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**Short Communication**

# Polymorphisms within *CEBPA, PRKAG3* and *SREBF1* Genes Associated with Fat Deposition in Fat-tail Altay Sheep

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**GX performed experiments and collected data. RD and YW collected data. SG and SL provided study materials. XW and WH analyzed data. QL and MC designed the experiments, supervised the project and wrote the manuscript.**

**Key words**
**Altay sheep, Fat tail, CEBPA, PRKAG3, SREBF1, SNP**

## ABSTRACT

The fat tail/rump is considered as an adaptive selection under harsh challenges which serves as a fat store for the animal. However, the mechanism of fat deposition in tail is unclear. The polymorphisms of candidate sheep *CEBPA*, *PRKAG3* and *SREBF1* genes and their relationship with fat deposition between fat-tailed (rumped) and thin-tailed breeds were investigated. Two and one SNPs were identified for *PRKAG3* and *SREBF1* respectively. Genotyping method was used to analyze genotypes among Altay sheep (fat-rumped breed) and White Suffolk (thin-tailed breed) by Sequenom MassArray. For *PRKAG3* gene, a c.1744C>T SNP and a c.1840C>T SNP have been genotyped. For *SREBF1* gene, an unknown synonymous c.2878A>G SNP was detected. The genotype distributions in those two loci were significantly different between fat tail and thin tail breeds by *chi*-square test (P<0.05).

There is a spectrum of phenotypically diverse populations of sheep in the worldwide due to their adaptability to poor nutrition diets, tolerance to extreme climatic conditions and their manageable size (Mohammad *et al*., 2012). The fat tail/rump is considered as an adaptive selection under harsh challenges which serves as a fat store for the animal.

To date, the next-generation sequencing platforms have been employed to explore candidate genes/region associated with fat deposition in thin and fat tail sheep breeds. A genome-wide scan was performed between Iranian thin and fat tail sheep breeds, and three novel regions located on Chromosomes 5, 7 and X were identified to associate with fat deposition in thin and fat tail sheep breeds (Mohammad *et al*., 2012). *De novo* transcriptome sequencing was used to compare sheep adipose tissue transcriptome profiles between fat-tailed and short-tailed breeds, and 646 differentially expressed genes and amounts of functional pathways were identified (Wang *et al*., 2014). In general, the genes affecting fat deposition in fat tails of sheep are still unknown.

CCAAT/enhancer binding protein, alpha (*CEBPA*) possesses many of the characteristics required for such a "master regulator", which can coordinately activate transcription of many adipocyte genes (MacDougald *et al*., 1995). Protein kinase, AMP-activated, gamma 3 non-catalytic subunit (*PRKAG3*) encodes regulatory γ subunit of adenosine monophosphate activated protein kinase (*AMPK*) whose mutations have been correlated with increased glycogen content and fatty acid uptake (Ryan *et al*., 2012). Sterol regulatory element binding transcription factor 1 (*SREBF1*) encodes a transcription factor that binds to the sterol regulatory element-1 (SRE1), which is a decamer flanking the low density lipoprotein receptor gene involved in sterol biosynthesis (Alvarez *et al*., 2014). In this study, the polymorphisms of sheep *CEBPA*, *PRKAG3* and *SREBF1* genes and their association with fat deposition between fat- rumped and thin-tailed breeds were investigated.

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*Materials and methods*

All procedures involving animals were approved by the animal care and use committee at the respective institutions where the experiment was conducted. All procedures involving animals were approved and authorized by the Chinese Ministry of Agriculture.

For SNP analysis study, 200 ewes of two different breeds reared in China were selected. The animals were distributed as follows: 100 Altay sheep in Fuyun Breeding Farm (Fuyun County, Xinjiang Uygur Autonomous Region, P.R. China), 100 White Suffolk in Beijing Aoxin Stud Farm Co. Ltd. (Beijing, P.R. China). All the sheep were in a good state of health and nutrition. Ear tissue taken from each Altay sheep was immersed in 70% ethanol under 4°C and stored at -20°C pending for DNA isolation. Venous jugular blood of White Suffolk was collected using acid citrate dextrose as an anticoagulant. Genomic DNA was extracted from ear tissue or whole blood by the phenol-chloroform method, and then dissolved in TE buffer (10 mmol/l Tris-HCl (pH 8.0), 1 mmol/l EDTA (pH 8.0)) and kept at -20℃.

