Association of the KRT33A (formerly KRT1.2) gene with live-weight and wool characteristics in yearling Perendale sheep

RMW Sumner[ab]*, RHJ Forrest[c], H Zhou[d], HV Henderson[a] and JGH Hickford[d]

[a]AgResearch Ruakura, Private Bag 3123, Hamilton 3240, New Zealand; [b]Retired; [c]Faculty of Sport and Health Sciences, Eastern Institute of Technology, Private Bag 1201, Napier 4142, New Zealand; [d]Faculty of Agriculture and Life Sciences, PO Box 85084, Lincoln University, Canterbury 7647, New Zealand
**Corresponding author. Email: roland.sumner@xtra.co.nz*

Abstract

DNA samples from 691 mixed-sex Perendale sheep, born between 1998 and 2001, were typed for variation in the KRT33A gene using polymerase chain reaction - single-strand conformational polymorphism. The sheep were the progeny of two lines selected for either decreased (L), or increased (H) core bulk at yearling shearing, crosses between the lines and back-crosses. Five variants (*A–E*) of *KRT33A* were identified with a frequency of 0.07, 0.04, 0.07, 0.35 and 0.47, respectively. The proportion of *A* decreased and *B* increased, with a progressive increase in crimp frequency, fibre curvature and core bulk and a progressive decrease in fleece weight, staple length and washing yield with each step-wise increase in the proportion of genes derived from the H line. An effect of the presence of *A* on the measured wool characteristics was found, but was less than the effect with direct selection for core bulk. There was no consistent effect on body growth. While none of the variants of *KRT33A* were strongly predictive of variation in core bulk, other genes on Chromosome 11 may be implicated in the differential expression of characteristics associated with core bulk.

Keywords: Perendale; sheep; *KRT33A*; live-weight; wool characteristics

Introduction

Perendale sheep produce wool in the 31 to 37 micron range (Wools of New Zealand 1994) and with a wide range in compressional properties, which makes the wool suitable for use in carpets (Carnaby & Elliott 1980; Sumner et al. 1991). Approximately 85% of the variation in the compressional property, referred to as bulk, can be accounted for by variation in mean fibre curvature and mean fibre diameter (Sumner & Upsdell 2001), with bulk being related to the proportion of ortho- and para-cortical cell types within the individual fibres (Sumner et al. 1993).

Proteins are major components of wool and bestow structural and mechanical properties on individual fibres. These wool proteins can be divided into two types: keratin intermediate-filament proteins (KRTs) and keratin-associated proteins (KAPs). KRTs form the skeletal structure (microfibrils) of the wool fibre and are embedded in a matrix of KAPs (Powell 1997).

Studies have described variation within both the KAP and KRT genes (*KRTAP*s and *KRT*s respectively) with both affecting wool characteristics. For example, Parsons et al. (1994) and Beh et al. (2001) reported associations between variation in mean fibre diameter and KAPs in Merino sheep, while Rogers et al. (1994) reported an association between staple strength in Romney sheep and Ovine Chromosome 11 in the region spanning *KAP1.1*, *KAP1.3* and *KRT1.2*. The latter gene was subsequently called *KRT33* when Itenge et al. (2010) reported associations between five variants of the gene in Merino sheep and variation in staple length, fibre diameter and staple length. Two KRT33 genes, *KRT33A* and *KRT33B*, have now been identified in sheep (Yu et al. 2011).

Wool bulk (cm^3/g), measured as the bulk of a subsample drawn by a core-bore tube and referred to as core bulk, and its associated fibre characteristics, are highly heritable and respond to phenotypic selection (Sumner et al. 2007). With the availability of tissue samples collected from the flock used to derive those heritability estimates, an investigation was undertaken to ascertain whether variation in *KRT33A* (GenBank accession number HQ283079) was associated with variation in any of the measured wool characteristics and to further explore the role of *KRT33A* in controlling wool fibre structure.

