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Characterization and SNP identification of goat *TYRP1* gene*

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As a member of tyrosinase-related family, tyrosinase-related protein-1 (TYRP1) has been reported to play an important role in melanin biosynthetic pathway in some species, but little is known about its role in goat. In this study, almost complete goat *TYRP1* gene (17554 bp, HMO70243) encompassing complete CDS was identified, the coding region amounting to 1614 bp, distributed in 7 exons (2-8), and intron 5 was found to possess GC-AG as its splice sites. Dozens of SNPs as well as simple sequence repeat (SSR) motifs were detected in goat *TYRP1* gene, in which 4 SSR motifs in intron 5 are combined in a tandem. Correlation analysis showed the allele A to be advantageous compared to C at site *g.1263A>C* and allele C compared to T at *g.1428C>T*, implyimg that haplotype AC is in favour of eumelanin biosynthesis, and haplotype CC is in favour of pheomelanin formation. Marked deficiency of heterozygotes occurred in Nanjiang Yellow Goat Black strain and Nanjiang Yellow Goat Fast Grow strain, indicating the high inbreeding in both strains.

KEY WORDS: coat colour / goat / SNP / TYRP1

Coat colour has fascinated animal breeders and geneticists for years. Classic breeding experiments told us much about the inheritance of coat colours and patterns in the early to mid nineties [Schmutz and Berryere 2007]. Only in these last years, have the underlying genes been discovered. It has been proposed that mammalian pigmentation is mainly controlled by the concerted action of *TYR*, *TYRP1* and *TYRP2*

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producing eumelanin (black, brown or blue-gray) and/or pheomelanin (red, tan, or yellow) in melanocytes. It has also been suggested that the coordinate expression of *TYR* and *TYRP1* genes caused eumelanin biosynthesis [Girardot *et al.* 2006, Kuzumaki *et al.* 1993].

Mutations of *TYRP1* have been reported to be associated with Rufous oculocutaneous albinism in southern African Blacks [Manga *et al.* 1997], with chocolate (aka chestnut) and cinnamon (aka red) coat colour in domestic cats (Lyons *et al.* 2005), with brown coat colour in dogs [Schmutz *et al*. 2002; Cargill *et al.* 2005], dun in Dexter cattle (Berryere *et al*. 2003), with coat-colour polymorphism in a free-living population of Soay sheep [Gratten *et al*. 2007], showing that TYRP1 is of critical importance for eumelanin biosynthesis [Box *et al.* 1998; Rieder *et al.* 2001]. To extend our knowledge of *TYRP1* gene and to provide some useful information for breeders we identified almost complete goat *TYRP1* (encompassing complete CDS), and looked for correlation between two SNPs (both before the start codon) and coat colour based upon 156 individuals from 4 goat breeds/strains *via* Cleaved Amplified Polymorphic Sequences (CAPS or PCR-RFLP) and Derived Cleaved Amplified Polymorphic Sequences (dCAPS) assay [Neff *et al.* 1998].

Material and methods

Animals

Used were the following goat populations:

1. Chengdu Ma Goat (CDM, n=40, brown);
2. Nanjiang Yellow Goat Fast Grow Strain (NJF, n=34, tan);
3. Nanjiang Yellow Goat High Fertility Strain (NJH, n=44, tan)
4. Nanjiang Yellow Goat Black Strain (NJB, n=38, black).

All animals were from Sichuan province of China. Nanjiang Yellow goat was bred from Chengdu Ma goat as the paternal side, while NJB is a new strain selected for black coat colour and separate from NJF and NJH with tan coat colour [Yu and Zhang 2005].

DNA extraction, primer design and PCR amplification

Genomic DNA from blood samples was isolated according to the standard phenol: chloroform extraction method. Pairs of primers (including CAPS and dCAPS) were designed using Primer Premier 5 version 5.00 (http://www.brezosoft.com/softwarelib/soft2356.htm) (Tab. 1) following the conserved genomic domain of cattle (NC_007306.3) and sheep (EU760771.1) *TYRP1* genes, and the published goat *TYRP1* mRNA sequence (AF136926.2).

