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Three SNPs Identified in the *MC1R* Gene of Nubian Goats may Relate to the Wool Color

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ABSTRACT

MC1R gene is the key gene that determines the color of animal hair. The melanocortin receptor gene of Nubian goat (white, pure black and brown flower) was cloned and analyzed by systematic bioinformatics. The result showed that the coding region of the MC1R gene of Nubian goat was 954 bp in length and encoded 317 amino acids. The results of multiple sequence comparison showed that the clones were 99%, 87%, 86%, 85%, 84%, 81%, and 77% similar to the published pig, cow, human, dog, sheep, mouse, and chicken, respectively. It showed that MC1R gene had a high conserved type among different species. The goat MC1R protein has a molecular mass of 34.65 ku, an isoelectric point of 8.70, which is weakly alkaline, and contains seven transmembrane domains typical of cell membrane receptor proteins. Sequencing analysis of the black, brown and white different color MC1R genes of Nubian goats revealed that there are three SNPs in the gene sequence, which are 219, 712 and 1160, respectively. The C/T mutation did not cause amino acid mutations; base A/G mutations occurred at position 712 and caused amino acid mutations in pure black and brown goat samples, which were mutated from tryptophan to cysteine. The results of this study have important reference significance for the future correlation analysis between MC1R gene and Nubian goat wool color traits and the color genetic mechanism of Nubian goats.

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Authors' Contribution
KC designed the study. QM took blood samples. TF cloned and analyzed the gene. CL analyzed the structure of MC1R. ZZ wrote the article. LS and LQ helped in writing the article.

Key words
Nubian goat, MC1R; Clones, Gene, SNPs

INTRODUCTION

The animal color is mainly related to the quantity, type, synthesis and distribution of true melanin (brown/black) and pseudo-melanin (red/yellow) produced by melanocytes (Chen *et al*., 2013). The key sites controlling the synthesis and regulation of these two pigments are the extension site E (Extention) and the mouse gray site A (Agouti) (Houde *et al*., 2014). Therefore, the bioinformatics analysis of the Nubian goat MC1R gene system was carried out to provide a reference for exploring the molecular mechanism of the black hair color formation in Nubian black goats. Extention-encoded melanocortin receptor-1 (MC1R) has seven transmembrane domains on its surface, which belongs to the superfamilies of G-protein coupled receptors. MC1R is activated by binding to α-MSH. cAMP increases the content of cAMP in the cells, thereby activating the melanin synthesis restriction enzyme (tyrosinase), resulting in an increase in melanin content (Yuhong *et al*., 2012). The ASIP encoded by Agouti protein belongs to a paracrine signal molecule and can compete with α-MSH for binding to MC1R, causing a decrease in cAMP levels, resulting in a decrease in true melanin content and a relative increase in pseudomelanin content (Hongtao *et al*., 2013).Mutations in the MC1R gene changes in animal coat color, such as pigs (Kijas *et al*., 2001), horses (Marklund *et al*., 1996), cattle (Guastella *et al*., 2007), humans (Zalfa *et al*., 2000), canine (Schmutz *et al*., 2003), chicken (Kerje *et al*., 2003), rabbit (Fontanesi *et al*., 2006), sheep (Vage *et al*., 1999). Nubian goats are long-term adaptation and evolution under the geographical and climatic conditions characteristic of southern China. There are three coat colors of white, pure black and brown flowers, related to Nubian. The goat MC1R study has not been reported, and its function related to coat color is not yet clear. This study will use the Nubian goat as a model to clone and analyze the MC1R gene sequence, which will provide a theoretical basis for the future research of Nubian hill wool color.

MATERIALS AND METHODS

Experimental animals

Three healthy adult Nubian goats with white color, pure black and brown flowers were randomly selected. The blood samples were taken at-80°C and stored immediately.

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PCR amplification of MC1R *gene*

DNA was isolated by using the genomic DNA extraction kit was purchased from TianGen Biotechnology Co., Ltd.

A pair of primers were designed with reference to the gene sequence of the common goat MC1R (Gen Bank accession number FM212940) landing in GenBank. The primer sequence is:

MC1R-F: 5'-CCTGAGAGCAAGCACCCTTTCC-3'

MC1R-R: 5'-GAGCGGGACTTCCCACAGC-3'

PCR amplification mixture (30 μL) comprised MasterMix 12 μL, upstream and downstream primers were each (10 μmol/L) 1.5 μL, template DNA (50-100 ng/μL) 1.5 μL, and ultrapure water 15 μL.

The following PCR reaction procedure was adopted pre-denaturation at 95 ° C for 3 min; denaturation at 95°C for 30 s, annealing at 66 °C for 40 s, 35 cycles; finally at 72 °C for 7 min, then cooled to 4 °C for storage. The PCR product was electrophoresed on a 1% agarose gel (including GoldView's accounting dye) and analyzed by a gel imaging system. The PCR amplification products were sequenced. The length of the amplified Nubian goat MC1R gene fragment is expected to be about 1300 bp.

