Preliminary linkage studies in sheep of keratin and keratin-associated protein genes with fleece weight, wool fibre diameter and fibre curvature

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Abstract

A linkage study was conducted to detect variations in the genes for keratins and keratin-associated proteins (KRTAP) that might affect wool traits. To date 93 such genes have been identified in sheep: 85 of them (~91%) are clustered in three chromosomal regions. Twenty-one genes are grouped within a 4-Mbp region on sheep chromosome 1 (OAR1), 28 within a 1-Mbp segment on chromosome 3 (OAR3), and 36 are clustered within a 1.3-Mbp site on chromosome 11 (OAR11). The DNA markers used in this work were single nucleotide polymorphisms (SNPs) dispersed throughout these keratin and KRTAP gene clusters. Markers were designed as three SNP-multiplexes: a 25-SNP, a 15-SNP and a 19-SNP multiplex for keratins/KRTAP regions on OAR1, OAR3 and OAR11, respectively. Resource animals came from eleven sire families whose progeny had been phenotyped for greasy fleece weight, washing yield, fibre curvature and diameter. Between 29 and 45 progeny per sire were genotyped with the three SNP-multiplexes. Linkage analyses on co-segregation of SNP alleles from sires with wool phenotypes of progeny were done across all the sires and within sire groups. Based on theoretical genome-wide threshold (F-value 10.13 and nominal P = 0.0016 for within-sire suggestive level), one family showed suggestive quantitative trait loci for greasy fleece weight on OAR1 and fibre diameter variability on OAR3. For these experimental animals, the results show no strong evidence of involvement of keratin and KRTAP genes in the wool characteristics analysed.

Keywords: keratins; keratin-associated proteins; SNP; wool traits; linkage analysis; quantitative trait loci

Introduction

Keratin proteins (K) and keratin-associated proteins (KAPs) constitute about 90% of the total content of wool fibre (Powell & Rogers 1994); these proteins are encoded by the keratin genes (KRT) and keratin-associated protein genes (KRTAP), respectively. Keratin proteins are found in two places in wool, namely the cuticle and the cortex; in the latter they are in the structures known as microfibrils which are in turn embedded in a matrix of KAPs. The keratin proteins belong to either of two types, keratin type I (K31-40) or keratin type II (K81-87), which are coded by multi-gene families (Schweizer et al. 2006). KAPs, on the other hand, fall into three major groupings based on their primary protein structures. Briefly, there are the high glycine-tyrosine KAPs which are encoded by KRTAP6.n, KRTAP7 and KRTAP8 genes, the high sulphur KAPs coded by the KRTAP1.n, KRTAP2.n and KRTAP3.n multi-gene families, and lastly the ultra-high sulphur KAPs specified by the KRTAP4.n and KRTAP5.n gene families (Powell & Rogers 1994). It is plausible that variations in the KRT and KRTAP genes which alter protein products could affect the characteristics of wool fibres. In eleven sire families that have been phenotyped for greasy fleece weight, washing yield, fibre curvature and fibre diameter, we tested the single nucleotide polymorphism (SNP) markers in the KRT and KRTAP gene regions for linkage to these wool traits.

Materials and methods

Resource animals

All work involving animals was carried out under the approval of AgResearch Animal Ethics Committee. There were eleven sire groups used in this study (Table 1) from a flock of composite sheep described by Scobie et al. (2007). Animal 159/06 was the grandsire of both 245/08 and 322/08. Animals 263/07, 136/08 and 175/08 were the sires of 276/09, 333/09 and 240/09, respectively. We theorized that fibre curvature would be a trait likely to be prone to variations in the primary structures of keratin and KAP proteins. Thus, within each sire family, about equal numbers of progeny were chosen from the upper and lower quartiles of mean fibre curvature measurements for this experiment. The number of progeny tested ranged between 29 and 45 animals per sire, which equates to 26 – 63% progeny of each sire family (Table 1). It should be noted that though they were animals extreme in mean fibre curvature, they were not necessarily extreme with respect to the other wool traits measured.

Blood samples of animals were collected by venepuncture from the jugular vein when they were approximately 8 months of age. DNA was extracted from white blood cells using the high-salt method of Montgomery and Sise (1990).