PCR amplification was carried out on a Programmable Thermal Controller (German BIOMETRA) with a total volume of 25 μL solution containing 50~100 ng genomic DNA, 2.5 μl 10× PCR reaction buffer (Mg^{2+} plus), 400 pmol/l of each forward and reverse primer, 200 pmol/μl dNTPs, and 2 U Taq DNA polymerase (TIANGEN

BIOTECH, Beijing). For PCR primers with products longer than 1.5 kb we used Taq10 DNA polymerase (ZEXING BIOTECH, Beijing). The PCR protocols were as follows: denaturation at 95° for 4 min followed by 35 amplification cycles (28 cycles for long fragment) of denaturation at 94° for 30 s, annealing at the corresponding temperature (shown in Tab. 1) for 30 s and extension at 72° for times that were developed based on the product length (basically 1 kb/min), followed by an extended elongation at 72° for 10 min. PCR products were detected on a 1-1.5% agarose gel including 0.5 μg/ml of ethidium bromide, photographed under UV light, and sequenced by Shanghai Sangon

Table 1. Primers used for amplification and sequencing of goat *TYRP1* gene

Primer no.	Primer sequence	Product base position	PCR annealing temp. (°)	Product size (bp)
P1	F: 5'-GGTGGTCTTAACAAGAGCCT-3' R: 5'-CACACTCTCTCGGGAACTGA-3'	<1-1599*	57.6	>1599*
P2	F: 5'-GAGCAGCAGAGAATGCCTTC-3' R: 5'-CGGCTATCACCACCTCACAT-3'	969-1719	55.1	751
P3	F: 5'-CACAAAGGGCTACAAACCAG-3' R: 5'-CGATGACAAACTGAGGGTGA-3'	1459-3391	60.0	1932
P4	F: 5'-TTTGAAGGTGGGTTGGGAAGG-3' R: 5'-AGGGCTGTGCAGGAAGCTCAT-3'	1342-2318	57.0	977
P5	F: 5'-ATTGCCACAAGGAGGTCAGAAG-3' R: 5'-CAGTAAGGGAGGGAGAAAGAAGG-3' S1: 5'-GTCTTCCAAGTTCCGAATGT-3'(+) S2: 5'-CTGAGCGTGCTGTTCTTTAG-3'(-) S3: 5'-GAGTAAGTTGGAGAAAGAGGGAG-3' (+) S4: 5'-AGGGATTAGCATAGGGAAAGAC-3' (-)	3388-6398	64.0	3011
P6	F: 5'-ACACCAGGCAGATAAGGC-3' (+) R: 5'-TGAAGCACCCCAGATTGT-3' (-)	5843-6723	55.5	881
P7	F: 5'-ATGGCGAGTAGTCTGCGAAT-3' R: 5'-GAACCAACTTCCAAGCACTGA-3' S1: 5'-CCATCTCCCTTGATACTAACAC-3'(+) S2: 5'-GATAGAGGTCCTTGTATTCGTT-3'(-) S3: 5'-GAGTCCAAACTCTGTTCT-3'(-)	6507-10069	58.0	3563
P8	F: 5'-ATCGCTCAGTGCTTGGAAGTT-3' R: 5'-GTATCGCCTCAGCCATTCATC-3' S1: 5'-CTTCAGCACCACAGTTCAAT-3'(+) S2: 5'-TGCTCTGACTGCCCTGAA-3'(-)	10044-13291	63.8	3248
P9	F: 5'-ACAGTGATCCCACAGGAAGGTA-3' R: 5'-CCAATAGGGGCATTTTCCAG-3' S1: 5'-AGGCTACTTTCTCTTCACTC-3'(+) S2: 5'-GCCACCTTTCATCTTTCTAC-3'(-)	13122-16273	60.0	3152
P10	F: 5'-CACTGGAAAATGCCCCTAT-3' R: 5'-CATTAGACCATAGACTGATTAGGG-3' S1: 5'-TGCCACAAACAGCATCAC-3' (+) S2: 5'-TGGCACGAATCAGACAAG-3' (-)	16252-17446	60.9	1195
P11	F: 5'-TACAGTGGAAGAGCACAGAA-3' R: 5'-GCACAGGAAGGAAGTAACAG-3'	16903->17554*	59.5	>652*
P12[a]	F: 5'-ACTTGCTAGAAATCTGCCTCCAGTT-3' R: 5'-GGAAAGAAAGCAGGACCAGGAACAT -3'	1084-1566	62	483
P13[b]	F: 5'-CATTTTGCTATTTTCTTTTAAG-3' R: 5'-ATTTCATTCTGCCTGGAG-3'	1403-1533	54	131

F – forward primer used in PCR amplification; R – reverse primer used in PCR amplification; S – sequencing primer (chromosome walking method) used when the PCR product was too long for single bidirectional sequencing; (+) – forward sequencing primer (-) – reverse sequencing primer.