Materials and methods

Sheep

Yearling sheep used in this study were part of a pedigree recorded flock of Perendale breeding ewes managed to study the inheritance of core bulk (Sumner et al. 2007). Foundation sheep in the flock were obtained from throughout the North Island. In 1987 sheep with a "low" core bulk measurement were allocated to a line to be subsequently selected for decreased core bulk (Low bulk – L), while sheep with a "high" core bulk measurement were allocated to a line to be subsequently selected for increased wool bulk (High bulk – H). The flock was closed in 1988.

Beginning in 1996, a portion of the ewes in each of the selection-lines were crossed with rams from the other line to create cross-line progeny. All the cross-line males were culled after being shorn as yearlings. Beginning in 1998, half the cross-line ewe progeny were back-crossed to the parent lines with

Table 1 Numbers of each genotype at the *KRT33A* locus for the 691 sheep that were DNA typed. Genotype is indicated by listing the first and second variants in alphabetical order. Homozygous individuals are shown on the diagonal in bold with heterozygous individuals in normal type.

Second variant	First variant				
	A	*B*	*C*	*D*	*E*
A	**2**	4	4	38	41
B		**0**	5	31	19
C			**3**	34	42
D				**72**	239
E					**157**

none of the progeny being retained after being shorn as yearlings (Sumner et al. 2007). The genetic background of each individual sheep shorn as a yearling was classified in terms of a pedigree-code indicating the line from which each grand-parent was sourced. LLLL and HHHH were individuals derived solely from either of the two parent lines, LLHH were cross-line individuals derived from crossing the two parent lines and LLLH and LHHH were back-crossed individuals derived from crossing cross-line ewes with rams from one of the parent lines. The sequence of letters in the pedigree-code took no account of whether the genetic contribution from each grandparent was derived from either the paternal or maternal side of the pedigree.

Between 1997 and 2004 the experimental flock was grazed at the Winchmore Research Station near Ashburton. The five pedigree-code groups of yearlings within gender, were managed as a single group, as were the breeding ewes, except over mating and lambing when the breeding ewes were separated into their pedigree-code groups. Breeding ewes were single-sire joined. The ewes within each selection-line were re-randomised to mating groups annually, avoiding half-sib or dam-offspring matings, and then lambed in groups based on the pedigree-code of the dam.

Measurements

Lambs were tagged within 24 hours of birth and their weight, gender and dam recorded. The weight of all lambs was also recorded at weaning when approximately 12 weeks of age. Lambs were shorn in mid-February to reduce the impact of a range of birth dates on yearling fleece weight. Subsequently in mid-September all yearlings were weighed and shorn, and the respective weights recorded. A mid-side fleece sample was collected. In 1998 to 2001 inclusive, a small notch of tissue was taken at yearling shearing from the tip of an ear for DNA extraction.

The mid-side fleece sample was measured for staple length, total number of crimps along the staple, washing yield at 16% regain, mean fibre diameter, fibre diameter variation and mean fibre curvature using an OFDA100 instrument (BSC, Ardross, Western Australia, Australia), and core bulk using an automatic core bulk tester (WRONZ, Lincoln, New Zealand). Production data are reported for the lambs born in 1998 to 2001 inclusive. Crimp frequency was calculated as the total number of crimps along the staple divided by the staple length.

The DNA sample was used to identify variation in *KRT33A*. DNA typing was undertaken by polymerase chain reaction (PCR) – single-strand conformational polymorphism (SSCP) analysis of the

Table 2 Frequency of each variant of the KRT33A gene (*A–E*), expressed as a proportion, within each pedigree-code group where the pedigree-code indicates the selection line(s) (L or H) from which each of the individual's four grandparents were sourced. The slope and its standard error, on the logit of the proportion scale across the pedigree-code groups, were calculated by logistic regression after the pedigree-code was expressed as the proportion of "H" in the code on a 0.0 to 1.0 scale. P values in bold text indicate significance at $P < 0.05$ and P values in italic text indicates significance between $P = 0.05$ and $P = 0.10$ (trends).