Measurements of wool traits

Wool samples were shorn with a conventional shearing hand piece from the mid-side region of each of the progeny at approximately 14 months of age. The yearlings had been shorn as lambs. Greasy fleece weight was recorded at shearing. A sample of the mid-side wool collected at shearing was scoured in aqueous detergent and dried to 16% regain, and washing yield was calculated as the percentage of clean wool in the greasy fleece sample. Clean samples were then measured for mean fibre diameter,

Table 1 Animal resource families and the progeny selected for use in the quantitative trait loci (QTL) studies. Mean fibre curvature (FCur), greasy fleece weight (GFW), washing yield (Yield), mean fibre diameter (FD) and the mean standard deviation of fibre diameter (FDSD) are reported for the high and low curvature groups.

			High curvature group					Low curvature group				
Sire family	Total progeny[a]	Number selected[b]	FCur (°/mm)	GFW (kg)	Yield (%)	FD (μm)	FDSD (μm)	FCur (°/mm)	GFW (kg)	Yield (%)	FD (μm)	FDSD (μm)
159/06	58	29	48.8	1.8	70.1	29.0	6.5	28.0	2.8	74.7	34.5	7.7
249/07	60	30	59.2	2.2	67.0	31.1	6.6	33.3	3.0	76.4	35.1	7.9
263/07	56	29	64.4	2.5	66.9	28.7	6.2	38.6	2.8	72.2	31.0	6.7
318/07	51	30	75.4	1.9	69.7	26.5	5.3	51.2	1.8	73.8	27.3	5.4
136/08	61	34	71.9	1.7	72.1	26.5	5.0	42.5	2.1	80.0	30.3	6.4
175/08	59	31	49.6	2.5	73.9	32.3	6.9	31.5	3.1	77.8	34.1	7.7
245/08	65	35	70.6	1.7	74.4	29.1	5.7	41.5	2.2	78.7	33.5	7.0
322/08	122	32	72.1	1.9	73.3	28.1	5.6	40.5	2.3	78.1	31.5	6.9
240/09	70	36	65.2	1.9	69.0	27.3	6.2	39.2	2.4	74.9	31.6	7.2
276/09	71	45	78.0	2.1	70.0	26.5	5.8	47.0	2.5	74.3	29.8	7.2
333/09	52	31	69.1	1.6	69.3	27.2	5.4	41.6	2.14	72.7	30.3	6.6

[a] The total number of progeny in each sire family. [b] The number of progeny used in the QTL studies. The animals were chosen from the upper quartile of mean fibre curvature measurements for the high curvature group, and from the lower quartile for the low curvature group. Approximately equal numbers of high and low curvature progeny were selected per family.

fibre diameter standard deviation and mean fibre curvature using an OFDA100 (Optical Fibre Diameter Analyser BSC, Ardross, Western Australia, Australia).

KRT/KRTAP genes, SNP markers and SNP-multiplex genotyping

Public databases were searched for KRT and KRTAP sequences. The sequences were then used to identify the locations of the genes on the Sheep Genome version 3. The SNP markers (from the Illumina OvineSNP50 BeadChip) that were dispersed amongst the sheep KRT and KRTAP genes were chosen for construction of SNP multiplexes, using the MassARRAY Assay Design 4.0 software (Sequenom Inc., San Diego, CA, USA).

Genotyping with the SNP-multiplexes was performed according to the Sequenom's recommended protocol. Reactions were carried out in 384-well plates using 25 ng of DNA per animal. The reaction products were transferred onto chips and analysed in a MassARRAY Compact 96 mass spectrometer.

Statistical analyses

Genetic positions of the markers were estimated by linkage analysis with CRI-MAP version 2.4 (Green et al. 1990), specifying marker order to be the same as on the Sheep Genome version 3. These genetic positions were then used to undertake quantitative trait loci (QTL) mapping, using the least-squares interval mapping method of Knott et al. (1996). Besides sire as a fixed effect and probability of inheriting a particular sire haplotype as a covariate, the models included birth year and sex combinations, birth-rearing rank and age of dam as fixed effects, and birth day of year as a covariate. F-statistic significance tests were conducted across sires to test for variants that were common in the population, and within sire to test for variants that might have been specific to one or a few sire families in this study. Genome-wide theoretical thresholds for the sheep genome were calculated (Lander & Kruglyak 1995) at the suggestive and P = 0.05 significance levels, which correspond to nominal P values of 0.00157 and 0.00005, respectively. Their respective F-threshold values were 2.83 and 3.73 for the across-all-sires analysis, and 10.13 and 16.89 for the within-sire analysis.

