Polymorphism of the KAP6-1 gene in Chinese Tan sheep and its effect on wool traits

Abstract

The keratin-associated protein KAP6-1 gene (*KRTAP6-1*) was previously investigated in NZ sheep and found associated with fibre diameter-related wool traits. This study investigated variation in this gene and analyzed association with wool traits measured in 529 Chinese Tan sheep. Two previously identified *KRTAP6-1* alleles (*A* and *B*) and two newly identified *KRTAP6-1* alleles (*D* and *E*) were detected with allele frequencies of 28.9%, 57.1%, 10.3% and 3.7% for alleles *A*, *B*, *D* and *E*, respectively. Interestingly, one *KRTAP6-1* allele (*C*) was not found in this study while reported previously in NZ sheep.

Allele *D* was found to be associated with an increase in straightened fibre-length at birth and crimped fibre-length at day-35 *post-partum*. A trend association was found between allele *D* with the degree of crimping and wool fibre growth rate from birth to day-35 *post-partum*. Sheep of *BD* had wool of longer straightened fibre-length at birth and longer crimped fibre-length at day-35 *post-partum*, and tended to have wool of more crimps at birth, when comparing to sheep of *BB*.

Allele *E* was found to be associated with an increase in straightened fibre-length at day-35 *post-partum*, the degree of crimping at day-35 *post-partum* and wool fibre growth rate from birth to day-35 *post-partum*. A trend of association was found between allele *E* and crimp number at day-35 *post-partum*.

The absence of allele *C* is consistent with improved crimping in Chinese Tan sheep.

These results suggest that variation in ovine *KRTAP6-1* may affect wool traits early in life.

Keywords: KAP6-1 gene (*KRTAP6-1*); variation; crimp; wool traits; Chinese Tan sheep

1. Introduction

Keratin associated proteins (KAPs) are a main structural component of wool fibres which form a matrix cross-linking with the intermediate filaments (Powell and Rogers, 1997). KAPs can be classified into three broad groups on the basis of their amino acid composition: the high glycine-tyrosine (HGT; 35- 60 mol% glycine and tyrosine) KAPs, the high sulphur (HS; ≤ 30 mol% cysteine) KAPs and the ultra-high sulphur (UHS; > 30 mol% cysteine) KAPs (Gong *et al.*, 2016). Of these, the HGT-KAPs are a group of proteins that are predominantly found in the orthocortex of wool fibres and their abundance varies considerably both between and within breeds (Rogers, 2006; Gillespie, 1990). Lincoln wool has less than 1% of HGT-KAPs while Merino wool has between 4% and 12% of HGT-KAPs (Gillespie, 1990). The Merion felting lustre (FL) mutant wool contains few HGT-KAPs (Gillespie and Darskus, 1971), and the expression of the HGT-KAP genes is found to be down-regulated in FL mutant follicles (Li *et al.*, 2009). Such a wide range in the content of HGT-KAPs in wool fibres raises intriguing questions how these proteins affect the properties of wool fibre.

To date three HGT-KAP families are identified in sheep: KAP6, KAP7 and KAP8 (Gong *et al.*, 2016). Sheep KAP6 is a diverse KAP family with five encoding genes having been identified in this species (Zhou *et al.*, 2016). Recently variation in a KAP6 gene (*KRATP6-1*) has been reported to affect wool fibre diameter-associated traits (Zhou *et al.*, 2015), suggesting that KAP6-1 may play a role in controlling wool traits.

Tan sheep are an indigenous breed in China and are known for producing long curled or crimped white wool, with four crimp patterns having been described by people in China and these being translated to English as “random curly”, “soft and big curly”, “walnut flower curly” and “Chinese string curly” (Tao *et al.*, submitted). The genetic basis underpinning these unique characteristics of Tan sheep wool is currently unknown. The objective of this study was

therefore to characterize *KRTAP6-1* in Chinese Tan sheep and investigate whether the variation if present, affects wool traits of economic value in China.

2. Materials and methods

2.1. Sheep investigated and wool traits recorded

Five hundred and twenty-nine Chinese Tan lambs from 23 sire-lines were investigated. The majority of the lambs were born as singles and only 14 of them were born as twins. These twins were removed from the association analysis and only the 515 single lambs were analysed.

