BRIEF COMMUNICATION: Association of variation in the ovine ADRB3 gene with weaning weight and post-weaning growth in New Zealand Suffolk sheep

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Introduction

β_3-adrenergic receptors (ADRB3s) are mediators of the lipolytic and thermogenic effects of catecholamine levels in brown and white adipose tissue. Studies have linked variation within the β_3-adrenergic receptor gene (*ADRB3*) to metabolic disease and obesity in humans (NCBI OMIM No. 109691), variation in loin eye muscle area in pigs (Hirose et al. 2009) and with dairy form in cattle (Connor et al. 2006).

In sheep, variation in *ADRB3* has been reported (Byun et al. 2008; Forrest et al. 2003; Yang et al. 2009) as well as associations of this variation with birth weight, weaning weight, pre-weaning growth rate, carcass traits and cold survival (Forrest et al. 2003, 2007; Horrell et al. 2009). Recently we reported variation in the 3'UTR of *ADRB3* in New Zealand Merino sheep and four Chinese breeds (Yang et al. 2011). Variation in the 3'UTR could cause variation in gene expression and thus function, as has been reported for other genes including *ADRB2* (Subramaniam et al. 2004).

In this study, we investigated the association of weaning weight and post-weaning growth rate with the intron variants and 3'UTR variants of the *ADRB3* in New Zealand Suffolk sheep.

Materials and methods

Eight hundred and eight Suffolk lambs from thirty six independent sires and eight studs within New Zealand were investigated. Lambs live weights were recorded at weaning and at ten months of age. The gender and birth rank of the lambs were recorded at birth. The post-weaning growth rate of the lambs was calculated as an average daily weight gain (g/day) from weaning to ten months. Blood was collected from lambs onto FTA® cards (Whatman®, Pittsburgh, Pennsylvania, USA). Sheep DNA was purified from the blood using a two-step procedure (Zhou et al. 2006).

Multiplex PCR amplification of both the intron and 3'UTR fragments was carried out in a single 15 µL reaction containing the genomic DNA on a 1.2 mm diameter punch of FTA paper, 0.5 µM of intron (Byun et al. 2008) and 3'UTR (Yang et al. 2011) primer pairs for ovine *ADRB3*, 150 µM of each dNTP (Eppendorf, Hamburg, Germany), 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× reaction buffer supplied with the polymerase, containing 1.5 mM $MgCl_2$. After initial denaturation at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 30 seconds were utilized, followed by a final five minute extension at 72°C.

A 0.7 µL aliquot of the PCR product was mixed with 7 µL of loading dye (98% formamide, 10 mm EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 94°C for five minutes, samples were rapidly cooled on wet ice and then loaded on 16 × 18 cm, 12% acrylamide / bisacrylamide (37.5:1) gels (Bio-Rad, Hercules, California, USA). Electrophoresis was performed using Protean II xi cells (Bio-Rad), at 290 V for 18 hours at 25°C in 0.5 × TBE buffer. Gels were silver-stained according to the method of Byun et al. (2009). Amplicons representative of the previously reported intron variants (Variants *A–M*; Forrest et al. 2003;Yang et al. 2009) and 3'UTR variants (Variants *a–f*; Yang et al. 2011) of ovine *ADRB3* were also included in each polyacrylamide gel and their banding patterns were used as standards for determining the genotypes in individual sheep.

All statistical analyses were performed using SPSS (Version 19. SPSS Science Inc., Chicago, Illinois, USA). General linear mixed-effects models were used to assess the effect of the presence/absence of a particular intron or 3'UTR sequence variant on weaning weight and post-weaning growth rate. Sire was fitted as a random factor in the models, and birth rank and gender were fitted as fixed factors. Sire was included in order to correct for management and individual environmental effects, as no sire was used on more than one property.

Single-variant models were performed first to ascertain which variants should be included in subsequent multi-variant models. The multi-variant models included any variant that had associations with weaning weight and post-weaning growth rate in the single-variant models with a P value of less than 0.2 ($P < 0.20$) and which could thus potentially impact on the weaning weight or growth rate. This statistical approach has been used previously by Forrest et al. (2009).

Table 1 Association between *ADRB3* intron and 3'UTR variants and weaning weight in New Zealand Suffolk sheep. P values in bold indicate significance at P <0.05. P values in italics indicate approaching significance between P = 0.05 and P = 0.10.

Model	Variant	Variant absent		Variant present		P value
		Number of lambs	Weaning weight (kg)	Number of lambs	Weaning weight (kg)	
Intron: Single-variant model	*A*	354	33.3 ± 1.2	454	31.5 ± 1.2	**<0.001**
	B	435	32.6 ± 1.2	373	32.1 ± 1.2	0.22
	C	594	32.0 ± 1.2	214	33.2 ± 1.2	**0.02**
	E	532	32.2 ± 1.2	276	32.8 ± 1.2	0.27
	F	668	32.1 ± 1.2	140	33.2 ± 1.2	*0.07*
Multi-variant model (A,C and F fitted)	*A*	354	33.5 ± 1.2	454	32.0 ± 1.2	**0.009**
	C	594	32.3 ± 1.2	214	33.2 ± 1.2	*0.08*
	F	668	32.4 ± 1.2	140	33.2 ± 1.3	*0.18*
3'UTR: Single-variant model	*a*	671	32.2 ± 1.2	137	33.3 ± 1.3	*0.09*
	b	373	33.2 ± 1.2	435	31.5 ± 1.2	**0.001**
	c	425	32.6 ± 1.2	383	32.1 ± 1.2	0.28
	e	362	31.9 ± 1.2	446	32.7 ± 1.2	*0.09*
Multi-variant model (a, b and e fitted)	*a*	671	32.1 ± 1.2	137	33.0 ± 1.3	0.21
	b	373	33.3 ± 1.2	435	31.8 ±1.2	**0.01**
	e	362	32.3 ± 1.2	446	32.8 ± 1.2	0.34

