Journal of Animal and Veterinary Advances 10 (7): 894-899, 2011
ISSN: 1680-5593

Analysis on the Genetic Variation of *KAP3.1* and *KAP6.1* Gene in 6 Rabbit Populations

[1]Wu Tian-Wen, [2]Li-Ting, [3]Feng-Kai, [1]Mao-Liu-Liu, [1]Li Bi-Chun and [1]Wu Xin-Sheng
[1]Animal Science and Technology College, Yangzhou University, 225009 Yangzhou, China
[2]Jinling Rabbit Farm, 211103 Nanjing, China
[3]Rabbit Farm of the Institute of Animal Science and Veterinary,
Fujian Academy of Agricultural Science, 350003 Fuzhou, China

Abstract: Researchers had scanned the complete CDS of rabbit *KAP3.1* and *KAP6.1* gene for the 1st time by PCR-SSCP and detected that their homology with mouse, chimpanzee, human, sheep and goat were >80%, suggesting that the two genes among different species kept high conservation in evolution. In this study, three genotypes and two alleles were found at KAP3.1-1 locus having one silent mutation (C91T) and there were two alleles and three genotypes in 5 un-translated region of *KAP6.1* gene with the A 755 G mutation of the enhancer region. Herein, the distribution of these genotypes in all populations was consistent with the Hardy-Weinberg equilibrium and showed intermediate or low diversity. The result suggested a theoretical reference to the breeding of rabbit wool quality whether *KAP3.1* and *KAP6.1* gene could be a molecular marker or not.

Key words: Rabbit, *KAP3.1* gene, *KAP6.1* gene, genetic variation, Hardy-Weinberg equilibrium, China

INTRODUCTION

In the wool type animal, its wool is an important economic trait. Scanning the candidate gene relatived to animal wool quality has been the target of many studies. Most of current researches focused on human and sheep and it was few studies of rabbits that restricted the rabbit breeding greatly.

As the fundamental composition of pelage, Keratin-Associated Proteins (KAPs) have been classified as ultra-high-sulfur, high-sulfur and glycine/tyrosine-rich proteins. In human, the number of individual KAPs appears to be very large (>80). KAPs are currently divided into 23 families, the majority of which has been characterized in human (Powell and Rogers, 1996; Rogers *et al*., 2001, 2002). KAPs can be subdivided into three groups based on their chemical properties: the high sulfur KAPs (<30 mol % cysteine content) containing the KAP1-KAP3, KAP10-KAP16 and KAP23 families; the ultrahigh sulfur KAP (>30 mol % cysteine content) containing the KAP4, KAP5, KAP9 and KAP17 families and the high glycine/tyrosine KAP containing the KAP6-8 and KAP18-22 families. Most of genes are small fragments without introns and the size is between 0.6~1.5Kb generally (Hui-Qin and Mei, 2007). Currently, studies on *KAP* gene family focused on human and sheep except rabbit. In this study, CDS sequences of the *KAP3.1* and *KAP6.1* gene were obtained and the genetic variation were detected for 6 rabbit populations. The study made a theoretical reference to the breeding of rabbit wool quality whether *KAP3.1* and *KAP6.1* gene could be a molecular marker or not.

Genomic DNA extraction and PCR condition: About 718 individuals were randomly selected. There were 60 Minxinan Black rabbits, 72 Jiuyishan rabbits, 209 Wanxi and 133 Suxi Angora rabbits, 143 Chinchilla and 101 White Rex rabbits. Minxinan Black rabbit and Jiuyishan rabbit were Chinese indigenous rabbit breeds. Wanxi and Suxi Angora rabbit were of coarse wool type Angora rabbit. Based on Germany Angora rabbit and New Zealand White rabbit, Wanxi Angora rabbit was bred by direct cross, backcross, grading, transversely breed and strict selection in Anhui academy of agricultural science. A formal appraisal was passed on October 1991 and Wanxi Angora rabbit was considered as the first coarse wool type Angora rabbit strain in China (HuiLing, 1996). Suxi Angora rabbit was bred by Jiangsu academy of agricultural science Germany containing the genealogy of France Angora rabbit, Germany Angora rabbit, Germany SAB rabbit and New Zealand White rabbit. Genomic DNA was extracted from ear tissues according to Molecular Cloning (Sambrook and Russell, 2001). PCR amplification was carried out in a mixture