*More than a dozen of nucleotides that followed the forward primer of P1 and the reverse primer of P11 couldn't be determined.

[a]Primers for Cleaved Amplified Polymorphic Sequences (CAPS) assay;

[b]Primers for Derived Cleaved Amplified Polymorphic Sequences (dCAPS) assay; the mispairing base is underlined.

Biological Engineering Technology and Services (Shanghai). The sequencing strategy used for large PCR fragments was the chromosome walking method (sequencing primers are listed in Tab. 1).

DNA sequence structure analysis and SNP identification

The sequenced fragments were connected using SeqMan software (version 7.1.0) as implemented in the DNAStar software package (http://www.dnastar.com/). Exons and introns were determined and numbered by aligning the connected sequence to *TYRP1* mRNA sequence (AF136926.2) using the Spidey programme (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/). Additionally, simple sequence repeats (SSR) were identified using SSR Tool (http://www.gramene.org/gramene/searches/ssrtool).

To identify SNPs of the goat *TYRP1* gene, we mixed PCR products from three individuals of each of the following breeds: Chengdu Ma Goat, Jining Gray Goat and Tangshan Dairy Goat, and got them sequenced. Then heterozygous sites were screened and determined by ABI chromatogramme of BioEdit software (version 4.7.0) (http://www.mbio.ncsu.edu/BioEdit/page2.html).

CAPS and dCAPS assay

Of the 80 SNPs identified in this study, two (*g.1263A>C* in intron 1 and *g.1428C>T* in exon 2) were analysed *via* CAPS and dCAPS, respectively. For *g.1263A>C*, the A→C mutation created one restriction site (R/GATCY) for *Psu*I restriction endonuclease. PCR products of the 483bp fragment (1084-1566) amplified using the primers P12 shown in Table 1 were digested with *Psu*I enzyme. The restriction endonuclease reaction was carried out in a total reaction volume of 10 µl solution containing 3 µl PCR product, 0.2 µl restriction enzyme (5 U/µl, MBI FERMENTAS), 1 µl 10×buffer B and 5.8 µl ddH_2O at 37 o (for 3 h). The digested products were analysed on a 2% agarose gel stained with 0.25 µg/ml ethidium bromide at 100 V for 30 min. The gel was examined under UV-transilluminator and finally documented by the gel photography system.

In dCAPS assay, a mismatch in PCR primer (Tab. 1) was used to create one *Hind*III (restriction endonuclease)-sensitive polymorphism based on the target mutation (*g.1428C>T*); C→T mutation abolishes the restriction site (A/AGCTT) created by the mispairing primer (P13R). Digestion reaction procedure of the 131bp fragments (1403-1533) was basically the same as CAPS, except for *Psu*I enzyme replaced by *Hind*III and 10×buffer B replaced by 10×buffer R. Digested products were analysed on 3% agarose gel stained with 0.25 µg/ml ethidium bromide at 100 V for 40 min and the gel was examined under UV-transilluminator and finally documented by the gel photography system.

Statistical

PopGene32 software (Version 1.32) was used to perform the Hardy-Weinberg equilibrium test and neutral test for *g.1263A>C* and *g.1428C>T*, and to calculate the Hom, Het, Ne, Fis, and I values (http://www.ualberta.ca/~fyeh/). Estimates of the

population haplotype frequencies for *g.1263A>C* and *g.1428C>T*, and respective standard errors (SE) were obtained *via* Bayesian statistics method implemented in PHASE 2.1 programme [Stephens *et al.* 2001].

