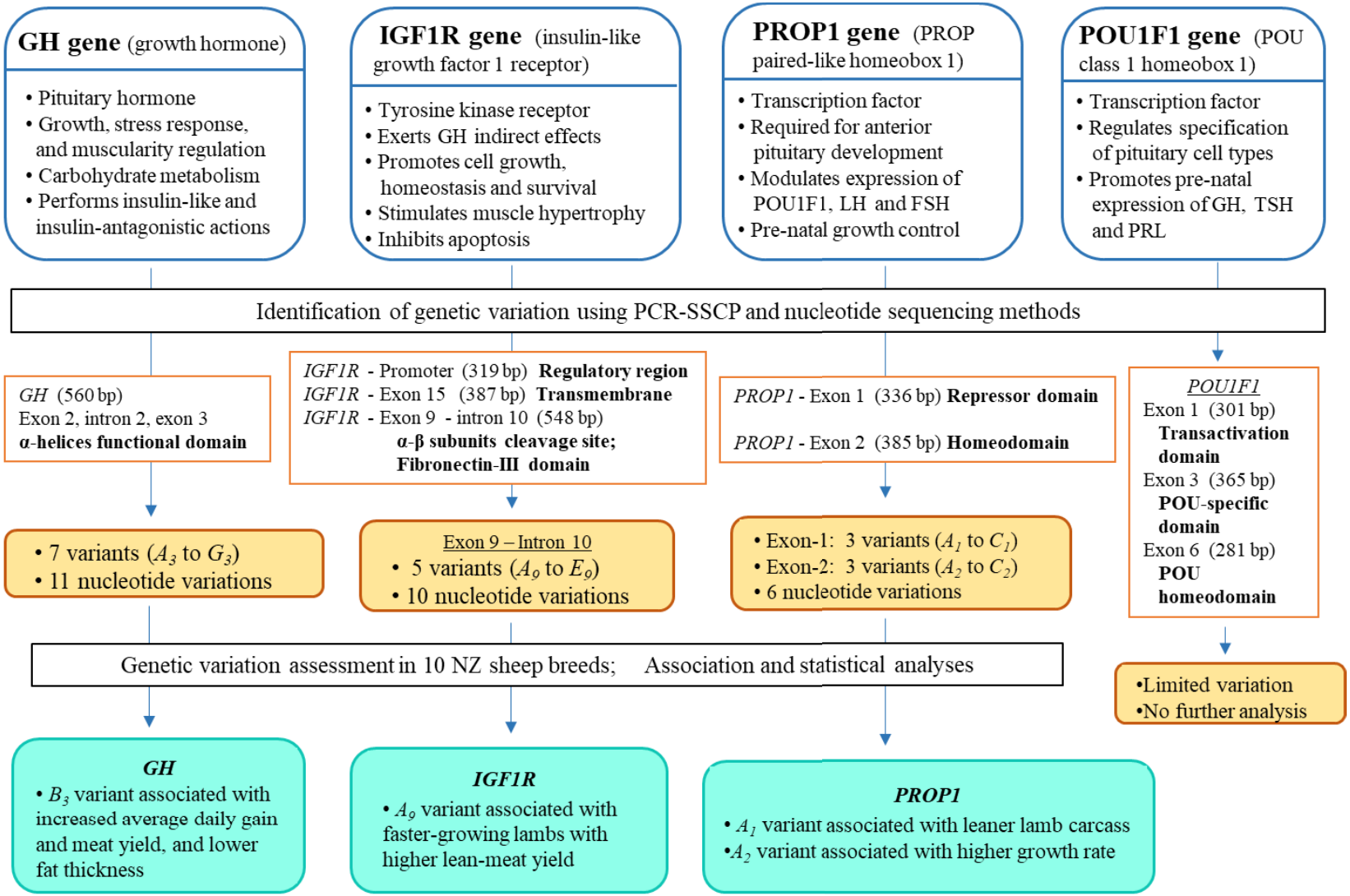
In this respect, breeding for faster-growing sheep with lean-carcasses by means of genetic technologies would benefit the sheep industry, enabling them to better meet consumer expectations and better financially reward farmers and breeders. High quality and premium meat products would also ensure that New Zealand retains its stake in the competitive global sheep meat market, bringing in important export revenue.

Although the influence of genetics on animal growth and carcass traits is well established, there still exists a dearth of information on genetic control in small ruminant animals. Future work, therefore, needs to examine other key genes involved in growth and metabolic pathways, with the aim of developing a multi-gene breeding tool that improves the accuracy and speed of genetic selection.

As promising as the early findings from this thesis are, further research is required to replicate and confirm the reported associations before they can be applied in breeding selection approaches. These studies could be undertaken using more advanced genetic

tools, such as clustered regularly interspaced short palindromic repeats (CRISPR), to drive greater genetic variation, by studying a wider range of sheep breeds and other livestock species such as cattle, goats and pigs. Information obtained from this research would ultimately improve the accuracy of choices made when a breeding stock is selected.

Diagrammatic summary of this thesis



References

Abuzzahab, M.J., Schneider, A., Goddard, A., Grigorescu, F., Lautier, C., Keller, E., Kiess, W., Klammt, J., Kratzsch, J. and Osgood, D. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *New England Journal of Medicine* **349** (2003), pp. 2211-2222.

Adams, T.E., Epa, V., Garrett, T. and Ward, C. Structure and function of the type 1 insulin-like growth factor receptor. *Cellular and Molecular Life Sciences CMLS* **57** (2000), pp. 1050-1093.

Agarwal, G., Bhatia, V., Cook, S. and Thomas, P.Q. Adrenocorticotropin deficiency in combined pituitary hormone deficiency patients homozygous for a novel PROP1 deletion. *The Journal of Clinical Endocrinology & Metabolism* **85** (2000), pp. 4556-4561.

Akis, I., Oztabak, K., Gonulalp, I., Mengi, A. and Un, C. IGF-1 and IGF-1R gene polymorphisms in East Anatolian Red and South Anatolian Red cattle breeds. *Russian journal of genetics* **46** (2010), pp. 439-442.

Allen, R.E., Merkel, R.A. and Young, R.B. Cellular aspect of muscle growth: myogenic cell proliferation. *Journal of Animal Science* **49** (1979), pp. 115-127.

An, X., Hou, J., Wang, L., Li, G., Wang, J., Song, Y., Zhou, G., Han, D., Ling, L. and Cao, B. Novel polymorphisms of the growth hormone gene and their effect on growth traits in Chinese goats. *Meat science* **86** (2010), pp. 758-763.

Ardlie, K.G., Kruglyak, L. and Seielstad, M. Patterns of linkage disequilibrium in the human genome. *Nature reviews. Genetics* **3** (2002), p. 299.

Argetsinger, L.S., Campbell, G.S., Yang, X., Witthuhn, B.A., Silvennoinen, O., Ihle, J.N. and Carter-Su, C. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. *Cell* **74** (1993), pp. 237-244.

Argetsinger, L.S. and Carter-Su, C. Mechanism of signaling by growth hormone receptor. *Physiological Reviews* **76** (1996), pp. 1089-1107.

Bahrami, A., Miraei-Ashtiani, S., Mehrabani-Yeganeh, H., Banani-Rad, H. and Behzadi, S. The association between polymorphism of the GH1 gene and changes in protein structure and carcass traits in Mehraban sheep (Ovis aries). *Animal production science* **55** (2015), pp. 661-665.

Baker, J., Hardy, M.P., Zhou, J., Bondy, C., Lupu, F., Bellvé, A.R. and Efstratiadis, A. Effects of an Igf1 gene null mutation on mouse reproduction. *Molecular endocrinology* **10** (1996), pp. 903-918.

Banerjee-Basu, S. and Baxevanis, A.D. Molecular evolution of the homeodomain family of transcription factors. *Nucleic acids research* **29** (2001), pp. 3258-3269.

Barrett, L.W., Fletcher, S. and Wilton, S.D. Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. *Cellular and molecular life sciences* **69** (2012), pp. 3613-3634.

Barta, A., Richards, R.I., Baxter, J.D. and Shine, J. Primary structure and evolution of rat growth hormone gene. *Proceedings of the National Academy of Sciences* **78** (1981), pp. 4867-4871.

Bartke, A. The response of two types of dwarf mice to growth hormone, thyrotropin, and thyroxine. *General and comparative endocrinology* **5** (1965), pp. 418-426.

Barton-Davis, E.R., Shoturma, D.I., Musaro, A., Rosenthal, N. and Sweeney, H.L. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proceedings of the national academy of sciences* **95** (1998), pp. 15603-15607.

Barton, E.R., Morris, L., Musaro, A., Rosenthal, N. and Sweeney, H.L. Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. *The Journal of cell biology* **157** (2002), pp. 137-148.

Bastos, E., Ávila, S., Cravador, A., Renaville, R., Guedes-Pinto, H. and Castrillo, J.L. Identification and characterization of four splicing variants of ovine POU1F1 gene. *Gene* **382** (2006a), pp. 12-19.

Bastos, E., Santos, I., Parmentier, I., Castrillo, J.L., Cravador, A., Guedes-Pinto, H. and Renaville, R. Ovis aries POU1F1 gene: cloning, characterization and polymorphism analysis. *Genetica* **126** (2006b), pp. 303-314.

Baumann, G. Growth hormone heterogeneity: genes, isohormones, variants, and binding proteins. *Endocrine Reviews* **12** (1991), pp. 424-449.

Baxter, R.C. IGF binding proteins in cancer: mechanistic and clinical insights. *Nature Reviews Cancer* **14** (2014), pp. 329-341.

Beck, K.D., Powell-Braxtont, L., Widmer, H.-R., Valverde, J. and Hefti, F. Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. *Neuron* **14** (1995), pp. 717-730.

Beef + Lamb NZ: Domestic Trends and Measuring Progress against the Read Meat Sector Strategy, *Presentation To: Red Meat Sector Conference 2012* (2012).

Beef + Lamb NZ: Export statistics - red meat (2017).

Beef + Lamb NZ: Lamb Export Statistics (2018a).

Beef + Lamb NZ: New Season Outlook 2018-19 (2018b).

Bell, G.I., Pictet, R.L., Rutter, W.J., Cordell, B., Tischer, E. and Goodman, H.M. Sequence of the human insulin gene. *Nature* **284** (1980), p. 26.

Bergan-Roller, H.E. and Sheridan, M.A. The growth hormone signaling system: Insights into coordinating the anabolic and catabolic actions of growth hormone. *General and Comparative Endocrinology* **258** (2018), pp. 119-133.

Berger, M.F., Badis, G., Gehrke, A.R., Talukder, S., Philippakis, A.A., Peña-Castillo, L., Alleyne, T.M., Mnaimneh, S., Botvinnik, O.B. and Chan, E.T. Variation in

homeodomain DNA binding revealed by high-resolution analysis of sequence preferences. *Cell* **133** (2008), pp. 1266-1276.

Bhaumick, B., Bala, R. and Hollenberg, M. Somatomedin receptor of human placenta: solubilization, photolabeling, partial purification, and comparison with insulin receptor. *Proceedings of the National Academy of Sciences* **78** (1981), pp. 4279-4283.

Bobola, N. and Merabet, S. Homeodomain proteins in action: similar DNA binding preferences, highly variable connectivity. *Current opinion in genetics & development* **43** (2017), pp. 1-8.

Bodine, S.C., Stitt, T.N., Gonzalez, M., Kline, W.O., Stover, G.L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J.C. and Glass, D.J. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nature cell biology* **3** (2001), p. 1014.

Bodner, M., Castriilo, J.-L., Theill, L.E., Deerinck, T., Ellisman, M. and Karin, M. The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. *Cell* **55** (1988), pp. 505-518.

Böttner, A., Keller, E., Kratzsch, J.r., Stobbe, H., Weigel, J.F., Keller, A., Hirsch, W., Kiess, W., Blum, W.F. and Pfäffle, R.W. PROP1 mutations cause progressive deterioration of anterior pituitary function including adrenal insufficiency: a longitudinal analysis. *The Journal of Clinical Endocrinology & Metabolism* **89** (2004), pp. 5256-5265.

Bowie, J.U., Reidhaar-Olson, J.F., Lim, W.A. and Sauer, R.T. Deciphering the message in protein sequences: tolerance to amino acid substitutions. *Science* **247** (1990), pp. 1306-1310.

Bray, G.A. and Popkin, B.M. Dietary fat intake does affect obesity! *The American journal of clinical nutrition* **68** (1998), pp. 1157-1173.

Brinster, R.L., Allen, J.M., Behringer, R.R., Gelinas, R.E. and Palmiter, R.D. Introns increase transcriptional efficiency in transgenic mice. *Proceedings of the National Academy of Sciences* **85** (1988), pp. 836-840.

Brito, L.F., McEwan, J.C., Miller, S., Bain, W., Lee, M., Dodds, K., Newman, S.-A., Pickering, N., Schenkel, F.S. and Clarke, S. Genetic parameters for various growth, carcass and meat quality traits in a New Zealand sheep population. *Small Ruminant Research* **154** (2017), pp. 81-91.

Brooks, A.J., Dai, W., O'Mara, M.L., Abankwa, D., Chhabra, Y., Pelekanos, R.A., Gardon, O., Tunny, K.A., Blucher, K.M. and Morton, C.J. Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. *Science* **344** (2014), p. 1249783.

Buckingham, M., Bajard, L., Chang, T., Daubas, P., Hadchouel, J., Meilhac, S., Montarras, D., Rocancourt, D. and Relaix, F. The formation of skeletal muscle: from somite to limb. *Journal of anatomy* **202** (2003), pp. 59-68.

Byrne, C.R., Wilson, B.W. and Ward, K.A. The isolation and characterisation of the ovine growth hormone gene. *Australian journal of biological sciences* **40** (1987), pp. 459-470.

Byrne, T., Amer, P., Fennessy, P., Cromie, A., Keady, T., Hanrahan, J., McHugh, M. and Wickham, B. Breeding objectives for sheep in Ireland: a bio-economic approach. *Livestock Science* **132** (2010), pp. 135-144.

Byun, S., Fang, Q., Zhou, H. and Hickford, J. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Analytical Biochemistry* **385** (2009), pp. 174-175.

Byun, S., Zhou, H. and Hickford, J. Polymorphism of the ovine insulin-like growth factor I receptor (IGFIR) gene. *Molecular and cellular probes* **22** (2008), p. 131.

Byun, S.O., Forrest, R., Frampton, C.M., Zhou, H. and Hickford, J.G. An association between lifespan and variation in insulin-like growth factor I receptor in sheep. *Journal of animal science* **90** (2012), pp. 2484-2487.

Campbell, G.S. Growth-hormone signal transduction. *The Journal of pediatrics* **131** (1997), pp. S42-S44.

Campbell, I.D. and Spitzfaden, C. Building proteins with fibronectin type III modules. *Structure* **2** (1994), pp. 333-337.

Campbell, J.R. and Marshall, R.T., The science of providing milk for man. McGraw Hill Book Company. (1975).

Capriotti, E., Fariselli, P. and Casadio, R. I-Mutant2. 0: predicting stability changes upon mutation from the protein sequence or structure. *Nucleic acids research* **33** (2005), pp. W306-W310.

Cartegni, L., Chew, S.L. and Krainer, A.R. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nature reviews. Genetics* **3** (2002), p. 285.

Carter-Su, C., Rui, L. and Herrington, J. Role of the tyrosine kinase JAK2 in signal transduction by growth hormone. *Pediatric Nephrology* **14** (2000), pp. 550-557.

Carter-Su, C., Schwartz, J. and Smit, L. Molecular mechanism of growth hormone action. *Annual Review of Physiology* **58** (1996), pp. 187-207.

Chen, H.-T., Schuler, L.A. and Schultz, R.D. Growth hormone receptor and regulation of gene expression in fetal lymphoid cells. *Molecular and cellular endocrinology* **137** (1998), pp. 21-29.

Chen, J.C. and Goldhamer, D.J. Skeletal muscle stem cells. *Reproductive Biology and Endocrinology* **1** (2003), p. 101.

Chen, X., Van Valkenburgh, C., Fang, H. and Green, N. Signal peptides having standard and nonstandard cleavage sites can be processed by Imp1p of the mitochondrial inner membrane protease. *Journal of Biological Chemistry* **274** (1999), pp. 37750-37754.

Cheng, J., Randall, A. and Baldi, P. Prediction of protein stability changes for single-site mutations using support vector machines. *Proteins: Structure, Function, and Bioinformatics* **62** (2006), pp. 1125-1132.

Chia, D.J. Minireview: Mechanisms of growth hormone-mediated gene regulation. *Molecular Endocrinology* **28** (2014), pp. 1012-1025.

Chitnis, M.M., Yuen, J.S., Protheroe, A.S., Pollak, M. and Macaulay, V.M. The type 1 insulin-like growth factor receptor pathway. *Clinical cancer research* **14** (2008), pp. 6364-6370.

Choi, Y., Sims, G.E., Murphy, S., Miller, J.R. and Chan, A.P. Predicting the functional effect of amino acid substitutions and indels. *PloS one* **7** (2012), p. e46688.

Chorev, M. and Carmel, L. The function of introns. *Frontiers in genetics* **3** (2012).

Clemmons, D.R. Role of IGF-I in skeletal muscle mass maintenance. *Trends in Endocrinology & Metabolism* **20** (2009), pp. 349-356.

