https://doi.org/10.17221/224/2019-CJAS

Polymorphisms and association of *FAT1* gene with wool quality traits in Chinese Merino sheep

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Citation: Ma G.-W., You X., Yang H., Yan X.-H., Mou F., Chu Y.-K., Rong E.-G., Wang S.-Z., Wang Z.-P., Li H., Wang N. (2020): Polymorphisms and association of *FAT1* gene with wool quality traits in Chinese Merino sheep. Czech J. Anim. Sci., 65: 31–39.

Abstract: FAT atypical cadherin 1 (FAT1) is a member of the atypical cadherin superfamily and is involved in the planar cell polarity signalling pathway which regulates hair follicle morphogenesis, cycling, and orientation. The purpose of this study was to investigate the sheep *FAT1* gene expression, polymorphisms and its association with wool quality traits in Chinese Merino sheep. Quantitative real-time PCR analysis showed that the *FAT1* mRNA was differentially expressed in the adult skin of Chinese Merino and Kazak sheep. Seven SNPs (termed SNPs 1–7) were identified in exon 2 of sheep *FAT1* gene by Sanger sequencing. SNPs 2 and 7 (novel SNPs) were significantly associated with wool crimp ($P < 0.05$). SNPs 4 and 5 (rs161528993 and rs161528992) were significantly associated with wool fibre length ($P < 0.05$). SNP 7 was highly significantly associated with average wool fibre diameter ($P < 0.01$). Similarly, *FAT1* haplotypes were significantly associated with wool crimp ($P < 0.05$), and the haplotypes H1–H3 and H5 were significantly associated with higher wool crimp ($P < 0.05$). Bioinformatics analysis showed that the wool quality trait-associated SNPs (SNPs 2, 4, 5 and 7) might affect the pre-mRNA splicing by creating or abolishing the binding sites for serine/arginine-rich proteins, and in addition, SNPs 2 and 4 might alter the *FAT1* mRNA secondary structure. Our results suggest that FAT1 influences wool quality traits and its SNPs 2, 4, 5 and 7 might be useful markers for marker-assisted selection and sheep breeding.

Keywords: FAT atypical cadherin 1; tissue expression; SNP; wool crimp; wool fibre diameter

Atypical cadherin is a type of cadherin which is essential for the regulation of cell shape (Shapiro et al. 1995) and is involved in the formation and maintenance of diverse tissues and organs, such as the polarization of epithelial cells and cochlear hair cells (Gumbiner 2005). FAT atypical cadherin 1 (FAT1) is a member of the atypical cadherin superfamily and is widely expressed in epithelial tissues, including skin and renal glomerular epithelial cells (Cox et al. 2000; Matis and Axelrod 2013). FAT1 is involved in the planar cell polarity (PCP) signalling pathway whose components such as Fuz, Rac1 and Frizzled6 have been shown to play important roles in the regulation of mammalian hair follicle morphogenesis (Dai et al. 2011), cycling (Chrostek et al. 2006) and orientation (Wang et al. 2010).

Supported by the Domain-specific projects for transgenic biological breeding (2014ZX08009-002 and 2009ZX08009-160B).

https://doi.org/10.17221/224/2019-CJAS

Knockout mouse studies showed that keratinocyte-specific loss of Fuz reduced the number of hair follicles in the back skin and delayed the hair follicle morphogenesis in E18.5d mouse embryos (Dai et al. 2011), keratinocyte-specific loss of Rac1 resulted in hair loss at 2 months of age, and no hair regrowth was observed at 24 months of age (Chrostek et al. 2006), and keratinocyte-specific loss of Frizzled6 led to inconsistent hair follicle orientation on the back skin at postnatal days 0–4 (Wang et al. 2010).

Wool is an important economic product of sheep and a vital source of income for sheep farming. The value of wool is determined by its intrinsic quality, which includes a number of wool quality traits such as average wool fibre diameter and wool crimp. Wool quality traits are polygenic, and several genes were reported to be associated with sheep wool quality traits. For example, *FST* (Ma et al. 2017) and *MTR* (Rong et al. 2015) were reported to be associated with average wool fibre diameter, and *FST* (Ma et al. 2017), *KAP22.1* (Li et al. 2017), *DSG4* (Ling et al. 2014), and *DKK1* (Mu et al. 2017) were associated with wool crimp.

Merino sheep and Kazak sheep have striking differences in wool quality traits such as the average wool fibre diameter. Merino sheep produce the finest and softest wool, while the Kazak sheep produce coarse wool (Rong et al. 2015). Wool production relies on the proliferation and differentiation of hair follicle epithelial and dermal cells. Considering the expression of the *FAT1* gene in skin epithelial cells (Cox et al. 2000) and the role of the PCP signalling pathway in hair follicle morphogenesis (Dai et al. 2011), cycling (Chrostek et al. 2006) and orientation (Wang et al. 2010), it is reasonable to hypothesise that FAT1 might affect sheep wool quality traits. Therefore, in this study, we performed tissue expression, single nucleotide polymorphism (SNP) detection, and association analysis of *FAT1* SNP with wool quality traits in Chinese Merino sheep.