Results and discussion

Structure of determined goat *TYRP1* gene

Excluding a dozen of nucleotides following each endmost primer that couldn't be determined, we got one sequence of 17554 bp in total, which has been submitted to the GenBank (HM070243). The sequence was verified to be almost complete *TYRP1* gene (including 8 exons and 7 introns and encompassing complete CDS). The exons and introns were shown and numbered, *via* aligning it to the published goat *TYRP1* mRNA sequence (AF136926.2) – Table 2 and Figure 1.

Table 2. Specific position of exons and introns in goat *TYRP1* gene

Exon	Start position	End position	Length (bp)	Intron	Start position	End position	Length (bp)
Exon 1	984	999	16	intron 1	1000 (gt)	1427 (ag)	428
Exon 2	1428	1893	466	intron 2	1894 (gt)	3292 (ag)	1399
Exon 3	3293	3615	323	intron 3	3616 (gt)	6360 (ag)	2745
Exon 4	6361	6565	205	intron 4	6566 (gt)	9960 (ag)	3394
Exon 5	9961	10128	168	intron 5	10129 (gc)	13118 (ag)	2990
Exon 6	13119	13298	180	intron 6	13299 (gt)	16236 (ag)	2938
Exon 7	16237	16383	147	intron 7	16384 (gt)	17238 (ag)	855
Exon 8	17239	>17554	>316				

The first and last two nucleotides of each intron are parenthesed.

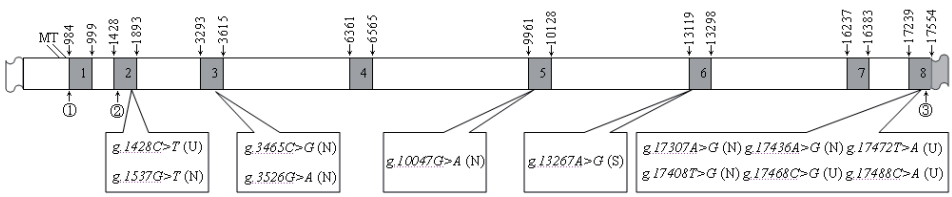

Fig. 1. Structural diagram of goat *TYRP1* gene with introns was shown in proportion. Exons (1-8) are specified by base position numbers. The SNPs found in exons are shown within attached rectangles (SNPs in introns are listed in Tab. 3). The corrugated segments at both ends are unsequenced part of *TYRP1* gene. S – Synonymous mutation; N – Nonsynonymous mutation; U – UTR mutation; ① – transcription start site (984); ② – translation initiation site (1509); ③ – stop codon site (17443); M – binding site (768-778) for Microphthalmia-associated transcription factor (MITF) (M-box: AA/GTCATGTGCT); T – binding site (944-950) for TATA binding protein (TBP) in the process of transcription (TATA box: TATAAAA).2

The length and position of identified exons and introns are shown in Table 2, with the first and last two nucleotides of each intron listed. The splice sites of intron 1, 2, 3, 4, 6 and 7 were in accordance with the canonical GT-AG rule, while intron 5 possessed GC-AG as its splice sites, which was the major splice site variant of the canonical GT–AG group in mammals [Kitamura-Abe *et al.* 2004], just like the corresponding intron

in *TYRP1* gene from *Bos taurus* (NC_007306.3), *Mus musculus* (NC_000070.5), *Homo sapiens* (NC_000009.11), *Rattus norvegicus* (NC_005104.2), *Equus caballus* (NC_009166.2), *Pan troglodytes* (NC_006476.2), *Macaca mulatta* (NC_007872.1) and *Sus scrofa* (NC_010443.1). The presence of high-frequency consensus sequence (AAGGT/CAAGT) around the 5' splice site of above-mentioned intron 5 indicates that this non-canonical GC-AG splice style in goat *TYRP1* gene could be accurately recognized by the splicing mechanisms [Kitamura-Abe *et al.* 2004].