Clemmons, D.R. Role of IGF binding proteins in regulating metabolism. *Trends in Endocrinology & Metabolism* **27** (2016), pp. 375-391.

Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibé, B., Bouix, J., Caiment, F., Elsen, J.-M. and Eychenne, F. A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nature genetics* **38** (2006), p. 813.

Cogan, J., Phillips 3rd, J., Sakati, N., Frisch, H., Schober, E. and Milner, R. Heterogeneous growth hormone (GH) gene mutations in familial GH deficiency. *The Journal of Clinical Endocrinology & Metabolism* **76** (1993), pp. 1224-1228.

Cogan, J.D., Wu, W., Phillips III, J.A., Arnhold, I.J., Agapito, A., Fofanova, O.V., Osorio, M.G.F., Bircan, I., Moreno, A. and Mendonca, B.B. The PROP1 2-base pair deletion is a common cause of combined pituitary hormone deficiency. *The Journal of Clinical Endocrinology & Metabolism* **83** (1998), pp. 3346-3349.

Cohen, L.E., Wondisford, F.E. and Radovick, S. Role of Pit-1 in the gene expression of growth hormone, prolactin, and thyrotropin. *Endocrinology and metabolism clinics of North America* **25** (1996), pp. 523-540.

Coleman, M.E., DeMayo, F., Yin, K.C., Lee, H.M., Geske, R., Montgomery, C. and Schwartz, R.J. Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. *Journal of Biological Chemistry* **270** (1995), pp. 12109-12116.

Collard, B., Jahufer, M., Brouwer, J. and Pang, E. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* **142** (2005), pp. 169-196.

Conway-Campbell, B.L., Wooh, J.W., Brooks, A.J., Gordon, D., Brown, R.J., Lichanska, A.M., Chin, H.S., Barton, C.L., Boyle, G.M. and Parsons, P.G. Nuclear targeting of the growth hormone receptor results in dysregulation of cell proliferation and tumorigenesis. *Proceedings of the National Academy of Sciences* **104** (2007), pp. 13331-13336.

Coolican, S.A., Samuel, D.S., Ewton, D.Z., McWade, F.J. and Florini, J.R. The mitogenic and myogenic actions of insulin-like growth factors utilize distinct signaling pathways. *Journal of Biological Chemistry* **272** (1997), pp. 6653-6662.

Corder, E., Saunders, A., Strittmatter, W., Schmechel, D., Gaskell, P., Small, G.a., Roses, A., Haines, J. and Pericak-Vance, M.A. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261** (1993), pp. 921-923.

Cunningham, B.C. and Wells, J.A. High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis. *Science* **244** (1989), pp. 1081-1085.

Cunningham, M.L., Horst, J.A., Rieder, M.J., Hing, A.V., Stanaway, I.B., Park, S.S., Samudrala, R. and Speltz, M.L. IGF1R variants associated with isolated single suture craniosynostosis. *American Journal of Medical Genetics Part A* **155** (2011), pp. 91-97.

Curi, R.A., De Oliveira, H., Silveira, A.C. and Lopes, C. Association between IGF-I, IGF-IR and GHRH gene polymorphisms and growth and carcass traits in beef cattle. *Livestock Production Science* **94** (2005), pp. 159-167.

Curi, R.A., Palmieri, D.A., Suguisawa, L., Oliveira, H.N.d., Silveira, A.C. and Lopes, C.R. Growth and carcass traits associated with GH1/Alu I and POU1F1/Hinf I gene polymorphisms in Zebu and crossbred beef cattle. *Genetics and Molecular Biology* **29** (2006), pp. 56-61.

D'Ercole, J.A., Ye, P. and O'Kusky, J.R. Mutant mouse models of insulin-like growth factor actions in the central nervous system. *Neuropeptides* **36** (2002), pp. 209-220.

Daga, C., Paludo, M., Luridiana, S., Mura, M.C., Bodano, S., Pazzola, M., Dettori, M.L., Vacca, G.M. and Carcangiu, V. Identification of novel SNPs in the Sarda breed goats POU1F1 gene and their association with milk productive performance. *Molecular biology reports* **40** (2013), pp. 2829-2835.

Dalton, D. and Ackerley, L. Performance of sheep on New Zealand hill country: I. Growth and wool production of wethers of five breeds. *New Zealand journal of agricultural research* **17** (1974), pp. 279-282.

Dasen, J.S. and Rosenfeld, M.G. Combinatorial codes in signaling and synergy: lessons from pituitary development. *Current opinion in genetics & development* **9** (1999), pp. 566-574.

Davis, S., Castinetti, F., Carvalho, L., Ellsworth, B., Potok, M., Lyons, R., Brinkmeier, M., Raetzman, L., Carninci, P. and Mortensen, A. Molecular mechanisms of pituitary organogenesis: in search of novel regulatory genes. *Molecular and cellular endocrinology* **323** (2010), pp. 4-19.

Dawson, L. and Steen, R. Estimation of maintenance energy requirements of beef cattle and sheep. *The Journal of Agricultural Science* **131** (1998), pp. 477-485.

De la Rosa Reyna, X., Montoya, H., Castrellón, V., Rincón, A., Bracamonte, M. and Vera, W. Polymorphisms in the IGF1 gene and their effect on growth traits in Mexican beef cattle. *Genet Mol Res* **9** (2010), pp. 875-883.

De Vos, A.M., Ultsch, M. and Kossiakoff, A.A. Human growth hormone and extracellular domain of its receptor: crystal structure of the complex. *Science* **255** (1992), pp. 306-312.

Deladoëy, J., Flück, C., Büyükgebiz, A., Kuhlmann, B.V., Eblé, A., Hindmarsh, P.C., Wu, W. and Mullis, P.E. "Hot spot" in the PROP1 gene responsible for combined pituitary hormone deficiency. *The Journal of Clinical Endocrinology & Metabolism* **84** (1999), pp. 1645-1650.

Deladoëy, J., Flück, C., Büyükgebiz, A., Kuhlmann, B.V., Eblé, A.e., Hindmarsh, P.C., Wu, W. and Mullis, P.E. "Hot spot" in the PROP1 gene responsible for combined pituitary hormone deficiency. *The Journal of Clinical Endocrinology & Metabolism* **84** (1999), pp. 1645-1650.

Desta, Z.A. and Ortiz, R. Genomic selection: genome-wide prediction in plant improvement. *Trends in plant science* **19** (2014), pp. 592-601.

Di Stasio, L., Sartore, S. and Albera, A. Lack of association of GH1 and POU1F1 gene variants with meat production traits in Piemontese cattle. *Animal Genetics* **33** (2002), pp. 61-64.

Dickerson, G. Animal size and efficiency: basic concepts. *Animal Science* **27** (1978), pp. 367-379.

Dodds, K., Newman, S., Auvray, B. and McEwan, J.: Breeds of New Zealand sheep–as recorded or by genomic prediction, *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* (2013), pp. 274-7.

Dong, H., Sharma, M., Zhou, H.-X. and Cross, T.A. Glycines: role in α-helical membrane protein structures and a potential indicator of native conformation. *Biochemistry* **51** (2012), pp. 4779-4789.

Dott, N.M. and Fraser, J. The influence of experimental pituitary and thyroid derangements upon the developmental growth of bone. *QJ Exp Physiol (Suppl)* **13** (1923), pp. 107-108.

Duan, C., Ren, H. and Gao, S. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. *General and comparative endocrinology* **167** (2010), pp. 344-351.

Dunner, S., Sevane, N., García, D., Cortés, O., Valentini, A., Williams, J., Mangin, B., Cañón, J., Levéziel, H. and Consortium, G. Association of genes involved in carcass and meat quality traits in 15 European bovine breeds. *Livestock Science* **154** (2013), pp. 34-44.

Duquesnoy, P., Roy, A., Dastot, F., Ghali, I., Teinturier, C., Netchine, I., Cacheux, V., Hafez, M., Salah, N. and Chaussain, J.-L. Human Prop-1: cloning, mapping, genomic structure. *FEBS letters* **437** (1998), pp. 216-220.

Dwyer, C. The welfare of the neonatal lamb. *Small Ruminant Research* **76** (2008), pp. 31-41.

Dwyer, C.M. and Bünger, L. Factors affecting dystocia and offspring vigour in different sheep genotypes. *Preventive veterinary medicine* **103** (2012), pp. 257-264.

Efstratiadis, A., Posakony, J.W., Maniatis, T., Lawn, R.M., O'Connell, C., Spritz, R.A., Deriel, J.K., Forget, B.G., Weissman, S.M. and Slightom, J.L. The structure and evolution of the human β-globin gene family. *Cell* **21** (1980), pp. 653-668.

El-Magd, M., Abbas, H., El-Kattawy, A. and Mokhbatly, A. Novel polymorphisms of the IGF1R gene and their association with average daily gain in Egyptian buffalo (Bubalus bubalis). *Domestic animal endocrinology* **45** (2013), pp. 105-110.

Elmes, S.N.: An investigation of on-farm factors that may affect lamb growth, carcass characteristics and meat quality: a thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science at Massey University, Palmerston North, New Zealand. Massey University (2013).

Esmaiel, N.N., Fayez, A.G., Thomas, M.M., Khalaf, R.I., Salem, S.M., Ramadan, A., Helwa, I., Raouf, H.A., El-Bassyouni, H.T. and Ismaeil, S. The association of +1150A polymorphism with low GH level in isolated growth hormone deficiency (IGHD) patients. *Gene Reports* **14** (2019), pp. 118-123.

Evans, H.M. and Long, C.N.H. Important Recent Experiments On The Hormone Of The Anterior Hypophysis. *Endocrinology* (1924), pp. 118-120.

Evans, L., Grimes, A., Wilkinson, B. and Teece, D. Economic reform in New Zealand 1984-95: The pursuit of efficiency. *Journal of Economic Literature* **34** (1996), pp. 1856-1902.

Falaki, M., Prandi, A., Corradini, C., Sneyers, M., Gengler, N., Massart, S., Fazzini, U., Burny, A., Portetelle, D. and Renaville, R. Relationships of growth hormone gene and milk protein polymorphisms to milk production traits in Simmental cattle. *Journal of Dairy Research* **64** (1997), pp. 47-56.

Fang, P., Hi Cho, Y., Derr, M.A., Rosenfeld, R.G., Hwa, V. and Cowell, C.T. Severe short stature caused by novel compound heterozygous mutations of the insulin-like growth factor 1 receptor (IGF1R). *The Journal of Clinical Endocrinology & Metabolism* **97** (2012), pp. E243-E247.

Fang, P., Schwartz, I.D., Johnson, B.D., Derr, M.A., Roberts Jr, C.T., Hwa, V. and Rosenfeld, R.G. Familial short stature caused by haploinsufficiency of the insulin-like growth factor I receptor due to nonsense-mediated messenger ribonucleic acid decay. *The Journal of Clinical Endocrinology & Metabolism* **94** (2009), pp. 1740-1747.

FAO: Food and Agriculture Organization of the United Nations: Meat Market Review 2017, *Market Review*. Food and Agriculture Organization of the United Nations (FAO) (2018).

Fikes, J., Barkocy-Gallagher, G., Klapper, D. and Bassford, P.J. Maturation of Escherichia coli maltose-binding protein by signal peptidase I in vivo. Sequence requirements for efficient processing and demonstration of an alternate cleavage site. *Journal of Biological Chemistry* **265** (1990), pp. 3417-3423.

Florini, J.R., Ewton, D.Z. and Coolican, S.A. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocrine reviews* **17** (1996), pp. 481-517.

Flück, C., Deladoey, J., Rutishauser, K., Eblé, A.e., Marti, U., Wu, W. and Mullis, P.E. Phenotypic variability in familial combined pituitary hormone deficiency caused by a PROP1 gene mutation resulting in the substitution of Arg→ Cys at codon 120 (R120C). *The Journal of Clinical Endocrinology & Metabolism* **83** (1998), pp. 3727-3734.

Fofanova, Q., Takamura, N., Kinoshita, E., Parks, J., Brown, M., Peterkova, V., Evgrafov, O., Goncharov, N., Bulatov, A. and Dedov, I. Compound heterozygous deletion of the PROP-1 gene in children with combined pituitary hormone deficiency. *The Journal of Clinical Endocrinology & Metabolism* **83** (1998), pp. 2601-2604.

Freer, M. 16 The Nutritional Management of Grazing Sheep. *Sheep Nutrition* (2002), p. 357.

Frontera, W.R. and Ochala, J. Skeletal muscle: a brief review of structure and function. *Calcified tissue international* **96** (2015), pp. 183-195.

Fryklund, L., Uthne, K. and Sievertsson, H. Identification of two somatomedin A active polypeptides and in vivo effects of a somatomedin A concentrate. *Biochemical and biophysical research communications* **61** (1974), pp. 957-962.

Gage, P.J., Roller, M.L., Saunders, T.L., Scarlett, L.M. and Camper, S.A. Anterior pituitary cells defective in the cell-autonomous factor, df, undergo cell lineage specification but not expansion. *Development* **122** (1996), pp. 151-160.

Gardner, D., Buttery, P., Daniel, Z. and Symonds, M. Factors affecting birth weight in sheep: maternal environment. *Reproduction* **133** (2007), pp. 297-307.

Gaunitz, F., Heise, K. and Gebhardt, R. A silencer element in the first intron of the glutamine synthetase gene represses induction by glucocorticoids. *Molecular Endocrinology* **18** (2004), pp. 63-69.

Ge, W., Davis, M., Hines, H., Irvin, K. and Simmen, R. Association of a genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. *Journal of Animal Science* **79** (2001), pp. 1757-1762.

Ge, W., Davis, M., Hines, H., Irvin, K. and Simmen, R. Association of single nucleotide polymorphisms in the growth hormone and growth hormone receptor genes with blood serum insulin-like growth factor I concentration and growth traits in Angus cattle. *Journal of animal science* **81** (2003), pp. 641-648.

Giorgio, D., Di Trana, A. and Claps, S. Oligosaccharides, polyamines and sphingolipids in ruminant milk. *Small Ruminant Research* **160** (2018), pp. 23-30.

Girnita, L., Worrall, C., Takahashi, S.-I., Seregard, S. and Girnita, A. Something old, something new and something borrowed: emerging paradigm of insulin-like growth factor type 1 receptor (IGF-1R) signaling regulation. *Cellular and molecular life sciences* **71** (2014), pp. 2403-2427.

Giustina, A., Mazziotti, G. and Canalis, E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocrine reviews* **29** (2008), pp. 535-559.

Glass, D.J. Skeletal muscle hypertrophy and atrophy signaling pathways. *The international journal of biochemistry & cell biology* **37** (2005), pp. 1974-1984.

Golding, K., Kemp, P., Kenyon, P. and Morris, S. High weaned lamb live weight gains on herbs. *Agronomy New Zealand* **38** (2008), pp. 33-40.

Gooch, M. and Firth, S.: New Zealand Lamb Industry Fact Finding Mission Report. Canadian Sheep Federation (2007).

Gootwine, E., Ofir, R. and Yossefi, S. Characterization of PvuII polymorphisms between the ovine growth hormone GH2-N and GH2-Z gene copies. *Animal Biotechnology* **7** (1996), pp. 135-143.

Gootwine, E., Suttie, J., McEwan, J., Veenvliet, B., Littlejohn, R., Fennessy, P. and Montgomery, G. The physiological effects of natural variation in growth hormone gene copy number in ram lambs. *Domestic animal endocrinology* **14** (1997), pp. 381-390.

Gorlov, I.F., Kolosov, Y.A., Shirokova, N.V., Getmantseva, L.V., Slozhenkina, M.I., Mosolova, N.I., Bakoev, N.F., Leonova, M.A., Kolosov, A.Y. and Zlobina, E.Y. Association of the growth hormone gene polymorphism with growth traits in Salsk sheep breed. *Small Ruminant Research* **150** (2017), pp. 11-14.

Goulding, M., Lumsden, A. and Paquette, A.J. Regulation of Pax-3 expression in the dermomyotome and its role in muscle development. *Development* **120** (1994), pp. 957-971.

Greenwood, P., Gardner, G. and Hegarty, R. Lamb myofibre characteristics are influenced by sire estimated breeding values and pastoral nutritional system. *Australian Journal of Agricultural Research* **57** (2006), pp. 627-639.

Gu, Z., Rifkin, S.A., White, K.P. and Li, W.-H. Duplicate genes increase gene expression diversity within and between species. *Nature genetics* **36** (2004), p. 577.

Gupta, N., Ahlawat, S., Kumar, D., Gupta, S., Pandey, A. and Malik, G. Single nucleotide polymorphism in growth hormone gene exon-4 and exon-5 using PCR-SSCP in Black Bengal goats–A prolific meat breed of India. *Meat science* **76** (2007), pp. 658-665.

Gupta, P.K., Rustgi, S. and Kulwal, P.L. Linkage disequilibrium and association studies in higher plants: present status and future prospects. *Plant molecular biology* **57** (2005), pp. 461-485.

Guy, J.C., Hunter, C.S., Showalter, A.D., Smith, T.P., Charoonpatrapong, K., Sloop, K.W., Bidwell, J.P. and Rhodes, S.J. Conserved amino acid sequences confer nuclear localization upon the Prophet of Pit-1 pituitary transcription factor protein. *Gene* **336** (2004), pp. 263-273.