As shown in Supplementary Table S1, primers of *CEBPA*, *PRKAG3* and *SREBF1* genes were designed according to the mRNA sequences of sheep derived from GenBank database. Polymerase chain reactions and were carried out as previously described (Liu *et al*., 2015).

The PCR products were separated by electrophoresis on 2% agarose gels (Promega, Madison, WI, USA) in parallel with DNA marker Ⅰ (Tiangen, Beijing, P.R. China). Gels were visualized using a 1.5% agarose gel that contained ethidium bromide, photographed, and analyzed using an AlphaImagerTM 2200 and 1220 Documentation and Analysis Systems (Alpha Innotech Corporation, San Leandro, CA, USA).

For SNP analysis 10 individuals for each sheep breed were selected randomly. Genomic DNA from Altay sheep and White Suffolk sheep was used as template to amplify with primers as shown above and sequences were aligned to search for the base pair variations. PCR products were separated on 2% agarose gels and recovered using Geneclean Ⅱ kit (Promega, Madison, WI, USA). Each DNA fragment was sequenced in both directions using an automatic ABI 3730 sequencer (Perkin Elmer Applied Biosystems, Foster City, CA, USA) by SinoGenoMax Co. Ltd. (Beijing, China).

Sequence analysis, and amino acid determination were performed with the program DNAMAN version 9.0 and DNAstar lasergene version 7.1.

For genotyping analysis three SNPs were selected for genotyping by using 200 samples from both Altay and White Suffolk sheep. Genotyping was performed using primer extension chemistry and mass spectrometric analysis (iPlex assay, Sequenom, San Diego, CA) on the Sequenom MassArray according to the manufacturer's instructions (http://www.sequenom.com). Only those samples with a > 95% success rate and only those SNPs with a genotype success rate of > 95% were included in the analysis.

Allele and genotype frequencies were estimated by direct counting. Statistical analyses were performed by use of the SAS (Ver 8.1) (SAS Institute Inc., Cary, NC, USA). Differences between two groups of samples were accessed by *t*-tests assuming unequal variances. P values less than 0.05 were considered to be significant. Chi-square test was applied to analyze the statistical significance of loci genotype distributions of two sheep breeds.

*Results*

Zero SNP was identified for *CEBPA*. Two SNPs were identified and genotyped for *PRKAG3*, and one SNP was identified and genotyped for *SREBF1*. SNPs were selected for genotyping by using 200 samples from Altay and White Suffolk sheep on the Sequenom MassARRAY plateform (Gabriel *et al*., 2009). As shown in Supplementary Figure S1, for *PRKAG3* gene, a c.1744C>T SNP and a c.1840C>T SNP (GenBank accession no. NM_001122692; Both are synonymous) have been genotyped. For *SREBF1* gene, an unknown synonymous A>G SNP was also detected (c.2878A>G, GenBank accession no. XM_004013336).

The allele and genotype frequencies of *PRKAG3* and *SREBF1* genes in Altay and White Suffolk sheep were calculated respectively as shown in Table I after genotype detection. As shown in Table II, allele C is dominant allele at the c.1840C>T of *PRKAG3* gene in both two breeds, while in the c.2878A>G locus of *SREBF1* gene, allele G is dominant allele. It was also shown that the genotype distributions in above two loci were significantly different between fat tail and thin tail breeds by chi-square test ($P<0.05$).

*Discussion*

Altay sheep chosen for this study has a large rump composed entirely of white adipose tissue which is known for their ability to cope with harsh environmental conditions such as drought and famine in northern part of Xinjiang Uygur Autonomous Region. Due to improved forage availability and healthy issue, fat tail trait is commercially undesirable now. For this trait breeders are interested in looking for useful molecular markers to serve sheep breeding program via marker- assisted selection, so searching gene variants affecting the phenotypic expression of fat-tailed trait in sheep are becoming a hot topic in molecular genetics.

Up to now, there is little published information related to tail fatness especially for Chinese local breeds.