Corresponding Author: Wu Xin-Sheng, Animal Science and Technology College , Yangzhou University, 225009 Yangzhou, China

Table 1: The information of primer sequences

Locus	Primer sequence	Product size (bp)	Annealing temperature (°C)
KAP3.1-1	5'-AAGCATTGAGACACCAAGACAT-3' 5' -AGCAGGATGGCACATAGATTTC-3'	257	58
KAP3.1-2	5'-TGAGCCTGAAATCTATGTG-3' 5'-ATGGTGTAGGCAGAAGC-3'	221	58
KAP6.1-1	5'-GATTCTCCCACTGTGATTGTCT-3' 5'-GAGGAGGAATGTAGGTGACTTG-3'	252	64
KAP6.1-2	5'-TACATTCCTCCTCCTTCTCCAC-3' 5'-CCTGGGGTTCAGAACTGTTCAA-3'	347	60

containing 1 μL DNA template (100 ng μL^{-1}), 2 μL 10×PCR Buffer, 1.5 μL dNTP (10 m mol L^{-1}), 1 μL of each primer (10 μmol L^{-1}) and 0.2 μL Taq DNA polymerase (5 U μL^{-1}). Double-distilled water was added to a final volume of 20 μL. After a denaturing step of 5 min at 94°C, samples were processed through 35 cycles of 40 sec at 94°C, 40 sec at an optimal annealing temperature and 40 sec at 72°C then the last elongation step was at 72°C for 10 min. After PCR an aliquot of 2 μL of PCR product was mixed with 7 μL denaturing solution in cubated at 98°C for 10 min and then chilled on ice. Denatured DNA was loaded on 10% PAGE gel in 1×TBE buffer with constant voltage 120 V for 14~16 h.

Primers and PCR amplification: About 4 pairs of primers were designed based on the *KAP3.1* and *KAP6.1* gene sequence of *Mus musculus*, human and rabbit in Genebank. Full-length CDS of *KAP3.1* gene was amplified by *KAP3.1-1* and *KAP3.1-2* primers, *KAP6.1-1* and *KAP6.1-2* primers were used to amplify full-length CDS of *KAP6.1* gene. All of the primers were synthesized by the Shenggong biological engineering technology company. The information of primers was shown in Table 1.

Statistical analysis: Genotype frequency, allele frequency, Polymorphism Information Content (PIC), expected Heterozygosity (He) were calculated by Microsoft Excel according to Botstein *et al.* (1980). The distribution of these genotypes among all rabbit populations was analyzed using χ^2-test (χ^2-test calculator software v1.51). Align software was used for sequence alignment and SNP loci screening.

RESULTS AND DISCUSSION

Sequencing of rabbit *KAP3.1* and *KAP6.1* gene and its homology relationship with other species: All of the PCR amplification products were detected by 2% agarose and obtained the expected fragments with the clear and specific bands. One rabbits *KAP3.1* gene sequence (449 bp) and two *KAP6.1* gene sequences (585 and 584 bp) had been acquisited by bi-directional sequencing and sequence assembly in cluding 297 bp of the *KAP3.1* gene complete CDS and 237bp of the KAP6.1 complete CDS. For the *KAP3.1* gene, the homology were respectively 88, 84 and 83% between rabbit and mouse, chimpanzee, human. Compared with human, mouse, sheep and goat, the homology of rabbit *KAP6.1* gene sequence were 91, 90, 83 and 80%, respectively. The sequences had been submitted to the GeneBank database (HM147283, HM147284, HM068868 and HM068869).