MATERIAL AND METHODS

Animal resource population and phenotype. A total of 744 Chinese Merino ewes were used for *FAT1* SNP analysis in this study. These ewes came from Xinjiang Academy of Agricultural and Reclamation Science and consisted of six different Chinese Merino sheep strains: strain A (n = 152), strain B (n = 103), prolific meat strain (strain PM, n = 134), prolific wool strain (strain PW, n = 138), superfine wool strain (strain SF, n = 181) and strain U (n = 36). All sheep were kept under the same environmental conditions and had free access to feed and water.

In addition, three ewes of strain SF and three Kazak ewes were used for *FAT1* gene expression analysis. Three ewes of strain SF were slaughtered at 240 days of age to collect samples of heart, liver, spleen, kidney, rumen, intestine, muscle, and body side skin. Three Kazak ewes were slaughtered at 240 days of age to collect body side skin samples. All samples were snap-frozen in liquid nitrogen and stored at –80 °C until analysed.

A total of five wool quality traits (average wool fibre diameter, wool fibre diameter standard deviation, coefficient of variation of wool fibre diameter, wool crimp, and wool fibre length) were measured and recorded according to the guidelines of the China Fibre Inspection Bureau and International Wool Textile Organization (Cottle 2010).

All animal work was conducted according to the guidelines for the care and use of experimental animals of the Ministry of Science and Technology of the People's Republic of China (Approval No. 2006-398) and was approved by the Laboratory Animal Management Committee of Northeast Agricultural University.

RNA extraction, reverse transcription, and quantitative real-time PCR analysis. Total RNA extraction and cDNA synthesis were performed as previously described (Ma et al. 2017). The primers used for the *FAT1* (NCBI: XM_015104568.1) and *GAPDH* (NCBI: NM_001190390.1) genes were synthesised by Invitrogen (Table 1). Quantitative real-time PCR was carried out using Applied Biosystems 7500 Fast Real-Time PCR System (Life Technologies, USA) using the *Taq* SYBR Green qPCR Premix (NOVA; Yugong Biolabs Inc., China). *FAT1* gene expression was normalised to the reference gene *GAPDH* by the $2^{-\Delta Ct}$ method, where $\Delta Ct = Ct_{FAT1} - Ct_{GAPDH}$. The quantitative real-time PCR was repeated three times and each sample was tested in triplicate.

SNP detection and genotyping. Genomic DNA extraction and SNP discovery were performed as previously described (Ma et al. 2017). Two partial regions of the sheep *FAT1* gene were amplified by PCR with high-fidelity DNA polymerase (M0530L, NEB) with primer pairs FAT1-1 and FAT1-2, respectively (Table 1). The PCR prod-

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Table 1. Primers used for gene expression analysis and genomic amplification

Primer pair	Primer sequence (5'- 3')	Product size (bp)	Purpose
FAT1	F: GAACACATATTAGAAGTTACTGTGAC R: GGGCGACCATTATCAACA	352	qPCR
GAPDH	F: CTGACCTGCCGCCTGGAGAAA R: GTAGAAGAGTGAGTGTCGCTGTT	153	qPCR
FAT1-1	F: GGAAAAAATACTAACGCTGAAGCACGA R: GAGACGGTAGTTATCTGCTCCCCGA	1 406	genomic amplification
FAT1-2	F: GCGTCGGGGAGCAGATAACTAC R: TTCAGGTTCCTCTGGTTCCATAC	1 692	genomic amplification

ucts were sequenced using Sanger sequencing (Invitrogen, USA). SNPs were identified by the presence of double peaks at a single position in a chromatogram. In this study, we only considered the common SNPs (minor allele frequency, MAF > 5%). Genotyping was performed using a 384-well plate format on the Sequenom MassARRAY platform as previously described (Ma et al. 2017).

Bioinformatics analysis. The functionality of SNPs was predicted using the online software: ESE Finder (http://krainer01.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home), and RNA SNP Web Server (https://rth.dk/resources/rnasnp/). For each identified synonymous SNP associated with wool quality traits, a 17-bp exonic sequence, with the SNP located in the middle (position 9), was used to predict the effect of each of the identified SNPs on the splicing of *FAT1* pre-mRNA using the ESE Finder (Smith et al. 2006), and the effect of each of these SNPs on the *FAT1* mRNA folding was analysed by submitting the full-length CDS of *FAT1* gene to the RNA SNP Web Server (Sabarinathan et al. 2013).

