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Short communication

Variation in the ovine trichohyalin gene and its association with wool curvature

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ABSTRACT

Trichohyalin (TCHH) is a protein found in ovine wool fibres. It is part of the interfilamentous matrix protein and cross-links both to itself and to the keratin intermediate filament proteins. TCHH is also believed to play a role in the regulation of calcium levels within the hair follicle. This study used PCR-single stranded conformational polymorphism (PCR-SSCP) analysis to investigate variation in the ovine TCHH gene (*TCHH*). Three sequence variants (*A*, *B* and *C*) were detected in 210 New Zealand (NZ) Romney, Merino, Corriedale, Poll Dorset and cross-bred sheep, with frequencies of 59.0%, 36.9% and 4.1%, respectively. Two single nucleotide polymorphisms (SNPs) were identified in these variants, one located in the 3′ region of intron 2 (c.139-14) and the other one located in exon 3 (c.227). The SNP located in the exon is non-synonymous and would result in an amino acid residue change (p.76Ala/Glu). The association between variation in *TCHH* and wool traits was investigated in a large half-sib family, in which the sire was *AB* heterozygous. Progeny that inherited *A* produced wool with lower mean fibre curvature (MFC), than those that inherited *B* ($P = 0.026$). No effects on other wool traits were observed. These results suggest that variation in ovine *TCHH* affects wool curvature.

1. Introduction

Trichohyalin (TCHH) is a large α-helix-rich insoluble protein that is abundant in the inner root sheath (approximately one third of total protein) and the medulla of wool (Rogers et al., 1991). The protein is also found in other toughened epithelial tissues such as the hard palate and the filiform ridges of the tongue (O'Keefe et al., 1993). TCHH possesses an unusually high content of charged amino acid residues (Lee et al., 1993) and it is subject to post-translational modification by peptidylarginine deiminase, which converts many of its arginine residues to citrulline, and by transglutaminase, which introduces intra- and inter-protein chain crosslinks (Steinert et al., 2003). In wool TCHH provides an interfilamentous matrix by cross-linking to both itself and the head and tail domains of the inner root sheath keratin intermediate filaments. It also serves as a cross-linking protein in the cornified cell envelope of the inner root sheath cells, and does this by attaching to barrier proteins such as involucrin (Steinert et al., 2003). In addition, TCHH stabilises the linkage between the keratin filaments and the cell envelope and hence it provides mechanical strength in the mature fibre structure (Steinert et al., 2003).

TCHH possesses a pair of calcium-binding motifs in helix-loop-helix structural domains called EF-hands found at the amino terminus (Lee et al., 1993). These EF-hands are named after a calcium-binding motif formed by helices E and F in the crystal structure of parvalbumin (McPhalen et al., 1991). These structures are also typically found in the S100 calcium-binding proteins, a multigenic family of non-ubiquitous calcium-modulated proteins present in vertebrates (Santamaria-Kisiel et al., 2006). The EF-hand domains may be involved in the regulation of calcium levels within cells (Lee et al., 1993). It is therefore suggested that TCHH plays a role in the control of post-translational modification processes during terminal differentiation in wool fibres, as the activities of both peptidylarginine deiminases and transglutaminase are calcium-dependent.

The TCHH gene (*TCHH*) consists of three exons: exon 1 contains 52 bp of 5′ non-coding sequence, exon 2 contains 166 bp (28 bp of 5′ non-coding and 138 bp of coding sequence), and exon 3 contains 5529 bp (4512 bp of coding sequence and 1017 bp of 3′ non-coding sequence). These exons are separated by introns of 1808 bp and 586 bp,

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respectively (Fietz et al., 1993). The coding sequence of the first EF-hand domain is located in exon 2, while the coding sequence of the second EF-hand domain is at the 5′ end of exon 3 (Fietz et al., 1993). The overall gene structure is similar to other genes encoding S100 calcium-binding proteins, and the locations of the exon/intron boundaries are conserved across *TCHH* and the genes for the S100 proteins (Fietz et al., 1993). In sheep, the TCHH gene has been mapped to OAR1 (McLaren et al., 1997): a chromosome on which the high glycine-tyrosine keratin associated protein (KAP) genes and some high sulphur KAP genes are located (Gong et al., 2016).

Sequence variation in *TCHH* has been described in humans and a nonsynonymous single nucleotide polymorphism (SNP) in exon 3 has been reported to be associated with hair curliness in Europeans (Medland et al., 2009). There have been two studies describing variation in ovine *TCHH* (Fietz et al., 1993; McLaren et al., 1997). Fietz et al. (1993) described three single nucleotide changes in the non-coding sequences of exon 1, and five single nucleotide changes in the 3′ non-coding region. They attributed this variation to deriving their sequences from different “strains of sheep”. McLaren et al. (1997) used a restriction fragment length polymorphism (RFLP) technique, to describe restriction fragment length variation in sheep, but no specific sequence variation was described (McLaren et al., 1997).