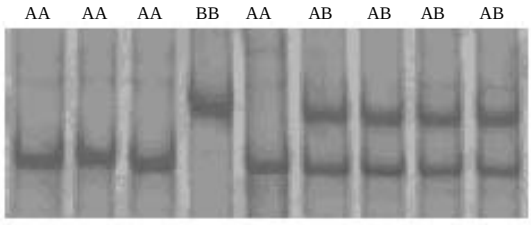

Fig. 1: The PCR-SSCP PAGE patterns of KAP3.1-1 locus

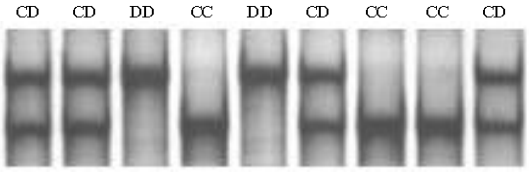

Fig. 2: The PCR-SSCP PAGE patterns of KAP6.1-1 locus

SSCP typing: The *KAP3.1* and *KAP6.1* gene were scanned by PCR-SSCP in 6 rabbit populations. The results showed that 3 genotypes and 2 alleles were found at both KAP3.1-1 and KAP6.1-1 loci and no mutation was found at KAP3.1-2 and KAP6.1-2 loci. Researchers named the alleles of KAP3.1-1 locus as A and B (Fig. 1), those of KAP6.1-1 locus as C and D (Fig. 2). According to the sequencing and sequence alignment, researchers found that C91T was a silent mutation in coding region at KAP3.1-1 locus (Fig. 3). Compared to the rabbit sequence in Genebank (M95718), 5 insertion mutations (675 (T), 689 (T), 692 (T), 711 (C) and 792 (T)) were found in both C and D alleles (Fig. 4) at KAP6.1-1 locus and there were an insertion mutation (694 (G)), a transition mutation (G 744 C), a transversion mutation (A 755G) between C and D allele (Fig. 5 and 6). About 6 basic radicals (CTATGG) were deleted at the position 1036-1041 of KAP6.1-2 locus. So that the original amino acid sequence is Gly (ggt) instead of Gly-Tyr-Tly (ggctatggt) in this study (Fig. 7).

Analysis of the genetic diversity of KAP3.1-1 and KAP6.1-1 loci in 6 rabbit populations: At KAP3.1-1 locus, A allele was a preponderant allele and the distribution of these genotypes in all populations was consistent with the Hardy-Weinberg equilibrium. About 5 rabbit populations were presented as low diversity (PIC<0.25) except for Suxi Angora rabbit with no diversity (Table 2).