Statistical analysis. Genotype frequencies, allele frequencies, observed heterozygosity and expected heterozygosity values were calculated, and the Hardy–Weinberg equilibrium test was performed using POPGENE (Version 1.31) (Yeh 1999). Haplotype analyses (D') were performed by the Haploview software (Version 4.2) (Barrett et al. 2005), and haplotype construction was performed in SAS Version 9.2 using the haplotype procedure, which uses the Expectation Maximization (EM) algorithm to generate maximum likelihood estimates of the haplotype frequencies.

Before analysing the association between the SNPs and wool quality traits, we performed data pre-processing: for all the tested population, if the contribution of a single genotype was less than 5% × the total number of samples, we removed the data for this genotype. In addition, the 744 Chinese Merino ewes were treated as a random population, because approximately 70.97% of the pedigree information was missing.

For all the tested population, the statistical models were:

Model 1:

$$Y = \mu + G + L + A + (G \times L) + (G \times A) + (A \times L) + e$$

Model 2:

$$Y = \mu + H + L + A + (H \times L) + (H \times A) + (A \times L) + e$$

Where: Y = phenotype value; μ = population mean; genotype (G), haplotype (H), line (L), and age (A) = fixed effects; ($G \times L$), ($G \times A$), ($A \times L$) = interaction effect of G by L, G by A, and A by L; ($H \times L$), ($H \times A$), ($A \times L$) = interaction effect of H by L, H by A, and A by L; e = residual effect.

Data were subjected to the GLM procedures of John's Macintosh Program (JMP Version 7.0, SAS Institute Inc.), which was used to examine the correlations between genotypes and haplotypes and continuous traits and to evaluate the Least Squares Means. For all the data, $P < 0.05$ was significant, and $P < 0.01$ was highly significant.

RESULTS

***FAT1* tissue expression in sheep.** Firstly, we investigated *FAT1* tissue expression in Chinese Merino sheep using quantitative real-time PCR. The results showed that the *FAT1* gene was more highly expressed in the body side skin than in the other tested SF sheep tissues except kidney ($P < 0.05$; Figure 1A). Furthermore, we investigated the skin *FAT1* expression in Kazak sheep, which have different wool quality traits, and the comparison

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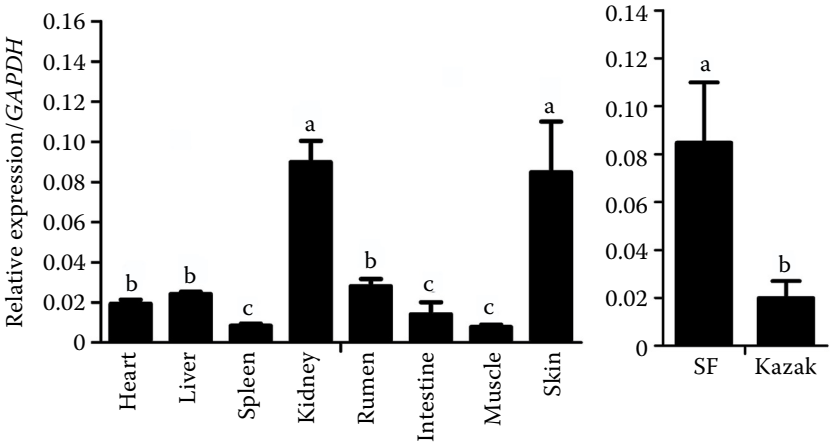

Figure 1. Expression of the *FAT1* gene in sheep: (**A**) *FAT1* gene expression in various tissues of superfine wool strain sheep (SF, $n = 3$); (**B**) *FAT1* gene expression levels in the body side skin between SF sheep ($n = 3$) and Kazak sheep ($n = 3$). The reference gene *GAPDH* was used as an internal control for qPCR analysis

$^{a–c}$significant difference ($P < 0.05$)

showed that skin *FAT1* expression was 4.26-fold higher in SF sheep than in Kazak sheep ($P < 0.05$; Figure 1B). These data suggest that *FAT1* may affect the development of sheep wool quality traits.

Identification of sheep *FAT1* gene SNPs. Two partially overlapping *FAT1* fragments (1 692 and 1 406 bp, respectively) were PCR amplified from sheep genomic DNA and mapped to Chr26. 15,259,862–15,261,553 and Chr26. 15,261,526–15,262,931, respectively. Sanger sequencing identified a total of seven SNPs (termed SNPs 1–7) in the two overlapping *FAT1* genomic fragments. These seven identified SNPs were all located in exon 2 of the sheep *FAT1* gene. Of these seven SNPs, SNP1 was a missense mutation (T213I) and the other six SNPs were all synonymous mutations (Table 2).