To date, no studies investigating the effect of variation in ovine *TCHH* on wool traits have been reported. In this study, we used polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) to search for variation in ovine *TCHH* and upon finding variation tested whether that variation was associated with variation in wool Greasy Fleece Weight (GFW), Clean Fleece Weight (CFW), Mean Fibre Diameter (MFD), Fibre Diameter Standard Deviation (FDSD), Coefficient of Variation of Fibre Diameter (CVFD), Mean Staple Length (MSL), Mean Fibre Curvature (MFC), Mean Staple Strength (MSS), Prickle Factor (PF; the percentage of fibres of diameter greater than 30 μm) and Yield (the percentage proportion of CFW to GFW). We describe an association between variation in ovine *TCHH* and wool curvature.

2. Materials and methods

2.1. Animals investigated and DNA extraction

To search for genetic variation in ovine *TCHH*, an initial investigation of 210 New Zealand (NZ) Romney (n = 40), Merino (n = 40), Corriedale (n = 30), Poll Dorset (n = 30) and cross-bred sheep (n = 70) was undertaken. Subsequently, association studies were carried out in a large half-sib family. The half-sib was produced by mating a Merino ram to 150 Merino ewes of mixed origin. Of the 169 lambs born from this sire, 126 lambs survived to one year of age and these were used in this study. All lambs were ear-tagged with unique identification numbers at birth and birth date, gender, birth rank and dam identity were recorded. At approximately one month of age, the lambs were tailed, and the ram lambs were castrated to limit hormonal influences on wool characteristics. The lambs were weaned at about three months of age and grazed on pasture. Blood samples from all lambs, ewes and sires were collected onto an FTA card (Whatman, Middlesex, UK). The blood was allowed to air dry and the cards stored in darkness at room temperature. For each blood sample, a disc of 1.2 mm in diameter was punched from the FTA card, and the genomic DNA on the card was then purified using a two-step method described by Zhou et al. (2006).

2.2. Wool sampling and measurements

All progeny were shorn for the first time at 12 months of age and a sample of wool was collected from the mid-side region of each animal. GFW was recorded at shearing and other wool traits were measured or calculated by the New Zealand Wool Testing Authority Ltd (Napier, NZ); including CFW, MFD, FDSD, CVFD, MSL, MFC, MSS, PF and wool Yield.

2.3. Variation screening and allele sequencing

Variation in two separate PCR amplified fragments of the ovine TCHH gene was investigated. The first fragment encompassed exon 2 that encodes the first EF-hand domain and was 340 bp in size. The second fragment of 215 bp was a portion of the exon 3 sequence, which encodes the second EF-hand domain. The exon 2 fragment was amplified using the PCR primers (ex2-up: 5′-cccaagatcagaaacagagt-3′ and ex2-dn: 5′-atggtgtagaactgtcatgg-3′), and the exon 3 fragment was amplified using the primers (ex3-up: 5′-ctacctgtaaatagcacattg-3′ and ex3-dn: 5′-tcacacttggcttctttctc-3′). These primers were designed based on the published ovine *TCHH* sequence (GenBank accession number Z18361). Amplification was performed in a 20-μL reaction containing the genomic DNA on one 1.2-mm punch of FTA paper, 0.25 μM of each primer, 150 μM of each dNTP (Eppendorf, Hamburg, Germany), 2.5 mM of Mg^{2+}, 0.5 U of Taq DNA polymerase (Qiagen, Hilden, Germany) and 1× reaction buffer supplied with the polymerase enzyme. 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 50 s at 72 °C, with a final extension of 5 min at 72 °C. Amplification was carried out in an iCycler (Bio-Rad, Hercules, CA, USA).

A 0.7-μL aliquot of each amplicon was mixed with 7 μL of loading dye (98% formamide, 10 mM EDTA, 0.025% bromophenol blue, 0.025% xylene-cyanol). After denaturation at 95 °C for 5 min, the samples were rapidly cooled on wet ice and then 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 380 V for 18 h at 5 °C in 0.5× TBE buffer. Gels were silver-stained according to the method of Byun et al. (2009). Variants of *TCHH* that were found in homozygous sheep were directly sequenced at the Lincoln University DNA Sequencing Facility. Those that were only found in heterozygous sheep, were sequenced using a PCR-SSCP-based approach described in Gong et al. (2011). Briefly, a band corresponding to the allele was excised as a gel slice from the first SSCP gel, macerated, and used as a template for re-amplification with the original primers to produce a SSCP gel pattern equivalent to a sheep homozygous for that allele. This second amplicon was then directly sequenced.

For each sheep, the ovine TCHH gene was genotyped using the PCR-SSCP approach described above. Amplicons representative of the allele sequences identified were included in each polyacrylamide gel, and their banding patterns were used as standards for determining the alleles present in individual sheep. For the half-sib family, the sire was also genotyped at *TCHH*, and for those progeny that typed the same as the sire, the dams were genotyped.

2.4. Statistical analysis

Half-sib (segregation) analysis was performed using SPSS version 15 (IBM, NY, USA). Progeny that typed the same as the sire and dam were removed from the data set, because it could not be determined which allele had been inherited from the sire or dam. A univariate analysis of variance was performed to assess the effect of the inherited sire allele on various wool traits. Birth rank (i.e. whether they were a single, twin or triplet) was found to affect wool weight traits (GFW, CFW and wool Yield) and was therefore included as a factor in the analyses of these wool weight traits. Gender was not found to affect wool traits and hence was not included as a factor in the analyses. Only main effects were tested.