Complete CDS of goat *TYRP1* gene was also deduced (1614 bp long, encoding a polypeptide of 537 amino acids with a molecular weight of 60.6 kDa). In human, minus the N-terminal signal sequence of about 20 amino acids, the remaining 58 kDa peptide plus 17 kDa modifying components (glycosyl-groups and Cu components) formed mature TYRP1 protein, the 75 kDa type-1 transmembrane glycoprotein [Jackman *et al.* 1991, Chen *et al*. 2001]

Variable sites and potential functional SSR motifs detected in goat *TYRP1* gene

Figure 1 shows the structural diagram of goat *TYRP1* gene with SNPs in exons. By sequencing the mixed PCR products from different breeds, a total of 80 SNPs were screened, of which 12 were in exons: *g.1428C>T*, *g.1537G>T*, *g.3465C>G*, *g.3526G>A*, *g.10047G>A*, *g.13267A>G*, *g.17307A>G*, *g.17408T>G*, *g.17436A>G*, *g.17468C>G*, *g.17472T>A* and *g.17488C>A*. Among the SNPs in exons, one was in the 5' untranslated region, three were in the 3' untranslated region, one was synonymous mutation, and seven were missense substitutions leading to the following amino acid mutations: *g.1537G>T* (*p.Gly10Val*), *g.3465C>G* (*p.Asn186Lys*), *g.3526G>A* (*p.Gly207Ser*), *g.10047G>A* (*p.Ala334Thr*), *g.17307A>G* (*p.Ile493Val*), *g.17408T>G* (*p.Asn526Lys*) and *g.17436A>G* (*p.Met536Val*). None of the seven missense substitutions affected the conserved amino acids as reported by Zheng *et al.* [2010].

Table 3. The SNPs distributed in introns and 5U of goat *TYRP1* gene

Region	Number of SNPs	SNPs
5U*	4	*g.105G>A, g.343A>T, g.352T>C, g.532C>T*
Intron 1	1	*g.1263A>C*
Intron 2	16	*g.1949T>C, g.1983G>T, g.1992T>C, g.1997C>A, g.2011G>C, g.2228A>T, g.2447G>A, g.2619T>G, g.2661G>A, g.2725A>G, g.2848T>C, g.3039C>T, g.3060G>A, g.3078G>C, g.3106A>G, g.3279T>C*
Intron 3	16	*g.4432A>G, g.4536T>C, g.4630A>G, g.4664C>T, g.4699C>T, g.5168A>G, g.5300A>G, g.5425A>C, g.5434A>C, g.5955C>T, g.2957A>T, g.6191C>A, g.6216A>C, g.6217T>G, g.6210delA, g.6280A>G*
Intron 4	10	*g.6976G>A, g.7017C>T, g.7280T>C, g.7440T>C, g.8787C>T, g.8793G>A, g.8807G>A, g.8885T>C, g.9449C>T, g.9527T>C*
Intron 5	8	*g.10812A>G, g.10895A>T, g.11604T>A, g.11729T>A, g.12356T>G, g.12414C>G, g.12776G>A, g.12969G>A*
Intron 6	9	*g.13635G>A, g.13947C>T, g.14038T>A, g.14055A>G, g.14215T>C, g.14263C>T, g.14532T>C, g.14600C>T, g.14751G>C*
Intron 7	4	*g.16758C>T, g.16869A>G, g.16979A>G, g.17130T>A*

*Represent the transcriptional regulatory region before exon 1.

Table 4. SSR motifs detected in goat *TYRP1* gene

Motif	No. of repeats	SSR start	SSR end
At	5	12013	12022
At	5	12361	12370
Tg	6	12370	12381
Ta	7	12382	12395
Ag	9	12395	12412
Ga	5	14056	14065
Ta	5	14246	14255

Except for those SNPs in exons, 68 SNPs in introns and 5'-untranscriptional region are listed in Table 3. In addition, based on the SSR Tool, five and two SSR motifs were detected in intron 5 and intron 6 of goat *TYRP1* gene, respectively, and it is noteworthy that 4 of the 5 SSR motifs in intron 5 are combined in a tandem (Tab. 4). Their potential functions need to be investigated further. Moreover, SNPs identified in this study should be confirmed with restriction analyses as presented for SNPs at 1263nt and 1428nt.