Results and discussion

Searches of the public databases (Swiss-Prot, InterPro, GO and KEGG) identified 92 genes for keratins and KAPs. All these genes were present on the Sheep Genome version 3. There were 23 genes on sheep chromosome OAR1, 29 genes on chromosome OAR3, 35 genes on chromosome OAR11, and a single gene on each of the OAR2, OAR7, OAR8, OAR14 and OAR16 chromosomes. Our study concentrated only on the 15-KRTAP (chromosomal position 123 – 124 Mbp) and 6-KRTAP (position 260 – 263 Mbp) gene clusters on chromosome OAR1, 28-KRT cluster (position 133 – 134 Mbp) on OAR3, and 25-KRT plus 10-KRTAP gene cluster (position 40 – 41.3 Mbp) on OAR11 (Table 2).

The 23 KRTAP genes on chromosome OAR1 were located in three separate regions which were 19 Mbp and 136 Mbp apart: our focus was on the 1-Mbp and 3-Mbp clusters of 15 and 6 genes, respectively (Table 2). The 29 KRT genes on chromosome OAR3 were in two groups, composed of 1 and 28 genes; the latter genes were clustered within a 1-Mbp segment (Table 2). All the 35 KRT and KRTAP genes on chromosome OAR11 fell within a 1.3-Mbp region; they are probably the most studied KRT and KRTAP genes in sheep to date.

The Illumina OvineSNP50 BeadChip has a total of 59,458 SNPs. In the totalled 4-Mbp KRTAP regions on chromosome OAR1, there were 33 SNPs which went into a multiplex design: we obtained a SNP-multiplex of 25 SNP markers which covered the 21 KRTAP genes (Table 2). Similarly, the 1-Mbp KRT region on OAR3 and the 1.3-Mbp KRT/KRTAP region on OAR11 have, respectively, 18 and

Table 2 The chromosomal segments carrying the keratin (KRT) and keratin-associated protein (KRTAP) genes. The multiplexes were designed to carry the maximum number of single nucleotide polymorphism (SNP) markers that were dispersed amongst the KRT and KRTAP genes.

Chromosome	KRT / KRTAP cluster	Cluster size (Mbp)[a]	Number of SNP markers[b]	SNP-multiplex	Mean number of heterozygous SNPs in sires
OAR1	15 KRTAP	1	10	Multiplex 1	4.0
	6 KRTAP	3	15		4.6
OAR3	28 KRT	1	15	Multiplex 3	4.9
OAR11	25 KRT and 10 KRTAP	1.3	19	Multiplex 11	9.1

[a] The chromosomal segment size, in million base-pairs (Mbp), containing the KRT and/or KRTAP genes. [b] The number of SNP markers within the gene clusters that were included in the SNP-multiplexes.

23 SNP markers. From these markers the multiplex design generated two multiplexes consisted of 15 and 19 SNPs; these SNP markers covered the 28 and 35 KRT/KRTAP genes on chromosomes OAR3 and OAR11, respectively (Table 2). In summary, the three SNP-multiplexes covered 84 of the 92 (~91%) KRT and KRTAP genes identifiable on the sheep genome.

In across-all-sires QTL analyses, no significant or suggestive QTL were detected for any of the wool traits. In reference to the genome-wide suggestive F-threshold of 2.83 (nominal P = 0.0016), there were two QTL approaching the suggestive threshold: namely washing yield on chromosome OAR1 and fibre diameter standard deviation on OAR3 (Table 3). With respect to the former result, in a separate wool QTL experiment involving three half-sib families of 200 progeny each, a suggestive washing yield QTL was detected in the same OAR1 chromosomal region (SH Phua, Unpublished data).

Table 3 The best across-all-sires quantitative trait loci (QTL) results are shown for each wool trait. The genome-wide suggestive F-threshold value is 2.83 with nominal P = 0.0016. The QTL for washing yield and fibre diameter standard deviation were near suggestive level.

Wool trait	QTL chromosome	Maximum F	Nominal P
Greasy fleece weight	OAR3	2.00	0.0281
Washing yield	OAR1	2.52	0.0047
Mean fibre diameter	OAR3	2.37	0.0080
Fibre diameter standard deviation	OAR3	2.65	0.0029
Mean fibre curvature	OAR3	1.95	0.0328