Pedigree-code	Number of sheep DNA typed	*KRT33A* variant					Total
		A	*B*	*C*	*D*	*E*	
LLLL	62	0.14	0.02	0.06	0.43	0.35	1.00
LLLH	195	0.09	0.04	0.05	0.34	0.48	1.00
LLHH	151	0.06	0.04	0.06	0.34	0.50	1.00
LHHH	197	0.04	0.05	0.07	0.37	0.47	1.00
HHHH	86	0.03	0.06	0.09	0.30	0.52	1.00
All pedigree groups	691	0.07	0.04	0.07	0.35	0.47	1.00
Slope across pedigree-codes ± standard error		-1.8 ± 0.4	1.0 ± 0.5	0.6 ± 0.4	-0.3 ± 0.2	0.4 ± 0.2	
P value		**<0.001**	**0.04**	*0.08*	0.17	*0.05*	

Table 3 Predicted mean values for the measured characteristics of the 691 yearling Perendale sheep with identified variants of the KRT33A gene sorted according to their pedigree-code group, minimum and maximum standard error of difference (SED) between the mean values, significance (P value) of the difference among pedigree-codes, slope across pedigree groups and significance (P value) of the slope. Pedigree-code indicates the selection line(s) (L or H) from which each of the four grandparents were sourced. Estimates derived from a mixed model with year born, gender, rearing status and age of dam as random effects in a group of four crops of mixed gender yearlings. P values in bold text indicate significance at P <0.05.

	Characteristic											
Pedigree score	Number of samples	Birth weight (kg)	Wean weight (kg)	Yearling live- weight (kg)	Yearling fleece weight (kg)	Staple length (mm)	Crimp frequency (Crimps/cm)	Washing yield (%)	Mean fibre diameter (μm)	Fibre diameter variation (μm)	Mean fibre curvature (°/mm)	Core bulk (cm^3/g)
LLLL	62	3.75	18.6	37.4	1.90	94	1.50	81.4	29.2	7.3	52.1	22.5
LLLH	195	3.88	19.7	37.5	1.87	88	1.73	80.5	28.9	7.2	57.5	23.7
LLHH	151	3.80	19.6	38.0	1.85	82	1.92	79.6	29.5	7.1	63.8	25.8
LHHH	197	3.89	20.0	37.7	1.79	74	2.33	76.8	30.0	7.2	72.2	28.4
HHHH	86	3.89	19.0	36.6	1.66	67	2.59	75.7	30.1	7.4	78.7	30.1
SED Minimum		0.08	0.3	0.5	0.03	1	0.05	0.4	0.3	0.1	1.0	0.2
Maximum		0.13	0.5	0.8	0.06	2	0.08	0.6	0.4	0.2	1.7	0.4
P value		0.64	**0.03**	0.34	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.31	**<0.001**	**<0.001**
Slope across pedigree-codes ± standard error		0.1 ± 0.1	0.3 ± 0.4	-0.4 ± 0.6	-0.21 ± 0.04	-28 ± 2	1.15 ± 0.06	-6.4 ± 0.5	1.4 ± 0.3	0.1 ± 0.1	28 ± 1	8.3 ± 0.3
P value		0.36	0.42	0.52	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.50	**<0.001**	**<0.001**

Table 4 Predicted mean values for the measured characteristics of the 691 yearling Perendale sheep with identified variants of the KRT33A gene, sorted according to either the absence or presence (one copy – heterozygous, two copies – homozygous) of each variant, minimum and maximum standard error of difference (SED) between the mean values, significance (P value) of the difference among presence codes, slope across presence codes ± standard error (Slope ± SE) and significance (P value) of the slope. Estimates derived from a mixed model with year born, gender, rearing status and age of dam as random effects in a group of four crops of mixed gender yearlings. P values in bold text indicates significance at $P < 0.05$ and italic text indicates significance between $P = 0.05$ and $P = 0.10$ (trends).