Table 2 Association between *ADRB3* intron and 3'UTR variants and post-weaning growth rate in New Zealand Suffolk sheep. P values in bold indicate significance at P <0.05. P values in italics indicate approaching significance between P = 0.05 and P = 0.10.

Model	Variant	Variant absent		Variant present		P value
		Number of lambs	Post-weaning growth rate (g/d)	Number of lambs	Post-weaning growth rate (g/d)	
Intron: Single-variant model	*A*	354	175 ± 12	454	172 ± 12	0.58
	B	435	179 ± 11	373	165 ± 12	**0.002**
	C	594	169 ± 11	214	185 ± 12	**0.002**
	E	532	172 ± 11	276	177 ± 12	0.44
	F	668	172 ± 11	140	178 ± 12	0.35
Multi-variant model (A,C and F fitted)	*B*	435	181 ± 11	373	170 ± 12	**0.02**
	C	594	169 ± 11	214	181 ± 12	**0.02**
3'UTR: Single-variant model	*a*	671	172 ± 11	137	186 ± 12	**0.02**
	b	373	176 ± 12	435	173 ± 12	0.50
	c	425	180 ± 11	383	167 ± 12	**0.005**
	e	362	172 ± 12	446	176 ± 11	0.36
Multi-variant model (a, b and e fitted)	*a*	671	171 ± 11	137	183 ± 12	*0.05*
	c	425	183 ± 11	383	171 ± 12	**0.01**

Results and discussion

Five intron variant sequences (*A*, *B*, *C*, *E* and *F*; GenBank accession numbers AF314200–AF314202, AF314204 and AF314205, respectively) and six 3'UTR variant sequences (*a*, *b*, *cd*, *e* and *f*; GenBank accession numbers HM776668–HM776673, respectively) (Yang et al. 2009, Yang et al. 2011) were identified. This was found to be associated with variation in weaning weight and post-weaning growth (Table 1 and Table 2).

In the single- and multi-variant models, the presence of *A* was associated with a decrease in mean weaning weight. Variant *C* was associated with an increase in mean weaning weight in the single-variant model, but this effect did not persist in the multi-variant model suggesting the effect was not an independent effect. These results appear to contrast two prior studies (Forrest et al. 2003; Horrell et al. 2009) where progeny inheriting *A* had higher pre-weaning growth rates than littermates inheriting *C*. Variant *b* was associated with a decrease in mean

weaning weight in the single- and multi-variant models, suggesting this is an independent allele effect.

In the single- and multi-variant models, the presence of *B* or *C* was associated with variation in post-weaning growth rate, with *B* being independently associated with a decrease in post-weaning growth and *C* being independently associated with an increase in post-weaning growth rates. Variant *c* was associated with a decrease in post-weaning growth rate in both the single- and multi-variant models, while the effect seen with *a* in the single-variant model only persisted as a trend in the multi-variant model.

The frequency of *A* (33.1%) was lower in these Suffolk lambs, when compared to 73.9% in the New Zealand Romney (Horrell et al. 2009) and 60% in the New Zealand Merino-cross sheep (Byun et al. 2008). Forrest et al. (2009) speculated that *A* probably represented more than one extended haplotype and this was confirmed by Yang et al.(2011) who described three intron-3'UTR haplotypes (*A-a*, *A-b* and *A-c*) of *A,* and two intron-3'UTR haplotypes (*C-a* and *C-e*) of *C* in New Zealand Merino sheep. It is therefore conceivable that the association previously reported with the New Zealand Merino and New Zealand Romney sheep is the result of a different intron-3'UTR haplotype than found here and/or the effect is moderated by some other genetic influence.

If the associations observed with the separate regions of *ADRB3* are taken together, then the results might suggest that New Zealand Suffolk sheep with an *A-b* haplotype have a reduced weaning weight and those with haplotype *B-c* have lower post-weaning growth rates, but this would need confirmation by haplotyping the lambs. Yang et al. (2011) have confirmed the presence of *A-b* and *B-c* haplotypes, but only in New Zealand Merino sheep and four Chinese sheep breeds.

Surprisingly, in a breed that is used as a terminal sire, *A*, which is associated with reduced weaning weight, is the most common variant in these stud Suffolk sheep; *A* (33.1%), *B* (25.5%), *C* (15.7%), *E* (14.9%) and *F* (10.8%). However, Forrest et al. 2007 described how *A* is associated with increased peri-natal survival in a large study of common New Zealand sheep breeds, including the Suffolk, while *C* is associated with reduced peri-natal survival. This may explain the variant frequencies observed in this study.

This study further supports the contention that *ADRB3* has a role in growth regulation that, if confirmed, may be of economic importance to sheep farmers.

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