The sequencing results were verified, corrected and the base length and base difference of the sequence were verified by DNAMAN. Bioinformatics software including phylogenetic trees and protein structure analysis is referenced in the article (Lui *et al*., 2020). We also blast the MC1R sequence in the NCBI database.

RESULTS

Figure 1 shows 1276bp amplification product. The sequencing results of PCR products showed that the DNA fragments of MC1R gene of three different coats of Nuby goats amplified in this study were all 1 276 bp in length, the start codon was -ATG, and the stop codon was -TGA, open reading frame. (ORF) is 954 bp long and encodes 317 amino acids (Fig. 2).

MC1R protein

The amino acid content of MC1R protein was analyzed, with the highest leucine content (17.35%), followed by alanine (9.78%) and valine (9.46%), and the lowest leucine content (1.26%). The molecular mass of MC1R protein was 34.65 ku, and the isoelectric point was 8.70, which was weakly alkaline.

The transmembrane domain is the main part of the membrane-bound protein and membrane lipid. It is anchored to the cell membrane and is generally composed of about 20 hydrophobic amino acids. The transmembrane helix region of the Nubian goat MC1R gene was predicted by the TMHMM Server.2.0 tool in Expasy software (Fig. 3). The predicted results show that the amino acid sequence of the Nu1 goat MC1R gene contains seven transmembrane domains typical of the cell membrane receptor protein, which is consistent with the characteristics of the cell membrane receptor protein.

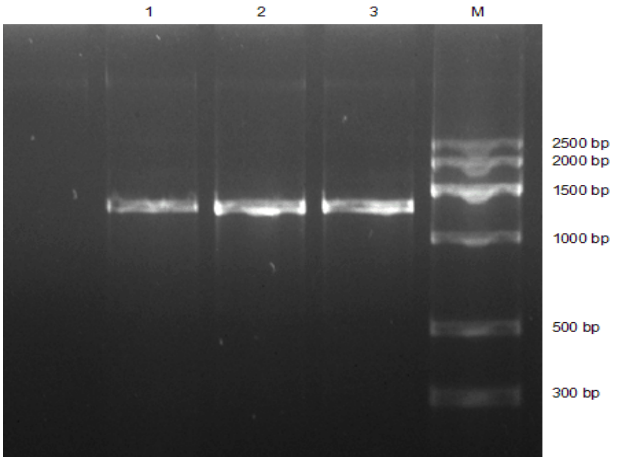

Fig. 1. White Nubian goat; 2: Black Nubian goat; 3: Brown Nubian goat; M: DNA Marker VII.

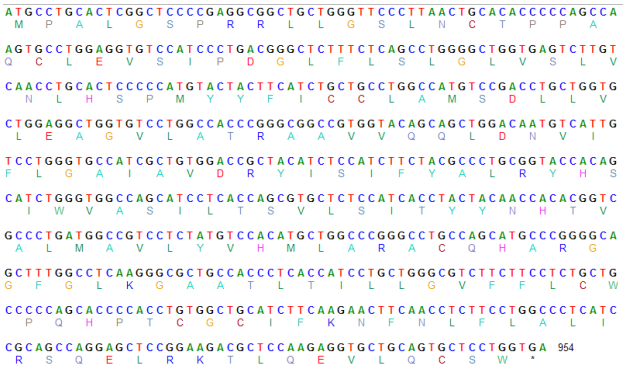

Fig. 2. MC1R gene sequence and amino acid sequence of Nubian goat.

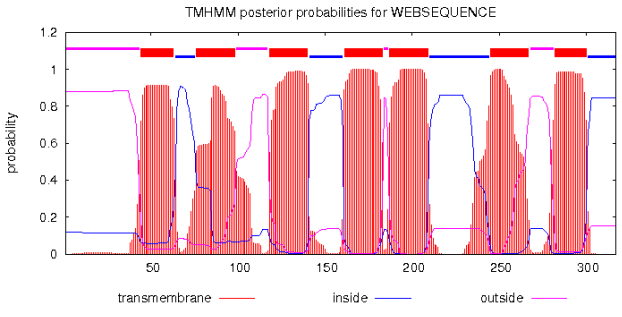

Fig. 3. The protein domain of Nubian goat MC1R.