		Characteristic										
Variant	Number identified	Birth weight (kg)	Wean weight (kg)	Yearling live weight (kg)	Yearling fleece weight (kg)	Staple length (mm)	Crimp frequency (Crimps/cm)	Washing yield (%)	Mean fibre diameter (µm)	Fibre diameter variation (µm)	Mean fibre curvature (°/mm)	Core bulk (cm^3/g)
A	602	3.58	18.9	36.8	1.81	80	2.05	78.6	29.6	7.2	66.6	26.6
Absent												
One copy	87	3.57	19.0	38.0	1.81	85	1.83	79.6	29.4	7.1	62.0	25.0
Two copies	2	3.26	19.8	40.1	2.18	99	1.57	79.8	30.0	6.4	53.4	23.8
SED		0.01	0.4	0.6	0.04	2	0.07	0.5	0.3	0.1	1.5	0.4
Minimum												
Maximum												
P value		0.60	2.3	3.5	0.25	11	0.43	3.0	1.9	0.6	9.5	2.3
Slope ± SE		0.87	0.89	*0.07*	0.32	**0.006**	**0.003**	0.13	0.73	0.16	**0.006**	**<0.001**
P value		-0.02 ± 0.09	0.2 ± 0.4	1.2 ± 0.5	0.01 ± 0.04	5 ± 2	-0.23 ± 0.07	0.9 ± 0.5	-0.2 ± 0.3	-0.2 ± 0.1	-4.7 ± 1.5	-1.6 ± 0.4
B	632	0.82	0.69	**0.02**	0.76	**0.002**	**<0.001**	**0.04**	0.50	*0.08*	**0.001**	**<0.001**
Absent		3.58	18.9	37.0	1.82	81	2.01	78.8	29.6	7.2	65.8	26.3
One copy	59	3.58	18.8	35.8	1.76	79	2.10	77.6	29.9	7.1	68.1	27.0
Two copies	0											
SED		0.11	0.4	0.7	0.05	2	0.08	0.6	0.4	0.1	1.8	0.4
P value		0.94	0.77	*0.05*	0.23	0.36	0.30	**0.03**	0.39	0.42	0.20	0.14
Slope ± SE		0.1 ± 0.1	-0.1 ± 0.4	-1.3 ± 0.6	-0.06 ± 0.05	-1 ± 2	0.08 ± 0.08	-1.2 ± 0.7	0.3 ± 0.4	-0.1 ± 0.1	2.3 ± 1.8	0.7 ± 0.5
P value		0.94	0.77	*0.05*	0.23	0.36	0.30	**0.03**	0.39	0.42	0.20	0.14
C	603	3.54	18.8	37.1	1.82	81	2.01	78.7	29.7	7.2	65.6	26.2
Absent												
One copy	85	3.73	19.6	36.6	1.77	77	2.09	77.8	29.5	7.2	68.5	27.1
Two copies	3	2.61	16.4	31.8	1.76	75	1.92	80.0	27.2	7.1	66.9	25.4
SED		0.10	0.4	0.6	0.04	2	0.07	0.5	0.3	0.1	1.6	0.4
Minimum												
Maximum												
P value		0.49	1.9	2.8	0.21	9	0.35	2.5	1.5	0.5	7.8	1.9
Slope ± SE		**0.02**	*0.06*	0.13	0.51	*0.07*	0.48	*0.09*	0.25	0.92	0.18	*0.06*
P value		0.1 ± 0.1	0.5 ± 0.4	-0.7 ± 0.5	-0.04 ± 0.04	-3 ± 2	0.07 ± 0.07	-0.8 ± 0.5	-0.3 ± 0.3	0.1 ± 0.1	2.6 ±1.5	0.7 ± 0.4
D		0.27	0.15	0.17	0.25	**0.02**	0.32	*0.07*	0.29	0.88	*0.08*	*0.05*
Absent	277	3.56	18.9	36.9	1.80	81	2.01	78.6	29.8	7.3	65.8	26.4
One copy	342	3.59	19.0	37.0	1.81	80	2.02	78.8	29.4	7.2	65.9	26.2
Two copies	72	3.63	18.7	37.4	1.88	82	2.09	78.6	29.4	7.2	66.6	26.7
SED		0.07	0.3	0.4	0.03	1	0.05	0.4	0.2	0.1	1.1	0.3
Minimum												
Maximum												
P value		0.11	0.4	0.6	0.05	2	0.08	0.6	0.3	0.1	1.8	0.4
Slope ± SE		0.79	0.81	0.76	0.18	0.69	0.60	0.81	0.15	0.64	0.89	0.59
P value		0.03 ± 0.05	-0.1 ± 0.1	0.2 ± 0.3	0.03 ± 0.02	0.2 ± 0.9	0.03 ± 0.04	0.1 ± 0.3	-0.2 ± 0.2	-0.05 ± 0.05	0.3 ± 0.8	0.1 ± 0.2
E		0.50	0.72	0.56	0.13	0.80	0.40	0.74	*0.07*	0.35	0.67	0.84
Absent	193	3.62	19.1	37.2	1.82	81	2.04	78.6	29.4	7.1	66.3	26.5
One copy	341	3.58	18.9	36.9	1.81	80	2.00	78.8	29.6	7.3	65.7	26.1
Two copies	157	3.52	18.9	37.1	1.81	81	2.04	78.8	30.0	7.3	66.1	26.6
SED		0.08	0.3	0.4	0.03	1	0.05	0.4	0.2	0.1	1.2	0.3
Minimum												
Maximum												
P value		0.09	0.4	0.5	0.04	2	0.06	0.5	0.3	0.1	1.4	0.4
Slope ± SE		0.56	0.78	0.74	0.82	0.78	0.69	0.88	*0.09*	**0.05**	0.85	0.23
P value		-0.05 ± 0.05	-0.1 ± 0.2	-0.1 ± 0.3	-0.01 ± 0.02	-0.2 ± 08	0.01 ± 0.03	0.1 ± 0.2	0.3 ± 0.1	0.09 ± 0.05	-0.1 ± 0.7	0.1 ± 0.1
		0.29	0.66	0.84	0.62	0.81	0.98	0.67	**0.03**	**0.05**	0.84	0.74
P value for adding genotype after additive alleles		0.30	0.30	0.15	0.85	0.62	0.77	0.20	0.96	**0.05**	0.44	0.50

