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An association between lifespan and variation in insulin-like growth factor I receptor in sheep[1]

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ABSTRACT: Longevity in livestock is a valuable trait. When productive animals live longer, fewer replacement animals need to be raised. However, selection for longevity is not commonly the focus of breeding programs as direct selection for long-lived breeding stock is virtually impossible until late in the reproductive life of the animal. Additionally the underlying genetic factors or genes associated with longevity are either not known, or not well understood. In humans, there is evidence that IGF 1 receptor (IGF1R) is involved in longevity. Polymorphism in the IGF1R gene has been associated with longevity in a number of species. Recently, 3 alleles of ovine *IGF1R* were identified, but no analysis of the effect of *IGF1R* variation on sheep longevity has been reported. In this study, associations between ovine *IGF1R* variation, longevity and fertility were investigated. Polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) was used to type *IGF1R* variation in 1,716 New Zealand sheep belonging to 6 breeds and 36 flocks. Ovine *IGF1R C* was associated with age when adjusting for flock (present 5.5 ± 0.2 yr, absent 5.0 ± 0.1 yr, $P = 0.02$). A general linear mixed effects model suggested an association ($P = 0.06$) between age and genotype, when correcting for flock. Pairwise comparison (least significant difference) of specific genotypes revealed the difference to be between *AA* (5.0 ± 0.1 yr) and *AC* (5.6 ± 0.2 yr, $P = 0.02$). A weak negative Pearson correlation between fertility and longevity traits was observed (r = -0.25, $P < 0.01$). The finding of an association between variation in *IGF1R* and lifespan in sheep may be useful in prolonging the lifespan of sheep.

Key words: fertility, insulin-like growth factor 1, longevity, polymerase chain reaction-single strand conformational polymorphism, sheep

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INTRODUCTION

In sheep production systems the cost of producing and maintaining replacement breeding ewes can be very high. What is more, those replacement ewes produce no output, other than wool (in wool breeds), until they reach sexual maturity, at which stage they may still be infertile. Consequently, strategies that might increase the longevity of breeding ewes may be valuable to sheep production.

In many species, the IGF1 receptor (**IGF1R**) has been implicated in regulating lifespan. Polymorphism in the IGF1R gene has been associated with longevity in species as diverse as nematodes (Braekman and Vanfleteren, 2007), fruit flies (Paaby and Schmidt, 2009), mice (Liang et al., 2003) and humans (Suh et al., 2008). A gene knockout model in mice (Holzenberger et al. 2003) revealed that heterozygous knockout mice (because null mutants are not viable) lived on average 26% longer than their wild-type littermates. Little is known about ovine IGF1R or *IGF1R* and whether it affects longevity.

The mammalian IGF is part of a complex metabolic system which mediates a wide range of biological activities including carbohydrate and lipid metabolism, cell growth, cell differentiation and aging (Richardson et al., 2004). Within the IGF system, IGF1R is responsible for initiating the IGF signaling pathway by interacting

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with insulin or IGF1. Structurally, IGF1R is composed of 2 extracellular α subunits and 2 transmembrane β subunits. The α subunits contain a cysteine-rich region that is involved in ligand binding, whereas the β subunits have a tyrosine kinase domain in their cytoplasmic region that is involved in signal transduction (Adams et al., 2000, Denley et al., 2005). Variation in the α and β subunits of IGF1R may affect its interaction with IGF1 or insulin, and result in alteration to signaling pathways.

A previous study has reported 3 allelic variants of ovine *IGF1R*. This variation occurred in a fragment of intron 2 and exon 3 (Byun et al., 2008), the latter encoding part of the α subunit of the receptor protein. In this study, associations between ovine *IGF1R* variation, longevity and fertility were investigated.

MATERIALS AND METHODS

The Lincoln University Animal Ethics Committee approved the collection of blood from animals used in this study.

Animals and Data Collection

In total, 1,716 New Zealand [Romney (n = 340, Corriedale (n = 318), Merino (n = 322), Polwarth (n = 127), Kelso (n = 173) and Coopworth (n = 436)] ewes from 36 different commercial breeding flocks were investigated. Blood samples were collected onto FTA cards (Whatman BioScience, Middlesex, UK). Sheep age was recorded in years as birth date data were not available. Fecundity records were available for many of the ewes with these data typically being obtained from Sheep Improvement Limited (**SIL**), the New Zealand national flock recoding system. Overall there were 766 young or replacement ewes (around 18 mo old) and 950 older ewes (3 to 16 yr of age). Fecundity data (number of lambs born each year) were available for 650 of the older ewes.

