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Research Article

Genetic Polymorphisms in New Zealand Sheep Breeds

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Abstract

Animal production is a booming industry with the improvement of economically desirable traits as its primary concern. Marker-assisted selection utilizes genetic variations within candidate genes that influence production traits as a means of guiding animal breeding and improving the traits of interest. Growth hormone (GH) plays a crucial role in pre-natal muscular and bone growth and development. GH brings about various physiological functions either directly by binding its receptor or indirectly by stimulating the release of insulin growth factor 1 (IGF1). Insulin growth factor 1 receptor (IGF1R) mediates its function on metabolism, homeostasis and development upon the binding of IGF1. The POU-domain class 1 transcription factor 1 (POU1F1) regulates the pre-natal development of cells of the anterior pituitary, including somatotrophs that produce GH. Reports have demonstrated associations between polymorphisms in these genes and animal production traits. This novel study examined the polymorphisms in the coding regions of candidate genes, GH2Z, IGF1R and POU1F1, in New Zealand (NZ) sheep. The sheep breeds investigated were NZ Romney and Merino, two commercially sought-after breeds. The results revealed two variants, AA and AB, for the exon 3 of POU1F1. The AA and AB genotypes had frequencies of 78% and 22% for Romney sheep, and 64% and 36% for Merino sheep respectively. All frequencies were in accordance with the Hardy-Weinberg Equilibrium ($P > 0.05$). The exon 2 of GH2Z revealed multiple variations while no variation was detected for the exon 15 of IGF1R..

Keywords: Marker-assisted selection; Polymorphism; Sheep; Gene

Introduction

The improvement of phenotypic traits such as growth rate, meat quality and milk production is at the core of the animal production industry. These production traits are dependent on a delicate interplay of genes. Research into finding variations in candidate genes that underpin production traits is becoming increasingly popular.

Growth is a complex physiological process involving a vast array of hormones and an interplay of signaling pathways and essential to all living organisms. Growth hormone (GH) or somatotropin is a 191-amino acid peptide hormone secreted by the anterior pituitary gland, which plays a prominent role in the control of growth and metabolism [1]. Ovine growth hormone is about 2.9 kbp long with five exons interspersed by four introns and is located on chromosome 11. Sheep GH differs from that of cattle and human GH but is similar to goat GH, in that it has two duplicate yet distinct alleles GH1 and GH2, the latter which is also a duplicate resulting in GH2-N and GH2-Z [2,3]. GH stimulates the somatic growth and development of essentially all body tissues, as well as regulates metabolism and body composition[2]. GH exerts its physiological effects directly or indirectly.

Insulin growth factor 1 (IGF-1) is a hormone structurally similar to insulin. IGF-1 secretion by the liver is regulated by GH hence IGF-1 serving as a chief mediator of GH effects by binding to its receptor, IGF1R [4]. When activated by IGF-1 or other similar ligand binding, IGF1R plays a crucial role in cell growth and metabolism by initiating a cascade of molecular interactions commonly via phosphotidylinositol-3-kinase/protein kinase B (PI3K-AKT) pathway [5,6]. In sheep, IGF1R gene is located on chromosome 11, comprising of 20 exons and 19 introns.

In sheep, POU1F1 is 17 kbp gene located on sheep chromosome 1, consisting of six exons and five introns and encoding a 33 kDa protein [7]. POU1F1 is a positive regulator of growth hormone, prolactin and thyroid-stimulating hormone β in mammals, and is crucial to the differentiation and survival of three anterior pituitary cell types: thyrotropes, somatotrophs and lactotrophs[8].

The aforementioned genes are all found in the vicinity of the quantitative trait loci (QTL) for economically beneficial traits [9]. The Romney and Merino breeds are two of the most popular breeds in New Zealand, the former bred for both meat and wool, while the latter is solely bred for wool. The meat and wool industry are major drivers of many economies in the world, including New Zealand [10]. The study aims to search for polymorphisms within the GH, IGF1R and POU1F1 genes, which could potentially serve as DNA markers.

Experimental Details

Animals and data collection

All research involving animals was performed in accordance with the Animal Welfare Act 1999 (NZ Government). Three hundred sheep breeds were examined in this study - Romney (n = 150) and Merino (n = 150). The lambs were progeny of non-consanguineous sires. Upon birth, each animal was given an identification number and details surrounding birth like birth weight, birth date and gender were recorded. Blood samples were collected from each lamb onto an FTA card (Whatman Bioscience, Middlesex, UK) by piercing the ears of the sheep. DNA was purified from the blood sample using a method where the disc containing blood sample is hydrolyzed with 200µl of sodium hydroxide at 60 0C, and subsequently rinsed with 200µl of TBE (Tris Borate EDTA) buffer [11].