5' un-translated and first coding exon of *KRT33A* as described by Itenge-Mweza et al. (2007). Standards for the five variants of the gene, *A–E* (GenBank accession numbers AY835598 through AY835602) were included on each gel.

Statistical analysis

The proportion of each variant of *KRT33A* within each pedigree-code group was analysed by logistic regression on the proportion of genes derived from the H line. Production data were analysed fitting a linear mixed model by residual maximum likelihood (REML) in GenStat (Payne et al. 2009) to assess either the effect of the proportion of genes derived from the H line, or the number of each variant present and the possible combinations of each of the five variants of *KRT33A*. As there were more than two levels in each analysis the slope across the level values was fitted to ascertain if there was a progressive trend across the set of factor groups. Year born, gender, rearing-rank and age of the dam for each lamb were fitted as random factors.

Results

A total of 789 yearlings were tissue sampled with 691 samples able to be DNA typed for *KRT33A*. All five variants of the gene, *KRT33A A–KRT33A E* corresponding to GenBank accession numbers AY835598 to AY835602 respectively, were identified. Numbers of animals within each "genotype" group are shown in Table 1. A total of 234 (34%) were classified as homozygous and 457 (66%) as heterozygous at the *KRT33A* locus.

The proportions of each variant present and the number of sheep DNA typed within each pedigree-code group and across all pedigree groups, are shown in Table 2. There was a decrease in the frequency of *A* and an increase in the frequency of *B* with an increasing proportion of H in the pedigree-code. Trends for the frequency of both *C* and *E* to increase with an increasing proportion of H in the pedigree code approached significance, while the frequency of *D* was unrelated to the proportion of H in the pedigree-code.