Frequencies of alleles and genotypes of sheep *FAT1* gene SNPs. We genotyped these seven SNPs in a total of 744 ewes (from the six tested strains A, B, PM, PW, SF, and U) of Chinese Merino sheep (Junken type) using a single base extension assay. The MAF of these seven SNPs ranged from 0.0587 to 0.4269 (Table 3). The observed heterozygosity and expected heterozygosity ranged from 0.1091 to 0.5045 and 0.1106 to 0.4898, respectively. The χ^2 test showed that the SNPs were all in Hardy–Weinberg equilibrium ($P > 0.05$, Table 3).

Association of sheep *FAT1* SNPs with wool quality traits. The association between the *FAT1* SNPs and wool quality traits was analysed using the linear model 1 in JMP 7.0 (Table 4). In the tested population, SNPs 2 and 7 were significantly associated with wool crimp ($P < 0.05$, Table 4), SNPs 4 and 5 were significantly associated with wool fibre length ($P < 0.05$, Table 4), and SNP 7 was highly significantly associated with average wool fibre diameter ($P < 0.01$, Table 4). SNP2-*GG*

Table 2. Summary of the seven identified SNPs in the sheep *FAT1* gene

SNP ID	Chromosome position[1] (bp)	Nomenclature[2]	db SNP rs # ID	Function class
SNP 1	Chr 26. 15,262,663	g.16025 *C* > *T*	rs161529005	T213I
SNP 2	Chr 26. 15,262,485	g.16203 *G* > *A*	novel	synonymous mutation
SNP 3	Chr 26. 15,262,227	g.16461 *A* > *G*	rs161528998	synonymous mutation
SNP 4	Chr 26. 15,262,134	g.16554 *G* > *A*	rs161528993	synonymous mutation
SNP 5	Chr 26. 15,262,125	g.16563 *G* > *T*	rs161528992	synonymous mutation
SNP 6	Chr 26. 15,261,306	g.17382 *A* > *C*	novel	synonymous mutation
SNP 7	Chr 26. 15,060,362	g.218326 *C* > *T*	novel	synonymous mutation

[1]nucleotides are numbered according to the *Ovis aries* genome [(ISGC Oar_v3.1/oviAri3), http://genome.ucsc.edu/cgi-bin/hgBlat] and located in chromosome 26 at 15,259,862 to 15,262,931 bp; [2]nucleotides are numbered according to the SNP position in relation to the transcription initiation site of *FAT1* gene ([ISGC Oar_v3.1/oviAri3], http://genome.ucsc.edu/cgi-bin/hgBlat); db = database; rs# = reference SNP

https://asia.ensembl.org/Ovis_aries/Gene/Variation_Gene/Table?g=ENSOARG00000008398;r=26:15156246-15263300;t=ENSOART00000009160

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Table 3. Summarized information for the seven identified SNPs in the sheep *FAT1* gene

SNP ID	Alleles[1]	MAF	Ho	He	HWE *P*-value
SNP 1	*C*/*T*	0.1289	0.2140	0.2248	0.1656
SNP 2	*G*/*A*	0.0587	0.1091	0.1106	0.9111
SNP 3	*A*/*G*	0.3656	0.4584	0.4642	0.8561
SNP 4	*G*/*A*	0.3448	0.4406	0.4521	0.6829
SNP 5	*G*/*T*	0.3657	0.4543	0.4642	0.6196
SNP 6	*A*/*C*	0.4269	0.5045	0.4898	0.5318
SNP 7	*C*/*T*	0.4232	0.4979	0.4886	0.6602

MAF = minor allele frequency; Ho = observed heterozygosity; He = expected heterozygosity; HWE = Hardy–Weinberg equilibrium

[1]*A*/*B* implies that *B* is the minor allele

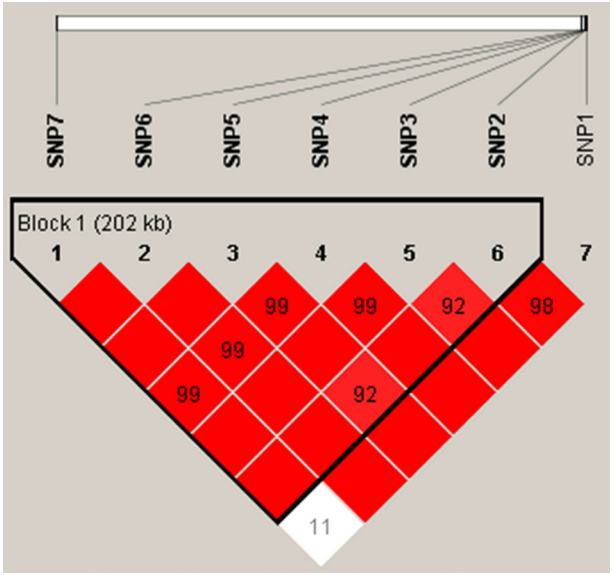

Figure 2. Linkage disequilibrium analysis of the seven identified *FAT1* SNPs

the number in each block means 100 × D' value (e.g. "11" means D' = 0.11); the red block with no number means D' = 1.00