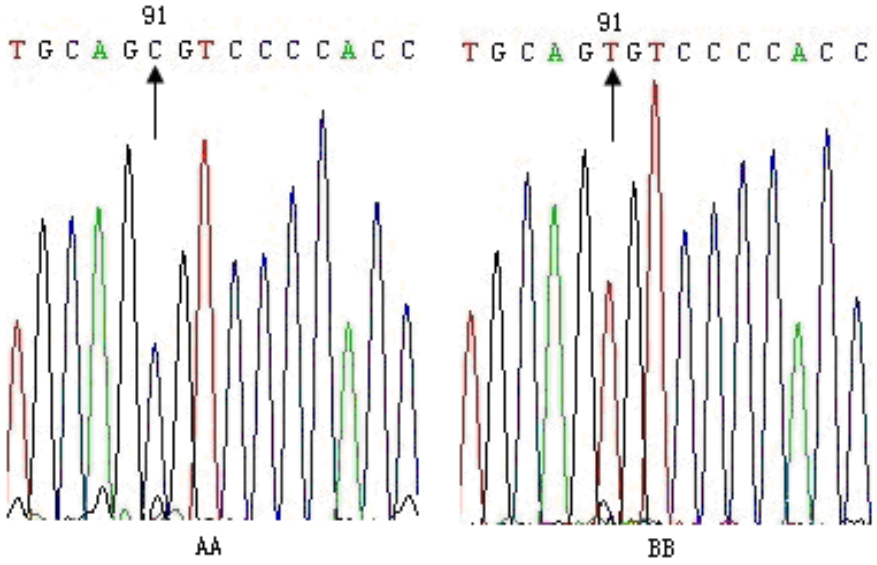

Fig. 3: The sequencing result of different genotypes at KAP3.1-1 locus

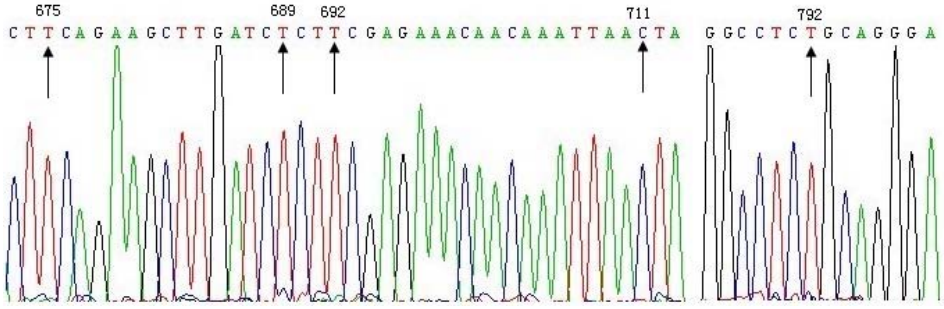

Fig. 4: The sequencing result of KAP6.1-1 locus

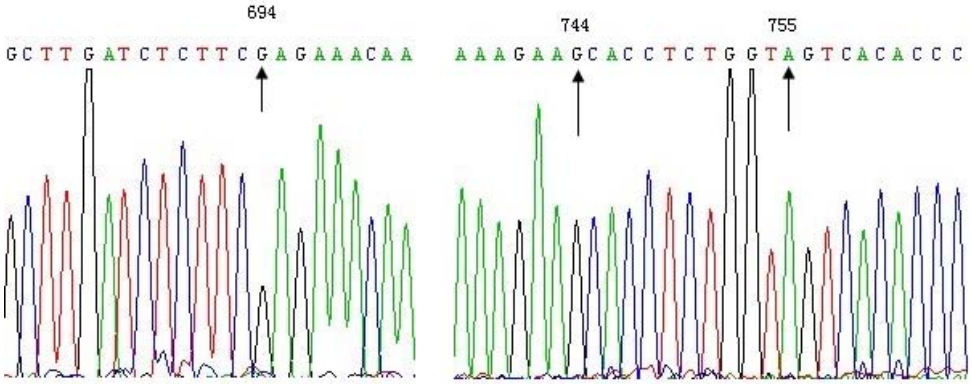

Fig. 5: The sequencing result of AA genotypes at KAP6.1-1 locus

There was a significant or very significant difference ($p<0.05$ or $p<0.01$) among each other population except for Minxinan Black rabbit and Jiuyishan rabbit, Minxinan Black rabbit and Wanxi Angora rabbit, Chinchilla and White Rex rabbit, Jiuyishan rabbit and White Rex rabbit, Jiuyishan rabbit and Wanxi Angora rabbit ($p>0.05$) (Table 3). At KAP6.1-1 locus, all of 3 genotypes were found in Minxinan Black rabbit and Jiuyishan rabbit but only CC and CD genotypes were in Chinchilla and White Rex rabbit and CC genotypes in Wanxi and Suxi Angora rabbit. The He and PIC value of Minxinan Black rabbit (He = 0.339 and PIC = 0.281) were higher than other

