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Original article

The red head and neck of Boer goats may be controlled by the recessive allele of the *MC1R* gene*

Zhao-Long WU[a,b,c,d], Xiang-Long LI[a,b]**, Yan-Qiang LIU[c], Yuan-Fang GONG[b], Zheng-Zhu LIU[b], Xiao-Juan WANG[b], Tian-Rong XIN[e], Qing JI[c]

[a] College of Animal Science and Technology, Hebei Agricultural University, Baoding, Hebei Province, 071001, P.R. China
[b] Department of Animal Science, Hebei Normal University of Science and Technology, Changli County, 066600, Hebei Province, P.R. China
[c] College of Life Science, Nankai University, Tianjin, 300071, P.R. China
[d] School of Medicine, Jishou University, Jishou, 416000, Hunan Province, P.R. China
[e] School of Life Science, Nanchang University, Nanchang, 330047, Jiangxi Province, P.R. China

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Abstract – The *Melanocortin-1-receptor* (*MC1R*) gene is an important candidate gene for the coat color trait. In order to understand the molecular genetic basis of the red head and neck of *Boer* goats, a comparative analysis of *MC1R* gene polymorphism in imported foreign breed *Boer* goats and another 26 goat populations including *Boe*r goat offspring backcrossed to *Tangshan Dairy* goats (F_1, F_2, F_3 and $F_{4)}$, 18 Chinese main indigenous goat breeds and four other imported foreign goat breeds (including a total of 319 individuals) were analyzed by PCR-RFLP. Two alleles of A and B, and three genotypes of AA, AB and BB were detected. The K226E (A676G) mutation of the *MC1R* detected by sequencing distinguished the B allele from the A allele. The only AA genotype found in *Boer* goats was complete in accordance with their red head and neck. The Chi-square test suggested that the red head and neck of *Boer* goats may be controlled by the recessive A allele of the *MC1R* gene. However, the K226E at the B allele may be a loss of function mutation associated with the whole white coat of goats.

***Boer* goat / red head and neck / *MC1R* gene / K226E mutation / white coat**

Résumé – La tête et le cou rouge chez la chèvre *Boer* seraient contrôlés par un allèle récessif de *MC1R*. Le récepteur 1 de la mélanocortine (*MC1R*) est un gène important pour le déterminisme génétique de la couleur du pelage. Pour comprendre la base moléculaire du caractère « tête et cou rouge » chez des chèvres *Boer* importées et chez 26 populations incluant des descendants améliorés par des croisements de cette chèvre avec celle de la variété laitière des Tangshan (F1, F2, F3, F4),

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** Corresponding author: lixianglongcn@yahoo.com

18 races indigènes chinoises et quatre autres races étrangères importées, représentant un total de 319 individus, a été analysé par PCR-RFLP. Deux allèles A et B et trois génotypes AA, AB, BB, ont été détectés. La mutation K226E (A676G) de *MC1R* détectée par séquençage a permis de distinguer les deux allèles A et B. Seuls les génotypes AA sont associés à « tête et cou rouge ». Le test de chi2 a montré que ce phénotype était contrôlé par l'allèle récessif A du gène *MC1R* alors que l'allèle B (K226E) correspondrait à une perte de fonction et serait associé à un pelage complètement blanc.

chèvre *Boer* / tête et cou rouge / gène *MC1R* / mutation K226E / pelage blanc

1. INTRODUCTION

It is generally acknowledged that the head and neck color of *Boer* goats is brown or red except for a white band in the region from the middle of the forehead to the extremity of the nose, and their body color is white. Li et al. [5] confirmed that the red head and neck of *Boer* goats are controlled by one recessive gene on an autosome that shows a simple Mendelian inheritance. It is regrettable that neither the identity of the recessive gene nor the molecular genetic basis of its phenotype have been reported so far.