The previously applied phenotypic selection-pressure for decreased or increased core bulk in the L and H lines respectively, resulted in significant changes among the predicted means of many of the measured phenotypic traits within each pedigree-code group (Table 3).

Predicted means of each of the measured phenotypic characteristics for individuals where each of the five identified variants of *KRT33A* were either absent, or present as one (heterozygous), or two (homozygous) copies of the variant, are given in Table 4.

Slopes for each characteristic across the number of copies of each variant present were fitted separately for each variant present to indicate the consistency of the observed responses, with each step-wise increase of each variant present in the genotype. There were no individuals with two copies of *B* and few individuals with two copies of *A* and *C*. Notwithstanding, the presence of *A* was associated with a longer staple length, a lower crimp frequency, lower mean fibre curvature and lower core bulk with a significant slope effect as the number of copies of the variant increased. The presence of *B* was associated with a lower washing yield and the presence of *E* with an increase in mean fibre diameter and an associated increase in fibre diameter variation.

In an additive allele model the only significant improvement from adding genotype was for fibre diameter variation (P = 0.05), although as one of 13 variables this could be significant by chance alone when we expect one in 20 (Table 5). The effect of possible interactions between the presence of individual variants in the genotype was examined by using a genotype model to compare predicted means among the observed genotypes associated with the five variants of *KRT33A*. Core bulk was the only characteristic where there was a significant difference (P = 0.004) among the genotypes. The basis for the significance of this effect for core bulk is shown in Table 5 as the difference between each genotype and the associated homozygote within each 'variant by genotype' subgroup, along with the standard error of the difference and the associated P value. There was a difference between *DD* and *DA* (same as *AD*) and between *EE* and *EA* (same as *AE*). The small group sizes and associated large standard-errors of difference for combinations of *A*, with *B* and *C*, limit the confidence that can be placed on the observed trends. With the predicted means for core bulk in *AD* and *AE* individuals being significantly lower than the equivalent homozygote, it may be suggested that the low values associated with the A variant in the groups with few individuals may be a real effect. None of the other identified heterozygotes associated with *C*, *D* and *E* were significantly different from the associated homozygote.

Discussion

Care is needed in interpreting results from closed flocks on account of possible founder effects present within the individuals selected to establish the flock as well as subsequent drift. In the case of the flock used in this study, it had been maintained for 16 years prior to being closed. While it was an "open" flock a new group of five rams were joined with all the ewes each year in a mass-mating scenario. The five new rams introduced each year were sourced from, and chosen by, five different breeders in the North Island of New Zealand. Each ram was used for one year. During this phase of the trial there was thus a wide genetic sampling of Perendale sheep available throughout the North Island. With many of the North Island breeders regularly exchanging rams with South Island breeders the generated flock thus

represented a fair sample of the national Perendale flock.

Trends for each of the measured phenotypic characteristics across the pedigree-code groups for individuals born between 1998 and 2001 (Table 3), where all pedigree-codes were represented in each year, were similar in direction to the trends reported as average breeding values over the period 1982 to 2002 by Sumner et al. (2007). Namely, an increasing proportion of genes from the H line resulted in a marked increase in core bulk with a concomitant increase in crimp frequency, mean fibre diameter and mean fibre curvature. The effect on mean fibre diameter was of limited practical significance. These effects were coupled with a decrease in yearling fleece-weight, staple length and washing yield. All the effects are biologically interrelated and associated with cell differentiation at the base of each growing wool fibre (Stobart & Sumner 1991).

All the variants of *KRT33A* previously reported in a sample of Merino sheep (Itenge-Mweza et al. 2007) were found in this sample of Perendale sheep, with a frequency of variants similar to that in an "All Breeds" reference flock reported by McKenzie et al. (2010).

Table 5 Number of individuals within the 691 yearlings DNA typed in each genotype group for the five identified variants of the KRT33A gene (*A–E*), predicted mean for core bulk of each genotype group, mean difference between each genotype group and each of the associated homozygotes, standard error of the difference and associated significance (P value). No *BB* individuals were identified. Homozygotes within each set of genotypes are indicated in bold italic. P values in bold text indicate significance at P <0.05.