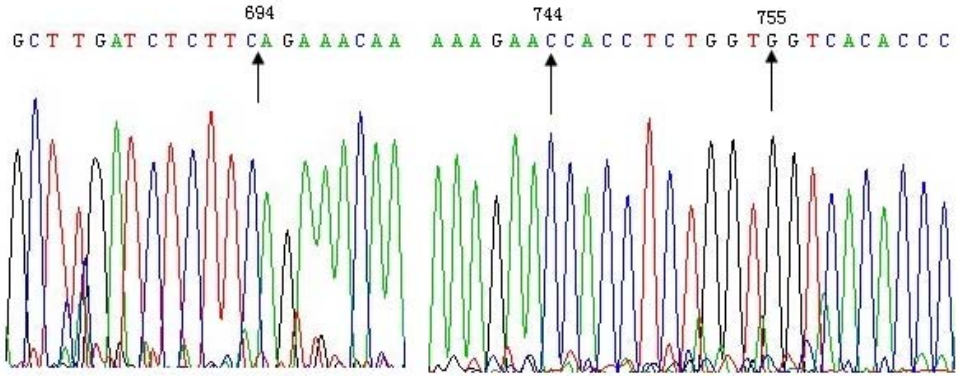

Fig. 6: The sequencing result of BB genotypes at KAP6.1-1 locus

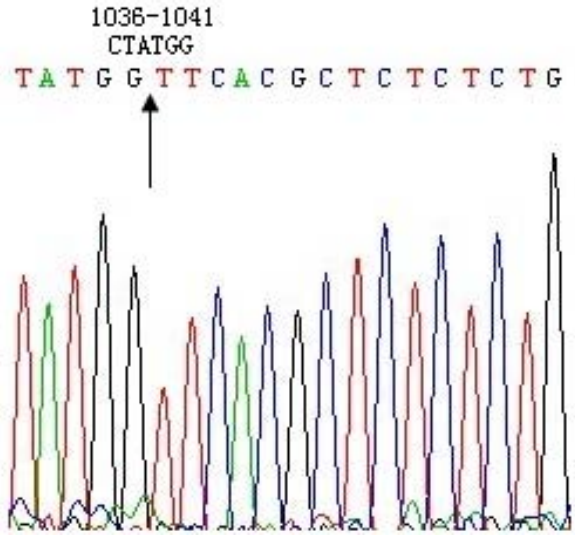

Fig. 7: The sequencing result of KAP6.1-2 locus

populations and presented as intermediate diversity while other populations showed low or no diversity. The distribution of these genotypes in all populations was consistent with the Hardy-Weinberg equilibrium. There was a significant or very significant difference ($p<0.05$ or $p<0.01$) among each other population except for between Minxinan Black rabbit and Jiuyishan rabbit, Wanxi and Suxi Angora rabbit ($p>0.05$). The results were shown in Table 4 and 5.

For recent years, studies on the KAP gene family has become a hot spot. Zhao (2008) detected the polymorphism of *KAP6.2* gene in the cashmere and found two alleles. A 24 bp deletion mutation was described for the first time in Shaanbei cashmere, three alleles at the KAP1.1 locus and 9 nine alleles at the KAP1.3 locus were detected in Merino sheep.

Shibuya *et al.* (2004) identified multiple unique sequences in the 21q22.3 region and predicted them to be a cluster of genes encoding hair-specific Keratin-Associated Proteins (KAPs), each *KAP5* gene showed about 50% homology (from 46-52%) except for 84% homology between hKAP5.4 and 5.5. Jun-Xing (2007) detected KAP6.1 mRNA expression by TQRT-PCR in two goat breeds (LiaoNing cashmere goat and KeLan local goat).

The results showed that mRNA differential expression can be found in the two breeds. Liu Gui-Fen (2007) found that AA and BB genotypes are significantly correlated with fine wool quality ($p<0.05$) among the high-glycine-tyrosine Keratin Associated-Protein (KAP6.1). The expression of *KAP6* and *KAP8* gene family in the cortex of sheep and human has a section of the vertical direction whereas, they are uniform distributed in mouse (Shimomura and Ito, 2005). In this study, researchers obtained the complete CDS of rabbit *KAP3.1* and *KAP6.1* gene and scanned them by PCR-SSCP for the first time. Compared with mouse, chimpanzee, human sheep and goat, both their homology were >80% in dicating that the genes of different species kept high conservation in evolution process.

At KAP3.1-1 locus, PIC of all populations were low but the PIC of meat-type rabbit (Minxinan Black rabbits, Jiuyishan rabbits) were higher than rex and angora rabbit at KAP6.1-1 locus. Resarchers deduced that it was because that angora and rex rabbit were originated in the meat-type rabbit and long-term selection process may result in decreased frequency of D allele at KAP6.1-1 locus but had no effect on KAP3.1-1 locos. Gene expression is the process in which an biologically active protein was synthesized after transcription and translation. It was based on the environment of individual normal growth and development.