Hai-nan, L., Hui-lin, L., Zi-qi, Z., Gan, L., Xue-qi, F. and Xin, Z. Cellular internalization and trafficking of 20 KDa human growth hormone. *General and comparative endocrinology* **270** (2019), pp. 82-89.

Hainan, L., Huilin, L., Khan, M., Xin, Z., YuJiang, Y., Hui, Z. and Naiquan, Y. The basic route of the nuclear translocation porcine growth hormone (GH)-growth hormone receptor (GHR) complex (pGH/GHR) in porcine hepatocytes. *General and comparative endocrinology* **266** (2018), pp. 101-109.

Harris, D.L. Breeding for efficiency in livestock production: defining the economic objectives. *Journal of Animal Science* **30** (1970), pp. 860-865.

Hawke, G.R., The making of New Zealand: An economic history. CUP Archive (1985).

Hayashizaki, Y., Hiraoka, Y., Endo, Y., Miyai, K. and Matsubara, K. Thyroid-stimulating hormone (TSH) deficiency caused by a single base substitution in the CAGYC region of the beta-subunit. *The EMBO journal* **8** (1989), p. 2291.

Hayes, B.J., Bowman, P.J., Chamberlain, A. and Goddard, M. Invited review: Genomic selection in dairy cattle: Progress and challenges. *Journal of dairy science* **92** (2009), pp. 433-443.

Hayes, B.J., Lewin, H.A. and Goddard, M.E. The future of livestock breeding: genomic selection for efficiency, reduced emissions intensity, and adaptation. *Trends in Genetics* **29** (2013), pp. 206-214.

Hazel, L.N. The genetic basis for constructing selection indexes. *Genetics* **28** (1943), pp. 476-490.

Hemmings, B.A. and Restuccia, D.F. Pi3k-pkb/akt pathway. *Cold Spring Harbor perspectives in biology* **4** (2012), p. a011189.

Hendriks-Stegeman, B.I., Augustijn, K.D., Bakker, B., Holthuizen, P., van der Vliet, P.C. and Jansen, M. Combined pituitary hormone deficiency caused by compound heterozygosity for two novel mutations in the POU domain of the Pit1/POU1F1 gene. *The Journal of Clinical Endocrinology & Metabolism* **86** (2001), pp. 1545-1550.

Herr, W., Sturm, R., Clerc, R., Corcoran, L., Baltimore, D., Sharp, P., Ingraham, H., Rosenfeld, M., Finney, M. and Ruvkun, G. The POU domain: a large conserved region in the mammalian pit-1, oct-1, oct-2, and Caenorhabditis elegans unc-86 gene products. *Genes Dev* **2** (1988), pp. 1513-6.

Hickford, J., Forrest, R., Zhou, H., Fang, Q., Han, J., Frampton, C. and Horrell, A. Polymorphisms in the ovine myostatin gene (MSTN) and their association with growth and carcass traits in New Zealand Romney sheep. *Animal genetics* **41** (2010), pp. 64-72.

Holder, A., Spencer, E. and Preece, M. Effect of bovine growth hormone and a partially pure preparation of somatomedin on various growth parameters in hypopituitary dwarf mice. *Journal of Endocrinology* **89** (1981), pp. 275-282.

Hopkins, D., Fogarty, N. and Mortimer, S. Genetic related effects on sheep meat quality. *Small Ruminant Research* **101** (2011), pp. 160-172.

Hopkins, D., Hegarty, R. and Farrell, T. Relationship between sire estimated breeding values and the meat and eating quality of meat from their progeny grown on two planes of nutrition. *Australian Journal of Experimental Agriculture* **45** (2005), pp. 525-533.

Hopkins, D., Safari, E., Thompson, J. and Smith, C. Video image analysis in the Australian meat industry–precision and accuracy of predicting lean meat yield in lamb carcasses. *Meat Science* **67** (2004), pp. 269-274.

Hribal, M.L., Nakae, J., Kitamura, T., Shutter, J.R. and Accili, D. Regulation of insulin-like growth factor–dependent myoblast differentiation by Foxo forkhead transcription factors. *The Journal of cell biology* **162** (2003), pp. 535-541.

Huang, W., Maltecca, C. and Khatib, H. A proline-to-histidine mutation in POU1F1 is associated with production traits in dairy cattle. *Animal genetics* **39** (2008), pp. 554-557.

Hunt, R.C., Simhadri, V.L., Iandoli, M., Sauna, Z.E. and Kimchi-Sarfaty, C. Exposing synonymous mutations. *Trends in Genetics* **30** (2014), pp. 308-321.

Hwa, V., Oh, Y. and Rosenfeld, R.G. The insulin-like growth factor-binding protein (IGFBP) superfamily 1. *Endocrine reviews* **20** (1999), pp. 761-787.

Iams, W.T. and Lovly, C.M. Molecular pathways: clinical applications and future direction of insulin-like growth factor-1 receptor pathway blockade. *Clinical Cancer Research* **21** (2015), pp. 4270-4277.

Igenity: Beef profiling application (2012).

Inagaki, K., Tiulpakov, A., Rubtsov, P., Sverdlova, P., Peterkova, V., Yakar, S., Terekhov, S. and LeRoith, D. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. *The Journal of Clinical Endocrinology & Metabolism* **92** (2007), pp. 1542-1548.

Inukai, S., Kock, K.H. and Bulyk, M.L. Transcription factor–DNA binding: beyond binding site motifs. *Current opinion in genetics & development* **43** (2017), pp. 110-119.

Irshad, A., Kandeepan, G., Kumar, S., Ashish, K., Vishnuraj, M. and Shukla, V. Factors influencing carcass composition of livestock: A review. *J. Anim. Prod. Adv* **3** (2013), pp. 177-186.

Isaksson, O., Eden, S. and Jansson, J. Mode of action of pituitary growth hormone on target cells. *Annual review of physiology* **47** (1985), pp. 483-499.

Jain, R.G., Rusch, S.L. and Kendall, D.A. Signal peptide cleavage regions. Functional limits on length and topological implications. *Journal of Biological Chemistry* **269** (1994), pp. 16305-16310.

Janchevska, A., Dimovski, A., Mironska, K., Tasic, V. and Gucev, Z. IGF1R Gene Alterations in Small for Gestational Age (SGA) Children. *Open access Macedonian journal of medical sciences* **6** (2018), p. 790.

Javadpour, M.M., Eilers, M., Groesbeek, M. and Smith, S.O. Helix packing in polytopic membrane proteins: role of glycine in transmembrane helix association. *Biophysical journal* **77** (1999), pp. 1609-1618.

Jia, J., Zhang, L., Wu, J., Ha, Z. and Li, W. Study of the correlation between GH gene polymorphism and growth traits in sheep. *Genet. Mol. Res* **13** (2014), pp. 7190-7200.

Jopson, N., Newman, S. and McEwan, J.: Developments in the sheep meat industry: Genetic evaluation of meat yield, *Proceedings of the New Zealand Society of*

Animal Production. New Zealand Society of Animal Production (2009), pp. 161-164.

Kannian, V.G. and Ryan, F.J.: Physiology of Growth Hormone in Fetus and Child. In: Huhtaniemi, I. and Martini, L. (Huhtaniemi, I. and Martini, L.(Huhtaniemi, I. and Martini, L.s), *Encyclopedia of Endocrine Diseases (Second Edition)*. Academic Press, Oxford (2019), pp. 10-18.

Kato, Y., Kimoto, F., Susa, T., Nakayama, M., Ishikawa, A. and Kato, T. Pituitary homeodomain transcription factors HESX1 and PROP1 form a heterodimer on the inverted TAAT motif. *Molecular and cellular endocrinology* **315** (2010), pp. 168-173.

Kawashima, Y., Higaki, K., Fukushima, T., Hakuno, F., Nagaishi, J.i., Hanaki, K., Nanba, E., Takahashi, S.I. and Kanzaki, S. Novel missense mutation in the IGF-I receptor L 2 domain results in intrauterine and postnatal growth retardation. *Clinical endocrinology* **77** (2012), pp. 246-254.

Kawashima, Y., Kanzaki, S., Yang, F., Kinoshita, T., Hanaki, K., Nagaishi, J.-i., Ohtsuka, Y., Hisatome, I., Ninomoya, H. and Nanba, E. Mutation at cleavage site of insulin-like growth factor receptor in a short-stature child born with intrauterine growth retardation. *The Journal of Clinical Endocrinology & Metabolism* **90** (2005), pp. 4679-4687.

Kelberman, D. and Dattani, M.T. The role of transcription factors implicated in anterior pituitary development in the aetiology of congenital hypopituitarism. *Annals of medicine* **38** (2006), pp. 560-577.

Kelberman, D., Rizzoti, K., Lovell-Badge, R., Robinson, I.C. and Dattani, M.T. Genetic regulation of pituitary gland development in human and mouse. *Endocrine reviews* **30** (2009a), pp. 790-829.

Kelberman, D., Turton, J., Woods, K., Mehta, A., Al-Khawari, M., Greening, J., Swift, P., Otonkoski, T., Rhodes, S. and Dattani, M. Molecular analysis of novel PROP1 mutations associated with combined pituitary hormone deficiency (CPHD). *Clinical endocrinology* **70** (2009b), pp. 96-103.

Kioussi, C., Carriere, C. and Rosenfeld, M.G. A model for the development of the hypothalamic–pituitary axis: transcribing the hypophysis. *Mechanisms of development* **81** (1999), pp. 23-35.

Kishimoto, M., Okimura, Y., Fumoto, M., Iguchi, G., Iida, K., Kaji, H. and Chihara, K. The R271W mutant form of Pit-1 does not act as a dominant inhibitor of Pit-1 action to activate the promoters of GH and prolactin genes. *European journal of endocrinology* **148** (2003), pp. 619-625.

Kissinger, C.R., Liu, B., Martin-Blanco, E., Kornberg, T.B. and Pabo, C.O. Crystal structure of an engrailed homeodomain-DNA complex at 2.8 Å resolution: a framework for understanding homeodomain-DNA interactions. *Cell* **63** (1990), pp. 579-590.

Klammt, J., Kiess, W. and Pfäffle, R. IGF1R mutations as cause of SGA. *Best practice & research Clinical endocrinology & metabolism* **25** (2011), pp. 191-206.

Knight, T., Lynch, P.R., Hall, D. and Hockey, H.P. Identification of factors contributing to the improved lamb survival in Marshall Romney sheep. *New Zealand Journal of Agricultural Research* **31** (1988), pp. 259-271.

Kopchick, J.J. and Andry, J.M. Growth hormone (GH), GH receptor, and signal transduction. *Molecular genetics and metabolism* **71** (2000), pp. 293-314.

Kosgey, I.S., Van Arendonk, J.A. and Baker, R.L. Economic values for traits in breeding objectives for sheep in the tropics: impact of tangible and intangible benefits. *Livestock Production Science* **88** (2004), pp. 143-160.

Kruglyak, L. Prospects for whole-genome linkage disequilibrium mapping of common disease genes. *Nature genetics* **22** (1999).

Kruis, T., Klammt, J.r., Galli-Tsinopoulou, A., Wallborn, T., Schlicke, M., Müller, E., Kratzsch, J.r., Körner, A., Odeh, R. and Kiess, W. Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. *The Journal of Clinical Endocrinology & Metabolism* **95** (2010), pp. 1137-1142.

Kumar, S., Stecher, G. and Tamura, K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution* **33** (2016), pp. 1870-1874.

Labad, J., Barbero, J., Gutierrez-Zotes, A., Montalvo, I., Creus, M., Cabezas, A., Solé, M., Algora, M., Garcia-Parés, G. and Vilella, E. Free thyroxine levels are associated with cognitive changes in individuals with a first episode of psychosis: a prospective 1-year follow-up study. *Schizophrenia research* **171** (2016), pp. 182-186.

Lacroix, M., Devinoy, E., Servely, J., Puissant, C. and Kann, G. Expression of the growth hormone gene in ovine placenta: detection and cellular localization of the protein. *Endocrinology* **137** (1996), pp. 4886-4892.

Lai, K.-M.V., Gonzalez, M., Poueymirou, W.T., Kline, W.O., Na, E., Zlotchenko, E., Stitt, T.N., Economides, A.N., Yancopoulos, G.D. and Glass, D.J. Conditional activation of akt in adult skeletal muscle induces rapid hypertrophy. *Molecular and cellular biology* **24** (2004), pp. 9295-9304.

Lan, X.-y., Zhao, H.-y., Li, Z.-j., Rui, Z., Pan, C.-y., Lei, C.-z. and Hong, C. Exploring the novel genetic variant of PITX1 gene and its effect on milk performance in dairy goats. *Journal of Integrative Agriculture* **12** (2013a), pp. 118-126.

Lan, X., Pan, C., Zhang, L., Zhao, M., Zhang, C., Lei, C. and Chen, H. A novel missense (A79V) mutation of goat PROP1 gene and its association with production traits. *Molecular biology reports* **36** (2009), p. 2069.

Lan, X., Peñagaricano, F., DeJung, L., Weigel, K. and Khatib, H. Short communication: A missense mutation in the PROP1 (prophet of Pit 1) gene affects male fertility and milk production traits in the US Holstein population. *Journal of dairy science* **96** (2013b), pp. 1255-1257.

Lande, R. and Thompson, R. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics* **124** (1990), pp. 743-756.

Lawrence, M.C., McKern, N.M. and Ward, C.W. Insulin receptor structure and its implications for the IGF-1 receptor. *Current opinion in structural biology* **17** (2007), pp. 699-705.

Lawrence, T.L.J., Fowler, V.R. and Novakofski, J.E., Growth of farm animals. CABI (2012).

Le Hir, H., Nott, A. and Moore, M.J. How introns influence and enhance eukaryotic gene expression. *Trends in biochemical sciences* **28** (2003), pp. 215-220.

Leal, A.C., Montenegro, L.R., Saito, R.F., Ribeiro, T.C., Coutinho, D.C., Mendonca, B.B., Arnhold, I.J. and Jorge, A.A. Analysis of the insulin-like growth factor 1 receptor gene in children born small for gestational age: in vitro characterization of a novel mutation (p. A rg511 T rp). *Clinical endocrinology* **78** (2013), pp. 558-563.

Lei, M., Peng, X., Zhou, M., Luo, C., Nie, Q. and Zhang, X. Polymorphisms of the IGF1R gene and their genetic effects on chicken early growth and carcass traits. *BMC genetics* **9** (2008), p. 70.

Lemmon, M.A., Treutlein, H.R., Adams, P.D., Brünger, A.T. and Engelman, D.M. A dimerization motif for transmembrane α–helices. *Nature structural biology* **1** (1994), p. 157.

Lemos, M.C., Gomes, L., Bastos, M., Leite, V., Limbert, E., Carvalho, D., Bacelar, C., Monteiro, M., Fonseca, F. and Agapito, A. PROP1 gene analysis in Portuguese patients with combined pituitary hormone deficiency. *Clinical endocrinology* **65** (2006), pp. 479-485.

LeRoith, D., Werner, H., Beitner-Johnson, D. and Roberts Jr, C.T. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocrine reviews* **16** (1995), pp. 143-163.

Lesniak, M.A., Gorden, P., Roth, J. and Gavin, J.R. Binding of 125I-Human Growth Hormone to Specific Receptors in Human Cultured Lymphocytes CHARACTERIZATION OF THE INTERACTION AND A SENSITIVE RADIORECEPTOR ASSAY. *Journal of Biological Chemistry* **249** (1974), pp. 1661-1667.

Lewis, R., Simm, G., Dingwall, W. and Murphy, S. Selection for lean growth in terminal sire sheep to produce leaner crossbred progeny. *Animal Science* **63** (1996), pp. 133-142.

Lewis, U., Singh, R., Tutwiler, G., Sigel, M., VanderLaan, E. and VanderLaan, W.: Human growth hormone: a complex of proteins, *Proceedings of the 1979 Laurentian Hormone Conference*. Elsevier (1980), pp. 477-508.

Li, S., Crenshaw, E.B., Rawson, E.J., Simmons, D.M., Swanson, L.W. and Rosenfeld, M.G. Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. *Nature* **347** (1990), p. 528.

Lind, V., Berg, J., Eilertsen, S.M., Hersleth, M. and Eik, L.O. Effect of gender on meat quality in lamb from extensive and intensive grazing systems when slaughtered at the end of the growing season. *Meat Science* **88** (2011), pp. 305-310.

Lindahl, A., Nilsson, A., Isgaard, J. and Isaksson, O.G.P. Mechanism of the Stimulatory Effect of Growth Hormone on Longitudinal Bone Growth*. *Endocrine Reviews* **8** (1987), pp. 426-438.

Liu, B.H., Statistical genomics: linkage, mapping, and QTL analysis. CRC press (1997).

Liu, J.-P., Baker, J., Perkins, A.S., Robertson, E.J. and Efstratiadis, A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* **75** (1993), pp. 59-72.

Liu, Q., Geng, C., Chu, M., Chen, H., Jin, M., Zhang, Y., Di, R., Feng, T. and Li, N. Polymorphism of Prophet of Pit-1 gene and its relationship with litter size of Small Tail Han sheep. *Journal of genetics* (2015), pp. 1-4.

Lobie, P.E., Mertani, H., Morel, G., Morales-Bustos, O., Norstedt, G. and Waters, M.J. Receptor-mediated nuclear translocation of growth hormone. *Journal of Biological Chemistry* **269** (1994a), pp. 21330-21339.