sheep had significantly higher wool crimp than SNP2-*GA* sheep ($P < 0.05$, Table 4). SNP4-*AA* sheep and SNP5-*TT* sheep had significantly longer wool fibre than SNP4-*GA*/*GG* sheep and SNP5-*TG*/*GG* sheep, respectively ($P < 0.05$, Table 4). SNP7-*TC* sheep had significantly lower average wool fibre diameter than SNP7-*TT* sheep, and higher wool crimp than SNP7-*CC* sheep ($P < 0.05$, Table 4).

Association of sheep *FAT1* gene haplotypes with wool quality traits. In the tested population, *FAT1* SNPs 2–7 were in strong linkage disequilibrium block (D' > 0.85, Figure 2). The association between the *FAT1* haplotypes (SNPs 2–7) and wool quality traits was analysed using the linear model 2 in JMP 7.0. The results showed that *FAT1* haplotypes (SNPs 2–7) were significantly associated with wool crimp ($P < 0.05$, Table 5). Of these identified haplotypes, the haplotypes H1–H3 and H5 were significantly associated with higher wool crimp ($P < 0.05$, Table 5).

Bioinformatics analysis. To understand the molecular mechanisms underlying the association between SNPs 2, 4, 5 and 7 and the wool quality traits, we predicted the effect of each of these

Table 4. Effects (Least Squares Means) of *FAT1* genotypes on wool quality traits (only traits associated with the identified SNPs are presented)

SNP ID	Traits	*P*-value	Genotype (animals *n*)		
			GG (642)	***GA*** (79)	
SNP 2	wool crimp, crimps/2.5 cm	0.0125*	12.23 ± 0.09[a]	11.40 ± 0.32[b]	
			AA (87)	***GA*** (308)	***GG*** (304)
SNP 4	wool fibre length (cm)	0.0167*	9.79 ± 0.16[a]	9.28 ± 0.08[b]	9.35 ± 0.09[b]
			TT (100)	***TG*** (328)	***GG*** (294)
SNP 5	wool fibre length (cm)	0.0253*	9.71 ± 0.15[a]	9.25 ± 0.07[b]	9.34 ± 0.09[b]
			TT (126)	***TC*** (360)	***CC*** (237)
SNP 7	average wool fibre diameter (μm)	0.0015**	21.21 ± 0.21[a]	20.41 ± 0.10[b]	20.74 ± 0.14[ab]
	wool crimp, crimps/2.5 cm	0.0494*	12.13 ± 0.24[ab]	12.38 ± 0.12[a]	11.92 ± 0.16[b]

[a,b]means in a row with different superscripts significantly differ ($P < 0.05$)

*$P < 0.05$; **$P < 0.01$;

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Table 5. Effects (Least Squares Means) of *FAT1* haplotypes (SNP2–7) on wool quality traits (only traits associated with the identified haplotypes are presented)

Haplotype (*n*)	Frequency (%)	Wool crimp, crimps/2.5 cm
H1: *GAGGAT* (636)	0.42	12.31 ± 0.09[a]
H2: *GGATCC* (534)	0.36	12.10 ± 0.10[a]
H3: *GAGGAC* (139)	0.08	12.26 ± 0.22[a]
H4: *AAGGAC* (84)	0.07	11.41 ± 0.32[b]
H5: *GAGGCC* (83)	0.06	12.78 ± 0.33[a]
P-value[1]		0.0201*

[1]*P*-value of the association between these haplotypes and wool quality traits; *$P < 0.05$

[a,b]means within the column with different superscripts differ significantly ($P < 0.05$)

four synonymous SNPs on pre-mRNA splicing by using the ESE Finder (Smith et al. 2006). The results showed that SNP 2 might abolish a binding site for serine/arginine-rich splicing factor 2 (SRSF2) and a binding site for SRSF5, SNP 4 might abolish a binding site for SRSF5, and SNP 7 might abolish a binding site for SRSF1 (IgM-BRCA1) and a binding site for SRSF6 (Table 6). In contrast, SNP 5 might create a binding site for SRSF2 and a binding site for SRSF5, and SNP 7 might create a binding site for SRSF1 and a binding site for SRSF6 (Table 6). The SNP effects on the local RNA secondary structure were predicted using the RNA SNP Web Server (Sabarinathan et al. 2013). The results showed that SNPs 2 and 4 might alter the *FAT1* mRNA secondary structure (Figure 3).