Nucleotide variation especially variation of the coding region may affect the expression and regulation of protein. In this study, C91T mutation was found at KAP3.1-1 locus and was a silence mutation (AGC-AGT). However, some literatures had been reported that although silence mutation could not result in the change of encoding amino acid but it may affectted the transcription processing, changed splice signal

Table 2: The characteristic of KAP3.1-1 locus in 6 rabbit populations

		Genotype frequency			Allele frequency				
Populations	Samples	AA	AB	BB	A	B	He	PIC	χ^2
Minxinan black rabbit	60	0.933 (56)	0.007 (4)	0.000 (0)	0.967	0.033	0.064	0.062	0.00
Chinchilla rex rabbit	143	0.769 (110)	0.203 (29)	0.028 (4)	0.871	0.129	0.225	0.200	0.31
White rex rabbit	101	0.772 (78)	0.208 (21)	0.020 (2)	0.876	0.124	0.217	0.194	0.03
Jiuyishan rabbit	72	0.903 (65)	0.097 (7)	0.000 (0)	0.951	0.049	0.093	0.089	0.00
Wanxi angora rabbit	209	0.962 (201)	0.038 (8)	0.000 (0)	0.981	0.019	0.037	0.036	0.00
Suxi angora rabbit	133	1.000 (133)	0.000 (0)	0.000 (0)	1.000	0.000	0.000	0.000	0.00

df = 1, χ^2 (0.01) = 6.63, χ^2 (0.05) = 3.84, df = 2, χ^2 (0.01) = 9.21, χ^2 (0.05) = 5.99 the same as follow table

Table 3: Chi-square (χ^2) test of the distribution of KAP3.1-1 locus genotypes in all populations

Population	Chinchilla rex rabbit	White rex rabbit	Jiuyishan rabbit	Wanxi angora rabbit	Suxi angora rabbit
Minxinan black rabbit	7.89*	7.20*	0.40	0.88	9.05**
Chinchilla rex rabbit		0.17	6.25*	31.27**	34.86**
White rex rabbit			5.47	27.80**	33.59**
Jiuyishan rabbit				3.68	13.39**
Wanxi angora rabbit					5.21*

Table 4: The characteristic of KAP6.1-1 locus in 6 rabbit populations

		Genotype frequency			Allele frequency				
Populations	No. of sample	CC	CD	DD	C	D	He	PIC	χ^2
Minxinan black rabbit	60	0.633 (38)	0.300 (18)	0.067 (4)	0.784	0.216	0.339	0.281	0.26
Chinchilla rex rabbit	143	0.867 (124)	0.133 (19)	0.000 (0)	0.934	0.066	0.123	0.116	1.03
White rex rabbit	101	0.970 (98)	0.030 (3)	0.000 (0)	0.985	0.015	0.030	0.029	0.00
Jiuyishan rabbit	72	0.806 (58)	0.153 (11)	0.041 (3)	0.882	0.118	0.208	0.187	1.65
Wanxi angora rabbit	209	1.000 (209)	0.000 (0)	0.000 (0)	1.000	0.000	0.000	0.000	0.00
Suxi angora rabbit	133	1.000 (133)	0.000 (0)	0.000 (0)	1.000	0.000	0.000	0.000	0.00

Table 5: Chi-square (χ^2) test of the distribution of KAP6.1-1 locus genotypes in all populations

Population	Chinchilla rex rabbit	White rex rabbit	Jiuyishan rabbit	Wanxi angora rabbit	Suxi angora rabbit
Minxinan black rabbit	18.91**	32.88**	4.95	83.46**	55.04**
Chinchilla rex rabbit		7.68**	6.31*	29.35**	18.98**
White rex rabbit			13.34**	6.27*	4.00*
Jiuyishan rabbit				42.77**	27.76**
Wanxi angora rabbit					0.00

sequence and some important base of the promoter region, reduced the conformation stability of mRNA and regulated gene expression finally (Kimchi-Sarfaty *et al*., 2007; Duan *et al*., 2003; Chamary *et al*., 2006).

CONCLUSION

The basic components of *KAP6.1* gene at 5 un-translated region (CAAT box, enhancer, CACCC box, TATA box) were similar to Ovis aries (Xu-Dong *et al*., 2007) so, we speculated that two kinds of animals had the same expression mechanism.