Distribution of *g.1263A>C* and *g.1428C>T* in different populations

Since none of the seven missense substitutions affected the previously reported conserved amino acids (Zheng *et al.* 2010), two SNPs (*g.1263A>C* and *g.1428C>T*) in the transcriptional regulatory region were selected for further association analysis, thereby checking their possible effect on this gene's transcription and then on the level of eumelanin biosynthesis. Genotype and allele distribution for these two SNPs in different populations were determined via CAPS and dCAPS, respectively (Fig. 2 and Tab. 5). The results showed that for *g.1263A>C*, allele A was mainly found in CDM (0.6250), allele C was mainly found in NJF (0.5882) and NJH (0.6136), while alleles A and C had the same frequency (0.5000) in NJB. We know that NJB is a novel strain selected for black coat colour and separate from NJF and NJH with tan coat colour. It

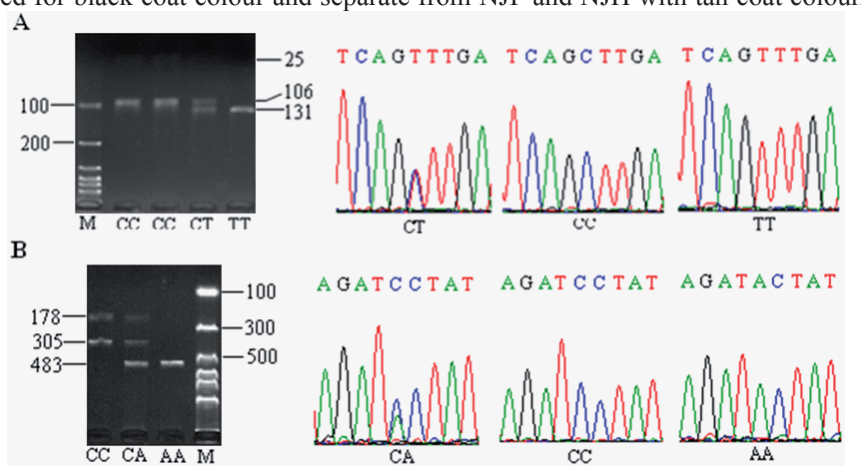

Fig. 2. Genotyping results from restriction endonuclease reaction (left) and direct sequencing (right). A – genotyping of g. 1428C>T; B – genotyping of g. 1263A>C.

Table 5. Genotype and gene frequency of *g.1263A>C a*nd *g.1428C>T* of *TYRP1* in goat populations

Population	Coat colour	Population size	SNPs	Number of genotypes			Gene Frequency	
				CC	CA and CT	AA and TT	C	A and T
CDM	Brown	40	*1263A>C*	5	20	15	0.3750	0.6250
			1428C>T	23	17	0	0.7875	0.2125
NJF	Tan	34	*1263A>C*	13	14	7	0.5882	0.4118
			1428C>T	29	4	1	0.9118	0.0882
NJH	Tan	44	*1263A>C*	16	22	6	0.6136	0.3864
			1428C>T	32	12	0	0.8636	0.1364
NJB	Black	38	*1263A>C*	4	30	4	0.5000	0.5000
			1428C>T	30	7	1	0.8816	0.1184

seems that selection for black coat caused an increase of the A allele frequency in NJB, implying that allele A at site *g.1263A>C* of goat *TYRP1* should be one factor among others contributing to eumelanin biosynthesis, *via* being in favour of TYRP1 gene's transcription.

For *g.1428C>T*, allele T frequency was remarkably low in each population (from 0.0000 to 0.2125), and only two individuals (in NJF and NJB, respectively) were detected to be of TT genotype, indicating that the mutation C→T might have little effect on the colour of the coat.

Hardy-Weinberg equilibrium test (chi-square) showed that the 4 involved populations were all in equilibrium for *g.1428C>T* at 0.05 rejection level, while for *g.1263A>C*, the population NJB (P = 0.000488) showed significant departure from equilibrium at 0.05 rejection, with other populations all in equilibrium (see Tab. 6). The artificial selection for black coat colour in NJB breeding must have affected variation of *g.1263A>C*, indicating some relation between this variant and dark coat. In addition, neutral test with 1000 simulated samples showed that the observed statistical values of evolutional power of all populations for both *g.1263A>C* and *g.1428C>T* were between the lower and upper boundaries of 95% confidence interval, but NJB for *g.1263A>C*, of which the value (0.5000) was below the lower 95% limit (0.5031), confirming some effect of artificial selection on site *g.1263A>C* in NJB breeding.