Genotype of Ovine IGF1R

For each sample, a single FTA card punch was placed in a 200-μL tube and the genomic DNA purified using a 2-step washing procedure as described by Zhou et al. (2006). The ovine IGF1R gene was genotyped using PCR-single strand conformational polymorphism (**PCR-SSCP**) as described previously (Byun et al., 2008).

Statistical Analyses

Data were analyzed using SPSS version 15 (SPSS Science Inc., Chicago, IL), with a significance level of $\alpha = 0.05$.

General linear mixed effects models (**GLMM**) were used to assess the effect of the presence of each of the *IGF1R* alleles on longevity and fertility, while correcting for flock (fitted as a random factor), which corrected for environmental, breed and management effects as each farm only had 1 breed. The GLMM were also used to explore the effects of the more common *IGF1R* genotypes (frequency ≥ 10%) on longevity and fertility. Where significant or if tending towards significance, these were further explored using pairwise comparisons (least significant difference).

Pearson χ^2 analysis was used to ascertain whether or not *IGF1R* genotype frequencies differed between older and younger ewes.

RESULTS

The average age of the ewes was 5.35 yr (ranged from 2 to 16 yr) and the average number of lambs born per year, per ewe, ranged from 0.71 to 3.00. The genotype frequencies for the 3 alleles of ovine *IGF1* is shown in Table 1. These frequencies were significantly different between the older ewes and the 18 mo old replacement ewes (χ^2, $P = 0.01$).

The presence/absence of *IGF1R C* was found to be associated with age (Table 2), with ewes that possessed *IGF1R C* having a mean age of 5.5 ± 0.2 yr whereas ewes that did not possess the allele having a mean age of 5.0 ± 0.1 yr ($P = 0.02$). To assess the effect of *IGF1R* genotype on lifespan, only the common genotypes *AA, AB* and *AC* were included (Table 3) as genotypes *BB* (n = 8), *BC* (n = 13) and *CC* (n = 23) were rare. The GLMM suggested an association, although not significant ($P = 0.06$), between age and genotype. A pairwise comparison revealed the difference to be between *AA* (5.0 ± 0.1 yr) and *AC* (5.6 ± 0.2 yr, $P = 0.02$).

The presence/absence of *IGF1R* variants and *IGF1R* genotype GLMM for fertility did not detect any associations (data not shown). A weak negative Pearson correlation between fertility and longevity traits was observed (r = -0.25, $P < 0.01$).

DISCUSSION

This is the first report suggesting a relationship between variation in *IGF1R* and lifespan in sheep. In this study the presence of the *C* allele of ovine *IGF1R* was associated with ewe longevity and therefore may affect the ovine IGF1 signaling pathway. Ovine *IGF1R C* has a synonymous substitution in exon 3 (Byun et al., 2008), compared with the *A* and *B* variants. Whereas this substitution does not result in an AA change, it may nevertheless be linked to other nucleotide changes in the coding regions, or to

Table 1. The *IGF1* genotype frequencies in older (3 to 16 yr) and younger (2 yr) ewe groups

	Genotype						
	AA	*AB*	*AC*	*BB*	*BC*	*CC*	All
Older	694	90	140	2	9	15	950
	73.1%	9.5%	14.7%	0.2%	0.9%	1.6%	100%
Younger	596	78	73	6	4	9	766
	77.8%	10.2%	9.5%	0.8%	0.5%	1.2%	100%
Total	1292	168	213	8	13	24	1716
Frequencies	75.2%	9.8%	12.4%	0.5%	0.8%	1.4%	100%

Table 2. Association of the ovine *IGF1* variants with ewe age in years

Variant		No.	Unadjusted model	Flock-adjusted model
A	Present	1671	5.3 ± 0.0	5.1 ± 0.1
	Absent	45	5.7 ± 0.2	5.0 ± 0.5
			($P = 0.51$)	($P = 0.82$)
B	Present	189	5.3 ± 0.2	5.6 ± 0.2
	Absent	1527	5.4 ± 0.1	5.1 ± 0.1
			($P = 0.81$)	($P = 0.90$)
C	Present	249	6.2 ± 0.2	5.5 ± 0.2
	Absent	1467	5.2 ± 0.1	5.0 ± 0.1
			($P < 0.01$)	($P = 0.02$)

sequence variation elsewhere in the gene. This may affect gene expression or the function or both of IGF1R and hence affect sheep longevity.