As most rural Chinese wool and pelt producers do not have access to internationally standardised wool testing services and methods, they have devised and used their own methods to measure the wool traits that they interested in, which are reliable relatively. These measures include: the straightened fibre-length (on the lambs shoulder or scapular) at birth, the straightened scapular fibre-length at day-35 *post-partum*, the crimped scapular fibre length at day-35 *post-partum*, the number of crimps at day-35 *post-partum*, the crimp frequency (expressed as the number of crimps per cm of length of straightened fibre) at day-35 *post-partum*, the degree of crimping (expressed as the ratio of the straightened length to the crimped length) at day-35 *post-partum*, and the wool fibre growth rate (expressed in mm/day) from birth to day-35 *post-partum*.

In this study, Fibre length was measured using a ordinary ruler and the number of crimps was counted by skilled farm staff. At birth, the straightened length of wool fibres on the shoulder was recorded. At day-35 *post-partum*, the straightened length on the shoulder, the crimped length of wool fibres from the shoulder and the number of crimps for wool fibres from the shoulder, were recorded.

Based on the above measurements, the crimp frequency and degree of crimping of wool fibres at day-35 *post-partum*, and the fibre growth rate from birth to day-35 *post-partum* were calculated.

Blood samples were collected from the lambs at four months of age. However, some of the ram lambs had been culled for fur or pelt production, and so blood samples could not be collected from these lambs. The blood was collected directly onto FTA cards (Whatman BioScience, Middlesex, UK) and DNA for analysis was purified from 1.2 mm punches from the cards, using the procedure described by Zhou *et al.* (2006).

2.2. PCR amplification of *KRTAP6-1* and SSCP analysis

Ovine *KRTAP6-1* was amplified using the polymerase chain reaction (PCR) primers described in Zhou *et al.* (2015). These primers were 5'-TCTACCCGAGAACAACCTC-3' and 5'-AGGCAAGTCTTTAGTAGGAC-3'. Primers were synthesised by Integrated DNA Technologies (Coralville, IA, USA) and the amplification was undertaken using the purified genomic DNA on one punch of the FTA paper, 0.25 μ M of each primer, 150 μ M of each dNTP (Bioline, London, UK), 2.5 mM of Mg^{2+} , 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1 $\times$ reaction buffer supplied in a 20 μ L reaction.

The thermal profile for amplification consisted of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 60 °C and 30 s at 72 °C, with a final extension time of 5 min at 72 °C. This was undertaken in S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA). Amplicons were visualized by electrophoresis in 1% agarose (Bioline) gels, using 1 $\times$ TBE buffer containing 200 ng/mL of ethidium bromide.

PCR amplicons were genotyping by single-strand conformational polymorphism (SSCP) analysis. A 0.7 μ L aliquot of each amplicon was mixed with 7 μ L of loading dye (98%

formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol) and after denaturation at 95 °C for 5 min, the samples were cooled rapidly on wet ice and loaded on 16 cm × 18 cm, 14% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels. Electrophoresis was performed using Protean II xi cells (Bio-Rad), at 340 V for 18 h at 18 °C in 0.5 × TBE buffer. The gels were silver-stained by the method of Byun *et al.* (2009). PCR amplicons of the three known alleles of ovine *KRTAP6-1* (Zhou *et al.*, 2015) were included as references in each gel.

2.3. Sequencing of allele variants

PCR amplicons representative of SSCP patterns different to known *KRTAP6-1* alleles (Zhou *et al.*, 2015) were selected for DNA sequencing using a rapid approach described previously (Zhou *et al.*, 2011). Briefly, a band corresponding to the allele was excised as a gel slice from the polyacrylamide gel, macerated, and then used as a template for re-amplification with the original primers. This second amplicon was then sequenced in both directions at the DNA Sequencing Facility at Lincoln University, New Zealand.

2.4. Statistical analyses

All statistical analyses were performed using SPSS (version 22; IBM). General Linear Models (GLMs) were used to assess the effect of the presence of a particular *KRTAP6-1* allele in a sheep's genotype on the various wool traits, including straightened wool fibre length at birth; straightened wool fibre length at day-35 *post-partum*, crimped wool fibre length at day-35 *post-partum*, crimp frequency at day-35 *post-partum*, degree of crimping at day-35 *post-partum* and wool growth rate from birth to day-35 *post-partum*. GLMs were also used to compare variation in wool traits between sheep with different genotypes.

Gender and sire were found to affect many of the wool traits and so they were included as factors in the GLMs. Straightened wool length at birth was also found to affect all the other wool traits and therefore it was also fitted in the models.