Analysis of nubian goat MC1R *gene*

The results of multiple sequencing and alignment results expression, fragments were similar to pig, cow, human, dog sheep, mouse and chicken with 99%, 87%, 86%, 85%, 84%, 81% and 77% identity respectively, indicating that MC1R gene had a relatively high conserved type among different species. The predicted amino acid sequence of the Nubian goat MC1R was compared with the sequences of other species (Figs. 4 and 5). The homology was sheep 315/317 (99%), cattle 308/317 (97%), and cat (83.91). %), canine (82.65%), horse (93.38%), human (80.13%), rabbit (76.34%), mouse (75.48%), chicken (61.83%). The results also showed that it was highly conserved in different species at the protein level, and also indicated that the predicted reading frame for the predicted MC1R amino acid sequence was correct.

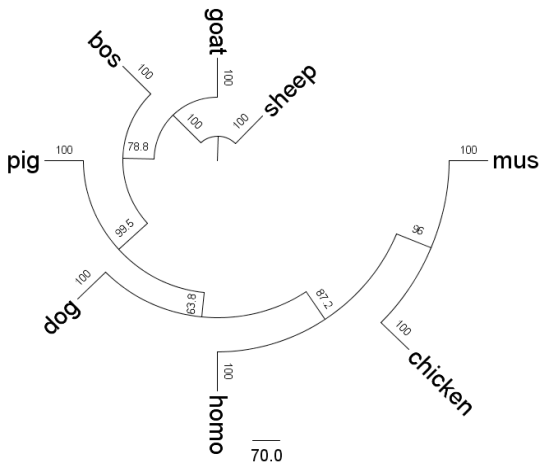

Fig. 4. Phylogenetic tree of deduced anino acid sequences of MC1R.

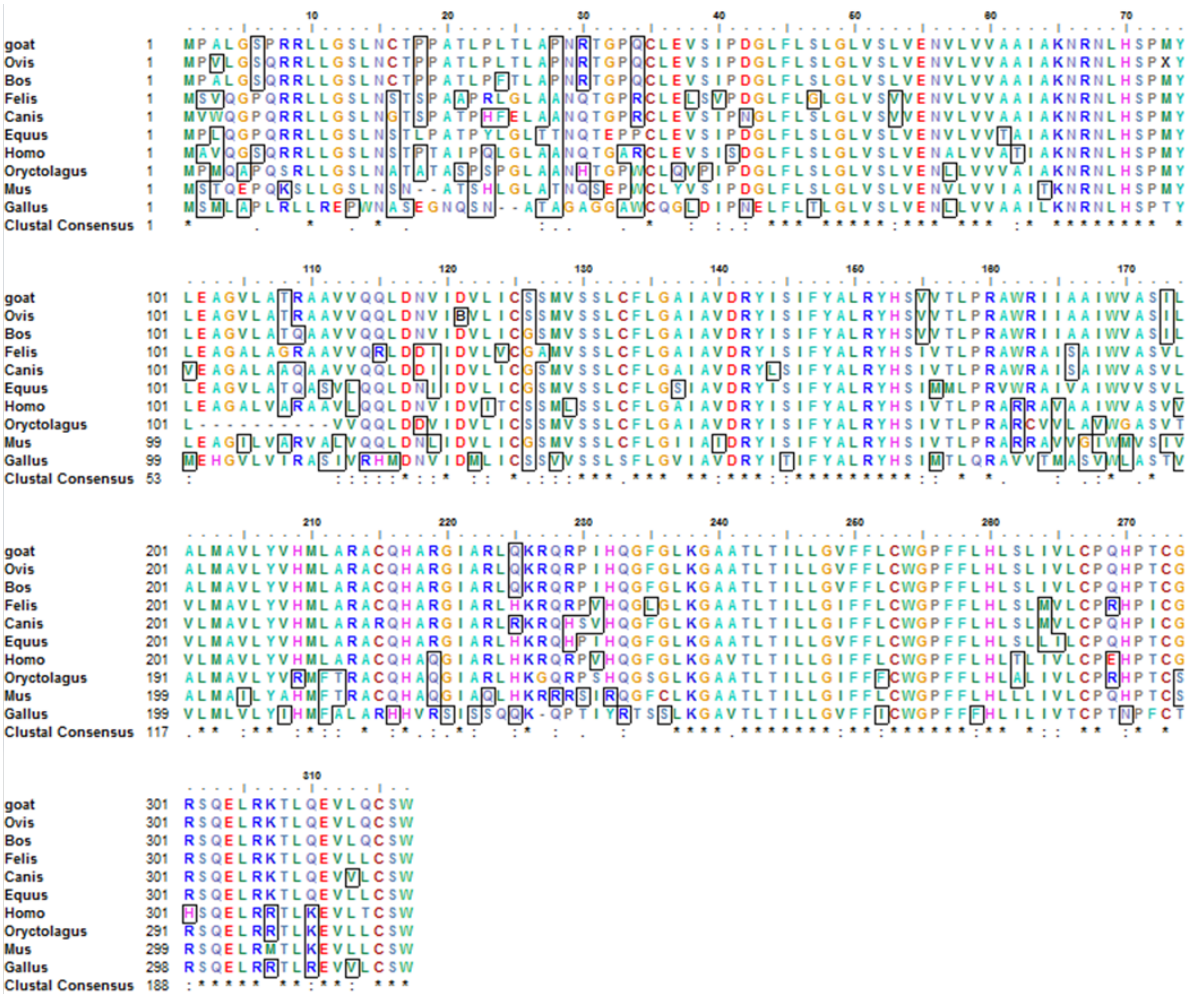

Fig. 5. Multiple alignment and analysis of deduced anino acid sequences of MC1R.