MC1R plays a critical role in the control of melanin synthesis [1]. It is a seven transmembrane, G-protein-coupled receptor that is activated by the melanocyte-stimulating hormone (MSH), leading to an increase in black/brown eumelanin production in melanosomes that are then transferred to the surrounding hair. In many mammals, a gain of function of *MC1R* variants is associated with an increase in the production of eumelanin, while loss of function variants is associated with an increase in red/yellow phaeomelanin production [2–4, 6, 9–12]. A loss of the function variant is also associated with the white coat color of bears [8]. Moreover, *MC1R* variants have been found to be overrepresented in humans with red hair [13]. However, the *MC1R* variants in goats have not been reported except for part of its sequence in GeneBank (AY292287), in which G259A was found to be the *Ear* I restriction site. In order to investigate the association of the candidate *MC1R* gene with the red head and neck of *Boer* goats, a comparative analysis of *MC1R* polymorphism was carried out in *Boer* goats and 26 other goat populations based on PCR-RFLP, with the intention of learning more on the genetic basis of the recessive color trait of the head and neck of *Boer* goats and providing experimental evidence on *Boer* goat pure propagation and their potentially improved cross to Chinese indigenous goat breeds.

2. MATERIALS AND METHODS

2.1. Samples and DNA extraction

A total of 319 goat samples were included in this investigation. The samples represented the imported breed *Boer* goats and their offspring backcrossed to *Tangshan Dairy* goats, 18 Chinese main indigenous goat breeds and four other imported breeds (*Angora* goat, *Toggenburg* goat, *Nubian* goat, *Saanen* goat), together with coat color recorded for each goat population. DNA extraction was conducted by the phenol extraction method.

2.2. Primer design, PCR amplification and sequencing

Since the complete sequence of the goat *MC1R* gene has not been sequenced, three primer pairs used to amplify the different fragments of the goat *MC1R* gene, E1, E2 and E3, were designed according to the homologous region between the complete *ovibos moschatus* sequence (GenBank Accession No. Y13958), part

of the goat sequence (AY292287) and the complete sheep sequence (GenBank Y13965). The primers were as follows: E1, forward 5' gtggaccgctacatctccat and reverse 5' ttgaagatgcagccacagg; E2, forward 5' gctgctgggttcccttaact and reverse 5' gggcgtagaagatggagatg and E3, forward 5' tgcctcgttggcttcttc and reverse 5'gcacctcttggagcgtctt. The E1 primer was used to amplify the 416bp fragment (bases from 418 to 833) of the *MC1R* gene (AY292287) in order to study the relationship between SNP and color variation. In order to find new SNP, the E2 and E3 primers were used to amplify the 422bp fragment (bases from 27 to 448) and the 367bp fragment (bases from 571 to 937) of the *MC1R* gene respectively from 10 goats representing five breeds with different coat colors (*Boer* goat, *Chengdu Brown* goat, *Nanjiang Brown* goat, *Shannan White* goat, *Leizhou* goat); each breed was comprised of two goats. PCR products amplified by E2 and E3 were sequenced by Bioasia biological and technology Co. Ltd. (Beijing, China), and PCR products of E1 were used to perform PCR-RFLP.

PCR was carried out in a PTC-100™ Programmable Thermal Controller (MJ-Research, Inc, U.S.A.) with a total volume of 30 μL reaction containing 4 μL (75 ng·μL^{-1}) of goat genomic DNA, 3 μL of 10×PCR standard reaction buffer, 2.4 μL dNTP (2.5 pmol·L^{-1} of each deoxynucleotide), 1.2 μL (10 pmol·L^{-1}) of each forward and reverse primer, 0.3 μL (5 U·μL^{-1}) of Taq DNA Polymerase (TaKaRa Biotechnology Co., Ltd., Dalian, China) and 17.9 μL of distilled water. After pre-denaturation for 3 min at 94 °C, the PCR profile consisted of a denaturation step at 94 °C for 45 s, an annealing step at 64 °C for 45 s, and an elongation step at 72 °C for 1 min for a total of 34 cycles, followed by a final extension of 10 min at 72 °C. The PCR products were run on 1.5% agarose gel including 10 mg·mL^{-1} of ethidium bromide, and were visualized and photographed with a gel automatic photographer under UV light.