Genetic diversity and differentiation of *g.1263A>C* and *g.1428C>T* in different populations

The genetic diversity of each population is summarized and illustrated in Table 6. The expected heterozygosity ranged from 0.4747 (CDM) to 0.5067 (NJB) for *g.1263A>C*, and from 0.1633 (NJF) to 0.3389 (CDM) for *g.1429C>T*, which were all lower than that based on mtDNA of Chinese indigenous goat breeds (0.676±0.191) - Li and Alessio [2004]. The Levene's expected heterozygosity ranged from 1.8824 (CDM) to 2.0000 (NJB) for *g.1263A>C*, and from 1.1918 (NJF) to 1.5031 (CDM) for *g.1429C>T*. Heterozygosity of *g.1263A>C* was significantly higher than that of *g.1428C>T* ($P < 0.01$).

Wright's Fixation Index values (Tab. 6) showed that deficiency of heterozygotes for *g.1263A>C* existed in NJF (0.1500), and of heterozygotes for *g.1429C>T* in NJB (0.1177) and NJF (0.2688), which might be due to their inbreeding history.

Table 6. Heterozygosis of *g.1263A>C* and *g.1428C>T* of *TYRP1* in goat populations

Population	Sample size	SNPs	Observed value		Expected value		Ne	F_{IS}	I	*P*
			Hom	Het	Hom	Het				
CDM	40	*1263A>C*	0.5000	0.5000	0.5253	0.4747	1.8824	-0.0667	0.6616	0.7322
		1428C>T	0.5750	0.4250	0.6611	0.3389	1.5031	-0.2698	0.5173	0.0994
NJF	34	*1263A>C*	0.5882	0.4118	0.5083	0.4917	1.9396	0.1500	0.6775	0.3357
		1428C>T	0.8824	0.1176	0.8367	0.1633	1.1918	0.2688	0.2984	0.0761
NJH	44	*1263A>C*	0.5000	0.5000	0.5204	0.4796	1.9018	-0.0545	0.6671	0.7753
		1428C>T	0.7273	0.2727	0.7618	0.2382	1.3081	-0.1579	0.3983	0.3183
NJB	38	*1263A>C*	0.2105	0.7895	0.4933	0.5067	2.0000	-0.5789	0.6931	0.0005
		1428C>T	0.8158	0.1842	0.7884	0.2116	1.2639	0.1177	0.3638	0.4004

Haplotype frequencies for *g.1263A>C* and *g.1428C>T* in different populations

Estimate of haplotype frequency for each population showed that haplotypes AC and CC were the mainly found haplotypes in all populations, with haplotype AC superior in goat breeds with black and brown coat, and haplotype CC superior in NJF and NJH with tan coat colour. Combining the implying of advantageous A compared to C at site *g.1263A>C* and advantageous C compared to T at *g.1428C>T*, it is implied that haplotype AC is in favour of eumelanin biosynthesis while haplotype CC is in favour of pheomelanin formation (see Tab. 7).

Table 7. Estimates of population haplotype frequencies for *g.1263A>C* and *g.1428C>T*

Population	Haplotype frequencies				Standard error
	CC	CT	AC	AT	
CDM	0.1792	0.1958	0.6082	0.0167	0.0081
NJF	0.5220	0.0662	0.3897	0.0220	0.0074
NJH	0.4806	0.1331	0.3831	0.0033	0.0083
NJB	0.4192	0.0808	0.4624	0.0376	0.0166

All in all, the 17554bp goat *TYRP1* gene was identified including 1614bp coding region, distributed in 7 exons (2-8) and intron 5. Dozens of SNPs as well as simple sequence repeat (SSR) motifs were detected in goat *TYRP1* gene, and 4 SSR motifs in intron 5 were connected in a tandem.

Correlation analysis showed that allele A is advantageous compared to C at site *g.1263A>C* and alle C is advantageous compared to T at *g.1428C>T*, implyimg that haplotype AC is in favor of eumelanin biosynthesis, and haplotype CC is in favour of pheomelanin formation. Genetic diversity analysis showed that marked deficiency of heterozygotes existed in Nanjiang Yellow Goat Black strain and Fast Grow strain, indicating high inbreeding within the goat populations in question.

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