Lobie, P.E., Wood, T., Chen, C.M., Waters, M.J. and Norstedt, G. Nuclear translocation and anchorage of the growth hormone receptor. *Journal of Biological Chemistry* **269** (1994b), pp. 31735-31746.

Lopez-Villalobos, N., Garrick, D., Blair, H. and Holmes, C. Possible effects of 25 years of selection and crossbreeding on the genetic merit and productivity of New Zealand dairy cattle. *Journal of Dairy science* **83** (2000), pp. 154-163.

Ma, Y., Fu, S., Lu, L. and Wang, X. Role of androgen receptor on cyclic mechanical stretch-regulated proliferation of C2C12 myoblasts and its upstream signals: IGF-1-mediated PI3K/Akt and MAPKs pathways. *Molecular and cellular endocrinology* **450** (2017), pp. 83-93.

Malveiro, E., Pereira, M., Marques, P., Santos, I., Belo, C., Renaville, R. and Cravador, A. Polymorphisms at the five exons of the growth hormone gene in the algarvia goat: possible association with milk traits. *Small Ruminant Research* **41** (2001), pp. 163-170.

Mangalam, H.J., Albert, V.R., Ingraham, H.A., Kapiloff, M., Wilson, L., Nelson, C., Elsholtz, H. and Rosenfeld, M.G. A pituitary POU domain protein, Pit-1, activates both growth hormone and prolactin promoters transcriptionally. *Genes & development* **3** (1989), pp. 946-958.

Marques, M.d.R., Santos, I., Carolino, N., Belo, C., Renaville, R. and Cravador, A. Effects of genetic polymorphisms at the growth hormone gene on milk yield in Serra da Estrela sheep. *Journal of dairy research* (2006).

Mathews, L.S., Hammer, R.E., Behringer, R.R., D'ercole, A.J., Bell, G.I., Brinster, R.L. and Palmiter, R.D. Growth enhancement of transgenic mice expressing human insulin-like growth factor I. *Endocrinology* **123** (1988), pp. 2827-2833.

McGinnis, W. and Krumlauf, R. Homeobox genes and axial patterning. *Cell* **68** (1992), pp. 283-302.

McPherron, A.C. and Lee, S.-J. Double muscling in cattle due to mutations in the myostatin gene. *Proceedings of the National Academy of Sciences* **94** (1997), pp. 12457-12461.

McRae, A., McEwan, J., Dodds, K., Wilson, T., Crawford, A. and Slate, J. Linkage disequilibrium in domestic sheep. *Genetics* **160** (2002), pp. 1113-1122.

Meadows, G., Sheep Breeds of New Zealand. Reed Publishing (NZ) (1997).

Meat Industry Association, M.: Export trends in selected markets up to March 2016, *Industry Statistics*. Meat Industry Association of New Zealand (Inc) (2016).

Meat Industry Association, M.: Meat Industry Association: Annual Report 2018, *Industry Statistics*. Meat Industry Association of New Zealand (Inc) (2018).

Meuwissen, T.H. and Goddard, M.E. Mapping multiple QTL using linkage disequilibrium and linkage analysis information and multitrait data. *Genetics Selection Evolution* **36** (2004), p. 261.

Miller, W.L. and Eberhardt, N.L. Structure and evolution of the growth hormone gene family. *Endocrine Reviews* **4** (1983), pp. 97-130.

Ministry of Primary Industries, M.: Situation and Outlook for Primary Industries - March 2019. Ministry for Primary Industries, New Zealand (2019).

Mohn, A., Marcovecchio, M., De Giorgis, T., Pfaeffle, R., Chiarelli, F. and Kiess, W. An insulin-like growth factor-I receptor defect associated with short stature and impaired carbohydrate homeostasis in an Italian pedigree. *Hormone research in paediatrics* **76** (2011), pp. 136-143.

Molina, L., Marino-Buslje, C., Quinn, D. and Siddle, K. Structural domains of the insulin receptor and IGF receptor required for dimerisation and ligand binding. *FEBS letters* **467** (2000), pp. 226-230.

Montaldo, H.H. and Meza-Herrera, C.A. Use of molecular markers and major genes in the genetic improvement of livestock. *Electronic Journal of Biotechnology* **1** (1998), pp. 15-16.

Moody, D., Pomp, D. and Barendse, W. Linkage mapping of the bovine insulin-like growth factor-1 receptor gene. *Mammalian Genome* **7** (1996), pp. 168-169.

Morikawa, M., Nixon, T. and Green, H. Growth hormone and the adipose conversion of 3T3 cells. *Cell* **29** (1982), pp. 783-789.

Musarò, A., McCullagh, K., Paul, A., Houghton, L., Dobrowolny, G., Molinaro, M., Barton, E.R., Sweeney, H.L. and Rosenthal, N. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nature genetics* **27** (2001), p. 195.

Musumeci, G., Castrogiovanni, P., Coleman, R., Szychlinska, M.A., Salvatorelli, L., Parenti, R., Magro, G. and Imbesi, R. Somitogenesis: from somite to skeletal muscle. *Acta Histochemica* **117** (2015), pp. 313-328.

Nakamura, Y., Usui, T., Mizuta, H., Murabe, H., Muro, S., Suda, M., Tanaka, K., Tanaka, I., Shimatsu, A. and Nakao, K. Characterization of Prophet of Pit-1 gene expression in normal pituitary and pituitary adenomas in humans. *The Journal of Clinical Endocrinology & Metabolism* **84** (1999), pp. 1414-1419.

Nakayama, M., Kato, T., Susa, T., Sano, A., Kitahara, K. and Kato, Y. Dimeric PROP1 binding to diverse palindromic TAAT sequences promotes its transcriptional activity. *Molecular and cellular endocrinology* **307** (2009), pp. 36-42.

Newman, S., Amyes, N., Cruickshank, G. and Young, M.: Sheep Improvement Limited-the national sheep performance recording scheme in New Zealand, *Proceedings of the Seventh World Congress on Genetics applied to Livestock Production (WCGALP), Montpellier, France* (2002), pp. 733-736.

Newman, S., Dodds, K., Clarke, J., Garrick, D. and McEwan, J.: The sheep improvement limited (SIL) genetic engine, *PROCEEDINGS-NEW ZEALAND SOCIETY OF ANIMAL PRODUCTION*. New Zealand Society of Animal Production; 1999 (2000), pp. 195-197.

Ng, P.C. and Henikoff, S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic acids research* **31** (2003), pp. 3812-3814.

Nielsen, H., Engelbrecht, J., Brunak, S. and Von Heijne, G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. *Protein engineering* **10** (1997), pp. 1-6.

Nose, O., Tatsumi, K., Nakano, Y. and Amino, N. Congenital combined pituitary hormone deficiency attributable to a novel PROP1 mutation (467insT). *Journal of pediatric endocrinology & metabolism: JPEM* **19** (2006), pp. 491-498.

Notter, D.R. and Brown, D.J. Effects of birth-rearing type on weaning weights in meat sheep are systematically associated with differences in mean performance among flocks. *Genetics Selection Evolution* **47** (2015), p. 57.

Nyberg, F. and Hallberg, M. Growth hormone and cognitive function. *Nature Reviews Endocrinology* **9** (2013), p. 357.

NZ Sheep Breeders Association, N.: Sheep Breeds in New Zealand (2019).

NZ Treasury: New Zealand Treasury: New Zealand Economic and Financial Overview 2010 (2010).

O'Neil, K.T. and DeGrado, W.F. A thermodynamic scale for the helix-forming tendencies of the commonly occurring amino acids. *Science* **250** (1990), pp. 646-651.

Ofir, R. and Gootwine, E. Ovine growth hormone gene duplication—structural and evolutionary implications. *Mammalian Genome* **8** (1997), pp. 770-772.

Ohanna, M., Sobering, A.K., Lapointe, T., Lorenzo, L., Praud, C., Petroulakis, E., Sonenberg, N., Kelly, P.A., Sotiropoulos, A. and Pende, M. Atrophy of S6K1−/− skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nature cell biology* **7** (2005), p. 286.

Ohno, S.: Gene duplication and the uniqueness of vertebrate genomes circa 1970–1999, *Seminars in cell & developmental biology*. Elsevier (1999), pp. 517-522.

Ohta, T. Simulating evolution by gene duplication. *Genetics* **115** (1987), pp. 207-213.

Oldham, C., Thompson, A., Ferguson, M., Gordon, D., Kearney, G. and Paganoni, B. The birthweight and survival of Merino lambs can be predicted from the profile of liveweight change of their mothers during pregnancy. *Animal Production Science* **51** (2011), pp. 776-783.

Olson, L.E., Dasen, J.S., Ju, B.G., Tollkuhn, J. and Rosenfeld, M.G. Paired-like repression/activation in pituitary development. *Recent progress in hormone research* **58** (2003), pp. 249-261.

Orian, J.M., O'Mahoney, J.V. and Brandon, M.R. Cloning and sequencing of the ovine growth hormone gene. *Nucleic acids research* **16** (1988), p. 9046.

Osorio, M.G., Kopp, P., Marui, S., Latronico, A.C., Mendonca, B.B. and Arnhold, I.J. Combined pituitary hormone deficiency caused by a novel mutation of a highly conserved residue (F88S) in the homeodomain of PROP-1. *The Journal of Clinical Endocrinology & Metabolism* **85** (2000), pp. 2779-2785.

Otto, A. and Patel, K. Signalling and the control of skeletal muscle size. *Experimental cell research* **316** (2010), pp. 3059-3066.

Owens, F.N., Gill, D.R., Secrist, D.S. and Coleman, S. Review of some aspects of growth and development of feedlot cattle. *Journal of animal science* **73** (1995), pp. 3152-3172.

Ozmen, O., Kul, S. and Unal, E.O. Polymorphism of sheep POU1F1 gene exon 6 and 3'UTR region and their association with milk production traits. *Iranian journal of veterinary research* **15** (2014), p. 331.

Paetzel, M., Dalbey, R.E. and Strynadka, N.C. Crystal structure of a bacterial signal peptidase in complex with a β-lactam inhibitor. *Nature* **396** (1998), p. 186.

Pan, C., Lan, X., Chen, H., Hua, L., Guo, Y.K., Zhang, B. and Lei, C. Five novel single nucleotide polymorphisms (SNPs) of the prophet of PIT1 (PROP1) gene in bovine. *Arch Tierz* **50** (2007), pp. 421-423.

Pan, C., Wu, C., Jia, W., Xu, Y., Lei, C., Hu, S., Lan, X. and Chen, H. A critical functional missense mutation (H173R) in the bovine PROP1 gene significantly affects growth traits in cattle. *Gene* **531** (2013), pp. 398-402.

Pannier, L., Gardner, G., O'Reilly, R. and Pethick, D. Factors affecting lamb eating quality and the potential for their integration into an MSA sheepmeat grading model. *Meat science* (2018).

Pannier, L., Pethick, D., Geesink, G., Ball, A., Jacob, R. and Gardner, G. Intramuscular fat in the longissimus muscle is reduced in lambs from sires selected for leanness. *Meat Science* **96** (2014), pp. 1068-1075.

Paracchini, R., Giordano, M., Corrias, A., Mellone, S., Matarazzo, P., Bellone, J., Momigliano-Richiardi, P. and Bona, G. Two new PROP1 gene mutations

responsible for compound pituitary hormone deficiency. *Clinical genetics* **64** (2003), pp. 142-147.

Park, Y., Juárez, M., Ramos, M. and Haenlein, G. Physico-chemical characteristics of goat and sheep milk. *Small ruminant research* **68** (2007), pp. 88-113.

Parks, J.S., Brown, M.R., Hurley, D.L., Phelps, C.J. and Wajnrajch, M.P. Heritable disorders of pituitary development. *The Journal of Clinical Endocrinology & Metabolism* **84** (1999), pp. 4362-4370.

Pearce, K.: Improving Lamb Lean Meat Yield, CRC for Sheep Industry Innovation and (2016).

Pereira, M., Risso, J., Santos, P., Mundim, T., Capparelli, F., Franco, R., Queiroz, L., Silva, H., Benedetti, E. and Goulart, L.: Análise do polimorfismo do gene IGF-1R sobre a produção de leite em bovinos da raça sintética Girolando, *Proceedings of the 49° Congresso Nacional de Genética, Águas de Lindóia, SP, Brasil* (2003), pp. 350.

Pfäffle, R., DiMattia, G., Parks, J., Brown, M., Wit, J., Jansen, M., Van der Nat, H., Van den Brande, J., Rosenfeld, M. and Ingraham, H. Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia. *Science* **257** (1992), pp. 1118-1121.

Piechaczyk, M. and Blanchard, J.-M. c-Fos proto-oncogene regulation and function. *Critical reviews in oncology/hematology* **17** (1994), pp. 93-131.

Pierpaoli, W. and Besedovsky, H. Role of the thymus in programming of neuroendocrine functions. *Clinical and experimental immunology* **20** (1975), p. 323.

Proskura, W.S. and Szewczuk, M. The polymorphism in the IGF1R gene is associated with body weight and average daily weight gain in Pomeranian Coarsewool ewes. *Pak. Vet. J* **34** (2014), pp. 514-517.

Putnam, T.J., Benedict, E.B. and Teel, H.M. Studies in acromegaly: VIII. Experimental canine acromegaly produced by injection of anterior lobe pituitary extract. *Archives of Surgery* **18** (1929), pp. 1708-1736.

Radovick, S., Nations, M., Du, Y., Berg, L.A., Weintraub, B.D. and Wondisford, F.E. A mutation in the POU-homeodomain of Pit-1 responsible for combined pituitary hormone deficiency. *Science* **257** (1992), pp. 1115-1118.

Rashidi, A., Mokhtari, M.S., Jahanshahi, A.S. and Abadi, M.M. Genetic parameter estimates of pre-weaning growth traits in Kermani sheep. *Small Ruminant Research* **74** (2008), pp. 165-171.

Rauw, W., Kanis, E., Noordhuizen-Stassen, E. and Grommers, F. Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock production science* **56** (1998), pp. 15-33.

Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I. and Chilliard, Y. Composition of goat and sheep milk products: An update. *Small ruminant research* **79** (2008), pp. 57-72.

Rehfeldt, C., Fiedler, I., Dietl, G. and Ender, K. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. *Livestock Production Science* **66** (2000), pp. 177-188.

Reich, D.E. and Lander, E.S. On the allelic spectrum of human disease. *TRENDS in Genetics* **17** (2001), pp. 502-510.

Renehan, A.G. and Brennan, B.M. Acromegaly, growth hormone and cancer risk. *Best Practice & Research Clinical Endocrinology & Metabolism* **22** (2008), pp. 639-657.

Reynaud, R., Barlier, A., Vallette-Kasic, S., Saveanu, A., Guillet, M.-P., Simonin, G., Enjalbert, A., Valensi, P. and Brue, T. An uncommon phenotype with familial central hypogonadism caused by a novel PROP1 gene mutant truncated in the transactivation domain. *The Journal of Clinical Endocrinology & Metabolism* **90** (2005), pp. 4880-4887.

Reynaud, R., Gueydan, M., Saveanu, A., Vallette-Kasic, S., Enjalbert, A., Brue, T. and Barlier, A. Genetic screening of combined pituitary hormone deficiency: experience in 195 patients. *The Journal of Clinical Endocrinology & Metabolism* **91** (2006), pp. 3329-3336.

Riepe, F.G., Partsch, C.-J., Blankenstein, O., Mönig, H., Pfäffle, R.W. and Sippell, W.G. Longitudinal imaging reveals pituitary enlargement preceding hypoplasia in two brothers with combined pituitary hormone deficiency attributable to PROP1 mutation. *The Journal of Clinical Endocrinology & Metabolism* **86** (2001), pp. 4353-4357.

Rodriguez, S., Gaunt, T.R. and Day, I.N. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *American journal of epidemiology* **169** (2009), pp. 505-514.

Rojas-Downing, M.M., Nejadhashemi, A.P., Harrigan, T. and Woznicki, S.A. Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management* **16** (2017), pp. 145-163.

Rommel, C., Bodine, S.C., Clarke, B.A., Rossman, R., Nunez, L., Stitt, T.N., Yancopoulos, G.D. and Glass, D.J. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI (3) K/Akt/mTOR and PI (3) K/Akt/GSK3 pathways. *Nature cell biology* **3** (2001), p. 1009.

Rosenbloom, A.L., Almonte, A.S., Brown, M.R., Fisher, D.A., Baumbach, L. and Parks, J.S. Clinical and biochemical phenotype of familial anterior hypopituitarism from mutation of the PROP1 gene. *The Journal of Clinical Endocrinology & Metabolism* **84** (1999), pp. 50-57.

Rosenfeld, R.G. Insulin-like growth factors and the basis of growth. *New England Journal of Medicine* **349** (2003), pp. 2184-2186.

Rosenfeld, R.G. Molecular mechanisms of IGF-I deficiency. *Hormone Research in Paediatrics* **65** (2006), pp. 15-20.

Sadeghi, M., Jalil-Sarghale, A. and Moradi-Shahrbabak, M. Associations of POU1F1 gene polymorphisms and protein structure changes with growth traits and blood metabolites in two Iranian sheep breeds. *Journal of genetics* **93** (2014), p. 831.

Safari, E., Fogarty, N. and Gilmour, A.R. A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. *Livestock Production Science* **92** (2005), pp. 271-289.