2.3. PCR-RFLP of *MC1R* and genotype determination

The PCR-RFLP was performed using *Ear* I (Sino-American Biotechnology Company, Beijing, China) recognizing GAAGAG and cutting at position 671 according to the alignment between Y13958 and the sequenced results. The digestion solution with a total volume of 10 μL containing 5 μL of PCR products, 1 μL (10 U·μL^{-1}) of *Ear* I, 3 μL of distilled water and 1.0 μL of 10×buffer, was incubated at 37 °C for 2 h in the PTC-100™ Programmable Thermal Controller. Genotyping was completed by running digested products on 2% agarose gel including 10 mg·mL^{-1} of ethidium bromide. Homozygote AA was defined when base A existed at position 676 forming GAAGAG being recognized by *Ear* I; homozygote BB was defined when base G existed at this position forming GGAGAG not being recognized by *Ear* I, and heterozygote AB was defined when A and G existed at the same position of the homologous chromosome.

2.4. Statistical test of inheritance of head and neck color of *Boer* goats

A chi-square test of SPSS software (version 11.5) was performed to test the inheritance of head and neck color of F_2 offspring of *Boer* goats backcrossed to F_1 and of F_3 offspring of *Boer* goats backcrossed to F_2 goats with white heads and necks.

2.5. Alignment of *MC1R* protein sequences in mammals

In order to analyze the lysine (K) conservation of *MC1R* protein in mammals,

Table I. *MC1R* genotype and coat color in *Boer* goats and their offspring.

Groups	Number of samples			Head and neck color		Body color
	Total	AA	AB	AA	AB	
Boer goats	41	41	—	red	—	white
F_1	7	—	7	—	white	white
F_2	53	26	27	red	white	white
F_3	56	44	12	red	white	white
F_4	6	6	—	red	—	white

Note: F_3 were the offspring of *Boer* goats to F_2 (including red, white head and neck individuals).

eight complete sequences (317aa) were obtained from goats (SWISSPROT Accession No. P56444), *ovibos moschatus* (SWISSPROT P56447), sheep (SWISSPROT O19037), cows (SWISSPROT P47798), red deer (SWISSPROT P56445), horses (NCBI AAK70924), cats (NCBI NM_001009324) and humans (SWISSPROT Q01726), respectively, aligned by MEGA software (Version 2.1).

3. RESULTS

3.1. Polymorphism of *MC1R* nucleotide sequences of goats

Six *MC1R* nucleotide sequences were successfully obtained, including four sequences amplified by the E2 primer and two by the E3 primer. According to the goat *MC1R* protein sequence (SWISSPROT P56444) and the sequenced results, 5 SNP were found in five of six sequences; the remaining sequence amplified by the E2 primer was not found to have an amino acid variant. Amongst 5 SNP, the R9L (25CTG27, at the first extracellular domain of *MC1R*) variant was detected in one *Boer* goat and one *Shannan White* goat; G49W (145TGG147, at the first transmembrane region) was found in one *Chengdu Brown* goat, and F250V (748GTC750) and G234D (700GAC702) variants were identified in the other *Boer* goat, which were at the sixth transmembrane region and the third cytoplasmic domain, respectively. Meanwhile, this *Boer* goat was identified as an AA homozygote at the 676 site, whereas the other *Shannan White* goat with white coat color was identified as a GG homozygote at the same site, and the 676A→G transition resulted in a 226K→E variant (at the third cytoplasmic domain) in this goat.