The finding of an association between variation in *IGF1R* and lifespan in sheep is consistent with reports in other species. Insulin-like growth factor1 has been implicated in attenuation of longevity from lower animals to mammals (Sepp-Lorenzino, 1998; Babrieri et al. 2003; Richardson et al., 2004;). Genetic mutant (Liang et al., 2003) and caloric restricted (Masoro, 2005) rodent models have revealed that a reduction in insulin/IGF1 signaling and/or a reduction in plasma concentrations of insulin/IGF1 appears to be correlated with increased longevity and extended aging.

The IGF1R is a key regulator of IGF 1 signaling and is activated by the binding of either IGF1 or insulin (Adams et al., 2000). Accordingly mutations in *IGF1R* may alter the function of the receptor and result in variation in IGF1R signaling, thereby increasing lifespan. This is supported by a rodent model in which heterozygous *IGF1R* knockout mice (null mutants are not viable) live on average 26% longer than their wildtype litter mates (Holzenberger et al. 2003). Furthermore, in humans, certain *IGF1R* variants have been associated with altered IGF1 signaling and increased lifespan (Bonafè et al., 2003; Suh et al., 2008).

It is interesting to speculate why the variation reported here in *IGF1R* occurs or persists or both in the population of sheep studied. These sheep were selected for analysis and hence a population-based analysis of Hardy-Weinberg equilibrium would be inappropriate. However, the results suggest that despite *IGF1R C* being more commonly found in older ewes, the *IGF1R A* variant is proportionally more common in the younger ewe population. The *A* variant is also the most common variant in the ewes studied, suggesting that *A* confers some advantage for ewes, especially younger ewes.

Table 3. Association of the ovine *IGF1* genotype with ewe age in years

Genotype	No.	Unadjusted model	Flock adjusted model
AA	1292	5.2 ± 0.1[a]	5.0 ± 0.1[a]
AB	168	5.3 ± 0.3[a]	5.1 ± 0.3[ab]
AC	212	6.3 ± 0.2[b]	5.6 ± 0.2[b]
		($P < 0.01$)	($P = 0.06$)

[a,b] Different superscripts indicate the pair of means was significantly different ($P < 0.05$) in pairwise comparisons (least significant difference)

The knockout mouse models suggest why this may be, as heterozygous knockout mice were typically slower growing that their non-transgenic litter-mates (Holzenberger et al. 2003). It could be speculated then that the *C* variant is associated with slower growth rates in sheep, which would be an anathema to sheep production, and the *IGF1R A* variant is associated with faster growth rates, although this would require further study.

The lifespan of a domestic ewe reflects a number of factors including health, reproductive performance and their productivity. If any of these factors is compromised then the ewe is likely to be culled, thus having an artificially shorter lifespan. According to the 'trade-off' theory, enhanced longevity is achieved at the expense of reduced fertility (Holliday, 2006). Hence any gene impacting on longevity may negatively impact on reproductive performance. However, longevity in livestock also reflects the ability of animal to maintain health and performance, and which would also impact on increasing a number of offspring during their life time. Thus any gene associated with lifespan in female breeding stock would not be expected to be associated with a large cost to fertility.

In this study a weak negative correlation was observed between longevity and fertility. Interestingly, and in contrast to the findings reported here, the heterozygous *IGF1R* knockout mice had normal energy metabolism, nutrient uptake, activity and fertility (Holzenberger et al. 2003).

As older ewes are culled and require replacement, some or all new-born female lambs are kept until they reach reproductive maturity. During this time, replacement ewes produce no output, other than wool (in wool breeds), and once sexual maturity is achieved they may be infertile. Thus the cost of producing and maintaining replacement breeding ewes can be very high, and maintenance of older ewes beneficial. Exploiting the

variation in the *IGF1R* as a gene marker for longevity may be valuable to sheep production.

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