3. Results

3.1. Polymorphism of KRTAP6-1 in Chinese Tan sheep

PCR-SSCP analysis of PCR amplicons identified four alleles of *KRTAP6-1* in the Chinese Tan sheep, including two known (*A* and *B*) and two new (*D* and *E*) alleles. The previously identified allele *C* was not found in the Tan sheep population investigated in this study. Allele *D* could be resulted from the combination of three previously known single nucleotide polymorphisms (SNPs) located downstream of the coding region, while allele *E* was resulted from a new SNP upstream of the coding region.

In total nine *KRTAP6-1* genotypes were detected observed among 515 Chinese Tan sheep. These genotypes and their frequencies were *AA* (9.3%), *AB* (30.5%), *AD* (6.4%), *AE* (2.1%), *BB* (35.0%), *BD* (10.0%), *BE* (3.9%), *DD* (1.6%) and *DE* (1.2%). This gives frequencies of 28.9%, 57.1%, 10.3% and 3.7% for alleles *A*, *B*, *D* and *E*, respectively.

3.2. Association between KRTAP6-1 variants with wool traits

The presence of variant *D* was found to be associated with an increase in straightened fibre-length at birth (absent: 48.4 ± 1.2 mm; present: 50.0 ± 1.3 mm; $P = 0.016$) and crimped fibre-length at day-35 *post-partum* (absent: 48.4 ± 1.3 mm; present: 50.1 ± 1.5 mm; $P = 0.022$) (Table 1). There was a trend association between variant *D* and the degree of crimping (absent: 1.52 ± 0.06 ; present: 1.47 ± 0.07 ; $P = 0.088$), and between variant *D* and wool fibre growth rate from birth to day-35 *post-partum* (absent: 0.68 ± 0.05 mm/d; present: 0.63 ± 0.06 mm/d; $P = 0.078$) (Table 1).

The presence of variant *E* was associated with an increase in straightened fibre-length at day-35 *post-partum* (absent: 72.0 ± 1.5 mm; present: 75.3 ± 1.9 ; $P = 0.006$), the degree of crimping at day-35 *post-partum* (absent: 1.50 ± 0.06 ; present: 1.62 ± 0.08 ; $P = 0.018$) and wool fibre growth rate from birth to day-35 *post-partum* (absent: 0.66 ± 0.05 mm/d; present: 0.77 ± 0.06 mm/d; $P = 0.010$) (Table 1). A trend of association was found between variant *E* and crimp number at day-35 *post-partum* (absent: 6.5 ± 0.1 crimps; present: 6.7 ± 0.2 crimps; $P = 0.063$) (Table 1).

3.3. Comparison of wool traits between *KRTAP6-1* genotypes

Of the nine genotypes identified in the Chinese Tan sheep, four genotypes (*AE*, *BE*, *DD* and *DE*) were detected at a frequency lower than 5%. These genotypes were therefore removed from the comparison analyses.

To confirm the effect of the presence of variant *D* on wool traits, genotype comparisons between *AA* ($n = 48$) and *AD* ($n = 33$), and between *BB* ($n = 180$) and *BD* ($n = 51$), were carried out. No differences in wool traits were detected between *AA* and *AD*, however, differences in wool traits were observed between *BB* and *BD*. Lambs of *BD* had wool of longer straightened fibre-length at birth and longer crimped fibre-length at day-35 *post-partum*, and tended to have wool of more crimps at birth, when comparing to lambs of *BB* (Table 2).

To confirm the effect of the presence of variant *E*, a genotype comparison between *BB* ($n = 180$) and *BE* ($n = 20$) was carried out, and differences in wool traits were detected between these two genotypes. Wool of *BE* lambs had a higher degree of crimping at day-35 *post-partum* and a higher growth rate from birth to day-35 *post-partum*, and tended to have a longer straightened length at day-35 *post-partum*, comparing to *BB* lambs (Table 2).

4. Discussion

This study describes the detection of four *KRTAP6-1* alleles in Chinese Tan sheep including two newly identified alleles, and reports association of variation in this gene with some wool traits of importance for wool of this sheep breed. The identification of two additional alleles at this gene locus brings the number of *KRTAP6-1* alleles from three to five, suggesting *KRTAP6-1* is a polymorphic gene in sheep and its effect on wool characteristics warrant investigation.