3.2. *MC1R* genotype and coat color in *Boer* goats and their offspring

Table I gives the *MC1R* genotype and coat color in *Boer* goats and their backcrossed offspring to *Tangshan Dairy* goats. Genotype AA, AB and BB could be identified from the electrophoresis of restriction fragments (Fig. 1). Only the AA genotype was found in 41 *Boer* goats that had red heads and necks and only the AB genotype was found in F_1 animals with white heads and necks that were the offspring of *Boer* goats and *Tangshan Dairy* goats with white head and neck color. There were two types of head and neck color observed in F_2 offspring of *Boer* goats backcrossed to F_1. One type was the red head and neck observed in 26 homozygous AA goats, and the other type was characterized as the white head and neck in 27 AB heterozygotes. The red and white heads and necks were also observed in F_3 of *Boer* goats backcrossed to F_2, and 12 of 44 red head

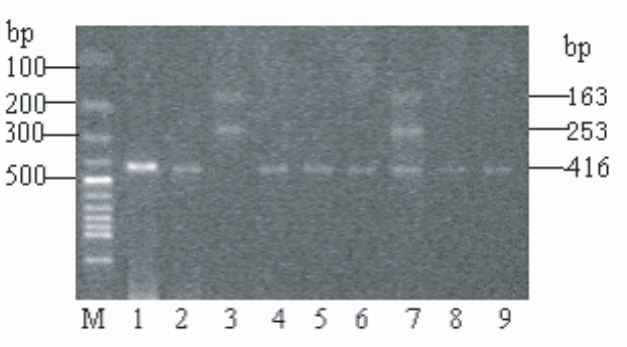


Figure 1. RFLP of *MC1R* gene digested by *Ear* I. Note: M refers to DNA Marker (*λ*DNA/*Ecor* I+*Hind* III); lane 1 illustrates PCR production of *MC1R*; lane 3 illustrates the AA genotype with bands of 253bp and 163bp; lane 7 illustrates the AB genotype with bands of 416bp, 253bp and 163bp, respectively; the remaining lanes illustrate the BB genotype with only one band of 416bp.

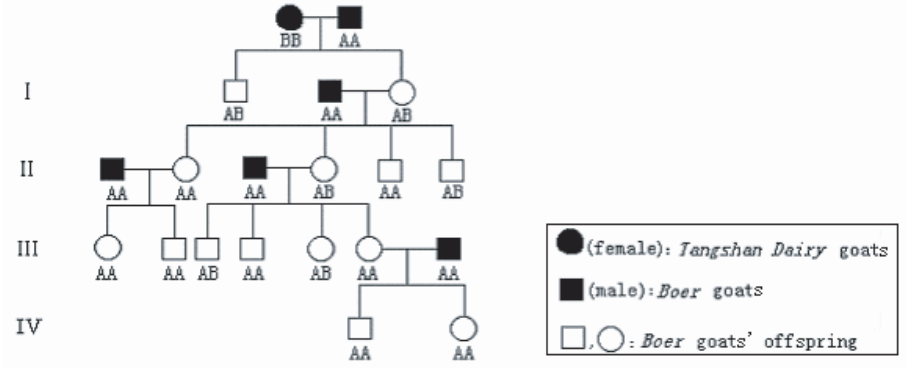


Figure 2. The family of *Boer* goats and their upgrading offspring to *Tangshan Dairy* goats.

and neck goats in F_3 were the offspring of *Boer* goats backcrossed to the white head and neck goats in F_2, and the other 32 individuals were the offspring of *Boer* goats backcrossed to the red head and neck of goats in F_2. Since the BB genotype was not detected in *Boer* goats and their offspring, it was deduced that the *Tangshan Dairy* goats should have the BB genotype in light of family lineage (Fig. 2). A chi-square test of inheritance of head and neck color for *Boer* goats is shown in Table II.

3.3. *MC1R* genotype and coat color in Chinese and imported breeds

Table III gives the *MC1R* genotype and coat color in 18 Chinese main indigenous goat breeds and four imported foreign goat breeds. Eighty-nine individuals from 11 breeds with white coat, except for one *Chuangdong White* goat and one *Duan* goat that were AA homozygote and the other two *Duan* goats that were AB heterozygote, all presented the BB genotype. *Nubian* goats with red coat presented the same AA genotype as the *Boer* goats with red heads and necks. Within the group of imported breeds (*Angora* goat, *Toggenburg* goat, *Nubian* goat and *Sannen* goat), the frequencies of the A and B allele were 35.3% and 64.7%, of AA respectively. The frequencies of the AB and BB genotypes were 35.3%, 0% and 64.7%, respectively; the corresponding values within the group of Chinese indigenous goat breeds were 7.6% and 92.4%, and 4.3%, 6.5% and

Table II. Chi-square test of inheritance of head and neck color of *Boer* goats.