Genotype	Number identified	Core bulk (cm^3/g)	Difference from associated homozygote	Standard error of difference	P value
AA	***2***	***23.4***	0		
AB	4	25.4	2.0	2.8	0.48
AC	4	26.3	2.8	2.8	0.32
AD	38	24.4	1.0	2.4	0.68
AE	41	24.9	1.5	2.4	0.53
BA	4	25.4	-		
BB	***0***	-			
BC	5	26.0	-		
BD	31	27.4	-		
BE	19	25.9	-		
CA	4	26.3	1.1	2.5	0.65
CB	5	26.0	0.8	2.4	0.72
CC	***3***	***25.1***	0		
CD	34	27.2	2.0	2.0	0.31
CE	42	26.8	1.7	2.0	0.39
DA	38	24.4	-2.0	0.7	**0.002**
DB	31	27.4	1.0	0.7	0.15
DC	34	27.2	0.7	0.7	0.30
DD	***72***	***26.4***	0		
DE	239	25.9	-0.5	0.4	0.22
EA	41	24.9	-1.5	0.6	**0.01**
EB	19	25.9	-0.5	0.8	0.52
EC	42	26.8	0.4	0.6	0.52
ED	239	25.9	-0.5	0.3	0.12
EE	***157***	***26.4***	0		

Differences in the phenotypic expression of wool characteristics between individual sheep grouped according to the presence of each of the five variants of the *KRT33A* gene (Table 4) were considerably less than when the individuals were grouped according to their pedigree-code. Phenotypic differences between individuals were even less when the individual sheep were grouped according to their genotype, with only core bulk displaying differences (Table 4). Effects associated with the presence of *A* were directly opposed to those arising from selection for increased core bulk, whereas effects associated with the presence of *B* and *C* were aligned with the selection response associated with selection for increased core bulk. While *D* and *E* were the most numerous within the sampled population, their presence was unrelated to the phenotypic expression of any of the measured wool characteristics, except for the presence of *E* being associated with a slightly higher mean fibre diameter and associated fibre diameter variation. A more balanced group of sheep with respect to variant frequency needs to be analysed to confirm these preliminary findings of additivity between the five identified variants of *KRT33A*.

While the difference in any of the measured characteristics for the presence of any given variant was insufficient to be of practical significance during wool processing and subsequent end-product performance (Hunter 1980), it is nevertheless noteworthy and suggests the role of *KRT33A* in wool fibre structure warrants further investigation.

In the case of body growth, selection for core bulk had a small but significant indirect effect on birth weight, a marginal effect on weaning weight and no significant effect on yearling weight. Presence of any of the variants was only associated with yearling weight through a small positive effect associated with the presence of *A,* and a small negative effect associated with the presence of *B*. The measured effects would be of limited practical

significance in a commercial flock, with no association between *KRT33A* variants and body growth being reflected in live-weight at different ages.

There was a reduced phenotypic response in the measured characteristics associated with the presence of each variant of *KRT33A* and among genotypes (Table 4), relative to the phenotypic response of the same characteristics when grouped by pedigree-code (Table 3). This reduction in the magnitude of the associated responses suggests that the phenotypic responses that have occurred following selection for core bulk are not directly related to the action of *KRT33A* and/or the gene is not wholly responsible for variation in the measured characteristics. However, seventeen other KRT and KAP genes have been identified on Ovine Chromosome 11 near *KRT33A,* with *KRT33A* expressed only in the cortex (Yu et al. 2011). Given this clustering of genes that are potentially all expressed in the wool fibre, and with many of the genes existing as multiple alleles, it will potentially be difficult to unravel their independent actions. Although many of the characteristics included in this study have a moderate to high heritability (Sumner et al. 2007), and are of importance in processing and end-product performance (Sumner et al. 1991), it remains unlikely that a simple gene-marker will be found that is directly related to a specific wool characteristic associated with wool bulk, such that it will be suitable for use in a commercial breeding programme.