**Table I. Allele and genotype frequencies of *PRKAG3* and *SREBF1* genes in two sheep breeds.**

| Genotype | Altay | Suffolk |
|---|---|---|
| ***PRKAG3* c. 1744C>T SNP** | **n=97** | **n=97** |
| Genotype frequency | CC 0.443 (43) | CC 0.515 (50) |
| | CT 0.474 (46) | CT 0.392 (38) |
| | TT 0.083 (8) | TT 0.093 (9) |
| Allele frequency | C 0.68 | C 0.711 |
| | T 0.32 | T 0.289 |
| H–W test $\chi^2$ | 0.793 | 0.206 |
| P | 0.373 | 0.650 |
| ***PRKAG3* c. 1840C>T SNP** | **n=99** | **n=96** |
| Genotype frequency | CC 0.505 (50) | CC 0.552 (53) |
| | CT 0.434 (43) | CT 0.281 (27) |
| | TT 0.061 (6) | TT 0.167 (16) |
| Allele frequency | C 0.722 | C 0.693 |
| | T 0.278 | T 0.307 |
| H–W test $\chi^2$ | 0.674 | 11.1 |
| P | 0.412 | 0.000884** |
| ***SREBF1* c. 2878A>G SNP** | **n=87** | **n=94** |
| Genotype frequency | AA 0.023 (2) | AA 0.021(2) |
| | AG 0.356 (31) | AG 0.117 (11) |
| | GG 0.621 (54) | GG 0.862 (81) |
| Allele frequency | A 0.201 | A 0.08 |
| | G 0.799 | G 0.92 |
| H–W test $\chi^2$ | 1.029 | 3.88 |
| P | 0.310 | 0.0490* |

Note: The numbers in the brackets are the genotype individuals. * P<0.05; ** P<0.01 ($\chi^2_{0.05}$,5.99; $\chi^2_{0.01}$,9.21)

**Table II. Test of difference of loci genotype distributions of *PRKAG3* and *SREBF1* in Altay and Suffolk sheep breeds. GenBank accession numbers for these SNPs can be found in Supplementary Table S1.**

| Breed | Suffolk sheep | | |
|---|---|---|---|
| | **SNP locus** | $\chi^2$ | **P** |
| Altay sheep | *PRKAG3* c. 1744C>T SNP | 1.348 | 0.51 |
| | *PRKAG3* c.1840C>T SNP | 8.246 | 0.016** |
| | *SREBF1* c.2878A>G SNP | 14.675 | 0.001*** |

Note: * P < 0.05, ** P < 0.01, *** P <0.001

Several candidate genes have been studied to associate with fat deposition and lipid metabolism in domestic animals. PPARG and its target genes is one factor leading to greater intramuscular fat deposition in cattle (Moisa *et al*., 2014). *FABP3* gene plays in fat deposition and the regulation of fatty acid metabolism in the Lanzhou fat-tailed sheep (Bai *et al*., 2013). *FABP4* gene mRNA and protein have no significant differences between control and continuous starvation groups which means that *FABP4* may not be the key gene in fat depositon in Altay sheep (Ruixia *et al*., 2015). The mRNA abundance of G-protein coupled receptor 41 (*GPR41*), Adiponectin receptors 1 and 2 (ADIPOR1/2) and LEPTIN are divergent in different fat depots from sheep (Lemor *et al*., 2010). There were novel associations of *DGAT1* gene in which the C allele had a positive effect on fat-tail weight and backfat thickness in fat-tailed sheep (Mohammadi *et al*., 2013). *CAST* gene being a potential candidate gene for growth and meat quality traits has been detected for novel SNPs and breed-specific haplotypes, and *CAST*-10 and *CAST*-8 might be breed-specific haplotypes that distinguish between fat-tailed and thin-tailed sheep breeds (Aali *et al*., 2014).

It has been reported that *CEBPA* highly expressed in fat-rumped sheep while lower expressed in thin-tailed sheep breeds which had significant correlations with fat deposition in tail tissues of sheep (Wei *et al*., 2014). Polymorphisms of sheep *CEBPA, PRKAG3* and *SREBF1* genes and their association with fat deposition between fat-tailed (rumped) and thin-tailed breeds were firstly investigated in the current study. New polymorphic sites of *PRKAG3* gene (c.1744C>T SNP and c.1840C>T SNP) and *SREBF1* gene (c.2878A>G SNP) were detected in our study. Genotype distributions were significantly different between fat tail and thin tail breeds. It may indicate that those two loci may be associated with fat deposition in fat-tail breed.

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*Supplementary material*

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20190121080154

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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