African Journal of Biotechnology Vol. 9 (16), pp. 2328-2332, 19 April, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1427
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Study on transition of g.*11584A>G* of goat *melanophilin* gene in different populations

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Accepted 24 November, 2009

Though the *melanophilin* (*MLPH*) gene has been characterized as one of the candidate genes for coat color dilution in human, mice and dog, little is known about this gene in sheep and goat. In this study, a missense mutation of g.*11584A>G* in exon 10 of goat *MLPH* gene was determined according to the sequence EU316218 previously obtained. Meanwhile the PCR-RFLP of this mutation was performed in 304 individuals from 9 goat breeds/strains, with different coat colors, widely distributed in China in order to investigate its association with coat color and its differentiation among populations. The results showed that allele A was the superior allele, while allele G was mostly found in Chengdu Ma goat and Nanjiang Brown goat (including three strains), in which homozygote GG was only found. It could be inferred that the allele G might be a candidate site for the particular dilute coat color (tan) found in Nanjiang Brown goat and Chengdu Ma goat. Hardy-Weinberg equilibrium test showed that the 9 subpopulations were all in equilibrium at 0.05 rejection level. Chengdu Ma goat, Nanjiang Brown goat (High fertility strain) and Nanjiang Brown goat (Black strain) performed plus values of Wright's fixation index. Genetic diversity of this mutation site was lower than that based on mtDNA reported.

Key words: Melanophilin, goat, transition, populations

INTRODUCTION

Melanophilin, together with myosin Va and Rab27A in mammals, are characterized to form a tripartite protein complex, taking responsibility for transferring melanosomes from the cell bodies to the tips of their dendrites by an actin-dependent movement (Matesic and Yip, 2001). Defects in the transfer process can cause pigment dilution in the skin and hair in human diseases (e.g. Gris celli syndrome) (Kuroda and Fukuda, 2002; Ho and Menasche, 2003; Itoh and Kuroda, 2005) and the corresponding coat-color mutant mice (e.g. dilute, ashen and leaden) (Provance and James, 2002; Fukuda and Kuroda, 2004). Among the three candidate gene (*MLPH*, *Rab27a* and *Myo5a*) for dilute coat color phenotype, mutation in *MLPH* gene was responsible for color dilution without any further impairment in human GS3 patients or leaden mice, thus it was considered as the most suitable candidate gene for color dilution (Philipp and Hamann, 2005; Yip et al., 2001; Ho et al., 2003).

The association between *MLPH* gene mutation and coat color dilution has been reported in mice (Ho et al. 2003), cats (David and Ishida, 2006) and dogs (Philipp and Hamann, 2005; Quignon and Philipp, 2005; Philipp and Drogemuller, 2007). No paper has been published on *MLPH* gene of ruminant. In order to extend knowledge of *MLPH* gene and provide some useful information for coat and hair research in goat, a missense mutation g.*11584A>G* in exon 10 of goat *MLPH* gene was determined according to the sequence EU316218 previously obtained. Meanwhile the PCR-RFLP of this mutation was performed in 304 individuals from 9 goat breeds/strains, with different coat colors, widely distributed in China in order to investigate its association with coat color and its differentiation among populations.

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Figure 1. Geographical distribution of the nine goat populations in China. A= Chengde Polled goat; B = Chengdu Ma goat; C = Jining Gay goat; D = Leizhou Black goat; E = Liaoning cashmere goat; F = Nanjiang Brown goat including 3 *strains*; G = Tangshan dairy goat.

Table 1. Genetype and gene frequencies of *g.11584A>G* in goat populations.

Goat population	Coat color	Population size	Individual number of genotype			Gene frequencies	
			AA	AG	GG	A	G
Chengde Polled goat	Black	36	26	10	0	86.11%	13.89%
Chengdu Ma goat	Brown (Dilute)	24	10	9	5	60.42%	39.58%
Jining Gray goat	**Gray (Dilute)	33	32	1	0	98.48%	1.52%
Leizhou Black goat	Black	31	30	1	0	98.39%	1.61%
Liaoning cashmere goat	White	35	35	0	0	100.00%	0.00%
Nanjiang Brown goat (Black *strain*)	Black	49	27	18	4	73.47%	26.53%
Nanjiang Brown goat (Fast grow *strain*)	Brown (Dilute)	34	16	16	2	70.59%	29.41%
Nanjiang Brown goat (High fertility *strain*)	Brown (Dilute)	35	20	11	4	72.86%	27.14%
Tangshan dairy goat	White	27	26	1	0	98.15%	1.85%
In total	-	304	221	68	15	83.88%	16.12%

Note:* Not strict, several individuals exhibited mottle or other colors.