Salemi, S., Besson, A., Eblé, A., Gallati, S., Pfäffle, R.W. and Mullis, P.E. New N-terminal located mutation (Q4ter) within the POU1F1-gene (PIT-1) causes recessive combined pituitary hormone deficiency and variable phenotype. *Growth hormone & IGF research* **13** (2003), pp. 264-268.

Sañudo, C., Sierra, I., Olleta, J., Martin, L., Campo, M., Santolaria, P., Wood, J. and Nute, G. Influence of weaning on carcass quality, fatty acid composition and meat quality in intensive lamb production systems. *Animal Science* **66** (1998), pp. 175-187.

Satozawa, N., Takezawa, K., Miwa, T., Takahashi, S., Hayakawa, M. and Ooka, H. Differences in the effects of 20K-and 22K-hGH on water retention in rats. *Growth Hormone & IGF Research* **10** (2000), pp. 187-192.

Sauna, Z.E. and Kimchi-Sarfaty, C. Understanding the contribution of synonymous mutations to human disease. *Nature Reviews Genetics* **12** (2011), p. 683.

Schaeffer, L. Strategy for applying genome-wide selection in dairy cattle. *Journal of animal Breeding and genetics* **123** (2006), pp. 218-223.

Scully, K.M. and Rosenfeld, M.G. Pituitary development: regulatory codes in mammalian organogenesis. *Science* **295** (2002), pp. 2231-2235.

Sepp-Lorenzino, L. Structure and function of the insulin-like growth factor I receptor. *Breast cancer research and treatment* **47** (1998), pp. 235-253.

Sheng, H.Z., Moriyama, K., Yamashita, T., Li, H., Potter, S.S., Mahon, K.A. and Westphal, H. Multistep control of pituitary organogenesis. *Science* **278** (1997), pp. 1809-1812.

Sheng, H.Z., Zhadanov, A.B., Mosinger, B., Fujii, T., Bertuzzi, S., Grinberg, A., Lee, E.J., Huang, S.-P., Mahon, K.A. and Westphal, H. Specification of pituitary cell lineages by the LIM homeobox gene Lhx3. *Science* **272** (1996), pp. 1004-1007.

Showalter, A.D., Smith, T.P., Bennett, G.L., Sloop, K.W., Whitsett, J.A. and Rhodes, S.J. Differential conservation of transcriptional domains of mammalian Prophet of Pit-1 proteins revealed by structural studies of the bovine gene and comparative functional analysis of the protein. *Gene* **291** (2002), pp. 211-221.

Skowronska-Krawczyk, D., Scully, K.M. and Rosenfeld, M.G.: Chapter 5 - Development of the Pituitary∗. In: Jameson, J.L., De Groot, L.J., de Kretser, D.M., Giudice, L.C., Grossman, A.B., Melmed, S., Potts, J.T. and Weir, G.C. (Jameson, J.L., De Groot, L.J., de Kretser, D.M., Giudice, L.C., Grossman, A.B., Melmed, S., Potts, J.T. and Weir, G.C.(Jameson, J.L., De Groot, L.J., de Kretser, D.M., Giudice, L.C., Grossman, A.B., Melmed, S., Potts, J.T. and Weir, G.C.s), *Endocrinology:*

Adult and Pediatric (Seventh Edition). W.B. Saunders, Philadelphia (2016), pp. 71-90.e5.

Sloop, K.W., Meier, B.C., Bridwell, J.L., Parker, G.E., Schiller, A.M. and Rhodes, S.J. Differential activation of pituitary hormone genes by human Lhx3 isoforms with distinct DNA binding properties. *Molecular Endocrinology* **13** (1999), pp. 2212-2225.

Smith, C. and Simpson, S. The use of genetic polymorphisms in livestock improvement. *Journal of Animal Breeding and Genetics* **103** (1986), pp. 205-217.

Snabboon, T., Plengpanich, W., Buranasupkajorn, P., Khwanjaipanich, R., Vasinanukorn, P., Suwanwalaikorn, S., Khovidhunkit, W. and Shotelersuk, V. A novel germline mutation, IVS4+ 1G> A, of the POU1F1 gene underlying combined pituitary hormone deficiency. *Hormone Research in Paediatrics* **69** (2008), pp. 60-64.

Sornson, M.W., Wu, W., Dasen, J.S., Flynn, S.E., Norman, D.J., O'connell, S.M., Gukovsky, I., Carrière, C., Ryan, A.K. and Miller, A.P. Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. *Nature* **384** (1996), p. 327.

Southdown Sheep Society NZ: Meat Cuts (2019).

Stanford, K., Jones, S. and Price, M. Methods of predicting lamb carcass composition: A review. *Small Ruminant Research* **29** (1998), pp. 241-254.

Stats NZ: Livestock Slaughtering: Total New Zealand by kill by animal type (Annual-Sep) September 2018 (2018).

Stats NZ: Indicators: Livestock Numbers (2019).

Stitt, T.N., Drujan, D., Clarke, B.A., Panaro, F., Timofeyva, Y., Kline, W.O., Gonzalez, M., Yancopoulos, G.D. and Glass, D.J. The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Molecular cell* **14** (2004), pp. 395-403.

Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terzaghi, E. and Inouye, M.: Frameshift mutations and the genetic code, *Cold Spring Harbor Symposia on Quantitative Biology*. Cold Spring Harbor Laboratory Press (1966), pp. 77-84.

Stringleman, H. and Peden, R.: Sheep farming - New Zealand sheep breeds, *Te Ara - the Encyclopedia of New Zealand* (2008).

Sturm, R.A. and Herr, W. The POU domain is a bipartite DNA-binding structure. *Nature* **336** (1988), p. 601.

Sugita, H., Ardiyanti, A., Yokota, S., Yonekura, S., Hirayama, T., Shoji, N., Yamauchi, E., Suzuki, K., Katoh, K. and Roh, S.-G. Effect of single nucleotide polymorphisms in GH gene promoter region on carcass traits and intramuscular fatty acid compositions in Japanese Black cattle. *Livestock Science* **165** (2014), pp. 15-21.

Szewczuk, M. Polymorphism in exon 2 encoding the putative ligand binding pocket of the bovine insulin-like growth factor 1 receptor affects milk traits in four different cattle breeds. *Journal of Animal Breeding and Genetics* **134** (2017), pp. 34-42.

Szewczuk, M., Zych, S., Wójcik, J. and Czerniawska-Piątkowska, E. Association of two SNPs in the coding region of the insulin-like growth factor 1 receptor (IGF1R) gene with growth-related traits in Angus cattle. *Journal of applied genetics* **54** (2013), pp. 305-308.

Tahmoorespur, M., Taheri, A., Gholami, H. and Ansary, M. PCR-SSCP variation of GH and STAT5A genes and their association with estimated breeding values of growth traits in Baluchi sheep. *Animal biotechnology* **22** (2011), pp. 37-43.

Takahashi, S., Shiga, Y., Satozawa, N. and Hayakawa, M. Diabetogenic activity of 20 kDa human growth hormone (20K-hGH) and 22K-hGH in rats. *Growth Hormone & IGF Research* **11** (2001), pp. 110-116.

Takuma, N., Sheng, H.Z., Furuta, Y., Ward, J.M., Sharma, K., Hogan, B., Pfaff, S.L., Westphal, H., Kimura, S. and Mahon, K.A. Formation of Rathke's pouch requires dual induction from the diencephalon. *Development* **125** (1998), pp. 4835-4840.

Tang, K., Bartke, A., Gardiner, C.S., Wagner, T.E. and Yun, J.S. Gonadotropin secretion, synthesis, and gene expression in human growth hormone transgenic mice and in Ames dwarf mice. *Endocrinology* **132** (1993), pp. 2518-2524.

Tatsumi, K.i., Kikuchi, K., Tsumura, K. and Amino, N. A novel PROP1 gene mutation (157delA) in Japanese siblings with combined anterior pituitary hormone deficiency. *Clinical endocrinology* **61** (2004), pp. 635-640.

Teara: Meat and Wool: Page 7 Wool Exports and Marketing (2008).

Tejeda, J.F., Peña, R.E. and Andrés, A.I. Effect of live weight and sex on physico-chemical and sensorial characteristics of Merino lamb meat. *Meat science* **80** (2008), pp. 1061-1067.

Todeschini, A.-L., Georges, A. and Veitia, R.A. Transcription factors: specific DNA binding and specific gene regulation. *Trends in genetics* **30** (2014), pp. 211-219.

Treier, M., Gleiberman, A.S., O'Connell, S.M., Szeto, D.P., McMahon, J.A., McMahon, A.P. and Rosenfeld, M.G. Multistep signaling requirements for pituitary organogenesis in vivo. *Genes & development* **12** (1998), pp. 1691-1704.

Turton, J.P., Mehta, A., Raza, J., Woods, K.S., Tiulpakov, A., Cassar, J., Chong, K., Thomas, P.Q., Eunice, M. and Ammini, A.C. Mutations within the transcription factor PROP1 are rare in a cohort of patients with sporadic combined pituitary hormone deficiency (CPHD). *Clinical endocrinology* **63** (2005), pp. 10-18.

Vacca, G., Dettori, M., Balia, F., Luridiana, S., Mura, M., Carcangiu, V. and Pazzola, M. Sequence polymorphisms at the growth hormone GH1/GH2-N and GH2-Z gene copies and their relationship with dairy traits in domestic sheep (Ovis aries). *Molecular biology reports* **40** (2013), pp. 5285-5294.

Valinsky, A., Shani, M. and Gootwine, E. Restriction fragment length polymorphism in sheep at the growth hormone locus is the result of variation in gene number. *Animal Biotechnology* **1** (1990), pp. 135-144.

Vallette-Kasic, S., Barlier, A., Teinturier, C., Diaz, A., Manavela, M., Berthezene, F., Bouchard, P., Chaussain, J., Brauner, R. and Pellegrini-Bouiller, I. PROP1 gene

screening in patients with multiple pituitary hormone deficiency reveals two sites of hypermutability and a high incidence of corticotroph deficiency. *The Journal of Clinical Endocrinology & Metabolism* **86** (2001a), pp. 4529-4535.

Vallette-Kasic, S., Pellegrini-Bouiller, I., Sampieri, F.o., Gunz, G., Diaz, A., Radovick, S., Enjalbert, A. and Brue, T. Combined pituitary hormone deficiency due to the F135C human Pit-1 (pituitary-specific factor 1) gene mutation: functional and structural correlates. *Molecular Endocrinology* **15** (2001b), pp. 411-420.

Van der Werf, J., Kinghorn, B. and Banks, R. Design and role of an information nucleus in sheep breeding programs. *Animal Production Science* **50** (2010), pp. 998-1003.

van Kerkhof, P., Sachse, M., Klumperman, J. and Strous, G.J. Growth hormone receptor ubiquitination coincides with recruitment to clathrin-coated membrane domains. *Journal of Biological Chemistry* **276** (2001), pp. 3778-3784.

Venselaar, H., te Beek, T.A., Kuipers, R.K., Hekkelman, M.L. and Vriend, G. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC bioinformatics* **11** (2010), p. 548.

Vieira, T.C., Dias da Silva, M.R., Cerutti, J.M., Brunner, E., Borges, M., Arnaldi, L.T., Kopp, P. and Abucham, J. Familial combined pituitary hormone deficiency due to a novel mutation R99Q in the hot spot region of Prophet of Pit-1 presenting as constitutional growth delay. *The Journal of Clinical Endocrinology & Metabolism* **88** (2003), pp. 38-44.

Vignal, A., Milan, D., SanCristobal, M. and Eggen, A. A review on SNP and other types of molecular markers and their use in animal genetics. *Genetics Selection Evolution* **34** (2002), p. 275.

von Heijne, G. The signal peptide. *Journal of Membrane Biology* **115** (1990), pp. 195-201.

Voss, J.W. and Rosenfeld, M.G. Anterior pituitary development: short tales from dwarf mice. *Cell* **70** (1992), pp. 527-530.

Vostry, L., Milerski, M., Schmidova, J. and Vostra-Vydrova, H. Genetic diversity and effect of inbreeding on litter size of the Romanov sheep. *Small Ruminant Research* **168** (2018), pp. 25-31.

Voutetakis, A., Maniati-Christidi, M., Kanaka-Gantenbein, C., Dracopoulou, M., Argyropoulou, M., Livadas, S., Dacou-Voutetakis, C. and Sertedaki, A. Prolonged jaundice and hypothyroidism as the presenting symptoms in a neonate with a novel Prop1 gene mutation (Q83X). *European journal of endocrinology* **150** (2004), pp. 257-264.

Wada, M., Uchida, H., Ikeda, M., Tsunekawa, B., Naito, N., Banba, S., Tanaka, E., Hashimoto, Y. and Honjo, M. The 20-kilodalton (kDa) human growth hormone (hGH) differs from the 22-kDa hGH in the complex formation with cell surface hGH receptor and hGH-binding protein circulating in human plasma. *Molecular Endocrinology* **12** (1998), pp. 146-156.

Walenkamp, M.J., van der Kamp, H.J., Pereira, A.M., Kant, S.G., van Duyvenvoorde, H.A., Kruithof, M.F., Breuning, M.H., Romijn, J.A., Karperien, M. and Wit, J.M.

A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *The Journal of Clinical Endocrinology & Metabolism* **91** (2006), pp. 3062-3070.

Wallborn, T., Wuller, S., Klammt, J., Kruis, T., Kratzsch, J., Schmidt, G., Schlicke, M., Muller, E., Schmitz van de Leur, H. and Kiess, W. A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. *The Journal of Clinical Endocrinology & Metabolism* **95** (2010), pp. 2316-2324.

Walling, G., Visscher, P., Wilson, A., McTeir, B., Simm, G. and Bishop, S. Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. *Journal of Animal Science* **82** (2004), pp. 2234-2245.

Wallis, M. Mechanism of action of prolactin. *New Comprehensive Biochemistry* **18** (1988), pp. 295-319.

Wallis, M., Lioupis, A. and Wallis, O. Duplicate growth hormone genes in sheep and goat. *J Mol Endocrinol* **21** (1998), p. 5.

Walsh, K. and Perlman, H. Cell cycle exit upon myogenic differentiation. *Current opinion in genetics & development* **7** (1997), pp. 597-602.

Wang, W., Ouyang, K., Su, X., Xu, M. and Shangguan, X. Polymorphism of insulin-like growth factor 1 receptor gene in 12 pig breeds and its relationship with pig performance traits. *Asian-australasian journal of animal sciences* **19** (2006), pp. 1541-1545.

Wapinski, I., Pfeffer, A., Friedman, N. and Regev, A. Natural history and evolutionary principles of gene duplication in fungi. *Nature* **449** (2007), p. 54.

Ward, C., Lawrence, M., Streltsov, V., Garrett, T., McKern, N., Lou, M.Z., Lovrecz, G. and Adams, T. Structural insights into ligand-induced activation of the insulin receptor. *Acta physiologica* **192** (2008), pp. 3-9.

Ward, C.W., Garrett, T.P., McKern, N.M., Lou, M., Cosgrove, L., Sparrow, L.G., Frenkel, M.J., Hoyne, P.A., Elleman, T.C. and Adams, T.E. The three dimensional structure of the type I insulin-like growth factor receptor. *Molecular Pathology* **54** (2001), p. 125.

Ward, C.W. and Lawrence, M.C. Ligand-induced activation of the insulin receptor: a multi-step process involving structural changes in both the ligand and the receptor. *Bioessays* **31** (2009), pp. 422-434.

Ward, R.D., Raetzman, L.T., Suh, H., Stone, B.M., Nasonkin, I.O. and Camper, S.A. Role of PROP1 in pituitary gland growth. *Molecular Endocrinology* **19** (2005), pp. 698-710.

Waters, M.J. The growth hormone receptor. *Growth Hormone & IGF Research* **28** (2016), pp. 6-10.

Waters, M.J. and Brooks, A.J. JAK2 activation by growth hormone and other cytokines. *Biochemical Journal* **466** (2015), pp. 1-11.

Watkins-Chow, D.E. and Camper, S.A. How many homeobox genes does it take to make a pituitary gland? *Trends in Genetics* **14** (1998), pp. 284-290.

Webster, A.: The energetic efficiency of growth, *Annales de génétique et de sélection animale*. BioMed Central (1980), pp. 122b.

Wegner, M., Drolet, D.W. and Rosenfeld, M.G. POU-domain proteins: structure and function of developmental regulators. *Current opinion in cell biology* **5** (1993), pp. 488-498.

Weiss, K.M. and Clark, A.G. Linkage disequilibrium and the mapping of complex human traits. *TRENDS in Genetics* **18** (2002), pp. 19-24.

Wilson, D., Sheng, G., Lecuit, T., Dostatni, N. and Desplan, C. Cooperative dimerization of paired class homeo domains on DNA. *Genes & development* **7** (1993), pp. 2120-2134.

Wilson, D.S., Guenther, B., Desplan, C. and Kuriyan, J. High resolution crystal structure of a paired (Pax) class cooperative homeodomain dimer on DNA. *Cell* **82** (1995), pp. 709-719.

Wolberger, C. Homeodomain interactions. *Current opinion in structural biology* **6** (1996), pp. 62-68.

Wolfenden, R., Andersson, L., Cullis, P. and Southgate, C. Affinities of amino acid side chains for solvent water. *Biochemistry* **20** (1981), pp. 849-855.