DISCUSSION

Hair follicles are parts of the skin which grow wool and determine wool characteristics. Considering the significant difference in wool quality traits between Merino and Kazak sheep, the differential skin expression of *FAT1* gene between SF and Kazak sheep indicates that FAT1 might be involved in wool quality trait formation.

In the present study, a total of 744 Chinese Merino ewes were used for the association study. These sheep consisted of six strains differing in the production and wool quality traits. To avoid false or ambiguous conclusions, the sheep strain (line, *L*) was included as a fixed effect in our statistical model (Bentsen 1991). Furthermore, the interaction effects of *G* (genotype) × *L* (line), *H* (haplotype) × *L* (line), and *A* (age) × *L* (line) were taken into account (Wagner 2015).

There is a known negative correlation between average wool fibre diameter and wool crimp in sheep (Naylor 1995). In our sheep population, SNP 7 was significantly associated with both wool fibre diameter and wool crimp. SNP7-*TC* sheep had significantly lower average wool fibre diameter than SNP7-*TT* sheep, and higher wool crimp than SNP7-*CC* sheep ($P < 0.05$, Table 4). These

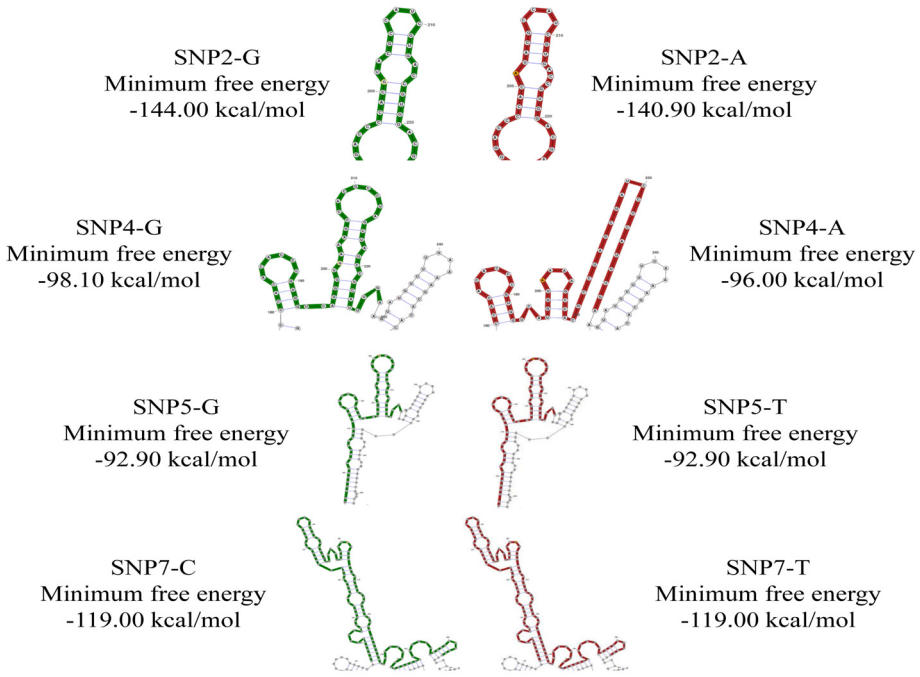

Figure 3. Computational predictions of the effects of the synonymous SNPs on *FAT1* mRNA secondary structure

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Table 6. Computational predictions of the effects of the wool quality trait-associated SNPs on the *FAT1* pre-mRNA splicing

SNP[1]	ESE Finder[2]	ESE creating[3]	ESE abolishing[4]
SNP 2 (*G* > *A*)	changed	no effect	binding site for SRSF2, binding site for SRSF5
SNP 4 (*G* > *A*)	changed	no effect	binding site for SRSF5
SNP 5 (*G* > *T*)	changed	binding site for SRSF2, binding site for SRSF5	no effect
SNP 7 (*C* > *T*)	changed	binding site for SRSF1, binding site for SRSF2	binding site for SRSF1 (IgM-BRCA1), binding site for SRSF6

ESE = exon splicing enhancer; SRSF1/2/5/6 = serine and arginine-rich splicing factor 1/2/5/6
[1]*A* > *B* implies the change from allele *A* to allele *B*; [2]ESE Finder: http://krainer01.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi?process=home; [3]ESE creating and [4]ESE disrupting imply that the effects of *A* > *B* by using the ESE Finder program

data suggested that SNP 7 could be a molecular marker for marker-assisted selection (MAS) for high-quality wool in sheep. However, this needs further validation in larger populations.