Offspring	Head and neck color	Number of female (♀)		Number of male (♂)		Chi-square test
		Obs.	Exp.	Obs.	Exp.	
F_2	red	17	15	9	11.5	♀$\chi^2 = 0.53$, $P > 0.05$ ♂$\chi^2 = 1.09$, $P > 0.05$
	white	13	15	14	11.5	
	total	30	30	23	23	
F_3	red	4	5	8	7	♀$\chi^2 = 0.40$, $P > 0.05$ ♂$\chi^2 = 0.29$, $P > 0.05$
	white	6	5	6	7	
	toatl	10	10	14	14	

Note: F_3 were the offspring of *Boer* goats crossed to the white head and neck individuals in F_2.

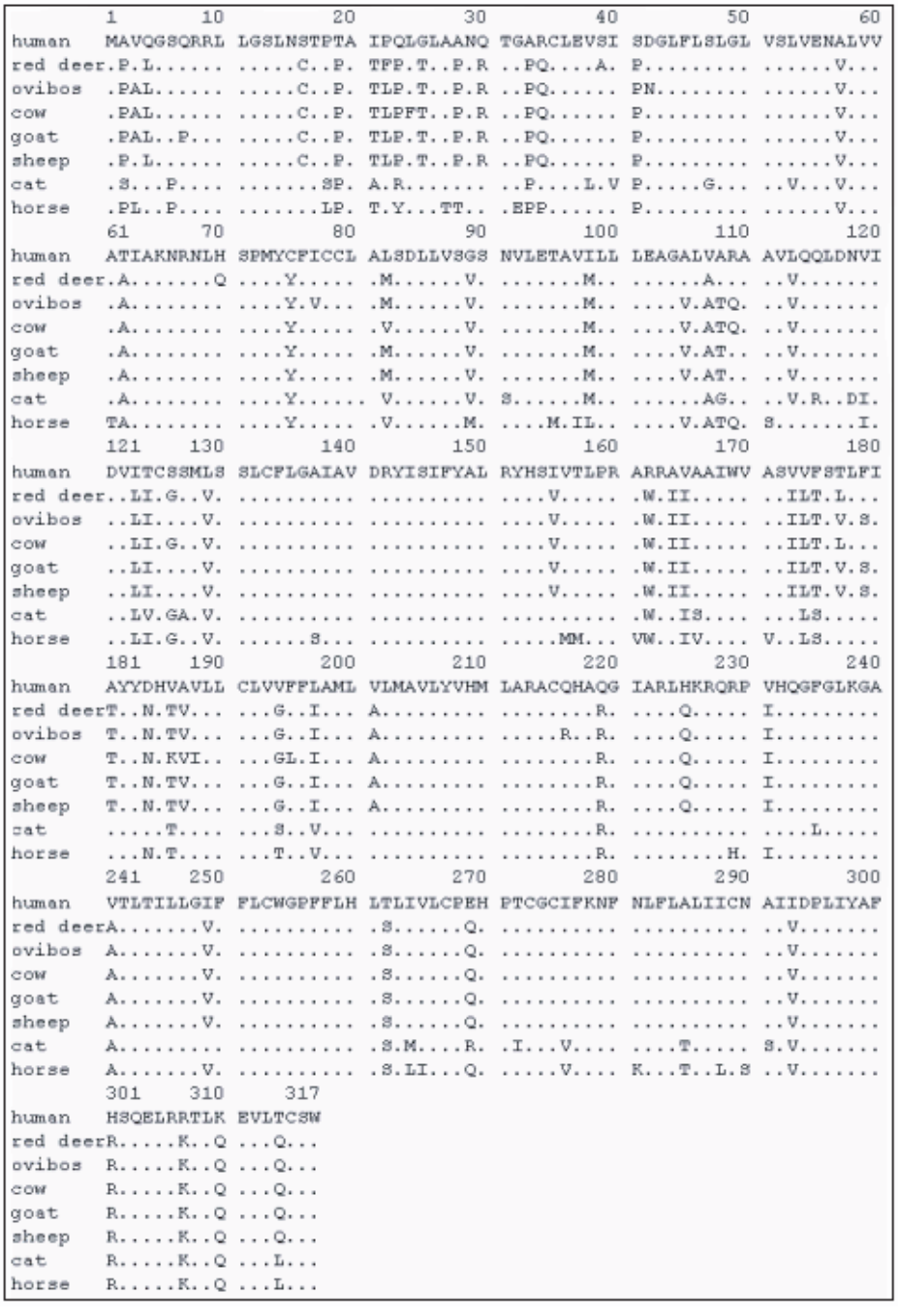
Table III. *MC1R* genotype and coat color in Chinese main indigenous goat breeds and imported goat breeds.