MATERIALS AND METHODS

Goat population used and PCR amplification

In this study, 304 goat from 9 breeds/strains with detailed coat color record were used. They were distributed in Hebei province, Sichuan province, Shandong province, Hainan province, Liaoning province and Chongqing city in China. The individual number of each population and their geographical locations are shown in Figure 1 and Table 1, respectively.

Genomic DNA from blood sample of goat was isolated according to the standard phenol : chloroform extraction method. According to the goat sequence information previously obtained (Genebank accession number: EU316218), the primers (Forward: 5' GATGCTGTTAGGATCTTTAGG 3' and reverse: 5' CTGGGTGTTTGGTCTGCTG 3') were designed to amplify a 680

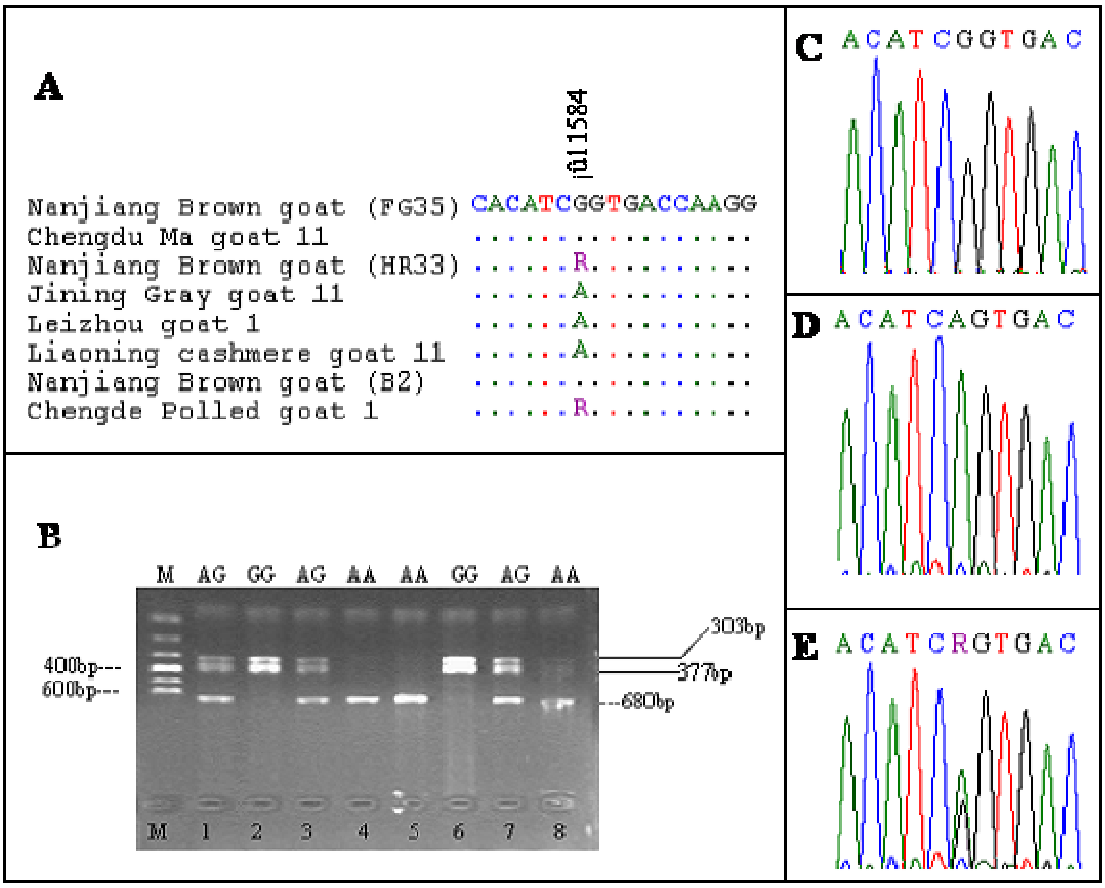

Figure 2. Alignment of varied individuals, SNP identification and PCR-RFLP by EcoO65 I. Note: A = Alignment of varied individuals; B = Result of PCR-RFLP by EcoO65 I. M refers to DNA Marker. Lanes 4, 5, and 8 illustrate genotype AA with bands of 680 bp. Lanes 1, 3 and 7 illustrate genotype AG with bands of 680 bp, 303 bp, and 377 bp, respectively. Lanes 2 and 6 illustrates genotype GG. C, D and E are SNP identification for genotype GG, AA and AG (R), respectively.

bp fragment that contained exon 10.