There were 6 basic radical deletion at position 1036-1041 (CTATGG) in KAP6.1-2 locus compared to the rabbit sequence in Genebank (M95718) an insertion mutation (694 (G)), a transition mutation (G 744 C), a transversion mutation (A 755G) between C and D allele in KAP6.1-1 locus. It was possible that the mutation had reduced the gene expression of KAP6.1 at the position of 755 in the enhancer region. According to the description in NCBI, the 5 flanking region of *KAP6.1* gene not only belonged to KAP6.1 but also to KAP7 and KAP8 high glycine/tyrosine genes so that those variations could also influence the gene expression of KAP7 and KAP8. Further study will be conducted on the relationship between all of the mutation positions and the rabbit wool quality.

ACKNOWLEDGEMENT

This study was supported by the earmarked fund for Modern Agro-industry Technology Research System (Grant no. nycytx-44).

REFERENCES

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Chamary, J.V., J.L. Parmley and L.D. Hurst, 2006. Hearing silence: Non-neutral evolution at synonymous sites in mammals. Nat. Rev. Genet., 7: 98-108.

Duan, J., M.S. Wainwright, J.M. Comeron, N. Saitou, A.R. Sanders, J. Gelernter and P.V. Gejman, 2003. Synonymous mutations in the human dopamine receptor D2(DRD2) affect mRNA stability and synthesis of the receptor. Hum. Mol. Genet., 12: 205-216.

Hui-Qin, K. and J. Mei, 2007. Research progress of cashmere proteome differential expression in domestic and overseas. AnHui Agric. Sci. Bull., 13: 46-47.

HuiLing, Z., 1996. Wan-III line angora rabbit. Anhui Agric., 3: 18-18.

Jun-Xing, Z., R. You-She and Y. Wen-Bin, 2007. Differential expression of KAP6.1 gene in two goat breeds. Grass Feed. Livestock, 4: 4-6.

Kimchi-Sarfaty, C., J.M. Oh, I.W. Kim, Z.E. Sauna, A.M. Calcagno, S.V. Ambudkar and M.M. Gottesman, 2007. A silent polymorphism in the MDR1 gene changes substrate specificity. Sciences, 315: 525-528.

Liu, G.F., K.C. Tian, E.P. Zhang, X.X. Huang and Y.H. Zhang, 2007. Candidate gene analysis of high quality merino sheep. Hereditas, 29: 70-74.

Powell, B.C. and G.E. Rogers, 1996. The role of keratin proteins and their genes in the growth, structure and properties of hair. Exp. Suppl. Format. Struct. Hum. Hair, 78: 59-148.

Rogers, A.M., L. Lutz, W. Hermelita, E. Claudia, P. Silke and S. Jurgen, 2002. Characterization of a first domain of human high glycine-tyrosine and high sulfur keratin-associated protein (KAP) genes on chromosome 21q22.1. J. Biol. Chem., 277: 48993-49002.

Rogers, M.A., L. Langbein, H. Winter, C. Ehmann, S. Praetzel, B. Korn and J. Schweizer, 2001. Characterization of a cluster of human high/ultrahigh sulfur keratin-associated protein genes embedded in the type i keratin gene domain on chromosome, 17q12-21. J. Biol. Chem., 276: 19440-19451.

Sambrook, J. and D.W. Russell, 2001. Molecular Cloning: A Laboratory Manual. 3rd Edn., Cold Spring Harbor Laboratory Press, New York, pp: 49-56.

Shibuya, K., I. Obayashi, S. Asakawa, S. Minoshima, J. Kudoh and N. Shimizu, 2004. A cluster of 21 keratin-associated protein genes within introns of another gene on human chromosome 21q22.3. Genomics, 83: 679-693.

Shimomura, Y. and M. Ito, 2005. Human hair keratin-associated proteins. J. Investigative Dermatology Symposium Proc., 10: 230-233.

Xu-dong, G., M. Shu-Yan, B. Ming-Tao,Y. Jun and B. Shorgan, 2007. Molecular cloning of 5 flanking region of ovine keratin associated protein 6-1 gene and comparison of the sequences. Scientiarum Naturalium Universitatis NeiMongol, 38: 58-63.

Zhao, M., X. Wang, H. Chen, X.Y. Lan and Y.K. Guo *et al*., 2008. The PCR-SSCP and DNA sequencing methods detecting a large deletion mutation at KAP6.2 locus in the cashmere goat. Small Ruminant Res., 75: 243-246.