Woods, K.A., Camacho-Hübner, C., Savage, M.O. and Clark, A.J. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *New England Journal of Medicine* **335** (1996), pp. 1363-1367.

Woollard, J., Tuggle, C. and de Leon, F.P. Rapid communication: localization of POU1F1 to bovine, ovine, and caprine 1q21-22. *Journal of animal science* **78** (2000), p. 242.

Woychik, R., Camper, S., Lyons, R., Horowitz, S., Goodwin, E. and Rottman, F. Cloning and nucleotide sequencing of the bovine growth hormone gene. *Nucleic Acids Research* **10** (1982), pp. 7197-7210.

Wu, W., Cogan, J.D., Pfäffle, R.W., Dasen, J.S., Frisch, H., O'Connell, S.M., Flynn, S.E., Brown, M.R., Mullis, P.E. and Parks, J.S. Mutations in PROP1 cause familial combined pituitary hormone deficiency. *Nature genetics* **18** (1998), p. 147.

Wuliji, T., Dodds, K., Land, J., Andrews, R. and Turner, P. Selection for ultrafine Merino sheep in New Zealand: heritability, phenotypic and genetic correlations of live weight, fleece weight and wool characteristics in yearlings. *Animal Science* **72** (2001), pp. 241-250.

Yang, L., Xu, D.-d., Sun, C.-j., Wu, J., Wei, H.-y., Liu, Y., Zhang, M.-y. and Luo, F.-h. IGF1R variants in patients with growth impairment: Four novel variants and genotype-phenotype correlations. *The Journal of Clinical Endocrinology & Metabolism* **103** (2018), pp. 3939-3944.

Yao, J., Aggrey, S.E., Zadworny, D., Hayes, J.F. and Kühnlein, U. Sequence variations in the bovine growth hormone gene characterized by single-strand conformation polymorphism (SSCP) analysis and their association with milk production traits in Holsteins. *Genetics* **144** (1996), pp. 1809-1816.

Yoshizato, H., Fujikawa, T., Soya, H., Tanaka, M. and Nakashima, K. The growth hormone (GH) gene is expressed in the lateral hypothalamus: enhancement by GH-releasing hormone and repression by restraint stress. *Endocrinology* **139** (1998), pp. 2545-2551.

Yu, M., Wang, H., Xu, Y., Yu, D., Li, D., Liu, X. and Du, W. Insulin-like growth factor-1 (IGF-1) promotes myoblast proliferation and skeletal muscle growth of embryonic chickens via the PI3K/Akt signalling pathway. *Cell biology international* **39** (2015), pp. 910-922.

Zeng, X.-C., Chen, H.-Y., Jia, B., Zhao, Z.-S., Hui, W.-Q., Wang, Z.-B. and Du, Y.-C. Identification of SNPs within the sheep PROP1 gene and their effects on wool traits. *Molecular biology reports* **38** (2011), pp. 2723-2728.

Zhang, L., Liu, J., Zhao, F., Ren, H., Xu, L., Lu, J., Zhang, S., Zhang, X., Wei, C. and Lu, G. Genome-wide association studies for growth and meat production traits in sheep. *PloS one* **8** (2013), p. e66569.

Zhao, G., Monier-Faugere, M.-C., Langub, M.C., Geng, Z., Nakayama, T., Pike, J.W., Chernausek, S.D., Rosen, C.J., Donahue, L.-R. and Malluche, H.H. Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. *Endocrinology* **141** (2000), pp. 2674-2682.

Zhou, H., Hickford, J. and Fang, Q. A two-step procedure for extracting genomic DNA from dried blood spots on filter paper for polymerase chain reaction amplification. *Analytical Biochemistry* **354** (2006), pp. 159-161.

Zhu, X., Gleiberman, A.S. and Rosenfeld, M.G. Molecular physiology of pituitary development: signaling and transcriptional networks. *Physiological Reviews* **87** (2007), pp. 933-963.

Zhu, X., Zhang, J., Tollkuhn, J., Ohsawa, R., Bresnick, E.H., Guillemot, F., Kageyama, R. and Rosenfeld, M.G. Sustained Notch signaling in progenitors is required for sequential emergence of distinct cell lineages during organogenesis. *Genes & development* **20** (2006), pp. 2739-2753.

Appendix A

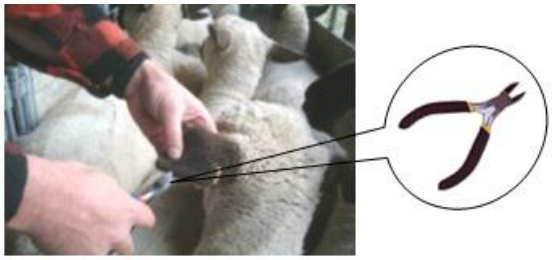
Sheep Blood Collection Instructions

LINCOLN UNIVERSITY
Te Whare Wānaka O Aoraki

Gene-Marker Laboratory
Agriculture & Life Sciences Division
PO Box 84, Lincoln University
Canterbury, NEW ZEALAND
Ph: 0064 3 3253803
0508 FOOTROT
Fax: 0064 3 325 3851

Step 1: Make a small cut about ½ cm into the bottom edge or tip of the ear of the sheep using electrical side-cutters.

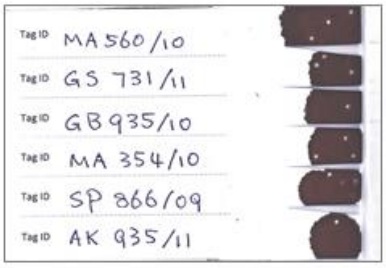
Clean the side-cutters in large amount of water if they are contaminated with blood from previous animals.



Step 2: Collect blood from the cut onto the strips on the collection cards. **Ensure blood soaks through.**

*One card has **SIX** collection strips and each strip is for one sample.*

Step 3: Label the each strip clearly with a unique ID (e.g. a brass tag number and year) for each blood spot. WRITE YOUR NAME ON EACH CARD.



Step 4: Blood samples are air-dried and then can be easily posted (together with a current New Zealand Ministry for Primary Industries import permit if from overseas) to the address above.

Appendix B

DNA sequencing protocol

Sequencing reaction and Clean-up protocols used by the Bio-Protection Research Centre Sequencing Facility

DNA samples were sequenced on an ABI Prism 3130xl Genetic Analyser with a 16 capillary 50cm array installed and using Performance Optimized Polymer 7 (POP7).

Cycle sequencing reaction Protocol :

Final reaction volume = 10µl
1/8 dilution

BDT*	0.5 µl
Seq. buffer**	2.0 µl
Primer (pmol)	1.0 µl
Template	variable
H_2O	to 10µl

* ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (P/N 4336917)
** BigDye® Terminator v3.1 5x sequencing buffer (P/N 4336697)

Thermal cycler conditions:

96 degrees, 1 minute, 1x

96 degrees, 10 seconds \
50 degrees, 5 seconds \ 25x
60 degrees, 2 minutes /

4 degrees, indefinitely

BigDye® Terminator v3.1 Cycle Sequencing Kit Protocols can be found in the 'Product and Service literature' library within the Applied Biosystems web site at www.appliedbiosystems.com

In particular:
https://www.thermofisher.com/content/dam/LifeTech/Documents/PDFs/sequencing_handbook_FLR.pdf

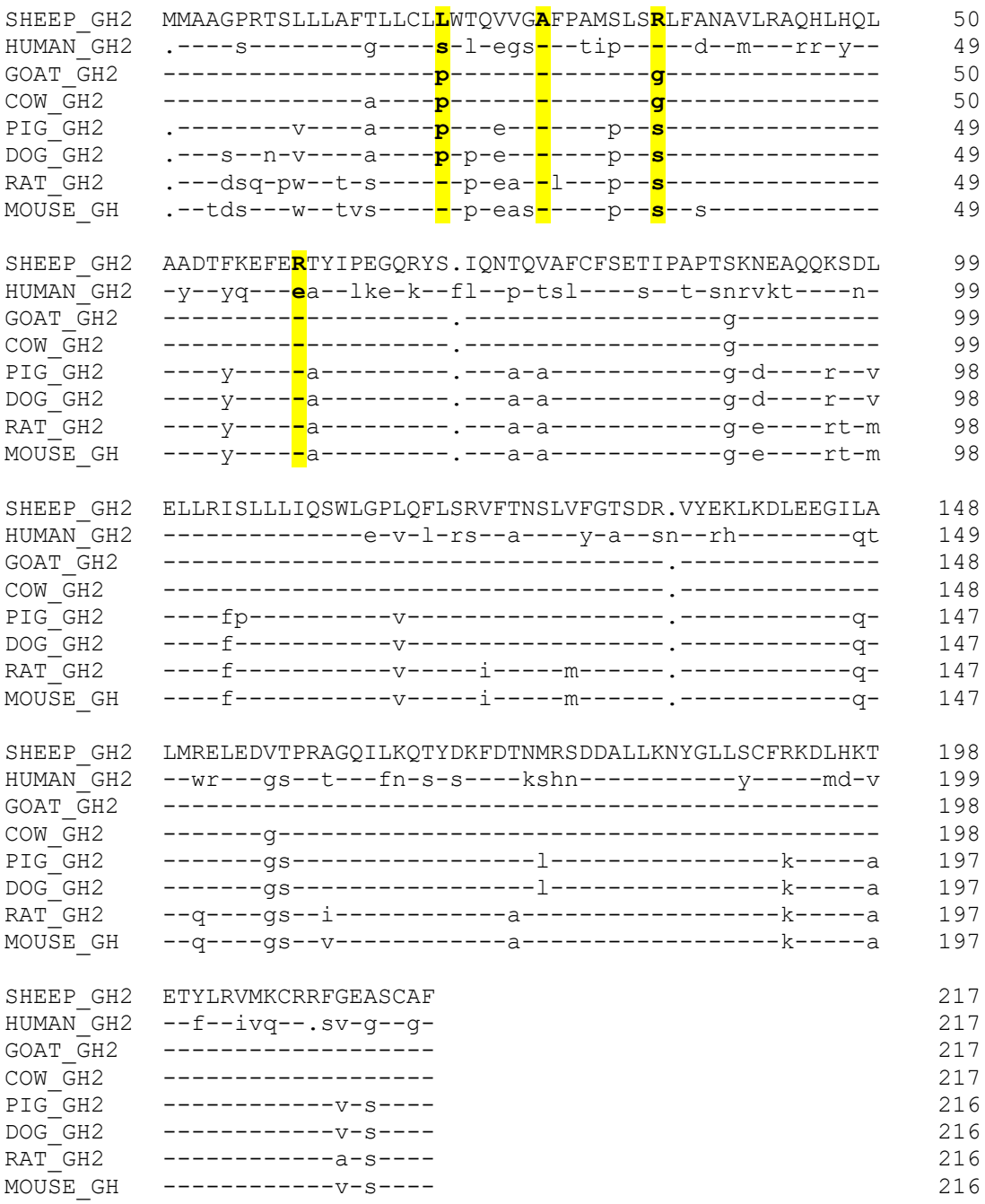
Post sequencing reaction clean-up:

The post sequencing reaction clean-up uses HighPrep™ Dye Terminator Removal Clean Up from MAGBIO®. HighPrep™ DTR uses magnetic bead based chemistry. (Magnetic particles in an optimized binding buffer selectively capture sequencing extension products. Unincorporated dyes, nucleotides, salts and contaminants are removed using a magnetic plate and a simple washing procedure.) Applications notes for HighPrep™ DTR can be found at:http://www.magbiogenomics.com/image/data/Literature/Protocols/HighPrep%20DTR%20Protocol.pdf

Appendix C

GH

GH protein sequence alignment across species



(a)

```
A_allele GPRTSLLLAFTLLCLLWTQVVGAFPAMSLSGLFANAVLRAQHLHQLAADT     5-54
B_allele ---------------p-------------------------------     5-54
C_allele ---------------p-------------------------------     5-54
D_allele ---------------p-------------------------------     5-54
E_allele ---------------p-------------r-------------------     5-54
F_allele ----------------------t-------r-------------------     5-54
G_allele ---------------p-------------------------------     5-54

A_allele FKEFERTYIPEGQRYSIQNTQVAFCFSETIPAPTGKNEAQQKS           55-97
B_allele ------------------------------------------           55-97
C_allele ------------------------------------------           55-97
D_allele -----h------------------------------------           55-97
E_allele ------------------------------------------           55-97
F_allele ------------------------------------------           55-97
G_allele ------------------------------------------           55-97
```

(b)

```
SHEEP_GH2  MMAAGPRTSLLLAFTLLCLLWTQVVGAFPAMSLSRLFANAVLRAQHLHQL     50
HUMAN_GH2  .----s--------g----s-l-egs---tip-----d--m---rr-y--     49
GOAT_GH2   -------------------p-------------g---------------     50
COW_GH2    --------------a----p-------------g---------------     50
PIG_GH2    .--------v----a----p---e-------p--s---------------     49
DOG_GH2    .---s--n-v----a----p-p-e-------p--s---------------     49
RAT_GH2    .---dsq-pw--t-s------p-ea--l---p--s---------------     49
MOUSE_GH   .--tds---w--tvs------p-eas-----p--s--s------------     49

SHEEP_GH2  AADTFKEFERTYIPEGQRYS.IQNTQVAFCFSETIPAPTSKNEAQQKS      97
HUMAN_GH2  -y--yq---ea--lke-k--fl--p-tsl----s--t-snrvkt----      97
GOAT_GH2   ---------------------.------------------g--------     97
COW_GH2    ---------------------.------------------g--------     97
PIG_GH2    ----y-----a---------.---a-a------------g-d----r-     97
DOG_GH2    ----y-----a---------.---a-a------------g-d----r-     97
RAT_GH2    ----y-----a---------.---a-a------------g-e----rt     97
MOUSE_GH   ----y-----a---------.---a-a------------g-e----rt     97
```

Coding of variables

Present = 1, Absent = 0

Geno	A_3	B_3	C_3	F_3	c.51C>T	c.59C>T	c.79G>A	c.103G>C	c.174+132A>G
AA	1	0	0	0	CC	TT	GG	GG	AA
AB	1	1	0	0	CC	CT	GG	GG	AA
AC	1	0	1	0	CC	CT	GG	GG	AA
AF	1	0	0	1	CT	TT	AG	CG	AG

Birth weight and *GH2* variants

Dependent variable: Birth weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Birth Wt	766	3.00	8.50	5.6821	.93899

Single-variant model

Estimated Marginal Means 2: A

Estimates

A	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
1.0	5.0886	.20324	4.6903	5.4870

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	5.0589	.20316	4.6608	5.4571
1.0	5.2043	.21009	4.7925	5.6161

Overall Test Results

Wald Chi-Square	df	Sig.
4.362	1	.037

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	5.0976	.20308	4.6996	5.4956
1.0	4.9686	.22022	4.5370	5.4002

Overall Test Results

Wald Chi-Square	df	Sig.
1.975	1	.160

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 2: A

Estimates

A	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
1.0	5.0859	.20826	4.6777	5.4940

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	5.0200	.20644	4.6154	5.4246
1.0	5.1518	.21594	4.7285	5.5750

Overall Test Results

Wald Chi-Square	df	Sig.
3.467	1	.063

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	5.1344	.20358	4.7354	5.5334
1.0	5.0374	.22280	4.6007	5.4741

Overall Test Results

Wald Chi-Square	df	Sig.
1.083	1	.298

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	5.0976	.20308	4.6996	5.4956
CT	4.9686	.22022	4.5370	5.4002

Overall Test Results

Wald Chi-Square	df	Sig.
1.975	1	.160

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	5.2424	.20825	4.8343	5.6506
TT	5.0434	.20257	4.6463	5.4404

Overall Test Results

Wald Chi-Square	df	Sig.
9.257	1	.002

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.79G>A

Estimates

c.79G>A	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
AG	4.9686	.22022	4.5370	5.4002
GG	5.0976	.20308	4.6996	5.4956

Overall Test Results

Wald Chi-Square	df	Sig.
1.975	1	.160

The Wald chi-square tests the effect of c.79G>A. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.103G>C

Estimates

c.103G>C	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CG	4.9686	.22022	4.5370	5.4002
GG	5.0976	.20308	4.6996	5.4956

Overall Test Results

Wald Chi-Square	df	Sig.
1.975	1	.160

The Wald chi-square tests the effect of c.103G>C. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.174+132A>G

Estimates

c.174+132A>G	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
AA	5.0976	.20308	4.6996	5.4956
AG	4.9686	.22022	4.5370	5.4002

Overall Test Results

Wald Chi-Square	df	Sig.
1.975	1	.160

The Wald chi-square tests the effect of c.174+132A>G. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Tailing weight and *GH2* variants

Dependent variable: Tailing weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Covariate: Tailing Age

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Tailing Wt	766	5.5	26.0	12.872	3.0026
Covariate	T.Age	766	5.0	55.0	31.808	10.6432

Single-variant model

Estimated Marginal Means 2: A

Estimates

			95% Wald Confidence Interval	
A	Mean	Std. Error	Lower	Upper
1.0	11.961	.5500	10.883	13.039

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Estimated Marginal Means 2: B

Estimates

			95% Wald Confidence Interval	
B	Mean	Std. Error	Lower	Upper
.0	11.860	.5489	10.784	12.935
1.0	12.350	.5671	11.239	13.461

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Overall Test Results

Wald Chi-Square	df	Sig.
6.928	1	.008

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	11.994	.5479	10.920	13.068
1.0	11.375	.5951	10.209	12.542