Analysis of mutation sites of MC1R *gene in different hair color*

Multiple sequence alignment of the gene sequences revealed that the MC1R gene of the Nubian goat and the goat standard gene in the Gene Bank database had an Identity=99.16%. Analysis of different coat color sequencing peak maps (Fig. 6 and 7) revealed that there are three SNPs loci in the gene sequence, which are 219, 712 and 1160, respectively. Among them, base C/T mutation occurred at 219 locus, which did not cause amino acid mutation; base A/G mutations occurred at position 712 and caused amino acid mutations in pure black and brown coat goat samples, which were mutated from tryptophan to cysteine. So, we predicted that the mutation of 712 (A/G) caused the black Nubian goat changing to brown coat color, this will be used to breed different economically valuable coat color in Nubian goat.

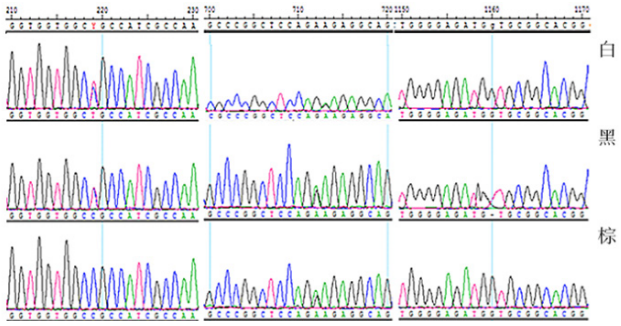

Fig. 6. The different coat color's mutation site of Nubian goat MC1R; Note: White and Black 219(C/T); Black and Brown 712(A/G); Black 1160(G-).

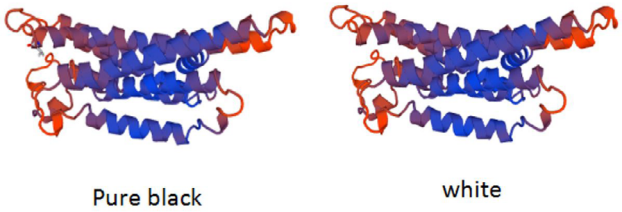

Fig. 7. The tertiary structure of Nubian goat MC1R protein.

DISCUSSION

Many studies have shown that mutations, deletions or insertions of the MC1R gene result in changes in the ratio of black/brown pigments, leading to changes in animal coat color (Demenais *et al*., 2010). In this study, the 1276 bp Nubian goat MC1R gene fragment was cloned for the first time, and the coding region was 954 bp, encoding 317 amino acids. The analysis of the composition of the encoded amino acids revealed that the proportion of hydrophobic amino acids such as leucine and alanine is relatively high, which is beneficial to increase the heat resistance of the protein and reduce the probability of inactivation of the MC1R protein due to environmental heat. Hydrophobin can also resist cell damage caused by the overheating of the skin caused by the absorption of sunlight by pure black hair. By using multiple comparison analysis the MC1R in different animals have high conservative, Nubian goats MC1R gene and NCBI standard goat MC1R gene homology is as high as 99.16%, found three SNP loci (219 loci, 712 loci and 1160 loci), of which 219 loci and 712 loci were caused base mutation, and can be observed clearly double fengfeng figure, at the same time, 1160 samples of pure black base G is missing, whether these SNPS loci directly affects the MC1R gene mutations, missing or insert, lead to changes in the black/brown grain percentage. Further studies are needed to investigate the phenotype differences of Nubian goat hair color (Grasberger *et al*., 1986).

By comparing the goat MC1R gene sequence in NCBI GenBank and consulting relevant literature (Vaiman *et al*., 1996), the results showed that there were base mutations at the MC1R base sites in these three goats, which further indicated that the predicted MC1R amino acid sequence was in the correct reading frame.

CONCLUSION

In this paper, we found that there are three SNPs in the MC1R coding region of different hair color Nubian goats, resulting in two base abnormalities and one base deletion phenomenon. The results have important reference significance for the correlation analysis of MC1R gene with Nubian goat hair color traits and the study on the hair color genetic mechanism of Nubian goat.

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Statement of conflicts of interest

The authors declare that they have no conflict of interest.

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