In within-sire analyses, the best results for each wool trait are presented in Table 4. Based on the genome-wide within-sire suggestive F-threshold of 10.13 (nominal P = 0.0016), the sire 175/08 family showed suggestive QTL for two wool traits: greasy fleece weight on chromosome OAR1 and fibre diameter standard deviation on chromosome OAR3 (Table 4). The greasy fleece weight QTL is in the 6-KRTAP gene cluster region. These results differed from that of Parsons et al. (1994) who found significant linkage of KRTAP6 and KRTAP8 genes on chromosome OAR1 with fibre diameter in one of eight merino half-sib families tested. Our results are also different from that of Gong et al. (2015) who reported significant association of KRTAP1.2 gene on chromosome OAR11 with washing yield, grease and clean fleece weights using 383 merino cross sheep from six sires. Although Beh et al. (2001) reported a yield QTL on chromosome OAR3 in one sire group out of five tested, they found no strong evidence for segregation of wool traits in their merino flock. However, our greasy fleece weight QTL on chromosome OAR1 coincides with the washing yield QTL detected in a separate study; the washing yield QTL was suggestive across-all-sires but was significant in one sire family out of three analysed (SH Phua, Unpublished data). Sumner et al. (2013) investigated one KRT gene on chromosome OAR11, namely KRT33A, and found no association with core bulk, a wool trait which is a manifestation of the effects of fibre curvature and fibre diameter. We found no QTL for mean fibre curvature which we first thought likely to be prone to variations in the composition and structures of keratin and KAP proteins.

Table 4 The best within-sire quantitative trait loci (QTL) results are shown for each wool trait. The genome-wide suggestive F-threshold value is 10.13 with nominal P = 0.0016. In the sire 175/08 family, the QTL for greasy fleece weight and fibre diameter standard deviation all reached suggestive threshold.

Wool trait	QTL chromosome	Sire ID	Maximum F	Nominal P
Greasy fleece weight	OAR1	175/08	11.51	0.0008
Washing yield	OAR11	175/08	7.72	0.0058
Mean fibre diameter	OAR1	245/08	7.63	0.0061
Fibre diameter standard deviation	OAR3	175/08	13.07	0.0004
Mean fibre curvature	OAR3	240/09	8.18	0.0045

In another QTL study on wool, using 36 microsatellite markers from only OAR1, OAR3, OAR4 and OAR11 chromosomes against ten merino sheep families, Roldan et al. (2010) detected QTLs for mean fibre curvature, clean wool yield, greasy fleece weight and clean fleece weight on chromosome OAR1; the QTLs are at least 40 Mbp away from the 15-KRTAP and 6-KRTAP clusters shown in Table 2. They also reported QTLs for fibre diameter coefficient of variation, greasy fleece weight and clean wool yield on chromosome OAR11; these QTLs are in the 25-KRT/10-KRTAP cluster region listed in Table 2.

Besides the KRT and KRTAP genes, the above results suggest that there are possibly other genes that affect wool traits. Using a candidate gene approach, Forrest et al. (2009) used a single-strand conformational polymorphism marker of beta3-adrenergic receptor gene on chromosome OAR26 to genotype 695 merino lambs. When analysed against wool traits, the marker showed an association with mean staple strength and yield. Similarly, Zeng et al. (2011) used SNP markers in the PROP paired-like homeobox 1 gene on chromosome OAR5 to test for association with wool traits in 345 Chinese merino sheep; they reported that the gene was significantly associated with fibre diameter characteristics.

Further, Wang et al. (2014) used genome-wide association studies approach to identify significant SNP loci for wool traits. They used the Illumina OvineSNP50 BeadChip to genotype 765 Chinese merino sheep which had been phenotyped for different fibre characteristics. They detected 29 genome-wide significant SNPs for fibre diameter, fibre diameter coefficient of variation, fineness dispersion and crimp. The two SNPs for fibre diameter and one SNP for fibre diameter coefficient of variation were located in the 6-KRTAP cluster region on chromosome OAR1, and the one SNP for crimp on chromosome OAR11 was in the vicinity of the 25-KRT/10-KRTAP genes (Table 2). The remaining 25 significant SNP loci for wool traits indicate that there are many other genes, besides KRT and KRTAP, which affect fibre characteristics.

This is our preliminary linkage study of KRT and KRTAP genes with wool traits. The best results came from the within-sire analyses which showed suggestive QTLs for greasy fleece weight and fibre diameter standard deviation on, respectively, OAR1 and OAR3 chromosomes. Interestingly, both these QTLs were identified in one sire family (Table 4). We detected no QTL for mean fibre curvature. Greasy fleece weight and mean fibre diameter are traits that strongly influence the value of the fleece, and therefore, farm income, though mean fibre curvature is a wool quality trait which also influences wool price. Overall the results do not provide convincing evidence for involvement of KRT and KRTAP genes in the variation of the wool traits studied, at least not in the resource animals used in this experiment. Perhaps the next avenue of work will be to concentrate on the non-KRT/KRTAP genes that may affect wool traits.

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