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Overall Test Results

Wald Chi-Square	df	Sig.
6.334	1	.012

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 2: A

Estimates

A	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
1.0	11.853	.5620	10.751	12.954

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	11.644	.5571	10.552	12.736
1.0	12.062	.5823	10.920	13.203

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Overall Test Results

Wald Chi-Square	df	Sig.
4.892	1	.027

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	12.111	.5487	11.036	13.186
1.0	11.594	.6014	10.415	12.773

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Overall Test Results

Wald Chi-Square	df	Sig.
4.299	1	.038

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	11.994	.5479	10.920	13.068
CT	11.375	.5951	10.209	12.542

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Overall Test Results

Wald Chi-Square	df	Sig.
6.334	1	.012

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	12.339	.5643	11.233	13.445
TT	11.856	.5486	10.781	12.931

Covariates appearing in the model are fixed at the following values: T.Age=31.808

Overall Test Results

Wald Chi-Square	df	Sig.
7.555	1	.006

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Weaning weight and *GH2* variants

Dependent variable: Weaning weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Covariate: Weaning Age

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Weaning Wt	766	12.5	50.5	31.238	4.7419
Covariate	W.Age	766	63.0	106.0	88.625	5.9194

Single-variant model

Estimated Marginal Means 2: A

Estimates

			95% Wald Confidence Interval	
A	Mean	Std. Error	Lower	Upper
1.0	29.274	.9645	27.383	31.164

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Estimated Marginal Means 2: B

Estimates

			95% Wald Confidence Interval	
B	Mean	Std. Error	Lower	Upper
.0	28.913	.9488	27.053	30.772
1.0	30.677	.9811	28.754	32.600

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Overall Test Results

Wald Chi-Square	df	Sig.
29.481	1	.000

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	29.421	.9475	27.564	31.278
1.0	27.133	1.0287	25.117	29.149

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Overall Test Results

Wald Chi-Square	df	Sig.
28.425	1	.000

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 2: A

Estimates

A	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
1.0	28.877	.9614	26.993	30.762

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	28.130	.9529	26.262	29.998
1.0	29.625	.9968	27.671	31.579

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Overall Test Results

Wald Chi-Square	df	Sig.
20.978	1	.000

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	29.839	.9392	27.998	31.680
1.0	27.916	1.0292	25.898	29.933

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Overall Test Results

Wald Chi-Square	df	Sig.
19.933	1	.000

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	29.421	.9475	27.564	31.278
CT	27.133	1.0287	25.117	29.149

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Overall Test Results

Wald Chi-Square	df	Sig.
28.425	1	.000

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	30.663	.9729	28.756	32.570
TT	28.872	.9461	27.018	30.726

Covariates appearing in the model are fixed at the following values: W.Age=88.625

Overall Test Results

Wald Chi-Square	df	Sig.
34.356	1	.000

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Growth rate and *GH2* variants

Dependent variable: Tailing weight

Model: (Intercept), RamID, Sex, Rearing rank, Variant

Covariate: Birth weight

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Growth rate	766	92.0	444.7	288.516	47.5343
Covariate	Birth Wt	766	3.00	8.50	5.6821	.93899

Single-variant model

Estimated Marginal Means 2: A

Estimates

			95% Wald Confidence Interval	
A	Mean	Std. Error	Lower	Upper
1.0	257.375	10.4606	236.872	277.877

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Estimated Marginal Means 2: B

Estimates

			95% Wald Confidence Interval	
B	Mean	Std. Error	Lower	Upper
.0	253.593	10.3143	233.377	273.809
1.0	270.969	10.6276	250.139	291.799

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Overall Test Results

Wald Chi-Square	df	Sig.
25.984	1	.000

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	264.061	10.4131	243.652	284.471
1.0	242.992	10.7634	221.896	264.087

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Overall Test Results

Wald Chi-Square	df	Sig.
21.862	1	.000

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 2: A

Estimates

A	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
1.0	258.407	10.2818	238.255	278.559

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	250.915	10.2383	230.848	270.981
1.0	265.900	10.6064	245.112	286.688

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Overall Test Results

Wald Chi-Square	df	Sig.
19.065	1	.000

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	267.164	10.3104	246.956	287.372
1.0	249.651	10.7407	228.599	270.702

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Overall Test Results

Wald Chi-Square	df	Sig.
14.978	1	.000

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	264.061	10.4131	243.652	284.471
CT	242.992	10.7634	221.896	264.087

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Overall Test Results

Wald Chi-Square	df	Sig.
21.862	1	.000

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	269.970	10.5573	249.278	290.662
TT	253.121	10.3109	232.912	273.330

Covariates appearing in the model are fixed at the following values: BirthWt=5.6821

Overall Test Results

Wald Chi-Square	df	Sig.
27.339	1	.000

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

HCW and *GH2* variants

Dependent variable: Hot carcass weight

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Single-variant model

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	17.035	.0760	16.886	17.184
1.0	17.445	.1260	17.198	17.692

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
9.451	1	.002

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	17.190	.0726	17.047	17.332
1.0	16.562	.1732	16.223	16.902

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
12.570	1	.000

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.831	.1001	16.634	17.027
1.0	17.174	.1526	16.875	17.473

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
6.532	1	.011

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	17.279	.0804	17.122	17.437
1.0	16.725	.1838	16.365	17.085

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
9.639	1	.002

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	17.190	.0726	17.047	17.332
CT	16.562	.1732	16.223	16.902

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
12.570	1	.000

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	17.448	.1174	17.218	17.678
TT	17.011	.0774	16.860	17.163

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
11.868	1	.001

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

V-GR and *GH2* variants

Dependent variable: V-GR

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Single-variant model

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	4.244	.1132	4.022	4.466
1.0	3.758	.1876	3.391	4.126

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
5.975	1	.015

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	4.050	.1080	3.838	4.262
1.0	4.891	.2576	4.386	5.396

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
10.197	1	.001

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	4.522	.1492	4.230	4.815
1.0	4.129	.2274	3.683	4.574

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
3.878	1	.049

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	3.947	.1198	3.713	4.182
1.0	4.704	.2739	4.167	5.241

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
8.087	1	.004

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	4.050	.1080	3.838	4.262
CT	4.891	.2576	4.386	5.396

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
10.197	1	.001

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	3.649	.1743	3.308	3.991
TT	4.308	.1149	4.083	4.534

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
12.298	1	.000

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Leg yield and *GH2* variants

Dependent variable: Leg yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Single-variant model

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	21.4528	.05708	21.3409	21.5646
1.0	21.7496	.09459	21.5643	21.9350

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
8.792	1	.003

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	21.5590	.05464	21.4519	21.6661
1.0	21.1603	.13029	20.9049	21.4156

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
8.968	1	.003

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	21.3260	.07534	21.1783	21.4736
1.0	21.5813	.11481	21.3562	21.8063

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
6.392	1	.011

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	21.6257	.06046	21.5072	21.7442
1.0	21.2815	.13829	21.0105	21.5526

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
6.568	1	.010

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	21.5590	.05464	21.4519	21.6661
CT	21.1603	.13029	20.9049	21.4156

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
8.968	1	.003

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	21.7856	.08792	21.6133	21.9580
TT	21.4240	.05798	21.3103	21.5376

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
14.544	1	.000

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Loin yield and *GH2* variants

Dependent variable: Loin yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Single-variant model

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	14.5422	.03976	14.4643	14.6201
1.0	14.7170	.06589	14.5879	14.8462

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
6.285	1	.012

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	14.6073	.03803	14.5328	14.6818
1.0	14.3483	.09067	14.1706	14.5260

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
7.812	1	.005

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	14.4584	.05250	14.3555	14.5613
1.0	14.6057	.08001	14.4489	14.7625

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
4.385	1	.036

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	14.6458	.04213	14.5632	14.7284
1.0	14.4183	.09637	14.2294	14.6072

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
5.908	1	.015

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	14.6073	.03803	14.5328	14.6818
CT	14.3483	.09067	14.1706	14.5260

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
7.812	1	.005

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	14.7451	.06127	14.6250	14.8652
TT	14.5229	.04040	14.4437	14.6021

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
11.304	1	.001

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Shoulder yield and *GH2* variants

Dependent variable: Shoulder yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Single-variant model

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.8106	.04185	16.7286	16.8927
1.0	16.9988	.06936	16.8628	17.1347

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
6.568	1	.010

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.8847	.03996	16.8064	16.9630
1.0	16.5680	.09528	16.3813	16.7548

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
10.579	1	.001

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Multi-variant model

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.7061	.05517	16.5979	16.8142
1.0	16.8599	.08408	16.6951	17.0247

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
4.329	1	.037

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.9249	.04427	16.8381	17.0117
1.0	16.6411	.10128	16.4426	16.8396

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
8.327	1	.004

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

SNP model

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	16.8847	.03996	16.8064	16.9630
CT	16.5680	.09528	16.3813	16.7548

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
10.579	1	.001

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	17.0018	.06464	16.8751	17.1285
TT	16.7992	.04263	16.7157	16.8828

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
8.440	1	.004

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Total yield and *GH2* variants

Dependent variable: Loin yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Single-variant model

Estimated Marginal Means 2: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	52.805701770000000	.116148710000000	52.578054480000000	53.033349060000000
1.0	53.466340310000000	.192483526000000	53.089079530000000	53.843601080000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
10.515	1	.001

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	53.051484590000000	.111013534000000	52.833902060000000	53.269067120000000
1.0	52.075413940000004	.264698391000000	51.556614620000000	52.594213250000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
13.019	1	.000

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 3: B

Estimates

B	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	52.489988590000000	.152950491000000	52.190211130000000	52.789766040000000
1.0	53.047072030000000	.233093111000000	52.590217920000000	53.503926130000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
7.385	1	.007

The Wald chi-square tests the effect of B. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 4: F

Estimates

F	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	53.197019800000000	.122736686000000	52.956460310000004	53.437579280000000
1.0	52.340040810000000	.280752034000000	51.789776940000000	52.890304690000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
9.879	1	.002

The Wald chi-square tests the effect of F. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.51C>T

Estimates

c.51C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CC	53.051484590000000	.11101353 4000000	52.833902060000000	53.269067120000000
CT	52.075413940000004	.26469839 1000000	51.556614620000000	52.594213250000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
13.019	1	.000

The Wald chi-square tests the effect of c.51C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: c.59C>T

Estimates

c.59C>T	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
CT	53.533348860000004	.178869937 000000	53.182770230000000	53.883927490000000
TT	52.746171290000000	.117948696 000000	52.514996090000004	52.977346480000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7598; DraftAgedays=132.29

Overall Test Results

Wald Chi-Square	df	Sig.
16.649	1	.000

The Wald chi-square tests the effect of c.59C>T. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

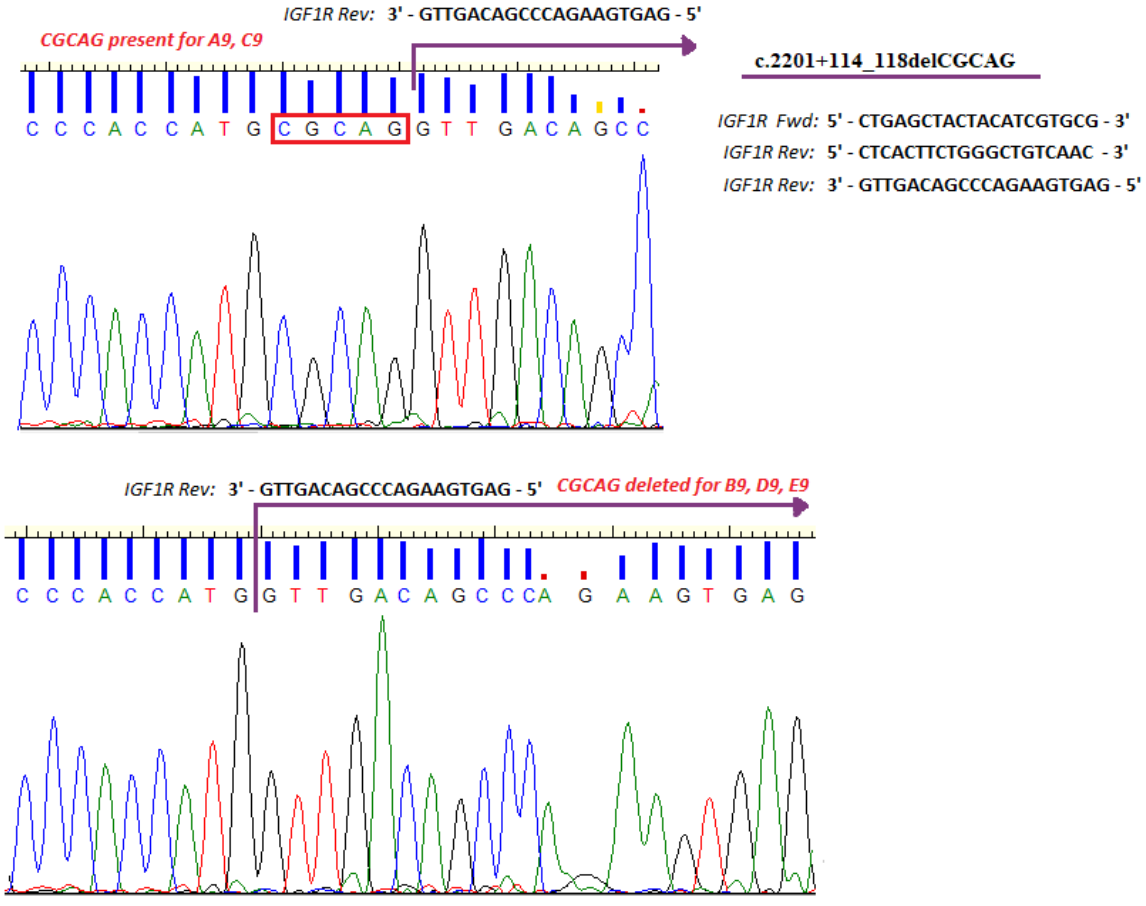
Appendix D

IGF1R

Coding of variables

Present = 1, Absent = 0

Geno	*A9*	*B9*	*E9*	c.2019C>T	c.2037C>T	c.2201+28A>C	c.2201+114_118delCGCAG
AA	1	0	0	CC	CC	AA	CGCAG/CGCAG
BB	0	1	0	TT	TT	AA	del/del
EE	0	0	1	CC	CC	CC	del/del
AB	1	1	0	CT	CT	AA	CGCAG/del
AE	1	0	1	CC	CC	AC	CGCAG/del
BE	0	1	1	CT	CT	AC	del/del



Birth weight and *IGF1R* variants

Dependent variable: Birth weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	5.0349150 68000000	.20733732 9000000	4.6285413 71000000	5.4412887 66000000
1	5.1306553 22000000	.20588319 9000000	4.7271316 66000000	5.5341789 77000001

Overall Test Results

Wald Chi-Square	df	Sig.
2.069	1	.150

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means: B9

Estimates

B9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	5.1297435 82000001	.20892991 9000000	4.7202484 64000000	5.5392386 99000000
1	5.0726328 44000000	.20487301 6000000	4.6710891 10000001	5.4741765 78000001

Overall Test Results

Wald Chi-Square	df	Sig.
.810	1	.368

The Wald chi-square tests the effect of B9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means 2: E9

Estimates

E9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	5.1061943 30000000	.20415738 7000000	4.7060532 04000001	5.5063354 56000000
1	4.9979943 04000001	.21282041 8000000	4.5808739 49000000	5.4151146 59000000

Overall Test Results

Wald Chi-Square	df	Sig.
2.228	1	.136

The Wald chi-square tests the effect of E9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Tailing weight and *IGF1R* variants

Dependent variable: Tailing weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Covariate: Tailing Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	11.752	.5615	10.652	12.853
1	12.142	.5580	11.048	13.236

Covariates appearing in the model are fixed at the following values: T.Age=31.915

Overall Test Results

Wald Chi-Square	df	Sig.
4.756	1	.029

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means: B9

Estimates

B9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	12.201	.5658	11.092	13.310
1	11.875	.5551	10.787	12.963

Covariates appearing in the model are fixed at the following values: T.Age=31.915

Overall Test Results

Wald Chi-Square	df	Sig.
3.675	1	.055

The Wald chi-square tests the effect of B9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means: E9

Estimates

E9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	12.035	.5533	10.951	13.119
1	11.609	.5766	10.479	12.739

Covariates appearing in the model are fixed at the following values: T.Age=31.915

Overall Test Results

Wald Chi-Square	df	Sig.
4.791	1	.029

The Wald chi-square tests the effect of E9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Weaning weight and *IGF1R* variants

Dependent variable: Weaning weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Covariate: Weaning Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	28.625	.9691	26.726	30.524
1	29.756	.9622	27.870	31.642

Covariates appearing in the model are fixed at the following values: W.Age=88.665

Overall Test Results

Wald Chi-Square	df	Sig.
13.234	1	.000

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means: B9

Estimates

B9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	29.926	.9780	28.009	31.843
1	28.998	.9589	27.119	30.878

Covariates appearing in the model are fixed at the following values: W.Age=88.665

Overall Test Results

Wald Chi-Square	df	Sig.
9.773	1	.002

The Wald chi-square tests the effect of B9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means: E9

Estimates

E9	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	29.450	.9550	27.578	31.322
1	28.272	.9957	26.320	30.224