Acknowledgements

To field and technical staff at Winchmore Research Station for management of the sheep, and assistance in the collection of field data, Denis O'Connell for collecting the tissue samples and to Grant McKenzie and Norma Merrick at Lincoln University for typing variation in the *KRT33A* gene in the collected tissue samples Maintenance of the flock was funded by the Foundation of Research Science Technology.

References

Beh KJ, Callaghan MJ, Leish Z, Hulme DJ, Lenane I, Maddox JF 2001. A genome scan for QTL affecting fleece and wool traits in Merino sheep. Wool Technology and Sheep Breeding 49: 88–97.

Carnaby GA, Elliott KH 1980. Bulk: A wool trait of importance to the carpet industry. Proceedings of the New Zealand Society of Animal Production 40: 196–204.

Hunter L 1980. The effects of wool fibre properties on processing performance and yarn and fabric properties. Proceedings of the 6th International Wool Textile Research Conference, Pretoria, South Africa. 1: 133–193.

Itenge TO, Hickford, JGH, Forrest RHJ, McKenzie GW, Frampton C 2010. Association of variation in the ovine KAP1.1, KAP1.3 and K33 genes with wool traits. International Journal of Sheep and Wool Science 58(1): 1–20.

Itenge-Mweza TO, Forrest RHJ, McKenzie GW, Hogan A, Abbott J, Amoafo O, Hickford JGH 2007. Polymorphism of the KAP1.1, KAP1.3 and K33 genes in Merino sheep. Molecular and Cellular Probes 21: 338–342.

McKenzie GW, Abbott J Zhou H, Fang Q, Merrick N, Forrest RH, Sedcole JR, Hickford JG 2010. Genetic diversity of selected genes that are potentially economically important in feral sheep in New Zealand. Genetics Selection Evolution 42: 43. http://www.gsejournal.org/content/42/1/43 [accessed 30 November 2012].

Parsons YM, Cooper DW, Piper LR 1994. Evidence of linkage between high-glycine-tyrosine keratin gene loci and wool fibre diameter in a merino half-sib family. Animal Genetics 25: 105–108.

Payne RW, Murray DA, Harding SA, Baird DB, Soutar DM 2009. GenStat for Windows, 12th Edition. Introduction. Hemel Hempstead, UK: VSN International.

Powell BC 1997. Molecular genetics of sheep. In: Piper L, Ruvinsky A ed. The genetics of sheep. Wallingford, Oxford, UK: CAB International. Pg. 149–181.

Rogers GR, Hickford JGH, Bickerstaffe R 1994. A potential QTL for wool strength located on ovine chromosome 11. Proceedings of the 5th World Congress on Genetics Applied to Livestock Production, University of Guelph, Guelph, Ontario, Canada. 21: 291–294.

Stobart RH, Sumner RMW 1991. Bulk and its structural basis: A review. Proceedings of the New Zealand Society of Animal Production 51: 339–346.

Sumner RMW, Clarke JN, Cullen NG 2007. Effect of divergent selection for wool bulk on live weight and wool characteristics in Perendale sheep. Proceedings of the New Zealand Society of Animal Production 67: 180–186.

Sumner RMW, Maddever DC, Clarke JN 1991. Effect of selecting Perendale hoggets for loose wool bulk on fleece characteristics and wool end-product performance. Proceedings of the New Zealand Society of Animal Production 51: 347–351.

Sumner RMW, Upsdell MP 2001. Factors associated with the prediction of core bulk from fibre diameter and fibre curvature of individual fleeces. Wool Technology and Sheep Breeding 49: 29–41.

Wools of New Zealand 1994. New Zealand sheep and their wool. Fifth edition. Wools of New Zealand, Wellington, New Zealand. Pg. 39.

Yu Z, Wildermoth JE, Wallace OAM, Gordon SW, Maqbool NJ, Maclean PH, Nixon AJ, Pearson AJ 2011. Annotation of sheep keratin intermediate filament genes and their patterns of expression. Experimental Dermatology 20: 582–588.