Breeds	Number of samples				Coat color
	Total	AA	AB	BB	
Angora goat	8	—	—	8	white
Saanen goat	2	—	—	2	white
Nubian goat	3	3	—	—	red
Toggenburg goat	4	3	—	1	brown
Liaoning Cashmere goat	10	—	—	10	white
Jining Grey goat	7	—	—	7	grey
Chengde Polled goat	10	—	—	10	black
Taihang Mountain goat	3	—	—	3	black
Jianchang Black goat	7	—	—	7	black
Inner Mongolia Cashmere goat	8	—	—	8	white
Chengdu Brown goat	6	—	2	4	brown-yellow
Tibetan goat	8	—	—	8	black
Nanjiang Brown goat	5	—	—	5	tan
Shannan White goat	6	—	—	6	white
Chuandong White goat	10	1	—	9	white
Guizhou White goat	13	—	—	13	white
Yichang White goat	10	—	—	10	white
Longlin goat	6	—	—	6	white
Matou goat	9	—	—	9	white
Duan goat	7	1	2	4	white
Guizhou Black goat	3	—	1	2	black
Leizhou goat	11	4	4	3	black

89.2%, respectively. The results showed that both Chinese breeds and imported breeds demonstrate a comparatively high B allele and BB genotype frequency; the A allele, AA and AB genotype frequency for them was lower.

3.4. Conservative lysine of mammal *MC1R* protein sequences

The alignment of the *MC1R* protein sequences of eight mammals was shown in Figure 3. The 226 site, at the cytoplasmic