Most wool quality traits are quantitative characters and are controlled by multiple genes (Bolormaa et al. 2017). A single SNP might have only a small effect on the related traits (Vandeputte et al. 2007). Linkage disequilibrium analysis could examine possible interactions amongst SNPs, and therefore haplotype analysis could provide more accurate information on the relationship between SNPs and traits (Stephens et al. 2001). In the present study, the *FAT1* haplotypes (H1–H3 and H5) were significantly associated with higher wool crimp ($P < 0.05$, Table 5), suggesting that the haplotypes H1–H3 and H5 could be molecular markers for MAS for high-quality wool in sheep. However, these need to be validated in the future.

Generally, synonymous mutations are assumed to cause no changes in the function of coded proteins. However, accumulated evidence has suggested that some synonymous mutations can affect alternative and constitutive splicing (Pagani and Baralle 2004; Kimchisarfaty et al. 2007) and mRNA secondary structure (Jubao et al. 2003), leading to an alteration in gene function and phenotype. In the present study, the four synonymous mutations (SNPs 2, 4, 5 and 7) were associated with wool quality traits (Table 4), and our bioinformatics analysis showed these four SNPs might affect the pre-mRNA splicing by creating or abolishing the binding sites for serine/arginine-rich splicing factors (SRSFs), such as SRSF1, SRSF2, SRSF5, and SRSF6 (Table 6), which are essential for pre-mRNA splicing (Zuo and Manley 1993; Kim et al. 2009), and RNA secondary structure analysis using the RNA SNP Web Server showed that SNPs 2 and 4 might alter the *FAT1* mRNA secondary structure (Figure 3). To date, there has been no evidence that SRSFs, RNA structure and wool quality traits have some relationship with each other. To determine whether these SNPs are causative mutations, much work has to be done in the future. For example, the regulatory roles of SRSFs and RNA secondary structure on *FAT1* gene expression and the functions of FAT1 and SRSFs in wool quality trait formation should be fully investigated.

Wang et al. performed the genome-wide association study (GWAS) on the same population using the Illumina ovine 50K SNP array, but the GWAS results did not detect the association between the *FAT1* gene polymorphisms and wool quality traits (Wang et al. 2014). This discrepancy might be explained by two reasons. First, the SNPs used for the two studies were different. The Illumina ovine 50K SNP array used in Wang's GWAS analysis had only two *FAT1* SNPs (rs407652318 and rs430442466), while in our present study, seven *FAT1* SNPs were used to perform the association analysis, and there were no SNPs in common between Wang's GWAS and our present study (Table 2). Second, GWAS generally generates high false-negative results due to the conservative algorithm (Moran 2003; Li et al. 2016). In Wang's GWAS analysis, due to a lack of 70.97% of the population pedigree information, the Bonferroni correction method was used to reduce the false-positive rate (Wang et al. 2014), which may lead to a further increase in the false-negative rate (Strucken et al. 2014).

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CONCLUSION

In summary, the *FAT1* gene was differentially expressed in the skin between Merino and Kazak sheep, and four synonymous *FAT1* SNPs (SNPs 2, 4, 5 and 7) and haplotypes (H1–H3 and H5) were associated with wool quality traits. These identified *FAT1* SNPs and haplotypes could be used as molecular markers for MAS for high-quality wool in Chinese Merino sheep.

Conflict of interest. The authors declare no conflict of interest.

REFERENCES

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263-265.

Bentsen HB, Klemetsdal G. The use of fixed effect models and mixed models to estimate single gene associated effects on polygenic traits. Genet. Sel. Evol. 1991;23:407-419.

Bolormaa S, Swan AA, Brown DJ, Hatcher S, Moghaddar N, Werf JHVD, Goddard ME, Daetwyler HD. Multiple-trait QTL mapping and genomic prediction for wool traits in sheep. Genet. Sel. Evol. 2017;49:62.

Chrostek A, Wu X, Quondamatteo F, Hu R, Sanecka A, Niemann C, Langbein L, Haase I, Brakebusch C. Rac1 is crucial for hair follicle integrity but is not essential for maintenance of the epidermis. Mol. Cell. Biol. 2006; 26:6957-6970.

Cottle DJ. International Sheep and Wool Handbook. Nottingham, UK: Nottingham University Press; 2010.

Cox B, Hadjantonakis AK, Collins JE, Magee AI. Cloning and expression throughout mouse development of *mfat1*, a homologue of the *Drosophila* tumour suppressor gene *fat*. Dev. Dyn. 2000;217:233-240.

Dai D, Zhu H, Wlodarczyk B, Zhang L, Li L, Li AG, Finnell RH, Roop DR, Chen J. Fuz controls the morphogenesis and differentiation of hair follicles through the formation of primary cilia. J. Invest. Dermatol. 2011;131:302-310.

Gumbiner BM. Regulation of cadherin-mediated adhesion in morphogenesis. Nat. Rev. Mol. Cell. Biol. 2005;6:622-634.

Jubao D, Wainwright MS, Comeron JM, Naruya S, Sanders AR, Joel G, Gejman PV. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. Hum. Mol. Genet. 2003;12:205-216.