Covariates appearing in the model are fixed at the following values: W.Age=88.665

Overall Test Results

Wald Chi-Square	df	Sig.
12.071	1	.001

The Wald chi-square tests the effect of E9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Growth rate and *IGF1R* variants

Dependent variable: Tailing weight

Model: (Intercept), RamID, Sex, Rearing rank, Variant

Covariate: Birth weight

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
0	249.588158 699999980	10.5708831 30000000	228.869608 500000000	270.306708 900000000
1	260.348450 100000000	10.3511972 10000000	240.060476 299999980	280.636423 800000000

Covariates appearing in the model are fixed at the following values: Birth Wt=5.686406460296090

Overall Test Results

Wald Chi-Square	df	Sig.
11.054	1	.001

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Estimated Marginal Means: B9

Estimates

B9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
0	264.911507 400000000	10.5654510 90000000	244.203603 800000000	285.619411 000000000
1	254.778842 699999980	10.3413829 70000000	234.510104 499999980	275.047580 800000000

Covariates appearing in the model are fixed at the following values: Birth Wt=5.686406460296090

Overall Test Results

Wald Chi-Square	df	Sig.
10.884	1	.001

The Wald chi-square tests the effect of B9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

HCW and *IGF1R* variants

Dependent variable: Hot carcass weight

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.744	.1155	16.517	16.970
1.0	17.266	.0776	17.114	17.419

Covariates appearing in the model are fixed at the following values: BirthWt=5.7671; DraftAgedays=132.09

Overall Test Results

Wald Chi-Square	df	Sig.
17.523	1	.000

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

V-GR and *IGF1R* variants

Dependent variable: V-GR

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	4.440	.1751	4.096	4.783
1.0	4.046	.1176	3.816	4.277

Covariates appearing in the model are fixed at the following values: BirthWt=5.7671; DraftAgedays=132.09

Overall Test Results

Wald Chi-Square	df	Sig.
4.314	1	.038

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Leg yield and *IGF1R* variants

Dependent variable: Leg yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	21.3480	.08780	21.1759	21.5201
1.0	21.5613	.05900	21.4457	21.6770

Covariates appearing in the model are fixed at the following values: BirthWt=5.7671; DraftAgedays=132.09

Overall Test Results

Wald Chi-Square	df	Sig.
5.049	1	.025

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Loin yield and *IGF1R* variants

Dependent variable: Loin yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	14.4531	.06115	14.3332	14.5729
1.0	14.6210	.04109	14.5405	14.7016

Covariates appearing in the model are fixed at the following values: BirthWt=5.7671; DraftAgedays=132.09

Overall Test Results

Wald Chi-Square	df	Sig.
6.456	1	.011

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Shoulder yield and *IGF1R* variants

Dependent variable: Shoulder yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	16.6574	.06380	16.5323	16.7824
1.0	16.9243	.04287	16.8402	17.0083

Covariates appearing in the model are fixed at the following values: BirthWt=5.7671; DraftAgedays=132.09

Overall Test Results

Wald Chi-Square	df	Sig.
14.972	1	.000

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Total yield and *IGF1R* variants

Dependent variable: Loin yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means: A9

Estimates

A9	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
.0	52.4592169800000000	.1780619560000000	52.1102219600000004	52.8082120000000005
1.0	53.1066180100000000	.1196392280000000	52.8721294300000000	53.3411065900000000

Covariates appearing in the model are fixed at the following values: BirthWt=5.7671; DraftAgedays=132.09

Overall Test Results

Wald Chi-Square	df	Sig.
11.310	1	.001

The Wald chi-square tests the effect of A9. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Appendix E

PROP1

Coding of variables

PROP1 Exon 1	A_1	B_1	C_1	c.45A>G	c.109+40T>C	c.109+207C>T
AA	1	0	0	GG	CC	CC
AB	1	1	0	AG	CT	CC
AC	1	0	1	GG	CC	CT
BB	0	1	0	AA	TT	CC
BC	0	1	1	AG	CT	CT
CC	0	0	1	GG	CC	TT

Present = 1, Absent = 0

PROP1 Exon 2	A_2	B_2	C_2	c.189G>C	c.237G>A	c.342+62A>T
AA	1	0	0	GG	AA	TT
AB	1	1	0	GG	AG	AT
AC	1	0	1	CG	AG	AT
BB	0	1	0	GG	GG	AA
BC	0	1	1	CG	GG	AA
CC	0	0	1	CC	GG	AA

PROP1 protein sequence alignment across species

```
SHEEP_PROP1 MDTEGRSEQAKQAKEQVCSSLWPEGYPAAETVTSSVDMNTQPYRNLSGVR     50
HUMAN_PROP1 -ea-r-rqae-pk-gr-g---l--rh--tg-p-tt--ssap-c-r-p-ag     50
MOUSE_PROP1 -eaqr--h-e--t-gha-grsl--prv-sg-li-t--rssea--r---te     50
RAT_PROP1   -eaqr--q-e--t-gp--grsl--sqa-sg-li-t--rspetskr---tg     50
PIG_PROP1   -ea---r--g-pr-gr-------------g-l-ar--is-r--------g     50
DOG_PROP1   -ea-----rg--k-grl-g----ds----g-l--t--vsp--sk-----g     50
COW_PROP1   -----s---g--t-------------------------------------     50
GOAT_PROP1  -------------------------------------------------     50

SHEEP_PROP1 VGRPKLSLQGGQRGRPHSRRRHRTTFSPAQLEQLESAFGKNQYPDIWARE    100
HUMAN_PROP1 g--srf-p-------------------v----------r----------    100
MOUSE_PROP1 l-----cp-...--------------n------------r----------     97
RAT_PROP1   l-----cp-...--------------n----g-------r-------v--     97
PIG_PROP1   a---r--p--------------------------------r----------    100
DOG_PROP1   -r--r--p-------l----------n-g------t---r----------    100
COW_PROP1   ----------v-----------------------------r----------    100
GOAT_PROP1  a-------------------------------------------------    100

SHEEP_PROP1 SLAQDTGLSEARIQVWFQNRRAKQRKQERSLLQPLAHLSPATFSGFLPEP    150
HUMAN_PROP1 ---r-------------------------------------a--s----s    150
MOUSE_PROP1 g---------------------------------i----t---------s    147
RAT_PROP1   g---------------------------------t------------s-s    147
PIG_PROP1   g--r-----------------------------------------------    150
DOG_PROP1   g--r-----------------------------------------------s    150
COW_PROP1   -------------------------------------------------    150
GOAT_PROP1  -------------------------h-----------------------    150

SHEEP_PROP1 PSCPYPYPTPPPPMTCFPHPYNHALPSQPSTGSSFARPYQSEDWYPNLHP    200
HUMAN_PROP1 ta---s-aa----v-------s----------ga--lsh-------t---    200
MOUSE_PROP1 say--t-g-----ap------s-s-------aa-l-l-p-p-----t---    197
RAT_PROP1   spy--t-t-----vp--------------c--a-ltl-a-p-----t---    197
PIG_PROP1   -a---s--------------------------g----hp-------t---    200
DOG_PROP1   -a---s--------------------------g-----h-------t---    200
COW_PROP1   -----s---------------------------------------------    200
GOAT_PROP1  -------------------------------------------------    200

SHEEP_PROP1 TPAGHLPCPPPPPMLPLSLEPPKSWN                          226
HUMAN_PROP1 a--------------------s----                           226
MOUSE_PROP1 a-t-----------f-----t-----                           223
RAT_PROP1   -ht---a-------fs----t-----                          223
PIG_PROP1   --t-------a--v------------                          226
DOG_PROP1   p-t---------t-------------                          226
COW_PROP1   ------a------------------                           226
GOAT_PROP1  -----------------g--------                          226
```

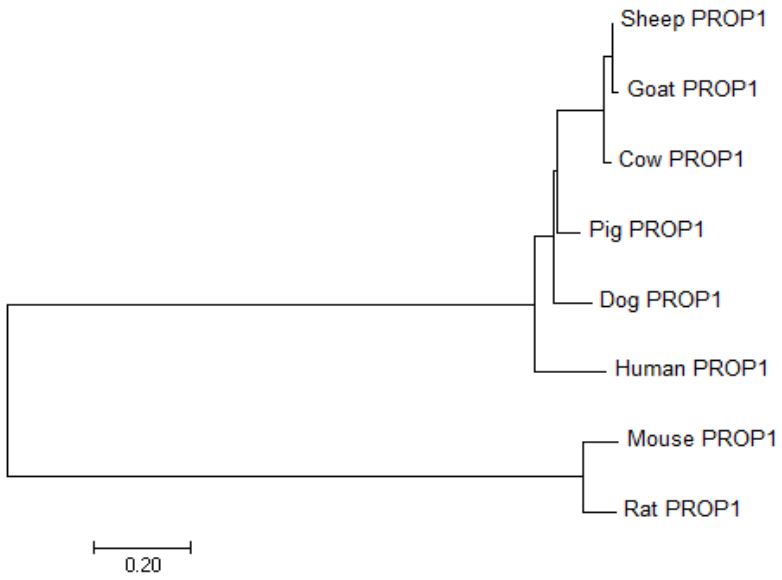
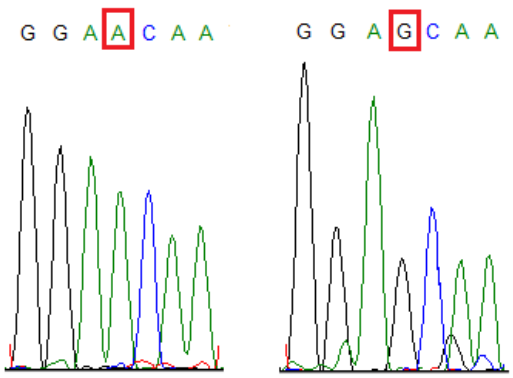


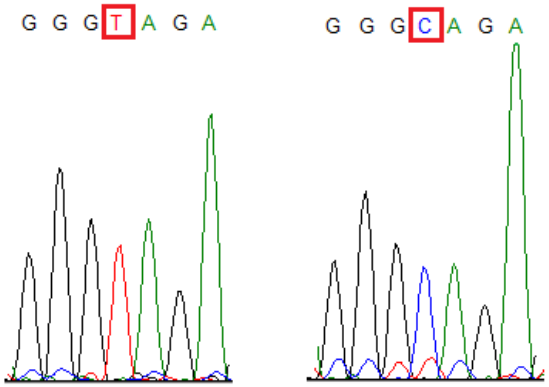
Figure E1. Phylogenetic tree of PROP1 protein sequences from various species. The tree depicts the phylogenetic relationships between mammalian PROP1 proteins, and was constructed using the maximum-likelihood algorithm based on the JTT-matrix model in the Mega7 package (Kumar et al., 2016) Branch lengths represent the number of substitutions per site. The amino acid sequences were extracted from Genbank with the following accession numbers: Human, *Homo sapiens* (AF076215); Sheep, *Ovis aries* (AY533709); Goat, *Capra hircus* (XM_005682787); Cow, *Bos taurus* (XM_015472155); Pig, *Sus scrofa* (XM_013987527); Dog, *Canis familiaris* (NM_001020807); Rat, *Rattus norvegicus* (XM_008767664) and Mouse, *Mus musculus* (U77946).

Chromatogram of *PROP1* Sequence

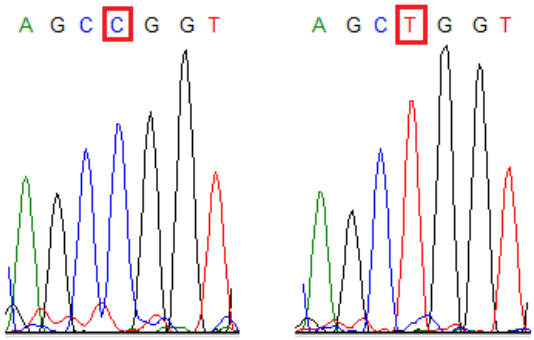
c.45 A>G ggaGcaa



c.109+40 T>C gggCaga



c.109+207 C>T agcCggt



Birth weight and *PROP1* variants

Dependent variable: Birth weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Estimated Marginal Means 2: A1

Estimates

A1	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	5.476534888000001	.22309982900000	5.039267259000001	5.913802517000001
1	5.540537251000000	.21226214400000	5.124511094000001	5.956563407000000

Overall Test Results

Wald Chi-Square	df	Sig.
.692	1	.406

The Wald chi-square tests the effect of A1. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Tailing weight and *PROP1* variants

Dependent variable: Tailing weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Covariate: Tailing Age

Estimated Marginal Means 2: A1

Estimates

A1	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	11.086	.6047	9.901	12.271
1	12.167	.5773	11.036	13.299

Covariates appearing in the model are fixed at the following values: Tailing Age=30.446

Overall Test Results

Wald Chi-Square	df	Sig.
27.152	1	.000

The Wald chi-square tests the effect of A1. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Weaning weight and *PROP1* variants

Dependent variable: Weaning weight

Model: (Intercept), RamID, Sex, Birth rank, Variant

Covariate: Weaning Age

Estimated Marginal Means 2: A2

Estimates

A2	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	28.284	1.0285	26.268	30.300
1	30.849	.9686	28.950	32.747

Covariates appearing in the model are fixed at the following values: Weaning Age=88.356

Overall Test Results

Wald Chi-Square	df	Sig.
43.341	1	.000

The Wald chi-square tests the effect of A2. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Growth rate and *PROP1* variants

Dependent variable: Tailing weight

Model: (Intercept), RamID, Sex, Rearing rank, Variant

Covariate: Birth weight

Estimated Marginal Means 2: A2

Estimates

A2	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	247.9125 1620000 0000	10.47903 3000000 000	227.3739 8900000 0000	268.4510 4349999 9970
1	275.4492 1610000 0000	10.22751 2700000 000	255.4036 5949999 9970	295.4947 7260000 0000

Covariates appearing in the model are fixed at the following values: Birth Wt=5.806111111111114

Overall Test Results

Wald Chi-Square	df	Sig.
46.886	1	.000

The Wald chi-square tests the effect of A2. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

HCW and *PROP1* variants

Dependent variable: Hot carcass weight

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means 2: A1

Estimates

A1	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
0	16.618	.1493	16.325	16.911
1	17.212	.0869	17.042	17.382

Covariates appearing in the model are fixed at the following values: Draft Age (days)=130.93; Birth Wt=5.793507462686569

Overall Test Results

Wald Chi-Square	df	Sig.
16.141	1	.000

The Wald chi-square tests the effect of A1. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

V-GR and *PROP1* variants

Dependent variable: V-GR

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means 2: A1

Estimates

A1	Mean	Std. Error	95% Wald Confidence Interval Lower	Upper
0	4.734399 8480000 01	.2180509 6200000 0	4.307027 8150000 01	5.161771 8810000 00
1	4.176791 9350000 01	.1269025 1400000 0	3.928067 5780000 00	4.425516 2930000 00

Covariates appearing in the model are fixed at the following values: Draft Age (days)=130.93; Birth Wt=5.793507462686569

Overall Test Results

Wald Chi-Square	df	Sig.
6.665	1	.010

The Wald chi-square tests the effect of A1. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Leg yield and *PROP1* variants

Dependent variable: Leg yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means 2: A1

Estimates

A1	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	21.209750770000000	.109124412000000	20.995870850000000	21.423630690000000
1	21.548790080000000	.063508833300000	21.424315060000000	21.673265110000000

Covariates appearing in the model are fixed at the following values: Draft Age (days)=130.93; Birth Wt=5.793507462686569

Overall Test Results

Wald Chi-Square	df	Sig.
9.838	1	.002

The Wald chi-square tests the effect of A1. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Loin yield and *PROP1* variants

Dependent variable: Loin yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means 2: A2

Estimates

A2	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	14.206174840000001	.0810557216000000	14.047308540000000	14.365041130000000
1	14.610912560000001	.0440437590000000	14.524588380000000	14.697236740000001

Covariates appearing in the model are fixed at the following values: Birth Wt=5.793507462686569; Draft Age (days)=130.93

Overall Test Results

Wald Chi-Square	df	Sig.
24.332	1	.000

The Wald chi-square tests the effect of A2. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Shoulder yield and *PROP1* variants

Dependent variable: Shoulder yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means 2: A2

Estimates

A2	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	16.57879052000002	.08854665040000	16.40524228000000	16.75233877000000
1	16.83056424000000	.04811415220000	16.73626224000002	16.92486625000000

Covariates appearing in the model are fixed at the following values: Birth Wt=5.793507462686569; Draft Age (days)=130.93

Overall Test Results

Wald Chi-Square	df	Sig.
7.890	1	.005

The Wald chi-square tests the effect of A2. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

Total yield and *PROP1* variants

Dependent variable: Loin yield

Model: (Intercept), RamID, Variant

Covariate: Birth weight, Draft Age

Estimated Marginal Means 2: A2

Estimates

A2	Mean	Std. Error	95% Wald Confidence Interval Lower	95% Wald Confidence Interval Upper
0	52.147185730000004	.241259159000000	51.674326470000004	52.620045000000005
1	52.959571190000000	.131094512000000	52.702630660000004	53.216511710000000

Covariates appearing in the model are fixed at the following values: Birth Wt=5.793507462686569; Draft Age (days)=130.93

Overall Test Results

Wald Chi-Square	df	Sig.
11.065	1	.001

The Wald chi-square tests the effect of A2. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.