PCR amplification was carried out in a PTC-100TM PCR instrument (MJ Research, Inc., Massachusetts, USA) with a total reaction volume of 50 µL solution containing 150 ng DNA, 400 pmol/L each forward and reverse primer, 2×GC buffer from ZEXING BIOTECH Co., Ltd (Beijing, China), 200 pmol/µL dNTPs and 2 U Taq DNA polymerase from TIANGEN BIOTECH Co., Ltd (Beijing, China). The PCR protocols followed was: denaturizing at 95°C for 4 min, followed by 35 amplification cycles comprising denaturizing at 94°C for 30 s, annealing at 56°C for 30 s and extension at 72°C for 1 min, followed by an extended elongation at 72°C for 10 min. PCR product was detected on 1.5% agarose gel including 0.5 µg/mL of ethidium bromide, photographed under UV light, and sequenced by Shanghai Sangon Biological Engineering Technology Biological and Technology and Service Co., Ltd. (Shanghai, China).

PCR-RFLP of goat *MLPH* and genotype determination

In order to identify the SNP of goat *MLPH* gene, eight sequenced individuals were aligned by ClustalW program as implemented in BioEdit software (Version 7.0.5.2). The transition of g.*11584A>G* (according to EU316218) was defined after alignment and checking for sequencing results (A, C, D and E in Figure 2). The PCR-RFLP was carried out according to this variation site. EcoO65 I (TaKaRa Biotechnology Co., Ltd. Dalian, China) recognizing GGTNACC and cutting at position 11584 (position 303 according to the 680 bp PCR product) was selected using Webcutter 2.0 (http://users.unimi.it/~camelot/tools/cut2.html). The digestion solution with a total volume of 10 µl containing 6.5 µl of PCR products, 0.3 µl (5 U/µl) of EcoO65 I, 1.2 µl of distilled water 1.0 µl 0.1% BSA and 1.0 µl of 10×buffer, was incubated at 37°C for 3 h in the programmable thermal controller. Genotype was detected by running digested products on 1.5% agarose gel including 0.5 µg/ml of ethidium bromide. Homozygote AA was defined (one 680 bp band) when base A existed at position 11584 forming AGTGACC not recognized by EcoO65 I (B in Figure 2). Homozygote GG was defined (one 303 bp band and one 377 bp band) when base G existed at position 11584 forming GGTGACC recognized by EcoO65 I and heterozygote CT was defined (one 680 bp band, one 303 bp band and one 377 bp band) when allele A and G both existed at the same position of the homologous chromosomes.

Statistical analysis

PopGene32 software (Version 1.32) was used to do Hardy-Weinberg equilibrium test for g.*11584A>G*. The genetic diversity of each population was also estimated using PopGene32 software. The genetic differentiation of g.*11584A>G* among different

Table 2. The heterozygosis of *g.11584A>G* in different goat breeds.

Breeds/ strains*	Sample size	Observed value		Expected values**		Nei's H***	Ne	F_{IS}	I
		Hom	Het	Hom	Het				
CDP	36	0.7222	0.2778	0.7574	0.2426	0.2392	1.3144	-0.1613	0.4029
CDM	24	0.6250	0.3750	0.5115	0.4885	0.4783	1.9168	0.2160	0.6713
JNG	33	0.9697	0.0303	0.9697	0.0303	0.0298	1.0308	-0.0154	0.0785
LZB	31	0.9677	0.0323	0.9677	0.0323	0.0317	1.0328	-0.0164	0.0826
LNC	35	1.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000
NJB	49	0.6327	0.3673	0.6061	0.3939	0.3898	1.6389	0.0577	0.5785
NJF	34	0.5294	0.4706	0.5786	0.4214	0.4152	1.7101	-0.1333	0.6058
NJH	35	0.6857	0.3143	0.5988	0.4012	0.3955	1.6543	0.2054	0.5847
TSD	27	0.9630	0.0370	0.9630	0.0370	0.0364	1.0377	-0.0189	0.0922
In total	304	0.7796	0.2204	0.7314	0.2686	0.2682	1.3664	0.1782	0.4389

*Breeds/strains: CDP = Chengde Polled goa; CDM = Chengdu Ma goat; JNG = Jining Gray goat; LZB = Leizhou Black goat; LNC = Liaoning cashmere goat; NJB = Nanjiang Brown goat (Black strain); NJF = Nanjiang Brown goat (Fast grow strain); NJH = Nanjiang Brown goat (High fertility strain); TSD = Tangshan dairy goat. **Expected homozygosity and heterozygosity were computed using Levene (1949); *** Nei's (1973) expected heterozygosity; The Ne = effective number of alleles; F_{IS} = Wright's fixation Index; I = Shannon information index.

populations was also investigated.