Kim DJ, Oh B, Kim YY. Splicing factor ASF/SF2 and transcription factor PPAR-γ cooperate to directly regulate transcription of uncoupling protein-3. Biochem. Biophys. Res. Commun. 2009;378:877-882.

Kimchisarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MM. A "silent" polymorphism in the *MDR1* gene changes substrate specificity. Science. 2007;315:525-528.

Li H, Yuan Z, Ji J, Xu J, Zhang T, Zhang X, Xue F. A novel Markov Blanket-based repeated-fishing strategy for capturing phenotype-related biomarkers in big omics data. BMC Genet. 2016;17:51.

Li S, Zhou H, Gong H, Zhao F, Wang J, Liu X, Luo Y, Hickford JGH. Identification of the ovine keratin-associated protein 22-1 (KAP22-1) gene and its effect on wool traits. Genes. 2017;8:27.

Ling YH, Xiang H, Zhang G, Ding JP, Zhang ZJ, Zhang YH, Han JL, Ma YH, Zhang XR. Identification of complete linkage disequilibrium in the *DSG4* gene and its association with wool length and crimp in Chinese indigenous sheep. Genet. Mol. Res. 2014;13:5617-5625.

Ma GW, Chu YK, Zhang WJ, Qin FY, Xu SS, Yang H, Rong EG, Du ZQ, Wang SZ, Li H, Wang N. Polymorphisms of FST gene and their association with wool quality traits in Chinese Merino sheep. PLoS ONE. 2017;12:e0174868.

Matis M, Axelrod JD. Regulation of PCP by the Fat signaling pathway. Genes Dev. 2013;27:2207-2220.

Moran MD. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos. 2003;100:403-405.

Mu F, Rong E, Jing Y, Yang H, Ma G, Yan X, Wang Z, Li Y, Li H, Wang N. Structural characterization and association of ovine *Dickkopf-1* gene with wool production and quality traits in Chinese Merino. Genes. 2017;8:400.

Naylor G. Does the fibre diameter distribution of wool consist of a mixture of two components? Wool Technol. Sheep Breed. 1995;43:3.

Pagani F, Baralle FE. Genomic variants in exons and introns: identifying the splicing spoilers. Nature Rev. Genet. 2004;5:389-396.

Rong EG, Yang H, Zhang ZW, Wang ZP, Yan XH, Li H, Wang N. Association of methionine synthase gene polymorphisms with wool production and quality traits in Chinese Merino population. J. Anim. Sci. 2015;93:4601-4609.

Sabarinathan R, Tafer H, Seemann SE, Hofacker IL, Stadler PF, Gorodkin J. The RNAsnp web server: predicting SNP effects on local RNA secondary structure. Nucleic Acids Res. 2013;41:W475-W479.

Shapiro L, Fannon AM, Kwong PD, Thompson A, Lehmann MS, Grubel G, Legrand JF, Als NJ, Colman DR, Hendrickson WA. Structural basis of cell-cell adhesion by cadherins. Nature. 1995;374:327-337.

Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, Krainer AR. An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. Hum. Mol. Genet. 2006;15:2490-2508.

Stephens JC, Schneider JA, Tanguay DA, Choi J, Acharya T, Stanley SE, Jiang R, Messer CJ, Chew A, Han JH. Haplotype

https://doi.org/10.17221/224/2019-CJAS

variation and linkage disequilibrium in 313 human genes. Science. 2001;293:489-493.

Strucken EM, Schmitt AO, Bergfeld U, Jurke I, Reissmann M, Brockmann GA. Genomewide study and validation of markers associated with production traits in German Landrace boars. J. Anim. Sci. 2014;92:1939-1944.

Vandeputte M, Dupontnivet M, Chavanne H, Chatain B. A polygenic hypothesis for sex determination in the European sea bass *Dicentrarchus labrax*. Genetics. 2007;176:1049-1057.

Wagner GP. Two rules for the detection and quantification of epistasis and other interaction effects. Methods Mol Biol. 2015;1253:145-157.

Wang Y, Chang H, Nathans J. When whorls collide: the development of hair patterns in frizzled 6 mutant mice. Development. 2010;137:4091-4099.

Wang Z, Zhang H, Yang H, Wang S, Rong E, Pei W, Li H, Wang N. Genome-wide association study for wool production traits in a Chinese Merino sheep population. PLoS ONE. 2014;9:e107101.

Yeh FC. POPGENE Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis. Available at: https://sites.ualberta.ca/~fyeh/popgene_download.html

Zuo P, Manley JL. Functional domains of the human splicing factor ASF/SF2. EMBO J. 1993;12:4727-4737.

Received: October 28, 2019
Accepted: January 24, 2020