The presence/absence GLMs revealed that variation in *KRTAP6-1* affect a number of wool traits, including straightened fibre-length at birth, straightened and crimped fibre-lengths and degree of crimping at day-35 *post-partum*, and wool growth rate from birth to day-35 *post-partum*. Lambs carrying the *D* allele of *KRTAP6-1* had wool of longer straightened fibre-length at birth, longer crimped wool-length at fibre at day-35 *post-partum*, but tended to have a lower degree of crimping and a low wool growth rate from birth to day-35 *post-partum*. Lambs carrying the *E* allele of *KRTAP6-1* had wool of longer straightened fibre-length and a high degree of crimping at day-35 *post-partum*, and a higher wool growth rate from birth to day-35 *post-partum*. These are consistent in general with the results obtained from pair-wise comparisons between genotypes, which show that *BD* lambs had wool of longer straightened fibre-length at birth and longer crimped fibre-length at day-35 *post-partum* than *BB* lambs, and that *BE* lambs had wool of a higher degree of crimping at day-35 *post-partum* and a high growth rate from birth to day-35 *post-partum*, and tended to have a longer straightened fibre-length at day-35 *post-partum* than *BB* lambs. The failure to detect differences in wool traits between *AA* and *AD* is probably due to the insufficient samples for these two genotypes.

It is interesting to note that a previously identified allele (allele *C*) of *KRTAP6-1* found in New Zealand sheep was not detected in the 515 Chinese Tan sheep investigated. This alleles has a 57-bp deletion in the coding region and would result in the loss of two GCGY repeats and an 11-peptide sequence in the central region of the protein. The allele is found to be associated

increased mean fibre diameter (MFD), fibre diameter standard deviation (FDSD) and prickly factor (PF) with (Zhou et al., 2015). The absence of this allele may indicate that this allele may have been heavily selected against for in the Chinese Tan sheep. The current selection programs with the Tan sheep are focused on crimping and crimped fibre length, with the objective of producing wool with a crimping number not less than 6 and a crimped length of wool fibres of over 70 mm at day-35 *post-partum*. Curvature was reported to be correlated moderately negatively with MFD (Taylor et al., 1999) although a low correlation was reported in Merino sheep (Gong et al., 2015). This seems consistent with the selection for improving crimping in Chinese Tan sheep result in the absence of *KRTAP6-1* allele *C*, which leads to decreased MFD.

The two new alleles (*D* and *E*) of *KRTAP6-1* identified in Chinese Tan sheep were found to favor various wool traits of importance in China. For example, the presence of *D* was associated with increased crimped fibre-length at day-35 *post-partum*, while the presence of *E* was associated with increased degree of crimping at day-35 *post-partum* and increase wool growth rate from birth to day-35 *post-partum*. The emerging of these two alleles is consistent with the selection programs for the Chinese Tan sheep operated in China.

A recent study in Chinese Tan sheep report that variation in another HGT-KAP gene called *KRTAP8-2* affect wool crimping and growth (Tao et al., submitted). Although both *KRTAP8-2* and *KRTAP6-1* encode HGT-KAPs and are clustered on the same chromosome (Gong et al., 2016), the effects detected for these two genes are similar but different. Both genes affect wool length and growth, however, *KRTAP8-2* affect fibre crimp frequency (Tao et al., 2016) whereas *KRTAP6-1* affect the degree of crimping. These findings suggest that one certain wool trait is under the control of multiple genes and one certain gene may have different effects on various wool traits. In the context of this, *KRTAP6-1* may be one of the genes that does affect wool crimping and growth.

It also remains possible that the association between *KRTAP6-1* variation and wool traits reported in this study may be due to variation in other *KRTAPs* that are clustered with *KRTAP6-1*. There are many *KRTAPs* clustered with *KRTAP6-1* on sheep chromosome 1, including the genes encoding KAP6, KAP7, KAP8, KAP11, KAP13 and KAP24 families (Gong et al., 2012; Zhou et al., 2012; Gong et al., 2014; Zhou et al., 2016). As a result of the highly clustered nature of the *KRTAPs*, it would be difficult to determine the independent effects of the individual *KRTAPs* especially if they are in close proximity to *KRTAP6-1*. Nevertheless the associations found in this study suggest that *KRTAP6-1* could be used as a gene-marker for selection for wool traits that are of value in the Chinese market.

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Fig.1. Four crimp patterns of the wool from the Chinese Tan sheep. (A): “random curly”; (B): “soft and big curly”; (C): “walnut flower curly”; and (D): “Chinese string curly”. These descriptions are literal translations of the Chinese names.

Fig.2. PCR-SSCP of ovine *KRTAP8-2* in Chinese Tan sheep. Two PCR-SSCP patterns representing two alleles (*A* and *B*) are shown.