RESULTS AND DISCUSSION

SNP of goat *MLPH* gene and its distribution in different populations

According to the alignment of 8 sequenced individuals, the substitution of g.*11584A>G* was detected. Genotyping results using PCR-RFLP with EcoO65 I for individuals in different populations are listed in Table 1. The results showed that allele A was superior. Frequencies of allele G ranged from 0 - 39.58%, with the four breeds/strains inhabited in Sichuan province and Chongqing city, which were adjacent in the western mountain area in China, having the highest G frequencies: Chengdu Ma goat 39.58%, Nanjiang Brown goat (Fast grow strain) 29.41%, Nanjiang Brown goat (High fertility strain) 27.14% and Nanjiang Brown goat (Black strain) 26.53%. And the homozygous GG was also only found in the four breeds /strains, indicating that the allele G might be a recessive site responsible for the specific brown coat color (different from other dilute coat colors) of Chengdu Ma goat and Nanjiang Brown goat. It is known that Nanjiang Brown goat black strain is a new strain selected for black coat color and separated from the other two strains with brown color without significant allele G frequency difference. Considering that the SNP *g.423G>T* found in *Agouti* Gene exon 4 exhibited significant allele T frequency difference between black strain and fast grow strain (91.41% VS 75.66%) (Tang and Li, 2007), it could be inferred that coat color of the black strain was not affected by the SNP here and most likely due to the variation of *g.423G>T* in *Agouti* Gene as Chunjuan Tang and Xianglong Li reported (2007).

For the site of *g.11584A>G*, only allele A was found in Liaoning cashmere goat, while allele G exhibited very low gene frequencies, without existence of GG genotype, in Jining Gray goat, Leizhou Black goat and Tangshan dairy goat.

Generally, goat coat color was controlled by the following loci: agouti locus, locus of color dilution factor, locus of the interaction white, and white spot-locus as well as extension locus (Chang, 1999). For locus of color dilution factor, Chang (1999) showed that two alleles, dominant D controlling dark color and recessive allele d diluting dark coat color to diluted grey, existed at this locus. An epistatic gene "I" at the locus of interaction white could result in the white coat phenotype in goat. The exiguous allele G content conserved the selected black coat color breed (Leizhou Black goat) in decades. Two white breeds (Liaoning cashmere goat and Tangshan dairy goat) were consistent with the epistatic gene "I" theory. Kinds of coat colour were observed in Jining Gray goat and no one single locus could clearly account for that so far. Likelihood ratio (G2) tests and Chi-square test for Hardy-Weinberg equilibrium via POPGENE software (Version 1.32) showed that the 9 subpopulations were all in equilibrium at 0.05 rejection level.

Genetic diversity and differentiation of *g.11584A>G* in different populations

The results of genetic diversity for each population are summarized in Table 2. The observed heterozygosity ranged from 0.0000 (Liaoning cashmere goat) to 0.4706 (Nanjiang Brown goat fast grow strain). The Levene and Nei's expected heterozygosity ranged from 0.0000 (Liaoning cashmere goat) to 0.4885 (Chengdu Ma goat) and 0.0000 (Liaoning cashmere goat) to 0.4783 (Chengdu Ma goat), respectively. The two kinds of

expected heterozygosity accorded with each other well, with little difference from observed heterozygosity in the nine Chinese goats breeds/strains. Chengdu Ma goat, Nanjiang Brown goat (High fertility strain) and Nanjiang Brown goat (Black strain), the three of four breeds/strains having the highest allele G frequencies and inhabiting adjacently in the western mountain area in China, showed plus values of Wright's fixation index, manifesting a deficiency of heterozygosis in these breeds /strains, which might be due to their inbreeding history. The relative degree of genetic variation of *g.11584A>G* for each population could be shown as CDM>NJF>NJH>NJB> CDP>TSD>LZB>JNG >LNC based on the Shannon index value shown in Table 2, which was basically the same with that based on *g.423G>T* in goat *Agouti* gene exon 4 (Li et al., 2007). It was noticed that most of the goat breeds investigated were poor in genetic diversity at *g.11584A>G*. This might be due to the directional artificial selection for coat color. The genetic diversity of this mutation site was lower than that based on goat mtDNA (0.676 ± 0.191) (Li and Valentini, 2004).

ACKNOWLEDGEMENT

This work was funded by Natural Science Foundation of Hebei (C2010000775) and development foundation of scientific research of Agricultural University of Hebei.

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