Materials and primer design

Oligonucleotide primers were designed for the genes, POU1F1, GH2Z and IGF1R based on NCBI database on sheep (Table 1). The primers were manufactured by Integrated DNA Technologies, IA, USA.

Table 1: Primer oligonucleotide sequences, NCBI accession numbers and PCR conditions.

Gene (Accession no.)	Gene Region	Oligonucleotide Sequence (5' to 3')	Amplicon Size (bp)	Annealing Temp (°C)	SSCP Conditions
GH2Z (DQ461615)	Exon 2	F:AGAGTGTGAATGCCCCAAAC R: GACCCCTCTGTCCTCCTTG	330	63 °C	300 V, 20 °C, 19 h
IGF1R (NC_019475.2)	Exon 15	F: CTCAGCTCCGGCCTCTTCC R: GCCCTGCACCACCTCTGG	387	59 °C	270 V, 30 °C, 19 h
POU1F1 (NC_019458.2)	Exon 3	F: ACTGGCCTTCACAGAACAATC R: GACTTTGCAGATGGGGTTGT	365	58 °C	320 V, 25 °C, 13 h

Polymerase chain reaction (PCR) and single-nucleotide conformational polymorphism (SSCP)

PCR was amplified in 15µl reaction tubes containing 1.2 mm punch of the FTA card, 0.25µM of each primer, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany), 150µM of each deoxyribonucleoside triphosphate (dNTP) (Bio line, London, UK), 2.5 mM Mg^{2+}, and deionized water (dH_2O) to make up to volume. Bio-Rad S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA) were used to perform DNA amplifications. The amplification conditions included an initial denaturation at 94 °C for 2min, followed by 34 cycles of 94 °C for 30s, annealing for 30s (temperature differs for genes, Table 1), and 72 ºC for 30s, and a final extension step at 72 °C for 5min.

The success of PCR was first confirmed using agarose gel electrophoresis. 1µl of ethidium bromide was added to 1% agarose, with wells into which 2µl of sample mixed with 8µl of bromophenol blue dye was added.

The amplified PCR products were then subjected to SSCP (single-nucleotide conformational polymorphism) analysis. A 0.7µl aliquot of the amplicons was added to 7µl of loading dye containing 10mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanole, 98% formamide. Initial denaturation at 95°C for 5min was followed by rapid cooling of the samples on wet ice. The samples were then loaded onto 16 cm X 18 cm, acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels. Electrophoresis was carried out at varying conditions using Protean II xi cells (Bio-Rad) (Table 1).

Silver staining of the gels was carried out after electrophoresis using a method described by Byun et al (2009) [12]. Homozygous and heterozygous patterns were identified from SSCP banding patterns.

Data analysis

IBM SPSS Statistics (Version 23, IBM, NY, USA) was used to perform the data analysis. Variant and genotypic frequencies were calculated using the Pop Gene 3.2 software. The variant and genotypic frequencies were tested for the deviation from the Hardy-Weinberg equilibrium (HWE) using Chi-squared analysis (Table 2). DNAMAN 5.2.10 Lynn on Bio soft was used to construct the phylogenetic trees. All p values at $P < 0.05$ were considered statistically significant.

Results and Discussion

SSCP patterns

The exon 1 of POU1F1 exhibited limited heterozygosity with variants, AA and AB (Table 2). Fifty samples were investigated for the Romney breed, and another 50 for the Merino breed. For the two both sheep breeds, the major variant was the A 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.

Table 2: Variant and genotype frequencies for POU1F1 variants in NZ sheep breeds.

Gene (Breed)	Variant	Variant Frequency	n	Genotype	Genotype Frequency	HWE
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	

POU1F1 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[13]. Previous research has linked polymorphisms in the exon 6 of POU1F1 with milk yield, and milk fat and lactose content [14]. Another study found association between POU1F1 and wool weight and fiber diameter [15].

Exon 15 of IGF1R was homozygous for both Romney and Merino breeds, with no variations detected (Figure 1). This result could be attributed to the small sample size or could be due to the highly conserved nature of IGF1R owing to its function as an important housekeeping gene [16]. IGF1R plays an important role in growth, anabolism and homeostasis in mammals. Genes that are critical to the biological function of an organism are usually highly conserved in certain regions [17]. In contrast to this study, significant association has been reported between a polymorphism in IGF1R and body weight and average daily gain of coarse-wool ewes [18].