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        1       10        20        30        40        50        60
human   MAVQGSQRRL LGSLNSTPTA IPQLGLAANQ TGARCLEVSI SDGLFLSLGL VSLVENALVV
red deer.P.L..... .....C..P. TFP.T..P.R ..PQ....A. P......... ......V...
ovibos  .PAL..... .....C..P. TLP.T..P.R ..PQ..... PN........ ......V...
cow     .PAL..... .....C..P. TLPFT..P.R ..PQ..... P......... ......V...
goat    .PAL..P... .....C..P. TLP.T..P.R ..PQ..... P......... ......V...
sheep   .P.L..... .....C..P. TLP.T..P.R ..PQ..... P......... ......V...
cat     .S...P.... .......SP. A.R....... ..P....L.V P.....G... ..V...V...
horse   .PL..P.... .......LP. T.Y...TT.. .EPP..... P......... ......V...
        61      70        80        90       100       110       120
human   ATIAKNRNLH SPMYCFICCL ALSDLLVSGS NVLETAVILL LEAGALVARA AVLQQLDNVI
red deer.A.......Q ....Y..... .M......V. .......M.. ......A... ..V.......
ovibos  .A........ ....Y.V... .M......V. .......M.. ....V.ATQ. ..V.......
cow     .A........ ....Y..... .V......V. .......M.. ....V.ATQ. ..V.......
goat    .A........ ....Y..... .M......V. .......M.. ....V.AT.. ..V.......
sheep   .A........ ....Y..... .M......V. .......M.. ....V.AT.. ..V.......
cat     .A........ ....Y......  V......V. S......M.. ......AG.. ..V.R..DI.
horse   TA........ ....Y..... .V......M. ....M.IL.. ....V.ATQ. S.......I.
        121     130       140       150       160       170       180
human   DVITCSSMLS SLCFLGAIAV DRYISIFYAL RYHSIVTLPR ARRAVAAIWV ASVVFSTLFI
red deer..LI.G..V. .......... .......... ....V..... .W.II..... ..ILT.L...
ovibos  ..LI....V. .......... .......... ....V..... .W.II..... ..ILT.V.S.
cow     ..LI.G..V. .......... .......... ....V..... .W.II..... ..ILT.L...
goat    ..LI....V. .......... .......... ....V..... .W.II..... ..ILT.V.S.
sheep   ..LI....V. .......... .......... ....V..... .W.II..... ..ILT.V.S.
cat     ..LV.GA.V. .......... .......... .......... .W..IS.... ...LS.....
horse   ..LI.G..V. ......S... .......... .....MM... VW..IV.... V..LS.....
        181     190       200       210       220       230       240
human   AYYDHVAVLL CLVVFFLAML VLMAVLYVHM LARACQHAQG IARLHKRQRP VHQGFGLKGA
red deerT..N.TV... ...G..I... A......... ........R. ....Q..... I.........
ovibos  T..N.TV... ...G..I... A......... .....R..R. ....Q..... I.........
cow     T..N.KVI.. ...GL.I... A......... ........R. ....Q..... I.........
goat    T..N.TV... ...G..I... A......... ........R. ....Q..... I.........
sheep   T..N.TV... ...G..I... A......... ........R. ....Q..... I.........
cat     .....T.... ...S..V... .......... ........R. .......... ....L.....
horse   ...N.T.... ...T..V... .......... ........R. ........H. I.........
        241     250       260       270       280       290       300
human   VTLTILLGIF FLCWGPFFLH LTLIVLCPEH PTCGCIFKNF NLFLALIICN AIIDPLIYAF
red deerA.......V. .......... .S......Q. .......... .......... ..V.......
ovibos  A.......V. .......... .S......Q. .......... .......... ..V.......
cow     A.......V. .......... .S......Q. .......... .......... ..V.......
goat    A.......V. .......... .S......Q. .......... .......... ..V.......
sheep   A.......V. .......... .S......Q. .......... .......... ..V.......
cat     A........ .......... .S.M....R. .I...V.... ....T..... S.V.......
horse   A.......V. .......... .S.LI...Q. .....V.... K...T..L.S ..V.......
        301     310     317
human   HSQELRRTLK EVLTCSW
red deerR.....K..Q ...Q...
ovibos  R.....K..Q ...Q...
cow     R.....K..Q ...Q...
goat    R.....K..Q ...Q...
sheep   R.....K..Q ...Q...
cat     R.....K..Q ...L...
horse   R.....K..Q ...L...
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Figure 3. Alignment of *MC1R* protein sequences of eight mammals.

domain of *MC1R*, is a conservative lysine (K) among the eight mammals. Thus the K226E variant found in one *Shannan White* goat sequence was confirmed to be a missense mutation, since this mutation just located at the *Ear* I recognition site allows distinguishing the B allele from the A allele. In addition, the eight mammals were observed with the conservative K at sites 65, 238 and 278, in which 65K and 238K were at the cytoplasmic domain of *MC1R* and only 278K was at the extracellular domain. The conservative 307K observed in seven mammals was also located at the cytoplasmic domain, but the nonconservative mutation of K→R at the same site has been observed in humans.

4. DISCUSSION

4.1. The red head and neck of *Boer* goats may be controlled by the *MC1R* gene

The AA genotype was perfectly in accordance with the red head and neck of *Boer* goats, suggesting that the A allele of the *MC1R* gene was strongly associated with such a phenotype. If the recessive A allele controls the red head and neck of the *Boer* goat, and the dominant B allele controls the white head and neck of the *Tangshan Dairy* goat, then the F_1 of *Boer* goats crossed to *Tangshan Dairy* goats should be AB heterozygote; the red head and neck individuals in both F_3 and F_4 should be AA homozygote; this deduction was well proven by the genotype identification described above. The intercross between *Boer* goats and F_1, and between *Boer* goats and white head and neck individuals in F_2 equals to a backcross, thus, theoretically, the ratio of red head and neck individuals to white head and neck individuals both in F_2 and F_3 from such an intercross should be 1:1. A chi-square test showed that the difference between the number of red head and neck individuals and of white head and neck individuals both in females and males of F_2 was not significant, thus the ratio between them was in accordance with the expectation value of 1:1, as in F_3. The statistical results suggest that the red head and neck of *Boer* goats may be mainly controlled by the recessive A allele of the *MC1R* gene, supporting the opinion that this color trait belongs to autosome inheritance and could be explained by Mendelian law [5]. Here, it must be pointed out that the conservative 226K of the A allele may be indispensable for forming the red head and neck of *Boer* goats, and does not exclude the possibility that there be variants at other sites of the A allele, for example, R9L, F250V and G234D found in *Boer* goats. But this deduction should be supported by sequencing based on more samples and correlated statistical tests.

4.2. Speculation on the genetic basis of the *Boer* goats' white body

The head and neck color of *Boer* goats is red, whereas their body color is white. Two distinctly different coat colors distributed in the same breed implies that the white body of *Boer* goats may not be controlled by the *MC1R* gene but probably by other gene (s) as follows: firstly, some gene inhibits melanocyte cell differentiation or migration from the neural crest to the body; secondly, some gene makes melanocytes lose its ability to deposit melanosomes in body hair; thirdly, a few genes directly inhibit the enzymes and related proteins from being produced, which are responsible for melanogenesis; fourthly, some genes with mutants inhibit the interaction of α-melanocyte stimulating hormone with the target melanocytes, and possibly all of these genes interact to give the final *Boer* goats' white body.

4.3. Association of the K226E mutation with the whole white coat of goats

Since nearly all individuals were identified as the BB genotype among 11 whole white coat breeds, the B allele is potentially associated with such a color phenotype. As Figure 3 shows, there are four conservative Ks at the cytoplasmic domain in goat *MC1R* protein sequences; consequently, the conservative 226K at the third cytoplasmic domain may play an important role in determining the function of *MC1R*. The non-conservative K226E mutation at the B allele is a replacement of a positive charged K with a negative charged E, and this kind of K→E mutation may be a loss of function mutation, resulting in the loss of melanin production and leading to the formation of the whole white coat of goats. Conversely, the kind of E→K mutation seems to be a gain of function mutation, which is strongly associated with melanism of birds [7] and mice [9], the chicken's black coat is also possible due to this kind of mutation [2]. It is noteworthy that the K→E mutation found in this investigation was not at the second transmembrane region, which is apparently a key function part in *MC1R*, since many mutations associated with coat color variation in chickens [2], mice [9], cattle [4], and horses [6] are located at the second transmembrane; but at the third and the seventh transmembrane region, even at the cytoplasmic domains, for example, at the third and the fourth cytoplasmic domain, there are also some mutations related to the coat color difference in animals [3, 8, 10, 11], implying that the coat color variation in different animals is possibly determined by mutations at different *MC1R* regions. Some other goat breeds, even if they had markedly various coat color (for example, the black of *Chengde Polled* goats, grey of *Jining Grey* goats and tan of *Nanjiang Brown* goats) also presented the BB genotype; such a phenomenon where genotype does not match the phenotype, on the one hand revealed the K226E mutation was not an important mutation associated with coat color variance among these breeds; on the other hand, that it may be due to different genetic background among them.

The following could be concluded: the red head and neck of *Boer* goats may be controlled by the recessive A allele of the *MC1R* gene, and the K226E mutation of *MC1R* was potentially associated with the whole white coat of goats. The B allele variant of *MC1R* overwhelmingly distributed in the Chinese goat population and imported foreign goat population contributes to the understanding of the evolution of the goat *MC1R* gene.

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