Multiple non-specific SSCP patterns were observed for GH2-Z (Figure 1). This is likely due to the gene duplication observed in ovine growth hormone. Many similar regions exist between GH1, GH2-N and GH2-Z.Combined GH2-N and GH2-Z variations have been demonstrated to bring about an approximate increase in milk production by 25% [19].

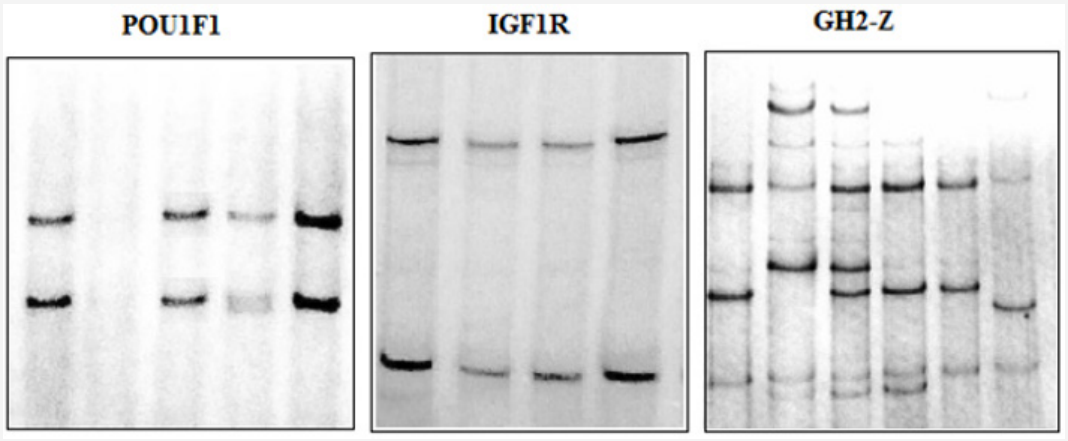

Figure 1: PCR-SSCP results of the genes POU1F1, IGF1R and GH2-Z.

Allele and genotypic diversity

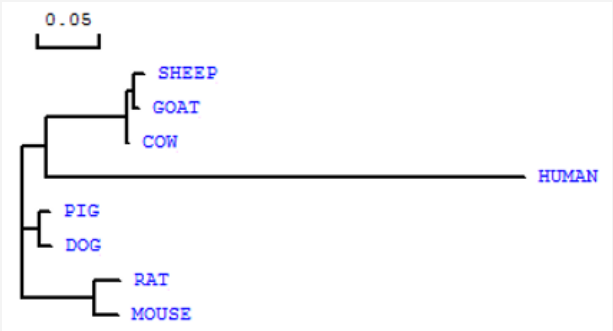

Figure 2: Phylogenetic tree of GH2-Z protein sequences from different species.

The evolutionary relationship between different species varies for different genes. For growth hormone, the genetic similarity between small ruminants, sheep and goat, are much closer than the link with the other mammalian breeds (Figure 2).

The limited diversity observed for POU1F1 in this study comes as no surprise as previous reports have shown coding region of ovine POU1F1 to be highly conserved as evidenced by its similarity with the bovine (98.2%), human (91.2%) and murine (86.2%) counterparts [20].

[The tree was constructed using the DNAMAN 5.2.10 Lynn on Bio soft package, based on NCBI accession numbers: Human, Homo sapiens (AY890602); Sheep, Ovisaries (DQ461615); Goat,

Capra hircus (GU355687); Cow, Bos Taurus (KP221576); Pig, Sus scrofa (AY536527); Dog, Canisfamiliaris (AF069071); Rat, Rattus norvegicus (U62779) and Mouse, Mus musculus (NM_008117.3)]

Traits such as growth rate, meat and milk quality, and wool quality, dictate consumer choices and preferences. The animal breeding industry has undergone tremendous transformations in recent years, with marker-assisted selection superseding traditional methods of 'visual appraisal' [9]. This study provides insight into the nature of variability of three genes across two New Zealand sheep breeds. The GH2-Z gene was highly variable, POU1F1 was slightly variable, and IGF1R displayed no variability. Further work that could be done in this area with a larger sample size, as well as performing nucleotide sequencing of the variant patterns to ascertain the precise nature of the variation. Association studies can also be carried out to test possible links between variants and phenotypic traits.

Conclusion

Several chromosomes in sheep have been identified as QTLs for production traits such as growth and carcass traits, and milk production. Present study investigated three candidate genes found within these QTLs and their variant frequencies. The number of polymorphisms detected differed across the three genes studied, with GH2-Z having the most polymorphisms and IGF1R displaying no variations. Further research needs to be conducted into validating these variations and determining their associations with various production traits.

Acknowledgement

None.

Conflict of Interest

No Conflict of Interest.

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